The Dawning of Primate Optogenetics

Tamara K. Berdyyeva[1,*] and John H. Reynolds[1,*]
[1]Systems Neurobiology Laboratory, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037-1099, USA
*Correspondence: tamara@salk.edu (T.K.B.), reynolds@salk.edu (J.H.R.)
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In this issue of *Neuron*, Han and colleagues bring optogenetic methods into use in nonhuman primates by demonstrating the feasibility of achieving cell-type-specific photoactivation of macaque neocortical neurons. The use of optogenetic approaches in nonhuman primates promises to revolutionize our understanding of the neural circuitry that mediates complex cognitive functions.

Major scientific breakthroughs often stem from key technological advances. For example, the development of gene targeting techniques has revolutionized biology by providing a set of tools that can be used to visualize, measure, and systematically change the spatiotemporal processes that define living systems. Within neurobiology, these techniques have been used to study the detailed structure and function of neural circuits. In recent years, new optogenetic methods have made it possible to target and modulate the activity of genetically distinct groups of neurons with pulses of light (Luo et al., 2008; Zhang et al., 2007). Optogenetic methods often involve cell-type-specific expression of a light-responsive channel—channelrhodopsin-2 (ChR2), a transmembrane protein derived from the green algae *Chlamydomonas*. ChR2 contains a chromophore which, upon absorption of blue light, undergoes a conformational change that causes the transmembrane channel to open. The influx of cations caused by the opening of the channel leads to neuronal depolarization and generation of action potentials. The great advantage of this approach is that ChR2 can be expressed in selected types of neurons, allowing one to establish a causal link between their activity and resulting changes in the properties of the system within which they are embedded, such as changes in physiological properties or behavior (Adamantidis et al., 2007). Viral methods have previously been used to enable suppression of neuronal activity in the monkey (Tan et al., 2006), and photoactivation of neurons expressing ChR2 has successfully been used to probe neural circuits not only in vitro (Boyden et al., 2005) and in invertebrate animals (Nagel et al., 2005), but also in the brains of awake behaving rodents (Zhang et al., 2007). Photoactivation has not, however, been used in the awake primate, until now.

Han and colleagues (2009) made stereotactic injections of lentivirus carrying the gene for ChR2, expressed under the CaMKII promoter, in macaque frontal cortex. They then used histological techniques to demonstrate convincingly that the procedure resulted in ChR2 expression predominantly in excitatory pyramidal neurons. It is worth noting that cell-specific targeting of neurons achieved in this study is not likely due solely to the use of a cell-specific promoter but also to the use of lentivirus, which tends to infect mainly excitatory pyramidal neurons (Nathanson et al., 2009). Using in vivo optical stimulation combined with electrophysiological recordings, the authors showed that it is possible to influence the activity of neurons in the infected area with millisecond temporal precision.

Although ChR2 was expressed in excitatory pyramidal neurons, Han and colleagues (2009) found that the activity of many neurons was suppressed following exposure to light. In addition, even neurons that increased their firing rates during photostimulation exhibited reduced levels of activity immediately after light cessation. The authors concluded that these reductions in firing rate likely resulted from recruitment of inhibitory interneurons that were activated by ChR2 expressing pyramidal neurons. This is consistent with previous studies employing optogenetic techniques to target pyramidal neurons in rodents and underscores the importance of considering the effects of targeted neuronal activation within the context of the broader neural network.

One possible concern when using optogenetic techniques in animal models is the possibility that the technique might damage the very circuitry to be studied. Therefore, Han and colleagues (2009) took pains to test whether viral infection, ChR2 expression, or photostimulation resulted either in direct tissue damage or an immune response. They found that it did not. Repeated electrophysiological recordings and subsequent histological examination of the infected tissue did not show signs of abnormalities in neural activity or cellular architecture. They also found no evidence of a productive immune response to ChR2. Taken together, their findings demonstrate the safety and efficiency of the optogenetic technique in primates over a period of months.

Successful photostimulation of a class of neurons in the rhesus macaque brain constitutes a substantial step forward because it opens the door to the use of optogenetic approaches to dissect the neural circuits underlying human cognitive functions that are best studied in the nonhuman primate model. The nonhuman primate is advantageous for several reasons. First, the macaque brain has been the subject of investigation for decades and is consequently one of the most well-studied model systems in neurobiology. Within the visual system alone, over thirty distinct visual areas have been identified and their anatomical interconnections thoroughly mapped (Felleman and Van Essen, 1991). The anatomy and functional connectivity of the local cortical circuits within some areas have been studied in exquisite detail (Callaway, 1998). This wealth of knowledge about the macaque brain has enabled researchers to pose highly refined questions about perception and cognition.

Second, the rhesus macaque can be trained to perform behavioral tasks similar

to those used to test human cognitive abilities. It has thus been possible, using conventional electrophysiological and imaging techniques, to identify and describe in depth the changes in neuronal response properties that accompany cognitive processes such as attention, decision making, and memory. These observations have, in turn, led to the development of detailed mathematical models of the neural mechanisms underlying human cognition and perception (Gold and Shadlen, 2007; Reynolds and Heeger, 2009).

Finally, close parallels between the organization of the human brain and that of the nonhuman primate mean that discoveries made about cognitive mechanisms in one species can directly illuminate the homologous structure in the other. For example, fMRI studies in humans have mapped out a network of areas that are involved in the allocation of attention (Corbetta and Shulman, 2002; Yantis and Serences, 2003). Single-unit recording and microstimulation studies in the macaque have then examined how feedback signals from these areas modulate neuronal responses in visual cortices (Awh et al., 2006).

Although these advances demonstrate the central importance of the nonhuman primate as a model organism for human cognition and perception, we remain limited in our ability to establish a causal relationship between the activity of specific classes of neurons and particular cognitive and perceptual processes. These limitations are a consequence of the fact that the tools available for perturbing primate neural circuits have not, until now, provided a means of targeting specific classes of neurons for control with high temporal resolution. For example, while microstimulation can be used to evoke neuronal activity with great temporal precision, it activates neurons nonspecifically and can activate fibers of passage, resulting in activation outside the area of interest. Pharmacological agents can locally modulate neurons, especially when combined with expression of heterologous receptors using viral vectors (Tan et al., 2006) and can with the use of promoters be restricted to a particular class of neurons. However, they lack temporal precision and thus cannot be used to modulate neuronal responses on the time scale of key cognitive processes, such as attention, which can shift from one object to another in less than a second. Optogenetic techniques overcome these limitations. Genetic targeting provides cell-class specificity. Spatial precision is achieved by limited spread of the virus and the narrow focus of the laser source used to activate ChR2. Temporal precision is achieved by high temporal resolution of the laser and the fast kinetics of the ChR2 channel. The successful use of this technique in a primate means that we can have our cake and eat it too: we can now leverage the power of molecular biology in the non-human primate to dissect the neural mechanisms of cognitive function.

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