Part I

Optogenetics in Model Organisms
1 Introduction to Optogenetics: From Neuronal Function to Mapping and Disease Biology

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In our quest to move the enterprise of science forward we should never forget that we do not know where this long march is taking us or what we will need to get there.


The brain, a highly heterogeneous organ, consists of many individual cell types. Understanding the properties of these cell types is critically important in forming a collective appreciation of how the brain works, and how various diseases and disorders arise when the components that constitute the brain are impaired or their normal functions altered. Toward this end, it is vital to develop and employ methods for precisely manipulating specific cell types within the intact brain. For hundreds of years, humanity has tried to understand the nervous system. Since the pioneering electrophysiology studies of Italian scientist Luigi Galvani in the late eighteenth century (1791), in which he developed the theory of electrical excitation of neurons while studying the muscles in frogs, advances in techniques, methods and knowledge have brought us closer to understanding the mechanisms governing neuronal activity (Goldensohn, 1998). In 1979, Francis Crick suggested that the major challenge facing neuroscience was the need to control one type of cell in the brain while leaving others unaltered. He later speculated:

One of the next requirements is to be able to turn the firing of one or more types of neuron on or off in the alert animal in a rapid manner. The ideal signal would be light, probably at an infrared wavelength to allow the light to penetrate far enough. This seems rather far-fetched but it is conceivable that molecular biologists could engineer a particular cell type to be sensitive to light in this way. (Crick, 1979, 1999)

He proposed that a light might have the necessary properties to serve as a control tool, but no one had experimentally demonstrated it.

It took almost three decades to obtain experimental evidence, which was provided for the first time in 2002 by Gero Miesenböck (then at Yale, now at Oxford), (Zemelman et al., 2003), and in 2005 by Edward Boyden (then at Stanford, now at MIT) and Karl Deisseroth (of Stanford) using the microbial opsin proteins discovered by the German scientists Ernst Bamberg, Peter Hagemann and Georg Nagel.
(Boyden et al., 2005). In this context, it is very important to mention the origin and biology of these opsins, as they significantly contributed to the development of the new field of optogenetics. In the early 1970s, bacteriorhodopsin (a microbial equivalent of the vertebrate rhodopsin protein), a retinal-containing 7-helical transmembrane protein, was first discovered in the archaeal halophiles by Walther Stoeckenius and Dieter Oesterhelt, both then at the University of California, San Francisco (Oesterhelt and Stoeckenius, 1971). In the early 1980s, James Spudich and his colleagues identified these bacterial proteins as the light sensors (each tuned to a different wavelength) that produce complex photactic behavior in the extremophiles, which includes ionic homeostasis, energy storage, phototaxis, flagellar motor rotation and photosynthesis (Bogomolni and Spudich, 1982; Spudich, 2006).

Although not the focus here, it is worth mentioning that the biochemistry of both bacteriorhodopsin and bovine rhodopsin were significantly studied for a period of two decades using synthetic biology approaches by my post-doctoral mentor Nobel laureate H. Gobind Khorana’s colleagues and several others (Sakmar, 2002; Kim et al., 2005). During this time, in the early 2000s, scientists from Germany, including Ernst Bamberg, Peter Hegemann (then at the University of Regensburg, now at the Humboldt University of Berlin), Georg Nagel (then at University of Frankfurt) and their colleagues, discovered channelrhodopsins (ChR1 and ChR2), another class of microbial light-gated ion channel proteins, and demonstrated their use in studying rapid behavioral responses in excitable cells of Caenorhabditis elegans (Nagel et al., 2002, 2003, 2005). These bacterial proteins act as a single-component ion pump that can be briefly activated by photons of green light – a remarkable all-in-one molecular machine (Deisseroth, 2010).

As mentioned to me by Edward Boyden in a private conversation (in the context of organizing a theme conference on the topic of optogenetics) in the summer of 2012, quoting his paper (Boyden, 2011), during 2004, Karl Deisseroth, a young physician-scientist in Robert Malenka’s lab at Stanford, teamed up with his graduate student Feng Zhang and Edward Boyden (who was also a graduate student in Jennifer Raymond and Richard Tsien’s lab at Stanford) and in collaboration with the previously described German team that discovered channelrhodopsin, introduced ChR2 into mammalian cell cultured neurons for the first time. Using pulses of visible light, they were able to attain reliable, millisecond-precision control over the patterns of firing of action potentials in the cells (Boyden et al., 2005). In 2005, two seminal articles (Lima and Miesenböck, 2005; Boyden et al., 2005) launched the era of optogenetics. This groundbreaking American–German collaborative work laid the foundations for the new field of optogenetics. Soon, several other groups had also demonstrated the same phenomenon in other model organisms. Most of these bacterial proteins were later proven to turn neurons on or off in response to light or photons. Since then, many groups have developed optogenetic tools, pushed opsin molecular engineering forward, and revolutionized our ability to study the processes in neuronal circuits, all with the promise of potential new...
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approaches for the treatment of different diseases (Montgomery et al., 2016; Vann and Xiong, 2016; Song and Knopfel, 2016; Hunter, 2016).

The term ‘optogenetics’ first appeared in a mini-review (Deisseroth, et al., 2006), and several definitions of optogenetics exist: Ernst Bamberg, a German biochemist, defines optogenetics as “the use of genetically encoded light-activated proteins for manipulation of cells in an almost non-invasive way by light,” (Adamantidis et al., 2015) whereas Michael Häusser, a British neuroscientist, felt that “any approach that combines optical interrogation with genetic targeting qualifies as optogenetic, and that includes the use of genetically encoded activity sensors” (Adamantidis et al., 2015). Richard Tsien, an American neuroscientist, considered optogenetics to be the “use of genetically encoded molecules to excite and inhibit neurons” (Adamantidis et al., 2015). However the definitions vary, optogenetics is now more than ten years old, and it follows from the Nobel techniques such as Patch-Clamp (by German biophysicist Erwin Neher and German physiologist Bert Sakman) and the Nobel discovery of fluorescent proteins (by American biochemists Osamu Shimomura, Martin Chalfie, and Roger Tsien) that revolutionized the fields of neurophysiology and biological imaging. Although optogenetics arose from neuroscience, it addresses a much broader unmet need in the study of biological systems that extends beyond neuroscience to muscle, cardiac, and embryonic stem cells. Disease models have also been explored, including for Parkinson’s disease, anxiety, retinal degeneration, respiration, addiction, and depression. It is hoped that a combination of viral optogenetic therapy and optical-fiber implants could provide treatments to combat specific neural abnormalities in neurodegenerative diseases. The present book details the historical perspectives of the field of optogenetics, its use in model organisms, the technology platform, applications in neurobiology for psychiatric and behavioral diseases, synaptic plasticity, and the restoration of vision, as well as its application for conditions relating to memory, learning and sleep.

Scope of This Book

This text consists of thirty-two chapters, grouped into six parts, starting with the biology and techniques, leading to descriptions of the applications as follows:

Part I: Optogenetics in Model Organisms

This section consists of five chapters. As Zemelman describes in the second chapter, directed manipulation of brain function can be traced back decades. Specifically, working with large mammals and primates, Delgado demonstrated that many properties attributed to the primitive brain – sleep, nurture, hunger, aggression – could be modified by stimulating narrowly circumscribed groups of neurons, including by “remote control” (Delgado 1964). Consequently, these studies motivated the performance of localized electrical stimulation to diagnose, condition, and treat human subjects suffering from behavioral and psychiatric disorders. Crick’s prediction that “the ability to probe defined groups of neurons with [light] will hold the key to an understanding of neural systems” succinctly
framed the revolution in experimental neuroscience that followed fifteen years later. Subsequently, Zemelman’s initial and seminal experiments on studying ion currents in *Drosophila melanogaster* in the presence of retinal and visible light laid the foundation for what several years later became the field of optogenetics (Zemelman and Miesenböck, 2003). *Caenorhabditis elegans* is one of the best-suited model organisms in which to use light stimulation to control gene expression. Most importantly, because of its transparent body, stimulatory light is readily delivered to the target cells, its “connectome biology” is well known, but the neuronal dynamics of its neural circuit signaling have not yet been mapped out. Therefore, in Chapter 3, Kimura and Busch describe the functional studies of chemical synapse function *in vivo*, using optical interrogation studies to understand the functional circuitry, and behavioral analyses. The complete “wiring diagram” of the nervous system in *C. elegans* is highly conserved, and exhibits a rich repertoire of quantifiable behavior and simple forms of associative learning. In fact, *C. elegans* was the first animal in which optogenetic tools based on microbial rhodopsins were successfully implemented and behavior was remotely controlled (Nagel et al., 2005). In Chapter 4, Nagpal highlights the diversity of optogenetic tools, primarily the actuators that have been applied in *C. elegans* and the insight they have provided in the field of neuroscience.

*Xenopus laevis* (the African clawed frog) is an important model organism for the study of developmental biology due to its exceptional resistance to infection and the external development of its eggs in larger numbers. Importantly, its oocytes are used as expression systems in which to determine the characteristics of channels and pumps, thus techniques for successful expression of exogenous ion translocators are well established. In Chapter 5 Adams et al. show that optogenetics can be useful for studying ion-flux dependent developmental processes *in vivo*, and demonstrated the potential utility of optogenetics for regenerative medicine. They describe the use of the light-activated hydrogen pump Archaerhodopsin to initiate full regeneration of a complex vertebrate appendage, especially to study the role of $V_{\text{mem}}$ during normal processes and the role of misregulation of $V_{\text{mem}}$ in disease.  

**Part II: Opsin Biology, Tools, and Technology Platform**

This section consists of six chapters. The proton-pumping and anion-pumping rhodopsins have been known for a long time. Therefore, it is natural that in addition to channelrhodopsin which is used to fire neurons, proton and chloride pumps were also used as optogenetics tools to silence neurons. For different reasons, sodium and, in particular, potassium light-driven pumps were long-sought tools of optogenetics. However, the cation (sodium) pumps were only described in 2013. In Chapter 6, Shevchenko et al. describe a family of sodium light-driven pumps, their functional, structural properties, and the applications of potassium-pumping proteins in optogenetic experiments. In combination with the light-activated channelrhodopsin 2, which is a well-known molecular off-switch, the KR2 potassium pump would form a perfect pair of tools for the
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precise control of nerve cell activity; those are described in detail by Shevchenko et al. in Chapter 6. Optogenetics was combined with electrophysiology in order to evaluate the effects on neuronal activity. Since the field of electrophysiology spans many different preparations, from tissue culture to live animals, different technical approaches had to be incorporated or invented when combined with optogenetics. Therefore, Katz and Lampl (Chapter 7) review the current methods for simultaneous electrophysiology and optogenetics in vivo. Depending on the electrophysiological requirements, different probes for both extracellular recordings, and intracellular recordings were developed.

During development, fundamental neuronal circuits are formed by a genetically defined program, and the fine connections are modified by neuronal activity (Katz and Shatz, 1996). Such refinement processes are essential to establish functional connectivity in the brain. A key issue is how neuronal activity influences axonal growth and branching. To address this issue, it is essential to manipulate the firing activity of the axons that are observed morphologically. Electrical stimulation with fine electrodes has been widely used for the manipulation. However, it is difficult to specify which cells or fibers are stimulated via the conventional method. The optogenetic method can be adopted to overcome this problem. This method is useful not only for stimulus-based physiological experiments, but also for morphological and developmental studies. An interesting question is how different patterns of spontaneous neuronal activity affect axonal growth, branching, and the refinement of neural circuits in general. The remote control of neuronal activity by light combined with morphological observation provides a new avenue for investigating the implication of activity in neuronal circuit formation. Thus, in Chapter 8, Malyshnevskaya and Yamamoto demonstrate the methodological efficiency of the optogenetic technique for developmental studies of neuronal circuit formation, focusing on axonal growth of cortical neurons which was regulated by firing activity.

In recent years, many different tools have been developed that use light to influence protein activity by regulating synthesis, localization, activity, or stability, and these have been described in depth in several reviews. In Chapter 9, Renicke and Taxis discuss practical aspects of using the different psd module variants in yeast, and provide guidelines for experimental design and applications. Overall, photoactuators from the optogenetic toolbox offer ample opportunities to influence at the molecular level and to interfere with regulatory processes in a non-invasive way. Over the last decade, the use of seven-transmembrane opsin proteins as optogenetic tools has been extended to structurally similar proteins, including archaeal halorhodopsin. Folcher (Chapter 10) reviews the recent advances in the bioengineering of photo-activatable nucleotide cyclases, especially secondary messenger-based optogenetic actuators, in the context of their fields of application. It is possible to fine-tune secondary messenger signalling at the single cell level or in a whole animal experiment by expressing the optogenetic transgene under the control of a tissue-specific promoter. Moreover, as described elegantly by Folcher, the knowledge-based protein engineering strategies targeting enzyme activities or domain organization have further broadened the possible strategies...
for adapting optogenetic devices to specific application scenarios. Folcher is hoping to develop an implantable, wirelessly piloted machine-cell interface, based on a light communication channel, which will become an innovative strategy for tomorrow's therapeutics.

Comprehensive interrogation of neuronal circuits requires acute as well as chronic manipulations of spatially defined subpopulations as well as entire populations dispersed over the brain. Thus, there is a need to combine optogenetic and chemogenetic approaches to allow the use of both modes of interrogation in the same brain circuit, and ideally through the same actuator molecule, thereby facilitating the comprehensive study of neuronal systems. Although a number of groups have presented the combined toolbox of chemogenetic and optogenetics to address pertinent questions, in Chapter 11 Hochgeschwender introduces the basic building blocks for bioluminescence-driven optogenetics. These novel classes of tools can be improved and extended in numerous ways and find applications beyond photonic control of neurons in modifying many cell types and cellular processes.

Part III: Optogenetics in Neurobiology, Brain Circuits, and Plasticity
This section consists of five chapters. Compared to electrical stimulation, optical stimulation offers the potential for superior spatial control over what portion of brain tissue is affected by the stimulation. As with electrical stimulation, optical stimulation can be applied without the pharmacological side effects and dosing considerations that invariably accompany drug interventions. Current neuromodulation therapies for epilepsy may be a good first choice for the application of optogenetics to a central nervous system disorder. In Chapter 12, Kaemmerer describes his group’s modified approaches to deep-brain stimulation. Over the past couple of decades, it has become increasingly clear that astroglia play a fundamental role in neurotransmission, not only by fuelling it and removing the waste, but also by integrating, amplifying, and modulating neuronal signals. Since astrocytes express a wide variety of receptors for neurotransmitters and can release gliotransmitters in response to stimulation, they are intimately linked with local neuronal activity. In Chapter 13, Teschemacher and Kasparov summarize the invaluable role of optogenetic approaches in the study of astrocytes and their natural signaling modes.

In recent years, viral techniques have been combined with optogenetics, which allowed study of the modifications of activity in different brain regions and circuits. Recently, several studies have focused on viral/optogenetic manipulation of “non-classical” neurotransmitters – neuromodulators and neuropeptides. In Chapter 14, Tang et al. provide an excellent overview of viral and transgenic techniques applied to specific targeting of hypothalamic neurons, expressing oxytocin–neuropeptide orchestrating social behavior. Using viral delivery methods in combination with optogenetics, Tang et al. have achieved the physiology of peptidergic neurons and peptidergic signaling mechanisms in the brain. To date, 5 out of 100 neuropeptidergic brain circuits have only been explored by optogenetics; that leaves the opportunity to explore the fundamental cell biology of “post-synaptic” signaling.
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The ability to manipulate specified cell types in a targeted manner is essential for decoding the contribution of individual neuronal populations in various physiological states. Deciphering patterns of functional synaptic connectivity is the main focus of both basic and clinical neurosciences. The evolution of optogenetic methods to target neurons and their circuits for activity manipulations has provided unprecedented opportunities to investigate functional connectivity in previously unimaginable detail. In Chapter 15, Herman et al. review the current optogenetic “toolbox” that is available to investigate brain circuitry, and further highlight the various genetic methods used to express optogenetic reporters with cell-type specificity. The mapping of neural circuits allows the high-throughput determination of the anatomical rules that govern the connections between the different nerve cells in a neural network. Although the invention of optical mapping of neural circuits pre-dated the advent of optogenetics by a decade, the use of optogenetic tools for circuit mapping did not come easily to the field. This was primarily because of the difficulty in localizing optogenetic excitation to the somato-dendritic compartment as opposed to axons and synapses. Accordingly, in Chapter 16, Kohl and Kätzel discuss strategies surrounding how this feature has either been harnessed to map long-range connections, or was overcome technologically to map local circuits.

Part IV: Optogenetics in Learning, Neuropsychiatric Diseases, and Behavior

This section consists of five chapters. Traditional methods have provided a strong groundwork for understanding the neural correlates of drug addiction. However, the development of optogenetic approaches has significantly advanced the study of addiction and reward-related learning by affording the ability to more closely interrogate neural circuitry and pathway-specific synaptic adaptations with far greater temporal and spatial precision than was possible in the past. In Chapter 17, Gutman and LaLumiere summarize the use of optogenetic techniques in understanding the role of the mesocorticolimbic system in learning, and to investigate this in the context of drugs of abuse. The contributions of optogenetic studies to the field’s understanding of the neural correlates of addiction will undoubtedly inform the identification and development of treatments for this disease. In the last sixty years, research into the biological basis of neuropsychiatric disorders has progressed tremendously because of technological precision in studying cellular neurobiology, advances in the scale of genome-wide studies, refined animal models, and the tuned dissection of neural circuits. However, with the success of deep-brain stimulation to treat major depressive disorder (MDD) and substance-use disorders – a technique that uses chronically implanted stimulating electrodes to specifically target brain regions in the reward system for electrical stimulation – there is an ever-evolving hypothesis that neuropsychiatric disorders are a neural circuit disorder (Volkow and Koob, 2015). Optogenetics has offered researchers the unique ability to selectively target genetically distinct cell populations or neural circuits for temporally precise stimulation or inhibition in awake and behaving animal models. This has shed light on the neural circuit mechanisms involved in depression and substance abuse. In Chapter 18, Juarez
et al. describe the optogenetics, in conjunction with validated animal models for neuropsychiatric disorders, which have dramatically altered how researchers approached and interpreted the study of depression and substance-use disorders.

In recent years, there has been a rapid expansion of insights into brain function related to reward learning and goal-directed behavior through the use of optogenetics technologies. In addition, we also have a sense of the brain circuits and neural dynamics related to reward and goal-directed behavior in learning and motivational processes, but little understanding of how specific patterns of neural activity or specific circuits control these behaviors. However, optogenetics has begun to help researchers circumvent this gap by employing highly precise interventions in neural activity as applied to specific questions. In Chapter 19, Crego et al. outline several advantages and considerations for using optogenetics in answering behavioral questions, especially in understanding the neural basis of goal-directed learning and reward-related behavior.

Epilepsy is one of the most common neurological disorders, affecting up to 60 million people worldwide and potentially occurring at all ages. Importantly, epilepsy is not a single disease, but is considered to be a family of disorders with different etiologies (Shorvon, 2011). Epilepsy was among the first diseases to be explored using optogenetics approaches to intercept the neural activity underlying seizures. Tønnesen et al. introduce optogenetic strategies for the first time to treat epilepsy, as described in Chapter 20, but they are still facing obstacles prior to initiating clinical trials. The optogenetic therapy for epilepsy does not seem to provide either a cure or disease modification; rather, it offers a prosthetic approach by suppressing seizures when they are about to occur or once initiated.

The major function of the nervous system in all higher organisms is to receive and process sensory information from the environment and, in turn, to produce an appropriate response to adapt to changes in the environment. In most cases, the elicited response takes the form of movement or ‘motor function’. Almost all human behavioral output, ranging from locomotion and articulated hand movement to speech and expression of emotions, is underpinned by motor functions. The neural circuits that govern motor function are extremely complex, and damage to almost any part of the motor system, either as a result of trauma or disease, can result in paralysis, i.e. an inability to control muscles. In Chapter 21, Bryson and Greensmith focus on two such insults that can critically affect the motor system: traumatic spinal cord injury and the progressive neurodegenerative disease, amyotrophic lateral sclerosis. Additionally, they emphasize the translational application of stem cell–based neural replacement techniques and the artificial control of motor function using optogenetics, as a therapeutic strategy to restore the lost motor function.

Part V: Optogenetics in Vision Restoration and Memory

This section consists of six chapters. The retina is our most complex sensory organ. Blindness often results from dysfunction of this complex neural network. At present, degenerative diseases of the retina remain incurable. Patients with
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these conditions suffer progressive visual decline resulting from the ongoing dysfunction or loss of retinal cells, with photoreceptors being particularly prone to degeneration.

One of the main goals of translational retinal research is to develop strategies to repair the retina. Given the great complexity of this tissue, any attempt to restore retinal function requires quantitative knowledge of retinal neural circuits as well as methods to target these circuits specifically. In Chapter 22, Nelidova and Esposti summarize the viral delivery of optogenetic tools to retinal cell types to restore natural vision. It is noteworthy that delivery of Adeno-associated virus to the retina of human patients has been shown to be safe in multiple clinical trials. All the pre-clinical reports thus far have demonstrated that optogenetic therapy is safe and is capable of inducing light-driven activity in mouse models of retina degeneration, irrespective of the cell type chosen for therapy. To date, the only therapy available for late-stage photoreceptor degeneration is based on electronic chips that are implanted in close proximity to the retina. The chips are costly and, due to their small size, recover vision only locally, with crude and non-physiological electrical activation. Because of this, many investigators have explored optogenetic therapies which collectively aim to render surviving inner retinal cells directly sensitive to light. Recently, optogenetic vision recovery has entered a new era, where light-sensing proteins are being engineered according to the need for a specific application. The potential advantages of optogenetic vision recovery treatment are that they are ambulant, long-lived, low-cost, and have the theoretical potential to recover high-resolution vision across the entire visual field. In Chapter 23, Sonja Kleinlogel discusses the use of such an optogenetic approach using opsin-based light sensors for potential vision restoration in blind patients suffering from photoreceptor degeneration.

Degeneration of photoreceptors (the cells responsible for the canonical image forming photo-transduction) is the cause of the two most common blinding diseases – retinitis pigmentosa and age-related macular degeneration – that affect millions of people worldwide. It would thus be highly beneficial to reintroduce light sensitivity, using optogenetic therapy in surviving cells, in order to restore vision. The use of genetically encoded light-activated proteins has revolutionized all fields of neuroscience by allowing the control of the excitation state of neurons with high spatial and temporal precision. These new powerful methods have had a major impact on every research field, and the optogenetic toolbox is still evolving as it becomes more highly optimized and diversified. In Chapter 24, Gauvain et al. describe pre-clinical tests in humans using optogenetics-based vision-restoration strategies. A retinal neuroprosthesis is a device aimed at providing an artificial sense of vision by translating visual scenes into appropriate spatio-temporal patterns of neuronal activity. A number of studies by several research groups have explored the fundamental feasibility of using ChR2 and other optogenetic probes in an optical retinal prosthesis, clearly suggesting that this technology may provide a viable path to vision restoration (Roska and Pepperberg, 2014). Combining optogenetics with advanced light projection technologies can potentially allow a relatively direct route toward non-contact...