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Complex relationship between BOLD-fMRI and electrophysiological signals in different olfactory bulb layers

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ABSTRACT

Blood oxygenation level dependent functional magnetic resonance imaging (BOLD-fMRI), one of the most powerful technologies in neuroscience, measures neural activity indirectly. Therefore, systematic correlation of BOLD signals with other neural activity measurements is critical to understanding and then using the technology. Numerous studies have revealed that the BOLD signal is determined by many factors and is better correlated with local field potentials (LFP) than single/multiple unit firing. The relationship between BOLD and LFP signals under higher spatial resolution is complex and remains unclear. Here, changes of BOLD and LFP signals in the glomerular (GL), mitral cell (MCL), and granular cell layers (GCL) of the olfactory bulb were evoked by odor stimulation and sequentially acquired using high-resolution fMRI and electrode array. The experimental results revealed a rather complex relationship between BOLD and LFP signals. Both signal modalities were increased layer-dependently by odor stimulation, but the orders of signal intensity were significantly different: GL > MCL > GCL and GCL > GL > MCL for BOLD and LFP, respectively. During odor stimulation, the temporal features of LFPs were similar for a given band in different layers, but different for different frequency bands in a given layer. The BOLD and LFP signals in the low gamma frequency band correlated the best. This study provides new evidence for the consistency between structure and function in understanding the neurophysiological basis of BOLD signals, but also reminds that caution must be taken in interpreting of BOLD signals in regard to neural activity.

Introduction

Blood oxygenation level-dependent functional magnetic resonance imaging (BOLD-fMRI) is one of the most powerful technologies for neuroscience because of its capability to non-invasively study any brain region with high spatiotemporal resolution (Bailey et al., 2013; Bandettini, 2012; Logothetis, 2008). It has been widely used not only to map brain activation, but also to study the dynamics of neural networks (Logothetis, 2008; Logothetis and Wandell, 2004; Mishra et al., 2011; Sanganahalli et al., 2013). Comprehensive studies have revealed the relationship among neural activity, hemodynamics, and BOLD signals in many brain regions, and found that the BOLD signals are affected by multiple factors, including the local neural activity, metabolic capacity, the blood vessel system (blood flow and volume), and the neuroanatomy of the examined regions (Huttunen et al., 2008; Logothetis et al., 2001; Mishra et al., 2011; Mullinger et al., 2013; Shmuel et al., 2006). Therefore, the BOLD signal measures local changes in brain hemodynamics and metabolism, and should be interpreted with regard to the specific brain area where it is measured.

Extracellular electrophysiological signals, especially single/multiple unit activity and local field potentials (LFP), which reflect the neural activities of single/a few neurons and cell assembly directly, are another type of the most commonly used methods in neuroscience (Buzsaki, 2010; Buzsaki et al., 2012; Panagiotaropoulos et al., 2012; Rasch et al., 2009). Compared with MRI, electrophysiological recordings have much higher sensitivity and temporal resolution. Instrumentally, they are simpler, less expensive and easier for maintenance. Functionally, they are capable of monitoring neuronal and neural activities. Thus they can be used to study the properties and functions of a specific neuron, circuit, and system under different conditions such as rest and stimulated, normal and diseased, developing and adult, learning and

Abbreviations: BOLD, blood oxygenation level dependent; fMRI, functional magnetic resonance imaging; LFP, local field potentials; OB, olfactory bulb; GL, glomerular layer; MCL, mitral cell layer; GCL, granule cell layer; IAA, iso-amyl acetate; OCT, octanal.

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learned, etc. (Buzsaki, 2010). Since they directly measure the electrical signals of neurons, it is generally accepted that the LFP and neuron firing from single/multiple units are gold standards for measuring the local neural activities (Buzsaki, 2010; Buzsaki et al., 2012).

It is critical to correlate the indirect measurement of neural activity by BOLD signals with a gold standard of neural activity, such as by LFP, to justify the usage of fMRI and to help with interpretation of the fMRI data. Therefore, large amounts of studies have amassed evidence on the relationship between BOLD and LFP signals from different species and brain regions (Herman et al., 2013; Lippert et al., 2010; Logothetis et al., 2001; Magri et al., 2012; Maier et al., 2008; Mishra et al., 2011; Young et al., 2011). The results have demonstrated that, generally, the BOLD signal is more correlated with the LFP (Logothetis, 2008; Logothetis and Wandell, 2004; Logothetis et al., 2001), although, in some other regions, it also correlates well with neuronal firing (Hyder et al., 2002; Maandag et al., 2007).

The olfactory bulb (OB), the first center in the olfactory system, has one of the most regular laminar structures in the brain (from the exterior to the interior): the olfactory nerve layer, the glomerular layer (GL), the external plexiform layer, the mitral cell layer (MCL), the internal plexiform layer, the granule cell layer (GCL), and the subependymal zone (Shipley and Ennis, 1996). Each layer has distinct molecular and cellular compositions, different physical and biological properties, and different functions (Shipley and Ennis, 1996). In addition, the OB is one of two regions containing a significant amount of newborn neurons in the adult brain (Alonso et al., 2012; Gheusi et al., 2000; Lazarini and Lledo, 2011). Therefore, the OB has served as a popular model for a variety of neuroscience topics (Shepherd and Charpak, 2008), including the correlation of vascular density, synaptic transmission, metabolism, and neurovascular coupling in optical imaging (Gurden et al., 2006; Petzold et al., 2008).

BOLD-fMRI has been successfully applied to the OB in both rats and mice. High resolution MRIs can clearly identify the OB layers (Xu et al., 2000; Yang et al., 1998), map the activity patterns evoked by odorants and pheromones (Martin et al., 2007; Schafer et al., 2006; Xu et al., 2000, 2003; Yang et al., 1998), and reveal the dynamics of neural responses and the property of adaptation (Schafer et al., 2005; Xu et al., 2005). These previous fMRI studies were basically focused on the GL, while the other layers, which play important roles in olfactory information processing and transmission, rarely received attention. Further, the underlying neural basis of the BOLD signals and the correlation of the BOLD and LFP signals in the OB layers remains to be explored. More importantly, the unique laminar structures of the OB can provide a unique model to correlate BOLD and LFP signals under different brain states.

In attempting to answer these questions, we sequentially obtained the BOLD and LFP signals evoked by different odorants in three major OB layers (GL, MCL and GCL) of the same rats. The results revealed that the relationships are distinct for BOLD and LFP signals in different OB layers, magnitudinally, temporally and spatially, leading to a rather complex relationship between the two signal modalities. These results can help us understand the neurophysiological basis and thus the interpretation of BOLD signals.

Materials and methods

Animal preparation and odorant delivery

Odor stimulation experiments were performed on 18 adult Sprague-Dawley rats (250–300 g). These animals were pre-anesthetized with ~2.5% isoflurane for surgery, and the skin on top of the OB was removed to expose the skull. After surgery, the anesthetic was switched to urethane (i.p., 1.5 g/kg), which was used to maintain stable anesthesia in the odor stimulation experiments. The odorants, iso-amyl acetate and octanal (IAA and OCT, Sigma, MO, USA) were delivered to freely breathing animals. The odorants were dissolved in paraffin oil to make the concentration at 50% or 5% (H-IAA at 50%, L-IAA at 5% and OCT at only 5%). A stream of charcoal-filtered, warm air flowed over the oil, and then was diluted to 1/5 by an olfactometer. The stimulation was synchronously controlled with the data acquisition system by a solenoid valve, which was driven by a digital to analog converter. Air (off) or odorized air (on) was delivered to the nose at a constant rate of 1 l/min to eliminate the effect of the airflow. Odor stimulation lasted 32 s with an inter-stimulation interval >6 min to minimize habituation, and was repeated at least 4 times for each odor. Of the 18 rats used in the present study, 9 were used for BOLD-fMRI only (4 with all the odorants, 4 with L-IAA and OCT, 1 with H-IAA and L-IAA. Five were used only for LFP recording with all the odorants. Four were sequentially used in BOLD-fMRI and LFP recording with H-IAA. Therefore, 9, 9 and 8 (9, 5 and 5) rats were used for H-IAA, L-IAA and OCT stimulation in BOLD-fMRI (LFP recording), respectively.

BOLD-fMRI data acquisition

The imaging experiments were performed on a horizontal-bore 7.0 T BioSpec (Bruker, Ettlingen, Germany). The procedures for animal preparation were similar to those previously reported (Xu et al., 2000, 2003, 2005). Briefly, the animal's head was placed in a head holder to minimize head movement, and the animal was laid on a water heating bed. The respiration rate was monitored by recording chest wall movements using a piezoelectric device. A circular transmitting and receiving surface coil (10 mm diameter) was centered on top of the OB between the eyes of the animal to maximize signal to noise ratio. Functional MRI data was mainly acquired using multi-segment gradient echo planar sequence (TR, 1000 ms; TE, 14 ms; flip angle, ~45°; FOV, 12.8×12.8 mm^2, spatial resolution, $0.1 \times 0.1 \times 0.5$ mm^3, 10 slices), which decreased the inflow effects compared with the previous fast low angle shot sequence (Gao and Liu, 2012; Liu et al., 2008). The segment number was 8 in order to minimize distortions caused by the EPI method, leading to a temporal resolution 8 s per frame. The rapid acquisition with relaxation enhancement (RARE) T2-weighted anatomical images were obtained for the same slices (TR, 2500 ms; effective TE, 60 ms; RARE factor, 4; matrix, 128×128; spatial resolution, $0.1 \times 0.1 \times 0.5$ mm^3, 10 slices; average number, 25). In order to construct global OB's topological graphs, higher inter-plane resolution ($0.2 \times 0.2 \times 0.25$ mm^3, 22 slices) was used.

LFP recordings

Each rat was placed prone on a stereotaxic holder on a vibration-free table inside a Faraday cage. The skull was exposed by an electric cranial drill and covered with mineral oil to prevent drying (~2 mm lateral to midline, ~8 mm anterior to Bregma). Then the linear array microelectrode (NeuroNexus, MI, USA), with 16-channel and 0.1 mm inter-channel spacing, was inserted into the OB (with an angle perpendicular to the surface) using a stereotaxic micromanipulator (Stoelting, IL, USA). The recording site was selected according to the activation maps of both odorants in fMRI studies (http://senselab.med.yale.edu/OdorMapDB/search.aspx), both odorants evoke strong responses in this area. A steel screw was fixed on the parietal lobe to record EEG signals. As the reference site for both LFP and EEG signals, the skin of the neck was hooked with a silver wire. Body temperature of the animal was maintained at 37 °C and the respiration rate was monitored by recording chest wall movements using a piezoelectric device. All the LFP, EEG and respiratory signals were amplified (×2000, PGA32, BW, Germany) prior to digitization (μ-1401; CED, Cambridge, UK, sampling rate: 4000 Hz).

Determination of the layers of interest

Based on the anatomic images, features of LFP signals and electric lesion marks, the sites of GL, MCL and GCL were determined (Fig. 1). In the MRI, these layers can be defined in anatomical images: the GL, MCL