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Functional Magnetic Resonance Imaging Study of Human Olfaction and Normal Aging

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Background. The function of human olfaction declines with advancing age. An important question centers on whether functional alterations to olfactory brain structures accompany age-related behavioral changes. In the present study, we tested the hypothesis that aged adults have intact though reduced activity in the central olfactory system using functional magnetic resonance imaging (fMRI).

Methods. University of Pennsylvania Smell Identification Test (UPSIT) was used to test the smell function of 11 young (23.9 ± 1.6 years) and 8 aged (66.4 ± 4.4 years) healthy participants. Then, the participants received fMRI at 3.0 T with lavender and spearmint as stimulants. After fMRI, the participants provided ratings for the odorants' intensity and pleasantness.

Results. The average UPSIT score of the aged adults was 34.1 ± 1.5, which was significantly lower than that of the young adults (37.3 ± 1.1) ($p = .0004$). Both age groups showed significant activation in major olfactory brain structures, including the primary olfactory cortex, entorhinal cortex, hippocampus and parahippocampal cortex, thalamus, hypothalamus, orbitofrontal cortex, and insular cortex and its extension into the inferior lateral frontal region. The aged adults showed less brain activity in olfactory structures ($p = .022$), consistent with lower ratings of odor intensity and UPSIT scores. Activation intensity in bilateral primary olfactory cortex areas and right insular cortex was also comparatively weaker ($p < .019$).

Conclusion. Results demonstrate that significant activation in aged adults can be observed in all the olfactory brain structures that are activated in young adults, but with lower activation volume and intensity. This finding provides a necessary baseline for further investigations in olfaction and aging.

THE function of human olfaction declines with advancing age (1–3). Morphological and neurophysiological changes have been observed in the central olfactory system (4–6). An important question centers on whether functional alterations to olfactory brain structures accompany age-related behavioral changes. Functional neuroimaging techniques, especially functional magnetic resonance imaging (fMRI), enable observation of olfactory activity in the human brain (7,8), and these techniques suggest that functional brain imaging may provide an important measure of age-related neural changes. However, characterization and quantification of aging-related central olfactory functional changes remain limited. Many olfactory structures did not show activation in aged adults (9,10). It is not clear whether the disappearance of activation in olfactory brain structures in these studies is related to actual diminished neural activity or to reduced imaging sensitivity of fMRI in these brain regions. We hypothesize that aged adults have intact though reduced activity in the central olfactory system. To test this hypothesis, we conducted a high field fMRI study with high spatial resolution to investigate the effects of aging on human brain olfactory activity.

Methods

Participants

Eleven young (5 men and 6 women, average age 23.9 ± 1.6 years, range 21–26 years) and 8 aged (5 men and 3 women, average age 66.4 ± 4.4 years, range 61–74 years), right-handed normal volunteers participated in the study. Participants had no history of medical, neurologic, psychiatric, or rhinal conditions. Before the fMRI scan, the olfactory function of the participants was evaluated with the University of Pennsylvania Smell Identification Test (UPSIT) (11); the participant was enrolled in the fMRI study only if the UPSIT score was within the age-adjusted normal limit. The investigation was reviewed and approved by the institutional review board of the Pennsylvania State University College of Medicine, and all participants gave informed written consent prior to participation.

Olfactory Stimulation

The odorant delivery system consisted of a set of Teflon tubing (DuPont, Wilmington, DE) that transported odorless, medical grade compressed air from a canister through a set of two odorant chambers to the participant's nose. The air was humidified before going into the odorant chambers. The odorant chambers were located next to the patient bed of the MRI system, allowing for rapid switching and timing between odor and no-odor conditions. The timing and switching of the odorants' delivery were automatically synchronized by TTL signals incorporated in the MRI pulse-timing program. The clean air with or without odorant was presented through a tube about 1 cm away from the participant's nose with a constant flow rate at 8 L/min. A ventilation pipe (20 cm in diameter) located superior to

Table 1. Ratings of Odor Stimuli Experienced During Functional Magnetic Resonance Imaging (fMRI) Using a 0–10 Likert-Type Scale

Rating	Young Group	Aged Group
Average odor intensity	7.4 ± 1.5	5.7 ± 1.2
Strongest odor intensity	8.4 ± 1.5	5.9 ± 1.6
Pleasantness	6.5 ± 3.1	9.5 ± 0.7

Notes: Two odorants were presented to the participants during fMRI. Each participant provided a rating(s) of the intensities of all the odorants perceived. Significant differences were observed between the two age groups in the ratings for the average odor intensity (two-sample two-tailed t test, $p = .015$), the strongest odor intensity ($p = .003$), and pleasantness ($p = .009$).

the head coil removed air in the magnet bore at a rate of 20 L/min. The two odorants used in this study were 10% lavender diluted in dipropylene glycol and 10% spearmint diluted in dipropylene glycol (Quest International Fragrances, Mount Olive, NJ).

The olfactory fMRI paradigm consisted of interleaved time intervals during which the odor stimulus was switched on and off without any auditory, visual, tactile, or thermal cues. Prior to the fMRI study, the participants were instructed to breathe normally without sniffing. After MRI scanning, participants provided ratings of the odorants with procedures similar to Van Toller and colleagues (12). These psychometric judgments were quantified on a 0–10 Likert-type scale for the dimensions of odor intensity and pleasantness.

fMRI Protocol

Participants lay supine in a dark environment with their heads fit in a head restrainer to minimize motion and provide precise positioning and comfort. Respiratory and cardiac rates were recorded through a chest belt and pulse plethysmograph interfaced with a personal computer (Biopac MP30 System; Biopac Systems, Goleta, CA) for monitoring of respiratory and sniffing patterns as well as any associated motions.

The studies were performed on a whole-body 3.0 T imaging spectrometer (MedSpec S300; Bruker BioSpin, Ettlingen, Germany). During execution of the olfactory paradigm, 188 T_2*-weighted echo planar imaging sets were acquired from a transverse brain section of 6.6 cm centered at the inferior frontal cortex with repetition time / echo time / flip angle = 2100 ms / 35 ms / 90°, field of view = 25 × 25 cm^2, matrix = 128 × 96, 15 axial slices, slice thickness = 4 mm with a 0.4-mm gap between slices. The olfactory paradigm consisted of five cycles of baseline and stimulation conditions. During the 25.2-second odor-stimulation period, the two odorants alternated every 5 seconds. The baseline period was 50.4 seconds. Finally, an anatomical image set was obtained from each participant with a three-dimensional gradient echo T_1-weighted sequence (repetition time / echo time / flip angle = 40 ms / 5 ms / 40°, field of view = 25 × 25 × 15 cm^3, matrix = 256 × 256 × 50).

Data Processing and Analysis

The time-series fMRI data were processed offline with SPM99 (the Wellcome Department of Cognitive Neurology, London, UK) (13). The first 10 images of each data set were discarded to remove the initial transit signal fluctuations. The subsequent images were re-aligned using sinc interpolation to remove any minor movements. The T_1-weighted anatomical images were co-registered and spatially normalized to the Montreal Neurological Institute brain template (14). Then the echo planar imaging time-series images were normalized using the same normalization parameters, re-sliced into 2 mm × 2 mm × 4 mm voxels, and then smoothed with a Gaussian kernel with 8 mm full width at half maximum in all three dimensions.

The statistical parametric map (SPM) of each participant was calculated by correlating voxel intensities of the time-series image set to the experimental paradigm convolved with the hemodynamic response function ($p < .001$, uncorrected, extent threshold = 3). Then, the SPM was overlaid to the corresponding anatomical images of the participant. Average olfactory activation maps of the two age groups were generated, and the fMRI activation maps from the two age groups were compared with two-sample Student's t tests.

Results

The average UPSIT score of the aged adults was 34.1 ± 1.5, which was significantly lower than that of the young adults (37.3 ± 1.1) (two-sample two-tailed t test, $p = .0004$). Both scores were within age-adjusted normal limits. Subjective ratings of the odorants presented during fMRI are summarized in Table 1.

Average olfactory activation maps of the young and aged groups are shown in three-dimensional renderings and in two-dimensional axial planes in Figure 1. In both young and aged samples, activity was observed in all the known olfactory anatomical structures, including the primary olfactory cortex (POC; encompassing piriform cortex, amygdala, and periamygloid cortex), entorhinal cortex, hippocampus–parahippocampal gyrus, thalamus, hypothalamus, orbitofrontal cortex, and insular cortex and its extension into the inferior lateral frontal region. The fMRI activation maps from the two groups are characterized and compared in terms of the locations, volumes, and peak intensities of activation clusters that are summarized in Tables 2–4. The activation locations between the two age groups in the olfactory structures were not significantly different, except in the left hippocampus and right thalamus (two-sample two-tailed t test, $p < .05$). The activation clusters at the entorhinal cortices moved laterally because of magnetic field inhomogeneity distortion. The activation volume of the olfactory structures were significantly smaller in the aged adults than in the young adults (387.1 ± 181.7 vs 759.7 ± 431.2 voxels, respectively; two-sample two-tailed t test, $p = .022$). In particular, the activation volume in the right POC was reduced more than threefold in the aged adults (two-sample two-tailed t test, $p = .035$). In addition, there was a smaller variation of activation volume in the aged group than in the young group (f test, $p = .032$). The peak activation intensities in the bilateral POC and right insular cortex from the aged adults were significantly weaker than those from the young adults (two-sample two-tailed t test, $p < .019$).

In the young group, the activation volume in the right POC was larger than that in the contralateral (paired

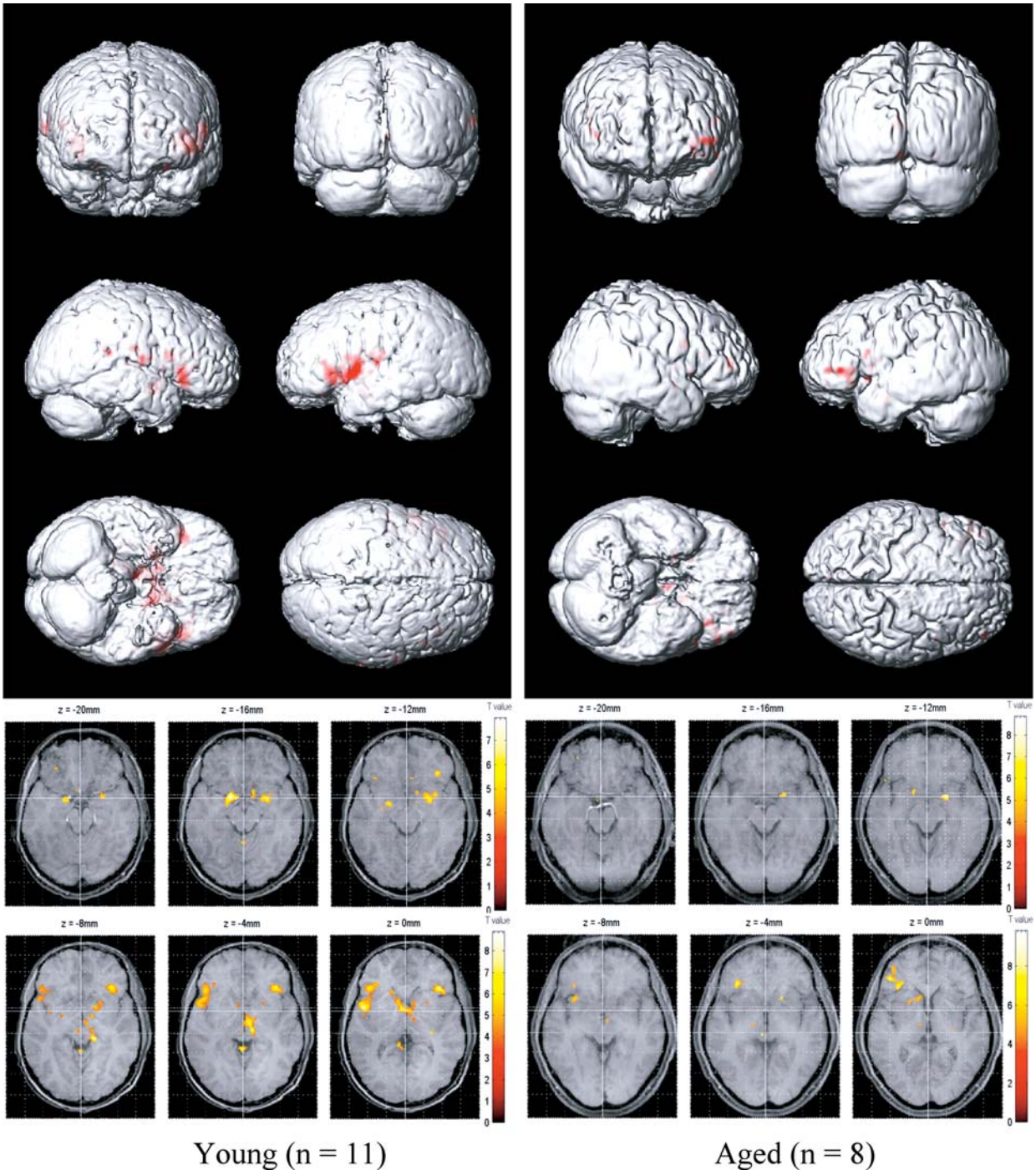

Figure 1. Olfactory functional magnetic resonance imaging (fMRI) activation maps from the young (left) and aged (right) groups. Larger activation in the major olfactory structures was observed in the young group than in the aged group (two-sample two-tailed t test, $p = .022$).

two-tailed t test, $p = .033$). However, no other significant lateralization effect was observed within either the aged or the young groups.

Discussion

Our results demonstrated that using fMRI, significant brain activity could be detected in all the major olfactory brain structures in both young and aged adults. The activation patterns detected in the aged adults were particularly important because they demonstrated the sensitivity of fMRI in evaluation of olfactory functional changes with the Blood Oxygenation Level-Dependent (BOLD) signal. Previous studies using 1.5 T MRI systems were unable to detect main olfactory structure activity in aged adults (9,10). The enhanced olfactory brain activation in this study can be attributed to the increased image signal-to-noise ratio, the

Table 2. Locations and Volumes of the Activation Clusters of Olfactory Brain Structures in the Young Adults*

Location	Left (L)/ Right (R)	Coordinates, mm x	y	z	Activation, Voxels	T Value
Primary olfactory cortex	R	−20 ± 4	3 ± 4	−17 ± 4	71.3	5.14
	L	25 ± 5	6 ± 6	−18 ± 5	46.4	3.57
Entorhinal cortex	R	−33 ± 2	−18 ± 6	−34 ± 5	3.5	1.07
	L	32	−12	−40	1.1	0.35
Hippocampus	R	−22 ± 5	−11 ± 19	−14 ± 6	11.8	2.13
	L	22 ± 7	−9 ± 4	−13 ± 4	11.5	1.99
Hypothalamus	R	−5 ± 1	−3 ± 9	−8 ± 4	7.6	1.18
	L	7 ± 4	−7 ± 4	−8 ± 5	5.4	1.74
Thalamus	R	−6 ± 3	−13 ± 4	5 ± 2	34.5	1.57
	L	10 ± 6	−19 ± 4	6 ± 8	26.1	1.78
Insular cortex	R	−38 ± 6	9 ± 11	−4 ± 8	125.8	4.36
	L	44 ± 5	10 ± 9	−3 ± 5	98.1	4.09
Orbitofrontal cortex	R	−26 ± 7	40 ± 4	−17 ± 4	71.5	3.69
	L	28 ± 7	37 ± 7	−22 ± 6	47.2	2.97
Inferior frontal gyrus	R	−42 ± 5	40 ± 10	6 ± 9	107.9	4.22
	L	48 ± 7	40 ± 13	9 ± 10	90.2	3.64

Note: *$p < .001$, uncorrected, extent threshold = 3.

BOLD contrast-to-noise ratio with higher field strength, and the reduced olfactory habituation with frequent alternation of odorants during the stimulation periods (8). These improvements provided the necessary sensitivity and dynamic range of BOLD contrast, and resulted in a more reliable neurobiological assay of the central olfactory system using activation maps. Such improvements are very important for quantitative evaluation of olfactory alterations, such as in aging and Alzheimer's disease, in which reduced neural activity is expected.

In normal aging, the olfactory processes show various attenuated capacities (1–3). The specific etiology of these changes is unclear and likely involves multifactorial effects on peripheral and central olfactory systems (15,16). In this study, the young and aged adults demonstrated age-appropriate odor identification scores on the UPSIT, with an approximately 10% decline in the older sample. Consistent with these measurements, the fMRI results indicated that the major olfactory brain structures in the aged adults could be activated as in the young adults, though at an attenuated level. Both the volume and intensity of olfactory activation were correlated with age-related decline in olfactory function as evaluated with the UPSIT. Among all the olfactory structures studied, the right POC yielded activation data that appeared to be most sensitive to aging decrements. This was demonstrated by the significant differences of both the activation volume and peak activation intensity in the right POC across the two age samples.

Table 3. Locations and Volumes of the Activation Clusters of Olfactory Brain Structures in the Aged Adults*

Location	Left (L)/ Right (R)	Coordinates, mm x	y	z	Activation, Voxels	T Value
Primary olfactory cortex	R	−20 ± 5	6 ± 8	−18 ± 3	19.4	2.91
	L	23 ± 4	1 ± 5	−17 ± 6	28.6	3.13
Entorhinal cortex	R	−31 ± 5	−6 ± 14	−33 ± 10	9.1	1.51
	L	39 ± 13	−5 ± 7	−36 ± 3	24.0	2.34
Hippocampus	R	−20 ± 11	−17 ± 4	−26 ± 3	2.3	0.85
	L	33 ± 1	−17 ± 4	−23 ± 6	3.6	1.38
Hypothalamus	R	−6	−6	−4	3.8	0.53
	L	12 ± 0	−5 ± 4	−10 ± 8	3.0	1.12
Thalamus	R	−4 ± 0	−12 ± 8	0 ± 0	4.5	1.00
	L	13 ± 6	−14 ± 4	4 ± 4	7.6	1.53
Insular cortex	R	−43 ± 7	12 ± 12	3 ± 4	54.5	3.44
	L	40 ± 5	19 ± 13	−7 ± 7	29.6	2.86
Orbitofrontal cortex	R	−28 ± 7	40 ± 4	−19 ± 4	40.1	3.94
	L	32 ± 10	40 ± 9	−21 ± 6	51.9	4.08
Inferior frontal gyrus	R	−44 ± 7	40 ± 11	−2 ± 8	46.3	4.85
	L	50 ± 5	40 ± 9	6 ± 7	58.9	3.32

Note: *$p < .001$, uncorrected, extent threshold = 3.

Table 4. Peak Activation Intensities of Major Olfactory Brain Structures*

Location	Left (L)/ Right (R)	Young Group	Aged Group
Primary olfactory cortex	R	2.28 ± 0.64	0.82 ± 0.75
	L	2.04 ± 1.04	0.93 ± 0.69
Insular cortex	R	2.03 ± 0.51	0.90 ± 0.66
	L	1.59 ± 0.99	0.91 ± 0.79
Orbitofrontal cortex	R	1.36 ± 1.17	0.85 ± 0.43
	L	1.16 ± 0.77	1.02 ± 0.57

Note: *Two-sample two-tailed *t* test showed that the differences between the activation in the right primary olfactory cortex (POC) ($p = .0003$), left POC ($p = .0182$), and right insular cortex ($p = .0006$) in the two age groups were significant.

We observed a significantly weaker BOLD signal at the bilateral POC and right insular cortex in the aged participants. Previous fMRI studies reported inconsistent observations of the aging effect on the BOLD signal at visual and motor cortices (17,18). Because fMRI is an indirect measurement of neural activity, there is an implication of the age-dependent changes in the coupling between neural activity and the BOLD signal. As demonstrated by the evoked potential studies, amplitude of olfactory neural activity decreased with age (5,6). Hence, the reduction of olfactory BOLD signals of our healthy older participants likely reflects the changes in associated neural activity. We also observed that the reduction of activation volume in the aged adults was accompanied by a smaller interparticipant variation. This observation may reflect a decreased dynamic range in the older brain's capacity to respond to sensory stimulation. It is conceivable that, as the brain becomes less responsive to stimulations with age, the variations in brain activation also decrease accordingly.

For this study, single stimulus intensity was specified for each odorant. Ratings of the odorants experienced during fMRI showed that perception of odor intensity decreased with age. Older participants rated the stimuli as less intense but more pleasant than did young adults. To understand the dynamics of age-related changes, future fMRI studies are needed to evaluate the effects of different odor intensities in a dose–response type of analysis. Furthermore, the relationship of fMRI findings to the olfactory threshold levels is

also important to establish. From an anatomical viewpoint, studies are needed to determine whether reduced brain activation volume is related to brain atrophy. In this study, the older adult sample showed a 49% reduction in activation volume in the olfactory brain structures. Although it is unlikely that their brain volumes could be reduced by such a proportion, the contribution of morphometric changes to the reduced activation volume needs to be evaluated in future studies.

Aging studies of the central olfactory system are particularly interesting because the accurate detection and characterization of the olfactory deficits occurring in early Alzheimer's disease can potentially provide preclinical markers for this disease. The olfactory activation patterns in Alzheimer's disease patients would be expected to decrease more significantly in certain olfactory brain structures because there is a disproportionate burden of plaques and tangles in these areas (19–21). Accurate differentiation of Alzheimer's disease versus normal aging using fMRI requires high sensitivity and dynamic range. Based on our study, high magnetic field olfactory fMRI may provide the necessary sensitivity and dynamic range for such evaluations. In addition, because participants are not required to perform precise or taxing cognitive tasks with this experimental paradigm, study of impaired persons such as those with Alzheimer's disease may yield more reliable results.

Conclusion

Our study demonstrated the sensitivity of high field olfactory fMRI in detection and evaluation of age-related brain activation changes. Brain activity can be observed within major olfactory brain structures in both young and aged adults with significant reductions in activation volume and intensity in the aged adults. This observation provides a necessary baseline for further investigations in olfaction and aging.

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