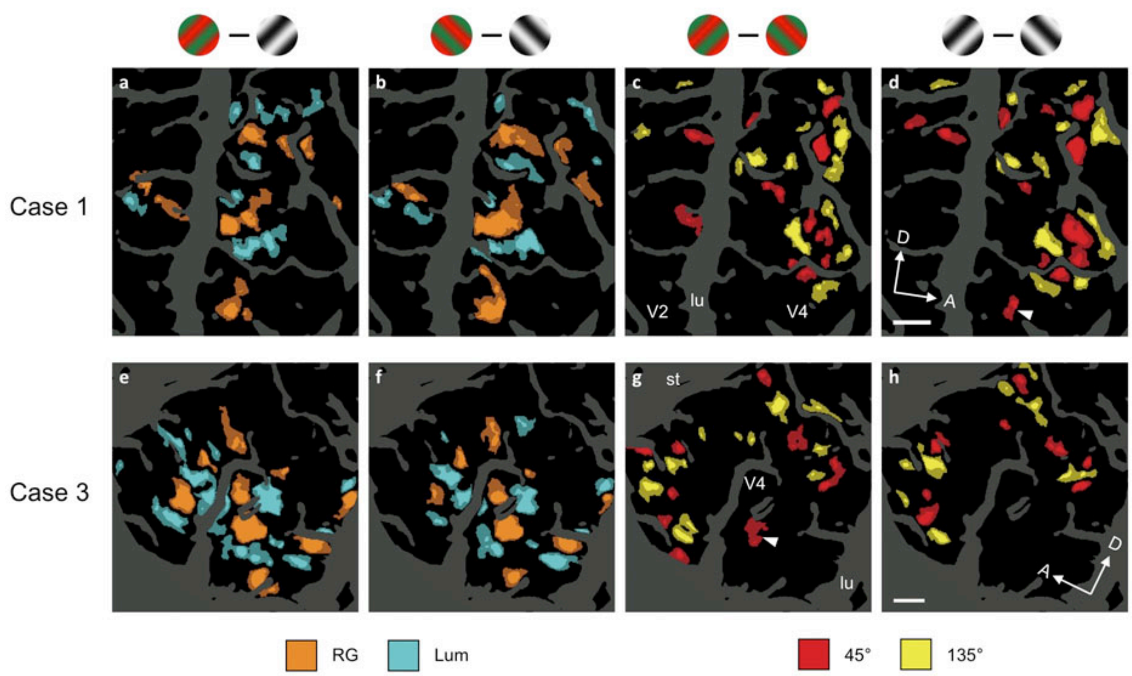
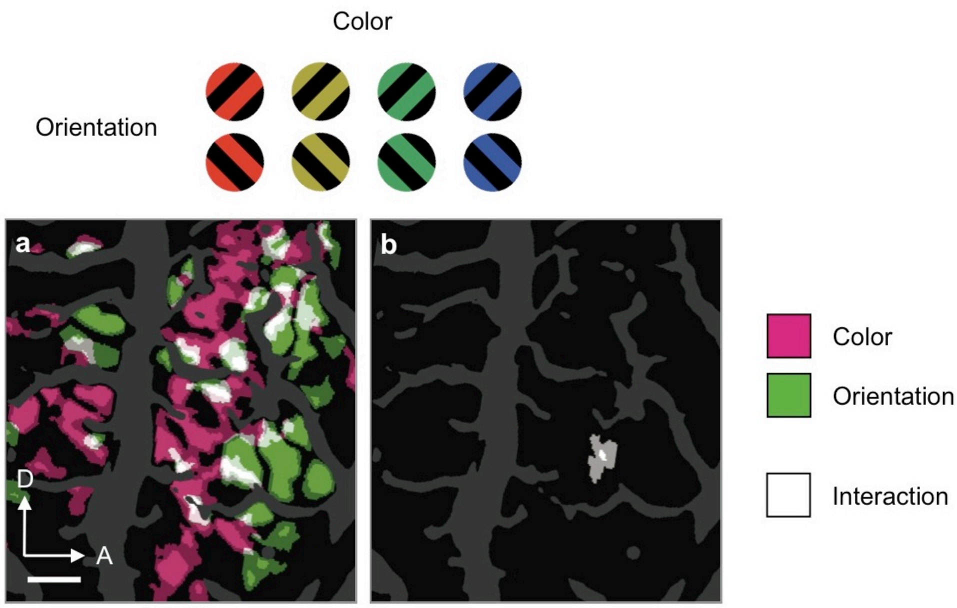
Functional organization for color and orientation in macaque V4

Hisashi Tanigawa, Haidong D. Lu, & Anna W. Roe

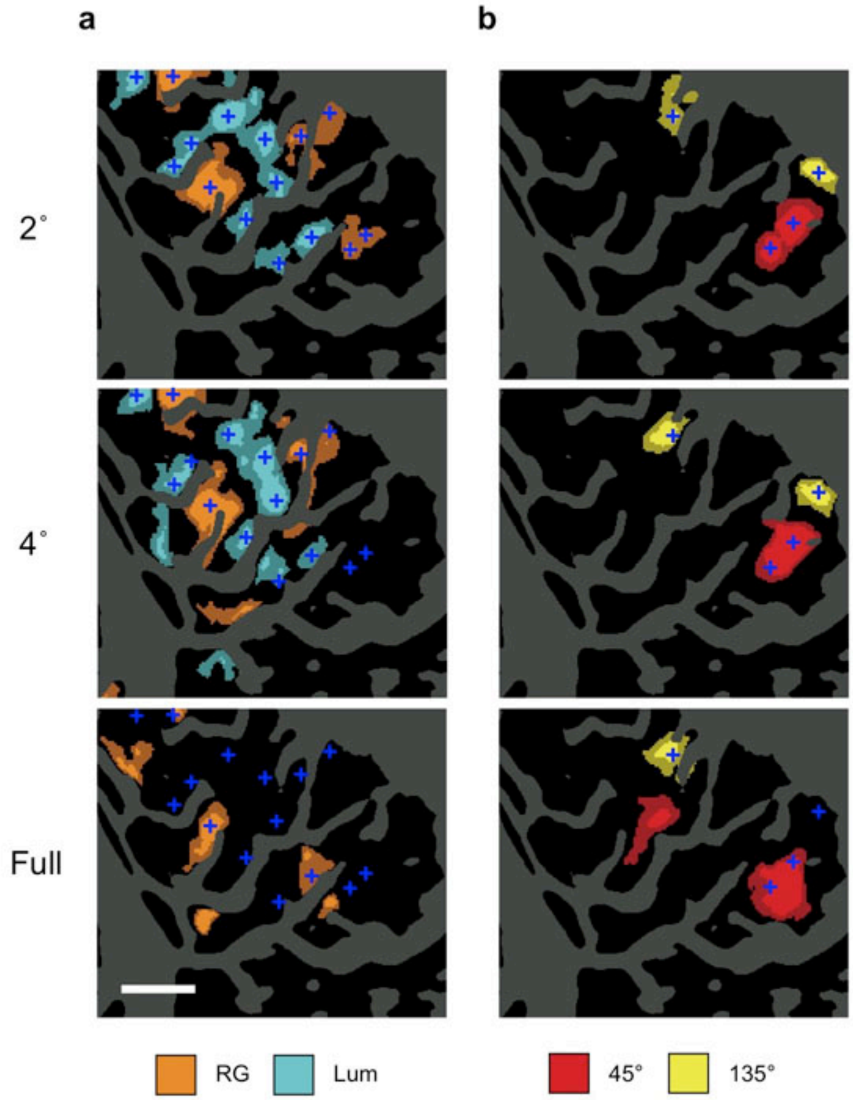
Supplementary Figures



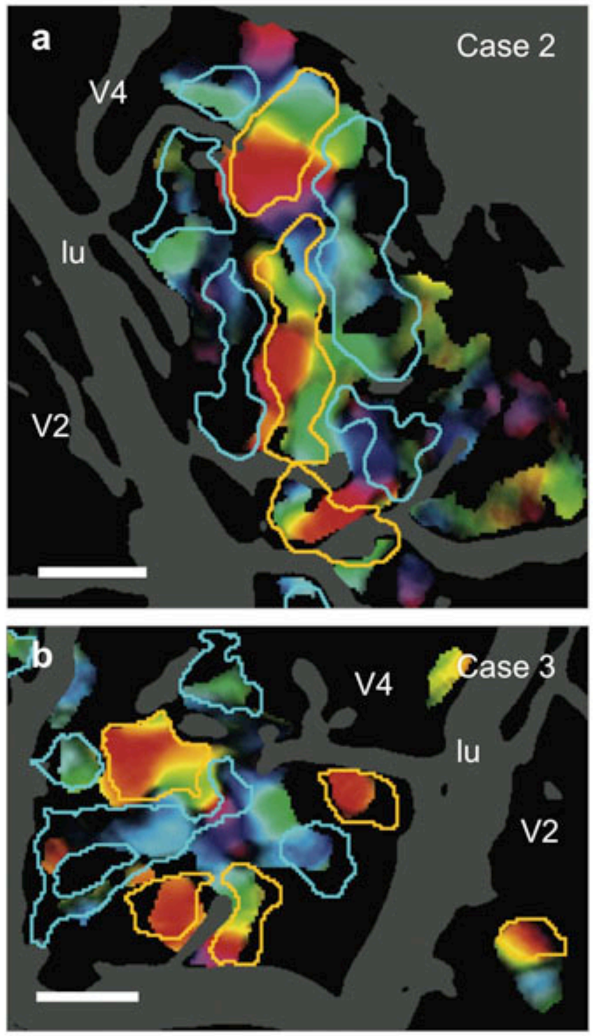
Supplementary Figure 1. Statistical maps of pairwise comparison. (**a**,**b**) Regions showing a significant difference in response to orientation-matched RG versus Lum (45° for **a** and 135° for **b**; case 1; two-tailed *t*-test, *n* = 208 and 206 trials for **a** and **b**, respectively). (**c**,**d**) Regions showing a significant difference in response to color-matched 45° versus 135° in the same imaging case (RG for **c** and Lum for **b**; n = 200 and 214 trials for **c** and **d**, respectively). (**e**–**h**) Data from the left hemisphere of another animal (case 3; *n* = 120, 116, 121, and 115 for **e**–**h**, respectively). Conventions are as for **a**–**d.** Note that some orientation-preferring regions (arrowheads in **d**,**g**) were observed in only RG (**g**) or Lum (**d**) stimulus condition and also showed a significant interaction between the two factors (RG/Lum and orientation) in the two-way ANOVA (**Fig. 2l**,**n**). Scale bar, 1 mm.



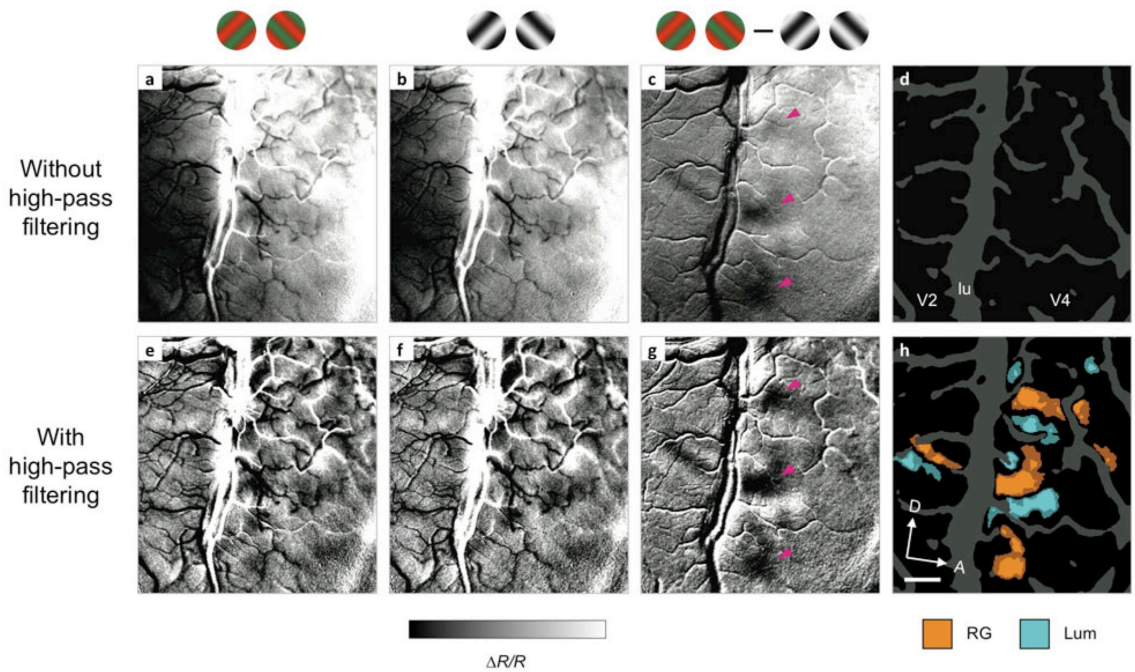
Supplementary Figure 2. Functional segregation of color and orientation domains revealed by a different set of stimuli. (**a**,**b**) Statistical maps revealed by two-way ANOVAs, based on the data obtained from case 1 but acquired on a different day, show color-sensitive (**a**, magenta) and orientation-sensitive (**a**, green) regions and regions with an interaction effect (**b**, white). In this imaging, the stimuli consisted of grating patches with the same luminance contrast of four different colors (red, yellow, green, blue) and two different orientations (45° and 135°), as shown above the maps. Mapping conventions are as for **Figures 2k**,**l.** Scale bar, 1 mm.



Supplementary Figure 3. Size-related attenuation of stimulus-evoked optical response preference. (**a**) Regions with a significant difference in response to RG minus Lum with a different stimulus size (case 2; two-tailed *t*-test, n = 118, 122, and 126 trials for top to bottom panels, respectively). The diameter of the stimulus used is indicated in the right. 'Full' means full screen gratings (24° in width). (**b**) Regions with a significant difference in response to 45° minus 135°. These maps were obtained from the same data set as used for **a**. To facilitate comparisons, blue crosses mark significant feature-sensitive regions, determined from the maps by 2° diameter stimuli. In RG versus Lum maps, the responses were attenuated in the full screen stimulation condition. The pattern of the responses to 45° versus 135° appeared less attenuated by full screen condition. Conventions are as in **Figure 2g**,**h**. Scale bar, 1 mm.

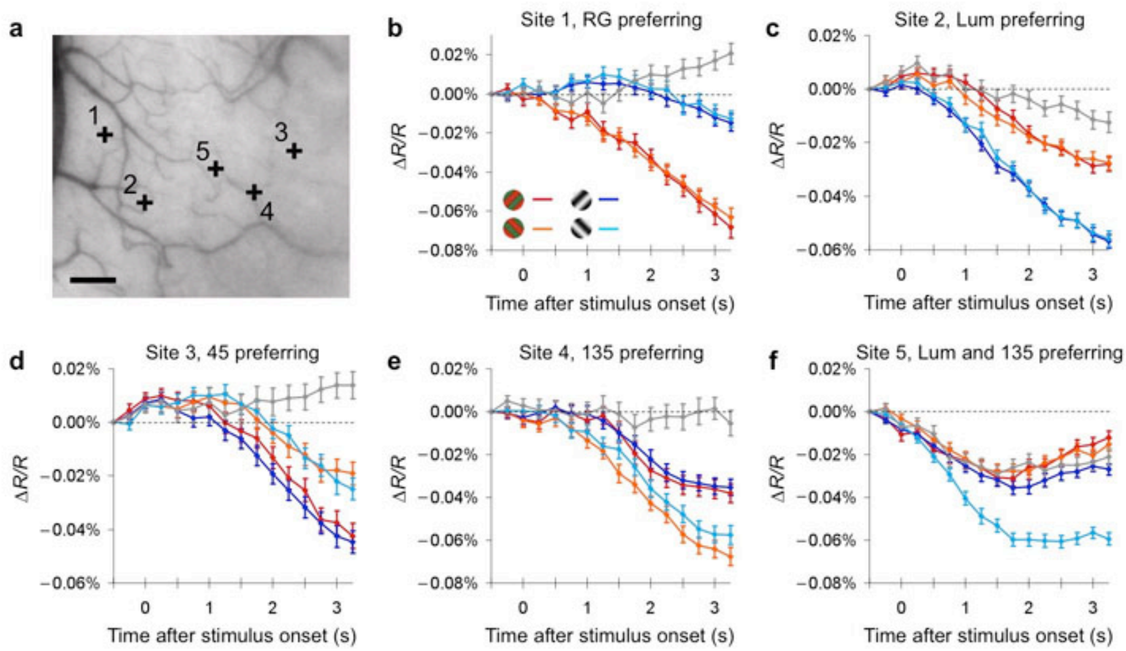


Supplementary Figure 4. Overlay of RG/Lum-preferring regions and hue-preference maps. (**a**,**b**) The outlines of RG-preferring (orange outlines) and Lum-preferring (cyan outlines) regions (as revealed by imaging using RG and Lum gratings) are superimposed on polar maps of hue preference from case 2 (**a**) and case 3 (**b**). Other conventions are as in **Figures 6c**,**d**. Scale bar, 1 mm.

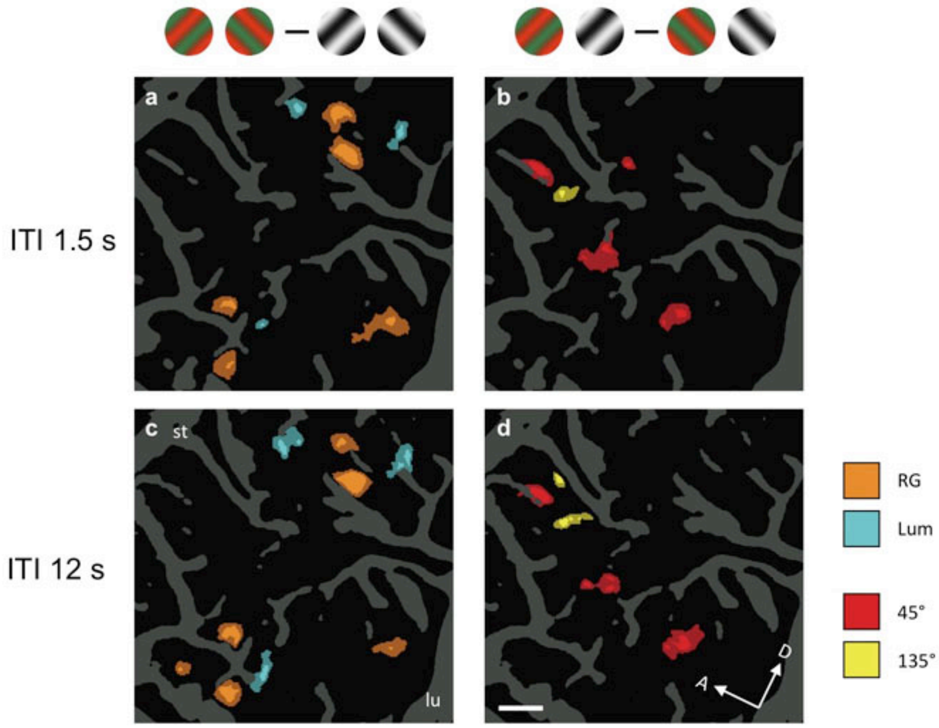


Supplementary Figure 5. Effects of high-pass filtering on imaging results. (**a**,**b**) Single condition maps in response to either RG (**a**) or Lum (**b**) from case 1, without high-pass filtering. These maps were obtained by averaging non-filtered maps of reflectance change ($\Delta R/R$) across trials (n = 200 trials for RG and 214 trials for Lum). (**c**,**d**) The difference map (**c**) and statistical map (**d**) of RG versus Lum from the data used in **a** and **b**. Even thought there appeared to be optical spots in the difference map (arrowheads in **c**), no region with a significant difference was observed without high-pass filtering (**d**, two-tailed *t*-test, $P > 0.05$, uncorrected) because of large trial-by-trial fluctuations of global signals. (**e**–**h**) The same analyses were performed using the same data as in **a**–**d** except that high-pass filtering was applied each $\Delta R/R$ map before averaging (using a 1.6 ×

1.6 mm median filter; see Methods). Optical spots are more visible in the difference map (arrowheads in **g**) and statistically significant (**h**). The range of $\Delta R/R$ indicated by the luminance key shown bottom is ± 0.1% for **a**,**b**, ± 0.05% for **c**, ± 0.04% for **e**,**f**, and ± 0.02% for **g**. Mapping conventions are as in **Figure 2c**,**g**.



Supplementary Figure 6. For **Supplementary Note**. Time courses of stimulus-evoked reflectance change in V4 without blank subtraction. (**a**–**f**) All conventions are as in **Figure 1** except that the $\Delta R/R$ values in the stimulation conditions (colored lines) were not subtracted by the ones in the blank condition. The time courses in the blank condition are shown by gray lines. Trends of reflectance changes in the $\Delta R/R$ values differ site by site. In some sites (**c**,**d**), the $\Delta R/R$ values initially increased a little, even in pre-stimulus periods, then started to decrease. In other sites, the values in the initial phase were relatively constant (**b**,**e**) or decreased from the beginning (**f**). Importantly, the trends of changes in the initial phase were similar among conditions at each site so these trend differences in the initial phase were almost removed after blank subtraction (**Fig. 1**).



Supplementary Figure 7. For **Supplementary Note.** Effects of inter-trial intervals (ITI) on imaging results. (**a**,**b**) Statistical maps of RG versus Lum (**a**) and of 45° versus 135° (**b**), obtained from case 4, using ITI of 1.5 s (two-tailed *t*-test, n = 146). (**c**,**d**) Statistical maps of RG versus Lum (**c**) and of 45° versus 135° (**d**), obtained from the same imaging region as in **a**,**b**, but using ITI of 12 s (two-tailed *t*-test, n = 153). Note that the distribution of revealed feature-sensitive regions are mostly conserved regardless of the difference in ITI. Conventions are as for **Figures 2g**,**h.** Scale bar, 1 mm.

Supplementary Table

Supplementary Table 1. Measurements of areas with significant effects of color type and orientation in the two-way ANOVA

Imaging location	color type factor[a]	Orientation factor[a]	Total area of regions (mm^2)[b]					
			With any effect or interaction	With a color type effect	With an orientation effect	With both color type and orientation effects	With both color type and orientation effects, expected by chance[c]	With an interaction effect
Case 1	RG, Lum	45°, 135°	9.88	6.22 (62.9%)	4.98 (50.4%)	1.37 (13.9%)	3.13 (31.7%)	0.35 (3.5%)
	R, Y, G, B	45°, 135°	13.7	9.52 (69.4%)	6.51 (47.4%)	2.31 (16.8%)	4.51 (32.91%)	0.32 (2.3%)
Case 2	RG, Lum	45°, 135°	9.74	6.26 (64.2%)	3.91 (40.2%)	0.43 (4.4%)	2.51 (25.8%)	0.00 (0.0%)
	R, Y, G, C, B, M	45°, 135°	14.2	10.5 (73.9%)	6.32 (44.4%)	2.61 (18.4%)	4.67 (32.8%)	0.65 (4.6%)
Case 3	RG, Lum[d]	45°, 135°	15.3	10.5 (68.8%)	5.27 (34.4%)	0.81 (5.3%)	3.62 (23.7%)	1.11 (7.2%)
	RG, Lum[d]	0°, 45°, 90°, 135°	18.7	13.5 (72.0%)	7.70 (41.2%)	2.59 (13.8%)	5.54 (29.7%)	1.46 (7.8%)
	R, Y, G, C, B, M	0°, 90°	17.5	13.3 (76.2%)	7.88 (45.2%)	3.96 (22.7%)	6.01 (34.4%)	2.07 (11.8%)
Case 4	RG, Lum	45°, 135°	3.16	1.97 (62.1%)	1.20 (37.9%)	0.00 (0.0%)	0.74 (23.5%)	0.00 (0.0%)

[a]See Methods for the details of the stimulus types. [b]Percentages indicate the ratio of the area to the total area of regions with any effect or interaction. [c]These expected values were estimated by multiplying the percentage of color type-sensitive areas by that of orientation-sensitive areas. [d]These two data sets were obtained from the same data set.

Supplementary Note

Inter-trial interval

The inter-trial interval (ITI) used in this study (1.5 s) was shorter than the ones used in other optical imaging studies (typically, 8.0–12.0 s or more)[47]. This interval was determined to meet our experimental requirements for presenting a variety of stimuli (up to 28 types) in a sufficient number of trials (40–100 trails) within a limited time of imaging session (about 1 hr). Because evoked optical signals need 12.0 s or more to return to baseline levels[47,49], response in preceding trials would affect optical signals in our imaging. Actually, we often found substantial changes of optical signals in blank (no stimulus) condition (gray lines in **Supplementary Fig. 6**), which may partly reflect remaining effects of evoked responses in the preceding trials. Regardless, this would not affect our findings because of the following reasons. First, responses from the preceding trials could be averaged across stimulus conditions by randomizing the stimulus sequence and removed from the response by blank subtraction or calculating differences between conditions. In **Supplementary Figure 6**, the trends of changes in blank condition is also observed in stimulation conditions, particularly in the initial phase of imaging period, and these trends are largely removed in **Figure 1**, in which blank subtraction was applied. Second, we conducted a supplementary experiment using different lengths of ITI (**Supplementary Fig. 7**). The functional maps obtained using either 1.5 s or 12 s ITI are similar to each other, indicating that the usage of a short ITI does not significantly affect the quality of functional mapping in this study.