

REPLY — In our recent article¹, we reported the use of high spatiotemporal resolution BOLD fMRI to detect early-negative BOLD signal changes and its application to map submillimeter iso-orientation columns in the cat visual cortex. Because the early-negative BOLD contrast is small and transient, the authenticity of the columnar layout derived from these data has been questioned by Dr. Logothetis. We would like to respond to his criticisms point by point.

Do functional maps based on the early-negative BOLD responses contain pixels from noise fluctuations? The answer is of course yes. In most functional data analysis methods, an arbitrary statistical threshold (that is, *p* value) is used to generate a functional map. Based on the statistical threshold used in our analysis, ~90% of the active pixels in the single-condition map (Fig. 1d in ref. 1) are expected to be caused by the visual stimulation, whereas the remaining ~10% of active pixels arise from random noise fluctuations. For example, the small number of early-negative response pixels without subsequent positive overcompensation (black contours in Fig. 1 above) can be attributed to noise fluctuation. The use of more sophisticated statistical methods might help to minimize noise contribution.

Why are the early-negative BOLD pixels also found around the sagittal sinus area? Functional MRI does not detect neural activity *per se*, but rather the resulting change in blood deoxyhemoglobin concentration. Deoxyhemoglobin drains from capillaries into venules and eventually into large veins. In fMRI⁷ and optical imaging⁶ studies, the negative BOLD signal changes induced by increase of deoxyhemoglobin concentration were observed initially in the active parenchyma, and later in the draining veins. Therefore, the presence of some negative response pixels around the sinus is not surprising. However, in our study, the negative response pixels around the sinus are clustered in patchy domains (Fig. 1d of ref. 1 and white contours in Fig. 1 above), in contrast to Logothetis' statement that these negative response pixels are 'largely overlapping' with the boundaries of the sagittal sinus. This, plus the fact that our imaging slice was ~0.5 mm below the cortical surface, indicates that the negative response pix-

els around the sinus originated predominantly from tissue areas (for example, columns from the suprasplenial gyrus on the medial bank) below the sagittal sinus. Nevertheless, our results clearly contradict the previous fMRI studies in monkeys by Logothetis *et al.*⁴, which claimed that the early-negative BOLD signal change was observed only in the tissue, and not in the sagittal sinus (Fig. 5 in ref. 4). This is puzzling, because optical imaging studies suggest that deoxyhemoglobin signals should be observed also in large draining vessels³. More importantly, in their paper⁴, the onset time (6 s) of positive BOLD signals from the tissue significantly lags behind the onset time (1 s) of positive signals from the sagittal sinus (Fig. 5c in ref. 4). This is counter-intuitive; given that blood drains from tissue to large veins, the signal in the tissue ought to precede the signal in the sinus. One possible explanation for the discrepancy between our data¹ and Logothetis *et al.*⁴ is that the signals that Logothetis *et al.*⁴ attribute to neural activation in their study might have been contaminated by large-vessel flow effects due to rapid radiofrequency pulsing; this so-called 'inflow effect' is a well-known source of artifact in the fMRI community⁸.

Are the columnar maps obtained from the negative BOLD signals genuine? Although our early-negative BOLD maps contain some pixels both from noise and from draining-vessel artifacts, the overall functional specificity of columnar maps is convincing. Unlike the positive BOLD response, the early-negative response is highly specific to the cortical columns (Fig. 2 in ref. 1). For example, the 45° columns have negative BOLD responses during the 45° stimulus, but not during the 135° (orthogonal) stimulus. More importantly, as noted in the accompanying News and Views by Grinvald *et al.*⁶, the single-condition columnar maps of orthogonal orientations (0° versus 90° and 45° versus 135°) are complementary. Moreover, topological features of the composite maps obtained from the negative BOLD signals (Fig. 4a in ref. 1) are consistent with those seen with optical imaging and single-neuron recording. Taken together, our results strongly support the authenticity of columnar architectures derived from the negative BOLD signals.

To further corroborate the functional validity of negative BOLD signals, more experiments are necessary, as discussed by Grinvald *et al.*⁶ and by Logothetis in his letter. We are actively working to improve the contrast-to-noise ratio, the reproducibility of single-condition columnar maps, and the cross-validation of fMRI maps with single and multi-neuron recordings.

Much work needs to be done before the early-negative BOLD technique can be applied for routine brain mapping at columnar resolution, especially in humans. The most important challenges are to increase the detectability of the negative BOLD signals (for example, by using high magnetic fields and better data processing methods) and to eliminate large vessel contributions (for example, by using different fMRI approaches such as spin-echo⁹ and perfusion-based¹⁰ MRI techniques). As with any new technique, this novel method for non-invasive visualization of cortical columns by fMRI will need further improvement and cross-validation. We believe, however, that our results lay the foundation for non-invasive mapping of brain functions at columnar resolution, thus opening new vistas for neuroscience research.

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