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Supplementary Information for

Functionally specific optogenetic modulation in primate visual cortex

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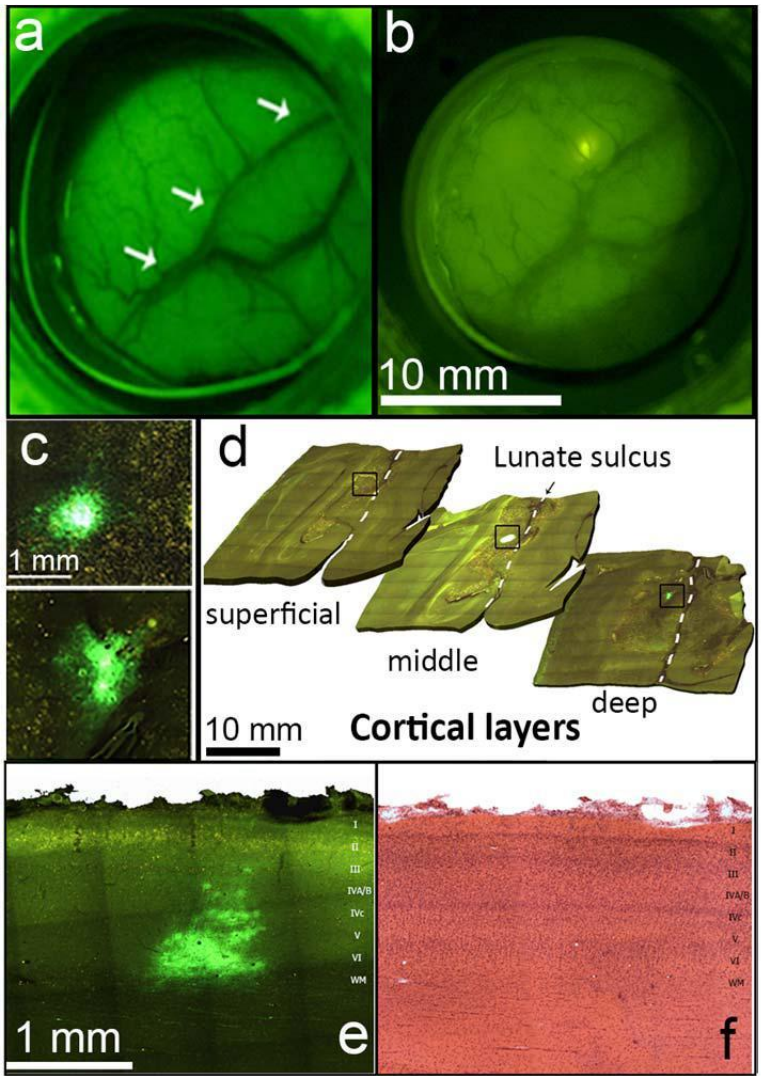

Fig. S1. Viral expression is focal.

ChR2 expression, inferred *in vivo* from reporter gene (eYFP) fluorescence, was first observed about 3 weeks post injection in Monkey B, reached maximum ~ 3 weeks later, and persisted for the duration of the study (up to 8 months). (**a**) and (**b**) show a view of the brain through a set of fluorescence filters for eYFP, before (**a**) and 4 weeks after (**b**) an injection of viral vector. Expression is approximately 1 mm in diameter, with a dimmer fluorescent halo around it. The injection is located in area V1 (as determined by ocular dominance imaging), posterior to the lunate sulcus (white arrows in **(a)**). (**c**) Subsurface expression in layers 4 (top) and layers 5-6 (bottom). (**d**) Slices taken parallel to the cortical surface at 3 different depths (300 microns, 800 microns and 1.4 mm). Box shows region shown in (**c**). (**e-f**) Case in which there was greater eYFP expression in deeper layers rather than at the surface, with relatively strong expression in layers IVc, V and VI, and more sparse expression in layer III (laminae determined via Eosin and Hematoxylin stain in (**f**). Scalebar **(e,f)**: 1 mm. Optical stimulation is likely to affect dendrites of pyramidal neurons in superficial and deep layers.

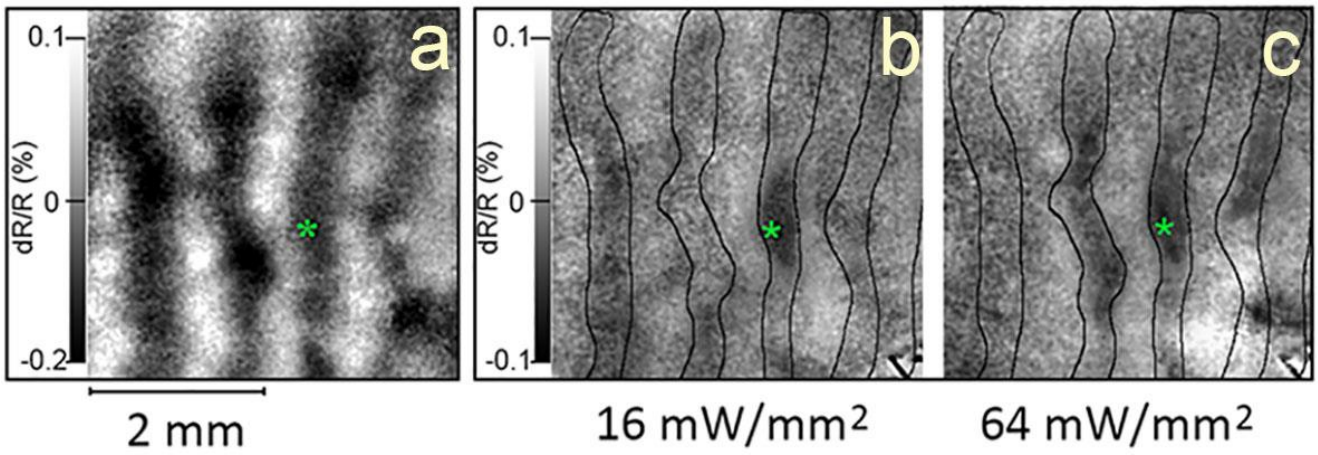

Fig. S2. Optical images from Figure 2d-f (Monkey C).
Panel (**a**) is from Fig 2d, showing location of OD columns. Panels (**b**) and (**c**) show activation patterns following optogenetic stimulation alone (600 ms, 24 Hz) with irradiances of 16 and 64 mW/mm^2, respectively (obtained in separate run than (**a**). The locations of OD columns are outlined in black. Green asterisk: viral expression site and center of opsin expression. Scale bar in **(a)** applies to **(a-c)**.

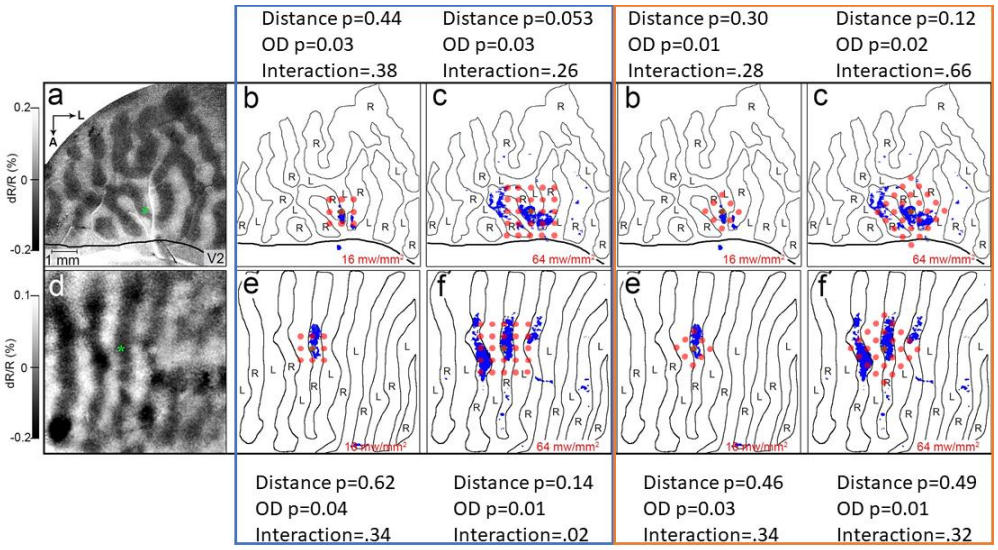

Fig. S3. Is domain location or distance the best predictor for optogenetically induced changes in intrinsic optical signal? To test this question, we created a grid of 250 micron diameter regions of interest spaced 0.5 mm apart. The grid was sized to cover approximately 90% of pixels that showed significant changes in intrinsic optical signal and centered on the stimulation location. A model was created with two inputs: a categorical input based on whether the ROI was in the same or opposite OD domain and a continuous distance variable based on the center of expression, the change in optical signal was the output. Two different grid orientations were tested. For each of four analyses (two cases, each at two stimulation intensities), OD was the better predictor of change in the intrinsic signal.

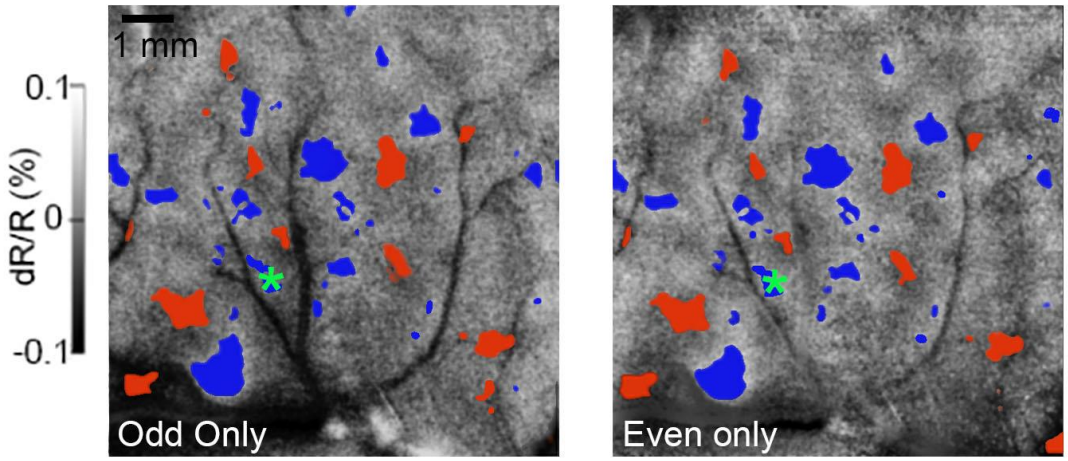

Fig. S4. Orientation maps are reproducible and stable. Analysis of odd (left), and even (right) blocks of trials produces very similar orientation maps. Blue: horizontal domains, red: vertical domains; $p < 0.05$. 40 blocks total.

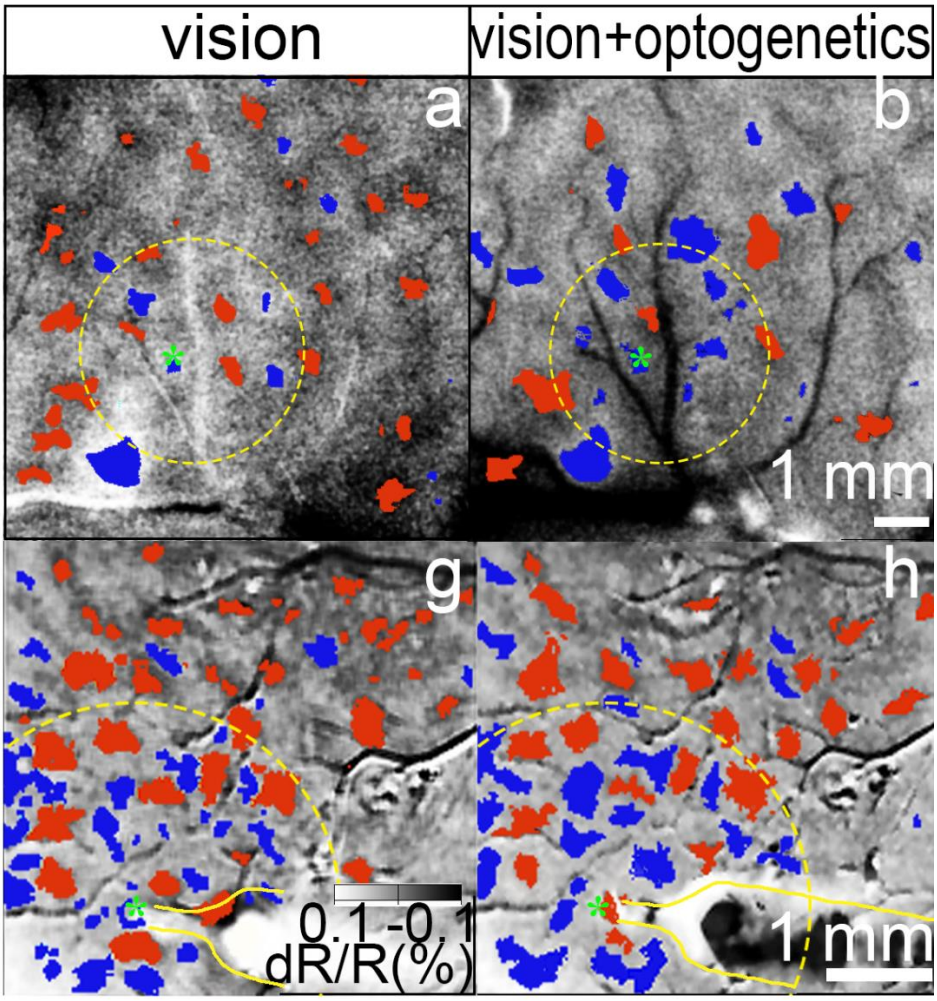

Fig. S5a. Larger reproduction of manuscript figure 4abgh.

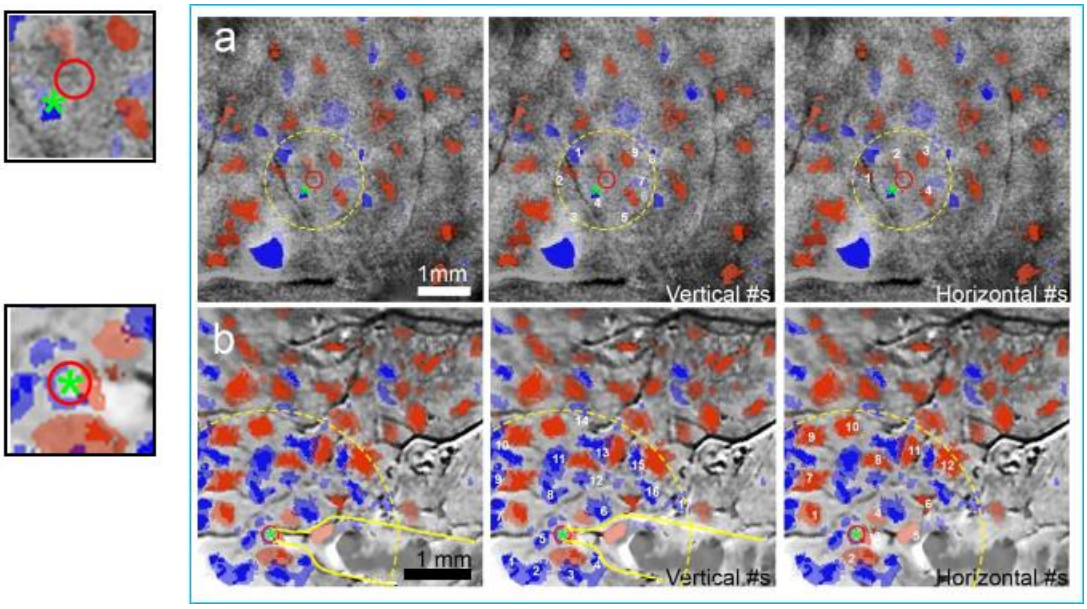

Fig. S5b. **Illustration of orientation domain changes with optogenetic stimulation**. The 3 images in a and in b are identical: vertical domains are numbered in the middle panel, horizontal domains are numbered in the rightmost panel. Panels show overlay of orientation maps with and without optogenetic stimulation for two cases (**a)**: Case 1 Monkey B, (**b)**: Case 2, Monkey C). For both cases, optogenetic stimulation (600 ms, 24 Hz, 64 mW/mm^2) targeted a vertical domain (Red circle and asterisk: center of opsin expression and target of laser stimulation, respectively). **Left panels:** Overlay of two orientation maps (V minus H) and (V+opto minus H+opto). Vertical domains: without (dark blue) and with (light blue) optogenetic stimulation. Horizontal domains: without (light red) and with (dark red) optogenetic stimulation. All t-maps: $p < 0.05$ Inset shows precise location of the stimulating fiber. With optogenetic stimulation of vertical domain: ***Middle panels*** Same maps with vertical domains numbered (**a)**: 1-9, (**b)**: 1-17). Vertical domains show increased number of pixels reaching significance (dark blue domains smaller than light blue domains). ***Right panels*** Same maps with horizontal domains numbered (**a)**: 1-4, (**b)**: 1-12). Horizontal domains show fewer pixels reaching significance (light red domains larger than dark red domains). Note that location of orientation domains before and after stimulation is stable. Inset at left: zoomed in view of stimulation site. Outline of the fiber is shown in yellow in bottom left and middle panels.

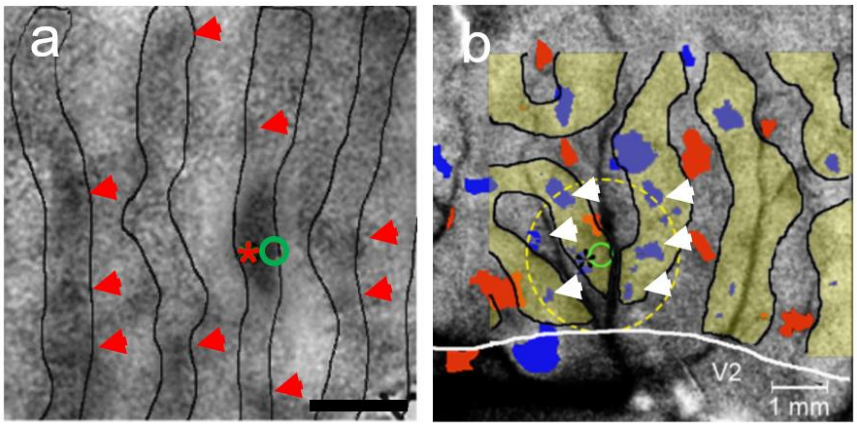

Fig. S6. Single site stimulation impacts both OD maps and orientation maps simultaneously. (**a**) Same image as Fig 2e and Supp Fig 2b (focal optogenetic stimulation alone 16mW/mm^2). Red arrows: patchy activations. (**b**) Optogenetic modulation of visual response is orientation and ocular dominance selective. Same as Fig 4b. White arrows: within 2 mm radius of stimulation site, orientation patches fall largely within same eye columns. Scalebars: 1 mm. Asterisks: location of optic fiber; green circle: center of the opsin expression.

Table S1. All imaging sessions from three animals and their contributions to figures in manuscript. Data was collected over a period of 2 years. Sessions, each lasting up to 24 hours, were separated by 2-4 weeks for each animal. We used one animal to develop the focal injection and activation method and to determine whether any effects could be observed (Monkey A). In Monkey B, one successful injection was made. This site was used to systematically determine optimal stimulation parameters (Figure 1, Supp Fig 1, 12 runs), and to examine effects relative to OD and orientation maps (Figures 2a-c, Fig 3, 4a-e, Supp Fig 2). In Monkey C, three successful injections were made. This provided data on effects relative to OD and orientation maps (Figures 2d-f and 4f-j). One site in Monkey C was used to collect electrophysiology data (Fig 5).

Animal ID	Total number imaging sessions (days)	Optogenetics w/o visual stimulation,	Vision+opto: OD	Vision + opto: orientation	Electrophysiology	Notes
A	3	9 runs collected over 2 sessions	0	0	No	Two expression sites but only one studied in detail
B Figures 1,2,3,4	6	25 runs collected over 4 sessions	3 runs collected over 2 sessions	2 runs collected over 2 sessions	3 sessions, but without combined visual stimulus presentation.	Data obtained from one injection site. Relatively weak responses to orientation
C Figures 2,3,4,5	6	12 runs collected over 2 sessions	3 runs collected over 2 sessions	2 runs collected over 2 sessions	3 sessions. Used to generate figure 5.	Three expression sites. One used for electrophysiology only

Table S2. Comparison of 3 optogenetic studies of macaque monkey V1.

	Ruiz et al, 2013[1]	Nassi et al, 2015[2]	This study
Injection numbers, volumes, locations	Large expression area achieved by clusters of 2 to 5 five injections per site.	1 injection at single location in V1 of 2 animals.	1 injection per site.
Transfection area	up to 4 mm^2	< 1mm^2	0.2 mm < 1mm^2
Fiber size (diameter)	0.6-2 mm	0.6mm	0.2 mm
Illumination	600 ms pulse train, 20 ms pulse width, 24 Hz (up to 128 mW/mm^2). Green light for C1V1, blue light for Chr2.	200-600 ms continuous illumination (up to 125 mW/mm^2). Green light	600 ms pulse train, 20 ms pulse width, 24 Hz (up to 128 mW/mm^2). Blue light
Viral type	AAV and Lentivirus, Carrying C1V1, ChR2.	Lentivirus carrying C1V1 gene. More extensive tissue penetration using green light.	Lentivirus carrying ChR2. Most focal activation with blue light.
Awake or anesthetized electrophysiology	Awake	Awake	Anesthetized

References

1 Ruiz, O. *et al.* Optogenetics through windows on the brain in the nonhuman primate. *J Neurophysiol* 110: 1455-1467, doi:jn.00153.2013 [pii] 10.1152/jn.00153.2013.

2 Nassi, J. J., Avery, M. C., Cetin, A. H., Roe, A. W. & Reynolds, J. H. Optogenetic Activation of Normalization in Alert Macaque Visual Cortex. *Neuron* 86:1504-1517, doi:S0896-6273(15)00479-1 [pii] 10.1016/j.neuron.2015.05.040.