Specific Smell Dysfunctions in Relapsing-Remitting Multiple Sclerosis

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Abstract

The present study assessed odour threshold, discrimination and identification in relapsing-remitting MS (RRMS) patients. We administered the Sniffin’ Sticks Extended Test (Burghart Messtechnik GmbH, Wedel, Germany) to 50 RRMS female patients and to 50 matched control subjects. Also, the number and the volume of MRI-visible demyelinating plaques within the inferior frontal and temporal lobes (IFTL complex), were quantified in a patient subgroup. The results indicated that up to 34% of the RRMS sample exhibited hyposmia, but none of the MS patients could be considered functionally anosmic. Odour identification and general olfactory performance significantly decreased with respect to the patients’ age. No significant correlations between olfactory scores and the number and the volume of plaques within the IFTL complex were found. These findings suggest that some specific forms of olfactory dysfunction do exist in MS patients and that the correlation between olfactory dysfunctions and structural brain damage might not be as strict as previously suggested.

Keywords: Multiple sclerosis; Olfaction; Magnetic Resonance Image; Sniffin’ Sticks

Introduction

A controversy within the neurological sciences concerns the presence of olfactory dysfunction in multiple sclerosis (MS) [1,2], the most common chronic disabling neurological disease in young adults, with particular reference to the female population [3]. Whereas some investigators reported that a decrease in odour perception can be one of the symptoms experienced by MS patients [4-10], others have failed to demonstrate any olfactory alterations in MS at all [11-13].

Preliminary evidence in favour of an olfactory dysfunction in MS patients comes from Wender and Szmeja [10], who reported an odour identification dysfunction in 18 out of 52 patients, i.e., 35% of the examined population. Subsequently, Pinching [9] administered a suprathereshold odour identification task and noticed the presence of anosmia or hyposmia in 10 out of 22 MS patients (i.e., 45.4%). More recent and compelling demonstrations of altered olfactory perception in MS have been reported in a series of studies using an objective and highly reliable standardized psychophysical test of olfactory functions, the University of Pennsylvania Smell Identification Test (UPSIT) [14-16] or a modified version of the same test [17-19]. In particular, Doty and colleagues [16] found that 23% of 31 patients obtained UPSIT scores significantly lower than the controls, a result which was confirmed in further studies by the same group which revealed an even higher percentage (38.5%) of micromic MS patients [5,6].

Other studies, however, failed to observe olfactory deficits in MS patients. For instance, Ansari [20] using serial binary dilutions of amyl acetate and nitrobenzene to evaluate odour detection thresholds, did not find any difference in the olfactory performance of MS patients compared with age- and sex-matched healthy controls. Similarly, MS patients scored as high as healthy controls in two further studies using the UPSIT [11, 13].

Previous research has also considered the MRI approach, which implies the correlation of olfactory test scores with the in vivo quantitative assessment of focal demyelinating areas (i.e., plaques) within primary olfactory regions by means of high-resolution magnetic resonance imaging (MRI). A strong negative correlation between the olfactory test scores (i.e., UPSIT) and the number of demyelinating plaques within the inferior frontal and temporal lobe (IFTLs) regions, which are involved in olfaction, has been reported [5,7]. Such correlations were not evident in brain regions unrelated to olfaction [5]. Similar evidence was reported in a study in which a robust correlation between a T2 lesion load within the IFTLs complex and smell loss was found [19]. From the above brief literature review it emerged that a clear consensus on this matter has yet to be reached. One of the factors which might have contributed to such controversial results is the nature of the olfactory test that was administered. Indeed, different – sometimes non-standardized tests – were used [9,10,20] and only odour identification was chiefly considered [4,6-8,13-17,20]. Also, even when the same test for the evaluation of odour identification was utilized (i.e., the UPSIT), the opposite results were found [5,13]. To date, the effect of MS on other measures of olfactory performance, such as odour threshold and discrimination, remain largely unknown.

Therefore the main aim of the present study was to elucidate whether RRMS patients are affected by a loss of smell, either global or task specific. A secondary aim was to evaluate the possible correlation between olfactory dysfunctions and the presence of demyelinating plaques within the inferior frontal and temporal lobes, the central brain structures associated with olfactory processing.

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Methods

Participants

Seventy-five female RRMS patients (age range 19 to 55 years; mean age = 37.5 years; SD = 8.3) and 64 controls (age range 16 to 55 years; mean age = 35.5 years; SD = 8.3) were enrolled in the experiment (Table 1). For the MS group the disease duration ranged from 1-28 years (mean 9.22 years). All patients were selected by three board certified neurologists after having their diagnosis verified on the basis of the revised McDonald criteria [25] and who were regularly followed at the MS Centre of the Veneto Region. In order to evaluate the disability of the RRMS patients the Expanded Disability Status Scale (EDSS) [26] was administered (EDSS mean = 1.80; SD = 1.21).

Both patients and controls were tested with the Mini Mental State Examination (MMSE) test [27] (patients: MMSE mean = 29.28; SD = 0.64; controls: MMSE mean = 29.73; SD = 0.52), the Beck Depression Inventory – II (BDI-II) [28] and the Beck Anxiety Inventory (BAI) [29] to exclude participants with cognitive impairment, depression and/or anxiety at the time of olfactory testing. Patients treated with immunosuppressants, prone to epilepsy or undergoing steroid therapy were excluded from the sample [30]. Finally, a questionnaire was administered to all participants to ascertain the previous history of nasal disease, smoking habits and the current status of olfactory functions. All patients and controls affirmed they were non-smokers. On the basis of these exclusion criteria, 25 patients and 14 controls were excluded. The final cohort consisted of 50 female RRMS patients (mean age = 37.4 years; SD = 8.7; see Table 1) and 50 female age-matched controls (mean age = 35.5 years; SD = 14.2).

All participants were naive as to the purpose of the investigation and gave informed written consent to participate in the study. The experimental procedures were approved by the Institutional Review Board at the University of Padova and were in accordance with the Declaration of Helsinki.

The Sniffin’ Sticks Extended Test

To test the olfactory functions we used the Sniffin’ Sticks Extended Test (Burghart Messtechnik GmbH, Wedel, Germany), a test devised to examine the three main aspects of olfactory functions, namely threshold, discrimination and identification, by means of pen-like odour dispensing devices presented alone (identification subtest) or in triplets (threshold and discrimination subtests) [21,22]. Normative data by age and gender for this test were used to determine the relative degree of general and specific olfactory loss which permitted a categorization of olfactory function in normal people and those with hyposmia and functional anosmia [31].

Procedure

The experimental session began with the collection of anamnestic data concerned with age, possible epilepsy and pharmacological history. Then the experimenter administered the 14-item questionnaire for testing the status of olfactory functions. During this phase participants were not allowed to eat and/or drink. Subsequently, the Sniffin’ Sticks Extended Test was administered. Please refer to Kobal and colleagues [22] for more details regarding the procedure administration. The experimental session lasted approximately 60 minutes.

Magnetic resonance images

The MR images for a subgroup of the tested patients (N = 13) were acquired within one month of administration of the olfactory test by means of a 1.5 T Philips Achieva (Philips Medical Systems, Best, Netherlands). The MR images were acquired on the same day as the Sniffin’ Sticks test was performed for five of the patients. No major hardware upgrades were applied to the scanner during the study and quality evaluation sessions took place weekly to guarantee measurement stability. The following sets of images were acquired:

(i) Fluid attenuated inversion recovery (FLAIR), a 2D sequence with 50 contiguous axial slices (TE 120 ms, TR 10000 ms, inversion time 2500 ms, slice thickness 3.0 mm, matrix 256 x 256, gap 0); (ii) Turbo-spin echo Dp/T2 (TSE) sequences. By using a semiautomatic thresholding technique, implemented in the software called Medical Images Processing, Analysis and Visualization (MIPAV) (http://mipav.cit.nih.gov) developed at the National Institute of Health (NIH), lesions were selected and segmented on the FLAIR images providing a white matter T2 (WMT2) hyperintense lesion volume (T2LV). The presence of possible FLAIR-related artifacts was controlled on proton density and T2 images. In addition we used a digital version of the Talairach and Tournoux atlas [32] to perform regional volumetric analyses The number and the volume (expressed in mm3) of demyelinating plaques within the inferior frontal and inferior temporal lobes and within the whole brain, excluding the IFTL complex, were calculated by means of MR examination. These brain regions contain the major zones of known central olfactory connections and include, respectively, (i)
the olfactory striae, subcallosal medial frontal lobe, paraterminal gyrus, orbitofrontal zone and gyrus rectus, and (ii) the prepiriform, entorhinal, amygdaloidal, hippocampal, and parahippocampal regions of the brain. The inferior frontal lobes were designated as being inferior and anterior to the body and genu of the corpus callosum, respectively. The inferior temporal lobes were considered as being superiorly bound by the plane of the Sylvian fissure. Plaques were counted and measured without knowledge of the scores obtained with the Sniffin’ Sticks Extended Test by two independent expert neuroradiologists. A reliability test revealed no significant differences between the two neuroradiologists with respect to plaque counting.

Statistical analyses

The following descriptive measures were calculated: mean, standard deviation and the minimum and maximum values together with the percentiles (see Table 2). A Chi-square test was used to compare global olfactory performance in the RRMS group and the control group. A between-subjects MANCOVA together with Spearman’s and Pearson’s correlations were used as appropriate. The MANCOVA was applied to compare olfactory performance in the RRMS and control group, with ‘group’ as the independent variable and each of the olfactory indexes as dependent variables. ‘Age’ was used as the covariate to statistically control for variance in olfactory performance. Spearman’s rank correlation coefficients were used to examine the relationship between olfactory Sniffin’ Sticks scores, MRI data (plaque numbers and volume within and outside the IFTLs) and clinical variables (i.e., MS onset). Partial correlation analysis was performed to remove the effect of potential confounding factors.

### Results

#### Hyposmia and functional anosmia

The Sniffin’ Sticks Extended Test ascertains the presence of both hyposmia and functional anosmia. Hummel et al. [21] defined the TDI cut-off point between normosmic patients and hyposmic patients as 30.3. Following this criterion, we found a percentage of hyposmic participants of 34% (17 out of 50) within our RRMS group and of 8% (4 out of 50) within our control group. A Chi-square test revealed that hyposmic participants were statistically more frequent in the patient group than in the control group ($\chi^2 = 8.04, P < 0.005$).

With respect to functional anosmia, which refers to a TDI score less than 16.5 [31,33], none of the RRMS or control participants were found to be totally functionally anosmic.

#### Comparing RRMS and control group olfactory performance

MANCOVA Wilks’ lambda revealed a significant main effect for ‘group’ ($F_{4,91} = 239.3, P < 0.0001$, $\eta^2 = 0.26$). The covariate analyses for ‘age’ were significant ($F_{4,91} = 4.29, P < 0.05$, $\eta^2 = 0.23$). Because the MANCOVA revealed a significant main effect of group, examination of univariate ANCOVA analyses of the dependent variables was performed to identify which dependent variable contributed to the overall effect. Significant group effects emerged for the discrimination and identification scores ($P = 0.01$, $\eta^2 = 0.10$ and $P = 0.001$, $\eta^2 = 0.20$, respectively). No significant group effect was found when the threshold and TDI scores were considered ($P > 0.05$, $\eta^2 = 0.05$). Significant covariate relationships were also observed. Age was significant for both the identification and the TDI scores ($P < 0.05$, $\eta^2 = 0.07$). Pearson’s correlations indicated that as age increased identification and TDI scores significantly decreased in both the RRMS and the control group ($r = -0.27, P < 0.01$).

#### Correlating the duration of MS with olfactory functions and neuropathological markers

As shown in Table 3, no significant correlation was found between

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**Table 2:** Sniffin’ Sticks scores for the MS patients and the considered female normative population.

<table>
<thead>
<tr>
<th>AGE GROUP</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-35 yrs</td>
<td>25</td>
<td>6.38</td>
<td>1.12</td>
<td>12.00</td>
<td>24.27</td>
<td>5, 10, 25, 75, 90</td>
</tr>
<tr>
<td>36-55 yrs</td>
<td>25</td>
<td>8.81</td>
<td>0.99</td>
<td>10.00</td>
<td>28.12</td>
<td>5, 10, 25, 75, 90</td>
</tr>
</tbody>
</table>

THR = Threshold; DIS = Discrimination; ID = Identification; TDI = The sum of THR, DIS and ID.
the duration of MS and the scores obtained with the Sniffin' Sticks
Extended Test, the number and the volume of the plaques within
the IFTL complex (Table 3) and the areas outside the IFTL complex
in terms of plaque numbers and plaque volumes (Table 3).

**Correlating the number and the volume of the plaques within
and outside the IFTL complex with olfactory indexes**

Correlations between the number and the volume of the plaques within the IFTL complex, in the whole brain excluding the
IFTL complex (e.g., outside IFTLs) and the olfactory indexes were
performed. The number and volume of plaque within the IFTL complex
did not negatively correlate either with any of the olfactory indexes
considered or with the areas outside the IFTL complex in terms
of plaque number and plaque volume. To remove the effect of potential
confounders such as age and disease duration, partial correlations
were also performed (Table 4). Thus, no association between Sniffin'
Sticks scores and neuropathological markers within and outside the
IFTL complex was demonstrated ($p > 0.05$). As an example of how the
relation between the number of plaques within the IFTL complex and
the olfactory indexes (i.e., TDI) might not be an optimal index, an
example of how the number of WMT2 lesions detected within the
regions of the frontal and temporal lobes involved in olfaction and the
olfactory indexes seemed to be significantly affected by disease-related
progression of the olfactory deficit.

**Discussion**

The main aim of the present study was to evaluate olfactory
functions in a carefully selected group of female RRMS patients.
Furthermore, we ascertained whether olfactory scores correlated
with the number and volume of WMT2 lesions within (and outside)
central eminent olfactory regions (IFTL complex).

Estimates of the prevalence of olfactory dysfunctions in MS
vary widely [5, 6, 8, 11, 20]. Previous studies only testing odour
identification on unspecified MS-subtypes samples reported olfactory
dysfunctions affecting 15% [8], 35% and 38% [5, 6, 10] and 45% [9] of the
considered MS populations. Various factors, such as the administered
olfactory test, the patients’ selection criteria (i.e., age, gender,
clinical form of MS), the interval of time since the last relapse/high-
dose steroid therapy and concomitant medication affecting olfactory
function (i.e., calcium-channel inhibitors, chemotherapeutics), might
have contributed to these discrepancies. As an example, a study by
Hawkes et al. [8] included patients who had experienced a recent
relapse and received a course of high-dose steroids, which could have
modified their performance either at the nose level or within the
central olfactory pathways. In our study, 34% of the RRMS participants
tested in our clinical setting exhibited a decreased olfactory ability
on smell testing, which is in line with previous reports [5,6,10] but,
importantly, was obtained from a carefully selected group of RRMS
patients: they were all females, had not received drugs known to
affect the olfactory system, did not smoke and they were far from the
last relapse/steroid course.

To the best of our knowledge, no previous research has
evaluated specific aspects of olfactory functions in order to obtain
detailed information on the smelling ability of MS patients. In this
perspective, our findings not only support a general loss in the sense
of smell of MS patients, but also suggest the presence of specific
olfactory dysfunctions. When comparing the scores for the odour
discrimination and identification tasks between the RRMS and the
control group, olfactory deficits within the RRMS sample did emerge.
The odour threshold scores did not significantly discriminate
between the RRMS and the control group. Taken altogether these
findings are in collusion with previous research reporting that odour
identification ability was, to a certain extent, compromised in MS
patients[5-10].

Another aspect of the present findings is that both the
identification and the TDI scores seemed to be significantly affected
by age-related effects. In other words, part of the amount of
variance for the global and identification components of olfactory
performance was accounted for by age, in the older participants,
for both the MS and the control group, who performed worse
than younger participants. Nevertheless, the significant MANCOVA
results, considering ‘age’ as a covariate, suggested that the specific
impairment in odour identification and general olfactory loss is a
disease-related progression of the olfactory deficit.

In neural terms, we expected a strong negative correlation
between the number of WMT2 lesions detected within the regions
of the frontal and temporal lobes involved in olfaction and the
scores obtained for the different components of the olfactory test
[6,7,19]. Although we adopted a methodological approach similar

![Figure 2: Panel A represents an axial T2-weighted MRI scan for a hyposmic patient (TDI = 29), a 30-year-old woman with a three-year history of MS. Panel B represents an axial T2-weighted MRI scan for a hyposmic patient (TDI = 29), a 39-year-old woman with an eight-year history of MS.](image-url)
to that of previous studies [5-7,19], we failed to demonstrate such a relationship. This result was also found for the patients who had the olfactory assessment and the MR scan on the same day. This is important because it might well be that from the time the MR assessment was conducted to the time the olfactory test was administered the number and volume of the WMT2 lesions might have changed. We acknowledge that due to the limited number of patients who underwent MRI scanning we might not be able to state definite conclusions. Further investigation in highly homogeneous samples of patients is needed. However, it is worth mentioning that the studies which reported a strong correlation between the number of plaques and the UPSIT score considered an even smaller sample [7].

The finding of a lack of correlation between the decrease in olfactory performance and structural changes in central olfactory areas might suggest two alternative explanations. On the one hand, the olfactory dysfunctions in RRMS might be linked to a central functional rather than a structural impairment. To this end, recent research has outlined a diffuse brain network activating task-specific regions while performing different olfactory tasks [34]. Specifically, odour threshold is linked to the activation of right thalamus, amygdala-paraform, cingulate, orbito-frontal and insular cortex whereas qualitative odour discrimination is known to engage thalamus, right caudate, subiculum, cingulate, orbito-frontal, pre-frontal, left insular and right cerebellar cortex [34]. Odour identification, though sharing part of the odour discrimination activation (thalamus, cingulate, orbito-frontal, pre-frontal, left insular and right cerebellar cortex), recruits, in addition, the pyriform cortex and sectors of the temporal and parietal cortex [34]. Given that an odour discrimination and identification loss is reported in our RRMS sample, it is tempting to speculate that the results of the present study might reflect the impairment of brain circuits engaged both in the odour discrimination and identification processes. Such an hypothesis seems to be supported by evidence from neurophysiological data considering the orbito-frontal cortex (OFC). As an example, Critchley and Rolls [35] demonstrated that the primate OFC responds in a highly selective manner to olfactory stimuli. In a similar vein, neuroimaging studies report that OFC lesions in the human brain account for specific smell disturbances, such as odour discrimination and identification impairment [36,37].

On the other hand, olfactory dysfunctions in RRMS might reflect a peripheral rather than a central deficit. There may be the possibility that the odour spatial map is primarily affected by the disease. In the olfactory system a spatial map for odour detection already exists in the periphery [38], in which each olfactory neuron expresses just one of the 350 (in human) odorant receptors [39] and olfactory receptors also play an instructive role in determining the central projections of the olfactory neurons in which they are expressed [40]. Since each receptor responds to several odour molecules as well as each odour molecule is capable to stimulate a variety of receptors, the mammalian olfactory system uses a combinatorial receptor coding scheme to identify and discriminate odours [41]. Thus, an impairment of such combinatorial coding may explain the deficits in identification and discrimination of odours in MS. In other words, slight alterations of this wiring diagram might be responsible for an impairment of olfactory performance in MS.

With respect to the considered plaques indexes, not only we took into account the number of plaques, but we extended this literature by performing analysis of the volume of plaques within the IFTIs. At present, this measurement is considered to be less prone to the subjective judgment of the operator than the counting of plaques. The plaque volume analysis did not find any significant correlations with the olfactory scores.

In this respect, it is of interest to draw a parallel between the present findings on RRMS patients and those obtained for people affected either by Parkinson’s disease (PD) or degenerative ataxias. The extensive literature on olfactory disturbances in PD [42] also suggests that the olfactory disorder is a sensitive sign of pathology. However, as found here, the olfactory loss in PD is unspecific and it does not correlate with disease severity [43]. Furthermore, the present findings are mindful of those reported for degenerative ataxias patients, in which smell test scores do correlate neither with a genetic severity marker (GAA trinucleotide repeats) nor with disease duration [44].

Finally, the limitations of conventional MRI in depicting MS pathology may explain the discrepancies between the findings of the present study and previous ones. Several reviews and consensus statements over the past few years have questioned the value of current MR measures (e.g., T2 lesion burden, number of T2 lesions) as surrogate markers by noting that the correlation between these MRI measures and disability has been relatively poor [45,46]. Indeed, WMT2 lesion burden does not reflect the complexity of MS pathology, which includes cortical demyelination and atrophy, spinal cord involvement and subtle biochemical alterations in the normally-appearing white matter [47,48].

Our data confirms and extends previous findings on the evaluation of olfactory functions in MS patients. They outline the importance of using highly reliable tests able to capture more finely-grained aspects of olfactory performance together with the use of more carefully selected population samples. Although no correlations were identified between the olfactory scores and the neuropathological markers, we suggest that future research on this issue should consider less operator-dependent measures such as plaques volume. Moreover, it will be of great interest to explore if (and to which extent) MS patients share the same functional activations in brain circuits engaged in olfactory performance when compared to control participants. Furthermore, having insights on the molecular basis of the peripheral functioning in MS patients might help in clarifying the causal relationship of the olfactory impairment found in this population. Future research should also consider testing of the olfactory ability in pre-clinical, primary and secondary progressive homogeneous MS samples in order to elucidate the features of olfactory loss throughout the natural course of MS. This might open to the possibility for the identification of some markers, either functional or molecular, as to improve patients’ outcomes. Since the sense of smell is fundamental for the quality of life, intended both in terms of survival mechanisms (e.g. detection and identification of potentially dangerous events signalled through smoke, the leaking of natural gas and spoiled foods) and in terms of daily well-being (e.g. appreciation of food, nutritional status, mood rate and social interactions), we suggest that a precise assessment of olfactory functions might be routinely performed in MS patients.

References


