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TIME COURSE OF ODORANT- AND TRIGEMINAL-INDUCED ACTIVATION IN THE HUMAN BRAIN: AN EVENT-RELATED FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

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Abstract—It is well known that most odorants stimulate the trigeminal system but the time course of the brain regions activated by these chemical stimulations remains poorly documented, especially regarding the trigeminal system. This functional magnetic resonance imaging (fMRI) study compares brain activations resulting from the contrast between two odorant conditions (one bimodal odor and one relatively pure olfactory stimulant) according to the duration of the stimulation (i.e. one inhalation, or three or six successive inhalations). The results show striking differences in the main brain regions activated according to these durations. The caudate nucleus and the orbitofrontal cortex are only involved in short-duration stimulations, and the posterior insular cortex and post-central gyrus (SI) are only activated by long duration stimulations. Different regions of the frontal, temporal and occipital lobe are activated depending on the duration but mainly during medium-duration stimulations. These results expand on the findings of previous studies and contribute to the description of temporal networks in trigeminal perception.

Key words: trigeminal, fMRI, olfaction, bimodal odor.

Most odorants are simultaneously perceived in humans by two sensory systems. They stimulate receptors of the olfactory epithelium, and thus the olfactory nerve (cranial nerve I), as well as free nerve endings and specific receptors in the nasal cavity, and thus the trigeminal nerve (cranial nerve V) (Doty et al., 1978; Hummel, 2000). Although the brain processes corresponding to their integration contribute to the various perceptions of odors and flavors (Albrecht et al., 2010), the interactions between these two sensory systems remain poorly understood.

Due to numerous studies in functional cerebral imaging, the brain areas affected by olfactory stimulation and perception are now well-known. Stimulation by any pure or relatively pure olfactory stimulant mainly activates primary olfactory regions (i.e. piriform cortex, amygdala and neighboring cortex) and secondary olfactory regions (hippocampus, orbitofrontal cortex and insula) (Zatorre et al., 1992; Royet et al., 2001; Gottfried et al., 2002; Savic, 2002). Asymmetry between the two hemispheres can be constant (e.g. the right orbitofrontal cortex is predominantly activated) or odor dependent (left amygdala appears to be more sensitive to unpleasant odors). Other brain areas may be involved depending on the subject's odor-related task: judgments of intensity, familiarity, memory or characteristics of hedonic valence (Royet et al., 2003). Nevertheless, studies on time course-induced activations have shown that some brain areas are successively affected by these processes. First and foremost, short bursts of stimulation activate the piriform cortex, hippocampus and part of the insula. These activities decrease in long duration stimulations which lead to a strong recruitment of the right orbitofrontal cortex (Sobel et al., 2000; Poellinger et al., 2001).

The literature on the functional neuroanatomy of trigeminal perception is less substantial. Contrasting or comparing brain activations due to odorants with different trigeminal properties is a common and fruitful approach to specifying the brain activations elicited by the trigeminal component of an odorant. Studies have shown additional activations of the insula, cingulum and cerebellum with bimodal (olfactory–trigeminal) stimuli compared to "pure" olfactory stimulants (Yousem et al., 1997; Bengtsson et al., 2001; Savic et al., 2002; Lombion et al., 2009). Hummel et al. (2005) and Boyle et al. (2007a,b) used CO_2 (a relatively selective trigeminal nerve stimulant) as a referent and found additional activations in the midbrain, caudate nucleus, middle cingulate and temporal and frontal gyri. Iannilli et al. (2007) investigated brain activations in anosmic subjects in response to CO_2 and mainly found activations in parts of the cerebellum and the temporal, parietal and frontal cortices. Nevertheless, the time course of activations for these areas involved in trigeminal perception remains largely unknown. Previous studies have used either relatively long durations, from 15 s to 30 s (Yousem et al., 1997; Bengtsson et al., 2001; Savic et al., 2002) or "puffs" of odors lasting 1 s or less (Boyle et al., 2007a,b; Iannilli et al., 2007). The present study aimed to define some of the elements of the time-dependent processes in response to trigeminal stimulation. As in a previous study (Lombion et

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Abbreviations: AA, isoamyl acetate; PEA, phenyl ethyl alcohol.

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al., 2009) we used phenyl ethyl alcohol (PEA), a rose-like odor, and isoamyl acetate (AA), a banana-like odor as odorants. PEA is a relatively pure olfactory stimulus and AA is a bimodal stimulus; they are detected by one of 15 and 15 of 15 total anosmics, respectively (Doty et al., 1978). Both are considered as slightly pleasant (Dravnieks et al., 1984; Hummel et al., 1997). Using these odorants, we therefore attempted to minimize any brain activations that could be due to obvious differences between sensations of pleasantness/unpleasantness elicited by the odorants used as noted in other studies (Savic et al., 2002; Boyle et al., 2007b).

EXPERIMENTAL PROCEDURES

Subjects

Twenty-five healthy undergraduate students (aged 20–24 years; 19 females and six males) were included in the study. The subjects were non-smokers, right-handed, free of head colds and screened for any possible olfactory dysfunctions prior to the study. The study was reviewed and approved by the local ethics committee and declared to the National authority (N° UF: 1013; DGS 2006/0494) in accordance with the Declaration of Helsinki on biomedical studies involving human subjects. Subjects' participation also required a written informed consent and medical screening.

Odor delivery

The odors were delivered via a multi-channel custom-built olfactometer (Andrieu et al., 2011). The olfactometer was suitable for the MRI environment and generated odors with a rapid and steady on-off time (400 ms). The change between odorant and non-odorant conditions did not produce any thermal, tactile or auditory cues. Under baseline conditions, a constant flow of odorless, humidified air at a constant temperature was delivered to the subject through two nasal cannula nosepieces (Pro-Flow Plus™ Nasal Oral Cannula, Pro-Tech®, Murrysville, PA, USA). The use of this air as a vector embedding the odor flow prevented the detection of odor delivery by sensory systems other than chemical (such as sensitivity to changes in pressure). The pressure of each air stream (vector and odor) was controled by a flowmeter, ensuring a constant flow rate of 591 ml/min for each one. The use of solenoid valves allowed the different odorant conditions to be generated by selecting the air flow passing through encapsulated gauze pads soaked either with 7 μl of PEA or 5 μl of AA (undiluted solution: Across Organics®, Gell, Belgium). The capsules (2 cm in diameter) were connected to the nosepieces by a tube which was short (10 cm) in order to ensure minimal adhesion and a square wave-like delivery of the odor. These supra-threshold concentrations were chosen following preliminary tests on a panel of five young women to obtain approximately the same self-ratings of intensity for the two odors and to ensure that both odors were constantly and correctly perceived for a sufficient amount of time corresponding to the MRI session duration. No sensation relating to trigeminal stimulation (Laska et al., 1997) was reported for PEA but a slight sensation of tickling or prickling was mentioned by four of the five subjects. The odor delivery was generated by a computer with E-Prime 2.0 (Psychology Software Tools, Pittsburg, USA) and synchronized with the onset of an inward breath by the subject (inhalation flow rate trigger). The delivery lasted 2 s to ensure that the odor would be smelled for the entire duration of the inhalation phase of the breathing cycle.

Before the scan session, subjects were debriefed on the purpose of the study and informed about the type of odors used. They were asked to breathe regularly through the nose only, without actively sniffing (sniffing has been shown to activate special brain processes [Sobel et al., 1998a]), and to focus on the odors without performing any others tasks. At the end of the scan, they were asked to describe their feelings about the odorants used.

Experimental paradigm

Subjects were scanned during a sequence of odorant stimulations, with either PEA or AA alternating with the non-odorant condition. Each of these odorants was delivered for either one inhalation, three successive inhalations or six successive inhalations. This procedure was repeated four times for each of the two odorants and for each of the three inhalation sequences (i.e. 1, 3, 6) corresponding to three different durations of continuous stimulation (Fig. 1). Each of these odorant stimulations (short, medium, long duration) was separated by a rest condition (odorless air flow) lasting at least 25 s, up until the inhalation trigger. The sequences of odor type and stimulus duration were randomly determined for each subject by a computer program, which continuously monitored the breathing cycle and controled the switching device of the olfactometer.

MRI data acquisition

Magnetic resonance images were collected on a 3-T scanner (G.E. Healthcare Signa, Milwaukee, WI, USA). First of all, a high-resolution T1-weighted (BRAVO FSPGR sequence) 3D anatomical scan with 134 slices, voxel size of 1×1×1 mm^3, 256×256 matrix and 256×256 mm^2 field of view (FOV) was recorded. Next, BOLD images were obtained covering the entire cerebrum and most of the cerebellum using an echo-planar imaging (EPI) sequence. Scan parameters included a 128×128 matrix, a repetition time (TR) of 2500 ms, a echo time (TE) of 35 ms and an FOV=256 mm^2. Thirty 4.5-mm thick slices were acquired for each of the volumes. They were acquired in an oblique orientation 30° to the anterior commissure–posterior commissure line to minimize sus-

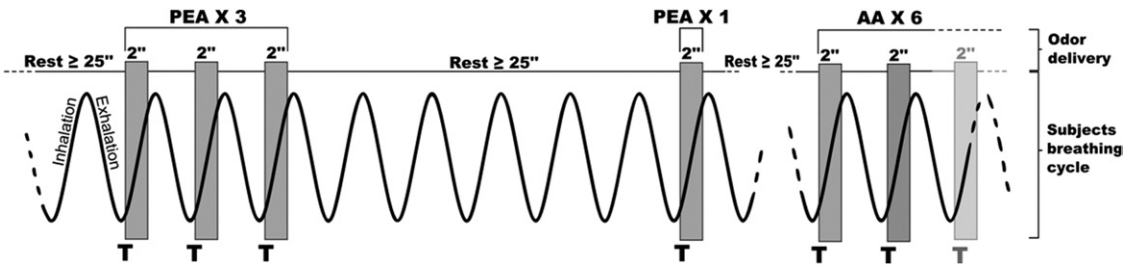

Fig. 1. Sample of the experimental procedure during the functional scan. Odorless epochs (rest) alternated with odorant stimulations (either with phenyl ethyl alcohol or isoamyl acetate: PEA and AA) synchronized with the beginning of the inward breath during one, three or six successive inhalations. This whole procedure was repeated four times in a random order during the scan. T=inhalation trigger.