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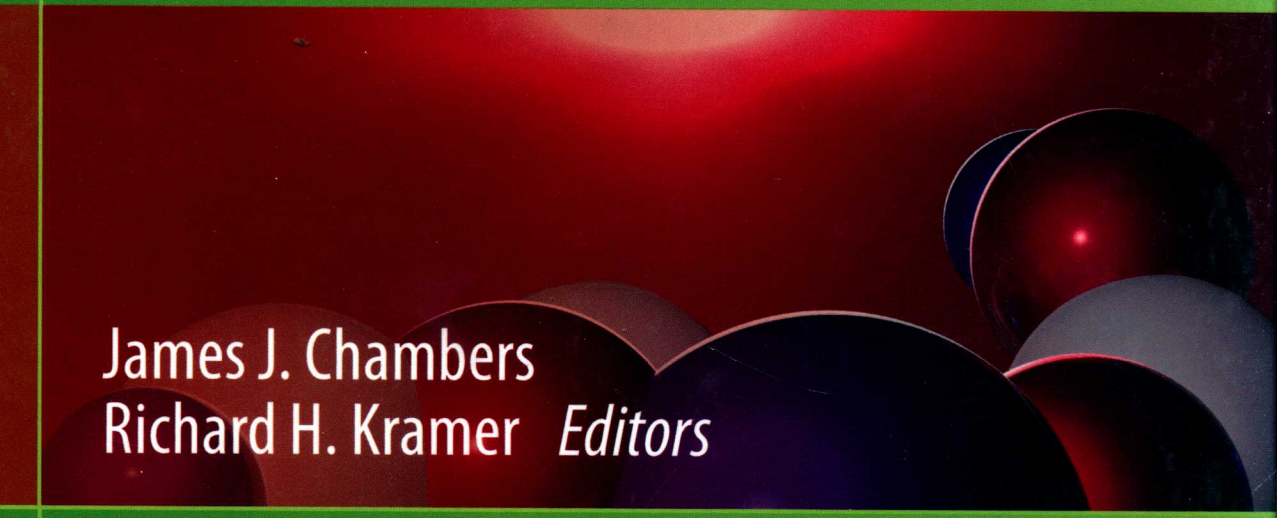

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Photosensitive Molecules for Controlling Biological Function

Humana Press

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NEUROMETHODS™

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Preface to the Series

Under the guidance of its founders Alan Boulton and Glen Baker, the Neuromethods series by Humana Press has been very successful since the first volume appeared in 1985. In about 17 years, 37 volumes have been published. In 2006, Springer Science + Business Media made a renewed commitment to this series. The new program will focus on methods that are either unique to the nervous system and excitable cells or which need special consideration to be applied to the neurosciences. The program will strike a balance between recent and exciting developments like those concerning new animal models of disease, imaging, in vivo methods, and more established techniques. These include immunocytochemistry and electrophysiological technologies. New trainees in neurosciences still need a sound footing in these older methods in order to apply a critical approach to their results. The careful application of methods is probably the most important step in the process of scientific inquiry. In the past, new methodologies led the way in developing new disciplines in the biological and medical sciences. For example, Physiology emerged out of Anatomy in the nineteenth century by harnessing new methods based on the newly discovered phenomenon of electricity. Nowadays, the relationships between disciplines and methods are more complex. Methods are now widely shared between disciplines and research areas. New developments in electronic publishing also make it possible for scientists to download chapters or protocols selectively within a very short time of encountering them. This new approach has been taken into account in the design of individual volumes and chapters in this series.

Wolfgang Walz

Preface

We have entered into a new and exciting era in the field of neurobiology. Myriad optical methods are changing the way neurobiological research is performed. Tried and true electrophysiological techniques are being challenged for their place on the stage of measuring and manipulating neuronal activity. This change is occurring rapidly and is in large part due to the development of new photochemical tools, some synthesized by chemists and some provided by nature. This book is focused on the three main classes of photochemical tools for the control of biological function. First, natural photoresponsive proteins, including channelrhodopsin-2 and halorhodopsin, can be exogenously expressed in cells and enable rapid photocontrol of action potential firing or silencing. Second, small molecule photosensitive protecting groups (cages) of neurotransmitters, including caged glutamate, are synthetic molecules that enable highly localized activation of neurotransmitter receptors in response to light. Third, synthetic small molecule photoswitches can also afford light sensitivity on native or exogenously expressed proteins, including K^+ channels and glutamate receptors, allowing photocontrol of action potential firing and synaptic events. These tools have developed at a rapid pace and are continuously being improved upon and new tools being introduced thanks to the powers of molecular biology and synthetic chemistry. The three families of photochemical tools have different capabilities and uses, but they all share in enabling precise and noninvasive exploration of neural function with light.

Beginnings

In the early days, neurophysiologists invented electrodes to learn about native electrical excitability and the functioning of neural circuits. However, it soon became apparent that the nervous system is much too complex to rely entirely on recordings from one, two, or even several neurons at a time. Even within an individual neuron, membrane potential and ion concentrations are certainly not homogeneous, limiting the usefulness of electrode-based methods that record from a single point in a cell. At least in theory, optical-based recording methods could provide a much more detailed view of the activities, either within the complex architecture of an individual neuron or across populations of neurons. The hunt for optically based neurophysiological methods was on.

The first breakthrough came from the development of optical methods for *monitoring* activity. Investigators developed a wealth of fluorescent dyes that report back on voltage, synaptic vesicle release, Ca^{2+} fluctuations, and other ions. These indicators opened new windows for observing different aspects of neuronal signaling within individual neurons and in neural circuits. Small molecule indicators, most notably for Ca^{2+}, have revolutionized our understanding of normal synaptic transmission. More recently, genetically expressed GFP-based indicators have been introduced. These reporter proteins have provided insights into many aspects of signal transduction. The search for new indicators continues at a fast pace, but there is still much room for improvement. Perhaps the most

pressing need is for a genetically expressed voltage indicator that can resolve single action potentials in individual neurons that are part of a native circuit. At the same time, new developments in microscopy are allowing investigators to peer into neural tissue deeper, faster, and with better spatial resolution than ever before, allowing us to see various aspects of neural activity in real time, and, more importantly, in vivo.

Until recently, optical methods for *manipulating* neural activity lagged behind methods for measuring activity. Recently, there has been a torrent of photochemical tools that can be used for controlling neurons, and these tools are the subject of this book. Most of the tools developed to date can be placed in one of three categories: *natural photosensitive proteins, caged neurotransmitters*, and *small molecule photoswitches* that bestow light sensitivity on ion channels and receptors. Each family of tools has its own unique advantages and limitations. When asking a particular neurobiological question, it is important to "choose the right tool for the right job." This book offers unprecedented access to the state-of-the-art for each tool, but it is important to note that this is a rapidly developing field, and we are cataloging the available toolkit at a moment in time, knowing full well that new tools with improved properties and different functionalities are right around the corner. Available at http://www.photobio.org

The Right Tool for the Right Job

It has been suggested that neurobiologists need a "Consumers Guide" to provide an unbiased comparison of the various photochemical tools currently available for controlling neuronal activity. The reality is that all of the tools covered in this book have merits. However, choosing the right tool depends entirely on the specific question and experimental system that is being explored.

A Common Challenge for All Photochemical Tools: Delivering Light to the Nervous System

All of the photochemical tools described in this book require the effective delivery of light to the part of the nervous system being targeted for control. Projecting light onto neurons in culture or in brain slices is straightforward, but delivering light onto neurons in vivo presents a major challenge. The brain is encased in an opaque cranium that presents a formidable barrier, physically and optically. Even after removal of cranial bone and the overlying dura, brain tissue tends to scatter light, and this limits spatial precision and makes it more difficult to affect structures far from the illuminated surface.

The retina is the one part of the nervous system that is normally exposed to light, making it a useful platform for testing photochemical tools. Of course, the retina is an interesting and important part of the central nervous system in its own right, and there is great clinical interest in developing tools that can impart light sensitivity on retinal neurons that are not normally photosensitive. Retinitis pigmentosa and macular degeneration are degenerative blinding diseases in which the normal rod and cone photoreceptors are destroyed, leaving the retina with no effective way to signal the visual cortex about light. Expression of ChR2 in either retinal ganglion cells or bipolar cells can restore visual sensitivity to retinas of animals with mutations that cause rods and cone degeneration.

Expression of melanopsin or halorhodopsin is also effective. Photoregulation of all of these tools require high intensity light, and azobenzene-based photoswitches require short wavelength illumination, which can be damaging over a prolonged time. For these reasons, there is a need for red-shifted photochemical tools that also have enhanced light sensitivity. Nevertheless, these studies provide hope that some neurological disorders might be treatable in a relatively noninvasive manner, using light to regulate activity in the parts of a neuron circuit that lie downstream from sites of damage or degeneration.

Despite the obvious difficulties, bioengineers have succeeded in delivering light into the brain with implanted fiber optics. Fiber-coupled systems have been used for optical measurement or manipulation of neural activity. Recent studies raise the possibility of substituting light for electrodes in "deep brain stimulation," a procedure that is being used increasingly for treatment of Parkinson's disease and other neuropsychiatric disorders.

Finally, the delivery of light for neural control involves an important but rarely discussed trade-off between effectiveness and precision. On one hand, a highly localized optical stimulus that illuminates part of a single neuron could ensure exclusive stimulation of only that cell. On the other hand, the light-regulated proteins are usually distributed over much of the cell surface, and more widespread illumination will activate more of these proteins resulting in a faster and more powerful effect. There has been considerable interest in developing photosensitive molecules that are highly sensitive to 2-photon illumination, because this would permit deeper and more precise photocontrol in neural tissue. However, the benefits of pinpoint accuracy will be offset by the asynchronous recruitment of photoactivated proteins as the 2-photon laser scans through a given focal plane within the tissue. New optical methods involving holographic illumination may help solve this problem by allowing simultaneous activation of distributed photosensitive molecules, with spatial and temporal precision that rivals 2-photon liberation of caged glutamate.

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Contents

Part I

Photoreactive Small Molecules for Affecting Biological Function

Chapter 1

Introduction to Part I: Caged Neurotransmitters

James J. Chambers and Richard H. Kramer

Abstract

The field of organic chemistry has provided neurobiologists with the ability to release biologically active neurotransmitters at precise locations and times of their choosing. These molecules are silent before the active molecule is released by photolysis, thus allowing for very accurate measurements of biological responses when temporally accurate data is required. Some of the newest caging groups have provided the added benefit of two-photon sensitivity, thus allowing for not only time, *x*-, and *y*-dimensional precision but now *z*-direction as well.

Key words: Caged neurotransmitters, Photorelease, Photolabile protecting groups, Two-photon excitation

Caged molecules contain a photolabile protecting group that is removed by exposure to light, liberating a biologically active compound. The most widely used caged molecules in the field of neurobiology have been caged agonists for neurotransmitter receptors, although studies have also utilized caged calcium buffers, caged nucleotides, and even caged peptides that can be used to influence intracellular signal transduction pathways. The first caged neurotransmitter agonists were ortho-nitrobenzyl derivatives of carbamoylcholine, an activator of acetylcholine receptors that was released in response to ultraviolet light exposure. These molecules enabled a rapid increase in agonist concentration in response to the externally supplied light, leading to a better understanding of the kinetics of acetylcholine receptor activation. But, it was the development of caged glutamate that truly revolutionized the use of caged neurotransmitters and, to this day, continues to have major impacts on neurobiology. Dalva and Katz were the first to use a laser to locally release glutamate in an intact brain slice. Laser-induced photorelease of glutamate at presynaptic neurons revealed that the pattern of connections to

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visual cortical neurons changes during development, a finding that would have been difficult, if not impossible, to obtain without local glutamate photorelease.

Unfortunately, however, light scattering inherently limits the spatial precision of laser photorelease. This problem motivated the development of caged molecules that could be readily photolysed by two-photon excitation, a method that can pinpoint in three-dimensional space the photorelease of neurotransmitter to individual neurons and *even individual dendritic spines.* MNI-caged glutamate (4-methoxy-7-nitroindolinyl-caged L-glutamate) has a fairly favorable two-photon cross-section, and because of this, it is now the most popular form of caged glutamate in neurobiology. Adding to its usefulness, MNI-caged glutamate has a very low rate of spontaneous glutamate liberation in the dark and the free "cage" that is formed as a photolytic reaction byproduct has no apparent effect on neuronal function. Two-photon release of MNI-glutamate has been used to trigger electrical responses that simulate the kinetics and magnitude of individual synaptic events on single dendritic spines. Fortunately, abundant and highly active glutamate transporters rapidly remove the liberated glutamate, minimizing spillover onto neighboring spines. Photo release at single spines allows for direct comparison of spine geometry and postsynaptic responsiveness, allows precise measurement of spatial summation across neighboring spines, and removes any ambiguity in attributing plastic changes in synaptic function to the presynaptic vs. the postsynaptic cell.

Highly localized and rapid photorelease of glutamate requires very bright light and a high concentration of caged compound (millimolar range). These requirements do present potential problems of phototoxicity and off-target effects on other receptors. The development of new flavors of caged glutamate with even more favorable two-photon cross-section may help alleviate these problems. At the same time, investigators are developing forms of caged glutamate that can be readily and rapidly released by exposure to visible light. These molecules are beneficial because the optical instrumentation required for their use is significantly simpler, less expensive, and widely available. However, two-photon sensitive caged molecules are still the best-suited reagents for ensuring spatial and temporal precision.

Caged versions of many other neurotransmitters have also been synthesized including new visible light-sensitive and two-photon-sensitive forms of caged GABA, glycine, and anandamides for local activation or inhibition of endocannabinoid receptors. Novel molecular tools for studying intracellular signaling include various types of caged Ca^{2+}, a caged IP_3 that is two-photon sensitive, and caged peptides that interfere with synaptic vesicle exocytosis.

A different type of photosensitive compound can irreversibly disrupt the function of certain types of glutamate receptors in response to light. ANQX is an azide-containing analog of commonly used AMPA receptor antagonists (e.g. CNQX and DNQX). Exposure of bound ANQX to UV light results in a high-energy species and then covalently attaches to the AMPA receptor, permanently preventing the binding of glutamate or other agonists. ANQX has been useful for probing the turnover of AMPA receptors in synapses between hippocampal neurons, a process that is thought to play a crucial role in long-term synaptic plasticity and learning and memory. So far, studies utilizing ANQX have been limited to neurons in culture, but compounds with different properties, including perhaps a more favorable two-photon cross-section and solubility profile, could enable ANQX to reveal receptor trafficking in more intact preparations including brain slices. Photocrosslinker-containing derivatives of antagonists of other neurotransmitter receptors might be used in a similar manner to explore receptor turnover and its possible activity dependence.