Laminar-resolution BOLD and CBV fMRI responding to layer-specific neural modulations in the olfactory bulb

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Target Audience: High-resolution fMRI scientists.

Introduction: High-resolution fMRI is of great interest for resolving layer-specific neural activity, but it is currently unclear whether the vasculature is regulated at a laminar scale. In order to investigate this issue, the olfactory bulb is an ideal model system because 1) layers are discrete and easily identifiable with anatomical MRI (Fig. 1A) and 2) layers can be preferentially activated with different stimuli (Fig. 1B). Axons from odor sensory neurons project through the olfactory nerve layer (ONL) and form excitatory synapses with the apical dendrites of mitral cells in the glomerular layer (GL). Mitral cell dendrites propagate through the external plexiform layer (EPL) and form dendro-dendritic synapses with the apical dendrites of granule cells, whose bodies are located in the granule cell layer (GCL). Finally, output from the mitral cell body layer (MCL) exits the bulb via the lateral olfactory tract (LOT). To preferentially activate different layers, odor stimulation and electrical stimulation of LOT were performed during BOLD and CBV-weighted fMRI. Odor stimulation preferentially activates superficial bulb layers, like GL[1], while LOT stimulation mainly evokes neural activity in the middle bulb layers, like EPL, via dendro-dendritic synapses[2].

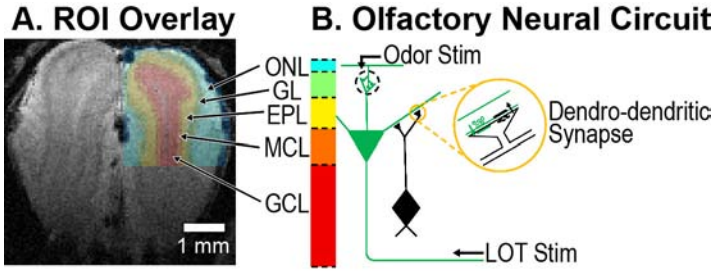

Figure 1: (A) Anatomical T_2-weighted image with ROI overlay to show laminar definitions. (B) Experimental paradigm in which superficial and middle bulb layers are preferentially evoked with odor and LOT stimulation.

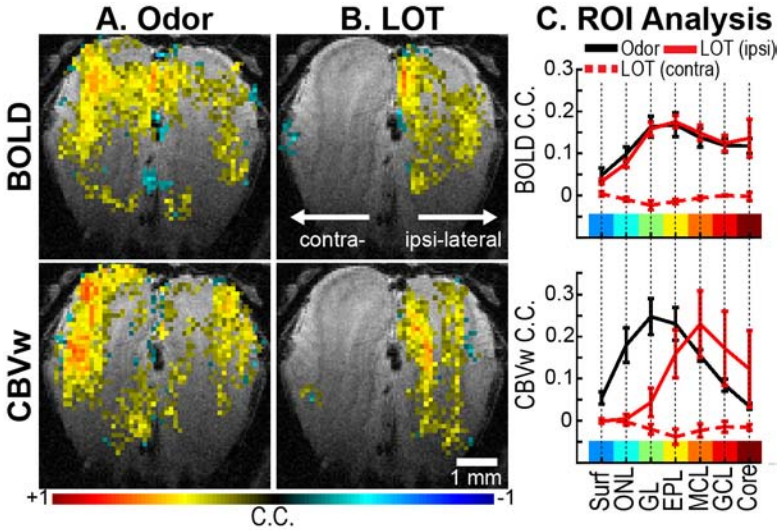

Figure 2: (A) Odor evokes activation in superficial layers for both BOLD and CBVw fMRI, while (B) LOT activation is unilateral and, for CBVw only, is localized to middle layers. (C) Average C.C. across 5 slices and 3 animals (mean ± s.e.m., n = 3). Activation laminar profiles are clearly dissociated for CBVw fMRI only.

Methods: Sprague-Dawley rats were induced with 45 mg/kg α-chloralose (40 mg/kg/hr maintenance) and a tungsten stimulating electrode was located to the right LOT. Odor delivery (5% amyl acetate) and LOT micro-stimulation (-200 μA, 200 μs duration, 40 Hz) were interleaved in a block design experiment (120-s off, 64-s on, 120-s off). For CBVw fMRI, 15 mg/kg MION was injected following BOLD fMRI. fMRI data were acquired at 9.4-T with a compressed-sensing, gradient-recalled echo technique[3]. Imaging parameters were T_R = 125 ms, T_E = 18 ms for BOLD and 8 ms for CBVw, 5 slices, 110 x 110 μm^2 in-plane resolution, 500 μm slice thickness, reduction factor of 4, and temporal resolution = 2 s. In one animal, ultra high-resolution CBVw fMRI images with LOT stimulation were acquired with 55 x 55 μm^2 in-plane resolution. The fMRI blocks were averaged and pixel-wise time courses were cross-correlated (C.C.) with the hemodynamic response functions. ROI analyses were performed by averaging all of the pixel-wise C.C. values for each ROI.

Results and Discussion: For odor stimulation, BOLD and CBVw activation maps (Fig. 2A) show focal activations in GL of the dorsal-lateral bulb and is consistent with previous fMRI[1] and 2-DG[4] studies of amyl acetate odor activation. LOT activation maps (Fig. 2B) show a unilateral response in the right olfactory bulb, which is ipsi-lateral to LOT stimulation, and is consistent with the neural activity evoked by this stimulation. Layer-dependent responses (Fig. 2C) show that BOLD (top) has a broad, similar activation pattern that peaks around GL and EPL during odor and LOT stimulations, while the CBVw response (bottom) peaks in GL for odor stimulation and shifts to middle layers (MCL) for LOT stimulation. Note that no significant change was observed in the olfactory bulb contralateral to the LOT stimulation (dashed lines in 2C). To further investigate the well-defined ring-shaped activation during LOT stimulation, ultra high-resolution (55 x 55 μm^2) CBVw images were acquired (Fig. 3). Activation is clearly observed to stay inside GL (outer green contour) and overlap with EPL and MCL (inner green contour). The activation line profile (Fig. 3B) shows distinct activation peaks in EPL and MCL with lesser activation in surrounding layers, likely due to layer-specific hemodynamic regulation at the site of dendro-dendritic synaptic activity[2].

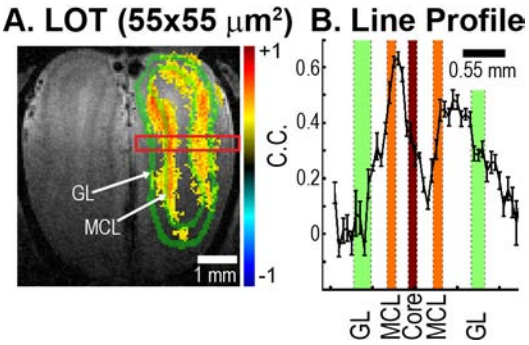

Figure 3: (A) Ultra high-resolution CBVw fMRI map showing the LOT ring of activation. (B) Line profile from red box confirms peak activation in middle bulb layers (mean ± s.e.m., n = 7 pixels in dorsal-ventral axis)

Conclusions: BOLD and CBVw fMRI responses were measured in olfactory bulb following odor and LOT stimulations. The layer-dependent BOLD fMRI response was less specific to the sites of synaptic activity compared to CBVw, possibly due to contributions of draining venous blood or to lower sensitivity to changes at capillaries. CBVw activation showed a clear shift of laminar-dependent fMRI responses corresponding to the location of increased neural activity. Our data provide definitive evidence that the hemodynamic response is regulated at the laminar level resulting from a single, non-differential stimulus.

References: 1. Poplawsky A and Kim S-K. Layer-Dependent BOLD and CBV-weighted fMRI Responses in the Rat Olfactory Bulb. NeuroImage. Submitted. 2. Rall W and Shepherd G. Theoretical Reconstruction of Field Potentials and Dendrodendritic Synaptic Interactions in Olfactory Bulb. J Neurophysiol. 1968;31:884-915. 3. Zong X, Lee J, Poplawsky A, et al. *In Vivo* Compressed Sensing fMRI using Conventional Gradient-recalled Echo and EPI Sequences. NeuroImage. Accepted. 4. Johnson B, Woo C and Leon M. Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb. J. Comp. Neurol. 1998;393(4):457-471.