Optogenetic mapping of brain circuitry

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ABSTRACT

Studies of the brain promise to be revolutionized by new experimental strategies that harness the combined power of optical techniques and genetics. We have mapped the circuitry of the mouse brain by using both optogenetic actuators that control neuronal activity and optogenetic sensors that detect neuronal activity. Using the light-activated cation channel, channelrhodopsin-2, to locally photostimulate neurons allows high-speed mapping of local and long-range circuitry. For example, with this approach we have mapped local circuits in the cerebral cortex, cerebellum and many other brain regions. Using the fluorescent sensor for chloride ions, Clomeleon, allows imaging of the spatial and temporal dimensions of inhibitory circuits in the brain. This approach allows imaging of both conventional "phasic" synaptic inhibition as well as unconventional "tonic" inhibition. The combined use of light to both control and monitor neural activity creates unprecedented opportunities to explore brain function, screen pharmaceutical agents, and potentially to use light to ameliorate psychiatric and neurological disorders.

Keywords: optogenetic, channelrhodopsin, Clomeleon, photostimulation, brain circuitry, local circuits, brain mapping, brain imaging

1. INTRODUCTION

One of the most fundamental challenges of human intellection is to determine how the brain works. This task is challenging because the brain consists of an enormous number of neurons (more than 100 billion) and these neurons form an ever larger number of synaptic connections (on the order of 100 trillion) that serve as the substrate for the circuits that process and store information within the brain.

To understand how the brain works, we need to map out the collective synaptic circuitry of the brain – termed the **connectome** - and to define how this circuitry functions to process and store information. We refer to this goal as **mapping the functional connectome**. The enormous challenges provided by the complexities of the functional connectome have not been met by conventional technical approaches.

Traditionally, neuroscientists have attempted to deduce brain circuitry via anatomic studies. While such studies describe the structure of neurons and their apparent synaptic connections, they offer no information on how brain circuitry functions. Physiological analyses can elucidate circuit function, but are greatly limited by our ability to stimulate defined populations of neurons and to record responses in their downstream targets.

Recent **optogenetic** approaches that merge **opti**cal and molecular **genetic** methods can tackle these challenges. Such optogenetic technologies provide powerful tools that are revolutionizing the study of brain circuitry. By taking advantage of genetic strategies that allow transgenes to be targeted to specific types of neurons, optogenetic approaches can unravel the tremendous cellular complexity of the brain. Because optogenetic probes either manipulate or report on neuronal function, they can also probe the functions of neurons and their synaptic connections. The recent development of two classes of optogenetic probes show great potential for functional mapping of brain circuitry (reviewed in refs. [1, 2]).

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2. OPTOGENETIC CONTROL OF NEURONAL ACTIVITY

One class of optogenetic probe is based on light-sensitive ion channels that allow optical control neuronal activity with remarkably high temporal and spatial control [3,4]. Specifically, the light-activated ion channel, channelrhodopsin-2 (ChR2), normally is found in the aquatic algae *Chlamydomonas*. Expression of this channel in neurons within the mouse brain [5-7] allows brief flashes of blue light to activate these neurons (Fig. 1A,B). Such "photostimulation" capability allows us to very selectively activate genetically-defined populations of neurons and thereby determine how the activity of these cells contributes to the function of brain circuits. Ever-improving versions of ChR2 offer enhancements in performance that increase the sophistication and power of optogenetic control of neuronal activity [8].

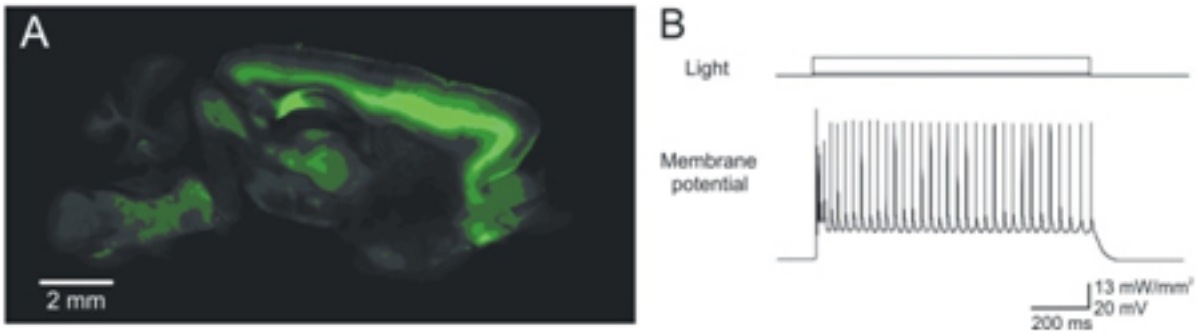

Figure 1. Optogenetic activation of neuronal activity. (A) Sagittal section through the brain of a ChR2 transgenic mouse; green color illustrates expression of ChR2 in various populations of neurons. (B) Illumination (top) of a neuron expressing ChR2 causes rapid depolarization and generation of a train of action potentials (bottom). From ref. [6].

By scanning small spots of light, it is possible to use ChR2 to map the location of presynaptic neurons innervating a given postsynaptic cell (Fig. 2). In contrast to conventional electrophysiological approaches, this optogenetic approach to circuit mapping is much more precise because the presynaptic neurons being stimulated are genetically defined. Equally important is that this approach is much more efficient: the map of cortical microcircuitry shown in Fig. 2C required approximately a half-day of experimental effort, while collecting the same data via conventional electrophysiology techniques would require approximately 1 year. Thus, optogenetic-based mapping is **high-throughput** because it increases experimental throughput rate by approximately 1,000-fold. In addition, this approach provides information about the spatial organization of presynaptic inputs; because the brain often processes information in a spatially precise manner, characterizing the spatial properties of circuits is fundamentally important. In sum, optogenetic circuit mapping provides unprecedented capabilities for mapping the brain functional connectome.

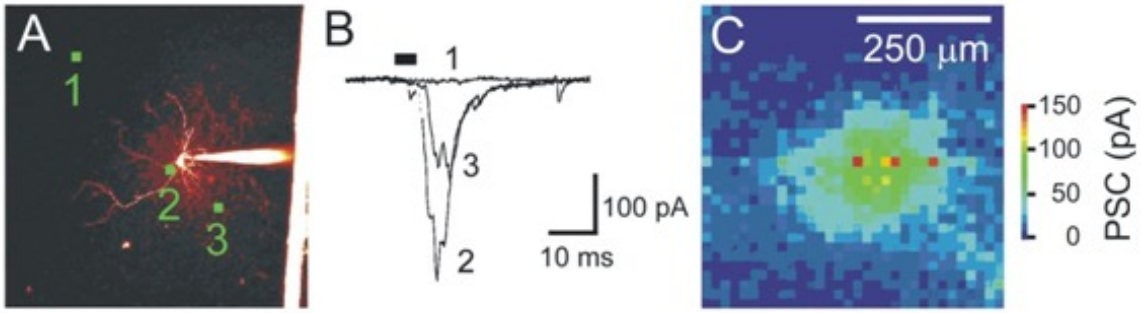

Figure 2 – Optogenetic circuit mapping. (A) Patch clamp recording from a dye-filled neuron. (B) Excitatory postsynaptic currents (PSCs) elicited by laser spots illuminating presynaptic neurons in the locations (numbered) indicated in (A). (C) Map of local excitatory circuitry, derived from measurements of the amplitude (pseudocolor scale) of PSCs evoked when the light spot was placed at each indicated position. From ref. [6].

3. OPTOGENETIC MONITORING OF NEURONAL ACTIVITY

A second, complementary set of optogenetic probes based on the green fluorescent protein have been developed to image neuronal activity [1, 2]. Perhaps the most popular optogenetic probes of neuronal activity are fluorescent reporters of intracellular calcium concentration. The rationale for using these to probe neuronal activity is that firing of action potential in neurons is associated with opening of voltage-gated calcium channels and a consequent rise in intracellular calcium concentration. Among many such probes, the most promising candidates are the GCaMPs [9] and GECOs [10]. In some cases, these probes reportedly have sufficient sensitivity to detect the firing of single action potentials in neurons. However, they are not appropriate for detecting subthreshold synaptic activity, in particular inhibitory synaptic responses. Other genetically-encoded fluorescent probes have been developed to monitor neuronal membrane potential, which changes during postsynaptic activity [1, 11-13]. While these sensors show great promise, at present their signal/noise limits detection of synaptic activity only under favorable circumstances.

Still other genetically-encoded fluorescent probes are valuable for monitoring the inhibition of postsynaptic neurons. Our laboratory has invented an important example of this type of probe: Clomeleon, the first genetically-encoded ratiometric indicator for chloride ions [14]. Clomeleon has been expressed in various populations of neurons in transgenic mouse lines [15] and has proven useful for a wide range of applications [16]. Such mouse lines allow fluorescent imaging of the spatial and temporal dynamics of inhibitory circuits in the brain (Fig. 3). Notably, detectable responses include not only conventional "phasic" synaptic inhibition, caused by signaling between neurons, but also unconventional "tonic" inhibition caused by communication between neurons and glial cells [17].

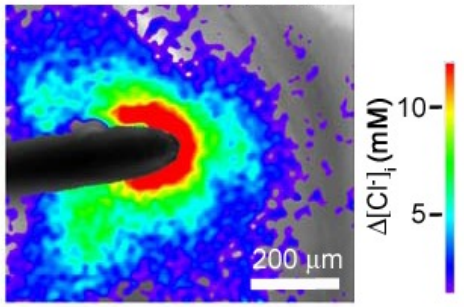

Figure 3 – Imaging synaptic inhibition with Clomeleon. Stimulation of presynaptic interneurons, via the electrode shown at left (black), causes a detectable increase in chloride concentration (pseudocolor scale at right) in postsynaptic CA1 pyramidal neurons. After ref. [15].

Regardless of the differences in signal modality that is detected, the use of optical techniques should improve the efficiency of data collection by at least two orders of magnitude in comparison to conventional electrophysiological approaches. During the time that is needed to record from a single postsynaptic neuron with electrophysiology techniques, on the order of 100-1000 neurons can be simultaneously imaged via an optogenetic sensor. Thus, optogenetic sensing also promises to improve throughput in brain functional mapping efforts. However, the properties of the current generation of optogenetic sensors are not optimal for this goal, so better probes must be developed and considerable effort is being in such pursuits.

4. CONCLUSIONS

It is clear that optogenetic probes are powerful enabling technologies that provide unprecedented vistas of brain function. Optogenetic actuators such as ChR2 allow temporally and spatially precise control of neuronal activity, while optogenetic sensors such as Clomeleon allow highly efficient parallel read-out of the activity of many neurons. While each of these probes is extremely valuable in their own right, we anticipate that combining both type of probe will prove decisive for mapping the functional connectome (Fig. 4). For example, simultaneously controlling the activity of a geneticallyp-defined population of presynaptic neurons with ChR2, while monitoring the resultant activation of postsynaptic via optogenetic activity probes such as Clomeleon will permit comprehensive optogenetic functional mapping of brain circuitry. In such studies, photoactivating a neuron expressing ChR2 will produce a response in a

Clomeleon-expressing neuron only if the two types of neurons are synaptically connected, thereby revealing a functional circuit. Because this optogenetic circuit-breaking strategy avoids the need for tedious electrophysiological recordings, a much higher rate of data acquisition is possible. Further, such experimental measurements require less technical training than required for electrophysiology, allowing more efficient manpower development. Together, these improvements in throughput will allow the database of brain circuit maps to increase at a rate that is orders of magnitude better (approximately 10^5-10^6 improvement) than has been possible in the past. Only such high-throughput approaches make it practical to decipher the vast complexity of the functional connectome.

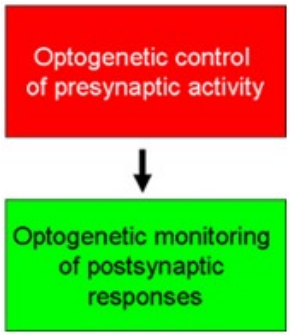

Figure 4 – Optogenetic circuit mapping strategy. Combining optical control of presynaptic activity with optical detection of postsynaptic responses enables high-throughput, neuron-specific circuit breaking.

Our laboratory is embarking on an effort to define the functional connectome of the mouse brain, a model system that offers the intrinsic complexity of a mammalian brain along with genetic tractability. Because of these advantages, the mouse has become a standard model system for studies of the mammalian brain. Although our goal is based in fundamental science, it is important to note that the project undoubtedly will yield information of immense practical application. For example, our optogenetic approach will allow subsequent definition of the changes in neural circuit function associated with various mouse models of human neurological and psychiatric disorders, termed "disease connectomics" [18]. Understanding the specific functional loci of these diseases will provide for much more reliable assays for drug screening, thereby providing many benefits for the pharmaceutical industry. Finally, it is also possible to imagine that optogenetic probes such as ChR2 could also provide a novel and more specific mechanism for "deep brain stimulation", an increasingly important therapeutic intervention for depression and other psychiatric disorders. Indeed, several start-companies have cropped up to exploit potential therapeutic opportunities associated with optogenetics [19].

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