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A stimulation method using odors suitable for PET and fMRI studies with recording of physiological and behavioral signals

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Abstract

A design for a semi-automatic olfactometric system is described for PET and fMRI experiments. The olfactometer presents several advantages because it enables the use of an 'infinite' number of odorants and the synchronization of stimuli with breathing. These advantages mean that the subject is recorded while breathing normally during olfactory judgment tasks. In addition, the design includes a system for recording the behavioral (rating scale) and physiological (breathing, electrodermal reaction (ED), plethysmography (PL)) signals given by the subject. Both systems present the advantage of being compatible with fMRI magnetic fields since no ferrous material is used in the Faraday cage and signals are transmitted via an optical transmission interface to an acquisition system.

Keywords: Olfactometric system; PET; fMRI; Breathing; Electrodermal reaction; Plethysmography

1. Introduction

Several methods of odorant stimulation have been used in a research context. The easiest way to present odorants is to use a piece of odorant saturated cotton. Whereas this method is directly usable in PET (Zatorre et al., 1992), the length of the tunnel in an fMRI imager means that a plastic rod at the end of which the cotton is placed must be used (Levy et al., 1997). This method, however, is not very reliable, because it does not allow the odor concentrations to be controlled, the time-course of the odors is imprecise and it can induce tactile stimulations if the experimenter touches the subject's nose. A more sophisticated method is that of dynamic olfactometry (e.g., Dravnieks, 1974; Kobal and Plattig, 1978; Doty, 1991; Warren et al., 1992; De Wijk et al., 1996). It is based on the use of a deodorized vector gas which conveys the odorant vapor towards the subject's nose after activating a solenoid valve. Several authors have employed this method in cerebral imaging studies (Yousem et al., 1997; Sobel et al., 1997, 1998a,b; Lorig et al., 1999; Gottfried et al., 2002a,b using Lorig's olfactometer). The main problem of olfactometers designed using this principle is that they use only a limited number of stimulation channels, giving rise to limited experimental designs, habituation, and then potential weakening of concentrations due to repeated sampling of the same liquid or gas. Olfactometers made in the laboratory can cost from a few thousand Euros to close to 100 KEuros depending upon the specific design and number of channels.

In most of the first cerebral imaging studies, subjects were not asked to perform a specific task during the actual scanning period. When subjects need to perform a cognitive task, it is recommended that experimenters use different stimuli to avoid the problems associated with sensory habituation or the performance of tasks in a routine manner (Démonet et al., 1993), but it is also interesting to record their behavioral responses on line. These may for instance be binary when subjects must judge whether the odor is intense or not, familiar or not, pleasant or unpleasant (Royet et al., 1999, 2001; Gottfried and Dolan, 2003). They can be graduated

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when subjects rate the levels of familiarity or the hedonic intensity using the visual rating scale principle (De Araujo et al., 2003; Royet et al., 2003). The used method can be then based on the finger-span (FS) technique (Cerf-Ducastel and Murphy, 2001; Royet et al., 2003). It may also be pertinent to record any indications of the subject's physiological activity inasmuch as this may reflect his reaction to a sensory stimulation. It is usually measurements of the autonomic nervous system that are made, such as the electrodermal (ED) reaction, plethysmography (PL), skin temperature and respiratory frequency (Van Toller et al., 1983; Dittmar, 1989; Vernet-Maury et al., 1999). However, no system adapted for the strong magnetic fields of an fMRI scanner is commercially available.

Our purpose was to perform cerebral imaging studies while subjects were performing olfactory judgment tasks. We propose a semi-automatic olfactometric system that can be used in both PET and fMRI environments, and that is based on new concepts. It was conceived to respect three main experimental conditions: the possibility of using an 'infinite' number of odorants; using the highest possible number of stimulations per scan or epoch; and synchronizing the stimuli with the subject's breathing. The first condition allows us to vary stimuli between olfactory tasks (Royet et al., 1999, 2001), and to avoid habituation phenomenona. Subjects can consequently concentrate on a given olfactory task while their brain activity is being recorded. The last two conditions involve the stimulation of subjects during each respiratory cycle, notwithstanding the well-known olfactory adaptation phenomenon (e.g., Cain and Engen, 1969; Cain and Moskowitz, 1974; Engen, 1982). Rather than using a video projector to project a visual message asking subjects to smell (Sobel et al., 1997; Suzuki et al., 2001), we chose to subordinate the odorous stimuli to subject's breathing. Subjects were thus tested during a regular breathing rhythm, avoiding sniffing. We have further conceived a system to record behavioral and physiological parameters during fMRI experiments. The two systems are compatible with fMRI magnetic fields, as they do not induce electromagnetic radiation and only materials with no ferrous metals are used within the Faraday cage.

2. Methods

The conception of odor stimulating apparatuses and behavioral and physiological parameter recording systems is conditioned by their more restrictive use in fMRI than PET due to magnetic fields. The use of magnetic material in the Faraday cage is proscribed for two reasons. Firstly, pieces of magnetic metal can be attracted into the magnet and present a potential risk for the physical integrity of the subject in the tunnel. Secondly, materials including magnetic pieces and/or producing electromagnetic wave radiation, such as a computer can modify field lines and induce image distortion. The use of plastic material or nonmagnetic metals in the Faraday cage avoids these problems. Other materials which include magnetic pieces are then placed in the control room. All the connections between both kinds of material are made with optical fibers.

2.1. Odor stimulating material

Odors are presented with an air-flow olfactometer, which allows synchronization of the stimulations with breathing. Instead of using several channels and solenoid valves that lead to over complexity, and of which the number is in any case limited, our stimulation method is based on the principle of directly delivering odors into a rapid airflow (to purge the system) and then to convey the odorized air to the subject via a tube made of very low adsorbent material (Teflon® Fluorinated Ethylene Propylene). This can be accomplished without drying the subject's nose because the odors are not delivered directly into the nostril, but into a facial mask and because a diffuser is used. In methods as used by Yousem et al. (1997) and Sobel et al. (1997), visual or auditory instructions are presented to the subject asking him to synchronously inspire with a stimulation. In the current method, while the subject is asked to breathe regularly, we locate his inspiration phases and deliver stimuli just at the end of an expiration. The subject must avoid sniffing or blocking his breathing. A session is performed before the scanning day to train the subject to keep a regular rhythm of breathing, to detect an odor delivered during inspiration, and to give a response before the following stimulation. Odors are presented at a supraliminal level and conveyed to the mask with a delay inferior to that needed for an inspiration phase (from 1.5 to 2 s as a function of the subjects).

The stimulating apparatus is composed of three parts (Fig. 1): an air treatment part including the electronic devices, a pneumatic part called the 'injection head' (air-dilution part), and a facial mask. In the frame of an fMRI study, these two parts can be dissociated in order to keep only the nonmagnetic pneumatic part near the magnet (2 m) while placing the air treatment and electronic part outside the Faraday cage.

2.1.1. The air treatment

Vector air (10 l/min) is pumped directly and continuously from the Hospital distribution network. It is treated with an activated charcoal filter, conveyed through a submicronic particle filter (Deltech 115 Leca, New Castle Delaware, US) towards the olfactometer where its pressure is checked by a manometer. Airflow is then fed into the mixing chamber of the injection head, and directed towards the nose of the subject. Since airflow is not directly delivered into the nostrils but into a mask, it is not necessary to warm and humidify the air. Airflow temperature and humidity are those of the ambient air.

2.1.2. The injection head and odor bottles

The injection head (50 mm high) is made in duralumin which is not magnetic. It is composed of two chambers: an

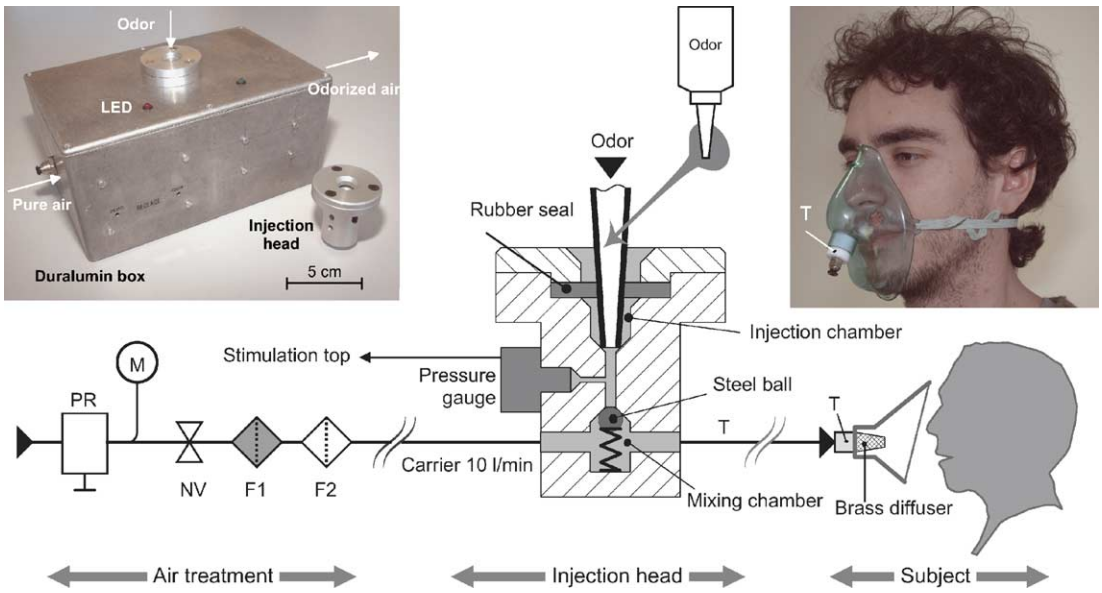

Fig. 1. Diagram of an olfactometer including the air treatment part placed outside the Faraday cage, and the injection head and odor mask situated in the Faraday cage. Left picture: box showing the superior part of the injection head and an injection head extracted from the box. Right picture: odor mask. F1, activated charcoal filter; F2, submicronic particle filter; LED, light emitted diode; M, manometer; NV, needle valve; PR, pressure regulator; T, Teflon®.

injection chamber and a chamber to mix the odor with the airflow. Airtightness between both chambers is maintained with a check valve made of a 5 mm stainless steel ball bearing (18/10 AISI 316) fitted to the conical superior part of the mixing chamber by a calibrated coil spring made of stainless steel (AISI 302, 9.5 spiral, 15 mm length, 0.2 mm wire diameter) (Cochet, France). The injection chamber is made airtight using a butyl (isobutylene isoprene-IIR) rubber seal which acts as an anti-leak valve. During stimulation, the experimenter introduces the dropper of a polyethylene bottle (Osi, France) into the injection chamber and rapidly squeezes it. The air, now saturated with the odorous product, induces a pressure over the steel ball, and enters the mixing chamber. A pressure gauge situated in the injection chamber allows the detection of any superpressure produced from the bottle. This signal is transmitted to the optical transmission interface, and thence to the acquisition system placed outside the Faraday cage. The injection head is enclosed in a die-cast aluminum box 222 mm × 146 mm × 106 mm in dimension (Boss, UK). A detailed scale drawing is given in Fig. 2.

2.1.3. The facial mask

The odorized air is delivered into a commercially available anesthesia mask (Respiron, Europe Medical, France). Different types of mask can be used as a function of the subject's facial morphology. A brass silencer (Pneumax G1/8 BSP, Italy) in the mask provides a rapid, homogenous diffusion of the odors, reduces decompression noise, and avoids tactile stimulation of the subjects. To allow for sufficient ventilation and odor elimination, a 15 mm-diameter opening is made on the lateral part of the mask. A Teflon® tube (Fluorinated Ethylene Propylene; 2.4 mm interior diameter and 3 m

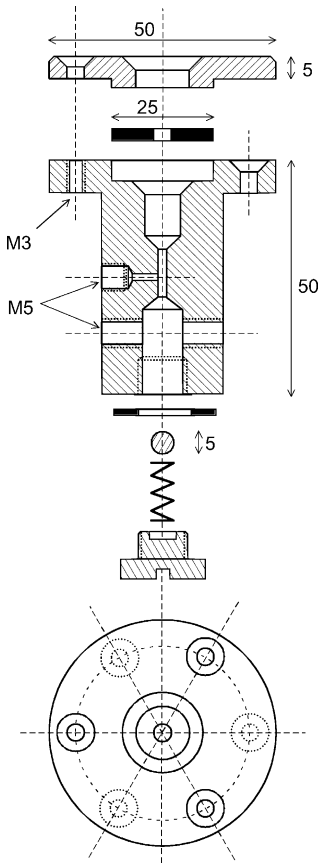

Fig. 2. Detailed drawing of the injection head. Dimensions are given in mm; M3, M5, dimensions of threads.

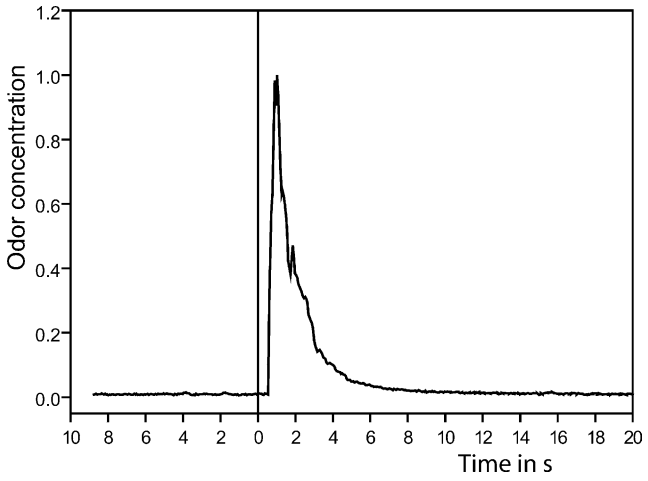

Fig. 3. Relative odorous concentration as a function of time for a stimulation with *n*-butanol. No units are given for concentration since the mass spectrometer had not been previously calibrated.

in length) links the stimulating apparatus to the mask via a custom-made Teflon® connection. The use of Teflon® allows us to minimize odor absorption and desorption phenomena.

2.1.4. Shape of the chemosensory stimulus

It is usually recommended that the shape of the concentration curve of a chemosensory stimulus as a function of time approximates a square wave (Evans et al., 1993). To rate the rise and fall times of odorous stimulation, we measured the concentrations of *n*-butanol in the facial mask by using a mass spectrometer (Agilent MS5973, US). The facial mask was placed over a mannequin head and connected to the injection head. The end of the mass spectrometer capillary was inserted into the mask 10 mm under the nose. Since the mass spectrometer filament can be destroyed by oxygen, nitrogen was used for the vector flow (10 l/min). The mass spectrometer allowed acquisition of a spectrum, from which we could detect the amount of analyte, every 20 ms. Fig. 3 shows data recorded after delivering an *n*-butanol stimulus. The odor was detected 430 ms after injection. Since the transfer time in the capillary is about 250 ms, the actual time for the odor to reach, then diffuse into the facial mask is therefore about 180 ms. The rise time was about 280 ms to reach 70% of maximum (as suggested by Evans et al., 1993). and the total stimulation time was approximately 4 s.

2.2. Materials of behavioral and physiological parameter recording

Devices used to record behavioral and physiological parameters must satisfy two conditions. Firstly, no metallic piece which could conduct electricity must be placed in contact with the subject's skin as high variations of magnetic field can induce Foucault's current that can heat up metallic pieces and burn the subject. Secondly, electrical connections between the inside and outside of the Faraday cage cannot be used because they produce antennas that can pick up electromagnetic waves from the outside and transmit them into the Faraday cage. The oxygen spin frequency is 100 MHz which corresponds to the waveband of commercial frequency modulation transmitters. To avoid this problem, optical fibers or pneumatic pipes can be used. A general schema of the stimulating and recording systems is illustrated in Fig. 4.

2.2.1. Breathing recording

The breathing rhythm of the subject is obtained by measuring variations in ventral breathing. It is recorded with the aid of a foot bellows in polyvinyl chloride (Herga Electric Limited, Suffolk, UK) held on the stomach with a flexible belt. Movements of the abdominal wall produce variations in the internal volume of the foot bellows which is linked via a polyethylene tube to a sensitive bi-directional mass airflow sensor (Honeywell AWM2100, US) transforming the flow into an electrical signal. This signal is amplified, and provides an image of the airflow during breathing, but not of breathing volume (i.e., it is represented by the derivative, not the integral of breathing). This signal is then transmitted via the optical transmission interface to the acquisition system and re-transmitted via the optical transmission interface to a headphone via a voltage to frequency converter. The experimenter then listens to the progressive variations in the sound with a treble frequency when the subject inspires and a bass frequency when the subject expires. This method makes respiration implicit for the experimenter and easy to control the delivery of an odorous stimulus. A regular respiration allows the experimenter to anticipate and to deliver a stimulus just at the end of an expiration phase.

2.2.2. Electrodermal signal

The ED response is recorded from two electrodes (size 15 mm × 10 mm) made of Maillechort (Cu, Ni, Zn) placed on the third phalanx of the forefinger and the middle finger of the non-dominant hand (Fig. 5A). The measurement method was previously described by Strong (1970). A direct current of 10 μA is used to measure ED resistance. To obviate the need to manually check the range of the signal variation during the experiment, we further compress its dynamic by converting the linear product of the ED resistance into a logarithmic function. The resistance is then measured between 68 kΩ and 3.3 MΩ. The signal is transmitted via the optical transmission interface to the acquisition system. The dynamics of the initial signal is re-obtained by applying an exponential function.

2.2.3. Plethysmographic signal

Changes in the peripheral blood flow response (Plethysmography) are recorded from an easy to attach sensor placed on the third phalanx of the thumb of the non-dominant hand (Fig. 5A). The photoelectric PL reflectance sensor contains a light source to illuminate the finger segment and a photodetector to monitor returning light. Living tissue is transparent to red and infrared light while nonhemolyzed blood is relatively opaque in this spectral range. As the blood volume

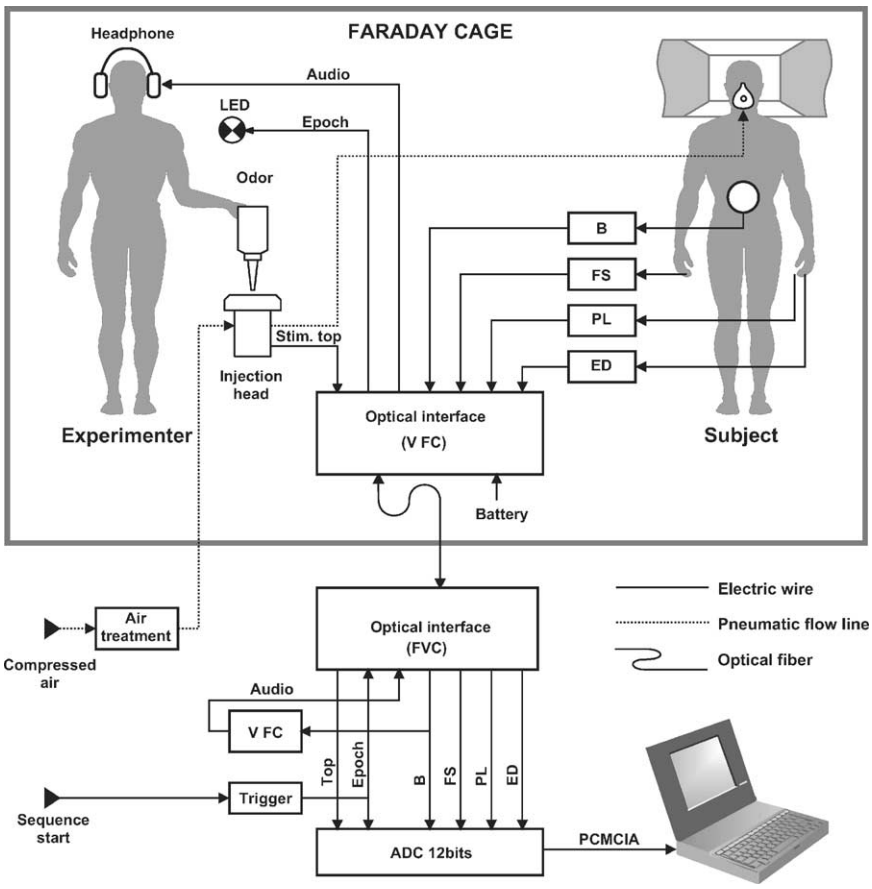

Fig. 4. General setup schema for stimulating the subject and recording his behavioral and physiological signals. See the text for a detailed description. ADC, analog-to-digital converter; B, breath; ED, electrodermal; FS, finger-span; FVC, frequency-to-voltage converter; LED, light emitted diode; PCMCIA, acquisition card (National Instrument); PL, plethysmography; VFC, voltage-to-frequency converter.

changes, the amount of light absorbed or reflected changes, and as a consequence, the electrical characteristics of the photodetector change as well. To reduce artifacts, we use a light source modulated in near infrareds, that is 900 nm. The reflected radiation is first filtered in the near infrareds before reaching the sensor. The photoelectric signal (processed by synchronous detection) is then amplified and transmitted, via the optical transmission interface, to the acquisition system.

2.2.4. Behavioral response

For a binary response, we use two key-press buttons (see Royet et al., 1999). For a graduated response requiring the use of a linear visual rating scale, the subject is instructed to rate olfactory sensations, such as hedonic intensity, through the distance between the thumb and forefinger according to the FS method (Berglund et al., 1978; Larson-Powers and Pangborn, 1978; Yamamoto et al., 1985) recently used in fMRI studies on gustation (Cerf-Ducastel and Murphy, 2001). Two long rectilinear potentiometers (Piher, Spain) are used with respectively 45 and 63 mm of sliding travel depending on a given subject's actual FS (Fig. 5B). The metallic parts of the potentiometers are suppressed and replaced by a custom-made Plexiglas® support (180 mm × 45 mm × 15 mm). Two Plexiglas® stops allow the subject to immobilize the thumb and the middle finger of the dominant hand while the forefinger moves a plastic slide. The potentiometer is connected to a 1 Hz low-pass filter, then to the optical transmission interface and the acquisition system.

2.3. Optical transmission interface and acquisition system

Transmission of the signal using optical pathways allows us to satisfy two conditions. Firstly, it avoids introducing radio-electric interference into the Faraday cage. Secondly, it presents a galvanic insulation between the subject and the acquisition system. The optical transmission interfaces are voltage-to-frequency (VFC) converters fitted with Nickel Cadmium batteries (750 or 1800 mA/h).

Behavioral and physiological data are recorded on line (100 Hz sampling rate) with a NEC PC computer and a multifunction DAQCard-500 characterized by eight inputs, a flow of 50 kS/s, and 12-bit resolution (National Instruments, US). LabView 5.0 software (National Instruments, USA) is used to acquire, store, and read these data.

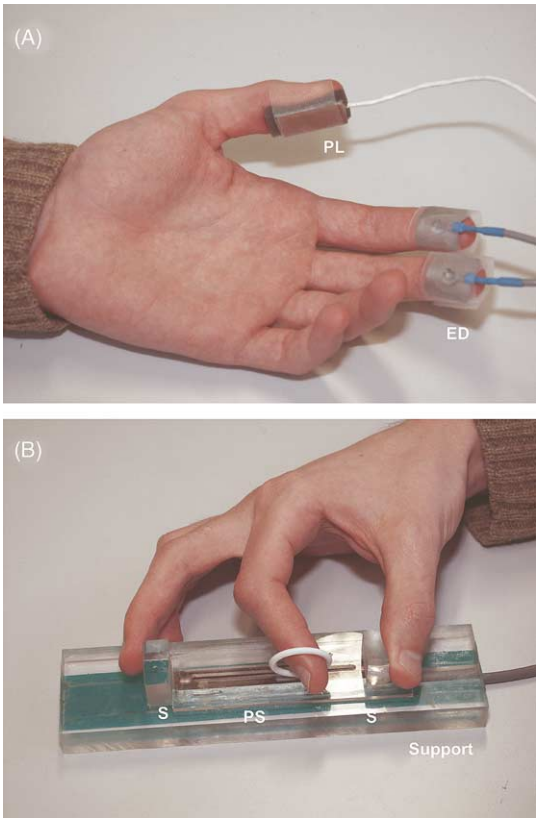

Fig. 5. (A) ED sensors placed on the third phalanx of the forefinger and the middle finger, and a PL sensor placed on the third phalanx of the thumb of the subject's non dominant hand. (B) FS device including a rectilinear slide potentiometer with 63 mm travel. ED, electrodermal; PL, plethysmography; PS, plastic slide; S, Plexiglas® stop.

3. Operational example

3.1. Procedures

The data presented in the current study were collected from a 24-year-old healthy right-handed non-smoking female subject. This subject had participated in a previous fMRI study in which 28 subjects performed hedonic judgments with pleasant and unpleasant odorants presented during one run of three epochs each (Royet et al., 2003).

Data processing and statistical analysis methods have already been described by Royet et al. (2003). Briefly, fMRI was performed using a 1.5-T imager (Philips NT). Twenty-five adjacent, 5 mm thick axial slices were imaged. The imaging volume covered the subject's whole brain and was oriented parallel to the bicommissural plane. The image planes were positioned on scout images acquired in the sagittal plane. A 3D PRESTO, less prone to the magnetic susceptibility artifacts than is the echo-planar imaging sequence (Liu et al., 1993), was used with the following parameters: TR = 26 ms, TE = 38 ms, flip angle = 14°, field-of-view = 256 mm^2 × 205 mm^2, imaging matrix = 64 × 51 (voxel size of 4 mm × 4 mm × 5 mm). A high-resolution anatomic 3D T1-weighted MR scan was also acquired.

The fMRI runs were analyzed using Statistical Parametric Mapping (SMP99, The Wellcome Department of Cognitive Neurology, London, UK; Friston et al., 1995a,b). Image processing included inter-scan realignment, spatial normalization to the stereotactic space as defined by the ICBM template provided by the Montreal Neurological Institute (MNI), and image smoothing using a three-dimensional Gaussian kernel (FWMH: 8 mm × 8 mm × 10 mm). A boxcar reference function was convolved with SPM99's 'canonical' hemodynamic response function. Global differences in blood oxygenation level dependent (BOLD) signal were covaried out of all voxels, and comparisons across conditions were effected with *t*-tests. The significance of signal differences was assessed through *Z*-scores in an omnibus sense (Friston et al., 1995b), using an uncorrected probability with a threshold of $P < 0.001$.

3.2. Results

A typical example of physiological and behavioral data recorded for a 60 s epoch when the subject is smelling and judging unpleasant odors is depicted in Fig. 6. Thirteen odorous stimulations were delivered in a 60 s epoch indicating that the subject's mean breathing was 4.62 s per cycle. For each breath cycle, an odorous stimulation was delivered just before an inspiration phase (see the square-box of Fig. 6). The subject provided a more or less ample response with the FS. For instance, odors of tar, butyl sulfide, pyrrole, 2,5-dimethyl pyrrole, and tetrahydrothiophene were judged as particularly unpleasant. Systematic variations of the ED response were observed after each olfactory stimulation. A short delay in the ED response attests to the low time constant of the ED response.

Variations of breathing could be expected according to whether the subject was recorded during rest epochs or when pleasant or unpleasant odors were delivered. Comparing inspiratory flows between these different epochs did not reveal significant differences [$F(2, 102) = 0.789$, $P = 0.457$]. Correlations between ED, FS and inspiratory flow values were also calculated for this subject when smelling pleasant or unpleasant odorants. A significant correlation was found between ED and FS when the subject smelt unpleasant odorants (Fig. 7), indicating that the more odors were rated as unpleasant, the higher the ED amplitude variations ($r = 0.472$, $t = 3.124$, $P = 0.0036$). No significant correlation was found between ED and FS for pleasant odorants ($r = 0.163$, $t = 0.964$, $P = 0.3417$), and between inspiratory flow and ED variations for pleasant and unpleasant odors ($r = 0.198$, $t = 1.180$, $P = 0.2461$ and $r = 0.261$, $t = 1.554$, $P = 0.1298$, respectively).

fMRI activation was determined by contrasting the unpleasant olfactory and rest conditions when the subject performed the hedonic judgment task. The results show BOLD signal variations in several brain regions commonly associated with odor processing, such as the orbitofrontal and piriform cortices, amygdala, and insula (Fig. 8).

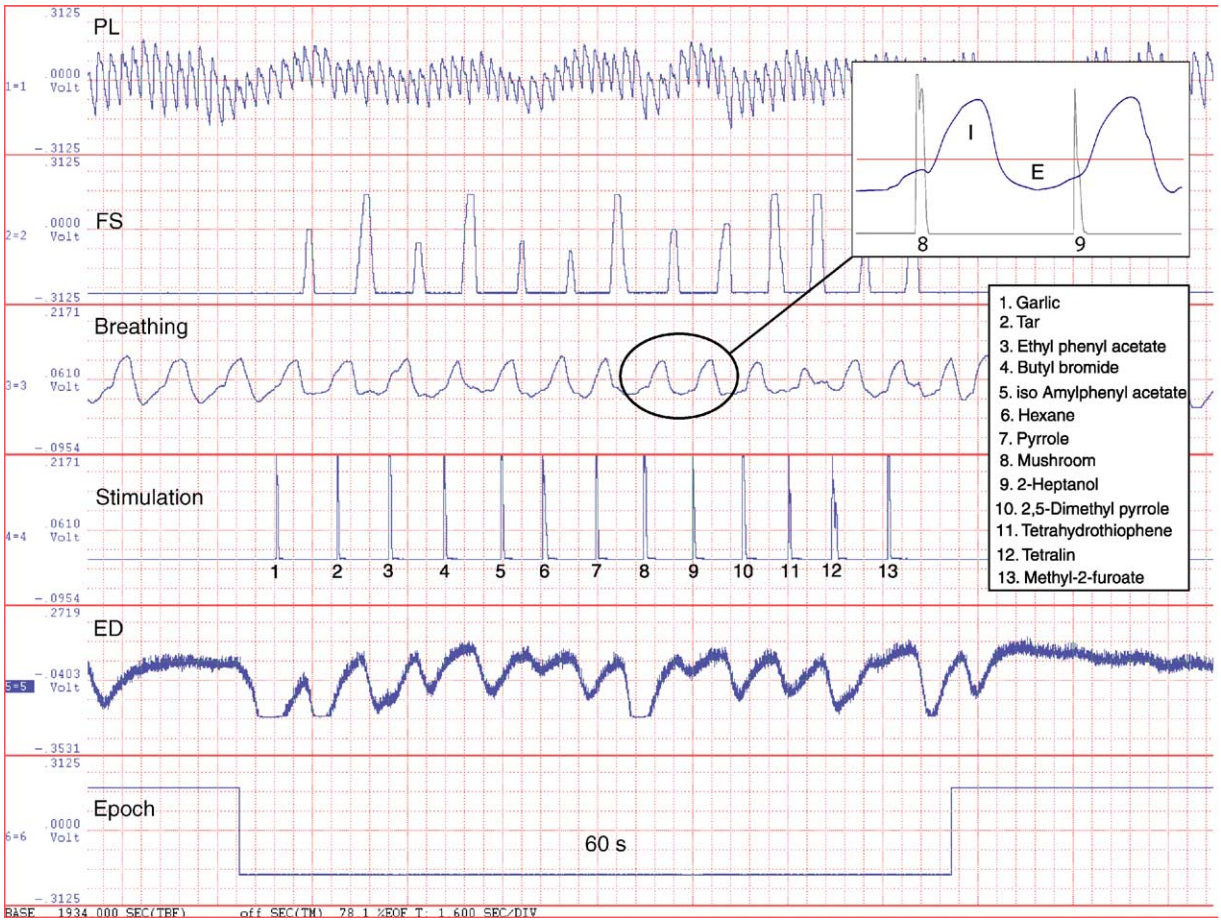

Fig. 6. Example of behavioral and physiological measurements recorded during an epoch for which 13 unpleasant odorants were synchronously delivered with the beginning of each inspiration. Square-box (top) shows two breathing cycles superimposed with stimulations 8 and 9. Inspiration and expiration phases are respectively represented by the superior and inferior parts of the horizontal reference line. ED, electrodermal; FS, finger-span; PL, plethysmography. Data were depicted with the WinDaq Waveform Browser 1.91 software (DataQ Instruments, Inc., USA).

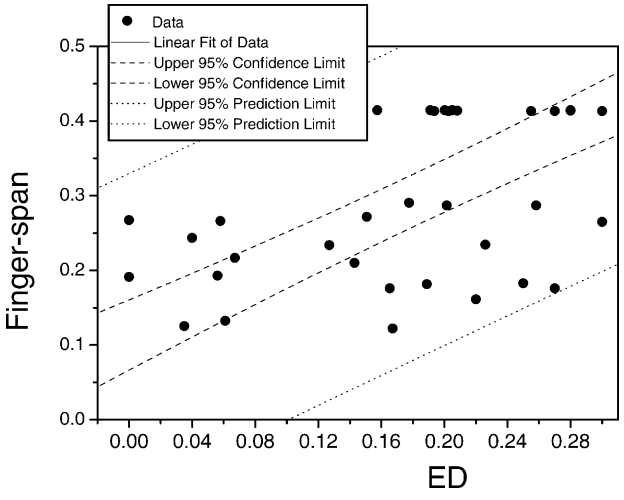

Fig. 7. Correlation between FS and ED data obtained during hedonic judgment of unpleasant odorants.

4. Discussion

The method of odor stimulation presented in the current study has proved its validity since regions activated in both our PET as fMRI studies (Royet et al., 1999, 2000, 2001, 2003) were reliable and consistent with those obtained by other authors (e.g., Zatorre et al., 1992; Sobel et al., 1997, 1998a; Zald and Pardo, 1997).

By synchronizing odorous stimulation with inspiration, our olfactometer allows the stimulation of the subject using a great diversity of odorants during the experiment and a high number of odorants during a scan or an epoch, which is not the case of olfactometers proposed by other researchers and based on a different principle (Sobel et al., 1997; Lorig et al., 1999). For instance, for a mean breath rhythm of about 4–5 s and 1 min of brain activity recording, 12–15 stimulations can be performed depending on the duration of the breath cycle. This advantage allows subjects to satisfactorily perform an olfactory task by improving their attention ability and increasing the signal by repeating stimulation while avoiding sensory habituation phenomenona. Although this frequency

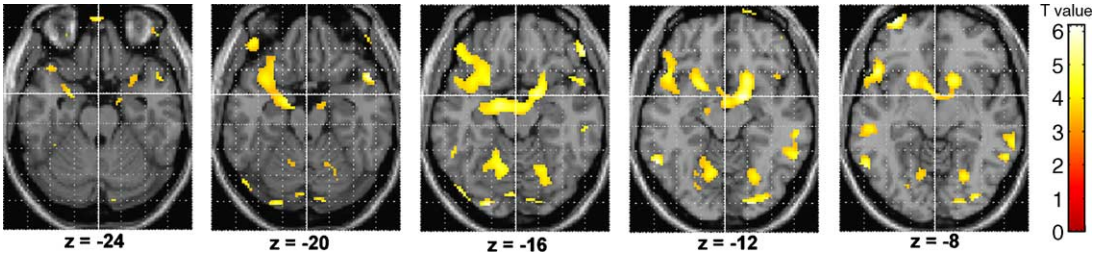

Fig. 8. fMRI activations covering regions of interest during hedonic judgment of unpleasant odorants. Activations were superimposed on axial sections (4 mm apart) from an anatomically normalized standard brain. Sections extended from −24 to −8 mm (MNI Z coordinates are given below the image). The gray scale indicates *T* values.

of sensory stimulation is lower than that employed in neuroimagery for other modalities, it is much higher than that currently used in olfaction when the experimenter wants to avoid the exacerbated phenomenon of odor adaptation (Cain and Moskowitz, 1974; Engen, 1982). However, the high flow of the vector air (10 l/min) avoids odors stagnating in the mask, and thus produces a more punctual odorous stimulation. When synchronizing odors with breathing, the number of odorous stimulations per scan or epoch depends on the individual (since the duration of the breathing cycle can vary from 2.5 s to more than 10 s), but can also vary for any one subject from one scan or epoch to another. To homogenize the number of stimulations between subjects, it therefore is necessary to select these subjects as a function of their respiratory cycle. When the duration of the breathing cycle is very short, the phenomenon of odor masking is increased. A cycle of long duration (low stimulation frequency) avoids these disadvantages, but the subject no longer needs to focus his attention on the task once he has responded, since several seconds will pass before the following stimulus is delivered.

Compared with other methods of stimulation, our olfactometer does, however, present some limitations. Whereas we can suppose that the control of odor concentration is reliable with olfactometers in which the airflow is imposed by solenoid valves, it seems more difficult to precisely control it with our method, because the quantity of odorant is manually delivered by squeezing the odor bottle. The quantity of odorant delivered may then vary from one stimulation to another. Reliability of stimulation timing was not rated in the present study, but it is also dependent on our manual method of stimulation that was conceived to synchronize stimuli with the subject's breathing. Therefore, reliability is mainly a function of the experimenter's ability to locate the subject's inspiration phases and to deliver the stimuli at the beginning of an inspiration.

Synchronizing the stimulations with inspiration allows the subject to be firstly in a regular respiration condition for testing without the need to sniff, and secondly, to keep his eyes closed and his ears plugged while performing the olfactory task since no visual or auditory messages are then needed. The first point avoids the recording of activations related to the motor activity of sniffing as previously observed by Sobel et al. (1998a,b). The second point allows the subject to be better concentrated on the olfactory task. It also induces activation patterns mostly limited to the olfactory neural networks by avoiding systematic activation of perceptual and semantic visual or auditory neural networks. The results clearly indicate that odors were delivered at the beginning of an inspiration, and that the subjects had time to respond before the following inspiration. The current study further demonstrates the possibility of simultaneously recording the behavioral responses of the subject, which is more efficient that re-measuring olfactory performance after scanning (Levy et al., 1997, 1998; Zald and Pardo, 1997; Cerf-Ducastel and Murphy, 2001; Crespo-Facorro et al., 2001; Savic et al., 2002). The present paper finally shows that the physiological parameters, such as breathing, ED and PL can be recorded online and the measurements then analyzed in different experimental conditions especially in studies linked to emotion. The significant correlations between the ED and FS responses testify to the reliability of these measurements.

The present olfactometric method was designed to be used in PET and fMRI experiments where the duration of the scans or epochs was 60 s. This method is, however, suitable for fMRI studies with shorter epochs (10 s) (Wicker et al., 2003) and in event-related fMRI experiments. Since stimulation timing of our olfactometer could be longer than that obtained with some other methods (e.g., Lorig et al., 1999), this may preclude its use for psychophysiological or rapid event-related studies. Nevertheless, we think that the crucial point in recording these responses in such paradigms is mainly to synchronise the stimulus with inspiration, and to start data acquisition at the beginning of an inspiration. Our olfactometer can satisfy such constraints. It thus appears that the inspiration cue represents the limiting factor for performing electrophysiological studies of olfactory evoked potentials, and that subordination of the stimulus to inspiration allows such approaches even when the odorants are presented directly under the nostrils with a bottle (Hudry et al., 2001, 2003).

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