



Primate optogenetics: Progress and prognosis

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Monkeys are a premier model organism for neuroscience research. Activity in the central nervous systems of monkeys can be recorded and manipulated while they perform complex perceptual, motor, or cognitive tasks. Conventional techniques for manipulating neural activity in monkeys are too coarse to address many of the outstanding questions in primate neuroscience, but optogenetics holds the promise to overcome this hurdle. In this article, we review the progress that has been made in primate optogenetics over the past 5 years. We emphasize the use of gene regulatory sequences in viral vectors to target specific neuronal types, and we present data on vectors that we engineered to target parvalbumin-expressing neurons. We conclude with a discussion of the utility of optogenetics for treating sensorimotor hearing loss and Parkinson's disease, areas of translational neuroscience in which monkeys provide unique leverage for basic science and medicine.

optogenetics | primate | monkey

The complexity of the primate brain poses a formidable challenge for science and medicine. Unraveling this complexity requires techniques for monitoring and manipulating neural activity at fine temporal and spatial scales. Substantial progress on this front was made in the early 2000s with the development of optogenetics (1, 2), an approach based on the expression of microbial opsins, which when illuminated modulate electrical activity in neurons. Optogenetic tools provide researchers unprecedented experimental control over neural activity, catalyzing discoveries and new therapeutic approaches.

The workhorse molecule of optogenetics, channelrhodopsin-2 (ChR2), is a moderately fast, blue light-gated channel that excites neurons (3). Other opsins, for example archaerhodopsins and halorhodopsins, suppress neurons (4, 5). Excitatory and suppressive opsins come in many types, with different kinetics, spectral sensitivities, conductances, and mechanisms of action (for reviews, see refs. 6–9). Most of these opsins have not yet been used in monkeys and are therefore not discussed in this article, but they continue to revolutionize studies in smaller model organisms and are of interest for future primate studies.

Optogenetics is now commonplace in the mouse, a standard model organism for vetting medical treatments before clinical use. However, the neurophysiology and behavior of rodents and primates differ in many ways, complicating the translation of discoveries in mice to treatments in humans. Moreover, standard strategies for expressing microbial opsins in mice are not directly applicable to primates (10). For optogenetic strategies to be used in monkeys or humans, alternative methods of gene delivery are needed.

Every primate optogenetic study to date has delivered opsin genes via viral vector. Two classes of viral vector have become de facto standards for optogenetics in monkeys due to their safety profile and ability to transduce postmitotic neurons efficiently: Adeno-associated viral vectors (AAV) and lentiviral vectors. The biology of these vectors and their application to the nervous system have been reviewed elsewhere (11, 12).

In this article, we summarize the progress that has been made in primate optogenetics over the past 5 y. Given the nascency of this field and the technical differences between studies, each study is summarized to provide a comprehensive overview. We highlight advances in viral vector-mediated gene delivery to

specific neuronal types, and we demonstrate the value of these advances with new data. We conclude with a perspective on the promise of primate optogenetics for translational research.

Analysis of Neural Circuits

Understanding the brain requires knowledge of how signals are processed by the neural circuits and cell types that compose it. Below, we summarize recent optogenetic studies of neural circuitry in macaque and marmoset monkeys.

I. Intraareal Signaling. The primate cerebral cortex has many distinctive functional specializations. Recent studies have used optogenetics to investigate the neural circuitry underlying these specializations in the visual cortex, as described below. The technical details of the vectors used are listed in *SI Appendix, Table S1*.

Functional connectivity. Neurons in area V1 of macaque monkeys are reciprocally connected via dense, long-range, horizontal connections (13). To examine the functional consequences of this connectivity, Chernov et al. (14) used a combination of optical stimulation and intrinsic signal optical imaging. They injected a lentiviral vector into area V1 to express ChR2 and covered the injection site with a transparent, artificial dura that provided optical access (15). To minimize the spread of optical stimulation, thin (200- μ m diameter) fibers were placed against the artificial dura and used to deliver blue light, which propagates only short distances through brain tissue. Optical stimulation of an ocular dominance column activated nearby columns with the same eye preference. Similarly, optical stimulation of an orientation column activated nearby columns with the same orientation preference and suppressed others. The study by Chernov et al. (14) demonstrates the feasibility of optogenetic control over cortical domains on the order of 200 to 400 μ m and lays the foundation for interrogating other local cortical circuits.

Nakamichi et al. (16) and Ju et al. (17) investigated V1 circuitry using similar techniques. In the study by Nakamichi et al. (16), optical stimulation was delivered to the V1–V2 border in one hemisphere to activate neurons in the corresponding region of the other hemisphere. In the study by Ju et al. (17), optogenetics was combined with 2-photon microscopy to stimulate and record individual neurons. Two-photon stimulation activated neurons less

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effectively than single-photon stimulation, but occasionally caused robust calcium signals, consistent with spiking activity. Collectively, these studies demonstrate the feasibility of using optogenetic stimulation at the scale of tens to hundreds of microns in the monkey brain, the challenges associated with single-neuron stimulation, and the benefits of combined optical stimulation and recording.

Excitation/inhibition balance and adaptation. Optogenetics has been used in monkeys to distinguish neurophysiological processes occurring within a local area from those inherited from upstream areas. For example, normalization, a process hypothesized to contribute to many brain functions, is likely mediated by feed-forward and intracortical mechanisms (18). To probe the role of intracortical circuitry in normalization, Nassi et al. (19) stimulated excitatory neurons in macaque V1, thereby indirectly exciting inhibitory neurons. Responses to combined visual and optical stimulation were smaller than the sum of responses to each stimulation mode alone, consistent with an intracortical contribution to normalization.

Intracortical inhibition is thought to be tightly linked to gamma power in the local field potential (20–22). Consistent with this association, Lu et al. (23) found that optogenetic stimulation of the macaque motor cortex increased gamma power in the local field potential. Cortical oscillations at gamma frequencies exhibited complex spatiotemporal dynamics, regardless of the temporal profile of stimulation, and vanished upon movement execution. Together, these results suggest that gamma frequency oscillations reflect the intrinsic dynamics of the neural circuit and are attenuated by descending commands during movement.

Repetition suppression, a form of stimulus-specific adaptation, has been characterized extensively, but its underlying mechanisms are poorly understood. To shed light on these mechanisms, Fabbrini et al. (24) used optogenetics in 2 ways: To probe the sensitivity of neurons in the inferotemporal cortex following visual stimulation and to fatigue these neurons by causing them to spike repeatedly. In contrast to visual responses, optically evoked responses were stereotyped whether presented after a visual stimulus, after an optical stimulus, or in isolation, demonstrating that the repetition suppression observed during visual stimulation is not due to firing rate fatigue of the stimulated neurons or the local circuit.

II. Interareal Signaling.

Thalamo-cortical pathways.

Visual. The lateral geniculate nucleus (LGN) of the thalamus contains 3 functionally distinct compartments. Neurons in the koniocellular compartment have diverse visual response properties and distinctive cortical projection patterns (25). To investigate the koniocellular inputs to V1, Klein et al. (26) injected macaque LGN with AAV vectors that biased ChR2 expression to the koniocellular compartment. Optical stimulation in the LGN produced short-latency responses primarily in the supragranular layers of V1, consistent with the anatomy of the koniocellular projection. In contrast, visual flicker stimulation, which modulates neurons in other LGN compartments, produced robust, short-latency responses primarily in the granular layer where most nonkoniocellular projections terminate.

Motor. The primary motor cortex (M1) and the motor thalamus are reciprocally connected. To study the projection from thalamus to cortex, Yazdan-Shahmorad et al. (27) injected an AAV vector carrying the gene for ChR2 into the motor thalamus of macaques. Thalamic transduction was extensive, due to the high-volume, high-pressure injection method (28). Transduced tissue appeared healthy, and modest anterograde and retrograde transduction of cortical neurons were noted. Optical stimulation of thalamic axons in M1 produced robust, short-latency postsynaptic responses, consistent with this thalamic–M1 projection operating as a primary driver of cortical activity (29).

In contrast, the reciprocal connection from M1 to the thalamus appears to play a modulatory role. Galvan et al. (30) injected AAV vectors into M1 and the premotor cortex of macaques to express ChR2 or C1V1. Optical stimulation of M1 axon terminals

in the motor thalamus induced small, long-latency responses that were excitatory or suppressive, in roughly equal proportion.

Cortico-cortical pathways.

Visual. Area V2 relays signals back to V1. To investigate the contribution of these feedback signals to visual responses in V1, Nurminen et al. (31) suppressed the V2–V1 pathway in marmosets optogenetically. They injected V2 with a mixture of 2 AAV vectors: one carried the gene for Cre-recombinase and the other carried the gene for the green light-sensitive, suppressive opsin, ArchT, in a Cre-dependent configuration. Linear multielectrode arrays were lowered into V1, and the axon terminals of transduced neurons were suppressed by optical stimulation at the recording site. Suppressing feedback signals from V2 decreased the responses of individual V1 neurons to stimuli within their receptive fields and reduced surround suppression.

The study by Nurminen et al. (31) demonstrates the utility of optogenetic projection targeting for suppressing the activity of cortico-cortical connections in monkeys, an approach that had previously been used in rodents (32–35). A key advantage of this approach is that activity suppression was restricted to the V2–V1 pathway, whereas classic approaches, such as pharmacological inactivation and cortical cooling, are less specific (36–38). On the other hand, optical suppression of axon terminals can have unintended consequences. The suppressive opsins currently available affect synaptic transmission in complex ways, especially when illuminated at high intensities and for extended periods (5, 35, 39–41).

Motor. M1 is reciprocally connected with the primary somatosensory cortex (S1). To investigate communication between these areas, Yazdan-Shahmorad et al. (42) expressed C1V1 in areas M1 and S1 of macaques, delivered optical stimulation through artificial dura, and measured neural activity using electrocorticography. Optical stimulation of the 2 areas simultaneously or in isolation strengthened the functional connectivity between them (43). These results show that optogenetics can be used to drive rapid, long-lasting changes in functional connectivity in macaques, and they highlight the power of electrocorticography paired with optogenetics for probing long-range intracortical connections.

Viral vector injections in the study by Yazdan-Shahmorad et al. (42) were made while the macaques were inside an MRI scanner. The spread of a contrast agent added to the vector predicted the spatial extent of the transduced region (44). This MRI-based approach to estimate the volume of transduction has advantages over direct neurophysiological or histological measurement: Opsin expression becomes detectable weeks after a viral vector injection is made, and knowing that an injection missed its target may motivate readministration of the vector. Making a second vector injection before an immune response to the first injection may be critical for efficient transduction, an issue we return to in the *Conclusion* (45, 46).

The Neural Basis of Behavior

Optogenetics can be used to reveal causal links between neural activity and behavior. Next, we review recent optogenetic studies of sensation, action, and cognition in monkeys.

I. Sensation.

Vision. Optogenetic suppression was used to investigate the contribution of neural activity to visual discrimination by Afraz et al. (47) and Fetsch et al. (48). Both studies capitalized on the spatial clustering of neurons with similar tuning properties and extended the results of electrical microstimulation studies (49, 50).

To investigate the neural basis of face perception, Afraz et al. (47) inactivated regions of the inferotemporal cortex using ArchT. Optogenetic suppression impaired the monkeys' ability to discriminate male from female faces, and the magnitude of this impairment correlated with neuronal face-selectivity at the stimulation sites. Control experiments demonstrated that the deficits could be reproduced with pharmacological inactivation.

To investigate the neural basis of motion perception and decision confidence, Fetsch et al. (48) inactivated regions of the middle

temporal area (MT) using the red light-sensitive suppressive opsin, Jaws. Macaques were trained to report the direction of motion in random dot fields by making a saccade to a choice target or, alternatively, to a sure-bet target that delivered a guaranteed but smaller reward. Low light levels and careful placement of the optical fiber restricted the manipulation to MT neurons with similar direction tuning. Optical stimulation reduced the number of choices in the preferred direction of neurons at the suppressed site, and it affected the frequency of choices to the sure-bet target in a manner consistent with a single mechanism underlying both effects. However, these results were only obtained during the first ~500 trials of each ~1,500 trial session and only during trials in which stimulation was brief (<350 ms). A compensatory mechanism, operating on the timescale of tens of minutes (the time required for a monkey to perform ~500 trials), thus appears to affect the read-out of signals from area MT.

Touch. Besides vision, the only sensory system in which optogenetics has been used to manipulate monkey behavior is somatosensation (51). May et al. expressed CIV1 in the hand and digit area of S1 of macaques that were trained to detect mechanical vibration of a finger tip. After training, mechanical stimulation was replaced with optical stimulation. Generalizing from mechanical to optical stimulation required more than 1,000 trials, but afterward the speed and accuracy of perceptual reports were similar in both conditions. Anecdotally, monkeys reacted to the optical stimulation initially by shaking, rubbing, and staring at the contralateral hand. This behavior is consistent with a somatosensory basis for detection, mitigating the concern that reports of optical stimulation were based on unintended cues (e.g., visual detection of light from the optical fiber).

II. Action. The frontal eye fields (FEF) contribute to saccadic eye movements. To investigate the role of this area in the production of memory-guided saccades, Acker et al. (52) suppressed FEF activity with Jaws at various time points with respect to target presentation and saccade execution. Red light, which activates Jaws strongly and penetrates tissue more efficiently than blue light, was delivered through a tapered and etched optical fiber, suppressing neurons across a large volume (~10 mm³). Optical stimulation reduced the number and accuracy of memory-guided saccades into the response fields of the transduced neurons regardless of stimulation timing.

The FEF projects strongly to the superior colliculus (SC). To probe the role of the FEF–SC pathway in the generation of saccadic eye movements, Inoue et al. (53) used the projection-targeting technique described earlier. ChR2 was expressed in the FEF, and optical stimulation was directed to FEF axon terminals in the SC. Although the monkeys were not incentivized to make eye movements, optical stimulation evoked saccades toward the response fields of the stimulated sites. This result contrasts with studies in which direct optogenetic stimulation of the FEF evoked saccades rarely (54, 55). This study marked the first use of pathway-specific optogenetic manipulation in monkey and provided strong evidence that the monosynaptic projection from FEF–SC produces saccades.

Optogenetic activation of axon terminals can evoke antidromic action potentials that may affect eye movements via indirect routes. Inoue et al. (53) did not test for antidromic action potentials and neither has any other optogenetic study in the monkey as of yet. In rodents, optogenetic stimulation of axon terminals evoked antidromic action potentials in some studies (56–59) but not others (33, 60–63). Light intensity and the pathway under study appear to be key factors. Antidromic action potentials are not a concern for suppressive projection targeting (e.g., ref. 31).

Optogenetics has also been used to perturb saccade accuracy through activation of the oculomotor vermis of the cerebellum (64). El-Shamayleh et al. expressed ChR2 in macaque Purkinje cells selectively, using a Purkinje cell-specific promoter in an AAV vector. Optical stimulation, which was brief and triggered by saccade initiation, systematically shifted saccade endpoints. The study by El-Shamayleh et al. showed that a single AAV vector can be used in primates to transduce a targeted cell type and perturb behavior on the millisecond timescale.

III. Cognition.

Attention. The lateral intraparietal cortical area (LIP) houses neurons that signal the salience of visual targets for saccadic eye movements (65, 66). To investigate the role of LIP in saccade target selection, Dai et al. (67) trained macaques to make a saccade to a visually distinct target among distractors. Optical stimulation of LIP neurons increased the number of saccades into the response fields of the stimulated neurons and decreased saccade latency.

Attention to a region of visual space increases behavioral sensitivity and reduces correlated response variability among neurons in area V4. This reduction in variability is restricted to neurons that represent attended locations and to low modulation frequencies. To probe the causal role of neuronal activity on visual performance, Nandy et al. (68) optogenetically induced low-frequency correlated activity in V4 while macaques performed an orientation change–discrimination task. Sinusoidal optical stimulation was delivered through an artificial dura at 5 or 20 Hz, and light intensities were kept low to maintain the average firing rate.

Low-frequency laser modulation impaired the monkeys' ability to detect orientation changes at the receptive fields of the stimulated neurons. In contrast, detection performance was unaffected when laser-modulation frequency was higher or when the stimulus appeared in the opposite hemifield. The study by Nandy et al. (68) showed that optogenetics can be used to change interneuronal correlations without changing the average firing rate and that low-frequency correlations in the activity of V4 neurons impair change detection.

Andrei et al. (69) showed a similar effect of V1 activation on visual stimulus detection. Excitatory neurons were targeted using the approach of Nandy et al. (68, 70). Monkeys were rewarded for reporting the appearance of a grating stimulus that, on half of the trials, coincided with optical stimulation. When the grating was low in contrast and matched to the preferred orientation of the stimulation site, the number of correct detections increased, and interneuronal correlations decreased. This result was explained using a model that includes weak interneuronal correlations and a read-out of V1 signals that decays with functional distance across the retinotopic map in V1.

Visual memory. The perirhinal cortex contains neurons that signal whether a visual stimulus is novel or familiar (71). To investigate the causal role of this activity in novelty judgements, Tamura et al. (72) familiarized macaques with a set of images and then trained them to discriminate these images from novel ones. Optical stimulation, delivered via 4 tapered fibers surrounding a microelectrode (73), caused animals to report novel images as familiar. Intriguingly, electrical stimulation produced the opposite effect at some sites, underscoring the need for a deeper understanding of the activation patterns achieved by both stimulation methods.

Value-based learning. Dopaminergic neurons signal reward prediction errors, and their activity contributes to value-based learning (74). To manipulate activity in these neurons selectively, Stauffer et al. (75) injected a mixture of 2 AAV vectors into the macaque midbrain. The first vector carried the gene for Cre-recombinase under the control of the dopamine cell-specific tyrosine hydroxylase promoter, and the second carried the gene for ChR2 under the control of the ubiquitous EF1 α promoter in a Cre-dependent configuration. This dual-vector approach drove strong ChR2 expression in dopaminergic neurons selectively (see also ref. 76). Monkeys were trained to make a saccade to 1 of 2 simultaneously presented visual stimuli. A saccade to either stimulus produced the same liquid reward, and a saccade to one, chosen at random at the start of the session, also triggered optical stimulation. After ~10 trials the monkeys made more choices to the stimulus that triggered optical stimulation, suggesting that increasing dopaminergic activity increases the subjective value of an associated visual stimulus.

Cell-Type-Specific Optogenetic Control

Promoters in viral vectors can drive opsin expression strongly, verging on exclusively, in a few targeted neuronal types in monkeys (26, 64, 75). To extend the range of neuronal types that

can be manipulated selectively using this approach, new cell-type-specific promoters must be discovered or engineered. Taking this step will require understanding gene regulation under normal conditions. Below, we provide a brief overview of advances in gene regulation that are likely relevant to primate optogenetics. We have reviewed several other strategies for targeting optogenetic manipulations in monkeys previously (77).

Promoters differ in selectivity and strength. How selective and strong a promoter must be to be useful for primate optogenetics depends on its intended use. Low levels of off-target ChR2 expression may be functionally insignificant because high levels are needed to affect neural activity (78, 79). On the other hand, low levels of Cre recombinase expression are sufficient to drive stable opsin expression from strong, nonspecific promoters (75, 80, 81).

A promoter, used in a viral vector, may include one or more enhancers. The distinction between promoters and enhancers (both referred to as gene regulatory elements) is subtle and becoming more so as commonalities between them are discovered (82–85). A key difference is that an enhancer, by itself,

drives expression weakly, if at all. To drive strong expression, an enhancer requires a minimal promoter in close proximity (86).

New gene regulatory elements can be incorporated in viral vectors to expand the range of neuronal types that can be targeted for optogenetic manipulation in monkeys. High-throughput functional assays are a powerful and direct way of finding these elements (87, 88). Similar to the DNA delivered by AAV vectors, the DNA delivered in these assays does not usually integrate into the genome of host cells (89, 90). Results from this type of functional assay may therefore be more predictive of AAV transduction patterns than other assays (91). Functional assays of enhancer activity are typically performed *in vitro*, but the development of *in vivo* versions of these assays, while challenging, has been facilitated by techniques for simultaneously identifying cell types based on gene expression and measuring enhancer activity in single cells (92–94).

Other assays take advantage of the facts that gene regulatory elements are often located in regions of open chromatin (95, 96), are transcribed (97, 98), and are bound to particular proteins (99). Each of these signatures is predictive of a role in gene regulation,

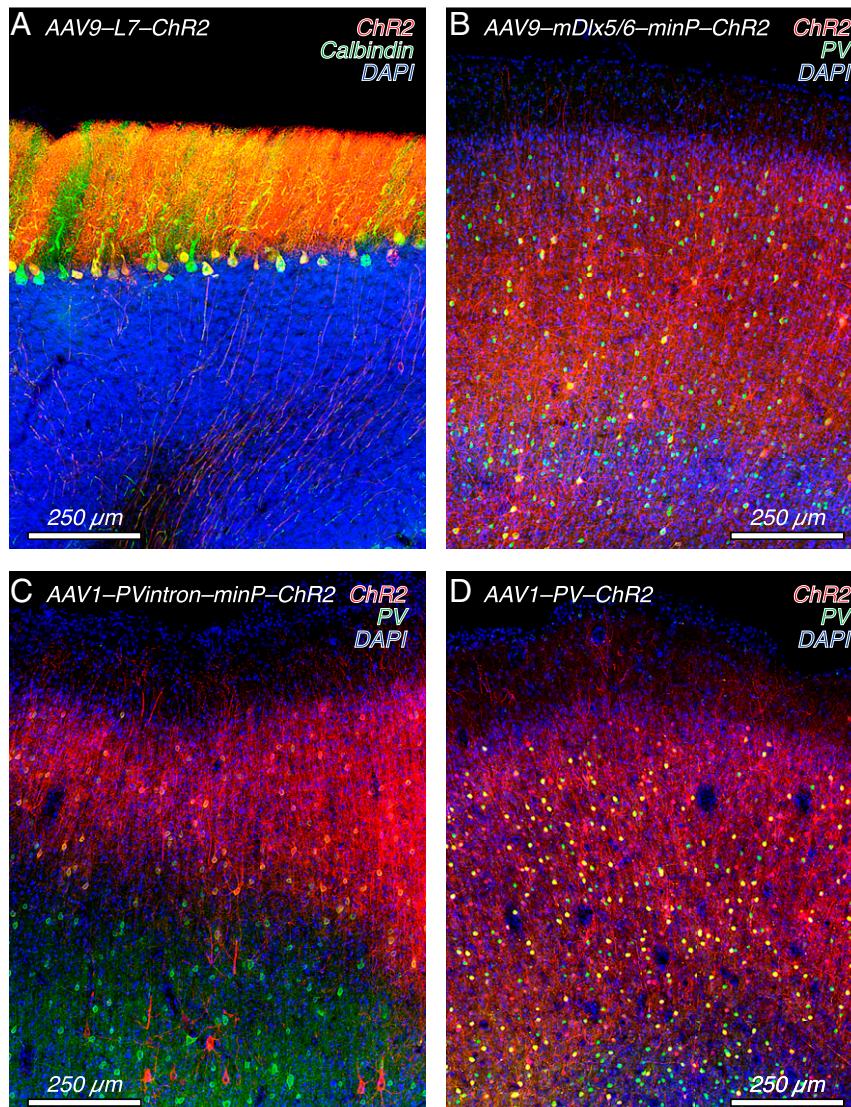


Fig. 1. Histological sections showing AAV vector-mediated opsin expression in the macaque cerebellar (A) and cerebral cortices (B–D). The vector constructs injected are listed in the title of each panel (minP, minimal promoter; PV, parvalbumin). Each section was processed using antibodies recognizing the fluorescent reporter encoded by the vector (red) and a marker gene for the targeted cell type (green) and counterstained with DAPI (blue). The primary antibodies were as follows: (A) mouse mCherry (Clontech 632543) and rabbit calbindin (Swant CB38), (B and C) rabbit mCherry (GeneTex GTX59788) and mouse parvalbumin (PV Swant 235), (D) chicken GFP (Abcam 13970-1000) and mouse parvalbumin (PV Swant 235).

but none is perfectly so. Some gene regulatory elements are silenced in the genome and thus lack these signatures, but nonetheless drive gene expression when delivered exogenously (100–102).

Many gene regulatory sequences are conserved across species (103, 104). The highly conserved L7 promoter, which has been used to create several lines of transgenic mice, drives expression in monkey cerebellar Purkinje cells when delivered by viral vector (Fig. 1A). A highly conserved gene regulatory sequence near the *DLX5* and *DLX6* genes (105), which are expressed in neocortical GABAergic cells (106–108) can also be used in AAV vectors to transduce neocortical GABAergic cells in several species, including marmoset monkeys (109). We confirmed that this vector was similarly selective in the macaque visual cortex (Fig. 1B). A related vector was coinjected with other vectors to target subsets of GABAergic neurons expressing parvalbumin, somatostatin, or neuropeptide Y (110).

To target parvalbumin-expressing inhibitory interneurons in the macaque neocortex using single AAV vectors, we tested 2 candidate macaque gene regulatory sequences. All procedures were approved by the University of Washington Institutional Care and Use Committee (protocol #4167-01). These sequences were identified on the basis of transcriptional and epigenetic profiling of macaque neurons using next-generation sequencing techniques. The first sequence (920 bp) is located in the last intron of the parvalbumin gene, is highly conserved across placental mammals, and is associated with an epigenetic mark of active enhancers in the macaque brain (111). As predicted, this sequence had enhancer activity, driving ChR2 expression from a minimal promoter (Fig. 1C). Selectivity for parvalbumin-expressing neurons was modest, however. The second sequence (1,100 bp) was immediately upstream of a transcription start site and drove expression more selectively (Fig. 1D). These data bode well for the prospect of harnessing regulatory elements near genes expressed selectively in cell types of interest to gain genetic access to them.

Clinical Applications

Optogenetics, while a relatively new technology, has already made valuable contributions to psychiatry and neurology (112, 113). Optogenetic suppression of compulsive cocaine-seeking in rats inspired a transcranial magnetic stimulation protocol that reduced cocaine use in humans (114, 115). Optogenetic studies of vision restoration in rodents laid the groundwork for 2 clinical trials to treat blindness (116–120). The field of primate optogenetics does not yet boast a similar clinical impact, but the advantages of monkey models for translational research are clear and, when combined with the power of optogenetics, will likely benefit medicine in many ways. Below, we discuss the promise that primate optogenetics holds for the treatment of 2 disorders: sensorineural hearing loss and Parkinson's disease.

Cochlear Implants. Sensorineural hearing loss is commonly caused by damage to hair cells in the cochlea. Damaged hair cells may no longer be capable of electrical signaling, but the downstream spiral ganglion neurons that innervate them can remain intact for many years, providing a suitable target for prostheses. An ideal implant would modulate each spiral ganglion neuron independently, but conventional cochlear implants usually contain <10 useful channels because the electrical currents they produce spread widely and affect large neuronal populations. An optogenetic cochlear implant might support as many as 100 independent channels because light can be directed with greater precision than electrical currents (121, 122).

Before any optogenetic device can be implanted in a human, it must be safe, reliable and more effective than a conventional device. This is a tall order. Conventional cochlear implants can mediate speech comprehension and have been implanted in nearly half a million people with minimal sequelae (123, 124). The adoption of any new technology, including optogenetics, will likely require implantation into macaques. Macaques can perform psychophysical tasks using acoustic or direct stimulation of the cochlea, and their psychophysical capacities are similar to those of

humans (125, 126). Macaque and human cochleae are sufficiently similar that a single device design can be used in either one (127).

The theoretical benefits of optogenetic stimulation for hearing restoration are substantial, but challenges remain. Techniques are needed to fabricate and accurately position light-delivery devices with the required intensity, spatial properties, and safety profile. Progress on this front includes the development of flexible arrays of μ LEDs that are small enough to fit inside the cochlea and bright enough to drive opsin-expressing neurons (128, 129). However, LEDs generate substantial heat and can damage tissue. An alternative to direct apposition of LEDs with tissue is to conduct light from distal LEDs into the tissue via waveguide, an approach that suffers from inefficient coupling but may be safer (130).

Methods are also needed to deliver opsin genes to spiral ganglion neurons safely and efficiently. AAV-mediated transduction of the spiral ganglion has been challenging (131) but was recently achieved in adult gerbils (132). The injection resulted in significant neuronal loss, but the deafened animals were able to detect optical stimulation of the surviving neurons and generalize auditory associations that they had learned prior to deafening. Transduction of spiral ganglion neurons has also been achieved in cynomolgus monkeys (133).

Opsins are needed that can modulate the firing of spiral ganglion neurons at high frequencies. Pitch perception is mediated in part by the phase-locking of spiking responses to the periodicity of sound up to \sim 300 Hz (134). Chronos, a fast ChR variant, can modulate spiral ganglion neurons up to 200 Hz, albeit weakly and with poor cycle-to-cycle reliability (135). How such a decrease in spiking reliability will affect sound perception is unknown. Conventional cochlear implants typically deliver electrical pulses at frequencies above the maximal neuronal firing rate to introduce stochasticity into the spike trains, so imperfect reliability may be beneficial in this context (136).

Parkinson's Disease. Parkinson's disease is a progressive, neurodegenerative disorder characterized by muscle rigidity, slowness of movement, and tremor. These symptoms are caused by the death of dopaminergic neurons that synapse onto 2 distinct neuronal populations in the dorsal striatum: one that directly inhibits the primary outputs of the basal ganglia and facilitates movement and one that indirectly excites these outputs and suppresses movement.

Experiments in monkeys were instrumental in relating the circuitry of the direct and indirect pathways to the symptoms of Parkinson's disease (137). An important step in this understanding was the discovery that the chemical MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) killed dopaminergic neurons and caused Parkinson's-like symptoms in humans and monkeys (138, 139). Like Parkinson's disease patients, MPTP-treated monkeys exhibit tremor, which is rarely seen in rodent models, and rigidity, which is difficult to measure in rodents. These symptoms can be relieved by the dopamine precursor L-DOPA, with side effects similar to those seen in humans. MPTP treatment does not induce Parkinsonism in rats and increases movement in some mouse strains (140, 141). Nevertheless, optogenetic studies in rodents continue to provide insight into the cellular and circuit-level basis of Parkinson's disease (142–144).

Optogenetic investigations of neural circuits in monkeys are at a relatively early stage but have already started to yield valuable new data. Neurons in the monkey basal ganglia, motor cortex, and motor thalamus have all been successfully transduced with viral vectors and stimulated optogenetically (23, 27, 30, 42, 55, 145–147). These are necessary first steps toward the functional interrogation of these structures in parkinsonian and nonparkinsonian animals.

Optogenetic studies of parkinsonian pathophysiology promise to reveal the therapeutic mechanisms of deep brain stimulation (148). Deep brain stimulation activates many circuit components (e.g., neuronal cell bodies, glia, and fibers of passage), with efficacy that is difficult to measure or predict (149, 150). The ability to manipulate each component selectively with optogenetics will

yield new insights that may guide the development of treatments with fewer side effects than conventional stimulation.

While primarily a motor disorder, Parkinson's disease also affects cognition, and the cognitive deficits in Parkinson's disease are more easily studied in monkeys than in rodents. Perceptual decision making, an experimentally tractable form of cognition, is an area of intense neurophysiological investigation in monkeys (151, 152). A major goal of these investigations is to develop quantitative models that describe the relationships among task demands, neural activity, and behavioral performance (153–155). These models provide a rigorous framework for breaking a decision into simpler components, some of which may map onto neural activity in specific brain regions. Investigations of decision-making in monkeys are starting to employ optogenetic techniques to investigate this mapping (47, 48, 67), and tasks used to study decision-making in monkeys are being applied to the study of Parkinson's disease (156, 157). The unification of optogenetics, modeling, and perceptual decision-making tasks may provide new insight into the cognitive deficits in Parkinson's disease.

Conclusion

The field of primate optogenetics is changing rapidly. A decade ago, optogenetics was first used to excite and suppress neural activity in monkeys (55, 145, 158). These early studies were followed by proof-of-principle uses of optogenetics to perturb monkey behavior (159–161). Over the past 5 y, the number of successful behavioral manipulations has more than doubled, and the field of primate optogenetics is expanding in many new directions. Optogenetics has been successfully merged with electrocorticography, optical imaging, and fMRI (162, 163). It has been combined with techniques for targeting specific neural pathways and cell types (53, 75, 77). It has also been used to probe the neural basis of sophisticated, primate-specific behaviors (48, 50). Through these innovations, optogenetic manipulations in monkeys are poised to provide valuable insights into primate brain function in states of health and disease.

Optogenetic studies in monkeys have unique challenges. Single animals are often used in multiple experiments, and some optogenetic manipulations can cause irreversible damage to the brain areas of interest. For example, light is typically delivered via optical fibers that are inserted into and removed from the brain area of interest each day. This simple approach allows an experimenter to sample different neuronal populations by moving the fiber but damages tissue. Sharpened optical fibers (52, 164, 165) or chronically implanted devices that direct light through multiple channels independently may mitigate this problem (166–169).

Sophisticated devices for light-delivery and neurophysiological recording used currently in rodents will likely be adapted for use in monkeys in the near future. These include arrays of optical wave guides and electrodes (170, 171), similar to the multielectrode arrays that are already widely used in monkey neurophysiology. Additional advances that may benefit primate optogenetics include semiconductors that can transmit light and

record electrical signals (172, 173) and the development of soft, flexible biocompatible materials (174, 175), thin, long-lasting biofluid barriers (176), bright small LEDs (177), and probes containing arrays of actuators and sensors spanning multiple modalities (178).

Another hurdle for primate optogenetics is that vector injections may trigger immune responses that reduce transduction efficiency. The blood–brain barrier protects the brain from circulating antibodies, but injections into the brain necessarily rupture this barrier and can elevate neutralizing antibody titers (46). Some anti-AAV antibodies are serotype-specific, motivating a policy of switching serotypes when multiple injections are made more than a few days apart, but others are not and may prevent AAV-mediated transduction broadly (179). Preexisting immunity to AAV, which is grounds for patient exclusion from gene therapeutic clinical trials, may be a practical criterion for excluding an animal from an optogenetics study. However, the relationship between neutralizing antibody titers and transduction efficiency *in vivo* is still unclear. This relationship is unlikely to be simple and probably depends on vector titer, volume, serotype, route of vector delivery, and technical details of the assay used to measure antibody titer.

Novel, engineered AAV capsids may be less susceptible to antibody-mediated neutralization than wild-type capsids (180). Some of these new capsids have the added advantage of allowing the vector to spread in ways that naturally occurring serotypes do not. For example, the novel AAV serotype, PhP.eB can transduce most of the cells in the mouse brain following a single retro-orbital injection into the venous sinus (93). However, the single published study using this vector in a marmoset monkey reported low transduction efficiency (181). Nevertheless, the techniques used to engineer PhP.eB, adapted to monkeys, may yield valuable new vectors for primate optogenetics.

Looking to the future, the potential for manipulating the activity of specific cell types optogenetically in monkeys using viral vectors remains an important frontier. New discoveries in gene regulation, technical advances for identifying gene regulatory elements, and recent successes using cell-type-specific promoters in monkeys bode well for this approach. Successful implementation will be assisted by the development of new vectors, methods of vector delivery, and techniques for assessing transduction non-invasively. Together these advances will spur progress in primate optogenetics, ushering in a new era of scientific discovery and medical breakthroughs.

Data Sharing and Availability. Data and reagents are available upon request.

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