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Functional ultrasound imaging reveals different odor-evoked patterns of vascular activity in the main olfactory bulb and the anterior piriform cortex

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ABSTRACT

Topographic representation of the outside world is a key feature of sensory systems, but so far it has been difficult to define how the activity pattern of the olfactory information is distributed at successive stages in the olfactory system. We studied odor-evoked activation patterns in the main olfactory bulb and the anterior piriform cortex of rats using functional ultrasound (fUS) imaging. fUS imaging is based on the use of ultrafast ultrasound scanners and detects variations in the local blood volume during brain activation. It makes deep brain imaging of ventral structures, such as the piriform cortex, possible. Stimulation with two different odors (hexanal and pentylacetate) induced the activation of odor-specific zones that were spatially segregated in the main olfactory bulb. Interestingly, the same odorants triggered the activation of the entire anterior piriform cortex, in all layers, with no distinguishable odor-specific areas detected in the power Doppler images. These fUS imaging results confirm the spatial distribution of odor-evoked activity in the main olfactory bulb, and furthermore, they reveal the absence of such a distribution in the anterior piriform cortex at the macroscopic scale in vivo.

Introduction

Processing of olfactory information is vital for the majority of vertebrates. The olfactory system is built in the same scheme across species, with only two synaptic relays to reach the olfactory cortex from the outside world (Bekkers and Suzuki, 2013). The wiring diagram of the olfactory circuits starts with the olfactory receptor neurons (ORNs), located in the main olfactory epithelium. In the rodent, each ORN, expressing one particular olfactory receptor out of ~1000 (Firestein, 2001; Mombaerts, 2004; Mori and Sakano, 2011), projects out to four glomeruli in the main olfactory bulb (MOB), the first central relay that codes the olfactory information (Shepherd and Greer, 1998). In turn, the MOB transmits the information to the anterior piriform cortex (aPC), the main output structure of the MOB among the primary olfactory cortices. The aPC receives direct and dense sensory input from the mitral/tufted cells (M/TCs) in the MOB from multiple glomerular sources through the lateral olfactory tract (LOT) projection (Apicella et al., 2010; Miyamichi et al., 2011). In addition, different tracing techniques have been used to show that M/TC axons from individual glomeruli were diffusely projecting throughout the aPC (Ghosh et al., 2011; Miyamichi et al., 2011; Sosulski et al., 2011).

Odor-induced spatial maps of the MOB have been characterized extensively (Mori et al., 2006; Murthy, 2011), in particular by in vivo optical imaging (Uchida et al., 2000) and magnetic resonance imaging (MRI) (Xu et al., 2000). A given odorant molecule can activate various subsets of ORNs, which results in a spatially invariant pattern of glomerular activity in the MOB (Belluscio and Katz, 2001; Soucy et al., 2009). Physiological studies have examined the transmission of odor-evoked activities from the MOB to the aPC (Litaudon and Cattarelli, 1996; Poo and Isaacson, 2009; Rennaker et al., 2007; Stettler and Axel, 2009; Suzuki and Bekkers, 2012). These studies have shown that odorants activate sparse groups of neurons that are largely disturbed in all layers of the aPC, without any apparent spatial preference. However, it is not known how odor-evoked activation of different odors is organized in the aPC at the macroscopic scale.

In this study, we used functional ultrasound (fUS) imaging (Macé et al., 2011), a new functional neuroimaging technique based on the use of ultrafast ultrasound scanners, to map odor-evoked activity in the aPC on a large scale. Ultrafast ultrasound scanners have already offered new insights in diagnostic imaging (Tanter et al., 2008) and blood flow imaging under the terminology of "Ultrafast Doppler Imaging" (Bercoff et al., 2011; Osmanski et al., 2012; Udesen et al., 2008). This concept relies on compounded plane wave transmission

Abbreviations: ORNs, olfactory receptor neurons; fUS, functional ultrasound; MOB, main olfactory bulb; aPC, anterior piriform cortex.

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(Montaldo et al., 2009), which can capture more than 10,000 frames per second compared to the usual 50 frames per second in conventional ultrasound scanners. Thus, Ultrafast Doppler has increased the sensitivity of blood flow measurements by 30-fold (Macé et al., 2013), making the detection of subtle hemodynamic changes in smaller vessels possible compared to conventional Doppler Ultrasound. Similar to other magnetic and optical functional neuroimaging techniques that depend on vascular oxygenation and dynamics (Pain et al., 2011), fUS relies on activity-dependent blood volume changes in small blood vessels to detect active neuronal assemblies in vivo (Macé et al., 2013; Rubin et al., 1995, 1997; Shung et al., 1976). Importantly, fUS was shown to reach an excellent spatiotemporal resolution (below 100 μm and 1 s for a single trial acquisition) in the field of deep brain imaging (Macé et al., 2011).

Using the technical advantages of fUS, we determined how distinct odorants were mapped in two interconnected stations along the olfactory pathway, the MOB and the aPC, on a macroscopic scale. We confirmed the existence of the well-described spatial maps evoked by odor stimulation in the MOB, and we further showed that the aPC is activated as a whole, across all layers, in response to different odorants.

Materials and methods

Animals, surgical procedures and odor delivery

Thirteen adult male Long–Evans rats (Janvier Labs; France) weighing 250–350 g were included in this study. They were housed in collective cages with free access to food and water and maintained under standard conditions (12/12 h light–darkness cycle, 22 °C). All experiments were conducted in accordance with the European Community Council Directive (86/609/EEC). The experimental protocol was controlled and approved by the University Paris-Sud Ethics Committee and the Direction of Veterinary Service (authorization #B91471101).

All rats were anesthetized by an intraperitoneal (i.p.) injection of a mixture of ketamine (60 mg/kg, Imalgene 500®, Merial; France) and medetomidine (0.4 mg/kg, Domitor®, Pfizer Santé Animale; France). Anesthesia was maintained by a periodic i.p. dosage using 1/3 of the initial dose. Body temperature was monitored and maintained at 37 °C using a heating blanket throughout the experiment. Animals were placed in a stereotaxic apparatus for imaging. After an incision in the cranial skin, the bone above the MOB or the aPC was thinned by a dental drill and carefully removed. All image recordings were conducted on freely breathing rats (constant breathing at 1–2 Hz in all rats).

A custom-modified version of a multivial perfusion system (ValveBank 8 II, AutoMate Scientific; USA) attached to an air compressor was used as an olfactometer. A precise volume of the diluted odor (50 μL) was loaded onto a filter paper and placed in a syringe reservoir. Pressure controlled air was delivered through the perfusion system, ensuring a constant rate of odorized air to the animal's nose during valve opening. A mask was placed in front of the rat's nostrils to deliver the odors.

A single activation trial lasted for 48 s. After 6 s of baseline recording under a constant deodorized airflow, one of the two odorants, hexanal 1% or pentylacetate 1% (Sigma-Aldrich; USA), was delivered for 15 s. Another 27 s of air delivery was allowed to recover the baseline value of the metabolic signal. Because the olfactory system is very sensitive to desensitization habituation in the case of repetitive odor stimulations, we allowed at least 3 min of inter-trial interval (ITI). Blank trials, with only air delivered throughout the 48 s, were performed between the odor trials. Four trials per odor were averaged together to obtain functional images of the MOB and the aPC.

To build a recording chamber for each structure, craniotomies were performed according to the MOB and aPC stereotaxic coordinates (Paxinos and Watson, 2007). In a group of five rats, a 5 mm window from 6.5 to 9.5 mm anterior to the bregma was made to give access to the entire MOB. In another group of 8 rats, a 10 mm window was made from 3 to 5 mm anterior to the bregma to give access to the aPC. A silicone gel was applied to the surface of the dura. The gel allows perfect coupling between the ultrasonic probe and the imaged brain region and makes the propagation of ultrasound wave fronts possible. Anteroposterior scans were performed, placing the ultrasound probe on top of these two regions (Fig. 1A). The choice of the ultrasonic image slice with the maximal signal to noise for the power Doppler signal was made taking the shape of the vasculature as a reference (see Fig. 2).

In vivo fUS: images and statistical analysis

fUS was performed using a linear ultrasound probe (192 elements, 20 MHz, 80 μm pitch, and 8 mm elevation focus, Vermon; France) driven by an ultrafast ultrasound scanner (Aixplorer, Supersonic Imagine; France). The biophysics and the technical procedure for fUS were fully explained in our previous reports (Macé et al., 2011, 2013). Briefly, to obtain an ultrasound image of the brain tissue, we used the following ultrafast imaging fUS sequence: i) insonify the brain tissue with a plane wave, ii) record the backscattered echoes coming from a wide-field view on the transducer array, and iii) beamform the raw data to produce an image. In order to ensure a high-quality ultrasound image while preserving an ultrafast frame rate (several 100 s of frames per second), we added several plane wave images coherently (with amplitude and phase) from successive transmissions of tilted plane waves (Montaldo et al., 2009). In this study, the plane wave compounding consisted of coherently adding the images of the brain tissue from 15 different tilted plane waves, with angles varying from −7° to 7° and a 1° step, to compute one high-quality ultrasound image (Fig. 1B). To sample blood flow changes, we repeated this sequence 200 times with a 500 Hz frame rate (corresponding to a 400 ms acquisition time) (Fig. 1C). Because blood moves faster than the tissue, its signal is higher in frequency and can be extracted by time filtering the data with a high-pass filter (4th order Butterworth with a 75 Hz cutoff). One image of power Doppler intensity (which is proportional to the cerebral blood volume, CBV) is obtained by the incoherent temporal mean of the blood signal. Because of the technological computing limitations of our fUS platform (6 CPU core unit, 24 GB RAM), a dead time of 1.1 s is needed to process the power Doppler signal (beamforming and high-pass filtering), resulting in a final temporal sampling rate of 1.5 s per image (Fig. 1D). At the end of this signal processing, we reliably recorded CBV variation in the imaging plane.

Maps of activated pixels were built showing the normalized correlation coefficient r between the local power Doppler signal obtained from fUS and the temporal binary pattern of the odor stimulus. Activation was considered significant for a correlation $r > 2\sigma$, where σ is the spatial standard deviation of the correlation map linked to the noise of the fUS technique. As the volume imaged by fUS covers more than just the brain tissue, σ was computed using at least 100 pixels of the correlation map located outside of the brain. Therefore, this thresholding method can be considered independent of brain activity. The time course for a given region was calculated by averaging the power Doppler signal over time for all pixels in the activated region ($r > 2\sigma$). The intensity of the power Doppler was represented as the percentage of change relative to the baseline in the activated region ± standard deviation (STD). Finally, raw (i.e., not thresholded) hexanal and pentylacetate activation maps in the aPC were compared using the Pearson correlation coefficient.

Results

fUS imaging in the olfactory system

In this study, we investigated the odor-evoked activation of the MOB and the aPC using the fUS technique. We achieved a spatial resolution of 100 μm × 100 μm in the imaging plane, with a slice thickness of 300 μm and a penetration depth (>2 cm) sufficient to image deep brain