Unirhinal Olfactory Testing for the Diagnostic Workup of Mild Cognitive Impairment

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Handling Associate Editor: Nan Wu

Accepted 14 April 2015

Abstract

Background: Olfactory dysfunction is associated with Alzheimer’s disease (AD), and already present at pre-dementia stage. Objectives: Based on the assumption that early neurodegeneration in AD is asymmetrical and that olfactory input is primarily processed in the ipsilateral hemisphere, we assessed whether unirhinal psychophysical and electrophysiological assessment of olfactory function can contribute to the diagnostic workup of mild cognitive impairment (MCI).

Methods: Olfactory function of 13 MCI patients with positive amyloid PET, 13 aged-matched controls (AC) with negative amyloid PET and 13 patients with post-infectious olfactory loss (OD) was assessed unirhinally using (1) psychophysical testing of olfactory detection, discrimination and identification performance and (2) the recording of olfactory event-related brain potentials. Time-frequency analysis was used to enhance the signal-to-noise ratio of the electrophysiological responses. Psychophysical and electrophysiological assessment of auditory and trigeminal chemosensory function served as controls.

Results: As compared to AC and OD, MCI patients exhibited a significant asymmetry of olfactory performance. This asymmetry efficiently discriminated between MCI and AC (sensitivity: 85%, specificity: 77%), as well as MCI and OD (sensitivity: 85%, specificity: 70%). There was also an asymmetry of the electrophysiological responses, but not specific for MCI. In both MCI and OD, olfactory stimulation of the best nostril elicited significantly more activity than stimulation of the worse nostril, between 3–7.5 Hz and 1.2–2.0 s after stimulus onset. Trigeminal and auditory psychophysical testing did not show any difference between groups.

Conclusion: MCI patients exhibit a marked asymmetry of behavioral olfactory function, which could be useful for the diagnostic workup of MCI.

Keywords: Alzheimer’s disease, EEG, evoked potentials, mild cognitive impairment, olfaction, smell

INTRODUCTION

Alzheimer’s disease (AD) is the most frequent cause of dementia, accounting for more than half of the cases of dementia [1]. The clinical symptoms of AD are characterized by a progressive deterioration of higher brain functions, involving memory loss and cognitive decline, affecting daily life activities [2, 3]. It is widely admitted that the disease progresses over years before reaching the dementia stage. The pathophysiological characteristics of AD are the progressive accumulation of neurofibrillary tangles and amyloid plaques in the...
central nervous system. Following Braak's staging, the accumulation of neurofibrillary tangles typically starts in the limbic and paralimbic regions of the brain [4]. As the disease progresses, neurofibrillary tangles and amyloid plaques tend to expand to neocortical areas, leading to global deficits in attention, memory, and behavior. Hence, dementia is present only in the later stages of the disease, and early diagnosis of AD is crucial to ensure optimal medical and social intervention.

Mild cognitive impairment (MCI) is characterized by progressive memory loss, with preserved global cognitive function and no major impact on daily life activities. Studies have shown that approximately 10% to 15% of MCI patients evolve toward AD each year, at least during the first years after the diagnosis of MCI [5, 6]. However, not all patients presenting with MCI evolve toward AD. Some patients remain stable or even recover a normal cognition [7–9]. Approximately 70% of MCI patients actually develop AD [10], and 30% of MCI patients do not present the neuropathological features of AD at autopsy [11]. Other neurological disorders, in particular, depression and age-related cognitive decline, are also potential causes of MCI [12]. Hence, the early diagnosis of AD on clinical grounds alone remains problematic.

In April 2011, new criteria for AD diagnosis were officially established by the National Institute of Aging (NIA) in three different publications. The first focused on the preclinical stage of AD [13]. The second focused on MCI [14]. The third focused on dementia [3]. It was proposed that AD is a continuum where the preclinical stage precedes MCI, which itself precedes AD dementia. The diagnoses are based on both clinical and pathological criteria. The authors emphasize on the importance of using accurate tests to characterize memory deficits. Biomarkers also contribute to the diagnosis: (1) biomarkers of amyloid-β deposit (low amyloid in the cerebrospinal fluid (CSF), amyloid ligand positron-emission tomography (PET)), and (2) biomarkers of neural degeneration (increased tau in CSF, medial temporal lobe atrophy on MRI, reduced glucose metabolism in parietal regions on fludeoxyglucose PET).

Over the last decades, the finding of an association between olfactory disorders and AD has generated interest in the scientific community. It is well known that olfactory function decreases with age. However, as compared to age-matched controls, AD patients exhibit a stronger loss of olfactory function. This difference is already present in the early course of the disease, coinciding or even preceding the onset of cognitive symptoms [15–21]. Studies have shown that patients with amnestic MCI present a deficit in the identification of odors [15, 22] and, most importantly, that olfactory deficit in amnestic MCI patients is predictive of evolution toward AD [23]. In addition, it has been suggested that a deficit in odor identification can be detected before patients are classified as cognitively impaired [24].

The neuropathological changes in AD patients are asymmetrical [25, 26] and result in different cognitive profiles [27]. Based on the assumption that early neurodegeneration in AD is asymmetrical and that olfactory input from each nostril is primarily processed in the ipsilateral hemisphere, it has recently been proposed that unihemispheric assessment of odor identification and detection could contribute to the evaluation of amnestic MCI and AD patients [28, 29]. Indeed, the asymmetrical progression of AD [25, 30, 31] may be expected to lead to a significant asymmetry in olfactory performance.

Assessment of olfactory function has thus been proposed as a tool for the early diagnosis of AD. Olfactory function can be investigated using various psychophysical tests, such as tests to assess odor detection threshold, discrimination, identification, recognition memory, and naming. These different abilities are thought to reflect different levels of olfactory processing [32–37]. The majority of studies have only assessed olfactory detection thresholds and odor discrimination, and the obtained results are conflicting. This could be due to the diversity of the psychophysical approaches used to assess olfaction, the low reliability of the methods used to assess olfactory detection threshold, and the small sample sizes [18, 39]. Another important issue is the use of different criteria to define MCI and AD, making between-study comparisons difficult.

A small number of studies have assessed olfactory function in AD by recording olfactory event-related potentials (OERPs) [22, 40–42]. Although this is a relatively unbiased technique requiring limited patient collaboration, published results are contradictory. Some studies found abnormal OERPs despite normal psychophysical function [42], and others reported normal OERPs despite abnormal odor identification scores [22, 40]. Moreover, a study showed that the latencies of OERPs are significantly increased in AD patients and that this increase in latency significantly correlates with...
The reasons for these conflicting results might be the heterogeneity of the studied populations and, most importantly, the fact that OERPs have a poor signal-to-noise ratio [43], particularly in aged subjects. In order to recruit more homogeneous samples of MCI patients that really present incipient AD changes it was recently proposed to use biological markers in addition to traditional cognitive evaluation [14].

The objective of the present study was to assess unirhinal olfactory function in MCI patients using (1) a validated psychophysical method to assess odor identification, threshold and discrimination abilities (Sniffin’ Sticks extended test) [44] and (2) the recording of brain responses to unirhinal olfactory stimulation using a novel approach based on the time-frequency analysis of electroencephalographic (EEG) signals, previously shown to markedly enhance the signal-to-noise ratio of OERPs [45, 46]. We hypothesized that asymmetry in olfactory function could be an early predictor of AD and, hence, that it could distinguish between MCI and age-matched healthy controls. Crucially, we selected a uniform population of MCI patients with positive amyloid PET, a biomarker currently considered as the hallmark of AD patients at a predemential stage of the disease [47] and compared them with a group of healthy, amyloid-negative age-matched controls. Furthermore, we also compared the MCI group to a group of patients presenting with a primary olfactory dysfunction (post-viral olfactory loss).

MATERIALS AND METHODS

Participants

13 amnestic MCI patients with positive amyloid PET (MCI), 13 age-matched controls with negative amyloid PET (AC), and 13 patients suffering from post-infectious olfactory loss (OD) were included in the current study. Written informed consent was obtained from all the participants. The investigations were approved by the local Ethics Committee and done in accordance with the Helsinki declaration of 1975. MCI and AC were recruited from a cohort studied to evaluate the usefulness of the combination of different biomarkers in the classification of non-demented patients attending a memory clinic [48]. MCI patients were at least 50 years old and complained of memory and/or cognitive problems. Evidence by a relative was sought and obtained for a majority of patients. The diagnosis of MCI was based on both clinical findings according to Petersen’s criteria [5], and biomarkers according to the NIA-AA criteria [14]. Dementia was excluded on basis of the DSM-IV-TR criteria [49]. A Mini Mental State Exam (MMSE) score of 24 or more was required [50]. AC subjects were healthy elderly without complaints of memory and/or cognitive problems, recruited by advertisement.

MCI and AC participants underwent the same assessment consisting in a comprehensive neuropsychological examination (see infra), apolipoprotein E genotyping, brain MRI, fluorine-18 fluoro-2-deoxyglucose ([18F]-FDG) PET scan and [F18]-flutemetamol PET scan ([F 18]-flutemetamol is an investigational product being studied clinically as an amyloid imaging agent; for details, see [51]). All MCI patients had an elevated amyloid deposition score ([F18]-flutemetamol SUVr (Standardized Uptake Value)) (see [48] for details on the method), exceeding the score reached by 90% of controls. All AC participants were below the same threshold for amyloid deposition score. Amyloid deposition scores were computed for the right and left neocortex, and the right and left orbitofrontal cortex. We did not explore amyloid load in mesial temporal structures because previous studies have shown that mesial temporal areas are relatively spared of amyloid deposition in the early stages of the disease [4, 52–56].

Patients with postinfectious olfactory loss were recruited from the ENT outpatient clinic. The diagnosis was made by two trained ENT specialists, based on patient history and clinical examination using both rhinoscopy and nasal endoscopy. All patients had a history of olfactory loss following symptoms of common cold.

For all groups, exclusion criteria also comprised major depression and other psychiatric diseases, past or present neurological condition, history of alcohol or drug abuse, and severe head injury. A rhinoscopy and nasal endoscopy was performed in all participants to exclude sino-nasal disease and to evaluate nasal patency of the left and right nostrils. Included subjects had no major asymmetries or obstruction of left and right nasal fossa.

Neuropsychological assessment

Neuropsychological assessment evaluated episodic memory, language, executive functions and visuospatial processing. Episodic memory was tested using the Free and Cued Selective Reminding Test (FCSRT) which requires memorization of 16 visually presented words belonging to 16 different semantic categories used as cues in order to control encoding and retrieval
Table 1
Cognitive performance of MCI patients and age-matched controls

<table>
<thead>
<tr>
<th></th>
<th>Aged controls (AC)</th>
<th>MCI patients</th>
<th>AC versus MCI</th>
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<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
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<tr>
<td><strong>Episodic memory (FCSRT)</strong></td>
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<tr>
<td>– free recall [0–48]</td>
<td>31</td>
<td>28–38</td>
<td>20</td>
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<tr>
<td>– total recall [0–48]</td>
<td>48</td>
<td>46–48</td>
<td>41</td>
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<td>– free delayed recall [0–16]</td>
<td>12.5</td>
<td>11.2–15.7</td>
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<td><strong>Language functions</strong></td>
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<tr>
<td>– LEXIS naming test [0–64]</td>
<td>61</td>
<td>60–64</td>
<td>58</td>
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<tr>
<td>– category fluency</td>
<td>35</td>
<td>32–43</td>
<td>25</td>
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<tr>
<td>– phonological fluency</td>
<td>28</td>
<td>22–35</td>
<td>19</td>
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<td><strong>Executive functions</strong></td>
<td></td>
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<tr>
<td>– TMT A (s)</td>
<td>34</td>
<td>25–42</td>
<td>50</td>
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<tr>
<td>– TMT B (s)</td>
<td>74</td>
<td>62–104</td>
<td>144</td>
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<tr>
<td>– TMT B-A (s)</td>
<td>40</td>
<td>26–43</td>
<td>104</td>
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<td><strong>Visuo-spatial functions</strong></td>
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<td>– clock drawing [0–8]</td>
<td>8</td>
<td>6–8</td>
<td>6</td>
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<td>– clock copy [0–10]</td>
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<td>10–10</td>
<td>10</td>
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<td>– CERAD [0–11]</td>
<td>11</td>
<td>10–11</td>
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IQR, Interquartile range (25–75); FCSRT, Free and Cued Selective Reminding Test; TMT, Trail Making Test.

Results of neuropsychological assessment are reported in Table 1. The detailed demographic results are reported in Supplementary Table 1.

Experimental sessions

Olfactory, trigeminal, and auditory functions were assessed using psychophysical and electrophysiological testing. The two tests were performed in separate sessions, organized on different days.

Psychophysical assessment of olfactory, trigeminal, and auditory functions

Before starting the experiment, subjects were familiarized with the experimental surrounding and the material used for the psychophysical assessment.

Psychophysical evaluation of olfactory function

Psychophysical evaluation of olfactory function was assessed unirhinally using the validated Sniffin’ Sticks test [61, 62]. The non-evaluated nostril was obstructed by asking the subject to close the nostril with the fingertip. The order of the tested nostril was counterbalanced across participants. Odors were presented to the subject using felt-tip pens placed approximately 2 cm in front the nostril. Based on previous studies, we assumed that the score of the best nostril reflected birhinal olfactory score [63, 64].

First, the olfactory threshold (T) was assessed using n-butanol presented by means of a single staircase, using stepwise dilutions in a row of 16 felt tip pens. Subjects were blindfolded. In each trial, three pens are presented in a randomized order, with two pens containing only the solvent and the third containing the odorant at a certain dilution. The task of the subject was to identify the odor-containing pen (three alternative forced choice; 3-AFC) [44].

Second, odor discrimination (D) was assessed by asking the subject to perform a 3-AFC using 16 pairs of odorants. While blindfolded, three pens are presented to the subject, two containing the same odorant and one containing a different odorant. Subjects were asked to identify the different odorant.

Third, odor identification (I) was assessed by asking the subject to identify 16 individual odors by performing a forced choice between four descriptors. To decrease the lexical demand required in the odor identification task, we developed a picture-based odor identification test. Instead of choosing between a list of four verbal descriptors such as in the original Sniffin’ Sticks test, the four descriptors were presented as pictures in front of the subjects. We ensured that subjects understood the meaning of every picture before starting the test. The pictures were named verbally by the experimenter.
simultaneous to their presentation. Subjects were asked
to designate the picture corresponding to the odor by
naming or pointing it. Participants were allowed to smell
the odor for as long as they desired before identify-
ing it. For olfactory discrimination and identification
tests, the order of the tested nostril and the sequence of
presentation were counterbalanced across participants.
Furthermore, the side of stimulation was changed every
8 items. Olfactory threshold (T), discrimination (D),
and identification (I) were summed to obtain the total TDI
score, used to evaluate global olfactory function [61].

To assess the asymmetry in olfactory function, we also
computed the difference (ΔT, ΔD, ΔI, ΔTDI) between
the “best” nostril (TMAX, DMAX, IMAX, TDIMAX) and
the “worse” nostril (TMIN, DMIN, IMIN, TDMIN).

Psychophysical evaluation of trigeminal
chemosensory function

Nasal trigeminal chemosensory function was
assessed using a lateralization test based on previous
studies [65, 66], using a device consisting in two par-
allel syringes (total volume 50 ml) with their spouts
angled so that the vapors from one were directed to
left nostril and the vapors of the other were directed
to the right nostril. One syringe contained 20 ml of
menthol diluted in propylene glycol (50%). The other
contained 20 ml of odorless propylene glycol. Air
from the headspace of the syringes was delivered in
a uniform manner by pressing the common bottom of
the syringes. Subjects were stimulated passively and
were blindfolded. They received 26 stimuli, counter-
balanced in a pseudorandom sequence. Subjects had to
indicate which nostril was stimulated with menthol.

Picture-based auditory-identification test

As a control to the odor picture identification test,
we developed a picture-based auditory identification
test. Sixteen animal noises were presented to subjects.
Subjects were asked to select the corresponding animal
within four pictures of animals presented in front of
them.

Electrophysiological assessment of olfactory,
trigeminal and auditory functions

Before the electrophysiological recording, subjects
were familiarized with the experimental surrounding,
as well as the olfactory, trigeminal and auditory stimuli
used to elicit chemosensory and auditory event-related
potentials (ERPs).

Chemosensory stimuli

Chemosensory stimuli were produced by an air-
dilution olfactometer (OM2S, Burghart Medical
Technology, Wedel, Germany). The device is able to
deliver brief pulses of odorant embedded within a con-
stant airflow. The rapid switching between the odor
and the control airflow is based on a vacuum line.
During the stimulation, the airflow (8 l/min), temper-
ature (36 °C) and humidity (80% relative humidity)
remain strictly unchanged, thus avoiding any con-
stantant stimulation of mechanical or heat sensitive
trigeminal receptors. Selective olfactory and trigem-
inal chemosensory stimulation was achieved using 2-
phenylethanol (50% v/v) and gaseous CO2 (55%
v/v), respectively [67, 68]. The stimuli were deliv-
ered through a Teflon™ tube placed in the nostril,
just behind the nasal valve, pointing toward the olfac-
tory cleft. Stimulus duration was 200 ms, with a rising
time of 20 ms. Each type of stimulus was repeated 30
times, and delivered in alternation using an interstim-
ulus interval varying randomly between 15 and 20 s.
Subjects were instructed to breathe through the mouth
and to perform velo-pharyngeal closure. The stimuli
were delivered to the right and left nostril, in two dif-
ferent sessions. The two sessions were separated by
a few minutes of rest. The order of the sessions was
counterbalanced across subjects.

Auditory stimuli

40 auditory stimuli (800 Hz tone) were delivered
binaurally through earphones at a comfortable hear-
ing level. Stimulus duration was 50 ms with a rising
and falling time of 10 ms. The inter-stimulus interval
was randomized, ranging between 3 and 6 s.

Electroencephalographic recording

Subjects were instructed to keep their eyes open
during the recording. The EEG was continuously
recorded from 64 Ag/AgCl electrodes placed on the
scalp according to the International 10/10 system
(Waveguard64 cap, Cephalon A/S, Denmark). Scalp
signals were recorded using an average reference. Ocu-
lar movements and eye-blinks were recorded using
two additional bipolar surface electrodes placed on
the upper-left and lower-right sides of the left eye.
Impedance was kept below 5 kOhm. Signals were
amplified and digitized at a 1000 Hz sampling rate
(64-channel ASA-LAB EEG system, Advanced Neuro
Technologies, The Netherlands). The recording of
auditory ERPs followed the recording of chemosensory ERPs.

Data preprocessing

All EEG processing steps were carried out using Letswave 5 (http://www.nocions.org/letswave/), running on Matlab (MathWorks, USA). The continuous EEG data was band-pass filtered using a 0.1–30Hz Butterworth zero phase filter, and segmented into 4 s epochs ranging from $-1.5$ to $+2.5$ s relative to stimulus onset. After baseline correction (reference interval: $-1.5$ to $0$ s), electrooculographic (EOG) artifacts were isolated using an Independent Component Analysis (ICA) using the runica algorithm [69]. Artifact-free EEG epochs were generated by removing independent components (ICs) capturing clear EOG artifacts (time course typical of eye blinks, frontal scalp topography) [70]. Finally, epochs with amplitude values exceeding $\pm 100\mu$V (i.e., epochs likely to be contaminated by an artifact) were rejected ($24\pm14\%$ of olfactory epochs, $26\pm16\%$ of trigeminal epochs, and $19\pm12\%$ of auditory epochs). Finally, signals were re-referenced to the average of the left (M1) and right (M2) mastoids.

Across trial averaging in the time-frequency domain

Similarly to our previous studies, a time-frequency (TF) representation based on the continuous Morlet wavelet transform (CWT) of EEG epochs was used to characterize the amplitude of oscillatory activity as a function of time and frequency [45, 46]. The Morlet wavelet consists in a complex exponential function localized in time by a Gaussian envelope. The initial spread of the Gaussian wavelet was set at $2.5/\pi \omega_0$ ($\omega_0$ being the central frequency of the wavelet). Explored frequencies ranged from 0.2 to 15 Hz in steps of 0.074 Hz.

To obtain a time-frequency representation of both phase-locked and non-phase locked EEG responses to olfactory, trigeminal and auditory stimulation, the time-frequency transform was applied to each single EEG epoch. For each subject and stimulus type, single-trial TF maps expressing signal amplitude were then averaged across trials (see [45, 46] for more details).

To assess the asymmetry of the EEG responses elicited by olfactory stimulation of the “best” versus the “worse” nostril (determined according to the results of the Sniffin’ Sticks test), the TF maps obtained from the “worse” nostril were subtracted from the TF maps obtained from the “best” nostril.

Statistical analyses

Statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). The level of significance was set at $p<0.05$.

Neuropsychological scores

A Mann-Whitney test was used to compare the results of neuropsychological testing between the AC and MCI groups.

Psychophysical scores of olfactory, trigeminal and auditory function

A Kruskall-Wallis test was used to compare the measures obtained in each of the three different groups (MCI, AC, OD). When significant, post-hoc Mann-Whitney tests corrected for multiple comparisons (Bonferroni) were performed.

For the different psychophysical scores showing significant group-level differences, Receiver Operating Characteristic (ROC) curves were constructed to evaluate their ability to distinguish between MCI, AC and OD groups at individual level. The area under the ROC curve (AUC) was used as an index of discrimination performance. An AUC of 0.5 indicates random performance, whereas an AUC of 1 and 0 denotes perfect performance. For each measure, the ability to distinguish between different groups of patients was assessed by examining whether AUC was significantly different from 0 [71, 72]. When significant, the cut-off value (J) associated with the greatest Youden index ($\gamma = \text{Sensitivity} + \text{Specificity} - 1$) was chosen as decision criterion [73, 74]. This cutoff value corresponds to the point on the ROC curve that is the farthest from the diagonal line.

EEG responses to olfactory, trigeminal and auditory stimulation

To assess significant differences between the TF maps of the EEG responses to olfactory, trigeminal and auditory stimulation obtained in the MCI, AC, and OD groups, point-by-point two-sample $t$-tests with
cluster-based thresholding were performed. Cluster-based thresholding is an approach commonly used in neuroimaging. This technique assumes that true neural activity will tend to stimulate signal changes over contiguous pixels of the TF maps [75, 76]. First, raw statistical TF maps were thresholded at \( p < 0.05 \) and \( p < 0.01 \) to identify the clusters of contiguous pixels which differed significantly between the two groups. Second, permutation testing (1000 permutations) was used to assess the distribution of clusters sizes in the permuted data. Cluster size threshold was set at \( Z > 3 \) standard deviations from the mean. This yielded, TF maps highlighting the regions where the EEG signal differed significantly between the two groups.

Correlation between olfactory testing, neuropsychological testing, and amyloid load

The correlations between the psychophysical olfactory scores, the magnitude of the EEG responses to olfactory stimulation, scores of neuropsychological testing and amyloid load were assessed in MCI patients using Spearmans’ correlation coefficient.

RESULTS

A total of 13 subjects were included in the MCI (5 women; mean age: 70.46 ± 5.97; 1 smoker), AC (7 women; mean age: 69.69 ± 8.35; 2 smokers), and OD (10 women, mean age: 52.00 ± 9.78; 2 smokers) groups. There was no significant difference regarding gender between the three groups (\( \chi^2 = 2.75, p = 0.252 \)). As expected, subjects in the OD group were, on average, significantly younger as compared to subjects in the MCI (\( p < 0.001 \)) and AC (\( p < 0.001 \)) groups. MCI and AC subjects were similar in terms of age (\( p = 0.812 \)). There was no difference regarding smoking status between the three groups (\( \chi^2 = 0.46, p = 0.795 \)).

Psychophysical assessment of olfactory performance

The results of individual olfactory psychophysical assessment are presented in Table 2. Global TDI scores of olfactory performance obtained from the best nostril (TDMAX, reflecting the scores which would have been obtained using bimodal stimulation) were 27.0 ± 3.7, 23.9 ± 7.7, and 21.8 ± 6.9 in the AC, MCI and OD groups, respectively (mean ± standard deviation) (Fig. 1). These differences were not significant (\( \chi^2 = 4.424, p = 0.110 \)). Global TDI scores of olfactory performance obtained from the worse nostril (TDIMIN) were 24.6 ± 4.1, 19.0 ± 6.5, and 19.1 ± 6.5 in the AC, MCI, and OD groups, respectively. These differences were significant (\( \chi^2 = 7.531, p = 0.023 \)). However, post-hoc test corrected for multiple comparisons did not show any significant difference. Regarding the left or right predominance for the best nostril, we observed that 6/13 AC, 8/13 MCI, and 3/13 OD had a right predominance, 1 AC had symmetrical performances. The predominant side was not significant between the three groups (\( \chi^2 = 4.703, p = 0.095 \)).

The asymmetry between the TDI scores obtained from the best and worse nostril (\( \Delta \)TDI) were 2.4 ± 1.8, 5.0 ± 1.7 and 2.7 ± 1.5 in the AC, MCI, and OD groups, respectively (Fig. 2). These differences were significant (\( \chi^2 = 11.968, p = 0.003 \)). Post-hoc comparisons revealed that the asymmetry in global olfactory performance was significantly greater in MCI patients as compared to both AC subjects (\( p = 0.006 \)) and OD patients (\( p = 0.009 \)).

Olfactory detection scores obtained from the best nostril (\( T_{\text{MAX}} \)) were 6.0 ± 2.0 in AC, 5.2 ± 2.7 in MCI and 3.0 ± 2.4 in OD (Fig. 1). These differences were significant (\( \chi^2 = 9.618, p = 0.008 \)). Post-hoc comparisons showed that OD had significantly lower \( T_{\text{MAX}} \) as compared to AC (\( p = 0.009 \)). However, there was no significant difference between MCI and AC subjects (\( p = 0.448 \)), nor between MCI and OD subjects (\( p = 0.066 \)). Olfactory threshold scores obtained from the worse nostril (\( T_{\text{MIN}} \)) were 4.6 ± 2.0 in AC, 3.4 ± 2.3 in MCI, and 2.3 ± 1.7 in OD. These differences were also significant (\( \chi^2 = 8.362, p = 0.015 \)). Post-Hoc tests showed that OD had lower \( T_{\text{MIN}} \) as compared to AC (\( p = 0.015 \)). However, there was no significant difference between MCI and AC subjects (\( p = 0.303 \)), nor between MCI and OD subjects (\( p = 0.507 \)).

The asymmetry of olfactory thresholds (\( \Delta T \)) was 1.4 ± 1.3 in AC, 1.8 ± 1.2 in MCI, and 0.7 ± 0.9 in OD (Fig. 2). These differences in threshold asymmetry were significant (\( \chi^2 = 6.773, p = 0.034 \)). Post-Hoc tests showed that MCI had greater asymmetry compared to OD (\( p = 0.048 \)). However, there was no significant difference between MCI and AC subjects (\( p = 0.922 \)), nor between AC and OD subjects (\( p = 0.216 \)).

Olfactory discrimination scores obtained from the best nostril (\( \Delta \text{D}_{\text{MAX}} \)) were 10.4 ± 1.9 in AC, 9.6 ± 2.3 in MCI, and 9.8 ± 2.2 in OD (Fig. 1). Olfactory discrimination scores obtained from the worse nostril (\( \Delta \text{D}_{\text{MIN}} \)) were 9.0 ± 1.6 in AC, 7.3 ± 2.2 in MCI, and 8.6 ± 2.3 in OD. These differences were not significant (\( \chi^2 = 0.818, p = 0.664; \Delta \text{D}_{\text{MIN}}: \chi^2 = 0.355 \)).
<table>
<thead>
<tr>
<th>Age</th>
<th>Olfactory disorder</th>
<th>Best TDmax</th>
<th>TDmin</th>
<th>Trax</th>
<th>Trmin</th>
<th>Dmax</th>
<th>Dmin</th>
<th>Imax</th>
<th>Imin</th>
<th>Auditory</th>
<th>Trigeminal</th>
<th>Amyloid neocortex</th>
<th>Amyloid orbitofrontal cortex</th>
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T, Threshold; D, Discrimination; I, Identification; R, Right; L, Left; S, Symmetric.
Fig. 1. Psychophysical scores of olfactory function measured using the Sniffin’ Sticks test in aged controls (AC), patients with mild cognitive impairment (MCI), and patients with post-infectious olfactory loss (OD). The TDI score (0–48) corresponds to the sum of the detection threshold (T: 0–16), discrimination (D: 0–16), and identification (I: 0–16) scores. Results obtained from the best and worse nostrils are shown in black and white bars, respectively (mean ± 95% confidence interval). Statistically significant differences between groups are represented by *$p<0.05$ and **$p<0.01$.

Fig. 2. The asymmetry of psychophysical olfactory performance in aged controls (AC), patients with mild cognitive impairment (MCI) and patients with post-infectious olfactory loss (OD) was assessed by computing the difference between the TDI ($\Delta$TDI), detection threshold ($\Delta$T), discrimination ($\Delta$D) and identification ($\Delta$I) scores obtained from the best and worse nostril (mean ± 95% confidence interval). Statistically significant differences between groups are represented by *$p<0.05$ and **$p<0.01$.

The asymmetry of olfactory discrimination ($\Delta$D) was 1.4 ± 1.1 in AC, 2.3 ± 1.0 in MCI, and 1.2 ± 1.1 in OD (Fig. 2). These differences in discrimination asymmetry were significant ($\chi^2 = 7.709, p = 0.021$). Post-hoc comparisons showed that MCI patients had a significantly greater asymmetry in discrimination as compared to OD ($p = 0.036$), but not as compared to AC ($p = 0.132$).

Picture-based olfactory identification scores obtained from the best nostril ($I_{\text{MAX}}$) were 11.9 ± 2.2 in AC, 9.5 ± 3.5 in MCI, and 9.2 ± 3.4 in OD (Fig. 1). These differences did not reach significance ($I_{\text{MAX}}$: $\chi^2 = 5.551, p = 0.062$; $I_{\text{MIN}}$: $\chi^2 = 2.661, p = 0.264$). The asymmetry of olfactory identification scores ($\Delta$I) was 2.1 ± 1.8 in AC, 1.5 ± 1.3 in MCI, and 1.2 ± 1.1 in OD (Fig. 2). These differences were not significant ($\chi^2 = 1.804, p = 0.406$).

Discrimination performance of the psychophysical measures of olfaction

The discrimination performance of the psychophysical measure of olfaction showing a significant group-level difference between MCI and AC ($\Delta$TDI) was assessed using ROC curves (Fig. 3). The asymmetry in the TDI scores obtained from the best and worse nostrils ($\Delta$TDI) was able to discriminate between MCI versus AC (AUC = 0.846, $p = 0.003$; sensitivity: 85%; specificity: 77%), MCI versus OD (AUC = 0.834, 95% confidence interval). Statistically significant differences between groups are represented by *$p<0.05$ and **$p<0.01$.  

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Correlation between psychophysical olfactory performance and neuropsychological evaluation

In MCI patients, we evaluated the correlation between the results of the different cognitive tests and ΔTDI. There was a small negative correlation between ΔTDI and the FCSRT free delayed recall test ($r = -0.648$, $p = 0.023$; corrected for multiple comparisons). Moreover, because of the known hemispheric lateralization of cognitive functions [77–79], we assessed the correlation between the results of the different cognitive tests and the difference of TDI scores obtained at the left and right nostril. No significant correlation was found. Detailed results are reported in Table 3.

Correlation between psychophysical olfactory performance and amyloid deposition degree

In MCI patients, we evaluated the correlation between the asymmetry of amyloid deposition degree and the asymmetry of olfactory performance. For the measures of amyloid load in the neocortex, there was no correlation between ΔTDI and the difference in amyloid load between the ipsilateral and contralateral hemisphere relative to the best nostril ($r = -0.091$, $p = 0.767$). For the measures of amyloid load in the orbitofrontal cortex, there was a non-significant trend toward a correlation ($r = 0.525$, $p = 0.065$).

In addition, we also evaluated the correlation between the right-left asymmetry of olfactory performance (right TDI–left TDI) and the right–left asymmetry of amyloid deposition degree. There was no significant correlation ($r = 0.178$, $p = 0.438$).

Table 3

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FCSRT, Free and Cued Selective Reminding Test. TMT, Trail Making Test.

Correlation between amyloid deposition degree and cognitive score

In MCI patients, there was no significant correlation between the different cognitive tests and the amyloid deposition degree.

Psychophysical assessment of trigeminal chemosensory performance

The individual results of the psychophysical assessment of trigeminal chemosensory performance are
presented in Table 2. Trigeminal lateralization scores were 16.5 ± 2.6 in AC, 15.5 ± 3.6 in MCI, and 16.2 ± 5.3 in OD. These differences were not significant ($\chi^2 = 0.931$, $p = 0.628$).

**Psychophysical assessment of auditory performance**

The individual results of the psychophysical assessment of auditory performance are presented in Table 2. The scores of the picture-based auditory identification test were 15.4 ± 0.7 in AC, 15.6 ± 0.7 in MCI, and 15.9 ± 0.3 in OD. These differences were significant ($\chi^2 = 6.221$, $p = 0.045$). However, post-hoc tests, corrected for multiple comparisons, did not show any significant differences between the different groups.

**EEG responses to olfactory stimulation**

Three subjects (1 AC, 1 MCI, and 1 OD) were excluded from these analyses because the recorded signals were contaminated by a large number of artifacts.

The group-level average TF maps obtained at electrode Cz in each group are shown in Fig. 4. Olfactory stimulation elicited a long lasting increase of EEG oscillations at very low frequencies (<1 Hz), maximal at electrode Cz. We were not able to identify a consistent response at higher frequencies, as previously reported in recordings obtained from young normosmic subjects [45]. Statistical comparisons of the TF maps obtained at electrode Cz revealed no significant difference between the TF maps of AC, MCI, and OD groups, following stimulation of the best and the worse nostril.

The group-level average TF maps of the difference between the responses elicited by stimulation of the best and worse nostril are shown in Fig. 5. In the AC group, there was no obvious difference between the two TF maps. In contrast, in the MCI group, stimulation of the best nostril elicited greater activity than stimulation of the worse nostril between 4 and 8 Hz and 1 to 2 s after stimulus onset, as well as between 1 and 2 Hz and 0.5 to 1.5 s after stimulus onset. A similar difference was observed in the OD group.

Point-by-point statistical comparisons of the difference maps obtained in AC and MCI revealed a significant cluster between 4 and 7.5 Hz and 1.2 to 1.8 s after stimulus onset (Fig. 5).
Fig. 5. Group-level average time-frequency (TF) maps expressing the difference between the responses obtained by stimulation of the best nostril and stimulation of the worse nostril in healthy aged controls (AC), patients with mild cognitive impairment (MCI) and patients with post-infectious olfactory loss (OD). In the AC group, there was no obvious difference between the responses elicited by stimulation of the best and worse nostril. In contrast, stimulation of the best nostril elicited significantly greater activity than stimulation of the worse nostril between 4–8 Hz and 1–2 s in the MCI group. A similar difference was observed in the OD group. Comparison of the difference TF maps obtained in AC versus MCI and AC versus OD was performed using point-by-point t-tests with cluster-based thresholding (see Methods). The significant clusters (Z > 3) between AC versus MCI and AC versus OD are shown in black (p < 0.05) and red (p < 0.01) contours.

There was also a significant difference between the difference maps of AC and OD between 3 and 6 Hz and 1.5 and 2.0 s after stimulus onset. There was no significant difference between the difference maps of MCI and OD.

Correlation between EEG responses to olfactory stimulation and psychophysical olfactory performances

At the whole group level, there was a significant correlation between the difference in magnitude of the EEG responses elicited by stimulation of the best and worst nostril at electrodes Fz, Cz and Pz, (ΔFz, ΔCz, and ΔPz) and the difference in TDI scores obtained at the best and worse nostril (ΔFz versus ΔTDI: r = 0.471, p = 0.004; ΔCz versus ΔTDI: r = 0.556, p < 0.001; ΔPz versus ΔTDI: r = 0.549, p = 0.001). In the AC group, there was a significant correlation between ΔPz and ΔTDI (r = 0.582, p = 0.047), but not between ΔFz and ΔTDI (r = 0.148, p = 0.646) and between ΔCz and ΔTDI (r = 0.339, p = 0.282). In the OD group, there was a significant correlation between ΔFz and ΔTDI (r = 0.589, p = 0.044), as well as between ΔPz and ΔTDI (r = 0.674, p = 0.016), but not between ΔCz and ΔTDI (r = 0.511, p = 0.090).
the MCI group, there was no significant correlation between the asymmetry of the EEG responses and the asymmetry of the psychophysical scores (ΔFz versus ΔTDI; r = 0.046, p = 0.888; ΔCz versus ΔTDI; r = 0.217, p = 0.498; ΔPz versus ΔTDI; r = 0.319, p = 0.313).

**Correlation between EEG responses to olfactory stimulation and cognitive scores**

In MCI patients, there was no correlation between the magnitude of the asymmetry of the EEG responses to olfactory stimulation and the different cognitive scores.

**Correlation between EEG responses to olfactory stimulation and amyloid deposition degree**

In MCI patients, there was no correlation between the magnitude of the asymmetry of the EEG responses to olfactory stimulation and the asymmetry of amyloid load measured in the neocortex (ΔFz: r = 0.147, p = 0.648; ΔCz: r = 0.140, p = 0.664; ΔPz: r = 0.063, p = 0.845), and the asymmetry of amyloid load measured in the orbitofrontal cortex (ΔFz: r = 0.102, p = 0.752; ΔCz: r = 0.201, p = 0.531; ΔPz: r = 0.071, p = 0.828).

**EEG responses to trigeminal chemosensory stimulation**

The group-level average TF maps obtained at electrode Cz in each group are shown in Fig. 4 (averaging of the best and worse nostrils). Such as in our previous study [45], trigeminal stimulation elicited a clear increase of activity between 1 and 12 Hz, peaking approximately 0.2 s after stimulus onset. There was no significant difference between the TF maps of AC, MCI and OD groups.

**DISCUSSION**

In the present study, using unilateral psychophysical testing of olfactory function, we show that MCI patients exhibit a marked asymmetry of olfactory function (ΔTDI) that is significantly greater than the asymmetry in age-matched healthy controls (AC) as well as in patients presenting with a primary olfactory disorder (OD). Furthermore, we show that ΔTDI is able to efficiently discriminate between MCI and AC (sensitivity: 85%, specificity: 77%), as well as between MCI and OD (sensitivity: 85%, specificity: 70%). Importantly, trigeminal and auditory testing did not show any significant difference between the three groups, indicating that the observed differences between MCI and AD are related to specific impairment of olfactory function. Taken together, our results suggest that unirhinal assessment of olfactory function could be useful for the diagnostic workup of MCI patients. Future longitudinal studies, including a greater number of patients are required to (1) determine a cutoff value defining the asymmetry of olfactory performance and to (2) evaluate whether this asymmetry could constitute an effective non-invasive biomarker for the early diagnosis of AD.

The usefulness of unirhinal evaluation of olfactory function in AD patients was first suggested by Bahar-Fuchs et al. [28], based on neuropathological findings showing an asymmetrical progression of AD-related neuropathological changes in left and right mesiotemporal structures. Some authors have reported that in its early course, AD predominantly affects the left hemisphere [30], whereas other authors have reported a predominant involvement of the right hemisphere [31]. Finally, Derflinger et al. [25] described asymmetry with no hemispheric predominance. Because it is admitted that olfactory information originating from a given nostril is primarily processed in the ipsilateral hemisphere [80], asymmetry of neurodegeneration in AD could be expected to manifest itself as an asymmetry in olfactory function. In their study, in which subjects performed a unirhinal odor identification task, Bahar-Fuchs et al. detected a significant difference between the olfactory performance of MCI and AD only when unirhinal results were considered. Indeed, when only the worst nostril of each participant was considered,
MCI patients performed better than AD patients. There was no difference between MCI and healthy controls. This contrasts with the results of the present study, in which we show that unirhinal testing using the Sniffin’ Sticks test—in particular, the asymmetry between the best and worst nostril—is able to discriminate between AC and MCI. This could be explained by the fact that we assessed not only identification but also threshold and discrimination performances. Moreover, the population of patients included in our study differed from the population studied by Bahar-Fuchs et al. since we included only amyloid positive MCI. Hence, the MCI group of the present study was probably more uniform and, most importantly, more likely to evolve toward AD [14, 81].

Stamps et al. [29] suggested that left-right naris differences in the distance at which an odorant source (peanut butter) can be detected could constitute a sensitive marker of AD. Indeed, they found that the distance at which the odorant can be detected from the left nostril was markedly shorter than the distance at which the same odorant can be detected from the right nostril in AD patients. In contrast, they found no systematic left lateralization in MCI patients. The notion that impairment of olfactory function is stronger on the left side in AD patients was questioned in a recent study by Doty et al. [82], who failed to show a significant asymmetry in smell in probable AD patients. A possible explanation for these contradictory findings could be differences in the methods used to assess olfaction, as well as differences in the studied populations (on average, AD patients had a lower MMSE score in the study of Doty et al.). In the present study, we found that MCI patients exhibit a significantly greater asymmetry of olfactory performance, but found no evidence for a systematic lateralization of that asymmetry.

As compared to several previous studies (i.e., [15, 83–85]), we found no significant difference regarding the odor identification performance of MCI and AC subjects. This might be explained by the fact that our odor identification test was adapted in order to decrease the lexical demand of the test (picture-based rather than word-based identification of odors). Hence, previous findings of a deficit in odor identification could have resulted, at least in part, from a cognitive impairment affecting lexical semantic memory [15, 83–85]. Of note, unirhinal studies found no significant difference between olfactory identification scores of the left and right nostril [28, 29, 82].

The TF distribution of the EEG responses to olfactory stimulation obtained in the MCI and AC group differed from that of the EEG responses obtained in young normosmic controls [45, 46]. Indeed, instead of an increase in activity predominant between 3–7 Hz, the response consisted in an increase at lower frequencies, mainly <2 Hz. This could be due to the physiological decline of olfactory function with age [38, 61]. Studies have consistently shown that latency and amplitude of CSERPs are affected by age, with longer latencies and lower amplitudes with aging [86–89]. Further studies investigating large population of healthy controls should be performed in the future to evaluate the TF distribution of the EEG responses to olfactory stimulation across age. Nevertheless, our EEG results confirm that MCI patients exhibit more asymmetrical EEG responses to olfactory stimulation as compared to aged-matched controls. Surprisingly, there was no significant difference between the difference maps of MCI and OD. Previous studies have shown that the magnitude of the EEG response to olfactory stimulation correlates with psychophysical testing of olfactory function [45, 46], and that the EEG responses to olfactory stimulation are dependent on the integrity of olfactory pathways [90–93]. However, it is important to keep in mind that little is known about the neural generators of the EEG responses to olfactory stimulation, and their functional significance. Several factors might explain the dissociation between asymmetry of olfactory performance and asymmetry of EEG responses to olfactory stimulation. First, it could be related to the fact that behavioral and EEG results were not obtained using the same chemosensory stimuli. Second, it could be related to differences in the cause of olfactory impairment in MCI and OD. In AD patients, slight asymmetries in the function of left and right olfactory structures/pathways could lead to measurable differences in the elicited EEG responses, without leading to marked differences of olfactory performance. A third explanation could be that the magnitude of the elicited EEG responses is more dependent on the number of activated olfactory receptors and on synchronization of that activity; factors which may be expected to strongly determine psychophysical olfactory detection scores (T), but may not be such a strong determinant of discrimination (D) and identification (I) scores. Future studies are clearly needed to investigate this dissociation.

We did not observe any difference in the EEG response elicited by auditory stimulation, indicating that the impairment in MCI patients is relatively specific of olfaction. However, we did observe a difference between the EEG responses to trigeminal stimulation, consisting in a less pronounced event-related desynchronization of alpha-band rhythms in MCI patients as compared to AC. Previous studies have shown that...
subjects with no sense of smell exhibit decreased trigeminal sensitivity [94, 95], interpreted as resulting from adaptive or compensatory interactions between olfactory and trigeminal systems.

In MCI patients, there was a significant negative correlation between the FCSRT free delayed recall, a neuropsychological test of episodic memory, and the asymmetry in olfactory performance (ATDI): patients which performed worse at this test of episodic memory tended to also show a stronger asymmetry in olfactory performance. The correlation was marginal, but nevertheless suggests a specific relationship between the two functions. Episodic memory deficit is the hallmark of amnestic MCI [96, 97]. It has been shown that episodic memory is closely linked to the function of the medial-temporal lobe (MTL) [98, 99]. Studies have shown that AD lesions start in the MTL [4]. This is in accordance with episodic memory deficit and with early olfactory dysfunction in the course of the AD. Hence, we may hypothesize that the negative correlation observed between episodic memory abilities and ATDI reflects the degree of involvement of MTL structures with the course of the disease.

Finally, we found no correlation between amyloid load and the results of the psychophysical and electrophysiological assessments of olfactory function. Similarly, Bahar-Fuchs et al. [100] used in vivo measures of amyloid burden using the Pittsburgh Compound B (PiB)PET and found no correlation between olfactory identification scores and the binding of PiB in amnestic MCI patients. Moreover, olfactory identification scores did not differ between amyloid-positive and amyloid-negative amnestic MCI patients. In an animal study, transgenic mice that overexpress the tau protein showed olfactory dysfunction [101]. Moreover, histopathological studies of human brains have shown that only the contribution of neurofibrillary tangles is significantly related with olfactory identification performance [102]. Taken together, these findings suggest that olfactory deficits are not associated with amyloid deposition but rather with markers of neural degeneration.

In conclusion, using unirhinal psychophysical assessment of olfactory function, we show that amyloid-positive MCI patients exhibit a marked and significant asymmetry of behavioral olfactory function as compared to age-matched controls and patients with post-infectious olfactory loss, suggesting that it could be used as an early biomarker for AD. Follow-up studies will be necessary to evaluate how this asymmetry in olfactory function progresses over time, as well as to determine its ability to predict actual evolution toward AD.

ACKNOWLEDGMENTS

Caroline Huart was supported by the Fund for Scientific Research (FRS-FNRS) of the French speaking community of Belgium. Lisa Quenon was supported by the Fund for Scientific Research (FRS-FNRS) of the French speaking community of Belgium.

Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/14-1494r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-141494.

REFERENCES


