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Functional imaging of olfaction by CBV fMRI in monkeys: Insight into the role of olfactory bulb in habituation

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ABSTRACT

Cerebral blood volume (CBV) fMRI with superparamagnetic iron oxide nanoparticles (USPIO) as contrast agent was used to investigate the odorant-induced olfaction in anesthetized rhesus monkeys. fMRI data were acquired in 24 axial slices covering the entire brain, with isoamyl-acetate as the odor stimulant. For each experiment, multiple fMRI measurements were made during a 1- or 2-h period, with each measurement consisting of a baseline period, a stimulation period, and a recovery period. Three different stimulation paradigms with a stimulation period of 1 min, 2 min, or 8 min, respectively, were used to study the olfactory responses in the olfactory bulb (OB). Odorant-induced CBV increases were observed in the OB of each individual monkey. The spatial and temporal activation patterns were reproducible within and between animals. The sensitivity of CBV fMRI in OB was comparable with the sensitivities reported in previous animal fMRI studies. The CBV responses during the 1-min, 2-min, or 8-min odor stimulation period were relatively stable, and did not show attenuation. The amplitudes of CBV response to the repeated stimuli during the 1- or 2-h period were also stable. The stable CBV response in the OB to both continuous and repeated odor stimuli suggests that the OB may not play a major role in olfactory habituation. The technical approach described in this report can enable more extensive fMRI studies of olfactory processing in OB of both humans and non-human primates.

Introduction

Functional magnetic resonance imaging (fMRI) (Belliveau et al., 1990; Ogawa et al., 1990) has been widely used to study neural processing, including olfactory processing, in the central nervous system. Using traditional blood oxygenation level dependent (BOLD) methods, fMRI activations due to odor stimulation have been reported in the olfactory bulb (OB) of rodents (Martin et al., 2007; Poplawsky and Kim, 2014; Schafer et al., 2005; Xu et al., 2000; Xu et al., 2005). However, in humans and non-human primates (NHP), no fMRI activations have been reported in the OB (Boyett-Anderson et al., 2003; Gottfried et al., 2002; Poellinger et al., 2001; Sobel et al., 1998; Sobel et al., 2000; Yang et al., 1997). The reason for this difference between rodents and humans is not clear. It may be caused either by the comparatively small size of the OB in humans and NHPs, or by the lack of attention to the OB in previous studies.

The olfactory processing pathway includes several structures (Kandel et al., 1991), with the OB being the first relay. The odorant molecules activate the olfactory receptors in the nostrils, and the activity is transmitted to the OB. From the OB, the activations are further transmitted to a number of higher brain regions, including the anterior olfactory nucleus, piriform cortex, medial amygdala, and entorhinal cortex. In general, fMRI should be able to detect olfactory activations in all these regions. However, habituation (adaptation to odor) may impact the ability of fMRI to observe the olfaction in these different regions. Habituation is characterized by the attenuation of responses to continuous or repeated stimulation. The ability of fMRI to detect olfaction depends on the repeatable responses during multiple fMRI measurements. Olfaction in some regions may become weakened after repeated stimulations due to habituation, rendering the fMRI signal to be too small to be detectable. Evaluation of the different sensitivities of fMRI in different brain regions to repeated odor exposure may provide useful information in understanding the roles of different brain regions in habituation and olfaction processing.

While BOLD fMRI is the most common fMRI method used in humans for measuring neural activity, cerebral blood volume (CBV) fMRI, which uses an intravascular contrast agent consisting of superparamagnetic iron oxide nanoparticles (USPIO or MION) has been widely used in animal fMRI studies (Kennan et al., 1998; Leite et al., 2002; Mandeville et al., 1998; van Bruggen et al., 1998; Zhao et al., 2006). CBV fMRI is sensitive to neural activation-induced cerebral blood volume (CBV)

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increases and has a higher sensitivity compared with BOLD fMRI (Zhao et al., 2006). CBV fMRI with contrast agent has also been reported in NHP and human fMRI studies (Jenkins et al., 2004; Leite et al., 2002; Qiu et al., 2012).

In this study, CBV fMRI was used to study the odorant-induced olfaction in anesthetized rhesus NHPs.

Materials and methods

Animal preparations

Ten female rhesus NHPs with body weight ranging from 4.9 to 8.2 kg (5.8 ± 0.92 kg, mean ± SD) were used with the approval from the Merck Research Labs Institutional Animal Care and Use Committee in three studies. Each study was performed in 5 or 6 NHPs. Each animal was initially anesthetized using ketamine (3 mg/kg, i.m.) and dexmedetomidine (40 μg/kg, i.m.) for catheterization and experiment setup. The areas for catheterization of vein were shaved, and two intravenous catheters were implanted into both saphenous veins for fluid support, dexmedetomidine, and contrast agent delivery. Each animal was then secured in the supine position in the MRI scanner, and the anesthesia changed to a continuous delivery of isoflurane (0.25%) and dexmedetomidine (i.v. infusion of 15 μg/kg/h). During the entire experiment session, the animal was kept under spontaneous respiration. Oxygen-enriched gas was used to maintain the blood oxygen saturation above 96%. Body temperature was measured by a probe placed in the axilla and maintained by a circulating warm-water blanket. The respiration was monitored by a pressure sensor connected to a balloon secured to the chest. Blood oxygen saturation and heart rate were monitored by an optical probe attached to a toe. At the end of the experiments, atipamezole HCl (0.25 mg/kg, i.m.) was administered to reverse dexmedetomidine anesthesia.

The USPIO contrast agent (Feraheme, AMAG pharmaceuticals, Cambridge, MA) was administered (10 mg/kg, i.v.) before the start of fMRI data acquisition. A preliminary study showed that the Feraheme has a half-life time of 12.12 ± 3.05 h (mean ± SD, $n = 6$) in NHPs, which is similar to the Feraheme half-life time of >15 h reported in the human CBV fMRI study (Srihasam et al., 2010). Since the fMRI data in this study were acquired only in 1- or 2-h after Feraheme injection, which is much shorter than the Feraheme half-life time, the impact of the Feraheme wash-out on the CBV fMRI signals should be negligible.

Odor stimulus

Olfaction was induced with the odorant isoamyl-acetate, and Fig. 1A displays a schematic for delivery of respiration gas and odor. A gas mixture (2 L/min medical air + 0.8 L/min O_2, with or without odor) was constantly delivered by two small tubes (PE 90), which were loosely placed in the nostrils at the depth of ~1 cm. The gas flow was split into two pathways, one of which received odorized gas through a bubbling bottle containing the odor solution. The two pathways join together prior to entering the nostrils. During the odor stimulation period, the odor is introduced by opening two valves at the inlet and outlet of the bubbling bottle through a control signal from MRI sequence. The concentration of isoamyl-acetate in the gas delivered was estimated to be ~2870 ppm (Xu et al., 2000).

MRI measurement

MRI measurements were performed on a 3-T, Siemens Trio system. A 16-channel head coil was used as the radiofrequency (RF) receiver. Scout images in three orthogonal directions were first acquired using fast low angle shot (FLASH) sequence. Based on the sagittal image, twenty-four consecutive axial slices were chosen (Fig. 2B) for the fMRI study. Previous studies show that it takes ~1 h after initiating the combined medetomidine-isoflurane anesthesia for physiological

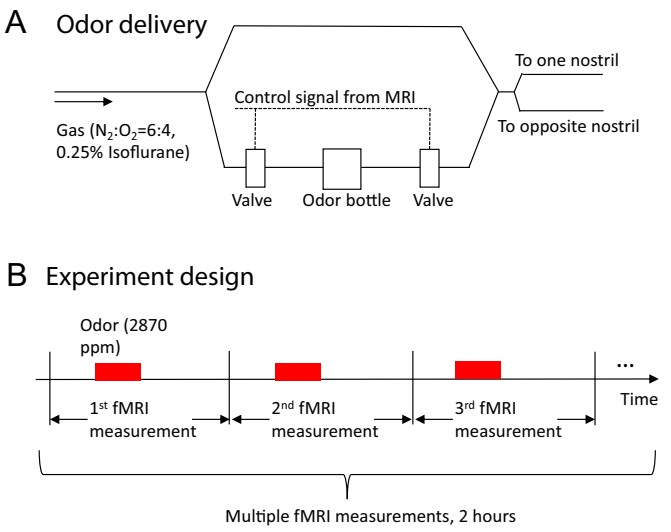

Fig. 1. Odor delivery and experimental design. (A) The gas flow is separated into two pathways with one pathway bubbling through the odor solution (vaporizer). With two on/off valves simultaneously controlling the inflow/outflow of the vaporizer, a sharp onset and shut-off of the odor was achieved. The two pathways are mixed at the subject end, and the gas is delivered to the bilateral nostril through two small tubes. (B) Each fMRI measurement includes a period of baseline, a period of odor delivery, followed by a period of recovery. Multiple fMRI measurements were performed for each animal during a 1- or 2-h period.

parameters and BOLD fMRI response to somatosensory stimulation to stabilize (Lu et al., 2012; Zhao et al., 2009). Therefore, fMRI data acquisition started ~1 h after initiating the combined medetomidine-isoflurane anesthesia. fMRI measurements were made during a 1- or 2-h period for each NHP. T_2*-weighted images were acquired using a single-shot gradient echo echo-planer imaging (GE EPI) sequence: matrix size = 64 × 64, field of view = 12 × 12 cm^2, slice thickness = 1.8 mm, repetition time (TR) = 3 s, and gradient echo time (TE) = 28 ms. The corresponding spatial resolution was 1.9 × 1.9 × 1.8 mm^3, and the acquisition time for imaging the entire volume was 3 s.

Three fMRI studies with different stimulation paradigms were performed. One study was with the 1-min stimulation paradigm: 1 min (baseline) + 1 min (stimulation) + 2 min (recovery). One single fMRI measurement required 4 min, with a total of 20 (baseline) + 20 (stimulation) + 40 (recovery) volume acquisitions. Thirty fMRI measurements were made for each NHP during a 2-h period in this study. The second study was with the 2-min stimulation paradigm: 2 min (baseline) + 2 min (stimulation) + 4 min (recovery). One single fMRI measurement in this study required 8 min, with a total of 40 (baseline) + 40 (stimulation) + 80 (recovery) volume acquisitions. Fifteen fMRI measurements were made for each NHP during a 2-h period. The third study was with the 8-min stimulation paradigm: 2 min (baseline) + 8 min (stimulation) + 8 min (recovery). One single fMRI measurement in this study required 18 min, with a total of 40 (baseline) + 160 (stimulation) + 160 (recovery) volume acquisitions. Three fMRI measurements were made for each NHP during a 1-h period.

fMRI data analyses

Data were processed using Stimulate (Strupp, 1996) and custom MATLAB routines (Mathworks, Natick, MA). For each animal, the data from the multiple fMRI measurements were averaged for detection of activation. Based on the averaged data, statistical *t*-value maps were computed by comparing the experimental fMRI data acquired during control and stimulation periods on a pixel-by-pixel basis. The control periods included the period of the pre-stimulus control and the period from 1 min after cessation of odor stimulation to the end of fMRI measurement; the first 1 min data after the cessation of odor were ignored