Neural source of laminar fMRI responses examined with temporal frequency visual stimuli

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Introduction Neuroscientists have been interested in layer-specific neural response properties for decades in an attempt to better understand inter-laminar sensory processing (1). If hemodynamic responses are sensitive to underlying changes in neural activity, then these laminar responses may be non-invasively probed with hemodynamic-based fMRI techniques. Our group and others have reported that under certain conditions, BOLD and relative cerebral blood volume (CBV) responses coincide with the layer of highest neural activity (2,3). However, it is still unknown whether layer-specific changes in neural activity give rise to layer-specific hemodynamic responses. To investigate this important issue, we intentionally modulate layer-dependent changes in neural activity during fMRI studies. Neural responses (as assessed by spiking activity) to temporal frequency of visual stimuli (i.e., tuning curves) have been shown to peak at ~3.5 Hz in the supragranular layer (upper-most of the three principal cortical layers), ~3.1 Hz in the granular (middle) layer and ~6.0 Hz in the infragranular (bottom) layer (4). Therefore, if layer-specific hemodynamic responses result from these layer-specific changes in neural activity, then tuning curves measured by high-resolution BOLD and relative CBV fMRI are expected to follow this same trend.

Materials and Methods Four adolescent cats were anesthetized with 1% isoflurane, and maintained within normal physiological range. All MRI experiments were carried out at 9.4 T with a 1.7-cm surface coil placed on the head. A 1-mm thick coronal slice through the primary visual cortex devoid of large vessels was prescribed based on scout images and a stereotaxic atlas (5). BOLD fMRI was performed with two-segment gradient-echo EPI (TR per segment= 0.5 sec, TE= 20 ms, FOV= 20.1×13.4 cm^2, matrix size= 96×64). CBV-weighted fMRI was performed with administration of iron oxide nanoparticles (MION) with acquisition parameters similar to BOLD fMRI studies except that TE= 10 ms. Before the Fourier reconstruction, k-space data were zero-filled to 128×128 (nominal resolution= 157×105 μm^2) and application of a Hamming filter. Visual stimuli of 24-s duration consisted of full-field moving sinusoidal gratings with spatial frequency of 0.15 cycles/degree and temporal frequencies of 1, 2, 10, and 20 Hz (pseudo-randomized within each run). T_1- and T_2-weighted anatomical images were acquired by inversion-recovery turbo-FLASH and fast-spin echo sequences respectively. Fig 1 shows the regions of interest (ROIs); the granular layer was defined by outlining the bright stripe (indicating high myelin content) in the middle of the cortex on the T_1-weighted image, and its assignment was corroborated by the presence of a dark stripe (indicating high microvascular-density) in the T_2-weighted image. After extracting the BOLD and CBV-weighted time courses from the ROI-based analysis, relative CBV was calculated with BOLD compensation for 4 temporal frequencies (6). The temporal frequency tuning curves were generated by averaging responses within a 24-s period (between 5 and 28 s after stimulus onset) for each temporal frequency and then normalizing to the maximum response.

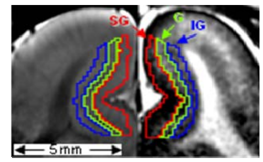

Fig 1. Laminar regions of interest for fMRI studies. ROIs within the supragranular layer (SG: red), granular layer (G: green) and infragranular layer (IG: blue) were defined on T_2-weighted (left) and T_1-weighted (right) images.

Results and Discussion The normalized and averaged BOLD and relative CBV fMRI temporal frequency responses for the three cortical layers and four temporal frequencies are shown in Fig 2. There are no significant differences in responses between layers for BOLD (ANOVA, $p>0.69$) or relative CBV (ANOVA, $p>0.35$) responses. Furthermore, all three laminar BOLD ($R>0.91$, $p<0.001$) and relative CBV ($R>0.94$, $p<0.001$) responses are highly correlated. The preferred temporal frequency of each layer can be estimated by logarithmic fitting with Gaussian function. For BOLD, the preferred frequency was 3.37, 3.28 and 3.21 Hz for supragranular, granular, and infragranular layer, respectively; for relative CBV, these were 3.13, 2.82 and 3.11 Hz. For supragranular and granular layers, the preferred frequencies estimated by BOLD and relative CBV fMRI are reasonably close to values measured by spiking activity (4). However, the preferred frequency for the infragranular layer estimated by fMRI was much lower than the value measured by electrophysiology, with an fMRI response similar to that of the upper two layers. These results imply that neither BOLD nor CBV fMRI responses followed layer-specific changes in neural activity, suggesting that hemodynamic responses across cortical layers may not fairly represent underlying neural activity. Possible explanations for these findings include: 1) layer-specific differences in tuning curves may not have been detectable due to the low signal to noise ratio (SNR) of our fMRI studies, 2) hemodynamic response maybe more correlated with LFP than spiking activity; if all layers have lower preferred frequency measured by LFP, then preferred frequency estimated by BOLD or relative CBV are also expected to be lower, and 3) the intrinsic architecture of the cortical vasculature may not give rise to layer-specific hemodynamic responses. The first explanation is unlikely since with similar hardware our group has successfully detected responses coinciding with sub-millimeter columnar structures, implying that our experimental configuration has adequate SNR to resolve microscopic fMRI signals (7). The second explanation is also unlikely because the preferred frequency measured by LFP is actually higher than the one measured by spiking activity in granular layer (8), and possibly in infragranular layer. The third explanation is more plausible, since a single cortical vasculature unit is known to be comprised of vertical penetrating arteries, laminar branching small vessels, and vertical returning veins; therefore, regulation of hemodynamic responses may occur along cortical columns with indistinguishable differences in hemodynamic response between each principal cortical layer. Nevertheless, it is currently very difficult for hemodynamic-based fMRI to differentiate layer-specific neural activity.

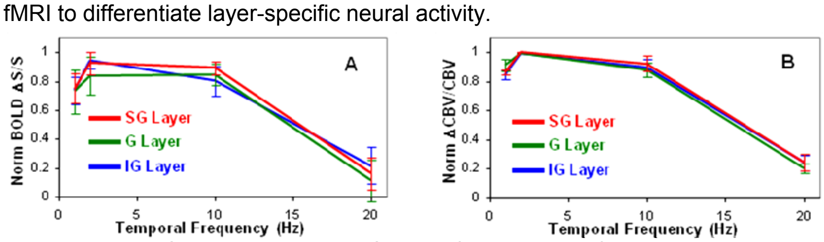

Fig 2. Laminar BOLD (A) and relative CBV (B) fMRI temporal frequency tuning curves. No significant difference in BOLD or CBV responses could be found between infragranular (IG: blue), granular (G: green) and supragranular (SG: red) layers at each frequency. Error bars: SEM of four animals

Acknowledgments We thank Ping Wang and Michelle Tasker for animal preparation and Kristy Hendrich for 9.4 T support. This work is funded by NIH grants EB003324, EB003375, NS44589

References 1. Hirsch, Martinez. Curr Opin Neurobiol 2006;16(4):377. 2. Zhao, et al. Neuroimage 2006;30(4):1149. 3. Logothetis, et al. Neuron 2002;35(2):227. 4. Leventhal, Hirsch. J Neurophysiol 1978;41(4):948. 5. Reinoso-Suárez. Darmstadt: E. Merck AG; 1961. 6. Kennan, et al. Magnet Reson Med 1997;37(6):953. 7. Moon, et al. Journal of Neuroscience 2007;27(26):6892. 8. Viswanathan, Freeman. Nat Neurosci 2007;10(10):1308.