Olfactory fMRI: Implications of Stimulation Length and Repetition Time

GEORGIOPOULOS, Charalampos, et al.

Abstract

Studying olfaction with functional magnetic resonance imaging (fMRI) poses various methodological challenges. This study aimed to investigate the effects of stimulation length and repetition time (TR) on the activation pattern of 4 olfactory brain regions: the anterior and the posterior piriform cortex, the orbitofrontal cortex, and the insula. Twenty-two healthy participants with normal olfaction were examined with fMRI, with 2 stimulation lengths (6 s and 15 s) and 2 TRs (0.901 s and 1.34 s). Data were analyzed using General Linear Model (GLM), Tensorial Independent Component Analysis (TICA), and by plotting the event-related time course of brain activation in the 4 olfactory regions of interest. The statistical analysis of the time courses revealed that short TR was associated with more pronounced signal increase and short stimulation was associated with shorter time to peak signal. Additionally, both long stimulation and short TR were associated with oscillatory time courses, whereas both short stimulation and short TR resulted in more typical time courses. GLM analysis showed that the combination of short stimulation […]

Reference

GEORGIOPOULOS, Charalampos, et al. Olfactory fMRI: Implications of Stimulation Length and Repetition Time. Chemical Senses, 2018, vol. 43, no. 6, p. 389-398

DOI : 10.1093/chemse/bjy025
PMID : 29726890

Available at:
http://archive-ouverte.unige.ch/unige:145438

Disclaimer: layout of this document may differ from the published version.
Original Article

Olfactory fMRI: Implications of Stimulation Length and Repetition Time

Charalampos Georgiopoulos¹,², Suzanne T. Witt², Sven Haller³,⁴, Nil Dizdar⁵, Helene Zachrisson⁶, Maria Engström²,⁷ and Elna-Marie Larsson⁴

¹Department of Radiology and Department of Medical and Health Sciences, Linköping University, Röntgenkliniken, Universitetssjukhuset, 581 85 Linköping, Sweden, ²Center for Medical Image Science and Visualization (CMIV), Linköping University, University Hospital, 581 85 Linköping, Sweden, ³Affidea CDRC Centre de Diagnostic Radiologique de Carouge SA, 1, clos de la Fonderie, CH-1227 Carouge, Geneva, Switzerland, ⁴Department of Surgical Sciences/Radiology, Uppsala University, Akademiska sjukhuset, 751 85 Uppsala, Sweden, ⁵Department of Neurology and Department of Clinical and Experimental Medicine, Linköping University, 581 83 Linköping, Sweden, ⁶Department of Clinical Physiology and Department of Medical and Health Sciences, Linköping University, 581 83 Linköping, Sweden and ⁷Department of Medical and Health Sciences, Linköping University, Sandbäcksgatan 7, 581 83 Linköping, Sweden

Correspondence to be sent to: Charalampos Georgiopoulos, Röntgenkliniken, Universitetssjukhuset, 581 85 Linköping, Sweden. e-mail: Charalampos.Georgiopoulos@regionostergotland.se

Editorial Decision 26 April 2018.

Abstract

Studying olfaction with functional magnetic resonance imaging (fMRI) poses various methodological challenges. This study aimed to investigate the effects of stimulation length and repetition time (TR) on the activation pattern of 4 olfactory brain regions: the anterior and the posterior piriform cortex, the orbitofrontal cortex, and the insula. Twenty-two healthy participants with normal olfaction were examined with fMRI, with 2 stimulation lengths (6 s and 15 s) and 2 TRs (0.901 s and 1.34 s). Data were analyzed using General Linear Model (GLM), Tensorial Independent Component Analysis (TICA), and by plotting the event-related time course of brain activation in the 4 olfactory regions of interest. The statistical analysis of the time courses revealed that short TR was associated with more pronounced signal increase and short stimulation was associated with shorter time to peak signal. Additionally, both long stimulation and short TR were associated with oscillatory time courses, whereas both short stimulation and short TR resulted in more typical time courses. GLM analysis showed that the combination of short stimulation and short TR could result in visually larger activation within these olfactory areas. TICA validated that the tested paradigm was spatially and temporally associated with a functionally connected network that included all 4 olfactory regions. In conclusion, the combination of short stimulation and short TR is associated with higher signal increase and shorter time to peak, making it more amenable to standard GLM-type analyses than long stimulation and long TR, and it should, thus, be preferable for olfactory fMRI.

Key words: fMRI, olfaction, smell, repetition time

Introduction

Olfaction is important for both pleasure and survival, and olfactory impairment is associated with depression and lower quality of life (Smeets et al. 2009). Olfactory neurotransmission has 4 main levels: the olfactory mucosa in the nose, the olfactory bulb, the olfactory cortex, and its main projections in the brain (Gottfried 2010; van Hartevelt and Kringelbach 2012). The piriform cortex is the largest and most distinct structure within the olfactory cortex, and it can be
divided, both anatomically and functionally, into 2 regions: the anterior piriform cortex, responsible for encoding odorant identity, and the posterior piriform cortex, responsible for encoding odor quality (Kadohisa and Wilson 2006). Olfactory projections beyond the olfactory cortex include the orbitofrontal cortex and the anterior insula. The role of orbitofrontal cortex in olfaction has not yet been well defined; it has been associated with odor discrimination and identification as well as olfactory attentional modulation (Zatorre et al. 1992; Jones-Gotman and Zatorre 1993; Plailly et al. 2008). The anterior insula is an important node in the salience network, with a proven response to olfactory stimulation; it is often distinctively activated after olfactory stimulation of negative valence (Zatorre et al. 1992; Small et al. 1999; Wicker et al. 2003; Seubert et al. 2010).

The above mentioned 4 main levels of odor neurotransmission have been increasingly studied in an effort to better understand the sensory processing and the neural substrates of human olfaction (Firestein 2001; Linsner et al. 2001; Cleland et al. 2002; Zelano et al. 2005; Gottfried et al. 2006; Plailly et al. 2008; Gottfried 2010). Olfactory impairment is often associated with aging, but it is also a clinical symptom of certain neurodegenerative diseases, such as Parkinson’s and Alzheimer’s disease (Hawkes et al. 1997; Barresi et al. 2012; Schapira et al. 2017). Functional magnetic resonance imaging (fMRI), measuring the Blood Oxygen Level Dependent (BOLD) response to neural activation in the olfactory cortex, has facilitated, in particular, the identification of cortical and subcortical brain structures that participate in olfactory processing (Li et al. 2008; Howard et al. 2009; Karunanayaka et al. 2014). fMRI has increasingly been employed in order to elucidate the neural basis of olfactory deterioration both in health and disease (Wang et al. 2005; Hummel et al. 2010; Moessnang et al. 2011; Vasavada et al. 2015; Pellegrino et al. 2016; Vasavada et al. 2017).

The collection of reliable olfactory fMRI data can be affected by various factors, both physiological and methodological, such as the participant’s respiration, magnetic susceptibility artifacts due to air-tissue interfaces at the skull base, the length of odorous stimulation, and the sampling rate (repetition time, TR) of the fMRI sequence (Yang et al. 1997; Poellinger et al. 2001; Dilharreguy et al. 2003; Wang et al. 2014). Prolonged odorous stimulation has long been associated with rapid adaptation (decrease of BOLD signal) of olfactory brain regions, especially the piriform cortex and the orbitofrontal cortex (Sobel et al. 2000; Poellinger et al. 2001; Li et al. 2006). To avoid adaptation, most olfactory fMRI studies employ either event-related designs with short odorous stimulation or block designs with short odorous pulses incorporated in each block of stimulation. Furthermore, short TR results in more accurate temporal resolution of the BOLD response, which has led to the development of novel acquisition techniques that achieve high temporal resolution by reducing the TR to <1 s (Dilharreguy et al. 2003; Lin et al. 2006; van der Zwaag et al. 2006; Feinberg et al. 2010; Posse et al. 2012; Witt et al. 2016). To our knowledge, the effects of fast sampling rate (short TR) on olfactory fMRI data have not been previously studied.

The current literature about olfactory fMRI displays high methodological diversity in both the experimental designs and the analytical approaches, leading sometimes to inconsistent results: 2 studies reported decreased activity in the olfactory cortex of patients with Parkinson’s disease after olfactory stimulation, whereas one study showed hyperactivation of the olfactory cortex (Westermann et al. 2008; Hummel et al. 2010; Moessnang et al. 2011). Therefore, establishing an optimal and reliable fMRI design is of high importance. This study aimed to investigate how the length of odorous stimulation and the TR influence the activation pattern in the olfactory cortex. For this purpose, 3 analytical approaches were employed: traditional General Linear Model (GLM) analysis, model-free Tensorial Independent Component Analysis (TICA), and Region of Interest (ROI) analysis by plotting the time courses of the BOLD signal. The overall aim was to establish a robust and reliable fMRI paradigm that can be employed in olfactory fMRI studies in healthy individuals and in patients with neurodegenerative disorders.

**Materials and methods**

**Participants**

Twenty-five participants (12 males) were recruited through advertisements at the Faculty of Medicine and Health Sciences at Linköping University. All participants underwent olfactory examination prior to fMRI examination. Inclusion criteria were age <40 years and normal olfaction (normosmic). Subjects with active colds or allergies, history of previous surgery in the nasal cavity, neurological disorders, mandibular implants, or magnetic/electromagnetic implants (such as pacemakers) were excluded. Smokers were also excluded. Written informed consent was obtained from all participants. The study was conducted in accordance with the 1964 Helsinki declaration and its later amendments and was approved by the Regional Ethical Review Board in Linköping, Sweden (registration number 2011/415–31).

**Olfactory evaluation**

All participants underwent olfactory examination with the University of Pennsylvania Smell Identification Test (UPSIT, Sensonics, Inc.). This test consists of 40 different odorants, microencapsulated in 4 different booklets. On each page, there is a multiple-choice question with 4 alternative responses and 1 odorant embedded in a microcapsule at the bottom of the page. Each odorant is released by scratching the microcapsule, and the tested subject is then required to choose the alternative that best corresponds to the odorant. The performance of each patient is presented as the UPSIT score, which has a range from 0 to 40. We used the norms provided by the manufacturer in order to classify the participants as normosmic, hyposmic, or anosmic.

Three participants (2 males) were classified as mildly hyposmic, based on their UPSIT score, and their fMRI data were excluded from the analysis. The remaining 22 participants (10 males) had a median age of 26.5 years (95% CI: 23 and 30.5 years) and median olfactory score 36 out of 40 (95% CI: 35 and 37).

**fMRI data acquisition**

fMRI was performed with a 3T scanner (Siemens MAGNETOM Prisma, Siemens AG) using a 20-channel head-neck coil. To test the effect TR, 2 different multiplex echo planar imaging (EPI) sequences were used. For the short TR, a sequence with the following parameters was used: TR/echo time (TE) = 90/30 ms; flip angle = 59°; simultaneous multi-slice (SMS) = 2; parallel acquisition technique (iPAT) = 2; EPI factor = 128; field of view (FOV) = 192 x 192 mm2; matrix = 64 x 64; # slices = 48; slice thickness (gap) = 3 (0) mm; voxel = 3 x 3 x 3 mm3; # time points = 705; total scan time = 635.205 s. For the long TR, the following parameters were used: TR/TE = 1340/30 ms; flip angle = 69°; SMS = 2; iPAT = 2; EPI factor = 128; FOV = 192 x 192 mm2; matrix = 64 x 64; # slices = 48; slice thickness (gap) = 3 (0) mm; voxel = 3 x 3 x 3 mm3; # time points = 474; total scan time = 635.16 s. Both sequences used an initial fat saturation pulse. Additionally, high-resolution 3D T1-weighted and T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) structural scans were acquired in all subjects to ensure that they did not have any obvious pathological changes in the brain.
fMRI experimental design

Natural coffee oil extract (Sigma-Aldrich), diluted (50% v/v) in odorless diethyl phthalate (Sigma-Aldrich) was used as odorant. Stimulation was administered in blocks of events. Two different stimuli lengths, 6 s and 15 s, were randomly embedded in 1 session (Figure 1), which included 10 blocks for each of the abovementioned stimulation lengths. A 20-s-long resting period, consisting of odorless air, separated the stimulation blocks from each other. To avoid habituation, each stimulation block consisted of 1-s-long odorous pulses, followed by 2 s of odorless air. The odorant was delivered simultaneously to both nostrils, using the OG001 Multistimulator (Burghart Messtechnik GmbH, Wedel, Germany), embedded in medical air stream (2.5 l airflow per nostril), through Teflon-tubing (4 mm inner diameter). To remove residual odorants, a constant, inverse airflow was maintained inside the magnet aperture. All subjects were instructed to breathe normally through the nose and avoid sniffing. All participants were asked to click a button with their index finger every time they could sense the smell of coffee in order to objectively confirm odor sensation. Registration of response was only open during stimulation; if the subject’s response were delayed, no response would be registered. The total task duration was 630 s. The same task design was repeated for each of the tested TRs (0.901 s and 1.34 s).

Data analysis

Extracting the event-related time course of brain activation in 4 olfactory brain areas (Figure 2) was the main analytical approach. Task-driven GLM analysis and model-free TICA were additionally performed as supportive analytical tools.

GLM analysis was carried out with SPM12 (Wellcome Trust Centre for Neuroimaging, University College London). All participants’ images were separately realigned and the translation and rotation correction parameters were individually examined to ensure that no participant had significant head motion larger than one voxel in any direction. No participants were excluded due to head motion. Thereafter, the realigned images of each participant were coregistered with the T1-weighted anatomical images. Spatial normalization into Montreal Neurologic Institute (MNI) space was initially performed on the mean functional image volume for each participant, and these normalization parameters were then applied to each respective functional image set. The normalized images were smoothed with an 8 mm full width half maximum (FWHM) Gaussian kernel. Separate GLM analyses were performed for each TR. In both cases, the stimulation blocks of the fMRI paradigm were modeled as regressors of interest, one for each stimulation length (6 s and 15 s). The resting periods were not explicitly modeled. The 6 motion parameters derived from the realignment step were included as covariates of no interest in both GLMs.

To assess the primary study aim, ROI analysis was performed on normalized data from the GLM analysis and it included plotting the Finite Impulse Response (FIR) event time courses for 4 different olfactory brain areas: the anterior piriform cortex, the posterior piriform cortex, the orbitofrontal cortex, and the anterior insula (Figure 2). ROI analysis was carried out with MarsBaR 0.44 toolbox for SPM. The ROIs were designed in accordance with a previously described statistical localization of the human olfactory cortex (Seubert et al. 2013). Prior to ROI analysis, the point with the peak activity was identified for each participant in each ROI, separately for each combination of TR and stimuli length, in both right and left hemispheres. The coordinates from all these points were averaged out to identify an average peak point for each ROI for the whole sample. This average point was then used as the center of 8 new spherical ROIs (5 mm diameter for anterior and posterior piriform cortex, 10 mm diameter for orbitofrontal cortex and insula). At last, the following 3 components of all time courses were calculated separately for each ROI: maximal signal change, time to peak, and estimate of oscillations. For the latter, we needed to estimate the total number of peaks for each time course, taking into account that this estimate varied for each brain region and each combination of stimulation and TR. We, therefore, chose to calculate the number of values that were greater than 25% of the maximal signal change, as this estimate would be proportional to the number of peaks and would, thus, depict oscillatory responses.

Finally, to further confirm the GLM- and FIR-based results, TICA was carried out with MELODIC version 3.14, part of FSL 5.0 (FMRIB Analysis Group, University of Oxford). TICA allows a model-free decomposition of the variance in the signal, into different activation and artefactual components, including their spatial maps and time courses (Beckmann and Smith 2005). The following data pre-processing was applied to the input data: masking of nonbrain voxels; voxel-wise de-meaning of the data; normalization of the voxel-wise variance; pre-processed data were whitened.
and projected into a 15-dimensional subspace using Principal Component Analysis. The whitened observations were decomposed into sets of vectors that describe signal variation across the temporal domain (time courses), the session/subject domain and across the spatial domain (maps) by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvarinen 1999). Estimated Component maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the histogram of intensity values (Beckmann and Smith 2004). Taking into consideration a study that proposed the choice of approximately 20 independent components to examine large-scale brain networks, we chose to separate the signal into 20 and into 15 independent components (Ray et al. 2013). There was no significant difference regarding the olfactory network when using 15 or 20 components, and we, therefore, chose to report the results from the analysis with the 15 components.

Statistics
The FIR event-related time courses represent the percentage of signal change by time. Repeated measures ANOVA with Bonferroni correction was employed separately for each ROI in order to estimate the effect of stimulation length and TR on the abovementioned components of these time courses: maximal signal change, time to peak (in seconds), and estimate of oscillations. Statistical significance for ROI analysis was set at $P < 0.05$ (Wilks’ Lambda test); statistical analysis was performed with IBM SPSS Statistics version 23. For the GLM analysis, whole-brain analysis was assessed at $P < 0.001$. Since GLM was performed as a supportive tool to the ROI analysis, no correction for multiple comparisons was employed here. The default threshold level of $P > 0.5$ was used for TICA in order to test the alternative hypothesis poststatistically. All plots were created in GraphPad® Prism 7. All results presented in the text represent the median value with lower and upper 95% confidence intervals (CI).

Results

Response tracking
The average of registered responses for the 6-s and the 15-s stimulations, from both TR sessions, was calculated for each participant. The identification rates were 83% and 97% respectively for the 6-s and 15-s stimulation.

Event-related time courses
As described above in the Methods section, to assess the primary study aim, the normalized fMRI data were analyzed by extracting the FIR event-related time courses for 4 olfactory brain areas: the anterior piriform cortex, the posterior piriform cortex, the orbitofrontal cortex, and the insula (Figure 3). Visually inspecting the event-related time courses, the short stimulation resulted in an almost typical BOLD response in all 4 ROIs, regardless of TR. Namely, the short stimulation resulted in a BOLD response that consisted of an initial dip, a peak within approximately 6 s, and a poststimulus undershoot below the baseline, being restored by the end of the resting period. However, the long stimulation resulted in an oscillating BOLD response: after reaching a peak, the BOLD signal continued with several lower peaks thereafter, without a distinct undershoot below the baseline in most ROIs.

The results from the statistical analysis of maximal signal change, time to peak and estimate of oscillations are summarized in Tables 1 and 2, separately for each ROI. TR had statistically significant effect on maximal signal change for all 4 ROIs bilaterally, with long TR resulting in lower maximal signal change in all ROIs. Stimulus length only had a statistically significant effect on maximal signal change in the left anterior piriform cortex and right orbitofrontal cortex, with the shorter stimulus length producing a higher peak signal. Stimulus length had significant effect in time to peak in the anterior piriform cortex and the insula bilaterally as well as in the right orbitofrontal cortex, with short stimulation being generally associated with faster time to peak. TR only affected time to peak in left anterior piriform cortex, with the long TR resulting in a shorter time to peak. TR had significant effect on the number of oscillations for all 4 ROIs bilaterally, with short TR being able to detect more oscillations compared with long TR. Similarly, the effect of stimulus length on oscillations was significant for most ROIs, except for the orbitofrontal cortex, with long stimulation resulting in a larger number of oscillations.

Task-driven GLM analysis
Data from both tested TRs (0.901 s and 1.34 s) were analyzed separately with 2 different contrasts: 6-s stimulation and 15-s stimulation, at $P < 0.001$ (uncorrected). As shown in Figure 4, the combination of short TR (0.901 s) and short stimulation (6 s) resulted in visually more extensive activation in olfactory brain areas bilaterally, namely the posterior piriform cortex, the orbitofrontal cortex, and the insula. The number of suprathreshold-activated voxels ($P < 0.001$) within the posterior piriform cortex was calculated in order to confirm this finding (Table 3). The combination of short TR and short stimulation resulted in the highest number of suprathreshold-activated voxels in both posterior piriform cortices, compared with all other combinations of TR and stimulus length. These brain areas were activated to a lesser degree with the combination of long TR (1.34 s) and short stimulation and even less with the combination of short TR and long stimulation (15 s). The combination of long TR and long stimulation activated the insula bilaterally and partly the right orbitofrontal cortex, but not the piriform cortex. The left motor cortex was activated in all cases, due to the response-tracking
task, where the subjects were asked to use their right index finger; however, the motor cortex is not illustrated in Figure 4.

Model-free TICA
The collected whole-brain signal from the tested TRs was separated into 15 independent components, using TICA. For the short TR (0.901 s), the second independent component was associated with a functionally connected network that included the anterior and the posterior piriform cortex, as well as insula and parts of the orbitofrontal cortex (Figure 5A). This component was responsible for 24.26% of the explained variance of the signal. The temporal representation of this component consisted of an oscillation with 20 distinct peaks, coinciding with the olfactory stimulation of the tested fMRI task design. Likewise, for the long TR (1.34 s), the third independent component showed a very similar spatial and temporal representation, interconnecting the abovementioned olfactory brain areas (Figure 5B). In this case, the component was responsible for 22.16% of the explained variance in the signal.

Discussion
The present fMRI study aimed to investigate the effect of stimulation length and TR on the BOLD response in 4 olfactory brain regions. Analyzing the event-related time course of activation in these regions
revealed that short TR is associated with more pronounced relative signal increase, compared with long TR. Long stimulation was associated with longer time to peak signal and oscillatory BOLD response. On the other hand, short TR and short stimulation resulted in a more typical BOLD response, making this combination better suited for traditional GLM analysis employing the canonical Hemodynamic Response Function (HRF). Traditional GLM analysis accordingly confirmed that the combination of short stimulation and short TR could result in visually more extensive activation within the olfactory cortex. The model-free TICA validated that the tested paradigm was spatially and temporally associated with a functionally connected network that included all 4 olfactory ROIs. Therefore, the results of the present study favored the choice of short stimulation length and short TR when designing olfactory fMRI studies.

A pioneering study by Sobel et al. demonstrated that odorous stimulation results in an early and sharp activation within the piriform cortex, followed by a rapid decrease of signal after continuous stimulation, explaining why early fMRI studies yielded small or no activation in the olfactory cortex (Sobel et al. 2000). Poellinger et al. tried to evaluate the effects of short (9 s) and long (60 s) continuous olfactory stimulation in olfactory related brain areas (Poellinger et al. 2001). Similar to the findings presented here, 9-s stimulation consistently activated the piriform cortex, the entorhinal cortex, and the amygdala, whereas 60-s stimulation resulted in an oscillating response in these brain areas, followed by a prolonged decline below baseline. However, in Poellinger’s experiment, the orbitofrontal cortex showed a sustained increase in activation after a long stimulation. Our fMRI paradigm differs in terms of both stimulus length and interstimulus interval, which can to certain extent explain the discrepancies between Poellinger’s experiment and our study. A more recent study by Li et al. confirmed the findings of Sobel and Poellinger and additionally demonstrated habituating activity (decreased BOLD signal) in the left orbitofrontal cortex (Li et al. 2006). To bypass the negative effects of long, continuous, olfactory stimulation, a broad range of olfactory fMRI studies employ stimulation in blocks of events, lasting 20 s or longer (Hummel et al. 2010; Moessnang et al. 2011; Bensafi et al. 2013; Croy et al. 2014; Pellegrino et al. 2017). Nevertheless, our study indicated that odor administration in blocks of events could still cause an oscillating response, when the blocks lasted for 15 s. This oscillating response, consisting of several peaks, is a potential explanation to our main finding of short stimulation being associated with shorter time to peak. Prolonged odor administration in short blocks of events resulted in an oscillating activity with several peaks of varying amplitude, as opposed to the single, distinct peak, which was the outcome of short stimulation. The presence of several peaks may to certain extent explain why the time to peak was longer when using long blocks as the highest peak was not necessarily the first one for all subjects.

The effects of sampling rate on the temporal properties of the HRF have long been studied, showing that the accuracy of HRF

| Right anterior piriform cortex | Maximal % signal change | Time to peak (s) |
|--------------------------------|-------------------------|-----------------|
| 6-s stimulus                   | TR 0.901 s 0.72         | Effect of stimulus 6-s stimulus TR 0.901 s 8.6 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.49         | ns              | TR 0.901 s 11.5 Effect of TR |
| Left anterior piriform cortex | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.62         | Effect of stimulus 6-s stimulus TR 0.901 s 9.0 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.67         | ns              | TR 0.901 s 10.4 Effect of TR |
| Right posterior piriform cortex | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.78         | Effect of stimulus 6-s stimulus TR 0.901 s 10.5 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.62         | ns              | TR 0.901 s 9.7 Effect of TR |
| Left anterior piriform cortex | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.84         | Effect of stimulus 6-s stimulus TR 0.901 s 7.9 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.84         | ns              | TR 0.901 s 10.6 Effect of TR |
| Right orbitofrontal cortex    | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.36         | Effect of stimulus 6-s stimulus TR 0.901 s 12.0 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.34         | ns              | TR 0.901 s 10.0 Effect of TR |
| Left orbitofrontal cortex     | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.74         | Effect of stimulus 6-s stimulus TR 0.901 s 8.2 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.61         | ns              | TR 0.901 s 14.6 Effect of stimulus |
| Right insula                   | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.81         | Effect of stimulus 6-s stimulus TR 0.901 s 9.6 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.65         | ns              | TR 0.901 s 8.8 Effect of stimulus |
| Left insula                    | Maximal % signal change | Time to peak (s) |
| 6-s stimulus                   | TR 0.901 s 0.69         | Effect of stimulus 6-s stimulus TR 0.901 s 11.8 Effect of stimulus |
| 15-s stimulus                 | TR 0.901 s 0.69         | ns              | TR 0.901 s 9.6 Effect of stimulus |

Mean values are presented. Statistically significant effects (P < 0.05) are highlighted with bold letters.
peak time determination increases with short TR (Miezin et al. 2000; Dilharreguy et al. 2003). Moreover, short TR is associated with better resolution of heartbeat related physiological signal fluctuations and reduced sensitivity to intrascan head motion (Posse et al. 2012; Smith et al. 2013). Therefore, several novel acquisition techniques have been developed, aiming to increase the sampling rate of the

![Table 2. Repeated measures ANOVA, with Bonferroni correction, was employed separately for each brain region of interest in order to estimate the effect of stimulus length and TR on resulting in oscillatory BOLD response](image)

| Brain Region                        | Stimulus Length | TR (s) | Oscillations | P-value |
|-------------------------------------|-----------------|--------|--------------|---------|
| Right anterior piriform cortex      | 6-s             | 0.901 s| 8.09         | P < 0.001|
|                                    | 15-s            | 0.901 s| 13.59        | P < 0.001|
|                                    | 15-s            | 1.34 s | 9.45         | P < 0.001|
| Left anterior piriform cortex       | 6-s             | 0.901 s| 9.45         | P < 0.010 |
|                                    | 15-s            | 0.901 s| 12.73        | P < 0.001|
|                                    | 15-s            | 1.34 s | 9.55         | P < 0.001|
| Right posterior piriform cortex     | 6-s             | 0.901 s| 8.95         | Effect of stimulus |
|                                    | 15-s            | 0.901 s| 13.18        | Effect of TR |
|                                    | 15-s            | 1.34 s | 10.36        | P = 0.002 |
| Left anterior piriform cortex       | 6-s             | 0.901 s| 8.45         | Effect of stimulus |
|                                    | 15-s            | 0.901 s| 14.09        | Effect of TR |
|                                    | 15-s            | 1.34 s | 10.36        | P = 0.002 |
| Right orbitofrontal cortex          | 6-s             | 0.901 s| 9.09         | Effect of stimulus |
|                                    | 15-s            | 0.901 s| 13.06        | Effect of TR |
|                                    | 15-s            | 1.34 s | 10.91        | P < 0.001 |
| Left orbitofrontal cortex           | 6-s             | 0.901 s| 9.91         | Effect of stimulus |
|                                    | 15-s            | 0.901 s| 9.64         | Effect of TR |
|                                    | 15-s            | 1.34 s | 8.46         | P = 0.014 |
| Right insula                        | 6-s             | 0.901 s| 10.18        | Effect of stimulus |
|                                    | 15-s            | 0.901 s| 14.55        | Effect of TR |
|                                    | 15-s            | 1.34 s | 10.36        | P < 0.001 |
| Left insula                         | 6-s             | 0.901 s| 9.95         | Effect of stimulus |
|                                    | 15-s            | 0.901 s| 14.82        | Effect of TR |
|                                    | 15-s            | 1.34 s | 10.05        | P < 0.001 |

For this purpose, an estimate of oscillations was employed (number of values greater than 25% of the maximal signal change). Mean values are presented. Statistically significant effects (P < 0.05) are highlighted with bold letters.

**General Linear Model Analysis**

![Figure 4. Thresholded maps for each combination of repetition time (TR) and stimulation length. The combination of short TR (0.901 s) and short stimulation length (6 s) resulted in visually more extensive activation in olfactory brain areas bilaterally, namely the posterior piriform cortex, the orbitofrontal cortex and the insula. Color bars are given in terms of T-statistic and maps are thresholded at P < 0.001 (uncorrected). Slices were created using Mango (http://ric.uthscsa.edu/mango; Jack L. Lancaster and Michael J. Martinez).](image)
BOLD signal and indicating significant benefits of collecting fMRI data with TR < 1 s (Lin et al. 2006; van der Zwaag et al. 2006; Feinberg et al. 2010; Posse et al. 2012; Witt et al. 2016). In particular, faster TRs achieved with multi-slice EPI sequences (as the sequence used in this study) can capture more information per time unit, allowing more accurate representation of the BOLD response (Chen et al. 2015). In contrast, with longer TR the response is averaged over longer time, which could lead to missing the actual peak and thereby lower signal increase and longer time to peak (Witt et al. 2016). This study confirms that a short TR value (0.901 s) is associated with more pronounced signal increase in all 4 olfactory brain regions, by giving more densely sampled information of the BOLD response. Shorter TRs should, thus, be preferred in olfactory fMRI studies. To the best of our knowledge, no previous studies have evaluated the effects of fast sampling rate on olfactory fMRI.

An inherent limitation of the GLM approach is the employment of the canonical HRF for all brain regions. However, the activation pattern of several brain regions may vary significantly from this a priori specified pattern of signal change, and Figure 3 indicates that this could also be applicable to the 4 olfactory brain regions studied here. This confounding factor can partly explain the variability in olfactory fMRI literature. Several studies have tried to overcome this problem and to efficiently model the HRF; however, this was beyond the scope of this study (Josephs and Henson 1999; Wager and Nichols 2003; Smith et al. 2007; Lindquist et al. 2009). To mediate this problem, we opted for a sequence with short TR, allowing fast sampling rate and, hence, more accurate estimation of the BOLD response.

Respiration is also an important factor that can influence the outcome of an olfactory fMRI study. Respiration-triggered odor delivery results in stronger activation of olfactory brain areas compared with fixed-timing odor delivery (Wang et al. 2014). However, this method demands a more complex experimental design and a subject with

### Table 3. Number of voxels within the posterior piriform cortex activated for the 4 different combinations of stimulus length and TR

| Stimulus Length | TR Value | Right posterior piriform cortex | Left posterior piriform cortex |
|-----------------|----------|---------------------------------|-------------------------------|
| 6-s stimulus    | TR 0.901 s | 123                             | 82                            |
| 15-s stimulus   | TR 0.901 s | 0                               | 54                            |
| 6-s stimulus    | TR 1.34 s  | 37                              | 49                            |
| 15-s stimulus   | TR 1.34 s  | 15                              | 19                            |

The number of voxels was extracted from the GLM analysis at $P < 0.001$ (uncorrected).

![Figure 3](https://academic.oup.com/chemse/article/43/6/389/4992038)

**Figure 5.** Results of the Tensorial Independent Component Analysis (TICA), including the thresholded maps and the temporal mode for each of the tested repetition times (TR). The whole-brain signal from all participants was separated into 15 independent components (IC) with TICA. For both the short TR (A) and the long TR (B), one independent component was associated with functional connectivity in the anterior and the posterior piriform cortex, the anterior insula, and parts of the orbitofrontal cortex bilaterally. The temporal mode of these components (red) coincided with the olfactory stimulation of the tested fMRI task design (blue). The default threshold of $P > 0.5$ was used for testing the alternative hypothesis poststatistically. Color bars are given in terms of T-statistic. Slices were created using Mango (http://ric.uthscsa.edu/mango; Jack L. Lancaster and Michael J. Martinez).
consistent respiration pattern (Wang et al. 2017). Thus, respiration-triggered paradigms can be compromised by poor synchronization between the odor delivery and the subject's inhalation. We, therefore, chose a simple and reproducible experimental design with fixed-timing odor delivery, which would also be easy to implement in less cooperative patients with, for example, neurodegenerative disorders.

Insular activation is often prominent in studies that require a task to be performed during olfactory stimulation (Seubert et al. 2013). The cohort of the present study was asked to confirm the presence of odor, by pressing a specific button. The results from this response tracking showed that the long stimulation was identified at a higher rate, compared with short stimulation. This can mainly be attributed to our experimental design: response tracking was only possible during stimulation, excluding all delayed responses. Future studies should allow a more generous timeslot for response registration, especially when using short odorous stimulation and when performed in patients with bradykinesia.

As with most fMRI studies in this field, our cohort is small and the results should be interpreted cautiously. Reproducibility was of high importance and we, therefore, employed broadly used, open-access software for the analysis of the data, instead of custom-made scripts. In order to ensure that the chosen ROIs correspond with both the anatomical brain regions and previously reported data, ROI localization was based on a previously published function-location meta-analysis of the human olfactory cortex (Seubert et al. 2013).

Robust, reliable, and reproducible fMRI paradigms are of great value in order to unveil the neural processes of human olfaction. The findings of this study support the choice of short TR and short stimulation length in order to achieve maximum signal increase and short time to peak. These 2 parameters should be taken into consideration during the design of future olfactory studies, both in healthy subjects and in patients with impaired olfaction.

Funding

This study was supported by Swedish Parkinson Foundation, Linköping University Hospital Research Fund and by ALF Grants from Region Östergötland.

Acknowledgements

The authors would like to thank Marcelo Perreira Martins for substantial contribution during data acquisition and Lars Valter from Forum Östergötland for statistical guidance.

References

Barresi M, Curileo R, Giacoppo S, Foti Cuzzola V, Celi D, Bramanti P, Marino S. 2012. Evaluation of olfactory dysfunction in neurodegenerative diseases. J Neurol Sci. 323:16–24.

Beckmann CF, Smith SM. 2004. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans Med Imaging. 23:137–152.

Beckmann CF, Smith SM. 2005. Tensorial extensions of independent component analysis for multisubject fMRI analysis. Neuroimage. 25:294–311.

Bensafi M, Iannilli E, Schreiner VA, Poncelet J, Seo HS, Gerber J, Rouby C, Hummel T. 2013. Cross-modal integration of emotions in the chemical senses. Front Hum Neurosci. 7:883.

Chen L, T Vu A, Xu J, Moeller S, Ugarbikl Y, Yacoub E, Feinberg DA. 2015. Evaluation of highly accelerated simultaneous multi-slice EPI for fMRI. Neuroimage. 104:452–459.

Cleland TA, Morse A, Yue EL, Linster C. 2002. Behavioral models of odor similarity. Behav Neurosci. 116:222–231.

Croy I, Schulz M, Blumrich A, Hummel C, Gerber J, Hummel T. 2014. Human olfactory lateralization requires trigeminal activation. Neuroimage. 98:289–295.

Dilharreguy B, Jones RA, Moonen CT. 2003. Influence of fMRI data sampling on the temporal characterization of the hemodynamic response. Neuroimage. 19:1820–1828.

Feinberg DA, Moeller S, Smith SM, Auerbach E, Ramanna S, Gunther M, Glasser MF, Miller KL, Ugarbikl Y, Yacoub E. 2010. Multiplexed echo planar imaging for sub-second whole brain fMRI and fast diffusion imaging. PLoS One. 5:e15710.

Firestein S. 2001. How the olfactory system makes sense of scents. Nature. 413:211–218.

Gottfried JA. 2010. Central mechanisms of odor object perception. Nat Rev Neurosci. 11:628–641.

Gottfried JA, Winston JS, Dolan RJ. 2006. Dissociable codes of odor quality and odorant structure in human piriform cortex. Neuron. 49:467–479.

Hawkes CH, Shephard BC, Daniel SE. 1997. Olfactory dysfunction in Parkinson’s disease. J Neurol Neurosurg Psychiatry. 62:436–446.

Howard JD, Plailly J, Grueschow M, Haynes JD, Gottfried JA. 2009. Olor quality coding and categorization in human posterior piriform cortex. Nat Neurosci. 12:932–938.

Hummel T, Flessbach K, Abel M, Okula T, Reden J, Reichmann H, Wallner U, Haelner A. 2010. Olfactory fMRI in patients with Parkinson’s disease. Front Integr Neurosci. 4:125.

Hyvarinen A. 1999. Fast and robust fixed-point algorithms for independent component analysis. IEEE Trans Neural Netw. 10:626–634.

Jones-Getman M, Zatorre RJ. 1993. Olor recognition memory in humans: role of right temporal and orbitofrontal regions. Brain Cogn. 22:182–198.

Josephs O, Henson RN. 1999. Event-related functional magnetic resonance imaging: modelling, inference and optimization. Philos Trans R Soc Lond B Biol Sci. 354:1215–1228.

Kadishna M, Wilson DA. 2006. Separate encoding of identity and similarity of complex familiar odors in piriform cortex. Proc Natl Acad Sci USA. 103:15206–15211.

Karananayaka P, Eslinger PJ, Wanj JL, Wettakamp CW, Molitoris S, Gates KM, Molenaar PC, Yang QX. 2014. Networks involved in olfaction and their dynamics using independent component analysis and unified structural equation modeling. Hum Brain Mapp. 35:2055–2072.

Li W, Howard JD, Parrish TB, Gottfried JA. 2008. Aversive learning enhances perceptual and cortical discrimination of indiscernible odor cues. Science. 319:1842–1845.

Li W, Luxenberg E, Parrish T, Gottfried JA. 2006. Learning to smell the roses: experience-dependent neural plasticity in human piriform and orbitofrontal cortices. Neuron. 52:1097–1108.

Lin FH, Wald LL, Ablfors SP, Hämäläinen MS, Kwong KK, Bolliveau JW. 2006. Dynamic magnetic resonance inverse imaging of human brain function. Magn Reson Med. 56:787–802.

Lindquist MA, Meng Loh J, Atlas LY, Wager TD. 2009. Modeling the hemodynamic response function in fMRI: efficiency, bias and mix-modeling. Neuroimage. 45:S187–S198.

Linstead C, Johnson BA, Yue E, Morse A, Xu Z, Hingco EE, Choi Y, Choi M, Messia A, Leon M. 2001. Perceptual correlates of neural representations evoked by odorant enantiomers. J Neurosci. 21:9837–9843.

Miezin FM, Maccotta L, Ollinger JM, Petersen SE, Buckner RL. 2000. Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. Neuroimage. 11:735–759.

Moessnang C, Frank G, Bodghain U, Winkler J, Greenlee MW, Klucken J. 2011. Altered activation patterns within the olfactory network in Parkinson’s disease. Cereb Cortex. 21:1246–1253.

Pellegrino R, Drechsler E, Hummel C, Warr J, Hummel T. 2017. Bimodal odor processing with a trigeminal component at sub- and suprathreshold levels. Neuroscience. 363:4–19.

Pellegrino R, Hähner A, Bojanowski V, Hummel C, Gerber J, Hummel T. 2016. Olfactory function in patients with hyposmia compared to healthy subjects - An fMRI study. Rhinology. 54:374–381.
van der Zwaag W, Francis S, Bowtell R. 2006. Improved echo volumar imaging.

Sobel N, Prabhakaran V, Zhao Z, Desmond JE, Glover GH, Sullivan EV, Smith SM, Johansen-Berg H, Jenkinson M, Rueckert D, Nichols TE, Miller KL, Smith SM, Beckmann CF, Andersson J, Auerbach EJ, Bijsterbosch J, Douaud Smeets MA, Veldhuizen MG, Galle S, Gouweloos J, de Haan AM, Vernooij J, Small DM, Zald DH, Jones-Gotman M, Zatorre RJ, Pardo JV, Frey S, Petrides Seubert J, Kellermann T, Loughead J, Boers F, Brensinger C, Schneider F, Habel Seubert J, Freiherr J, Djordjevic J, Lundström JN. 2013. Statistical localization of human olfactory cortex. Neuroimage. 66:333–342.

Seubert J, Kellermann T, Loughead J, Boers F, Brensinger C, Schneider F, Habel U. 2010. Processing of disgusted faces is facilitated by odor primes: a functional MRI study. Neuroimage. 53:746–756.

Small DM, Zald DH, Jones-Gotman M, Zatorre RJ, Pardo JV, Frey S, Petrides M. 1999. Human cortical gustatory areas: a review of functional neuroimaging data. Neureport. 10:7–14.

Smeets MA, Veldhuizen MG, Galle S, Gouweloos J, de Haan AM, Vernooij J, Visscher F, Kroese JH. 2009. Sense of smell disorder and health-related quality of life. Rehabil Psychol. 54:404–412.

Smith SM, Beckmann CF, Andersson J, Auerbach EJ, Bijsterbosch J, Douaud G, Duff F, Fjell AM, Griffanti L, Harms MP, et al.; WU-Minn HCP Consortium. 2013. Resting-state fMRI in the Human Connectome Project. Neuroimage. 80:144–168.

Smith SM, Johansen-Berg H, Jenkinson M, Rueckert D, Nichols TE, Miller KL, Robson MD, Jones DK, Klein JC, Bartsch AJ, et al. 2007. Acquisition and voxelwise analysis of multi-subject diffusion data with tract-based spatial statistics. Nat Protoc. 2:499–503.

Sobel N, Prabhakaran V, Zhao Z, Desmond JE, Glover GH, Sullivan EV, Gabrieli JD. 2000. Time course of odorant-induced activation in the human primary olfactory cortex. J Neurophysiol. 83:537–551.

van der Zwaag W, Francis S, Bowtell R. 2006. Improved echo volumar imaging (EVI) for functional MRI. Magn Reson Med. 56:1320–1327.