

# The association between olfactory bulb volume, cognitive dysfunction, physical disability and depression in multiple sclerosis

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## **Conflicts of interest**

ÖY has received lecture fees from Teva, Novartis and Bayer Schering (paid to the University Hospital Basel).

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EWR served on advisory boards for Novartis, Biogen Idec, Merck-Serono and Bayer Schering. He received travel support from Novartis, Biogen and Bayer Schering.

LK: The University Hospital Basel as the employer of LK received in the last 3 years and used exclusively for research support: steering committee, advisory board and consultancy fees from Actelion, Addex, Bayer Health Care, Biogen, Biotica, Genzyme, Lilly, Merck, Mitsubishi, Novartis, Ono Pharma, Pfizer, Receptos, Sanofi-Aventis, Santhera, Siemens, Teva, UCB, Xenoport; speaker fees from Bayer Health Care, Biogen, Merck, Novartis, Sanofi-Aventis, Teva; support of educational activities from Bayer Health Care, Biogen, CSL Behring, Genzyme, Merck, Novartis, Sanofi, Teva; royalties from Neurostatus Systems GmbH; grants from Bayer Health Care, Biogen, Merck, Novartis, Roche, Swiss MS Society, the Swiss National Research Foundation, the European Union, and Roche Research Foundations.

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## ABSTRACT

**Background:** Olfactory bulb atrophy is associated with cognitive dysfunction in Parkinson's and Alzheimer's disease, and with major depression. It has been suggested that olfactory bulb atrophy or dysfunction is therefore a marker of neurodegeneration. Multiple sclerosis (MS) is now also recognised as having a significant neurodegenerative component. Thus, the aim of this study was to investigate associations between physical and cognitive disability, depression, and olfactory bulb volume in MS.

**Methods:** In total, 146 patients with MS (mean age  $49 \pm 10.9$ , disease duration  $21.4 \pm 9.1$  years, median Expanded Disability Status Scale (EDSS) 3.0 (range 0-7.5), 103 relapsing-remitting, 35 secondary-progressive and 8 primary-progressive multiple sclerosis) underwent a standardised neurological examination, comprehensive neuropsychological testing and magnetic resonance imaging (MRI); **27 healthy people served as age- and gender matched control subjects.** The olfactory bulb was semi-automatically segmented on high-resolution 3D T1-weighted MRI.

**Results:** **Mean olfactory bulb volume was lower in MS than controls ( $185.6 \pm 40.1$  vs.  $209.2 \pm 59.3$ ;  $p=0.006$ ;  $p=0.018$  adjusted for intracranial volume).** Olfactory bulb volume (normalised to intracranial volume) was similar across clinical disease subtypes and did not correlate with cognitive performance, EDSS scores or total PD/T2 lesion volume. However, in progressive MS, the mean olfactory bulb volume correlated with depression scores (Spearman's  $\rho=-0.38$ ,  $p<0.05$ ) confirmed using a multivariate linear regression analysis including cognitive fatigue scores. This association was not observed in relapsing-remitting MS.

**Conclusion:** Olfactory bulb volume **was lower in MS than healthy controls**. It does not seem to mirror cognitive impairment in MS, however, it is associated with higher depression scores in progressive MS.

## INTRODUCTION

Cognitive impairment is common in multiple sclerosis (MS) with prevalence estimates ranging from 40% to 70% [1]. It is more frequent and pronounced in progressive forms of MS [1], and has a significant impact on quality of life [2].

The cognitive domains commonly affected in patients with MS include memory, processing speed, executive functioning, and visuospatial abilities [3]. In most MS MRI studies, measures of brain atrophy are more closely correlated with cognitive impairment than the WM lesion load on PD/T2 weighted images, suggesting a greater role for neurodegeneration over neuroinflammation in MS cognitive deficits [4].

An interesting feature of quintessentially neurodegenerative diseases is that they do not affect all parts of the brain equally, and in some instances involvement of the olfactory system may be an early feature. In Alzheimer's disease cognitive impairment is correlated with olfactory dysfunction [5] and in Parkinson's disease olfactory dysfunction may even precede the motor symptoms for many years [6].

Olfactory dysfunction has been described repeatedly in MS, particularly in secondary-progressive (SP)MS, and may even mark transition from relapsing-remitting to SPMS [7]. The mechanism underlying this is not known, but pathology in the subventricular germinal zone has been implicated [8]. This region plays a major role in the generation of stem cells, many of which migrate into the olfactory bulb following the rostral migratory stream [8]. In experimental autoimmune encephalomyelitis (an animal model of MS) oligodendrogenesis in the subventricular zone is increased, as is migration of stem cells into the brain parenchyma [9], however, stem cell migration to the



olfactory bulb is reduced, and this is associated with olfactory memory deficits in mice [10].

There is only one study demonstrating a moderate (Spearman rho coefficient = -0.4) correlation between olfactory bulb volume and cognitive dysfunction in MS using the Mini Mental Status Examination [11]. However, the Mini Mental Status Examination is not particularly sensitive or specific for MS associated cognitive impairments and this study excluded patients with significant depression (as assessed using the Beck Depression Inventory) but did not look for residual effects of lower levels of mood disturbance on Mini Mental Status Examination scores [12]. The lifetime risk of major depression in MS patients has been estimated to be as high as 50% compared to 10-15% in the general population [13] and is itself associated with cognitive impairments [14].

We sought to investigate systematically the association between olfactory bulb volume, neuropsychological impairment and depression in a large MS cohort.

We addressed the following questions:

*I. Does olfactory bulb volume differ between patients and **healthy control subjects and across clinical disease subtypes (relapsing-remitting (RR)MS, secondary progressive (SP)MS, primary progressive (PP)MS)?***

*II. What are the relative strengths of associations of olfactory bulb volume with measures of cognitive disability and depression in different subtypes of MS?*

We hypothesized that the olfactory bulb volume would be significantly lower in SPMS than RRMS. Moreover, we hypothesized that the olfactory bulb volume would correlate with the neuropsychological tests and depression scores and that these correlations would be stronger in SPMS compared with RRMS. To determine if any associations seen between olfactory bulb volume and cognitive impairment was specific, or part of a more generalised process, we also looked for correlation between olfactory bulb volume, brain volume and physical disability.

## **PATIENTS AND METHODS**

### **Participants**

Participants were recruited from an ongoing prospective, non-interventional cohort study on the phenotype-genotype characterisation of MS. Inclusion criteria of this study were: age 18-65 years, diagnosis of MS according to the McDonald criteria 2001 [15], EDSS 0-7.5 inclusively. Patients had to have neuropsychological testing and brain MRI scans performed in a 28-day period. In total, 167 MS patients were included in this substudy. Twenty-one patients were excluded due to insufficient image quality and, hence, data of 146 MS patients were included in this analysis. Medical history and clinical examination of the patients were not indicative of diseases of the central nervous system other than MS. **Concomitant systemic diseases (such as hypothyreosis and diabetes mellitus) and drugs which may affect ability to smell and olfactory bulb volumes have been documented.** MS subtypes were classified using the Lublin-Reingold criteria [16]. **Twenty-seven age and gender-matched healthy subjects served as controls.** All participants gave

written informed consent, and this study was approved by our local institutional ethics committee.

### **Assessment of cognitive impairment, fatigue and depression**

All patients were assessed for cognitive function, fatigue severity and depression. The cognitive test battery included the Paced Auditory Serial Addition Test 3 seconds (PASAT) [17] measuring cognitive processing speed in the auditory modality, Symbol Digit Modalities Test (SDMT) [18] measuring cognitive processing speed in the visual modality, Verbal Fluency Test [19], Interference Test for measuring mental flexibility [19], and an Immediate and Delayed Recall Test for measuring memory function [19]. The tests are described detailed in [Table 1](#).

The results of the neuropsychological tests were transformed into z scores, with normative data from literature serving as a reference. A z-score below -1.5 was considered as an abnormal test result [20]. Patients were considered to be cognitively impaired if they failed in two or more tests, a criterion used in previous studies [21]. Depressive symptoms were assessed using the German version of the Center for Epidemiologic Studies Depression Scale [22]. We measured fatigue, a potential co-founder for neuropsychological performance and depression [4] using the Fatigue Scale for Motor and Cognitive Functions (FSMC) [23].

### **MRI acquisition**

Brain MRI was obtained on a 1.5 Tesla system (MAGNETOM Avanto, Siemens Medical Solutions, Erlangen, Germany). The mean time difference

between the MRI and neuropsychological examination was 1.2 days (range 0-14 days). None of the patients had a relapse in-between the neuropsychological examination and MRI. The MRI protocol included proton density (PD)/T2-weighted sequences and spin-echo T1-weighted sequences (all acquired 2D in axial plane with 3 mm thick slices). The volumetric measures of the olfactory bulb were obtained from 3D T1-weighted Magnetisation Prepared Rapid Gradient Echo (MPRAGE) images with the following acquisition parameters: repetition time=1.9 sec; echo time=3.5 ms; inversion time=1.9 sec; flip angle 7°; isotropic resolution of 1 mm<sup>3</sup>; acquisition time: 7 min; no gap, acquired in sagittal plane. We used coronal reconstructions to minimise the impact of partial volume effects as previously proposed [24].

### **MRI analysis**

MS lesions (T2-weighted hyperintense and T1-weighted hypointense lesions), and the olfactory bulb on the MPRAGE scan, were segmented using the semi-automated thresholding tool in AMIRA (Version 3.1.1., Mercury Computer Systems Inc.). The olfactory bulb was segmented between the crista galli and the rostrum of the corpus callosum **on coronal reconstructions** as previously described ([Figure 1](#)) [5]. Olfactory bulb volumes were obtained by planimetric manual contouring (surface in mm<sup>2</sup>) and subsequent addition of all surfaces multiplied by the slice thickness. The intraclass correlation coefficient using this method has been previously shown to be >0.9 both for intra- and interobserver variation using the same MRI sequences [5]. **Olfactory bulb segmentation was carried out by TY and OY blinded to the clinical data and under the guidance of an expert neuroradiologist. The intra- and interrater**

variability were 0.90 and 0.73 (Cronbach's alpha), respectively. Olfactory bulb volumes as stated below represent the sum of both sides and mean of both consecutive measurements. Total intracranial and brain parenchymal volume were assessed by using NeuroQuant software package (CorTechs Labs, La Jolla/CA), a fully automated brain MRI segmentation software [25]. Brain parenchymal fraction was calculated as the ratio of brain parenchymal tissue volume to the total intracranial brain volume [26]. All MPRAGE images were reviewed for quality blinded to clinical data. The images of every tenth patient were re-reviewed by OY for artifacts and segmentation quality.

## **Statistics**

Demographic data and MRI results are presented as mean  $\pm$  standard deviation. EDSS values are presented as median (range). Inspection of clinical, neuropsychological and MRI results revealed evidence for non-normality for the EDSS, T2 and T1 Lesion volume, disease duration, fatigue and depression scores, Immediate and Delayed Recall Test, Verbal Fluency Test, SDMT and PASAT (all  $p < 0.05$ , Shapiro-Wilk Test). Results were compared between groups using general linear model or Mann-Whitney U Test depending on the normality of the data. Olfactory bulb volume was adjusted for total intracranial volume to control for inter-individual variation independent of MS disease effects, and compared across the groups using general linear model, covarying for age and gender. The correlations between olfactory bulb volume and neuropsychological test performance and depression scores were calculated using the Pearson's or Spearman's correlation test depending on normality of the data. Linear regression analysis (inclusion model) was performed to analyse the effect of potentially meaningful

covariates on the relation between olfactory bulb volume (adjusted to intracranial volume) and depression score. The results were confirmed by using bootstrap analysis (n=1000). Given the problems associated with formally correcting for multiple comparisons [27] we present results flagged using conventional ( $p < 0.05$ ) significance threshold.

We used SPSS (MAC version 21 SPSS, Chicago, IL, USA) for all statistical analyses.

## RESULTS

In total, 103 RRMS, 35 SPMS, 8 PPMS patients and 27 healthy control subjects were included in this study. Their demographics and clinical characteristics are given in [Table 2](#). Four patients had a hypothyroidism and further two patients diabetes mellitus, all treated for these concomitant conditions.

Drugs used at the time of study assessment are given in [Supplemental Table 1](#) (online only).

### Neuropsychological test performance

Results of the neuropsychological testing are given in [Table 3](#) and [supplementary Table 2](#) (online only). Thirty out of 146 participants (20.5%) were cognitively impaired, 45/146 (30.8%) had one abnormal neuropsychological test result and 71/146 (48.6%) were cognitively preserved. Patients with progressive MS were more likely cognitively impaired than those with RRMS (27.9 vs. 17.5%,  $p < 0.01$ , Chi Square Test). Test performance outside the normative range was most frequently observed with the SDMT,

being abnormal in 75/146 (51.4%) of the MS cohort, followed by the Delayed recall test (n=26), PASAT (n=15), Interference Test (n=11), Immediate Recall Test (n=8) and Verbal Fluency Test (n=5).

### **Depression**

Depression scores were available in 137/146 patients. Patients with progressive MS had significantly higher depression scores than patients with RRMS (16.4±11.0 vs. 11.5±10.1,  $p=0.008$ , Mann-Whitney test). In total, 27/137 (19.7%) patients had depression scores that exceeded the test threshold accepted as marking significant depression; 12 of these 27 patients with depressive symptoms had progressive MS and 15 RRMS. Depression scores correlated with cognitive fatigue scores (Spearman's  $\rho=0.637$ ,  $p<0.001$ ), physical fatigue scores (Spearman's  $\rho=0.524$ ,  $p<0.001$ ), Immediate Recall Test (Spearman's  $\rho=-0.283$ ;  $p=0.001$ ), Delayed Recall Test (Spearman's  $\rho=-0.270$ ;  $p=0.002$ ), Verbal Fluency Test (Spearman's  $\rho=-0.233$ ;  $p=0.007$ ), PASAT (Spearman's  $\rho=-0.239$ ;  $p=0.005$ ), and SDMT scores (Spearman's  $\rho=-0.386$ ;  $p<0.001$ ).

### **Fatigue**

Ninety five (65%) of the 146 patients with MS reported cognitive and 111 (76%) physical fatigue. Both physical and cognitive fatigue scores were higher in progressive MS compared with RRMS ( $p=0.029$  cognitive fatigue;  $p<0.001$  for physical fatigue; Mann-Whitney test).

### **Olfactory bulb volume**

Mean olfactory bulb volume was higher in MS than healthy controls (209.2±59.3 vs. 185.6±40.1 µl, p=0.006). This was true after adjusting for intracranial volume (p=0.018). In all participants, men had higher olfactory bulb volumes than women (204.9±52.0 vs. 178.8±37.0 µl, p=0.001). The difference was not significant after adjusting for intracranial volume (p>0.05).

Olfactory bulb volume correlated with intracranial (Pearson's r=0.354, p<0.001) and brain parenchymal volume (Spearman's rho=0.317, p<0.001).

In MS, mean olfactory bulb volume was similar values across the clinical disease subtypes. This was true after adjusting for intracranial volume (general linear model, [Table 4](#)).

Olfactory bulb volume was similar in 104 MS patients who were on disease modifying treatments compared with 42 patients without. Olfactory bulb volume (both raw and normalised to intracranial volume) did not correlate with total T2-weighted hyperintense or T1-weighted hypointense lesion volume. It also did not correlate with EDSS scores, fatigue scores, or any of the neuropsychological measures and did not differ between those with and without cognitive impairment. Olfactory bulb volume did also not correlate with depression scores in the total MS group.

However, in the progressive MS group, patients with depression (n=12) had significantly lower olfactory bulb volumes than those without depression (n=29, 169.17± 41.6 vs. 202.9 ± 44.8 µl, p=0.031, general linear model, [Figure 2](#), missing depression score in 2 progressive MS patients).

This remained lower after adjusting for intracranial volume (normalised olfactory bulb volume in progressive MS patients with depression 0.105±0.022 vs. 0.125±0.027, p=0.027, general linear model).



The proportion of patients taking drugs which might influence olfactory bulb volumes (see Supplemental Table 1) was similar between progressive MS patients with and without depression (72% vs. 60%,  $p=0.38$ , Chi Square Test). None of the patients in the progressive MS group had hypothyroidism or diabetes mellitus.

Correspondingly, in patients with progressive MS, olfactory bulb volume correlated with depression scores (Spearman's  $\rho=-0.378$ ,  $p=0.015$ ) also after adjusting for intracranial volume (Spearman's  $\rho=-0.414$ ;  $p=0.007$ , [supplementary Figure 1 \[online only\]](#)).

In contrast, brain parenchymal fraction and intracranial volume did not correlate with olfactory bulb volume (both  $p>0.05$ ). The association between normalised olfactory bulb volume and depression scores was confirmed in a multivariate linear regression model with depression score as the dependent variable and age, gender, disease duration, EDSS score, T1 hypointense lesion volume, PD/T2 hyperintense lesion volume. Moreover, the association between normalised olfactory bulb volume and depression remained after adding cognitive fatigue score as covariate into the model ( $p=0.025$  for olfactory bulb volume normalised to intracranial volume, adjusted  $R^2=0.151$ , for the whole model  $=0.403$ , [supplementary Table 3 \[online only\]](#)).

## DISCUSSION

In the present study, **olfactory bulb volume (adjusted to intracranial volume) was lower in MS than healthy controls**. However, we did not find any differences in olfactory bulb volume between patients with RRMS and SPMS or PPMS, and cognitive performance did not correlate with olfactory bulb

volume in the total cohort. However, patients with progressive MS and depressive symptoms had significantly lower olfactory bulb volumes (adjusted for intracranial volume) compared with those without depressive symptoms. Moreover, there was an inverse correlation between olfactory bulb volume and depression scores in the progressive, confirmed by the multivariate analysis including cognitive fatigue scores. We did not find this correlation in the RRMS group.

One previous study has investigated the olfactory bulb volume in MS [11].

The authors included a group of age- and gender matched healthy controls.

However, a comparison with our results is not possible as MRI results of healthy controls were not documented [11].

In contrast to our study, Göktas et al. described a significant correlation between the Mini Mental Status Examination and EDSS scores and olfactory bulb volume in 36 MS patients. We did not find such an association, in our larger cohort with a comprehensive cognitive battery, which includes a subset of those tests recommended by an international consensus committee for the use in MS [28]. Different techniques were used to measure the olfactory bulb volume, and it is possible that this has contributed, in part, to the discrepant results: Göktas et al. identified the olfactory bulb on coronal slices and traced consecutive slices until an abrupt decrease of the olfactory bulb area occurred, indicating the posterior border of the olfactory bulb [11]; we measured the olfactory bulb between the crista galli and on consecutive slices until the first appearance of the rostrum of the corpus callosum, which seems to represent a more clear-cut landmark [5]. However, there is no study comparing these methods, therefore their relative strength and weaknesses when applied in MS are unknown.

In patients with progressive MS, we found an association between depression scores and olfactory bulb volume, a relation that has not been described in MS up to now. Göktas et al. excluded patients with the Beck Depression Inventory test score of 15 or higher [11]. Our results are in line with a study demonstrating significant lower olfactory bulb volumes in non-MS patients with major depression [29].

Interestingly, in our study, in contrast to the olfactory bulb volume, brain parenchymal fraction did not correlate with depression scores in the progressive MS group suggesting a more specific involvement of olfactory networks in the pathophysiology of depression in progressive MS.

Although we did not assess olfactory bulb function in our study, previous work has shown a clear association between olfactory function and olfactory bulb volume after head injury or infection [30] and in MS [11].

Our results would therefore also appear to be concordant with a previous study showing a correlation between depressive symptoms and hyposmia in MS [31].

The mechanism underlying this possible association is unclear. The olfactory tracts connecting the olfactory bulb to higher cortical regions are bidirectional, and so processes in the entorhinal cortex, amygdala, septal nuclei, prepiriform cortex, hippocampus, subiculum, thalamus and frontal cortex may be reflected in olfactory bulb neurons [32] and so olfactory bulb volume [33].

Many of these regions form the limbic system, and so are linked with motivation and emotional processes [34]. However, another model of depression postulates a primary role for olfactory bulb dysfunction in depression, with reduced olfactory perception leading to amygdala disinhibition which in turn alters emotional responses [35].

## Limitations

In addition to those noted above, there are a few other study limitations worth mentioning. We assessed depressive symptoms using a standardised and widely used scale. However, as a self-reported outcome it is still at least in part subjective [36]. The participants included in this study had subjectively no olfactory dysfunction; however, they were not examined by an **Ear, Nose and Throat** specialist to exclude potentially confounding conditions that may affect olfactory bulb measures. **Theoretically, postviral or posttraumatic conditions may influence olfactory bulb volume even if olfactory dysfunction has not been complained by the participants. Six patients had concomitant conditions which are known to be able to affect the ability to smell [37]. However, none of them were in the progressive MS group. Moreover, patients took drugs which may affect olfactory bulb volume but the proportion of patients taking these drugs was similar in progressive MS patients with and without depression. However, this does not exclude that drug side effects may have had an influence on the study results. Furthermore,** the association between olfactory bulb volume and depression scores is derived from a subgroup analysis with a higher risk of type I error, and as such needs to be replicated in an independent larger cohort [38].

## Conclusions

Although olfactory bulb volume does not seem to mirror cognitive dysfunction in MS, our findings suggest an association between olfactory bulb volume and depression in progressive MS.



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