

## **REVIEW**

Back on the Scent: The Olfactory System in CNS Demyelinating Diseases

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### **Word Count**

4.927 excluding abstract, figures and references

### **Keywords**

Multiple Sclerosis, Smell, Myelin, Neuropathology, Neuroradiology

## **ABSTRACT**

Olfactory dysfunction is recognized across an ever broadening spectrum of neuropsychiatric conditions including CNS demyelinating diseases such as multiple sclerosis (MS) and neuromyelitis optica (NMO). In this review, we unravel the striking evidence highlighting how olfactory loss is a common clinical feature in MS and NMO. We provide an overview of the supportive psychophysical, electrophysiological, radiological, and pathological data that point to the anatomical substrate of olfactory deficits in these diseases. The pattern of underlying pathology affecting the olfactory system is shown to be complex, involving multiple structures that are affected in different ways throughout the course of the disease. This review is the first to synthesize the expanding body of literature on the topic, provides novel insight into the way in which the olfactory system is affected in CNS demyelinating diseases, and raises intriguing questions about the role of this system in the pathogenesis of these diseases.

## INTRODUCTION

Olfaction is the most ancient of the senses. Its evolutionary importance is highlighted by the vastness of the olfactory genome, extending over more than 1000 genes in humans. Whilst olfaction is no longer critical to survival in modern times, smell dysfunction can significantly impair quality of life and increase risk to hazardous events, such as gas-leaks and fires.[1] The association of smell loss with significant psychiatric co-morbidity,[2] and increased 5-year mortality in older adults,[3] further accentuates olfactory impairment as either a cause or consequence of underlying neuropsychiatric disease. Therefore, it is not surprising that this symptom, often overlooked by patients and clinicians, is receiving increasing attention.

Olfactory dysfunction is a recognized feature of a constellation of neuropsychiatric diseases, namely Parkinson's,[4] Alzheimer's,[5] Motor Neurone Disease,[6] depression,[7] schizophrenia,[8] bipolar disorder,[9] and anorexia nervosa.[10] This spectrum of olfactory involvement continues to broaden with emerging evidence to suggest that smell loss is a feature of central nervous system (CNS) demyelinating diseases, such as multiple sclerosis (MS).[11] This review aims to unravel the basic anatomy of the olfactory system and the anatomic substrate of olfactory dysfunction, the tools used in the clinic to assess its impairment, and typical manifestations and presentations of smell loss in MS and other CNS demyelinating disorders. The relevance of olfactory disturbance on the pathogenesis of CNS demyelinating disease will be highlighted.

## **OLFACTORY ANATOMY**

Olfactory anatomy is complex with peripheral and central nervous system contributions critical to function. To fully understand how the olfactory system is affected in neurological disease, an overview of the olfactory processing pathway is necessary. The three major components of olfactory anatomy, peripheral olfactory apparatus, olfactory bulb/tract, and olfactory cortex will be summarized here.

1. Peripheral olfactory apparatus: Volatile odorants are inhaled through the nostrils and pass through a cleft in the medial part of the superior turbinate to enter the nasal attic where they are absorbed into a layer of mucous secreted by abundant Bowman's glands. Odorants subsequently encounter the olfactory neuroepithelium, which contains the bipolar cell, the first order sensory neuron of the olfactory system where signal transduction of odorant input into electrical signals takes place. The axons of the neuroepithelium coalesce into ~50 nerves, which subsequently pass through clefts in the cribriform plate to synapse onto the olfactory bulb of CNS.
2. Olfactory bulb/tract: The olfactory bulb has a volume of roughly  $140\text{mm}^3$  in the healthy adult and is bordered by the skull base inferiorly and the inferofrontal cortex superiorly. The structure is the site of significant olfactory signal processing, being replete with GABA and dopaminergic inter-neurons, and contains the anterior olfactory nucleus, a part of the olfactory brain. The bulb contains a unique population of cells, the olfactory ensheathing cells, which play a role in neurogenesis and remyelination that extends into adulthood.[12] It is in the olfactory bulb

where the second order olfactory neurons extend into the olfactory tract to transmit their axons to the olfactory cortex where further processing takes place.

3. Olfactory cortex: It is important to make the distinction between the olfactory brain and the olfactory cortex. The former includes all brain regions in which olfactory processing occurs, including both olfactory cortex and grey matter nuclei in the olfactory bulb. Olfactory cortex specifically refers to a constellation of processing modules distributed predominantly throughout the mediotemporal and inferofrontal cortices, with right inferofrontal cortex being typically more involved in olfactory processing than the left.[13] More distant cortical structures, such as orbitofrontal cortex and cerebellum, are also involved in olfactory function.[14]

Olfactory structure is intrinsically linked to its function. However, the precise role these different component parts play in smell loss seen in neurologic disease is difficult to ascertain. This is, in part, related to functional interdependence of these regions in olfactory processing and the insensitivity of diagnostic tests to tease out the relative contribution of these anatomic structures to olfactory loss. That being said, clinical and paraclinical tools used to assess olfactory function have cast light onto the roles of the peripheral neuroepithelium, olfactory bulb/tract, and olfactory cortex on the ability to smell in health and in demyelinating disease.

## **OLFACTORY FUNCTION AND ITS ASSESSMENT**

Olfactory dysfunction can be manifested in several ways. Anosmia and hyposmia (also referred to as a microsmia) describe complete and partial loss of smell respectively. Parosmia refers to the inability to correctly identify specific odorants and phantosmias are olfactory hallucinations. Olfaction comprises three major functional components - olfactory detection threshold, identification and discrimination - which are defined and explained in more detail below.

1. Olfactory detection threshold: Minimum concentration at which a given odorant can be detected.[15] This component of smell is frequently affected in disease of the nasal apparatus and olfactory neuroepithelium,[15, 16] but is also altered by generalized neurodegeneration such as that seen in Alzheimer's Disease (AD).[17]
2. Olfactory Identification: The ability to specifically label, either verbally or non-verbally, a given odorant at a suprathreshold level.[15] Identification is thought to be mediated by neural pathways linking the olfactory bulb and tract to temporal cortices (the seat of olfactory semantic memory). Reductions in olfactory bulb and tract volume have been found to reduce olfactory identification across a number of studies.[18] A temporal lobe contribution to olfactory identification is exemplified by epilepsy syndromes with a temporal lobe focus, where explicit identification of smells is a feature. Frontal lobe function has also been implicated. [19]
3. Olfactory Discrimination: The ability to correctly distinguish between differing odorants at suprathreshold levels [20]. Discriminatory function is believed to be

especially dependent on frontal cortical activity, requiring attention and executive function to compare and contrast multiple inputs simultaneously.

Psychophysical tests provide objective measurement of overall olfactory function and of its various components. There is a wide range of commercially available tests, the most common being Sniffin' Sticks,[20] and University of Pennsylvania Smell Identification Test (UPSIT).[21] Sniffin' Sticks measure Threshold (T), Discrimination (D) and Identification (I) in order to create a composite 'TDI' score for overall olfactory function.[20] Threshold is measured in a 'single staircase protocol' in which the testee is presented with three sticks, two of which contains a solvent and the other a solvent plus an odorant, and has to pick out which contains the odorant. The concentration of the odorant is increased as the test progresses and the number of times that the testee accurately detects the correct stick is used to calculate a Threshold score. An Identification score is generated by presenting the testee with sticks containing specific odorants and calculating frequency at which odorants can be correctly assigned to a given verbal or non-verbal label. The Discrimination score is calculated by the number of times a testee correctly selects the odd one out from a selection of three odorant containing sticks, two of which contain the same odorant. The UPSIT is a pure test of identification [21]. The testee is provided a booklet of multiple-choice questions 'e.g. This odor smells most like: a.) chocolate; b.) banana; c.) onion or d.) fruit punch" with a corresponding strip of microencapsulated crystals containing the odorant of interest. The benefits of

psychophysical tests are that they are economic, quick and easy to administer. Their drawback is their limited capacity for discerning the anatomical/pathologic basis of a given presentation of smell loss.

Electrophysiological tests provide an alternative to olfactory psychophysics. To record an olfactory evoked potential (OEP), the subject is exposed to either gaseous H<sub>2</sub>S (an olfactory stimulator) or CO<sub>2</sub> (a trigeminal stimulant) in a manner that does not alter the thermal or chemical environment of the nose.[22] Electrodes at Cz and Pz are simultaneously used to record cerebral responses.[22] Increase in latency between stimulation and cerebral response are thought to reflect myelin loss within olfactory pathways, including bulb and tract. In contrast, reductions in amplitude reflect axonal loss in these structures.

Psychophysical and electrophysiological techniques have enabled measurement of the frequency and nature of olfactory disturbance in CNS demyelinating diseases. Data derived from these techniques combined with those obtained from radiological and pathological studies allow the evaluation of the specific anatomic substrate(s) for loss of smell in these diseases, as outlined below.



## **OLFACTORY DYSFUNCTION IN CNS DEMYELINATING DISEASES**

Olfactory disturbance in MS and other CNS diseases where demyelination is a feature, such as neuromyelitis optica (NMO) and acute disseminated encephalomyelitis (ADEM), has been relatively neglected in clinical and research studies. While this arises from this clinical symptom being often overlooked, a key factor lies in a few key historical reports, which suggested that the olfactory system is spared in MS.[23-26] It is only in the last two decades that the nature and extent of olfactory disturbance in MS and other demyelinating diseases is being appreciated. The next section of the review will dissect the clinical, electrophysiologic, radiographic, and pathological evidence for olfactory involvement in MS, NMO, and ADEM, where available.

In order to systematically review literature relating to olfactory dysfunction in the context of these conditions, searches for all combinations of the following words were performed on both Google Scholar and Pub Med up to 11<sup>th</sup> October 2015: 'multiple sclerosis', 'MS', neuromyelitis optica', 'NMO', 'Devic's', 'Acute Disseminated Encephalomyelitis', 'ADEM', 'Demyelination', 'Demyelinating' with either 'smell', 'chemosensory', 'olfaction', 'olfactory', 'odor', 'odorant', 'sinusitis', 'rhinosinusitis', 'sinus'. Relevant literature was read and included in this review if deemed relevant. If the article contained data relating to frequency of smell loss in demyelinating disease then it was included in the Supplementary Material, Table 1.

## **Clinical data**

The weight of available evidence suggests that olfactory symptoms are common in MS although reports have been conflicting. First noted in 1971, Wender and Szmeja observed that olfactory loss occurred in 35% of MS patients compared to controls.[27] Despite this remarkable finding, attention was drawn away from further pursuit of olfactory disturbance in MS after a key paper published by Ansari which refuted smell loss in the disease.[23] Around that time, an experimental report on human post-mortem tissue identified a paucity of myelin basic protein in the olfactory bulb/tract [24], suggesting decreased likelihood of these CNS structures being subject to immune attack in MS, adding further support to this clinical finding. While a report in 1977 by Pinching once again identified olfactory disturbance in MS patients and challenged the claim that there was a paucity of myelin within the bulb/tract,[28] it was only from the late 1980s that momentum gathered in the investigation of loss of smell in MS. To date, a total of 30 studies on olfactory function in MS and other demyelinating disease have been published.[19, 22-24, 27-53]. The message that smell loss not only occurs in MS, but is common is now clear (see Supplementary Material, Table 1).

Psychophysical testing has found the mean frequency of olfactory disturbance in MS patients to be 35.6% (11-50%), with considerable variability in the nature and extent of disturbances noted between studies. In part, this reflects studies that have assessed patients across the temporal and severity continuum of the disease. In MS, the most striking difference in psychophysical findings exists between early versus late disease stages. Olfactory loss can be detected at the earliest of disease

stages, namely at first presentation and in early relapsing-remitting (RR)MS.[41, 44, 47] Luterotti and colleagues observed olfactory impairments in 4/5 patients with clinically isolated syndrome.[41] Similar findings were found in a cohort presenting with monosymptomatic episodes of acute optic neuritis.[47] This patient group was also reported to have heightened awareness of their olfactory troubles relative to other disease stages.[47, 51, 52] In clinically isolated syndrome and early RRMS, increased disease activity within the preceding two years of olfactory assessment was associated with poorer olfactory function, especially in the threshold domain.[41, 44] In a similar vein, relapses negatively impact olfactory function, again with threshold disturbance being preferentially affected.[41] With disease evolution, olfactory impairment appears to increasingly affect other olfactory domains, namely identification and/or discrimination.[19, 35, 41, 44, 49]

Olfactory disturbance is also well documented in progressive MS. Approximately 60-90% of patients with progressive MS have objective olfactory loss.[47, 50] Not only is olfactory loss more common, it has been shown to be more severe in progressive and more advanced disease.[19, 35, 37, 41, 44, 47, 49] A common theme across studies in MS patients with progressive disease is impairment of olfactory discrimination and identification,[19, 35, 44, 49] similar to that seen in late RRMS.[41, 42, 44] The shift in the nature of olfactory disturbance between early and late disease stages raises the question as to whether different olfactory structures are affected at different disease stages.

Psychophysiologic data on olfactory function in other demyelinating diseases is sparse. NMO has been linked to smell impairment. Schmidt et al. (2013) report

olfactory loss in 50% (5/10) of their NMO cohort,[48] and Revis et al. (2014) found olfactory identification to be significantly more impaired in NMO than in MS.[43] In a larger study, Zhang et al. (2015) observed smell dysfunction in 53% (26/49) of patients with NMO spectrum disorder.[53] The frequency and extent to which olfactory dysfunction forms part of the clinical spectrum of other CNS demyelinating diseases, such as acute disseminated encephalomyelitis, myelin oligodendrocyte glycoprotein (MOG) encephalomyelitis, and other immune-mediated CNS disorders, is unknown and warrants further investigation.

### **Electrophysiological data**

Electrophysiological data has added an important objective element to olfactory testing. OEPs are deranged in MS.[22, 29, 31, 38, 39] In a cohort of 45 MS patients and 47 controls, Hawkes et al. found an increase in latency and a decrease in amplitude (of the H2S (olfactory)-related OEP in MS compared to controls, suggesting demyelination and axonal loss within olfactory bulb/tract and/or olfactory brain as an element of MS pathology.[22, 38] The extent of OEP disturbance in MS related to poor performance on the UPSIT score and to the extent of physical disability, as measured by EDSS, in the disease (see clinical association section below).[22, 38] Other independent groups have corroborated these OEP findings in MS, adding further credence to physiologic disturbance of these olfactory structures at some point in the clinical evolution of the disease.[29, 31, 39]

The value of OEPs as a diagnostic tool in MS was also evaluated by Hawkes et al. Whilst VEPs were more commonly abnormal than OEPs, there was a subgroup of patients within the studied cohort exhibiting deficits in OEP/ UPSIT but not in

relation to VEP, suggesting that addition of olfactory assessment might increase overall sensitivity of diagnostic investigations.[22, 38]

## **Radiological Data**

MRI has provided an excellent means of identifying structural correlates of olfactory dysfunction in MS patients. However, the small size of the olfactory bulbs and tracts and MRI signal artefacts generated by the sinuses in close proximity to these structures, have biased early studies to evaluate changes in the olfactory brain. In a landmark study, Doty et al demonstrated a significant positive correlation between reductions in UPSIT score with T2 lesion burden in the inferofrontal and medial temporal cortices of 26 MS patients, 10 of whom exhibited hyposmia (Figure 1A and 1B).[32, 33] This study was of particular significance because the authors highlighted that it was the first study to link clinical with radiologic abnormalities. Importantly, the contribution of olfactory bulb/tract was not considered.[32, 33] Several other clinico-radiographic studies have supported this work,[19, 34, 49, 51, 52] although two reports with large, well-characterized MS cohorts failed to replicate these findings.[42, 44] Differences in MS demographic and clinical features (i.e. disease course, duration, and severity), olfactory assessment techniques used, and MRI resolution may explain these discrepancies. The application of diffusion weighted MRI protocols has increased sensitivity to detect subtle pathological changes in the olfactory brain, which have been shown to correlate more significantly with reduced metrics of olfactory function, as measured by TDI.[19, 35] Temporal factors undoubtedly influence the relationship between olfaction and pathology in the MS brain. A longitudinal study over a 18-20 month period found

olfactory function fluctuated in a manner which mirrored disease activity in the olfactory brain adding support to this claim.[34]

Recent MRI studies have examined olfactory bulbs and tracts as possible contributors to olfactory disturbance in MS.[37, 39, 49] Through the measurement of olfactory bulb and tract volume, a Berlin based group have repeatedly demonstrated reductions in these structures in MS compared to controls,[37, 39, 49] and have shown that these changes relate to loss of smell and,[37, 49] OEP disturbance (Figure 1C).[39] Olfactory bulb volume and olfactory sulcus depth have also been shown to be inversely correlated with disease duration and attack frequency in MS.[54] Similarly, olfactory loss in NMO has been linked to reductions in bulb volume, alongside grey matter reduction within the piriform gyri.[53] The implication of these studies is substantial. Given that atrophy is likely a pathological surrogate of loss of myelin and/or axons, the demonstrated involvement of olfactory bulb/tract has challenged the view that the olfactory brain is solely responsible for olfactory deficits often encountered in these diseases. Pathological studies engender further support to this claim (see below).

### **Pathological Data**

As outlined in the previous sections of this review, the conflation of clinical and radiographic data confirms that olfactory dysfunction is a common feature in MS. However, the relative non-specificity of clinical, paraclinical, and radiographic tools limit their ability to decipher adequately the substrate of olfactory loss in MS. Pathological studies carefully evaluating post-mortem human tissue (olfactory bulb/tract and brain) have filled this important knowledge gap.[11, 55-57] Whilst

involvement of the olfactory bulb and tract has been only recently confirmed,[11] awareness of olfactory bulb and tract pathology in MS dates back to the 19<sup>th</sup> century. In his careful monograph entitled “Manual of Diseases of the Nervous System”, Gowers recognized the frequency of olfactory bulb/tract pathology noting, “...the nerve may be grey in its entire thickness or in part”. [55] Not surprisingly, his brief description was neglected for several decades thereafter. The failure of eminent neuropathologists contemporary to Gowers, such as Cruveilhier,[58] Charcot,[59] and Dawson,[60] to recognise olfactory bulb/tract pathology certainly contributed to the lack of interest in these structures in MS pathology. Zimmerman and Netsky’s failure to detect demyelination in the olfactory bulb/tract in MS post-mortem cases,[26] and the (inaccurate)[28] claim that these structures have a lower density of myelin basic protein,[25] further directed attention away from these anatomic components of the olfactory system.[23] In fact, for a number of years the misconception that these CNS structures are “spared” in MS abounded in scientific circles. A report in German,[57] and a fleeting reference to unpublished observations of olfactory bulb/tract pathology in MS,[56] certainly did not help dispel this belief.

A rekindled interest in the role of the olfactory bulb and tract in MS pathology has surfaced in recent years. Fundamental observations derived from clinical, electrophysiologic, and radiographic studies inspired careful pathologic re-examination of olfactory bulb and tract involvement in MS and other CNS demyelinating diseases. DeLuca and colleagues evaluated these olfactory

structures in a large post-mortem cohort of MS (n=17), NMO (n=3), and ADEM (n=7) cases and compared them to Herpes Simplex Encephalitis (HSE; n=3), Alzheimer's Disease (AD; n=4), and non-neurologic controls (n =8).[11] Olfactory bulb/tract demyelination was frequent in all demyelinating diseases (MS 12/17 (70.6%); ADEM 3/7 (42.9%); NMO 2/3 (66.7%)) but was absent in HSE, AD and non-neurologic controls (Figure 2).[11] The frequency of olfactory bulb/tract demyelination in MS is similar to other areas of the CNS preferentially affected in the disease, such as the optic nerve, corpus callosum, periventricular white matter, and cervical spinal cord. Inflammation was greater in the demyelinating diseases compared to non-neurologic controls.[11] Olfactory bulb/tract axonal loss was most severe in MS where it correlated significantly with the extent of demyelination ( $r = 0.610$ ,  $P = 0.009$ ) and parenchymal inflammation ( $r = 0.681$ ,  $P = 0.003$ ).[11] This study was the first to highlight the distribution of AQP-4 expression in the human olfactory bulb and tract in non-neurological controls and how its expression is altered in demyelinating diseases. The loss of AQP-4 in NMO lesions within the olfactory bulb/tract was consistent with the pattern seen in NMO lesions elsewhere in the nervous system (Figure 3). The extent of remyelination and neurogenesis in the olfactory bulb/tract of these CNS demyelinating diseases (and related animal models) remains unexplored and would be worthy of investigation.

### **Relationship between disease in olfactory bulb and tract and its neighboring structures**

Recent observations suggest that olfactory bulb and tract pathology might relate to that seen in neighboring olfactory brain structures. Anatomically, the olfactory bulb and tract are virtually juxtaposed to the inferior frontal cortex, with only a fine



meningeal layer separating these structures.[11] Given what is known about the tight relationship between cortical demyelination and meningeal inflammation [61], it might be inferred that inflammatory demyelination of one structure relate to that seen in the other. DeLuca et al. (2014) explored this possibility by evaluating the relationship between olfactory bulb/tract demyelination and that seen in the inferior frontal cortex in MS and ADEM, diseases wherein cortical demyelination is an established feature.[11] These investigators showed that the extent of olfactory bulb/tract demyelination correlated with that found in the adjacent inferior frontal cortex.[11] To exclude the possibility these relationships were not merely a reflection of global disease burden, the extent of demyelination in the more distant hippocampus of these MS and ADEM cases was not found to correlate with that found in olfactory bulb and tract (Figure 4).[11] These pathological findings are also not without radiographic support. An MRI study of 30 MS patients quantified olfactory bulb/tract atrophy and found that its extent correlated significantly with T2 lesion burden and atrophy in the anatomically proximate olfactory brain but not in non-contiguous brain regions.[49] Imaging studies relating olfactory bulb/tract and cortical pathology in ADEM are lacking. While cortical demyelination is not thought to be a feature of NMO,[62] recent evidence suggesting the occurrence of cortical neuronal loss in the absence of astrocyte loss and secondary demyelination in this disease support the cortical contribution to olfactory loss observed in NMO by Zhang and colleagues.[63]

## **Linking olfactory anatomy to substrate of loss of smell in demyelinating disease**

Pathologic and radiographic studies have provided a detailed topographical map of pathology along the olfactory system in CNS demyelinating diseases. However, there are limitations in separating out the contributions of the components of olfactory anatomy to loss of function in olfactory threshold, discrimination and identification. The reasons for this are multiple, including a relative lack of sensitivity and specificity of the batteries used to assess olfaction because of the interdependence of olfactory domains (i.e. threshold, discrimination, and identification) on one another, and the inherent subjective nature of most investigative tools used to assess loss of smell. That being said, studies of olfactory dysfunction in demyelinating disease can provide insight into which olfactory structures are affected at various time points (i.e., early versus late), clinical stages (i.e. relapsing-remitting versus progressive), and how smell loss relates to other symptoms often encountered in the disease (i.e., neuropsychiatric and disability measures).

As outlined above, loss of smell seems to differ in early compared to late disease stages. Clinically isolated syndromes, which represent the first clinical manifestation of demyelinating disease, have a surprisingly high frequency of smell loss, with patients typically being more subjectively aware of the olfactory deficits.[47] In particular, olfactory threshold seems to be prominently affected at this early disease stage, with relative sparing of olfactory discrimination and identification.[41, 44] The anatomic correlate of this observation is not clear. However, the link between

olfactory threshold and neuroepithelial and cortical function implicates early involvement of one or both of these structures.[14-17] The relative sparing of discrimination and identification function, olfactory domains dependent on olfactory cortex, challenges a contribution of cortical pathology to early threshold impairment in MS.[41, 44] The possibility that olfactory neuroepithelium could be affected in the early stages of disease is intriguing given epidemiological and radiographic evidence linking nasal sinusitis with MS and AON,[64-67] evidence linking nasal sinusitis with MS and AON. That obstructed aeration induced by nasal sinusitis could explain altered olfactory threshold that occurs early on is made less likely by the fact that olfactory studies in early MS have excluded patients with active or chronic sinus disease. The implication is that the olfactory neuroepithelium is pathologically affected in early MS, which is intriguing from a pathogenesis point of view (see below). This does not exclude the possibility that olfactory submodalities (i.e., threshold, discrimination, and identification) subserved by olfactory cortex may have different levels of susceptibility to injury. In NMO, the association of both anti-AQP4 seropositivity and radiologic abnormalities within the olfactory bulb and cortex with olfactory dysfunction is explained by the diffuse expression of AQP-4 throughout the olfactory system.[53] Involvement of the olfactory neuroepithelium in NMO cannot be precluded given expression of AQP-4 in this structure also.

Pathological studies point to early involvement of olfactory bulb and tract pathology in not only MS but also NMO and ADEM.[11] MS and NMO cases, which came to autopsy within only months of disease onset, demonstrate striking olfactory bulb and tract pathology suggesting early involvement of these structures.[11] Hyperacute involvement of olfactory bulb and tract structures is supported by extensive

inflammatory pathology in ADEM cases, which died within days of symptom onset.[11] As in MS, upper respiratory tract involvement has been linked to later emergence of ADEM,[68] once again possibly linking involvement of neuroepithelial structures with olfactory bulb and tract. While olfactory assessments in ADEM patients are impractical given the reduced level of consciousness that heralds diagnosis of this condition, we would predict prodromal dysfunction of olfactory threshold, with early impairment in olfactory discrimination and identification based on these pathological observations. Further, the association between olfactory bulb/tract and adjacent cortical demyelination provides reason to consider that the olfactory system may provide a conduit for the initiation and subsequent propagation of these diseases. It is interesting to note that the olfactory bulb/tract are juxtaposed to inferior frontal and temporal cortices, which are amongst the commonly affected cortical regions in MS (see Figure 5).[69] This would support the evolution of olfactory dysfunction that occurs throughout the course of MS where this has been extensively studied.

The nature of olfactory loss changes later in the disease course wherein olfactory identification and discrimination impairments becoming predominant features in established relapsing- remitting and progressive disease.[19, 41, 42, 44] The anatomical basis for problems in olfactory discrimination and identification is complex as both olfactory bulb/tract and cortex have been implicated in these olfactory domains. In comparison to olfactory threshold abnormalities which appear restricted to the neuroepithelial compartment, the involvement of olfactory bulb/tract and cortex in relapsing-remitting and progressive MS could merely reflect more diffusely distributed pathology as is recognized with disease evolution. This might also explain

why deficits in olfaction in MS have been linked to the extent of disability (as measured by the ambulatory-biased EDSS).[19, 31, 35, 37, 44, 46, 51, 52] Whilst the degree to which olfactory defects in MS relate to cognitive dysfunction, and depression remains an area of controversy,[31, 40, 51, 52] intriguingly, a contribution of olfactory bulb/tract pathology to cognitive problems and depressive symptoms warrants consideration based on mouse models wherein olfactory bulbectomy drives depressive behaviour and global cognitive dysfunction.[70]

### **Implications for disease pathogenesis**

Does pathological involvement of the olfactory bulb and tract in MS, ADEM and NMO have implications for the pathogenesis of these diseases? Several observations suggest that this may be so. The olfactory system connects the external world (olfactory receptor cells in the nasal mucosa) to the CNS (olfactory bulb/tract and subjacent meninges and cortex). Animal models have shown that olfactory structures are vulnerable to invasion by diverse pathogens, some of which can bypass the blood-brain barrier to penetrate deep regions of the brain and induce an inflammatory response to cause disease.[71-73] This has led some to hypothesise that environmental agents enter the brain via this route to cause and/or propagate diseases where anosmia is an early feature, such as Parkinson's and Alzheimer's (i.e. 'olfactory vector'/'nose to brain hypothesis').[11, 71, 72] As anosmia can similarly occur early in MS, pathological involvement of the olfactory bulb/tract and its relationship to adjacent cortical demyelination may not only explain this symptom but also may spin a different light onto observations which link chronic sinusitis to MS susceptibility, relapse risk and its demography (Figure 5).[64, 65] In ADEM where olfactory bulb/tract demyelination is highly inflammatory and symptom onset occurs within days, disease is typically preceded by contraction of an URTI.[68] Might the olfactory system play a role in permitting a nasopharyngeal agent into the CNS where it initiates a process of acute or chronic

neuroinflammation? Whilst such questions arise from evidence that is only circumstantial at this point in time, they highlight the importance of further investigation into the olfactory manifestations of CNS demyelinating diseases and suggest that the olfactory demyelination observed in these conditions might have implications, which extend far beyond loss of smell alone.

## **CONCLUSIONS**

The olfactory system is complex and functionally interdependent, comprising peripherally the nasal apparatus and the olfactory neuroepithelium, and centrally the olfactory bulb/tract and the olfactory cortex, situated in the inferofrontal and mesial temporal cortices. The conflation of clinical, psychophysical, electrophysiological, radiological, and pathological data suggest that the olfactory system is commonly affected in CNS demyelinating diseases and can have a significant impact on quality of life. The fact that olfactory pathology in CNS demyelinating diseases is frequent, can occur early, be highly inflammatory, and relates to pathology of closely juxtaposed cortex, raises intriguing questions about the role of the olfactory system in the initiation and propagation of these diseases. Further research focused on the deciphering the nature, extent, and underlying mechanisms of olfactory system involvement in MS, NMO, and ADEM could shed critical pathogenic insight into these enigmatic diseases.

## **LIST OF ABBREVIATIONS**

Multiple Sclerosis (MS), Neuromyelitis Optica (NMO), Acute Disseminated Encephalomyelitis (ADEM), Central Nervous System (CNS), Aquaporin-4 (AQP-4), Threshold (T), Discrimination (D) and Identification (I) – composite score TDI, University of Pennsylvania Smell Identification Test (UPSIT), Olfactory Evoked Potential (OEP), Visual Evoked Potential (VEP), Expanded Disability Status Score (EDSS), diffusion weighted Magnetic Resonance Imaging (dw – MRI), Acute Optic Neuritis (AON), Myelin Oligodendrocyte Positive (MOG), Herpes Simplex Encephalitis (HSE), Alzheimer’s Disease (AD), Demyelination (DM)

## **ACKNOWLEDGEMENTS**

We would like to acknowledge Professor Margaret Esiri for kindly offering to proof read the manuscript and Aimee Taylor, medical illustrator, for her assistance in creating Figure 5 entitled *Illustration of the ‘Nose to Brain Hypothesis’*.

## **COMPETING INTERESTS**

None declared

## **FUNDING STATEMENT**

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

## **AUTHORS' CONTRIBUTION**

AJ and GD jointly conceived of the idea for this review article. AJ carried out a review of literature as outlined in text and put together the figures. AJ and GD together interpreted the literature and wrote the manuscript. Both gave final approval for the submitted article and take joint responsibility for the accuracy and integrity of this work.



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## FIGURE LEGENDS

### Figure 1: MRI Studies: The Olfactory Cortex and Olfactory Bulb in MS and Relation to Smell Dysfunction

Smell loss (as measured by UPSIT) correlates with the extent of measures of MRI MS-related lesion burden in cortical regions responsible for olfaction (i.e. inferior and temporal cortices) (A) with such a relationship between smell loss and lesion burden in other (non-olfactory) brain regions not seen (B) (from Doty et al. 1997 with permission).[32] This seminal finding pointed to the olfactory brain as the main substrate of smell loss in MS. This view has been recently broadened to include a role for olfactory bulb pathology as an important contributor to smell loss in MS with lower mean olfactory bulb (bulbus olfactorius) volume observed in hyposmic compared to normosmic MS patients (C) (from Holinski et al. (2014) with permission).[39]

### Figure 2 - Pathological evidence of demyelination within Olfactory Bulb/Tract in MS

Olfactory bulb and tract demyelination in demyelinating diseases compared to non-neurologic controls. (A–H) Olfactory bulbs and tracts stained for myelin (proteolipid protein). High power views of boxed areas represent gray matter (A, C, E, G) and white matter (B, D, F, H) regions for each disease category. In controls, olfactory gray matter structures demonstrated a fine reticulated meshwork of myelin (A). In contrast, myelin in olfactory white matter structures (lateral, medial and intermediate striae) was more densely packed and linearly organized. In each of the



demyelinating diseases (multiple sclerosis (MS) (C, D), acute disseminated encephalomyelitis (ADEM) (E, F) and neuromyelitis optica (NMO) (G, H), olfactory bulb and tract demyelination affected both gray and white matter regions, the extent of which was consistently most severe in MS. Scale bars represent 100  $\mu$ m. Figure and figure legend adapted from De Luca et al. 2014 with permission.[11]

### Figure 3: Aquaporin-4 Staining in Non-Neurologic Controls and Demyelinated Lesions

Aquaporin-4 staining derived from regions outlined in corresponding myelin-stained (Proteolipid Protein) sections of the olfactory bulbs and/or tracts (A-F). In non-neurologic controls (A-C), aquaporin-4 staining is located throughout the parenchyma in both white (B) and grey (magnified view of small box on olfactory tract in Panel C) matter, being significantly more intense in the former especially in the subpial zone (Panel A and with magnified view of box area in Panel B). Intense staining was also noted around blood vessels. There is selective loss of aquaporin-4 staining within neuromyelitis optica (NMO) lesions (E) compared to lesions in multiple sclerosis (MS) (D) and acute disseminated encephalomyelitis (ADEM) (F) where AQP-4 staining is increased. Scale bars represent 1mm in Panel A, and 50  $\mu$ m in Panels B-F. Figure and figure legend adapted from DeLuca et al. (2014) with permission.[11]

### Figure 4: Relationship between the Olfactory Bulb/Tract and Olfactory Cortex in CNS Demyelinating Disease

Inferofrontal cortical demyelination was significantly greater in cases with high levels of olfactory bulb and tract demyelination (red bar) compared to cases with

low levels (blue bar) in demyelinating diseases where cortical demyelination is a feature (i.e. MS and ADEM). No such relationship was found between the extent of olfactory bulb/tract and hippocampal demyelination (From De Luca et al. (2014) with permission) (A).[11] Similarly, in B, olfactory bulb volume (here called bulbus olfactorius (OB)) correlated with the volume of lesions in the olfactory cortex (olfactory brain (BO)) (From Holinski et al. (2014) with permission).[39] These findings suggest that olfactory bulb/tract and adjacent cortical demyelination are associated with one another highlighting their combined importance in smell loss in demyelinating disease.

#### Figure 5: Schematic of the 'Nose to Brain Hypothesis' and Relationship to Olfactory Demyelination

Illustration of the 'Nose to Brain' hypothesis. An environmental agent (e.g virus, bacteria, cigarette smoke) (red particles) infiltrates the nasal passage and sinuses. In a genetically susceptible individual, this could establish an inflammatory milieu in the olfactory bulb (marked by \*), a structure in direct contact with the sinuses through the fenestrations in the cribriform plate. Microglial (blue star-like structures) and lymphocytic (indigo circles) activation leads to demyelination (orange) in the olfactory bulb and tract, which relates to cortical demyelination, possibly via meningeal inflammation (arrow). The establishment of meningeal inflammation in this region could generalize to other CNS areas leading to more diffuse pathology typically encountered in the disease.[11, 71, 72]

Supplementary File, Table: Summary of Studies of Olfactory Dysfunction in MS and NMO

Summary of publications evaluating olfactory (and gustatory) dysfunction in MS or NMO reviewed in this review. [19, 22-24, 27-53] Abbreviations not in text: EDSS = Expanded Disability Status Scale, dw-MRI = diffusion weighted Magnetic Resonance Imaging