The neural cascade of olfactory processing: A combined fMRI-EEG study

Yuri Masaoka¹, ², Ian H. Harding², Nobuyoshi Koiwa³, Masaki Yoshida⁴, Ben J. Harrison², Valentina Lorenzetti², ⁵, Masahiro Ida⁶, Masahiko Izumizaki¹, Christos Pantelis², Ikuo Homma⁷

1. Department of Physiology, Showa University School of Medicine, Tokyo, Japan
2. Melbourne Neuropsychiatry Centre, Department of Psychiatry, University of Melbourne and Melbourne Health, Melbourne, Victoria, Australia
3. Human Arts and Sciences Research Center, University of Human Arts and Sciences, Saitama, Japan
4. Department of Ophthalmology, Jikei Medical University, Tokyo, Japan
5. Monash Clinical & Imaging Neuroscience, School of Psychological Sciences and Monash Biomedical Imaging, Monash University, Melbourne, Victoria, Australia
6. Department of Radiology, Stroke Center, Ebara Tokyo Hospital, Tokyo, Japan
7. Tokyo Ariake University of Medical and Health Sciences, Tokyo, Japan

Address correspondence to Yuri Masaoka,
Department of Physiology, Showa University School of Medicine,
1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
Telephone: +81 3 3784 8113
Fax: +81 3 3784 0200
E-mail: faustus@med.showa-u.ac.jp

Conflict of Interest
The authors declare that they have no conflict of interests.
1. Introduction

The neurophysiological mechanisms underlying human olfaction are unique among the sensory modalities. Firstly, olfaction is the only sense that is directly dependent on a basic physiological process, relying on respiratory activity for the delivery of odorants to chemoreceptors in the nasal cavity (Sobel et al., 1998). The link between respiration and olfaction is evidenced explicitly by observed synchronization between respiratory cycles and neural activation of the olfactory circuit during odor perception in both animals (Curry and Uchida, 2010; Shusterman et al., 2011) and humans (Sobel et al, 1998, Masaoka et al., 2005, 2012). Secondly, primary olfactory areas in the brain exist outside of the cerebral cortex, and the neural pathways that subserve olfactory processes bypass the thalamus and ascend directly to olfactory-limbic areas (Yashurum and Sobel, 2010).

Upon inspiration, odorant molecules bind to olfactory receptors in the nasal mucosa, stimulating activity in the olfactory bulbs located on the ventral surface of the orbitofrontal cortex (OFC)(Tanabe et al., 1975). In turn, the olfactory bulbs innervate the piriform cortex (PIR), the anterior cortical nucleus of the amygdala (AMG), and the rostral entorhinal cortex (ENT) via the olfactory peduncle (Johnson et al., 2000). The ENT provides a subsequent gateway to the hippocampal formation (HI) (Ramus & Eichenbaum, 2000). This “neural cascade” is hypothesized to directly link olfactory perception with emotional salience processing in the AMG and memory systems in the ENT and HI (Masaoka et al., 2005, 2012). Outputs from ENT and AMG subsequently converge on the OFC where higher-order processing takes place, including smell identification and emotional labeling (Rolls, 2001).

Taking advantage of the time-locked nature of inspiration and olfaction, we have previously investigated the in vivo spatio-temporal profile of olfactory processing in the human brain (Masaoka et al., 2005, 2012). By simultaneously recording electroencephalography (EEG) and respiration during odor perception, we have identified olfactory potentials associated with the onset of inspiration. These inspiration-related olfactory potentials are referred to as inspiration phase-locked alpha band oscillations (I-alpha). On the basis of EEG dipole modeling (EEG/DT), the neural substrates of early I-alpha signals were isolated to primary olfactory areas, including the ENT, AMG, HI, while subsequent activity was localized to secondary olfactory areas within the OFC (Masaoka et al., 2005, 2012). These findings supported the neural cascade model of olfactory processing leading from primary olfactory regions to core limbic structures, and then subsequently, prefrontal cortex.

The principal strength of I-alpha EEG recordings lies in their millisecond-level temporal resolution (Masaoka et al., 2005, 2012). However, while dipole modeling (EEG/DT) approaches have been developed to anatomically localize the source generators of these brain signals with high precision (Fuchs et al., 2004; Homma et al., 2001), the validity of source localization procedures
remains controversial. Dipole modeling of I-alpha signals may therefore be complemented by the high spatial resolution afforded by functional magnetic resonance imaging (fMRI). Studies using fMRI to investigate olfaction have previously reported odor-induced activations in both primary and secondary olfactory areas (Gottfried et al., 2002; Rolls et al., 2003; Royet et al., 2003; Sobel et al., 1998). Yet, these investigations remain insensitive to the temporal profile of the underlying olfactory information processing as it moves from primary limbic areas to higher-order association cortices.

In this study, we therefore combined fMRI and EEG in order to re-examine the neural cascade model of human olfactory processing higher spatial and temporal resolution. To achieve this goal, we introduce a novel experimental apparatus that enables delivery of odorants to subjects during the inspiration-phase of respiration whilst undergoing fMRI. These same subjects also completed an EEG experiment based on our previously validated method (Masaoka et al., 2005, 2012). The first aim of the present study was to examine whether the inspiration-locked olfactory stimulator resulted in comparable neural activations to those reported in previous fMRI studies (Gottfried et al., 2002; Rolls et al., 2003; Royet et al., 2003; Sobel et al., 1998). Our second aim was to compare the results obtained by fMRI and EEG to determine whether the olfaction-related neural sources identified by EEG/DT mapped onto neural activations identified with fMRI.

2. Material and Methods

2.1. Subjects

Eight right-handed healthy volunteers (mean age: 28.5 ± 8.3; 4 male, 4 female) participated in this study. The olfactory acuity of each participant was within the normal range, as evaluated using the T&T olfaction test (Takasuna Co., Ltd, Tokyo, Japan; recognition: mean 0.35 ± 0.3; detection: mean -0.7 ± 0.3; (Masaoka et al., 2013). The study was approved by the ethics committee of Showa University School of Medicine and all participants provided informed consent.

2.2. Stimuli and subjective valence of the two odors

All subjects completed separate EEG/DT and fMRI sessions within a 1-week period with the order of experiments, counter-balanced across subjects. Within each session, participants were exposed to relatively pleasant (rose) odor and the least pleasant odor as opposed to the most pleasant odor of rose (chamomile) odors (indicated as an unpleasant odor in the text). These two stimuli were selected on the basis of subjective valence scores obtained from all participants prior to their first session with regard to the following distinct smells: chamomile, orange, lemon, rose and peach.
Subjective scores, rated on a scale from 1 (most unpleasant) to 100 (most pleasant) were as follows:
chamomile, 13.1 ± 2.2; orange, 41.5 ± 3.6; peach, 45.1 ± 4.3; lemon, 46.5 ± 3.7; and rose, 80.3 ± 2.5.
The subjective valence of the two odors presented during fMRI and EEG recording were re-assessed
following each session to confirm consistency of participants’ responses.

2.3. fMRI Experimental Design

The fMRI study was divided into 2 sessions/runs: (i) periods of pleasant odor (rose) interleaved with
unscented air, and (ii) periods of unpleasant odor (chamomile) interleaved with unscented air. Each
session comprised 5 unscented and 5 scented blocks, with a duration of 30s each. Subjects were
instructed to breathe normally throughout the experiment. This design is comparable to the EEG
session (below) and our previous work (Masaoka et al., 2005, 2012).

Odorants were administered using a custom-designed, MRI-compatible apparatus (Figure 1,
Arco System, Chiba, Japan). Subjects wore a nose mask (ComfortGel Blue Nasal Mask 1070038,
medium size, Philops Respironics, Pennsylvania, USA) fitted with a one-way valve apparatus to
ensure inspiration of air from the olfactory stimulator, and expiration out of the system. Odor stimuli
were controlled by a series of balloon valves in the stimulator that were controlled from outside the
scanner room (Figure 1). Stimulus presentation was manipulated by variously shrinking and
swelling these balloon valves, each connected to a pleasant odor cartridge, an unpleasant odor
cartridge, or the open environment. The odor cartridges consist of a disposable plastic round cassette
containing a liquid odorant that was absorbed into a mesh fabric at the mouth of the valve. During
odor conditions, inspired air is filtered through the mesh, delivering the stimulant to the nasal cavity.
Expired air is then directed out through the expiration valve. Inflation and deflation of the balloon
valves was controlled using in-house software via connection and control boxes. The control box
consisted of a gas pressure regulator, a valve driver, and a piezoelectric pressure transducer (Figure
1, bottom). The gas pressure regulator connected to an O2 gas cylinder outside the connection box
and three solenoid valves inside the control box. Each solenoid valve linked to one of the balloon
valves in the stimulator. Digital input/output (DIO) signals from the PC interfaced with the valve
driver, via the connection box. The valve driver subsequently operated the appropriate solenoid
valves, delivering or restricting pressurized gas to the desired balloon valves. The piezoelectric
pressure transducer was connected to the respiratory pressure sensor in the nose mask through a
urethane tube. The pressure signal measured inspiratory and expiratory flow, which was converted
from an analogue to a digital signal within the control box and stored in LabChart through PowerLab
(ML846, AD Instruments, Aichi, Japan).
2.4. fMRI data collection and analysis

MRI was performed in at Ebara Hospital (Tokyo, Japan) using a 3 Tesla MAGNETOM A Trio Tim scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. fMRI time-series (gradient echo, EPI) consisted of 120 whole-brain volumes/session comprising of 39 axial slices (Matrix size: 80×80; TR: 2.5 s; TE: 23 ms; FOV: 22 cm, thickness: 2.5 mm; Flip angle: 90º; voxel size: 2.75×2.75×2.75 mm). An anatomical MRI was additionally acquired using a 3D-magnetization-prepared rapid acquisition by gradient-echo T1-weighted sagittal sections.

Statistical analysis of fMRI data was performed using statistical parametric mapping (SPM8) software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 8 (Math Works Inc., Natrick, MA, USA). Image pre-processing included rigid-body motion correction (realignment and unwarping), co-registration of functional and structural images, normalization to standard space, and spatial smoothing using a 8mm FWHM Gaussian filter (Ashburner and Friston, 2003). For each session in each subject, fMRI time-series were modeled using a single regressor encoding the blocked on-off periods of the experiment, convolved with a canonical hemodynamic response function. A standard high-pass filter (< 1/128 Hz) removed low-frequency (thermal and physiological noise) fluctuations from the time-series. Group-level inference was undertaken using a fixed-effects framework befitting one-to-one mapping of fMRI to EEG results in the same group of participants. This approach takes advantage of the increased power inherent to our within-subject repeated-measures design. Furthermore, olfactory perception represents a set of basic neurophysiological processes that are shared (i.e., fixed) across the normal population. Separate analyses were performed for pleasant and unpleasant odors.

Based on prior fMRI studies of olfaction (Gottfried et al., 2002; Rolls et al., 2003; Royet et al., 2003; Sobel et al., 1998) and our own previous EEG/DT work (Masaoka et al., 2005, 2012), inference was restricted a priori to brain regions associated with olfactory processing. A composite brain mask was defined anatomically using the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002), and included bilateral olfactory areas, parahippocampus, AMG, HI, insula (Insula), frontal medial (Frontal_Med), middle (Frontal_Mid), inferior (Front_Inf) and superior (Front_Sup) orbitofrontal (Orb), and superior/middle temporal pole (Temporal_Pole_Sup). Inference was undertaken using one-sample T-tests within this mask and corrected for multiple comparisons (family wise error; FWE-corrected p < 0.05) based on minimum cluster-extent thresholds estimated using the AlphaSim permutation procedure (REST toolbox; http://pub.restfmri.net). Simulations were run with a voxel-level threshold of p<0.01, uncorrected, across 1000 permutations, resulting in a minimum required cluster-threshold of 122 voxels. In addition, we performed a further whole-brain analysis to examine potential effects outside of the region-of-interest mask. For this analysis, we applied a stringent voxel-wise correction of P < 0.05.
FWE across the whole brain volume.

2.5. EEG Experimental Design

The EEG session was analogous to the fMRI session (Masaoka et al., 2005, 2012). Two runs were performed, one for each of pleasant and unpleasant odors, in which 30s periods of odor presentation were interleaved with 30s blocks of normal, unscented air (Masaoka et al., 2005, 2012). Each Odor was presented 30 s on and 30 s off schedule to minimize adaptation to the olfactory stimuli (Ekman et al., 1967). Eight odor blocks were presented in each run.

The nose mask, one-way valve apparatus, and balloon-value olfactory stimulator used were the same model as described for the fMRI session, above. The mask was additionally connected to a transducer leading to a respiratory monitor (CPX, Arco System, Chiba, Japan) for measurement of breath-by-breath ventilation ($V_{E}$), tidal volume ($V_T$) and respiratory frequency ($f_R$). End-tidal CO$_2$ ($F_{ET,CO_2}$) and O$_2$ consumption ($V_{O_2}$) were also measured.

2.6. EEG data collection and EEG/DT analysis

EEG/DT was performed in the Department of Physiology, Showa University School of Medicine. Full details of the EEG/DT procedure are described elsewhere (Homma et al., 2001; Masaoka et al., 2005, 2012). In brief, 19 electrodes were attached to participants’ scalp according to the international 10–20 system, with the reference electrode on the right earlobe. An EEG and electro-oculogram were recorded and stored in a digital EEG analyzer (DAE-2100, Nihon Kohden, Tokyo, Japan). The EEG was sampled at 100 Hz through a 0.016–30 Hz bandpass filter. Impedances were kept below 10 kΩ. Signals of the onset of odor stimulation and respiratory flow were obtained simultaneously with EEG and oculogram recordings and stored in the EEG analyser. The onset of inspiration was used as a trigger for averaging potentials. All sniffing activity observed as quick inhalations (within 1.6 s for one breath)(Laing, 1983) was excluded from the averaging. Eye blinks or artefactual activity exceeding ±50 μV was also excluded. Potentials were averaged during odor presentation by the EEG Focus (Version 2.1, Nihon Koden, Tokyo, Japan) and the mean value of EEG during 100 ms pre-trigger point was subtracted from original EEG. The mean number of event averages (i.e., respiratory cycles) across individuals was 62.7 ± 3 for pleasant odor and 70.4 ± 2.4 for unpleasant odor. The averaged potentials were transferred to the EEG/DT software (Brain Space Navigator; BS-NAVI, Brain Research and Development, Tokyo, Japan).

Full details of the source localization algorithm are available elsewhere (Homma et al., 2001; Masaoka et al., 2005, 2012). In brief, the actual potential field distribution recorded from the 19 scalp electrodes ($u_{meas}$) was compared with the calculated field distribution ($u_{cal}$) for a properly
chosen equivalent current dipole (in the one-dipole estimation) or two equivalent dipoles (in the two-dipole estimation). The inverse solution (He et al., 1987) was used to determine the dipole location and orientation that best fitted the recorded data. The locations and vector moments of one or two current dipoles were iteratively changed within the head model until the minimal squared difference between $u_{\text{meas}}$ and $u_{\text{cal}}$ was obtained by the simplex method (Kowalik and Osborne, 1968). The degree of source concentration can be calculated in terms of goodness of fit (GOF). GOF of 100% is an ideal case; however, in practice it is usually less than 100% due to noise, electrode misalignment and non-dipole components of the electric sources. GOF greater than 99.5% in the absence of background EEG activity and greater than 98% in the presence of background EEG activity was observed in a prior study (Musha and Homma, 1990). In the present study, one dipole analysis was performed and GOF > 98% was adopted to indicate a concentrated source (Homma et al., 2001; Masaoka et al., 2005, 2012).

The dipoles localized for EEG/DT were superimposed on fMRI results using MRIcro software (http://www.sph.sc.edu/comd/rorden/mricro.html).

3. Results

3.1. Emotional scores

Comparisons of emotional scores related to each odor before and after the data collection sessions were analyzed with Wilcoxon’s signed rank tests. Emotional scores for each odor measured after each session replicated the pre-test measures (EEG/DT session: pleasant odor, 82.5 ± 9.2; unpleasant odor, 12.6 ± 5.6; fMRI session: pleasant score, 80.6 ± 7.7; unpleasant score, 12.5 ± 6.5). There was additionally no difference in pleasant and unpleasant scores between EEG/DT and fMRI sessions (p>0.05).

3.2. Respiratory measurements

Respiratory frequency ($f_R$) during the EEG/DT and fMRI sessions is shown in Figure 2. There was a significant effect of odor on $f_R$ ($F_{4,35}$=12.1, p<0.001). Post-hoc assessments indicated that pleasant odor stimulation induced slower respiration than normal air in both sessions (p<0.001) and relative to unpleasant odor in the EEG session (p < 0.001). There was no difference in $f_R$ between the EEG/DT and fMRI sessions for either odor (p>1). These findings indicate a comparable odor-related response in both sessions.

For the EEG/DT session, we additionally measured $V_E$, $V_T$, $\dot{V}O_2$ and $F_{ET,CO_2}$. There were no changes in $\dot{V}O_2$ during odor stimulation (Normal air, 309 ± 50 ml; pleasant odor, 310 ± 46 ml; unpleasant
odor, 318 ± 48 ml: F_{2,23}= 0.09, p= 0.91). This result suggests that the observed respiratory changes were not caused by differences in metabolic demand, but rather by the influence of olfactory stimuli.

There was change in V\textsubscript{T} (F_{2,23}=12.2, P < 0.001) during odor stimulation. V\textsubscript{T} during pleasant odor stimuli increased compared with normal air (normal air, 553 ± 62 ml, pleasant odor, 721 ± 73: p < 0.001) and unpleasant odor (unpleasant odor, 607 ± 71 ml; p < 0.05); no significant difference in V\textsubscript{T} was evident between normal air and unpleasant odor (p > 0.4). This change in V\textsubscript{T} shows the slowed breathing during pleasant odor stimulation was accompanied by deeper breaths.

There were no changes in either V\textsubscript{E} (normal air, 7.8 ± 0.8 l; pleasant odor, 8.2 ± 0.7 l; unpleasant odor, 8.3 ± 0.9 l: F_{2,23}=0.9, p=0.42) or \textit{F}_{\text{i} \text{ETCO}_2} (normal air, 4.8 ± 0.5 %; pleasant odor, 5.03 ± 0.53 %; the least pleasant odor, 5.03 ± 0.54: F_{2,23}=0.4, p=0.73) throughout the session. During pleasant odor stimuli, it was interesting to observe the combination of f\textit{R} decrease and V\textsubscript{T} increase hence V\textsubscript{E} remained unchanged. This adjustment of constant V\textsubscript{E} resulted in unchanged \textit{F}_{\text{i} \text{ETCO}_2}.

### 3.3. fMRI results superimposed on Source generators estimated by EEG/DT

Table 1 shows fMRI results during pleasant odor stimuli. Significant activations were observed in the left middle and superior OFC at corrected significance thresholds corresponding to the composite region-of-interest mask (p\textsubscript{FWE} < 0.05); Figure 3. These regions correspond to secondary olfactory areas. Our supplementary whole-brain analysis (p < 0.05 FWE) identified a further significant activation of the left middle temporal gyrus (Z score = 5.63; x, y, z = -60, -38, -12; 44 voxels) associated with pleasant odor stimulation.

Table 2 shows dipole localization estimated by EEG/DT during pleasant odor stimulation. EEG/DT was estimated from the I-alpha (Figure 3 top, left), and used to identify time-to-time changes in source generators beginning from the onset of inspiration. Dipoles first appeared in primary olfactory regions, including the left parahippocampal gyrus encompassing regions of the PIR and ENT (48-49 ms post-inspiration), the left superior temporal pole (50-56 ms), and the left AMG (60 ms). After these activations, dipoles appeared in secondary olfactory regions, including the inferior frontal OFC (148 ms-155 ms), the left superior OFC (156 ms), and the left gyrus rectus (157 ms). Higher-order association cortices were subsequently engaged, including dipoles detected in the left insula (250 ms), left inferior frontal OFC (255 ms) and left middle frontal OFC (300-310 ms).

Overlap between the EEG/DT and fMRI results was found within secondary olfactory and association cortices engaged from approximately 150 ms to 310 ms (indicated by asterisks in Table 2; indicated by yellow crosses in Figure 3).

Conversely, unpleasant odor was associated with dipoles converging in the right AMG from 50 ms to 80 ms (MNI coordinates, 17, -2, -12); however, the GOF of the model was less than 90%
(threshold = 98%), did not reach significant level. fMRI results showed no significant activations at corrected statistical thresholds (uncorrected p < 0.05: right AMG, MNI coordinates, 22, -8, -12).

4. Discussion

Using a novel method of odorant delivery, this study identified a partially convergent pattern of fMRI activation and EEG source generators underlying olfactory processing in the human brain. This convergence centered on association regions of the OFC that were activated from approximately 150ms to 300ms post-stimulus onset, highlighting the sensitivity of both techniques to temporally extended cognitive processes. The ability of EEG/DT to additionally localize antecedent neural activity in primary olfactory regions, beginning approximately 50ms after stimulus delivery, points to the sensitivity of EEG dipole modeling to transient activity in focal regions of the olfactory system.

Olfaction-related activations found in the OFC are consistent with previous fMRI research (Gottfried et al., 2002; Rolls et al., 2003; Sobel et al., 1998). However, primary olfactory areas including the PIR, ENT, and AMG were not significantly activated, contrary to the EEG/DT analysis. A potential reconciliation accounting for the lack of primary olfactory fMRI activations may lie in the rapid habituation of these regions to olfactory stimuli (Sobel et al., 2000). Repeated presentation of the same odorant may lead to decreased neural responses in the PIR, ENT, and AMG, and in turn, a decreased BOLD response. Although the primary olfactory areas habituate very quickly, downstream secondary areas of OFC continue to respond to recurrent odor presentation (Sobel et al., 2000; Poellinger et al., 2001). This characteristic profile of olfactory habituation has been suggested to play a role in ensuring sensitivity to new odorant stimuli (Masaoka and Homma, 2009). In addition, activity in these secondary regions is more temporally extended, with the onset of I-alpha signals throughout the OFC ranging from 148ms up to 310ms post-inspiration. The distinct temporal profiles governing primary versus secondary olfactory processing may therefore explain why the detection of robust fMRI activations was restricted to the OFC in this study.

On the other hand, EEG/DT was able to detect source generators in the PIR, including regions of the parahippocampus and AMG, despite comparable habituation of the olfaction-related electrophysiological responses (Masaoka et al, 2005, 2012). The differential sensitivity of the two imaging modalities to primary olfactory activity may be a function of methodological differences. While fMRI activations were statistically modeled as the average BOLD response across a block of time, EEG/DT was estimated from the averaged potentials measured from the point of inspiration onset. This distinction allows EEG/DT models to identify the more transient micro-volt changes within the PIR, despite the potential for habituation across repeated stimulations. It is relevant to note that fMRI activations were also observed in the AMG, although below the magnitude required
for robust and definitive statistical inference. However, taken together, this pattern of results suggests that fMRI studies of primary olfactory function may benefit from event-related studies of olfactory processing that are also time-locked to inspiration activity.

Dipoles estimated using EEG/DT additionally converged in the temporal pole very shortly after the onset of inspiration (~50ms). The temporal pole comprises para-limbic areas with an olfactory allocortical focus, and is thought to play a role in behaviors that require integration between extrapersonal stimuli and the internal milieu (Mesulam and Mufson, 1982). Anatomically, the PIR project directly to the dorsomedial agranular sector of the temporal pole, which also receives inputs from AMG and OFC (Morán et al., 1987). Temporal pole has been suggested to play role for object naming and object recognition (Tsapkini et al., 2011), as well as in the identification and naming of olfactory stimuli (Olofsson et al., 2013). In support, our own previous work demonstrated the localization of dipoles in the temporal pole when odors were presented at a recognition threshold (subjects were able to identify/name the odor), but not at a detection threshold (subjects were able to detect the odor but not identified/naming the odor) (Masaoka et al, 2005). Therefore, while the role of the temporal pole in olfaction remains indistinct, this region might be involved in the early stages of odor identification.

Although activation of middle and superior OFC has been consistently observed in fMRI studies of olfaction (Gottfried et al., 2002; Rolls et al., 2003; Royet et al., 2003; Sobel et al., 1998), questions remain about the laterality of findings observed in these studies. In earlier work, odor-induced pleasant emotions were observed to activate the right OFC (Gottfried et al., 2002), while unpleasant odor stimulation activated the left OFC (Rolls et al. 2003). By comparison, Royet et al. (2003) reported a left-dominant OFC response for pleasant odor stimulation and proposed a role for the left hemisphere in the conscious appraisal of the emotional quality of odors. Our previous EEG/DT study on odor-induced autobiographical memory implicated the right AMG, HI and OFC in odor familiarity and associated emotional arousal (Masaoka et al., 2012). We proposed that right lateralized olfactory processing may represent increased emotional arousal associated with spatial olfactory memory while right hemispheric activations may represent semantic olfactory memory. In the current study, we identified robust activation of the left posterior middle temporal gyrus in our supplementary analysis, which has been previously implicated in semantic based odor identification (Jones-Gotman et al., 1997). Taken together, one hypothesis is that the left dominant neural response observed here may represent processes aligned with the conscious identification of odors as opposed to high levels of emotional arousal induced by pleasant odor stimulation.

Globally, the results presented here provide an overall replication of our previous EEG/DT findings in response to pleasant odor stimuli (Masaoka et al, 2005, 2012). However, the response to unpleasant odor was not comparably robust in either the fMRI or EEG measures. Moreover, characteristic changes in breathing patterns that are typical in emotionally-valenced olfactory
perception (e.g., deeper, slower breathing in response to pleasant odors; shallow, rapid breathing in
response to unpleasant odors; Masaoka et al, 2005) were observed only for pleasant stimuli.
Therefore, the chamomile odor used as unpleasant odor likely did not elicit a strong olfactory or
emotional response in this study. Although we presented the least pleasant odor from among five
possibilities, this chamomile scent may not elicit the level of unpleasantness as odors that were used
in previous work, as reflected by less severe emotion scores (Masaoka et al, 2005). A less offensive
odor was chosen here due to the use of a hospital (as opposed to dedicated research) setting,
necessitating prevention of lingering unpleasant odors following the experimental procedure.
Notably, however, subjects did sense the chamomile odor during both experimental sessions. As
such, the areas that were responsive to odorant delivery might be related not only to olfactory
perception, but also to odor-induced emotional processing. However, it is quite difficult to
distinguish the neural areas activated by mere olfactory perception as compared to odor-induced
emotional responses due to the largely overlapping anatomy responsible for these functions and the
fact that emotions themselves alter breathing patterns (Boiten et al., 1994; Carnevali et al., 2013).
The isolation of brain activation differences in response to olfactory perception and emotional
processing, while controlling for respiratory changes, therefore represents a worthwhile avenue for
future study.

4.1. Limitations and Future Directions

In this study EEG/DT source generators were isolated based on average potentials time-locked to
inspiration onsets. Conversely, a block design was used for fMRI analysis. As mentioned above, the
incorporation of an event-related fMRI design should provide a more sensitive measure of olfaction-
related activations. Such a design was not feasible in this experiment due to the inexact measurement
of respiratory cycles during fMRI, which relied upon a rudimentary pressure sensor. Future work
should focus on providing more specific measures of inspiration onset in the MR environment.
Greater reliability may also be provided by simultaneous measurement of EEG and fMRI (e.g. Bak et
al., 2011). In addition, given that respiratory fluctuations are a known contributor of physiological
noise to fMRI-BOLD signal measurements (e.g., Birn et al. 2009), future olfaction related studies
should seek to better understand and control for their specific influence.
In conclusion, this study confirmed that the olfactory stimulator for fMRI resulted in comparable
neural activation with previous fMRI studies and our EEG/DT results. EEG/DT was found to be
more sensitive to transient activity in discrete neural regions that form the primary olfactory cortex.
However, activations of the secondary olfactory areas within the OFC, providing for perception,
identification, and emotional labeling of odor (Gottfried et al., 2002; Rolls et al., 2001, 2003; Royet
et al., 2003; Sobel et al., 1998), were consistent across both modalities. These results provide a
partial validation of current models of olfaction and EEG/DT source modeling techniques, and inform a variety of novel future directions in the investigation of human olfaction.

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References


Figure legends

Figure 1
Custom-designed MRI-compatible olfactory stimulator. Inside the scanner, a nose mask worn by the participant is connected to a one-way valve apparatus: three valves for inspiration (red arrows; two valves with odor cartridges and one valve for normal air) and one valve for expiration (blue arrow). Airflow is regulated by deflating or inflating a balloon within each valve using compressed O₂ sent from the control room via urethane tubes (pink lines). For example, by deflating the balloon within the valve connecting to an odor cartridge while the other two balloons are inflated, the odor is delivered to the participant upon inspiration. Respiratory rate is measured using a pressure sensor inside the nose mask and relayed to the control room. The flow of O₂ gas is regulated by digital signals that operate a set of solenoid valves within the control box.

Figure 2
Respiratory frequency (fR) during pleasant and unpleasant odor stimuli in EEG/DT and fMRI sessions. Error bars represent standard deviation of the mean. Significant (p < 0.05) differences relative to the normal air condition in each session are indicated using asterisks.

Figure 3
Grand averaged potentials triggered by inspiration onset (up to 400 ms post-stimulus onset) referred as I-alpha during pleasant odor stimuli (top left). RMS, Root Mean Square; Topographical map at 60 ms (left), 150 ms (middle) and 310 ms (right). Lt, left; Rt, right. Dipoles were converged during the times Dipole locations estimated by EEG/DT (green dots; Table 3) and fMRI results (red; Table 2) related to pleasant odor stimulation are overlaid onto representative sagittal (top right), axial (middle; slice numbers correspond to Table 3), and coronal (bottom) brain slices. Overlap between the EEG/DT and fMRI results was indicated by yellow crosses. A, anterior; P, posterior.
Abstract

Olfaction is dependent on respiration for the delivery of odorants to the nasal cavity. Taking advantage of the time-locked nature of inspiration and olfactory processing, electroencephalogram dipole modeling (EEG/DT) has previously been used to identify a cascade of inspiration-triggered neural activity moving from primary limbic olfactory regions to frontal cortical areas during odor perception. In this study, we leverage the spatial resolution of functional magnetic resonance imaging (fMRI) alongside the temporal resolution of EEG to replicate and extend these findings. Brain activation identified by both modalities converged within association regions of the orbitofrontal cortex that were activated from approximately 150ms to 300ms after inspiration onset. EEG/DT was additionally sensitive to more transient activity in primary olfactory regions, including the parahippocampal gyrus and amygdala, occurring approximately 50ms post-inspiration. These results provide a partial validation of the spatial profile of the olfactory cascade identified by EEG source modeling, and inform novel future directions in the investigation of human olfaction.

Keywords
Inspiration, olfaction, piriform, amygdala, electroencephalogram, fMRI
Highlights

- Temporal and spatial neural correlates of olfaction were examined using EEG and fMRI
- A novel MRI-compatible stimulator was created that links olfaction to inspiration
- EEG dipole modeling identified ‘early’ activations in parahippocampus and amygdala
- Both EEG and fMRI identified ‘late’ activations in orbitofrontal association cortex
- EEG may be more sensitive to transient activity in primary olfactory regions
- fMRI provides partial validation of EEG source localization in the olfactory system
Table 1 Regions showing significant increases in fMRI signal.

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<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
<td></td>
</tr>
<tr>
<td>Frontal_Mid_Orb_L</td>
<td>-.22</td>
<td>30</td>
<td>-.20</td>
<td>132</td>
</tr>
<tr>
<td>Frontal_Inf_Orb_L</td>
<td>-.28</td>
<td>22</td>
<td>-.22</td>
<td></td>
</tr>
<tr>
<td>Frontal_Mid_Orb_L</td>
<td>-.42</td>
<td>54</td>
<td>-10</td>
<td>232</td>
</tr>
</tbody>
</table>

Brain regions defined as Automated Anatomical Labeling (AAL) atlas. L, left.
Table 2 Regions showing dipole localizations by EEG/DT analysis

<table>
<thead>
<tr>
<th>Slice No.</th>
<th>Brain regions</th>
<th>MNI Coordinates</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ParaHippocampus_L</td>
<td>-26 -8 -32</td>
<td>48 ms</td>
</tr>
<tr>
<td></td>
<td>ParaHippocampus_L</td>
<td>-20 -10 -32</td>
<td>49 ms</td>
</tr>
<tr>
<td></td>
<td>Temporal_Pole_Sup_L</td>
<td>-18 10 -32</td>
<td>50 ms</td>
</tr>
<tr>
<td></td>
<td>Temporal_Pole_Sup_L</td>
<td>-20 16 -32</td>
<td>51 ms</td>
</tr>
<tr>
<td></td>
<td>Temporal_Pole_Sup_L</td>
<td>-28 18 -32</td>
<td>52 ms</td>
</tr>
<tr>
<td></td>
<td>Temporal_Pole_Sup_L</td>
<td>-28 24 -32</td>
<td>53 ms</td>
</tr>
<tr>
<td></td>
<td>Temporal_Pole_Sup_L</td>
<td>-36 24 -32</td>
<td>54 ms</td>
</tr>
<tr>
<td>2</td>
<td>Temporal_Pole_Sup_L</td>
<td>-36 12 -26</td>
<td>55 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Inf_Orb_L</td>
<td>-26 14 -26</td>
<td>148 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Inf_Orb_L</td>
<td>-30 24 -26</td>
<td>150 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Sup_Orb_L</td>
<td>-14 32 -26</td>
<td>156 ms</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>-8 14 -26</td>
<td>158 ms</td>
</tr>
<tr>
<td>3</td>
<td>Rectus_L</td>
<td>-8 14 -24</td>
<td>157 ms</td>
</tr>
<tr>
<td></td>
<td>Temporal_Pole_Sup_L</td>
<td>-34 12 -24</td>
<td>56 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Inf_Orb_L</td>
<td>-26 14 -24</td>
<td>152 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Inf_Orb_L</td>
<td>-30 24 -24</td>
<td>155 ms</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>-70 -34 -24</td>
<td>154 ms</td>
</tr>
<tr>
<td>4</td>
<td>AMG_L</td>
<td>-20 -4 -14</td>
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</tr>
<tr>
<td></td>
<td>Insula_L</td>
<td>-28 20 -14</td>
<td>250 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Inf_Orb_L</td>
<td>-24 22 -14</td>
<td>255 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Mid_Orb_L</td>
<td>-34 56 -14</td>
<td>300 ms</td>
</tr>
<tr>
<td></td>
<td>Frontal_Mid_Orb_L</td>
<td>-30 60 -14</td>
<td>310 ms</td>
</tr>
</tbody>
</table>

Brain regions defined as Automated Anatomical Labeling (AAL) atlas, L, left.
Author/s:
Masaoka, Yuri; Harding, Ian H.; Koiwa, Nobuyoshi; Yoshida, Masaki; Harrison, Ben J.;
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