# Contributions of excitatory and inhibitory neural activities to BOLD and CBV fMRI

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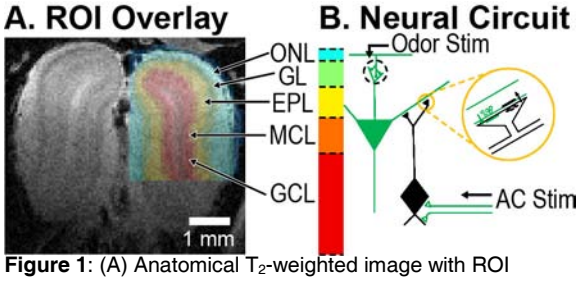


**Figure 1**: (A) Anatomical $T_2$-weighted image with ROI overlay to show laminar definitions. (B) Experimental paradigm in which excitatory mitral cells (green cell body) are preferentially evoked with odor stimulation and inhibitory granule cells (black body) are evoked by AC stimulation.

**Target Audience**: fMRI scientists and clinicians studying disease diagnosis.

**Introduction**: It is commonly assumed that increased excitatory neural processes increase the hemodynamic response measured by fMRI. However, it is currently unclear whether and how inhibitory neurons contribute to the hemodynamic response (e.g., a decrease or increase in BOLD fMRI). This issue is challenging to examine because it is difficult to independently control excitatory and inhibitory activities, and their individual responses cannot be precisely separated since they are co-localized at laminar resolutions in the cortex. To overcome these problems, we chose the olfactory bulb model because 1) excitatory and inhibitory neurons are organized to different layers that can be identified with anatomical MRI (Fig. 1A) and 2) excitatory and inhibitory neurons can be preferentially activated with different stimuli (Fig. 1B). Axons from odor sensory neurons project through the olfactory nerve layer (ONL) and activate the apical dendrites of excitatory mitral cells in the glomerular layer (GL). Mitral cell bodies, located in the mitral cell layer (MCL), are the primary projection neurons of the bulb. Inhibitory granule cells, located in the granule cell layer (GCL), are activated by cortical feedback fibers from the anterior commissure (AC). In addition, mitral and granule cells form reciprocal dendro-dendritic synapses in the external plexiform layer (EPL), whereby each cell type can exert either excitatory or inhibitory influence onto the other[1] (Fig. 1B, orange circle). To preferentially drive excitatory and inhibitory processes, odor stimulation and electrical stimulation of AC were performed, respectively, and the resultant hemodynamic responses were measured with BOLD and CBV fMRI.

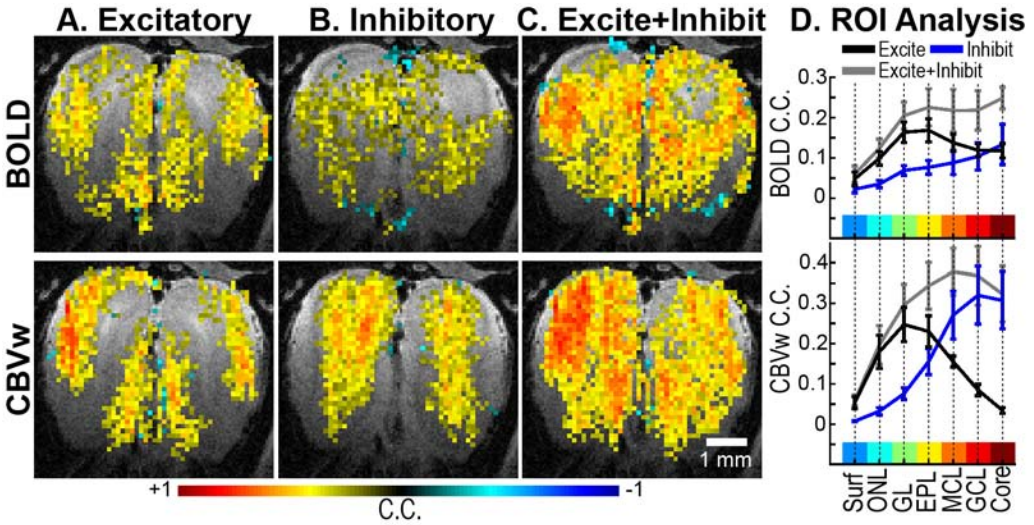


**Figure 2**: (A) Excitatory and (B) inhibitory neurons were preferentially evoked by odor and AC stimulations, respectively. The location of activation is consistent with the site of neural activity with excitation occurring in superficial layers and inhibition in deep layers. (C) Combined stimulation evokes activity across all layers. (D) The average C.C. across 5 slices and 3 animals (mean ± s.e.m., n = 3). The combined stimulation responses (gray) appear to be the addition of the individual stimuli (black + blue) for both fMRI contrasts.

**Methods**: Three male Sprague-Dawley rats were induced with 45 mg/kg α-chloralose (40 mg/kg/hr maintenance) and a tungsten stimulating electrode was positioned to the left AC. Odor delivery (5% amyl acetate), AC micro-stimulation (-200 μA, 200 μs duration, 40 Hz) and combined odor plus AC stimulation were interleaved in a block design experiment (120-s off, 64-s on, 120-s off). For CBV-weighted 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[2]. Imaging parameters were $T_R$ = 125 ms, $T_E$ = 18 ms for BOLD and 8 ms for CBVw, 5 slices, 110 x 110 $\mu m^2$ in-plane resolution, 500 μm slice thickness, reduction factor of 4, and temporal resolution = 2 s. 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**: Odor and AC stimulations were used to preferentially drive excitatory and inhibitory neural processes, respectively. Excitatory BOLD and CBVw activations (Fig. 2A) are primarily located to superficial bulb layers, like GL, while inhibitory activation (Fig. 2B) is observed mostly in deeper layers, like GCL. It is noted that inhibitory CBVw activation appears more concentrated in deep layers, while BOLD activation is more diffuse and includes superficial layers. AC stimulation data indicate that the increased inhibitory neural activity indeed increases hemodynamic responses. When both excitatory and inhibitory circuits are simultaneously stimulated by odor and AC stimuli, an increase in total (excitatory + inhibitory) synaptic activity is expected at dendro-dendritic connections in EPL and MCL, which results in a reduced spiking output in MCL. Combined stimuli increase both fMRI responses (Fig. 2C), and activations are similarly observed throughout the entire bulb. To further analyze contributions of excitatory and inhibitory activities, layer-dependent fMRI responses are plotted in Fig. 2D. Excitatory odor stimulation (black lines) induces a peak in GL and EPL for both BOLD (top) and CBVw fMRI contrasts (bottom), while inhibitory AC micro-stimulation (blue lines) induces a peak in GCL. When the combined stimuli (gray lines) were applied, BOLD and CBVw responses are similar to the sum of the individual excitatory (black line) and inhibitory (blue lines) stimulations. Of a particular importance, the largest separation between the combined and individual stimulations is observed in the middle layers, like EPL, where there is the strongest interaction of the combined stimuli. This observation indicates that both excitatory and inhibitory synaptic activities contribute to the hemodynamic response.

**Conclusions**: BOLD and CBVw fMRI responses increase in the olfactory bulb following inhibitory AC micro-stimulation, which is, to our best knowledge, the first *in vivo* study to demonstrate the role of inhibitory neurons to hemodynamics. Both excitatory and inhibitory neural activities contribute to the hemodynamic response; thus, the contributions of total synaptic activity must be considered for proper interpretation of increased BOLD fMRI.

**References**: 1. Mori K and Takagi S. Activation and inhibition of olfactory bulb neurones by anterior commissure volleys in the rabbit. J Physiol. 1978;259:589-604. 2. Zong X, Lee J, Poplawsky A, et al. *In Vivo* Compressed Sensing fMRI using Conventional Gradient-recalled Echo and EPI Sequences. NeuroImage. Accepted.