Odor-evoked fMRI maps are coupled to calcium-sensitive dye imaging patterns of input activity in the olfactory bulb

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TARGET AUDIENCE: Neuroscientists interested in understanding sensory processing of the brain, and imaging scientists interested in cellular basis of fMRI

PURPOSE: Complex neuropil events give rise to different functional imaging signals (e.g., fMRI, calcium imaging, etc) that are used to map activities of sensory systems at different spatiotemporal scales. Exogenous calcium-sensitive dyes are taken up by olfactory receptor neurons in the nose and are slowly transported to presynaptic terminals in glomeruli of the olfactory bulb. These presynaptic dyes report the input from the nose to the bulb [1]. On the other hand the fMRI signal, which is based the endogenous intravascular probe deoxyhemoglobin, is blood oxygenation level dependent (BOLD) and reflects an intricate coupling between metabolic and hemodynamic changes that support the electrochemical activities associated with bulk neuropil activity, not a particular class of neuronal activity. While the BOLD signal can measure glomerular-level activity patterns in the entire olfactory bulb, optical imaging of calcium-sensitive signals is usually localized to the dorsal layers of the glomerular sheet [2]. To improve the functional understanding of odor-evoked glomerular activity patterns revealed by the BOLD signal and to relate how the input activities of glomeruli reflected by calcium imaging relate to bulk neuropil activity of fMRI, we designed a study to image the same rats with fMRI first, followed by calcium imaging. Excellent correspondence between odor-evoked fMRI maps and calcium-sensitive dye imaging patterns of input activity suggests input activity is a dominant part of neuropil activity in glomeruli. While optical imaging may be used to feature the fast timing of dorsal glomerular clusters, fMRI may be used to map the entire glomerular sheet in the olfactory bulb.

METHODS: ***Animal preparation****:* Male Sprague-Dawley rats (250-350 g; Charles River, Wilmington, MA) were anesthetized with urethane (1.3 g/kg, intraperitoneal) with additional doses (0.13 g/kg) administered if necessary depending on the duration of the experiment. These rats were freely breathing. Physiology was monitored using Mouse Ox (www.starrlifesciences.com). ***fMRI (n=6):*** The fMRI data were obtained on a modified 9.4T system with Varian (Agilent Technologies, Santa Clara, CA) spectrometer using a custom-built ^{1}H surface coil. During fMRI recordings, the rat was positioned prone in a specially designed plastic holder so that a surface coil (diameter, 1.4 cm) was at the top of the rat's olfactory bulb [3, 4]. Whole olfactory bulb was shimmed using local B0 shim method [5]. The static field inhomogeneity was optimized until the half-height line width of water in the shimming voxel was less than 15-20 Hz. The neuroanatomy was imaged using fast spin echo with multi slice (fSEMS) sequence. For functional imaging of the dorsal bulb we used FLASH contrast and the following parameters: TR = 312 ms; TE = 12 ms; FOV = 1.56×1.56 cm^2; image matrix = 64×64; number of slices = 5; slice thickness = 300 μm. Odor delivery via an optimized flow tube was precisely time-locked to fMRI acquisition in a block design experiment (3-min OFF, 2-min ON, 3-min OFF) and was controlled through Spike-2 software. ***Calcium imaging (n=6)***: Three days after fMRI rats recovered, olfactory receptor neurons in the dorsal recess of the nasal cavity of rats were loaded bilaterally with dextan-conjugated calcium sensitive dye (Oregon Green BAPTA 488-1 dextran, Invitrogen) using a well-established protocol [1]. Odorant evoked calcium signals were imaged across the dorsal bulb during the presentation of odors, at 256x256 pixels and 25 fps. Raw images were converted to images representing the relative change in fluorescence (%ΔF/F) in each pixel and frame after stimulus application. ***Co-registration:*** Using Bioimage suite (www.bioimagesuite.org), co-registered all of the images by maximizing the similarity between the optical anatomical image and the MRI anatomical image.

RESULTS: We succeeded in concurrent imaging of the same rats for fMRI and calcium imaging during orthonasal odor stimuli. First we registered the axial image of the dorsal OB as shown in **Fig.1A**. Subjects were repeatedly exposed to odor stimuli and resultant patterns were compared. **Fig 1B** shows the bulbar responses (calcium maps and fMRI activity) of a subject exposed to two different odors across two rats. Odor evoked calcium maps show strong specificity for methyl valerate and ethyl butyrate. fMRI activation maps revealed similar activation patterns for both the odors. Calcium maps were later overlaid on the registered MRI anatomy. Similar regions in anterior OB were significantly activated under both imaging modalities (dotted circle). Excellent correspondence between odor-evoked fMRI and calcium maps was observed across trials and subjects (e.g., Rat1 and Rat2, **Fig 1B**) for each imaging modality. To assess inter-trial reproducibility; a subject was exposed to multiple stimuli of different odors. Cross correlation across trials for fMRI and calcium maps are shown in **Fig 1C**.

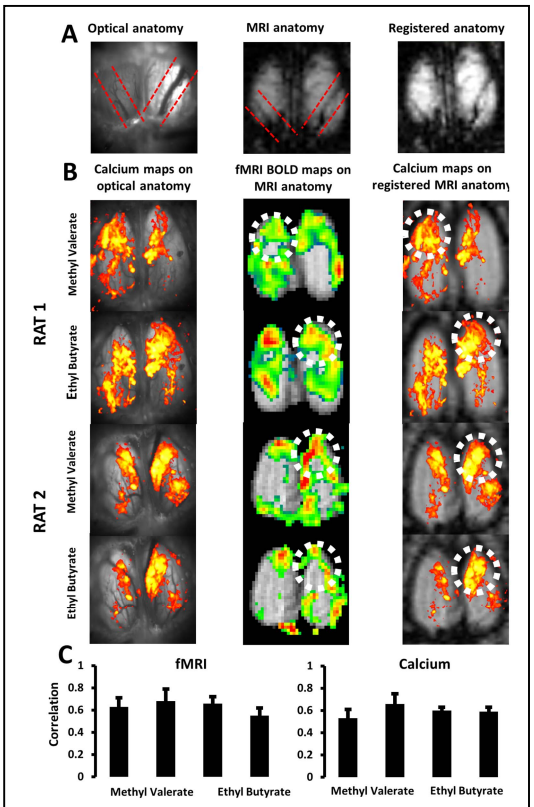

Figure 1. Axial anatomical images (optical and MRI) were collected from dorsal to ventral aiming for the vessel contrast. MRI anatomy is then co-registered with optical anatomy using the land mark (shown with dotted lines) (**A**). Odor induced calcium and fMRI BOLD maps were overlaid on their respective anatomy for two different odors methyl valerate and ethyl butyrate. Calcium maps (ΔF/F) are relative changes in fluorescence in each pixel and frame after the stimulus application. fMRI activation maps (ΔS/S) were obtained by applying Student's t test comparison of resting and stimulated data. Calcium activation maps were further overlaid on the registered MRI anatomy for the regional comparison (**B**). Cross correlation were assessed for both fMRI and calcium maps across trials for different odors using Pearson correlation (**C**)

DISCUSSION: Concurrent recordings of fMRI and calcium indicator signals provide opportunities for directly linking BOLD responses to underlying neural activity. Calcium maps obtained during methyl valerate and ethyl butyrate stimuli are representative of changes in presynaptic activity. Both calcium and fMRI BOLD maps showed clear focal areas of activation and which were odor-specific **(Fig 1B).** fMRI elicits slow responses of complex origin which are based on dynamic changes in oxy- and deoxy-hemoglobin, variations which are intricately tied high demand for electrochemical activities of neuronal function. fMRI can image the entire bulb, whereas calcium imaging is limited to the dorsal regions but with high spatiotemporal resolution. Mapping functional signals with fMRI involves a tradeoff between spatial resolution and sensitivity, which ultimately defines the temporal resolution. Our results show strong correspondence between odor-evoked fMRI and calcium maps, confirming the strong relationship between the bulk neuropil and presynaptic activities. This suggests that the input activity in glomeruli dominates glomerular functions. These in vivo results are in good agreement with an energy budget of glomerular activity, as the activated state is dominated by energy demands of action potential propagation in afferent olfactory sensory neurons and their synaptic input to dendritic tufts, whereas subsequent dendritic potentials and dendrodendritic transmission contribute only a minor share of energy costs [6]. In conclusion, multi-modal functional imaging of rat olfactory bulb with odorant stimulation provides new opportunities for gaining functional insights of complex neuropilar activities.

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