Laminar-Specific fMRI Changes in Rat Olfactory Bulb following Evoked Odor Stimulation

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Introduction: The olfactory bulb (OB) is an ideal model system to study laminar-specific activation because 1) layers are discrete and easily identifiable with anatomical MRI and 2) each layer has distinct neurophysiological roles in odorant encoding (fig. 1a). [^{14}C]-2-deoxy-D-glucose (2DG) methods utilize radioactive glucose and have been used to report metabolic increases in OB cells after exposure to an odor stimulus. Previous 2DG studies of OB showed large activation foci surrounded by lesser, global activity (fig. 1b) and metabolic profiles that differed across layers (fig. 3c) [1]. Blood oxygenation level-dependent (BOLD) fMRI changes are coupled to cellular metabolism and, similar to 2DG, showed large activation increases in the olfactory nerve (ONL) and glomerular (GL) layers. However, unlike 2DG, BOLD showed limited signal change in the external plexiform layer (EPL) and little to no change in mitral/tufted (M/T) and granule (GCL) cell layers [2]. The purpose of the current study is to investigate whether this laminar discrepancy between 2DG and BOLD studies is due to a dissociation in the location of metabolic and vascular responses in OB. Cerebral blood volume (CBV) fMRI was chosen due to its reduced signal contributions from large vessels and increased capillary sensitivity at locations specific to neural activity [3].

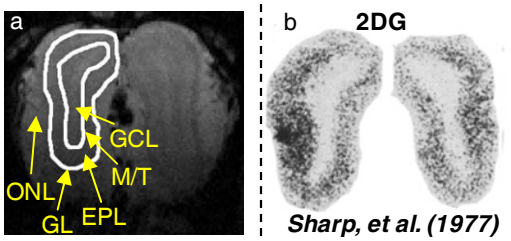

Figure 1: (a) Laminar distribution of OB. (b) 2DG study shows a discrete focus of activation surrounded by lesser, global activation.

Methods: Four male Sprague-Dawley rats, 344 ± 9 g (mean ± std), were induced with 1.3 g/kg urethane (0.1 g/kg/hr maintenance). Odor delivery (5% amyl acetate) was precisely time-locked to fMRI acquisition in a block design experiment (2-min OFF, 1-min ON, 2-min OFF). BOLD fMRI images were acquired on a 9.4-T magnet using a Fast Low-Angle Shot (FLASH) sequence with the following parameters: TR = 125 ms, TE = 18 ms, 5 slices, 110 x 110 μm^2 in-plane resolution, 500 μm slice thickness, # of blocks = 15. To achieve CBV-weighted images, 15 mg/kg MION was injected following BOLD fMRI. Acquisition parameters were identical, except TE = 8 ms. The fMRI blocks were averaged and a pixel-wise two-sample Student's t-test (odor ON vs. OFF) was performed. The valence of CBV t-values was reversed for comparison to BOLD.

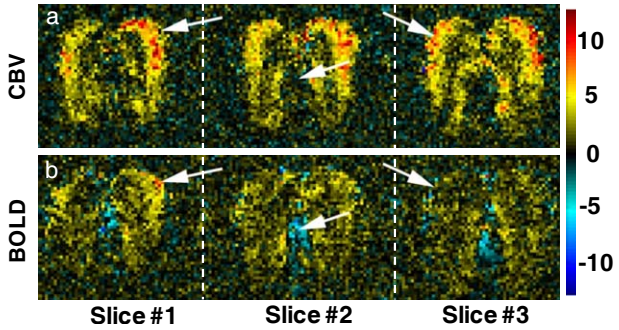

Figure 2: CBV t-value maps (no threshold) show activation foci (red pixel clusters) in ONL, synapse-rich GL, EPL and M/T (slice #3 arrow) while BOLD maps show activation in ONL, a layer devoid of synapses (slice #1 arrow), GL, and large blood vessels (slice #2, arrow). The activation foci and lesser, global activation in CBV (yellow pixels) resemble the 2DG image in fig. 1b.

Results and Discussion: Results from one rat are shown. Evoked odor signal changes were more significant (↑ t-value) in the CBV images (no threshold) compared to BOLD (fig. 2) and, therefore, CBV has increased sensitivity to detecting small signal changes. In CBV images, discrete activation foci (red pixel clusters) were located in ONL, GL, EPL and M/T (fig. 2, slice #3 arrow); in addition to a lesser, global activation (yellow pixels) that resembles the activation focus and diffuse global metabolism observed in the 2DG image (fig. 1b) [1]. Contrarily, activation in BOLD images is spurious and localized to large blood vessels (fig. 2, slice #2 arrow), ONL and GL (fig. 2, slice #1 arrow).

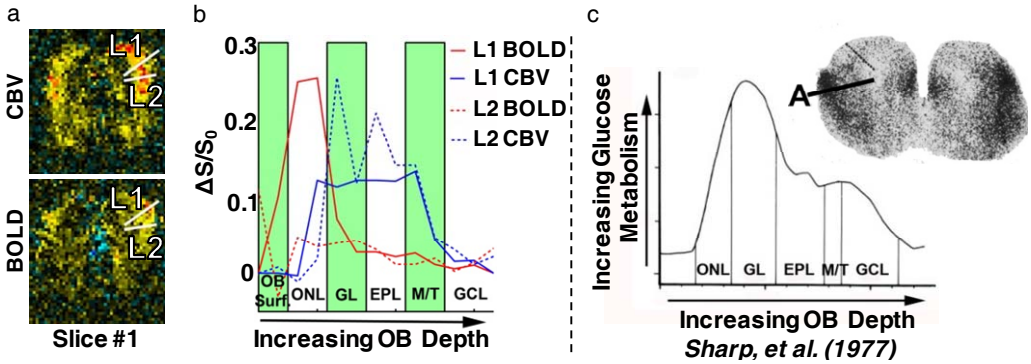

Figure 3: (a) Signal change along two lines: L1 passes through the focus of most significant activation for BOLD, while L2 passes through a local CBV maximum. (b) Greatest CBV signal change is observed in GL and is sustained as deep as M/T (blue), whereas, for BOLD, the greatest signal change is in ONL and rapidly decreases within GL (red). (c) 2DG laminar distribution along line A is similar to CBV.

Blood volume was observed to be greatest in ONL that progressively decreases in deeper layers (data not shown) and was consistent with optical imaging reports of large arterioles populating ONL, while capillaries first appeared in GL [4]. Because ONL is devoid of capillaries and only contains larger vessels, it is unclear if the hemodynamic response in this layer is spatially specific to neural activity for BOLD and CBV. Next, the laminar distribution of the CBV signal change is greatest in GL, but large changes are still observed in deeper layers like M/T; while BOLD has the greatest signal change in ONL and less discernible signal changes below GL (fig. 3a, b). A laminar profile of 2DG metabolism through a local focus of increased activation in rat OB (line A in fig. 3c) initially increases in ONL, peaks in GL and gradually decreases throughout deeper layers [1], which is similar to the observed CBV profile in our study.

Conclusions: CBV fMRI has increased sensitivity and spatial-specificity that is highly suitable for measuring changes in different lamina of OB. In addition, signal changes observed in deeper layers, like M/T and GCL, give CBV fMRI the ability to measure functional changes in OB output layers, which currently cannot be accomplished in rat optical imaging experiments. Finally, the t-value maps and laminar profiles of CBV images are similar to 2DG studies, which give confidence that CBV fMRI is measuring the vascular response at sites of metabolically active, odor-evoked cells.

References: [1] Sharp, et al. (1977) J Neurophysiol, 40:800-813. [2]Yang, et al. (1998) PNAS, 95:7715-7720. [3] Zhao, et al. (2006) Neuroimage, 30:1149-1160. [4] Chaigneau, et al. (2003) PNAS, 100:13081-13086.