Central Olfactory Dysfunction in Alzheimer’s Disease and Mild Cognitive Impairment: A Functional MRI Study

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Abstract

Background: Olfactory deficits are present in early Alzheimer’s disease (AD) and mild cognitively impaired (MCI) patients. However, whether these deficits are due to dysfunction of the central or peripheral olfactory nervous system remains uncertain. This question is fundamentally important for developing imaging biomarkers for AD using olfactory testing.

Objective: This study sought to use olfactory functional magnetic resonance imaging (fMRI) to further demonstrate the involvement of the central olfactory system in olfactory deficits in MCI and AD.

Methods: We investigated the central olfactory system in 27 cognitively normal controls (CN), 21 MCI, and 15 AD subjects using olfactory fMRI with an odor-visual association paradigm during which a visual cue was paired with lavender odorant (odor condition) or odorless air (no-odor condition).

Results: The CN subjects had significantly greater activated volume in the primary olfactory cortex during both the odor and no-odor conditions compared to either the MCI or AD groups (p < 0.05). No significant differences were observed between the odor and no-odor conditions within each group. No-odor condition activation in AD and MCI correlated with the cognitive and olfactory assessments.

Conclusion: The no-odor condition, allowing investigation of activation patterns when the peripheral olfactory system was not directly involved, elicited the same functional response as the odor condition for each of the three groups. Thus, the olfactory activation deficits present in AD and MCI patients are most likely caused by degeneration of the central olfactory nervous system.

Keywords: Alzheimer’s disease, functional magnetic resonance imaging, mild cognitive impairment, olfaction

INTRODUCTION

Patients with Alzheimer’s disease (AD) and mild cognitive impairment (MCI), a population at high risk for developing AD, present with olfactory deficits in threshold detection, odor memory, and/or odor identification [1, 2]. Olfactory tests such as the University
of Pennsylvania Smell Identification Test (UPSIT) and the Sniffin’ Sticks have consistently demonstrated these specific olfactory dysfunctions in AD and MCI patients compared with age-matched normal controls [3–13]. Furthermore, these olfactory symptoms correlate with cognitive decline and the progression of both MCI and AD. As such, it has been hypothesized that the olfactory deficits observed behaviorally in AD are the result of early pathological changes (plaques, neurofibrillary tangles, and atrophy) that occur in the olfactory bulb, anterior olfactory nucleus, piriform cortex, and olfactory epithelium in the central nervous system [14–20]. The pathology in these regions typically increases as the disease progresses and the symptoms worsen [3, 5, 8, 9, 20].

Though behavioral studies have consistently demonstrated olfactory deficits in MCI and early AD, it is still unknown whether these deficits derive from pathology within and/or degeneration of central olfactory regions or components of the peripheral olfactory system. While the peripheral olfactory system is primarily involved in the initial detection of odorants, the central part is involved in processing and integration of odorant information with other sensory cues, such as visual stimuli. The peripheral olfactory system includes the olfactory epithelium and olfactory nerve, whereas the central olfactory system includes the olfactory bulb, olfactory tract, anterior olfactory nucleus, piriform cortex, amygdala, olfactory tubercle, hippocampus, and the orbitofrontal cortex [21]. The olfactory receptors present in the olfactory epithelium project to the mitral cells of the olfactory bulb via the olfactory nerve. The axons from the mitral cells then travel to the brain via the olfactory tract and project primarily to the primary olfactory cortex (POC), olfactory tubercle, amygdala, and entorhinal cortex. The POC includes the piriform cortex, entorhinal cortex, anterior cortical nucleus of the amygdala, and the periamygdaloid cortex [22]. Neurons from here then send projections to the dorsomedial nucleus of the thalamus, the basal forebrain, the limbic system, and the hippocampus [9–21, 23].

The peripheral and central olfactory systems function together to fully assess, process, and evaluate odorants in the environment.

Despite clear evidence of olfactory deficits, very few studies have investigated the role of the central versus peripheral olfactory systems in AD and MCI. Specifically, the major question that remains unanswered is whether the dominant reason for olfactory deficits in AD is due to degeneration of the epithelium, which would denote a primarily peripheral problem affecting odor detection, and/or due to degeneration in the brain, which would constitute a mainly central deficit affecting odor processing. Interestingly, both behavioral and postmortem pathological studies have leaned toward suggesting a central rather than peripheral problem in AD patients. Specifically, based on lower performance on odor identification tests compared to threshold detection tests in early AD and MCI subjects, several studies have concluded a dysfunction of the central olfactory system is most likely, where processing and integration of the odor information is the issue [7, 9]. Based on neuron loss in the anterior olfactory nucleus, Ter Laak et al. concluded odor identification is processed in the central olfactory structures, again supporting the notion of a primarily central olfactory system problem [24]. However, the strongest evidence has been provided by several postmortem studies that indicate AD pathological changes were found preferentially in central olfactory structures compared to other sensory systems. Specifically, an autopsy study reported less severe pathology in the peripheral olfactory areas compared with the central olfactory areas, further suggesting a central problem [21, 25].

While these studies support the dominance of the central olfactory system degeneration hypothesis, postmortem studies are inconclusive overall in their findings due to most of these studies taking place when pathology was in the advanced stages. As such, they may not reflect the pathological status in early AD and MCI patients at the time the tests are given. Several other AD autopsy studies also report pathology in, and degeneration of, peripheral olfactory regions, including axons of the olfactory tract and olfactory epithelium [16, 17, 19, 26]. Furthermore, current literature has focused on the behavioral and pathological distribution to provide evidence for the involvement of the central olfactory system, but a direct link between these behavioral and postmortem studies is lacking. Recently, however, functional deficits of the central olfactory structures in AD have been detected using olfactory functional magnetic resonance imaging (fMRI) [22, 27, 28]. Furthermore, olfactory fMRI as a whole has become a promising technique for studying olfaction and olfactory deficits [29–34]. Nevertheless, these fMRI studies still require peripheral afferent information to be sent to the central olfactory structures and, therefore, cannot definitively rule out peripheral olfactory involvement. Thus, whether central or peripheral degeneration in the olfactory system is responsible
for the olfactory deficits in AD and MCI still remains an open question.

Therefore, in our study, we further investigated this question with an olfactory fMRI study using an odor-visual association paradigm that was specifically designed to isolate the central olfactory system from the peripheral components [35]. The paradigm included a visual cue accompanied by either an odor (odor condition) or fresh air (no-odor condition) presentation. The visual cue paired with an odor is used to establish an association with odor stimulation, such that olfactory activation can be observed in the central olfactory regions when the visual cue is presented alone subsequently. We specifically investigated the POC, which plays an important role in olfactory processing [36–40] and is consistently present in prominent AD pathology. We hypothesized that the olfactory deficits present in AD and MCI patients are due to central olfactory system dysfunction with both the no-odor and odor conditions showing similar activation patterns in the central olfactory regions.

MATERIAL AND METHODS

Study cohort

Sixty-three subjects, 15 AD subjects (Clinical Dementia Rating Scale (CDR) of 0.5 or 1), 21 MCI subjects (CDR of 0.5), and 27 age-matched cognitively normal controls (CN), were enrolled in this study (Table 1). The patient population, AD and MCI subjects, were recruited from local clinics and CN were recruited from the local community through advertisement. The study was approved by the Pennsylvania State University College of Medicine Institutional Review Board and all subjects provided written consent prior to participation. All participants were screened for neurologic and psychiatric conditions (other than dementia) to rule out any confounds that could adversely influence the study results, which included checking for complications specific to olfactory dysfunction (e.g., head trauma, viral infection, allergies) and for contraindications to MRI (e.g., not-MRI-safe metal implants, severe claustrophobia). The AD and MCI subjects were clinically diagnosed by a specialist in accordance with NINCDS-ADRDA criteria [41] and Peterson criteria [42], respectively. Fourteen AD and 12 MCI patients were being treated with a cholinesterase inhibitor (donepezil, galantamine, or rivastigmine) and/or memantine. There was no significant difference in age (p > 0.05, F = 0.77, df = 62), gender, or educational level (p > 0.05, F = 2.89, df = 62) between the three study cohorts.

| Table 1 Demographic and behavioral data of the study cohort |
|-------------------------|-------------------------|-------------------------|
|                         | CN (n = 27)             | MCI (n = 21)            | AD (n = 15)             |
| Male/Female             | 12/15                   | 10/11                   | 5/10                    |
| Age (y)                 | 69.5 ± 10.4             | 73.2 ± 9.0              | 71.9 ± 11.9             |
| Educational level (y)   | 16.0 ± 1.7              | 14.6 ± 2.9              | 14.3 ± 3.0              |
| UPSIT                   | 34.0 ± 4.2              | 24.2 ± 8.6*             | 15.5 ± 8.4*             |
| MMSE                    | 28.5 ± 1.5              | 26.5 ± 1.9              | 18.9 ± 5.4*             |
| CVLT-II                 | 62.6 ± 13.1             | 47.3 ± 12.7*            | 21.3 ± 14.1*            |
| DRS-2                   | 13.3 ± 1.6              | 9.6 ± 3.2*              | 3.9 ± 2.5*              |
| DRS-mem                 | 11.1 ± 1.7              | 7.0 ± 2.9*              | 2.5 ± 1.2*              |

CN, cognitively normal controls; MCI, mild cognitive impaired; AD, Alzheimer’s disease; UPSIT, University of Pennsylvania Smell Identification Test; MMSE, Mini-Mental State Examination; CVLT-II, California Verbal Learning Test-Short Form Version 2; DRS-2, Dementia Rating Scale 2; DRS-mem, memory portion of the DRS-2. Note: Mean ± standard deviation is reported. *p < 0.05, ANOVA when compared to CN. †p < 0.05, ANOVA when compared to MCI.

Behavioral tests

The University of Pennsylvania Smell Identification Test (UPSIT, Sensonics, Inc., Haddon Heights, NJ, USA) was administered to assess smell identification function, and clinical neurocognitive examinations [Mini-Mental State Examination (MMSE), Mattis Dementia Rating Scale 2 (DRS-2), and the California Verbal Learning Test-Second Edition Short Form (CVLT-II)] were administered to assess cognitive performance. The MMSE, commonly used in clinical practice, is a 30-point test of general cognitive ability. The DRS-2 is a more detailed measure of cognitive ability in five areas: attention, inhibition/perseveration, construction, conceptualization, and memory. The scores are given as age-corrected scaled sub-scores. The CVLT-II provides a comprehensive assessment of verbal learning and memory. The sex and age scaled short-term memory score is presented here. A medical history evaluation was also conducted for all participants.

Olfactory stimulation paradigm

The olfactory fMRI paradigm included a visual cue paired with an odor followed by the same visual cue without the odor in an interleaved fashion (Fig. 1). When the subject saw the word “Smell?” on the screen, they were asked to respond “yes” using the...
Fig. 1. The visual-odor association fMRI paradigm. The paradigm consists of interleaved “odor” and “no-odor” conditions. For the odor conditions, the visual cue was a display of the word “Smell?” when lavender odor was presented at the same time. For the no-odor conditions, only odorless air was given when the same visual cue appeared on the screen. When “Smell?” appeared on the screen, the subject provided responses using a button press device in each hand, with the left hand if they detected no smell and the right hand if they detected the odor. Four concentrations of lavender were presented. Each concentration was presented three times before moving on to the subsequent concentration. The paradigm was explained to each participant prior to the scan. We confirmed they would breathe normally throughout the paradigm, that they understood the task and could press the appropriate button, and could see the screen clearly. Respiration was monitored during the paradigm to see breathing patterns and confirm that the participant was breathing normally. Prior to the task for each volunteer, the technician confirmed that the olfactometer and paradigm were working and odorants were being delivered.

A programmable olfactometer (Emerging Tech Trans, LLC, Hershey, PA, USA) executed the odor stimulation paradigm by delivering the odorant to both of the subject’s nostrils simultaneously without any optical, acoustic, thermal, or tactile cues. The olfactometer delivered constant airflow (6 L/min) with 50% relative humidity at room temperature (22°C). Optical triggers from the MRI scanner were used to synchronize the odor stimulation paradigm and the MRI image acquisition. Respiration patterns of all subjects were monitored and recorded during the execution of fMRI paradigm via a chest belt by the olfactometer in order to confirm the subject was awake throughout the paradigm and breathing rate was normal.

Lavender oil (Givaudan Flavors Corporation, East Hanover, NJ, USA) diluted in 1,2-propanediol (Sigma, St. Louis, MO, USA) was used as the olfactory stimulant due to its effectiveness as an olfactory stimulant with minimal propensity to stimulate the trigeminal system and being perceived by North American individuals as being pleasant and familiar [44]. The stimulant was stored in six 300 ml glass jars with 50 ml of the odorant.

Imaging protocol

The imaging data were acquired on a 3 T MRI system (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany) with an 8-channel head coil. A blood-oxygen-level-dependent (BOLD) signal sensitive T2*-weighted echo planar imaging (EPI) sequence was used to acquire functional data with slices = 34, slice thickness = 4 mm, field of view (FOV) = 230 × 230 mm, acquisition matrix = 80 × 80 mm, echo time (TE) = 30 ms, repetition time (TR) = 2000 ms, flip angle (FA) = 90°, iPAT acceleration factor (GRAPPA) = 2, 234 repetitions, and acquisition time (TA) = 7 min 56 s. T1-weighted images with 1 mm isotropic resolution were acquired with MPRAGE method for structural assessment of the POC: TE = 2.98 ms, TR = 2300 ms, inversion time (IT) = 900 ms, FA = 9°, FOV = 256 mm × 256 mm × 160 mm, acquisition matrix = 256 mm × 256 mm × 160 mm, iPAT factor = 2, and TA = 6 min 21 s.

fMRI data processing and analysis

Imaging data was analyzed utilizing Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, University College London, UK). The first 10 images were discarded to remove initial transit signal fluctuations. The following standardized procedure was used to preprocess the fMRI data:
1) spatial realignment within the session to remove any minor head movements (movement <2 mm, rotation <1°); 2) co-registration with high-resolution anatomical image; 3) normalization to the Montreal Neurological Institute (MNI) brain template in a spatial resolution of 2 mm × 2 mm × 2 mm; and 4) smoothing with an 8 mm × 8 mm × 8 mm (full width at half maximum) Gaussian smoothing kernel [45–48]. A statistical parametric map was generated at the individual level by fitting the stimulation paradigm to the functional data with a default 128-s high pass filter, convolved with the canonical hemodynamic response function (uncorrected, \( p < 0.001 \), extent threshold = 6). Contrast files were produced at the individual level to model each experimental condition, including one accounting for all the odor presentations and one for the no odor conditions. Olfactory activation maps at the group level were generated using one-sample \( t \)-test (uncorrected, \( p < 0.001 \), extent threshold = 6) for odor and no odor conditions using the contrast files created at the individual level. The statistical threshold, \( p < 0.001 \) uncorrected, was used because when reporting the voxel-based fMRI data analysis results, nearly no activation cluster could survive in the MCI and AD group with a \( p = 0.05 \) FWE. One contributing factor for this is the large atrophy and related morphology changes in the medial temporal lobes in these cohorts, which rendered voxel-based analysis unreliable. Thus, we used a statistical threshold at \( p < 0.001 \) uncorrected with an extent threshold ≥6 to reduce the potential type I error within the manually-segmented ROI of each individual.

**Region of interest (ROI) analysis of the POC**

Bilateral manual segmentation of the POC on \( T_1 \)-weighted images from each subject was accomplished using FMRIB Software Library View (FSLview, Analysis Group, FMRIB, Oxford, UK). The POC was defined as including the anterior olfactory nucleus, olfactory tubercle, piriform cortex, anterior portion of the periamygdaloid cortex and amygdala, and anterior perforated substance [22]. The segmentation of the POC was performed by two trained investigators and reviewed by a neuroradiologist. The individuals participating in the image segmentation were blind to the group assignment of each subject. The ROI was normalized and coregistered to the fMRI maps to quantify the activated volumes within the ROI (\( p < 0.05 \), extent threshold = 6). The UPSIT scores and fMRI data were corrected for age effects. This data was analyzed using GraphPad Prism 6 (GraphPad Software San Diego, CA).

**RESULTS**

**Demographics and behavioral results**

Table 1 shows the demographic information and cognitive/behavioral test results of the three subject cohorts. The behavioral tests (MMSE, CVLT-II, DRS-2, and UPSIT) showed significant differences between the three groups (one-way ANOVA analysis, \( p < 0.0001 \)). For the UPSIT, CVLT-II, and DRS-2, multiple comparisons tests showed that the CN group had higher scores, indicating greater overall neurocognitive and smell identification functions. Most notably, prominent olfactory deficit in the MCI and AD subjects was indicated by the UPSIT scores. The average UPSIT score was 15.5 ± 8.4 out total score of 40 in the AD group, which was likely a result of choosing answers by chance. The MCI group, a mixed cohort including preclinical AD and subjects with memory deficits, exhibited a large variation in neurocognitive and olfactory performances, overlapping with scores from CN and AD groups.

**Age effect**

Aging effects were observed in olfactory scores and fMRI measurements. When examining all subjects, the UPSIT score (\( r = 0.33, p = 0.0094 \)) and activation in the POC (\( r = 0.36, p = 0.0035 \)) showed strong negative correlations with age. The CN group alone also showed significant aging effects in the UPSIT score (\( r = 0.64, p = 0.0003 \)) and activation in the POC (\( r = 0.45, p = 0.019 \)). The MCI group showed significant age effects in the UPSIT scores (\( r = 0.45, p = 0.041 \)) and activation in the POC (\( r = 0.49, p = 0.025 \)). However, the data for the AD subjects showed no significant aging effects, indicating that the predominant effect on these cognitive measurements is due to the disease. The measurements that showed age effects were corrected for subsequent analyses.

**Olfactory fMRI**

Figure 2 displays the olfactory activation maps within the POC from the three study groups for both the odor and no-odor conditions (one sample \( t \)-test,
Fig. 2. Olfactory activation maps. Activation maps (one sample t-tests, \( p < 0.001 \), uncorrected with extent threshold = 6) for both odor (A) and no odor (B). Activation is shown only in the average primary olfactory cortex (POC) from the cognitively normal controls (CN). The color scale indicates the significance of activation. The underlay image for each group is the mean T1-weighted image (Montreal Neuroimaging Institute (MNI) space, \( Z = –28 \) to \( –14 \)) of the subjects within the cohort.

\( p < 0.001 \), extent threshold = 6). In the CN group, strong activation was observed in the POC under both conditions; however, both MCI and AD groups yielded much less activation for both conditions.

The data from the olfactory fMRI paradigm was quantified in terms of activated voxels in the segmented ROI for each subject as shown in Fig. 3. No significant differences between the odor and no-odor conditions were found in the CN, MCI, or AD groups. During the odor conditions where the visual cue “Smell?” was paired with odorant, the activation in the POC showed significant group differences (one-way ANOVA, \( p = 0.0002 \)). A multiple comparisons test revealed that both patient groups had less than 50% activation when compared to the CN group, with both the MCI and AD groups presenting nearly the same level of reduction in fMRI activation in the POC. Similarly, during the no-odor condition where the visual cue “Smell?” was paired with odorless air, the activation in the POC (one-way ANOVA, \( p = 0.0007 \)) showed significant differences among the three groups. Specifically, the activation showed a stepwise descending trend with CN having significantly greater activation than MCI, and MCI than AD (did not reach statistical significance).

**Correlation between the behavioral and MRI results**

Positive correlations were demonstrated between the behavioral tests and activation in the POC during both the odor and no-odor conditions; greater functional activation was correlated with higher cognitive and olfactory scores (Table 2). When investigating the
Fig. 3: Activation in the primary olfactory cortex (POC, mean ± standard error) during odor and no odor conditions. The activation in the POC in mild cognitive impaired (MCI) and Alzheimer’s disease (AD) subjects was decreased by more than 50 percent than that of the cognitively normal controls (CN) during odor presentation. The no odor conditions showed a decrease in activation in a more stepwise fashion. *p ≤ 0.05, ANOVA when compared to CN- Odors. †p < 0.05, ANOVA when compared to CN- No Odor.

correlation between behavioral tests and activation in only the patient cohort (MCI and AD- both with significant cognitive loss), a significant correlation (p < 0.05) was found between the cognitive results and activation within the POC during the no odor condition only.

DISCUSSION

In this study, the use of a visual cue without direct olfactory afferent inputs allowed for separation of functional activations in the brain from the influence of the peripheral olfactory sensory system. Previously, we demonstrated that a preceding olfactory stimulus paired with the visual cue is necessary and sufficient to induce central olfactory activation by the subsequent visual cue during the no odor conditions [49]. By presenting a visual cue with interleaved odor and no-odor pairings, we established a task that requires constant updating of visual and odor association information. We demonstrated that a visual cue with odor and no-odor conditions elicited similar activation levels in the age-matched CN group, which suggested that brain activation in the central olfactory structures could be obtained without an afferent input from the peripheral olfactory system, but likely via a top-down mechanism that is initiated by an initial afferent stimulation during the odor conditions. Thus, the significant activation decline under the no-odor conditions in the POC in MCI and AD should be a result of, or at least dominantly caused by degeneration in the central olfactory structures. Our data support the hypothesis that the olfactory deficit observed in AD and MCI patients is contributed to by the degeneration of the central olfactory system.

It is important to note that the paradigm utilized in this investigation is not a simple olfactory sensory stimulation paradigm, but involved olfactory and visual associations, which requires working memory in the associative process. The pairing of visual and olfactory stimuli and their association enhance the activation in the central olfactory regions as we previously demonstrated in a young healthy cohort [35], which, in turn, improves the reliability of the olfactory fMRI data. As a result, this study found that the odor and no-odor conditions exhibited a similar activation volume in the POC and both conditions showed group differences.

Table 2: Correlations between behavioral and imaging measurements

<table>
<thead>
<tr>
<th></th>
<th>POC- All Subjects Odor Condition</th>
<th>POC- All Subjects No-odor Condition</th>
<th>POC- Patients Odor Condition</th>
<th>POC- Patients No-odor Condition</th>
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<tbody>
<tr>
<td>CVLT-II</td>
<td>p = 0.0002</td>
<td>p &lt; 0.0001</td>
<td>p = 0.021</td>
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<td></td>
<td>r = 0.39</td>
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<td>MMSE</td>
<td>p = 0.001</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
<td>p &lt; 0.002</td>
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<td></td>
<td>r = 0.40</td>
<td>r = 0.54</td>
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<tr>
<td>DRS-2</td>
<td>p = 0.0008</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
<td>p = 0.029</td>
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<td>r = 0.41</td>
<td>r = 0.54</td>
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<tr>
<td>DRS-mem</td>
<td>p = 0.0004</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
<td>p = 0.013</td>
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<tr>
<td></td>
<td>r = 0.43</td>
<td>r = 0.61</td>
<td>r = 0.41</td>
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<tr>
<td>UPSIT</td>
<td>p = 0.0002</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
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<td></td>
<td>r = 0.45</td>
<td>r = 0.48</td>
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POC, primary olfactory cortex; UPSIT, University of Pennsylvania Smell Identification Test; CVLT-II, California Verbal Learning Test- Short Form Version 2; not significant, NS; MMSE, Mini-Mental State Examination; DRS-2, Dementia Rating Scale 2; DRS-mem, memory portion of the DRS-2. Patients include all MCI and AD volunteers.
in AD and MCI patients during an odor-visual association task. The paradigm involved presentation of a visual cue that was accompanied either with an odor or with odorless air. The paradigm allowed for investigation of the differences between the three groups with and without an afferent olfactory stimulus. This is important because prior studies and UPSIT scores demonstrate olfactory deficits in AD and MCI patients; therefore, the odor condition provides a stimulus that is influenced by the status of the peripheral olfactory system of the three groups. As indicated, MCI subjects had significantly decreased activated volumes in the POC, as in AD. The condition with the visual cue without olfactory stimulation allowed investigation of activation patterns in the three groups when the peripheral olfactory system was not directly involved. For each group, no significant differences between the odor and no odor condition were observed. Both conditions showed significant group differences with the AD and MCI groups having less activation than the CN subjects; however, the trend of activation decrease in the MCI and AD group was different.

POC activation correlated positively with the cognitive and olfactory tests. Of note, the activation under the no-odor condition showed significant positive correlations with all cognitive tests and the UPSIT, suggesting that the odor-visual association paradigm may be sensitive in detection of early stage cognitive decline in AD, as it is involved in working memory and associative learning [49]. Specifically, when only investigating the patient cohorts, only the no-odor condition showed correlation between activation within the POC and cognitive tests. For the odor condition, while the MCI group has significantly higher performance on the behavioral tests, they have similar average POC activation to that of AD with a larger variability, rendering the correlation between cognition and activation to be nonsignificant. Whereas, during the no-odor condition the MCI group had greater activation than the odor condition (difference did not reach significance). This may reflect the cognitive decline in the MCI group when processing odor-visual association cues.

Our results are supported by previous findings in both behavioral and pathological studies. Central olfactory dominance for olfactory deficits in AD has been suggested by studies showing greater deficits in odor identification tests than odor detection tests [7, 9]. Identification of an odor requires memory functions and one of the key symptoms in AD is a memory functional deficit, suggesting a close relationship between olfaction and memory deficits. Postmortem and imaging studies report excessive amyloid plaques, neurofibrillary tangles, and atrophy in the central olfactory regions including the olfactory bulb and tracts, anterior olfactory nucleus, entorhinal cortex, and the piriform [21, 24, 25, 50–53]. To our knowledge, very few studies have reported on pathology within the POC specifically in humans [53]. While this study did not collect data regarding pathology within the POC from our volunteers due to the invasiveness of positron emission spectroscopy, we have reported decreased POC volumes within the AD and MCI groups compared to normal controls (same sample as current investigation) suggesting atrophy [28]. Even when controlling for the volume of the POC, significant activation changes were found in MCI and AD patients compared to normal controls. Further investigation of pathology in the POC would provide insight into how pathological findings relate to degeneration of the POC and how that influences olfaction and activation to olfactory stimulation.

Several limitations in our study should be addressed. First, the current study is limited by the relatively small sample size of the patient groups and our MCI group consisted of patients who may and may not convert to AD. Second, treatment was not controlled in the MCI group; however, MCI patients being treated with cholinesterase inhibitor and/or memantine did not significantly differ in cognitive and olfactory performance or POC activation from MCI patients not on a treatment regimen. We did not collect data regarding pathology within the POC from our volunteers. This data would provide further insight into how pathological findings relate to degeneration of the POC and how that influences olfaction. While gender was matched between the three cohorts, gender was not equally matched within the AD group (5 male and 10 females) due to difficulty in recruitment of AD patients. Non-parametric analysis showed no significant differences between gender within the AD group in behavioral performance and activation data. Moreover, it is also a possibility that the “Smell?” cue elicited POC activation; however, in a previous study using the same visual cue with no pairing of odor throughout the paradigm in a young healthy cohort, no activation of the POC was observed [49]. Therefore, the odorant is needed for activation in the POC by the visual cue. Another limitation of the current paradigm is
that it does not answer the question of how much activation is due to involuntary stimulation of the olfactory system versus how much activation is due to the active search in odor detection. Inclusion of a paradigm with a condition with odor but no visual cue would allow for a more quantitative separation of these two conditions. While response data was collected, we were not able to use the responses in the analysis of the fMRI data due to failure of the recording system for a majority of the volunteers. In addition, though respiration was monitored throughout fMRI data collection and patients were asked not to sniff and keep a normal breathing pattern, there still remains the possibility that activation during the no odor condition could have been due to the act of sniffing, which has been shown to cause activation in the POC [54]. In the current study, the POC was examined during an olfactory paradigm; however, it is possible that diminution in activation may be one that is global for the patient group rather than specific to olfaction. The visual cortex and motor cortex were investigated during the paradigm to confirm an olfactory effect rather than that of a global effect. Both visual and motor cortex activations showed no significant differences between the three cohorts, suggesting olfactory-specific effects and not global effects. Finally, while the results in this study demonstrated the involvement of the central olfactory system in the olfactory deficits in MCI and AD subjects, it cannot rule out the involvement of the peripheral system.

In summary, the novel visual-odor association paradigm utilized in this study allowed us to specifically examine the functional deficits of central olfactory nervous system, the site of early degeneration of AD. The results of our olfactory fMRI study demonstrated that the central olfactory system dysfunction may be the dominant reason for the olfactory deficits present in AD and MCI subjects.

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Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/17-0310r2).

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