Optogenetics in primates: monkey see monkey look

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Abstract

Optogenetics has emerged as a powerful tool for studying the neural basis of simple behaviors in rodents and small animals. In the primate model, however, optogenetics has had limited utility because optical methods have not been able to drive behavior. Here, we report that monkeys reliably shift their gaze toward the receptive field of optically driven channelrhodopsin-2-expressing V1 neurons. This result establishes optogenetics as a viable means for the causal analysis of behavior in the primate model.

In 2007, Aravanis and colleagues pioneered an optogenetic approach to control the behavior of an experimental animal [1]. They expressed channelrhodopsin-2 (ChR2) in the vibrissal motor cortex of the mouse and demonstrated that activation of ChR2 by blue light evoked whisker deflections. Since then, this approach has found numerous applications in the study of the neural circuitry underlying simple behaviors in rodents and lower animals [2, 3]. These successes bode well for the use of optogenetics in the analysis of more complex behaviors, cognition, and their disorders [4]. A key step towards this goal is to adapt this technology to non-human primates, both as a tool for analyzing neural function in more sophisticated models of behavior and as a stepping-stone toward clinical applications. Several groups have successfully used ChR2 and other light-sensitive proteins to influence neural activity in the primate brain [5-7], but attempts to manipulate primate behavior have been unsuccessful. Here we demonstrate the first use of optogenetics to evoke a behavioral response in the rhesus monkey (*Macaca mulatta*).

We expressed channelrhodopsin-2 (ChR2) in a small region of the primary visual cortex (V1), and asked whether ChR2-mediated neuronal activation produced a visual sensation at the location of the neurons' receptive fields (RFs). The ChR2 gene was delivered with an AAV vector (rAAV1-hSyn-ChR2(H134R)-mCherry) which was pressure injected at multiple depths, ~300 μm apart, spanning the thickness of the cortex. Five to seven weeks later, we verified ChR2 expression by monitoring the effect of optical stimulation on neural activity. In both monkeys, pulses of blue light directed at the injected V1 site, but not at other V1 sites, reliably modulated local single- and multi-unit activity.

A key question was whether ChR2-mediated activation of V1 neurons was sufficient to engage visuomotor behavior. We answered this question by exploiting the monkeys' natural tendency to orient toward flashed stimuli. Our behavioral paradigm, developed by Tehovnik and colleagues [8], consisted of two trial types, 'Fix' and 'Tar'. On Fix trials, monkeys received liquid reward for maintaining fixation on a central spot. On Tar trials, monkeys received reward for making a saccade to a visual target that appeared after fixation spot offset. Unbeknownst to the monkeys, on half of the trials in each category, the offset of the fixation point was followed by brief optical stimulation ('Op+Fix' and 'Op+Tar'). Importantly, reward contingencies were independent of stimulation: in both Fix and Op+Fix trials, monkeys received reward for maintaining fixation, and not for their oculomotor behavior after fixation point offset.

The main result was that in Op+Fix trials, following optical stimulation, monkeys shifted their gaze toward the RF location of the injection site (**Fig. 1b**). This behavior did not occur on Fix trials, which were identical except for the absence of optical stimulation (**Fig. 1a**). Across trials, saccade endpoints were significantly closer to the location of the RF when optical stimulation was applied than when it was not (Mann–Whitney, one-tailed; monkey 1: U=29082, $n1$=188, $n2$=189, p<1e-10; monkey 2: U=17124, $n1$=$n2$=155, p<1e-10).

In most sessions, the saccade target was presented in the RF of the ChR2-expressing neurons. This consistent geometry raises the possibility that the behavioral response we observed was due to a nonspecific association between optical stimulation and the rewards given for saccades into the RF. To control for this possibility, we moved the saccade target into the opposite hemifield (**Fig. 1, inset**). Importantly, in these trials, eye movements toward the RF of the optically driven neurons were never rewarded. Nevertheless, the monkeys continued to make saccades into the RF of the optically-stimulated neurons (**Fig. 1b, inset**), indicating that ChR2-mediated activity produced a sensation that was localized in visual space to the RF of the stimulated neurons.

Optogentic stimulation of V1 neurons produced oculomotor behavior similar to that produced by the appearance of a visual target, but it produced a rather different pattern of neural activity. At all V1 sites, the

onset of the saccade target had a modest excitatory effect on neural activity (**Fig. 2c**). The effect of ChR2-mediated activity on the Op+Fix trials (**Fig. 2b**), on the other hand, varied markedly between recording sites. At some sites, optical stimulation produced sustained excitation as expected by the biophysical properties of ChR2(H134R) (**Fig. 2b bottom row**). At other sites, the effect was either suppression (**Fig. 2b top row**) or a mixture of synchronized excitation followed by sustained suppression, which is consistent with a post-excitation recruitment of a dominant inhibitory circuit or depolarization block. On average, there was no correlation between visually-evoked and optically-induced activity across recording sites (Pearson's $r(8)= 0.43$, $p>0.25$).

In light of the clear dissociation between patterns of activity evoked by the optical stimulation and saccade target, we asked which of the two was more effective in driving responses in the Op+Tar trials, in which both visual and optical stimulations were present. As shown by the two examples in **Fig. 2d**, in the Op+Tar condition, responses were invariably dominated by the activity associated with optical stimulation. To quantify this effect across recording sites, we constructed a regression model to relate responses in Op+Tar trial to a linear sum of responses to the Op+Fix (optical stimulation alone) and Tar (target alone) conditions. The model, which predicted responses in the Op+Tar condition well ($r^2=0.96$, $p<0.001$) suggested that responses were dictated by the response to the optical stimulation alone ($\beta=1.02$, CI=[0.74 1.30]), and were independent of the responses associated with the saccade target ($\beta=-0.19$, CI=[-4.14 3.76]).

What factors contributed to the successful manipulation of primate behavior using a technique that has been unsuccessful previously? One key factor may be that we activated a sensory cortical area, whereas previous attempts to manipulate behavior targeted motor structures. It may seem paradoxical that manipulations of sensory signals could be more effective in driving behavior than those targeted directly at the motor structures, but there are three reasons why optically-induced signal could be more effective in sensory areas. First, signals initiated in sensory cortex undergo a complex series of processing stages, providing ample opportunity for amplifying weak signals so that they can become manifest in behavior [9]. In contrast, weak signals initiated in motor areas might not have the opportunity to be sufficiently amplified.

Indeed, the effectiveness of near-threshold signals in both sensory and motor areas in driving behavior is thought to depend on further cortical processing [10, 11]. Second, as evidenced by our analysis of firing rates, the patterns of activity induced by optical stimulation may be idiosyncratic and markedly different from native cortical signals (Fig. 2). In V1, such an unfamiliar pattern of activation may nonetheless draw attention and engage visuomotor circuits that would lead to an orienting behavior. In motor structures, on the other hand, only suitably structured patterns of activity might be able to drive behavior [12].Third, the dominant efferent pathway form V1 arises from neurons in the superficial layers, which are readily accessible to illumination. In contrast, the dominant efferent pathway from motor cortical areas arises from infragranular layers, which may be more difficult to illuminate. Finally, the AAV1-hSyn expression system we used to deliver ChR2 might be particularly well suited for driving healthy ChR2 expression in monkey cortex.

The promise of optogenetics comes from its potential to dissect function at the level of projections and cell-types of interest. An important next step is to develop and refine such targeting methods for use in the primate. Our work serves as a "proof of principle" that optogenetic stimulation is an effective technique for manipulating behavior in primates and sets the stage for future investigations into previously intractable components of the underlying neural circuitry.

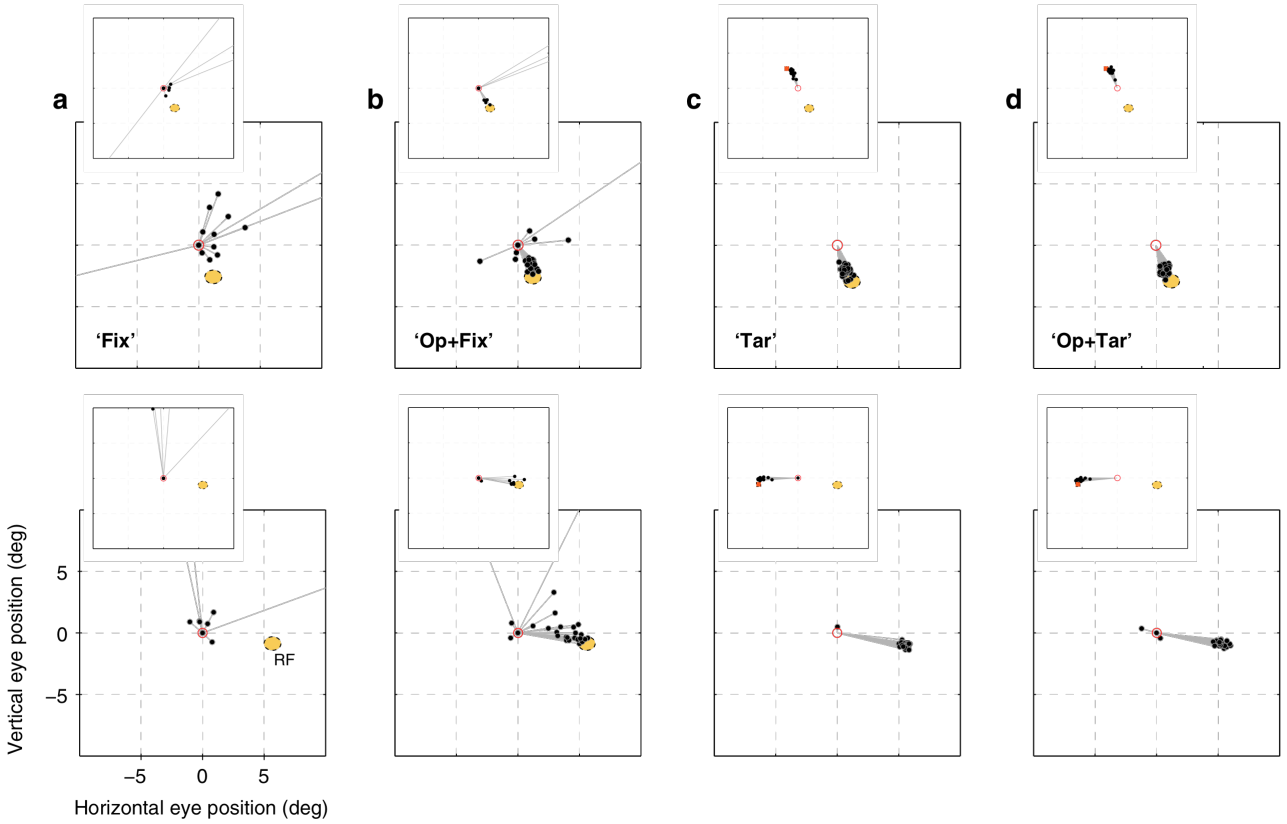

Figure 1. Saccadic eye movements (gray lines) after the offset of the fixation point (red circle) in the four experimental conditions. (**a**) In the Fix condition, saccade endpoints (black) were broadly distributed. (**b**) In the Op+Fix condition, saccade endpoints were concentrated near the RF of the injection site (yellow) even though no target was shown in these trials. In the Tar (**c**) and Op+Tar (**d**) conditions, saccade endpoints were directed toward the target (in the RF). The inset shows saccades in the control condition in which the target (red square) was presented in the hemifield opposite the site of injection. Saccades in this condition were directed towards the target when it was shown (Tar and Op+Tar trials) but into the RFs of the stimulated neurons when it was not (Op+Fix trials). Results for the two monkeys appear in separate rows.

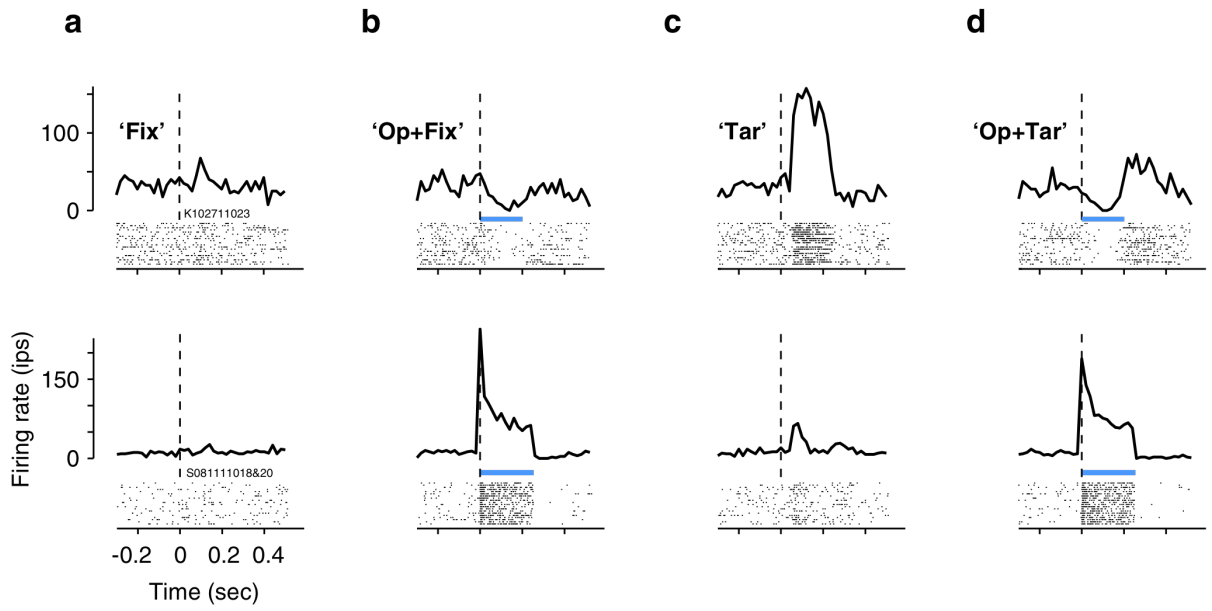

Figure 2. Multiunit neural activity associated with the four experimental conditions for two example sites in V1. In each panel, the raster plot shows spiking times (black ticks) of individual trials (rows), and the PSTH shows the average firing rates (bin width = 20 ms). Spikes times and the corresponding PSTHs for the Fix (**a**) and Tar (**b**) conditions were aligned to fixation point offset and target onset respectively. In the Op+Fix (**b**) and Op+Tar (**d**) conditions, trials were aligned to the onset of the optical stimulation (which followed target onset by 30 ms). The top and bottom rows show examples in which the optical stimulation reduced and increased firing rates respectively. At both example sites, Op+Tar responses (**d**) were more similar to Op+Fix responses (**b**) than to Tar responses (**c**). The blue bar indicates the duration of optical stimulation.

Methods

Two female rhesus monkeys (7.2 and 8.3 Kg) (*Macaca mulatta*) participated in this experiment. Behavioral protocols, animal care and surgical procedures were all in accordance with the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Washington Animal Care Committee.

Monkeys were surgically implanted with a titanium head-holding device and recording chamber. We characterized a target V1 site using standard electrophysiological techniques, and pressured injected 10-12 μL of the viral vector containing the ChR2 gene (rAAV1-hSyn-ChR2(H134R)-mCherry) over the course of 4-5 hours into that site using a cannula with a ~150 μm inner diameter. The viral vector was made via the helper-free triple-transfection procedure, dialyzed in PBS, and titered at 5.52×10^{11} particles/ml.

During experiments, monkeys were seated in primate chairs with their head fixed and viewed stimuli on a computer monitor (background luminance = 90 cd/m^2) binocularly from a distance of 100 cm. The behavioral task consisted of two randomly interleaved trial types. On Fix trials, the monkey fixated a black square (side = 0.2°, luminance > 2 cd/m^2) for 500-1000 ms and received a juice reward when the fixation point was extinguished. On Tar trials, a peripheral square target (side = 0.2-0.4° side, luminance = 49 cd/m^2) was displayed 100 ms after fixation offset, and the monkey was rewarded for making a saccade to the target within 300 ms after target onset. Trials were aborted without reward if the eye position deviated more than 1° from the fixation point before fixation offset. In the Tar condition, reward was delivered only of the saccade was within 1.8° of the target.

We recorded neural activity using tungsten electrodes and measured eye position with scleral search coils. Digitized gaze position signals, extracellular neural activity and other behavioral timing events were stored using a Plexon MAP system for offline analysis. Saccades were identified based on velocity criteria.

The recording electrode and the optical fiber were placed inside a common guide tube above the dura mater and were advanced independently using a custom microdrive. First, the electrode was advanced until neural activity was detected. Afterwards, the optical fiber was advanced until light pulses (473 nm, ≤ 50 mW)

clearly modulated neural activity. Neural responses were not modulated if the tip of the optical fiber was far from the where electrical activity was detected (> ~1 mm) or if both were located far (> ~1 mm) from the injection site. Data in Figure 1 are from trials in which the optical fiber was advanced to its terminal point (i.e., closest to the depth at which electrical activity was recorded).

In the main experiment, the saccade target was presented inside the RF of the neurons at the injection site, as measured from the multi-unit activity. In the control experiment, the saccade target was presented in the opposite hemifield. Optical stimulation was applied to the site of injection on a random half of trials of each category. In Opt+Fix trials, the optical stimulation was applied 130 ms after fixation offset. On Op+Tar trials the stimulation was applied 30 ms after target onset. We verified the effectiveness of light pulses of various durations (100-250 ms) and various frequencies (0-200 Hz) in eliciting saccades in the Opt+Fix condition.

To determine whether neural responses in the Op+Tar condition were predicted by responses to the target and optical stimulation alone, we used linear regression model in which regression coefficients β_1 and β_2 related firing rates during the Op+Fix and Tar trials (R_{Op+Fix} and R_{Tar} respectively) to firing rates in the Op+Tar condition (R_{Op+Tar}). The model also included a constant term (β_3):

$$R_{Op+Tar} = \beta_1 R_{Op+Fix} + \beta_2 R_{Tar} + \beta_3$$

We measured average firing rates within the 30 to 130 ms after fixation point offset and minimized the least squares error of the linear prediction and the data to fit the regression coefficients. Our conclusions were robust with respect to small changes in the interval from which firing rates were estimated.

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