Functional Magnetic Resonance Imaging of Odor Identification: The Effect of Aging

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Background. Sense of smell declines with age and impairment in olfaction has been observed in some neurodegenerative disorders such as Alzheimer’s disease. Functional neuroimaging techniques enable researchers to observe brain regions activated by olfactory stimuli.

Methods. We gave three mainly olfactory-mediated odors (limonene, methylsalicylate, and eugenol) to six young and six elderly subjects and observed the areas activated by using blood oxygen level dependent contrast functional magnetic resonance imaging.

Results. The group mapping of young subjects showed extensive activation in the orbitofrontal cortex, commonly believed to be the olfactory cortex, some limbic areas (the hippocampus and the thalamus), regions involved with gustatory sensation (the anterior insula and the inferior postcentral gyrus), superior and inferior temporal gyri, and cerebellum. In the elderly group, only the left inferior temporal gyrus and the primary visual cortex reached accepted significance levels.

Conclusions. We have therefore confirmed previous reports of brain regions involved in olfactory processing in young volunteers and demonstrated decreased activation in elderly volunteers.

Despite the significant impact of olfaction on human behavior (1), its functional neuroanatomy is not yet fully understood relative to other sensory systems. The acuity of the sense of smell declines with age (2–6), and dramatic olfactory dysfunction has been observed in some neurodegenerative disorders (4,7). It is commonly believed that the prepiriform cortex and orbitofrontal cortex play key roles in olfaction, particularly olfactory identification in the latter, which is thought to be responsible for higher processing of olfactory information rather than simple detection of smells (8–11). There have also been suggestions that the olfactory pathway is innervated from these secondary structures to various other regions, such as the hippocampus and thalamus (8). Most previous physiological and pathological data on olfaction have been gained mainly from studies using animal models or individual case studies in patients with brain lesions. Functional neuroimaging techniques now enable researchers to observe brain regions activated by olfactory stimuli in human subjects. To investigate the brain regions involved in olfactory processing and to test whether we would observe any age effect, we examined six healthy young and six healthy elderly individuals using functional magnetic resonance imaging (fMRI).

Methods
Six right-handed men (age 30 ± 5 years; mean ± SD) and six right-handed women (73 ± 5 years), who were screened to exclude conditions affecting olfactory function (e.g., history of head trauma or nasal disease or presence of respiratory infection, metabolic disorders, medication, or cognitive decline), were drawn from a volunteer database within the Clinical Age Research Unit of Guy’s, King’s, and St Thomas’ School of Medicine. Written informed consent was obtained after complete description of the study, which was approved by the Local Research Ethics Committee. Prior to the scanning, we administered two olfactory tests to assess the odor-detection threshold and olfactory identification. For the assessment of the odor-detection threshold, we modified the method used by Cain and colleagues (12), in which subjects are presented with a forced choice of two alternatives (odorant and blank). Ten dilutions of the odorant 1-butanol were prepared in deionized water, beginning with a 4% concentration by volume (Step 0) and decreasing stepwise in concentration. Each step was one third of the preceding dilution. Subjects sampled the saturated vapor phase of odorant solution birhinically, beginning with the lowest concentration (Step 9). Incorrect choice led to a one-step increase in concentration. Correct choice was confirmed over five trials at the same concentration, thereby defining detection threshold. Olfactory identification was assessed by the University of Pennsylvania Smell Identification Test (UPSIT), a widely used test of odor identification involving a scratch-and-sniff test of 40 microencapsulated odorants with a forced choice of four alternatives per item (13).
For the fMRI experiment, we used a custom-built olfactometer consisting of four 200-ml-capacity glass reservoir tubes, three of which were filled with different liquid odors (limonene, methylsalicylate, and eugenol). One glass reservoir was empty. Each glass reservoir had an inflow conduit of compressed room air and an outflow conduit that was connected to the face mask of the subjects by Teflon-coated tubing. Inflow of compressed air was controlled by the computer, which switched airflow into one of the four reservoir tubes; thus, odorized air or odorless air was sent to the face mask through outflow tubing. A vacuum suction device was connected to the face mask to remove the remaining odor immediately throughout the experiment. During the 5-minute experiment, the computer was programmed to produce alternating 30 seconds of resting condition (OFF), when odorless air was sent to the face mask, and 30 seconds of stimulating condition (ON), when the three odors were presented in a randomized sequence for 10 seconds each. This ON/OFF cycle was repeated five times.

The subjects were comfortably positioned with their head immobilized on the scanning table. After putting on the face mask, subjects were asked to press a response button with their right hand according to the instruction projected on the screen set at the bottom of the scanning table. Visual instruction was synchronized with odor presentation. During the OFF condition, the instruction “PRESS BUTTON” was projected once for 1 second; otherwise, the instruction “DON’T PRESS BUTTON” was projected throughout the period. The timing of the former instruction was randomized in each cycle. During the ON condition, the instruction “PRESS BUTTON WHEN YOU SMELL LEMON” was projected throughout the period. The subjects were expected to respond by pressing the button with their index finger when they smelled limonene. In both conditions, the visual cue “BREATHE” was projected onto the screen every 5 seconds for 1 second. The subjects inhaled odorless air during the OFF condition and odorized air during the ON condition through both nostrils without sniffing, maintaining regular respiratory rates in both conditions. The visual cue was aimed to ensure that airflow through the nostrils was equivalent across conditions.

Image acquisitions were performed using the 1.5 T GE Signa system (General Electric, Milwaukee, WI) retrofitted with an Advanced Nuclear Magnetic Resonance operating console (ANMR, Woburn, MA) in the MR Unit at the Maudsley Hospital, London. Fourteen noncontiguous 5-mm-thick axial slices with slice skip of 0.5 mm, parallel to the intercommissural (AC-PC) line, were acquired depicting blood oxygen level dependent (BOLD) contrast (14) with an in-plane resolution of 3 mm in 3 seconds. This gradient-echo planar imaging (EPI) sequence (time to echo [TE]: 40 milliseconds; time to repetition [TR]: 3000 milliseconds) was repeated 100 times during a total scanning time of 5 minutes. Each 2D image matrix was comprised of 128 × 64 pixels, each of which had a 16-bit integer value for signal intensity. An inversion recovery EPI dataset was acquired in the same scanning session at 43 axial 3-mm slices parallel to the AC-PC line (TE: 80 milliseconds; inversion time: 180 milliseconds; TR: 16 seconds). This dataset was used to register the fMRI datasets in each experiment in the standard stereotactic space of Talairach and Tournoux (15).

The difference between both groups on the performance of the detection threshold test and the UPSIT was assessed using a two-tailed Student’s t test. Effects of slight subject motion during image acquisitions were corrected before further analysis in each subject’s fMRI datasets by realigning images using tricubic spline interpolation. The realigned signal intensity time series was regressed on the concomitant lagged-time series, thereby estimating movement at each voxel (16). The power of periodic signal intensity change at the fundamental frequency of alternation between ON/OFF conditions was estimated by pseudogeneralized least-squares fitting of a sinusoidal regression model to the motion-corrected time series observed at each voxel on all images. The fundamental power quotient (FPQ) (the fundamental power divided by its standard error) at each voxel was estimated, and parametric maps representing FPQ at each voxel were constructed. Each time series was randomly permuted 10 times, which produced 10 parametric maps of FPQ in each plane for each subject estimated under the null hypothesis assuming that the FPQ was not determined by experimental design. All observed and random parametric maps of FPQs were registered in standard space of Talairach and Tournoux (15) and smoothed by a two-dimensional Gaussian filter with a standard deviation of 3 mm to accommodate individual variability in gyral anatomy. The median observed FPQ at each voxel in standard space was compared with a null distribution of median FPQ computed from randomized FPQ maps. For a one-tailed test of size α, voxels were considered to be generically activated if the observed median FPQ exceeded the (100 (1 − α)th percentile of the random distribution with voxel-wise probability of Type 1 error (α < .0004). Activated voxels during the ON condition were colored red and were displayed on the standard template image; thus, a generic brain activation map was constructed for each slice in both groups.

**Results**

There was no significant difference in the ability of both groups to detect 1-butanol. The average detection threshold for the young group was 6.3 ± 1.2, and the average threshold for the elderly group was 5.4 ± 0.9 (t = −1.43, p = .2). However, scores on the UPSIT did differ significantly by group; the average number of correct choices for the young group was 36.5 ± 1.2, and the average number of correct choices for the elderly group was 33.3 ± 2.9 (t = −2.36, p = .04).

In young subjects, we observed extensive activation in the insula bilaterally, right hippocampus, right thalamus (medial dorsal), right inferior postcentral gyrus (Brodmann area 43), right superior temporal gyrus (Brodmann area 22), right primary visual cortex (Brodmann area 18), left orbitofrontal cortex (Brodmann area 11), left inferior frontal cortex (Broca’s area), left cerebellum, and left inferior temporal fusiform cortex (Brodmann area 19). In the elderly group, the only areas of activation to reach statistical significance were the left inferior temporal gyrus (Brodmann area 19) and the left primary visual cortex (Brodmann area 18) (Table 1; Figure 1).
Table 1. Activated Brain Regions by the Olfactory Task

<table>
<thead>
<tr>
<th>Region</th>
<th>Brodmann Area</th>
<th>Side</th>
<th>Size (No. of Voxels)</th>
<th>Max FPQ</th>
<th>Tal(x)*</th>
<th>Tal(y)*</th>
<th>Tal(z)*</th>
<th>p Value</th>
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<tr>
<td>Insula*</td>
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<td>10</td>
<td>2.4</td>
<td>43</td>
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<td>−24</td>
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<tr>
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<td>−3</td>
<td>53</td>
<td>−13</td>
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<tr>
<td>Inferior temporal gyrus</td>
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<td>−23</td>
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<td>−13</td>
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<td></td>
</tr>
<tr>
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<td>Primary visual cortex</td>
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<tr>
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<td>Elderly subjects (n = 6)</td>
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</table>

Notes: Max FPQ = maximum fundamental power quotient.
*Talairach coordinates refer to the voxel with the FPQ in each regional cluster. Tal(x): medial–lateral position relative to midline (positive = right); Tal(y): anterior–posterior position relative to the anterior commissure (positive = anterior); Tal(z): superior–inferior position relative to the commissural line (positive = superior).
*Activated voxels were identified by a one-tailed test of the null hypothesis assuming median FPQ is not determined by experimental design. The probability threshold for false positive (type 1 errors) was p < .0004.
*Significantly different between groups on analysis of covariance constrained to areas showing significant activation in both groups (p < .004; voxel clusters >3).

**DISCUSSION**

Although an age-related decline in detection threshold had been reported before (2,4–6), the results might suggest that decrement by aging in odor identification may precede that of detection sensitivity, which is reminiscent of results from a study of Alzheimer patients (17).

In this study, many of the regions activated by the olfactory task in the young subjects showed laterality; that is, they were observed mainly in the right hemisphere. This may be due to relative predominance of the right hemisphere over the left in olfactory function, which has been shown not just in lesion studies and some olfactory tasks (10,18) but in functional imaging studies using positron emission tomography (PET) and fMRI (19–24). As for the orbitofrontal activation, we observed this only on the left side, which is not in keeping with some other olfactory fMRI studies (19,21–23). Although right orbitofrontal cortex dominance has been suggested in previous neuroimaging literature, possible reasons to interpret the conflicting results can be considered. Left-sided orbitofrontal activation in our results may be due to hedonic factors of the target odors we used. Many of the subjects reported that the smells they inhaled through the nostrils were not pleasant during the scan. Therefore, the subjective pleasantness of the odors may have affected the laterality of orbitofrontal activation, as was suggested by Zald and Pardo (25). Whereas other olfactory fMRI studies were carried out in a passive fashion, our study was designed to expect subjects to respond to the target smells. Therefore, we may have observed areas that were activated not by simple olfactory stimuli, but areas involved in processing information related to the target smells.

Of interest, we found relatively robust right-sided activation in the areas related to gustatory sensation (anterior insula and inferior postcentral gyrus). This corroborates the involvement of the gustatory cortex in processing olfactory information (23,26). The right hippocampal activation we observed is consistent with a previous PET study of an odor memory task. Because the odor identification task required the retrieval of odor memory, the regions involved with such memory could have been activated. Thus, the results may support the hypothesis that the hippocampus is involved in processing olfactory information, as suggested by Stäubli and colleagues (27). We also observed activation of the right medial dorsal nucleus of the thalamus, which is considered to receive afferent connections from the temporal prepiriform cortex (8). However, we did not detect activation of this area. This may be due to the effects of susceptibility artifacts, which could not be avoided with the fMRI sequence we applied in this study (22).

The difference between the two age groups we observed in this study is intriguing. Gender difference in olfaction (28) was already translated to the difference in the volume of activated brain by Yousem and colleagues in an odor-stimulated fMRI study of non-elderly subjects, which showed greater activation in women than in men (23). If the difference of activation by gender can be true in the elderly population as well, as we observed a difference in activated voxels between young male and elderly female subjects, it could be predicted that there may be even greater difference between young and elderly subjects within the same gender. However, the effect of age on gender difference in response to odor stimulation needs to be examined further. A parsimonious explanation of the results would be that they reflect decreased neuronal activity in response to olfactory stimuli, as has been reported in a previous odor-stimulated...
fMRI study (21) and an electrophysiological study of human olfactory event-related potentials (29–31). However, we speculate that there may be other factors that can account for the absence of activation in the elderly group. As suggested by Yousem and colleagues, possible delays between odor presentation and actual perception by subjects may undermine the ability of the BOLD signal change analysis (22). It is plausible that the delay in hemodynamic change, which is commonly observed in fMRI studies using BOLD contrast, may have been greater in the elderly subjects and may thus have affected our ability to detect activation. There could have been some difference between the two groups in the level of attention to the odors given, even though we confirmed that both groups identified the target odor (lemon) correctly by checking log files, which recorded button responses during the experiment. Because this study employed subtraction methodology, activations refer to increases in BOLD signal relative to the control conditions. Because we used a nonpassive fashion, which required subjects to respond by visual cues throughout both conditions, relative demand for recruiting areas of the brain to process visual data in the elderly subjects might have been greater than that of the young subjects. Thus, the difference might have affected subtraction analyses even though we aimed to match all factors in the task design except the presence of odorants throughout the conditions. Robust activation of the left inferior temporal gyrus in the elderly group compared with the young group suggests the possibility of an underlying age-related compensatory mechanism (32), in which elderly subjects may use a different neural network for categorical perception (33) as an alternative pathway to maintain their ability to identify odors.

In this fMRI study using olfactory stimulation, we observed extensive activation in the olfactory-associated cortex and limbic areas in young volunteers. Our results showed that multimodal sensory systems and limbic areas are involved in processing olfactory information. We have also provided the fMRI evidence of an age-related decline of neuronal activity in response to olfactory stimuli.

Acknowledgments

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References


Figure 1. Generic brain activation maps of odor identification. The results show group mapping of 6 young subjects and 6 elderly subjects in their responses to the target smell (limonene). Activated voxels are colored red.


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