Assessment of Human Olfaction:

Investigations into Clinical Practice and Neuroanatomical Correlates of Dysfunction

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Declaration

I, Katherine Lisa Whitcroft, confirm that the work presented in my thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

My thesis was originally submitted for examination in May 2025. My viva took place in September 2025. This final version of my thesis, which incorporates minor corrections, was submitted in December 2025.

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Abstract

Olfaction augments daily life. It guides and enriches feeding behaviours, signals environmental hazards, and facilitates social communication. Olfactory dysfunction (OD) is common – affecting at least one in five adults. Additional to effects on nutrition and quality of life, OD is associated with important healthcare outcomes, including neurodegeneration and death. However, historical data suggest clinicians infrequently assess olfaction. When assessed, controversy remains regarding how this should be done. My thesis has two broad aims: in Theme A, to characterise current clinical assessment practice through interrogation of clinician and end-user populations; in Theme B, to explore new ways in which olfaction could be assessed, through investigation of potential neuroanatomical correlates of OD.

In Theme A I gathered quantitative and semi-qualitative data from 465 clinicians and 576 end-users, from 17 and 33 countries respectively, providing the largest, most detailed analysis of current clinical practice and end-user preferences for assessment. These data suggest that most otorhinolaryngologists do not formally assess olfaction across different clinical scenarios. End-users prioritise tests of orthonasal identification and specialist assessment. Many patients are unhappy with the standard of assessment received, with issues relating to the emergent themes of 'knowledge', 'rigour', 'attitudes' and 'healthcare systems'.

In Theme B I provide the first multimodal longitudinal evidence of functionally significant structural plasticity within the central olfactory networks in association with improved olfactory function. In patients undergoing FESS for CRS, structural changes were observed within the hippocampus, parahippocampus, anterior cingulate cortex, orbitofrontal cortex, insula and temporal poles. Increased functional activity was demonstrated within the latter four regions. Functionally significant structural plasticity was replicated in these four regions in patients with non-CRS OD undergoing functional septorhinoplasty. Together, this work provides more robust evidence for these regions as neuroanatomical correlates of general OD than existing cross-sectional data. Interestingly, bidirectional changes in grey matter volume were observed.

Impact Statement

My thesis contains the most comprehensive available description of current practice in the clinical assessment of olfaction, and the first in-depth analysis of end-user experience and preferences for olfactory assessment. This is a unique resource for research and service planning, funding acquisition and education. It addresses one of the top ten priorities in 'Smell and Taste Disorders', recently published by the James Lind Alliance[1] and supports the ENT-UK GENERATE research agenda[2] and United States NIH/NIDCD strategic plan[3], in which improved assessment/access to assessment is a key goal. Following on from this, I am now supporting colleagues to gather further North American and international paediatric ENT practice data.

My neuroimaging results provide the first longitudinal, multimodal evidence of functionally significant structural plasticity within the central olfactory networks in association with improved olfaction, and stronger evidence for these regions as neuroanatomical correlates of OD. Interestingly, bidirectional changes in grey matter volume were observed, reflecting a more complex relationship between structure and function than previously suggested by cross-sectional data. This knowledge is important when considering such regions as potential future biomarkers of OD. Further research into the mechanisms driving bidirectional changes could prove exciting (e.g., could OD reflect peripherally modifiable neuroinflammation?). To my knowledge, I was the only person performing olfactory fMRI in the UK at the time of research, and the first to do so at the Lysholm Department of Neuroradiology. The protocols I established have now been successfully used in further studies at UCL.

I have published my PhD data in five, first author publications. I have published parts of my general introduction as a book chapter in the current and forthcoming editions of 'Cummings Otolaryngology, Head and Neck Surgery'. I have presented my work internationally: as an invited speaker at the AChemS (USA), ISOT (Iceland), the International Fragrance Association (UK), and 'Osmocosm: Global Machine Olfaction Technologies Conference' (MIT, USA); and as a delegate (oral presentations) at BACO International, and the ERS Congresses (Sweden/UK). I have also given teaching sessions on olfactory assessment at regional and international levels.

Indirectly, the knowledge and experience I have accumulated during my PhD has allowed me to author/co-author over 50 peer-reviewed publications on olfaction, in journals such as JAMA, Rhinology and Chemical Senses. These publications include two sets of international guidelines (The Position Paper on Olfactory Dysfunction, 2017 and 2023, the latter of which I am first author), and a set of national guidelines. Since starting my PhD, I have now personally amassed >4,000 citations and have an h-index of 25 and i10-index of 38. I am now a member of the Clinical Olfactory Working Group and I have acted as peer reviewer for journals including JAMA, JAMA Otolaryngology, BMJ, BMJ Medicine, Rhinology, IFAR, Chemical Senses, Behavioural Brain Research, Neurotrauma, and Cortex.

Finally, aiming to increase awareness of OD, I have presented my work to the public at the Cheltenham Science Festival and the Tate Modern, and written a piece for the digital magazine 'Aeon', which received 10,000 reads within one week of publication.

Acknowledgements

When I began this PhD, olfaction was a niche area of clinical and research interest. In this niche, I found a like-minded community of scientists and clinicians who were working at the interface of medicine, neuroscience, psychology, philosophy and art, with the common goal of improving the lives of patients who had too often been ignored. I am proud to be part of this community.

A heartfelt thank you to Prof Peter Andrews. Thank you for introducing me to olfaction and for giving me this opportunity. I would also like to thank Prof Jonathan Gale – for your generosity, kindness and for making me think laterally.

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Finally, my personal PhD journey has been deeply punctuated by life. This thesis has survived motherhood, a pandemic and boundless grief. It is dedicated to my crazy, wonderful family, my husband Malek and my children, Raphael and Clara. Thank you for all the encouragement and distraction, in almost equal measure.

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Abbreviations Used

ACC	Anterior Cingulate Cortex
ACIII	Adenylyl Cyclase III
AChemS	Association for Chemoreception Sciences
AI	Artificial Intelligence
ALE	Activation Likelihood Estimation
AR	Allergic Rhinitis
BACO	British Academic Conference of Otorhinolaryngology
BRS	British Rhinological Society
C19OD	COVID-19 associated Olfactory Dysfunction
cAMP	Cyclic Adenosine Monophosphate
CCT	Certification of Completion of Training
CN	Cranial Nerve
CNG	Cyclic nucleotide-gated
COWoG	Clinical Olfactory Working Group
CRS	Chronic Rhinosinusitis
CRSsNP	Chronic Rhinosinusitis without Nasal Polyposis
CRSwNP	Chronic Rhinosinusitis with Nasal Polyposis
CT	Computed Tomography
CTh	Cortical Thickness
DGH	District General Hospital
DICOM	Digital Imaging and Communications in Medicine
DNS	Deviated Nasal Septum
DT	Detection Threshold
DTI	Diffusion Tensor Imaging
EDI	Equality, Diversity and Inclusivity
EEG	Electroencephalography
EOG	Electro-Olfactography
ENT	Ear Nose and Throat
ERS	European Rhinologic Society

EUFOREA	European Forum for Research and Education in Allergy and Airway Diseases
FESS	Functional Endoscopic Sinus Surgery
fMRI	Functional MRI
fSRP	Functional Septorhinoplasty
GA	General Anaesthetic
HPC	Hippocampus
HRA	Health Research Authority
ICAS	International Clinical Assessment of Smell
IFAR	International Forum of Allergy & Rhinology
IOD	Idiopathic Olfactory Dysfunction
ISOT	International Symposium on Olfaction and Taste
MIT	Massachusetts Institute of Technology
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NIDCD	National Institute on Deafness and Other Communication Disorders
NIH	National Institutes of Health
NIfTI	Neuroimaging Informatics Technology Initiative
NSD	Nasal Septal Deviation
OB	Olfactory Bulb
OBP	Odourant Binding Protein
OC	Olfactory Cleft
OD	Olfactory Dysfunction
OE	Olfactory Neuroepithelium
OEC	Olfactory Ensheathing Cell
OFC	Orbitofrontal Cortex
OM	Olfactory Mucosa
OR	Olfactory Receptor
ORN	Olfactory Receptor Neuron

OSC	Olfactory Sustentacular Cell
OT	Olfactory Training
PC	Piriform Cortex
PEA	Phenyl Ethyl Alcohol
PET	Positron Emission Tomography
pHPC	Parahippocampus
pICAS	Patient International Clinical Assessment of Smell
PIOD	Post-Infectious Olfactory Dysfunction
PROM	Patient Reported Outcome Measure
PTOD	Post-Traumatic Olfactory Dysfunction
REC	Research Ethics Committee
ROI	Region Of Interest
RT	Recognition Threshold
SBM	Surface Based Morphometry
SIT	Smell Identification Test
SIT-40	Smell Identification Test-40
SnS	Single staircase procedure
SPM	Statistical Parametric Mapping
SRP	Septorhinoplasty
SS	Sniffin' Sticks
TAAR	Trace Amine Associated Receptor
TDI	Threshold, Discrimination and Identification
TRH	Tertiary Referral Hospital
UPSIT	University of Pennsylvania Smell Identification Test
URTI	Upper Respiratory Tract Infection
VAS	Visual Analogue Scale
VBM	Voxel Based Morphometry
VNO	Vomeronasal Organ
WHO	World Health Organisation

1 General Introduction

1.1 Summary

In the following sections I will provide a general overview of human olfaction in health and disease. In doing so, I will describe the anatomy, physiology and pathophysiology of smell and its disorders – what we know, and what we still don't. I will then describe in detail the available approaches for the clinical assessment of olfaction, and their limitations. In doing so, I will provide background and justification for my chosen research aims.

1.2 Statement of Contribution

Sections of this chapter were published as a book chapter: **Whitcroft KL, Hummel T (2021) Olfactory Function and Dysfunction. In: Flint P, Francis H, Haughey B, et al (eds) Cummings Otolaryngology, 7th ed. Elsevier, Philadelphia.**

For this book chapter, I performed the literature review, synthesised relevant information and wrote the manuscript. TH critically appraised the resultant manuscript for intellectual content and approved its final version. For the purposes of this thesis, sections have been expanded, reduced or moved, where relevant.

1.3 How Does it Work? The Anatomy and Physiology of Olfaction

The perception of smell requires coordinated interaction of peripheral and central olfactory systems. Peripherally, volatile chemicals must activate the olfactory nerve (CN I) within the nose. For most odours, this is accompanied by some degree of trigeminal (CN V) activation, which imparts sensations of heat, coolness, pungency or irritation[4]. Following appropriate activation in the periphery, signals must be transmitted to the central olfactory system where the odour percept is formed.

In non-human mammals, the olfactory system can be divided into main and accessory systems, which are anatomically and functionally distinct. The accessory system consists of a peripheral vomeronasal organ (VNO), which is connected to the accessory olfactory bulb via the vomeronasal nerve. This system is used to detect odourants with low volatility, and facilitates chemosignalling through detection of pheromones[5]. In humans, a VNO-like structure can be found in the septum of some patients. However, these are thought to be vestigial organs, as they have no apparent central connection[6,7]. For this reason, I will not consider the accessory olfactory system further in this research. The following sections therefore describe the anatomy and physiology of the main olfactory system.

1.3.1 Olfactory Neuroepithelium

The olfactory neuroepithelium or 'olfactory epithelium' (OE) is a specialised sensory epithelium found within the nose. It is a pseudo-stratified columnar epithelium, containing three main cell types: olfactory receptor neurons (ORN, also known as olfactory sensory neurons (OSN)), supporting (sustentacular) cells and basal cells (a stem cell population including horizontal and globose subtypes)[8]. Deep to the basement membrane, the underlying lamina propria contains Bowman's glands, olfactory ensheathing cells (OECs, a specialised form of glia that share Schwann (peripheral) and macroglia (central) properties) and olfactory nerve fibroblasts, surrounded by connective tissue and a dense vascular network. Overlying the OE is a thin layer of olfactory mucus, secreted from Bowman's glands, and which mixes with respiratory mucus produced by neighbouring goblet cells. To initiate signal transduction, odourants must enter the olfactory mucus where they must then access the ORN – a process facilitated by odourant binding proteins (OBPs). Together, the OE, basal membrane and lamina propria constitute the olfactory mucosa (OM)[9].

ORN are bipolar neurons, with cell bodies located within the OE. Apically, each ORN extends a single dendrite towards the epithelial surface, where it swells to form a dendritic knob. From here, up to 30 non-motile cilia extend into the overlying nasal mucus layer. These cilia contain olfactory receptors (OR) and their presence

therefore greatly increases available surface area for odourant binding. At their basal pole, each ORN extends a single, unmyelinated axon into the lamina propria. Together with ensheathing OECs, these axons form fascicles of increasing diameter, which in turn are enwrapped by perineural olfactory nerve fibroblasts[10]. These *fila olfactoria* then pass through the foramina of the cribriform plate, and collectively constitute the olfactory nerve. Once through the cribriform plate, individual ORN axons synapse with second order neurons in the ipsilateral olfactory bulb[11].

In this way, ORN are in simultaneous contact with the external environment and central nervous system. Consequent to this position, they are prone to damage from a variety of exogenous factors, including pathogens, wood and metal dusts and other toxins. However, as a possible compensatory response, ORN are (in health) capable of adult neurogenesis: under both homeostatic conditions and following injury, they are replaced from the basal cell population of the neuroepithelium. Accordingly, ORN present within the OE are at varying states of maturation. This process is facilitated by OECs, which are present in the OE as well as the OB, and help to guide axonogenesis[12]. Whilst the rate of ORN turnover in humans is unknown, it is likely affected by the presence of injury, its cause and associated degree[13]. However, with extensive damage or increasing age, the regenerative capacity of the OE may fail, resulting in respiratory or squamous-type metaplastic change[14,15].

The olfactory cleft is an anatomical region demarcated by the cribriform plate superiorly, superior turbinate laterally and superior septum medially. Whilst the location of the OE was traditionally thought to be limited to this region, at present, there is uncertainty about its extent within the adult human nasal cavity[16]. It is thought that the OE forms one continuous sheet at birth, and recent histological work has demonstrated proportionally similar distributions of OE within the embryonic and adult nose[17]. However, cumulative damage and failed neurogenesis appear to cause metaplastic change from as early as 2 years[18,14]. Consequently, the OE regresses with age in an anterior/ventral to posterior/dorsal direction[17]. The distribution of the adult OE therefore varies between subjects - with some cases displaying greater OE replacement, which can assume a checkerboard appearance[19].

Accordingly, cadaveric tissue from older adults only consistently contains OE in the area of mucosa directly underneath the cribriform plate[16].

This variability in location and metaplastic interspersion causes difficulty when attempting to biopsy the olfactory mucosa, particularly given that human OE does not appear macroscopically different to respiratory or metaplastic squamous epithelium. Biopsy at the level of the cribriform plate is unfeasible in most cases, due to risk of cerebrospinal fluid leak and associated sequelae. At present, there are no known markers that can be used to label human OE in vivo – meaning that the OE to non-OE tissue sampling ratio in most studies is poor[20–22]. Despite this, OE has been obtained from various locations, including the superior turbinate, superior septum and anterior insertion of the middle turbinate, with variable success[17,19,23–28]. Whilst the regenerative capacity of the OE may mitigate any potentially deleterious effect of OE harvest, the long-term effects of biopsy on olfaction – particularly in older adults – has yet to be established. In practice, these factors mean histological investigation of the OE for clinical purposes is uncommon, and the utility questionable. This is further compounded by variability and overlap in histological findings between different types of olfactory dysfunction[29].

1.3.2 Central Olfactory Network

The olfactory bulb (OB) is the first relay in the olfactory system and forms an important part of the primary olfactory brain network. These paired structures are found immediately dorsal to the cribriform plate and ventral to the orbitofrontal cortex.

Axons from ORNs enter the ipsilateral OB and synapse with second order neurons, mitral and tufted cells, within specialised regions called glomeruli. Axons from mitral and tufted cells then extend to regions of the 'primary olfactory network', regions that receive direct axonal input from the OB, and which have largely been delineated using neural tracing and anatomical studies in non-human animals[30]. The main target of mitral/tufted cell axons is the piriform cortex (PC), though other structures that receive direct axonal connections include the amygdala and the entorhinal cortex.

Odour processing also involves higher order ('secondary') brain areas, which are involved in olfaction, but which do not receive direct axonal input from the OB. This higher order network includes both cortical and subcortical structures, which have largely been delineated in humans through functional imaging studies. In 2013, a meta-analysis of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) demonstrated the highest probability for activation, when comparing odour to non-odour baseline, within the piriform cortex[31]. However, significant activation was also demonstrated within secondary regions including the orbitofrontal cortex, insula and anterior cingulate. More recently, probabilistic tractography with diffusion tensor imaging was used in an attempt to define a merged olfactory network based on both structural and functional evidence[32]. In addition to the piriform cortex, regions highlighted in this study included the anterior cingulate cortex, orbitofrontal cortex and gyrus rectus, caudate nucleus, amygdala, insula, putamen, pallidum, parahippocampus, hippocampus and temporal poles. Central olfactory processing therefore involves many structures of the limbic system. This is important when considering the sequelae of olfactory dysfunction, which will be discussed further in §1.4.5.

The clinical utility of structural and functional neuroimaging in olfactory assessment will be discussed in detail in §1.5.3.

1.3.3 Nasal Aerodynamics

Odourant access to the OE is facilitated by nasal airflow. This airflow may either be anteroposterior in direction, as occurs in orthonasal olfaction, or posteroanterior, as occurs in retronasal olfaction. Retronasal olfaction is required for formation of the flavour percept (where flavour is the multimodal experience of gustation – which transmits sweet, salty, sour, bitter, umami 'tastes' – combined with retronasal olfaction) and is facilitated by retrograde airflow during exhalation, or more markedly during eating and drinking. Unless otherwise stated, when discussing 'olfaction' in this thesis, I will be addressing the orthonasal route.

Assuming that the majority of OE is found within the classical confines of the olfactory cleft - during orthonasal flow, odourants must traverse the narrow nasal cavity to reach this remote area beneath the cribriform plate. Consequently, during normal breathing, less than 15% of the total inspired nasal air will reach the OE.[33–35] Furthermore, the combined irregularity of nasal cavity anatomy, and high velocity airflow results in nonlinear aerodynamics and complex odourant distribution patterns. Odourant access to ORN is further affected by their physicochemical properties and associated differential sorptive (i.e., adsorptive and absorptive) properties[36].

The nasal cycle describes the physiological and cyclical alternation of mucosal congestion between right and left nasal cavities. This can increase unilateral nasal resistance by up to four times[37], though several studies have demonstrated minimal effect on monorhinal olfactory function, as measured using odour threshold[38,39]. The nasal cycle has, however, been suggested to enhance olfactory range due to absorption-dependent differential odourant perception in nostrils with asymmetrical airflows: depending on their absorption odourants will have an optimal nasal flow rate at which they are perceived, meaning that differential flow rates in right and left nostrils will increase the range of odours perceived simultaneously during normal birhinal olfaction[36,40,41]. Taken together, odourant access to the OE is not 'all or nothing' but rather characterised by a unique, and likely variable, 'spatiotemporal fingerprint'.

Whilst profound nasal obstruction is intuitively associated with reduced olfactory function, some previous work has demonstrated that total nasal airflow or nasal resistance, as measured using standard techniques, may not be a sensitive measure of olfactory function[42–44]. Following on from this, evidence from anatomically accurate 3D modelling techniques has shown that small changes in nasal anatomy can lead to significant changes in airflow distribution without concurrent change in overall flow rate[45,46]. For example, small anatomical changes within pivotal regions (e.g. the nasal valve or olfactory cleft), that may not affect total nasal airflow/resistance, can result in marked changes in airflow within the olfactory cleft.[47] This is of particular relevance when considering surgical procedures aimed at

modifying the nasal valve, such as functional septorhinoplasty, or conditions such as chronic rhinosinusitis, in which mucosal congestion may cause oedema within the olfactory cleft.

Finally, when considering modelling techniques that allow more detailed measurement of airflow within specific regions, it should be kept in mind that the distribution of OE may be variable between patients. Clinically, whilst measures of overall nasal airflow are required for the assessment of obstruction, associated inferences regarding olfactory function should be made with caution, outside of the context of total or near total obstruction.

1.3.4 Olfactory Receptors and Signal Transduction

Human OE contains three types of receptor that are thought to be involved in olfaction: the olfactory receptor (OR), the trace amine-associated receptor (TAAR), and a putative pheromone receptor[48]. The largest and most functionally relevant of these groups is the OR.

OR are G-protein coupled receptors that are found in the surface membrane of the ORN's dendritic cilia. Odourant-OR binding initiates a cascade of downstream signalling that ultimately results in the firing of an action potential within the parent ORN. More specifically, following binding of an odourant within the OR binding pocket, an olfaction-specific G protein (G_{olf}) is activated, which then interacts with intracellular adenylyl cyclase III (ACIII), increasing its activity. Increased cytoplasmic cAMP concentrations in turn lead to opening of cAMP sensitive cyclic nucleotide-gated (CNG) channels, which allow influx of Ca^{2+} and Na^{+}. This cation influx results in depolarisation of the cell. As intracellular Ca^{2+} concentration rises, calcium-gated chloride channels open, resulting in an outward Cl^{-} current that modulates and potentiates cell depolarisation. Once depolarisation reaches threshold, an action potential is generated, which is then propagated down the ORN axon towards the OB[49,50]. Mice deficient in G_{olf}, ACIII or CNG are phenotypically anosmic, suggesting this is a canonical pathway in mammalian olfaction[51,52].

To allow for temporal encoding of a stimulus, as well as ORN adaptation to prolonged odourant exposure, several feedback inhibition pathways are initiated following OR activation. Two of these pathways are triggered by rising levels of intracellular Ca^{2+}: first, calcium–calmodulin interacts directly with cyclic nucleotide-gated cation channels, causing reduced channel sensitivity to cyclic nucleotides and so reduced cation influx[53–55]. Second, calcium-dependent phosphorylation of adenylyl cyclase causes reduced production of intracellular cAMP and thereby reduced cyclic nucleotide-gated cation channel activation[56–59]. Other mechanisms involved in feedback inhibition include removal of intracellular Ca^{2+} via the Na^{+}- Ca^{2+} exchanger[60] and degradation of intracellular cAMP by phosphodiesterases.

In addition to ORs, a wide variety of proteins are directly or indirectly involved in olfactory signal transduction, during odourant-receptor binding, downstream signal generation and termination, as well as the production and maintenance of cellular components. These 'auxiliary gene products' therefore have an important role in maintaining tissue function, and have been implicated in across-odourant sensitivity phenotypes (for example, congenital general anosmia or general olfactory sensitivity)[61].

1.3.5 Odour Encoding

The human OR receptor gene family is thought to contain more than 850 genes (873 list by HUGO Gene Nomenclature Committee at time of writing[62]), distributed across the genome except for chromosomes 20 and Y. Of these, it is thought that approximately 465 are non-functional pseudogenes and approximately 390 functional genes[63,64,65]. The OR gene superfamily is the largest within the mammalian genome, highlighting the evolutionary importance of olfaction[65,66]. However, recent estimates suggest only approximately 12% of OR have been deorphanised[67].

Olfactory receptor neurons express OR-genes in a monoallelic and mutually exclusive fashion[68]. Consequently, each neuron displays only one receptor type. However, due to broad molecular binding ranges, many ORs can detect multiple odourants. In turn, each odourant is recognised by multiple receptors. Importantly, as part of the

mechanism by which odour perception is neurally encoded, odourants are detected by a unique combination of OR (and therefore ORN)[69]. Such combinatorial encoding facilitates creation of 'neural-fingerprints' for odourants, so enabling the detection and discrimination of many more distinct odours than would otherwise be possible given the number of functional OR genes[49,66]. Combinatorial encoding is made more complex by ligand-binding behaviour, where some odourants act as agonists, some partial agonists[70] and some antagonists[71,72]).

The neural fingerprint of an odourant may be further enhanced through some degree of spatial encoding. In rodents, the main OE can be divided into four rough zones. In turn, ORN expression varies according to zone, though within a particular zone neurons expressing the same OR-type are distributed randomly[73]. This rudimentary 'rhinotopy' may contribute to odour discrimination, though zonal ORN distribution in humans has yet to be delineated[74]. The 'spatiotemporal fingerprint' (psychochemical properties of an odourant + nasal airflow, described in §1.3.3) overlays onto the neural fingerprint, creating a variable and dynamic representation of odour space. Voluntary modification of nasal airflow through sniffing may thereby potentially modulate odour encoding and associated perception[41,75] – a concept made further complex by the recent suggestion that olfactory receptors may act as mechanoreceptors, sensitive to shear stress[76–79].

Further spatial encoding has also been suggested at the level of the OB. Experimental work in rodents has shown that axons from ORNs expressing the same receptor type come together to synapse within one of two glomeruli in the ipsilateral OB (though in humans the ORN-type to glomeruli ratio may be up to 1:16[80]). Consequently, each glomerulus in the OB receives axonal input from only one type of OR. In this way, the spatial activation map of glomeruli within the bulb is dependent on the receptor binding properties of an odourant[81–83]. Temporal dynamics will also likely contribute to odour encoding within the OB, as well as complex inhibitory interneuron activity[84].

After initial encoding at the peripheral/OB level, olfactory signals are further integrated at the level of the primary and secondary olfactory brain networks, ultimately resulting in formation of the odour percept. Whilst the true discriminatory

ability of humans is unknown, estimates have ranged from the order of thousands to hundreds of thousands of different odours. This being said, people are often unable to smell specific odours, with otherwise intact olfaction. This is thought to be a normal physiological trait, and is termed specific anosmia[85].

1.4 Olfactory Dysfunction

1.4.1 Classification of Olfactory Dysfunction

Classification of olfactory dysfunction can be: 1 – perceptual; 2 – anatomical; 3 – aetiological.

Perhaps most meaningful to patients, olfactory dysfunction (OD) can be perceptually classified as either quantitative or qualitative. Quantitative dysfunction describes alteration in the perceived strength of odours, and includes hyposmia (reduced smell), anosmia (absent smell) and hyperosmia (increased smell)[86]. Qualitative olfactory function describes alteration in the perceived quality of odours and is divided into parosmia and phantosmia. Parosmia is experienced as alteration of odour quality (usually unpleasant) in the presence of an odour stimulus. Phantosmia is the experience of smell (again usually unpleasant) in the absence of a stimulus (an 'olfactory hallucination'). In practice, there may be significant overlap between these types of OD; whilst quantitative olfactory dysfunction may occur in the absence of qualitative dysfunction, it is less common for qualitative dysfunction to exist alone. Further, whilst parosmia and phantosmia often co-occur, and are frequently grouped together for research purposes, they are perceptually different, have varying presentation rates and divergent proposed pathophysiology.

Classification according to anatomical location of pathology has traditionally been used by ENT surgeons. OD consequent to sinonasal pathology, for example chronic rhinosinusitis with nasal polyposis, in which there may be physical obstruction of odourants to the OE is termed 'conductive' OD. OD as a result of pathology at the level of the ORN/olfactory nerve, as may be seen in post-infectious OD, is termed 'sensorineural'. Finally, OD caused by intracerebral pathology, e.g., neurodegenerative disorders such as Parkinson's Disease, is termed 'central'. However, such classification is problematic for several reasons. First, OD causing pathology may be simultaneously or sequentially located at several anatomical locations. For example, whilst cases of chronic rhinosinusitis with nasal polyposis may cause physical obstruction of odourants to the OE, there is now evidence that

associated inflammation at the level of the OE can cause initial OR-dysfunction[87,88] and shift in stem cell phenotype[89] with eventual metaplastic OE replacement[29,90] and upstream effects at the level of the OB[91,92]. Accordingly, diagnoses of obstructive, sensorineural and central OD could be applied, so highlighting the limitations of this classification system. Furthermore, the pathophysiology of many conditions remains unclear, meaning that reclassification may be required as new information is gathered.

Consequently, there has been a move in both the literature and clinical practice towards classification according to aetiology. Such classification encompasses both congenital and acquired, quantitative and qualitative OD, and is not limited by anatomical location of pathology. There are estimated to be approximately 200 different aetiological causes for olfactory dysfunction.[93] Prior to the COVID-19 pandemic, work from several specialist centres demonstrated that, when age-related dysfunction is excluded, approximately 2/3 of cases can be attributed to sinonasal, post-infectious or post-traumatic causes[94–98]. SARS-CoV-2 has now created the largest single-aetiology cohort of OD in history, affecting more than half of infected patients in some series.[99,100] However, it is as yet unclear how many of these will go on to develop long-term post-infectious OD.

1.4.2 Common Aetiological Types of OD

1.4.2.1 Olfactory Dysfunction due to Sinonasal Disease

Sinonasal disease is a common cause of quantitative olfactory dysfunction, and in the olfactory literature, most frequently refers to chronic inflammatory conditions of the nose and paranasal sinuses. Accordingly, quantitative olfactory dysfunction is a key diagnostic symptom for chronic rhinosinusitis (CRS), as outlined in both the European Position Paper on Chronic Rhinosinusitis and Nasal Polyps ('EPOS' guidelines), the American Academy of Otolaryngology-Head and Neck Surgery Guidelines and the International Consensus Statement on Allergy and Rhinology: Rhinosinusitis (ICAR:RS) 2021[101–103]. Given that a large proportion of the general population is thought to have CRS (e.g. 10.9% of the European population, ranging

from 6.9% in Finland to 27.1% in Portugal[104]), and that such conditions are often treated outside of the specialist chemosensory clinic (e.g. primary care or general ENT/rhinology clinics), it is likely that the true burden of sinonasal olfactory dysfunction is greater than is reflected in specialist case series[105,106]. Nevertheless, these series demonstrate that 7 to 56% of cases seen are due to sinonasal disease[107].

Chronic rhinosinusitis has traditionally been classified according to phenotype (e.g. CRS with nasal polyposis, CRS without nasal polyposis), and whilst many studies continue to use this terminology, current guidelines recommend classification according to: 1. origin (primary or secondary); 2. anatomical location (localised or diffuse); 3. endotype dominance (type 2 or non-type 2 inflammation)[103]. Using this system, OD is most severe in patients with type 2 inflammation, and accordingly has been linked to severe nasal polyposis, aspirin-exacerbated respiratory disease and tissue eosinophilia[108].

According to phenotype, the degree of olfactory impairment in sinonasal/airway *inflammatory diseases* is greatest in chronic rhinosinusitis with nasal polyposis, followed by chronic rhinosinusitis without polyps, non-allergic rhinitis, atrophic rhinitis, allergic rhinitis (AR) and chronic obstructive pulmonary disease[109–111]. Given its high prevalence in the general population (approximately 10 – 15% of children and 26% of adults in the UK[112]), and symptomatic overlap with CRS, it is worth noting that OD is much less common in AR than CRS. Indeed, the estimated prevalence of OD in AR is between 20% (approximately equal to the prevalence of OD in the general adult population – see §1.4.3) and 40% of patients, compared with 61-95% of those with CRS[103,109,113,114]. Further, the severity of OD appears milder in AR than CRS, and patients (particularly those with seasonal disease) may have long periods of normal olfactory function[113,115]. Nevertheless, care is needed when separating patients with AR from those with CRS.

Presentation of sinonasal OD is dependent on the underlying condition. Across the different subtypes of CRS, according to diagnostic criteria, OD must be accompanied by nasal obstruction or discharge (rhinorrhoea/post-nasal discharge) ± facial pain and either endoscopic or CT evidence of sinonasal inflammation. Left untreated, it is unlikely that sinonasal olfactory dysfunction will resolve spontaneously[116–118].

Treatment should be undertaken in line with current guidelines and involves an initial period of medical treatment (most often with intranasal ± systemic corticosteroids, nasal saline irrigation ± antibiotics) with progression to other medical and/or surgical treatments where required. Treatments with immune modulating monoclonal antibodies that target type 2 inflammation, and particularly dupilumab, have shown promising improvements in olfactory outcomes in severe CRSwNP[119].

It has been suggested that early intervention may lead to better outcomes in CRS with respect to health-care provider attendances and disease-specific medication use[120]. This is in keeping with current pathophysiological models, as will be discussed in the following section (§1.4.3).

1.4.2.2 Post-Infectious Olfactory Dysfunction (PIOD)

Prior to the COVID-19 pandemic, upper respiratory tract infections (URTI) accounted for between 18 and 45% of cases in specialist series[107]. The link between SARS-CoV-2 and OD was officially recognised by the World Health Organisation (WHO) in May 2020 and is now well established. With more than 650,000,000 cases of infection documented globally (at time of writing), COVID-19 related OD (C19OD) represents the largest single aetiological cohort in known history. The prevalence of OD in COVID-19 varies between studies, but a recent systematic review and meta-analysis demonstrated rates between 44% to 77% of patients, depending on assessment method used[121]. Work has also demonstrated geographical variation in prevalence rates – with higher burden of disease in people of European ancestry – thought to be related to geographical differences in polymorphisms of the UGT2A1/UGT2A2 locus (which encodes an odourant-metabolising enzyme, UDP glycosyltransferase)[122,123]. Rates of OD also appear dependent on prevalent strain, with multiple sources of evidence indicating less frequent chemosensory impairment during the omicron-dominant period, in comparison with early waves of infection[124–126].

Transient olfactory impairment may occur intercurrent to an acute URTI infection[127]. In cases of non-C19OD this is likely secondary to the effects of acute inflammation

including mucosal oedema (± obstruction of airflow and therefore odourant access to the OE) and changes in intranasal (respiratory ± olfactory) mucus[128]. Acute impairment is particularly marked in COVID-19 infection, likely due to divergent pathophysiological mechanisms, as will be described below[129]. Post-infectious olfactory dysfunction (PIOD) occurs when this impairment continues after other symptoms have resolved. In the case of COVID-19, however, 'long COVID' is defined where symptoms persist beyond 12 weeks – therefore, such 'post-infectious' C19OD may exist concurrent to other persistent symptoms. The proportion of patients who go on to develop PIOD after infection with SARS-CoV-2 or other pathogens has yet to be fully demonstrated. However, with respect to C19OD, longer term data is increasingly available, and has demonstrated a range of results – likely due to different assessment time points, assessment techniques and changes in prevalent strain. Several recent meta-analyses have themselves produced varying results. Tan *et al.,* projected persistent C19OD in 5.6% of cases, based on self-report[130]. However, Hu *et al.,* demonstrated persistent C19OD in approximately 1/3 of patients at both 6 months and 1 year, irrespective of assessment technique. In general, higher rates of persistent C19OD are demonstrated where psychophysical assessment techniques are used (a type of assessment that will be discussed in detail in §1.5.2).

Onset is typically sudden, and chronologically related to the causative infection, which patients may describe as particularly severe. Some patients may not, however, recall an offending infection (many URTI are thought to be asymptomatic[131]), which may lead to an incorrect diagnosis of idiopathic dysfunction. Typically, women are affected by PIOD more commonly than men, and are frequently middle aged or older at presentation[29,132]. The reason for this female preponderance is unknown, but possibly reflects the higher incidence of upper respiratory tract infections in women[133]. Increasing prevalence with age may be related to reduced regenerative capacity and/or accumulation of successive insults[134]. PIOD can cause both quantitative and qualitative dysfunction – with parosmia being particularly prevalent in both acute and persistent C19OD. In

addition to SARS-CoV-2 and other viruses, PIOD is less frequently caused by other pathogens, such as bacteria, fungi or rarer organisms such as microfilaria[135].

Spontaneous recovery from non-C19-PIOD has been demonstrated, and is thought to be more frequent than in other common aetiological subgroups (e.g., PTOD, see below)[97]. Spontaneous recovery from acute C19OD is described as above (with some work suggesting that most patients recovery within approximately one month[130]) though recovery rates from persistent C19OD are as yet unknown.

1.4.2.3 Post-Traumatic Olfactory Dysfunction (PTOD)

Olfactory dysfunction following head injury (post-traumatic olfactory dysfunction, PTOD) is another common cause of impairment, accounting for between 8 and 20% of patients presenting to specialist chemosensory clinics[107]. The incidence of PTOD after head injury in adults is between 5 and 10%[136–139]. In children, transient loss is reported in 3.2%, whilst permanent loss is reported in 1.2%[140]. Whilst head injury is more common in men[141], olfactory dysfunction is independent of sex or age at time of injury[142]. In general, olfactory loss is greater where the head injury is more severe[136,143], however, anosmia has been documented following relatively minor trauma[138]. Whilst frontal blows are common, occipital impact is more likely to cause worse olfactory loss[138,142]. Onset is usually immediately following injury, but may also be delayed, either reflecting progressive pathology, or lack of impairment awareness during the acute phase. Recovery of olfactory function in this group is less common than in other aetiological subtypes[132], but early treatment with systemic corticosteroids and olfactory training (a systematic programme of deliberate exposure to a range of odours over ≥3 months, shown to improve olfactory function) may both be of benefit[144–146].

1.4.2.4 Other Causes of Olfactory Dysfunction

In addition to the above, olfactory dysfunction is frequently associated with aging and neurodegeneration. Epidemiological studies have demonstrated impairment in up to 62.5% of people over the age of 80[147]. In the context of neurodegeneration,

idiopathic anosmia in older adults is associated with the development of mild cognitive impairment[148] and dementia[149]. Indeed, OD is well established in Alzheimer's and Parkinson's disease, where OD may predate other clinical signs of Parkinson's disease by many years[150,151]. Onset of dysfunction in patients with neurodegeneration is insidious and typically does not recover[152] .

Other causes of olfactory dysfunction are outlined in Table 1-1. Where a cause cannot be identified, a diagnosis of idiopathic olfactory dysfunction (IOD) can be made. However, this should only be done after careful exclusion of other diagnoses including sinonasal inflammation.

Classification	Examples
Neurological	Epilepsy, myasthenia gravis, multiple sclerosis, migraine, stroke, neurodegeneration (e.g., Parkinson's and Alzheimer's disease) [153,154,155,156,135,157–160]
Congenital	Isolated sporadic, syndromic (e.g., Kallmann syndrome)
Drugs and Toxins	Various medications, heavy metals, pesticides, herbicides and solvents[107]
Neoplasms: Intracranial	Pituitary tumours, suprasellar ridge meningiomas, olfactory groove meningiomas and frontal lobe gliomas[135]
Neoplasms: Intranasal	Olfactory neuroblastomas (esthesioneuroblastomas), adenomas, inverting papillomas and squamous cell carcinomas[161]
Iatrogenic Dysfunction	Surgical procedures: sinonasal (e.g. septoplasty, septorhinoplasty or ethmoidectomy) or neurosurgical (e.g. hypophysectomy, craniofacial resection, anterior craniotomy or focal cerebral excision).[162–164]
Psychiatric Conditions	Depression, schizophrenia.[165]

Table 1-1: Non-exhaustive list of other causes of olfactory dysfunction

1.4.3 Cellular Pathobiology of Acquired Olfactory Dysfunction

The exact pathobiological processes underlying many forms of OD remain to be elucidated. In the following section, I will describe the current state of knowledge for the more common types of acquired OD, including sinonasal disease (limited to CRS), PIOD (COVID-19 and non-COVID-19 related) and PTOD.

1.4.3.1 Olfactory Dysfunction due to Sinonasal Disease

In addition to mechanical obstruction of odourants to the OE caused by polyps and oedema, inflammatory changes within the OE contribute to both short and longer-term OD in CRS. Using a transgenic mouse model in which TNF-α is expressed locally within the OE, Lane and colleagues demonstrated progressive inflammatory infiltrates that resulted in loss of mature ORNs and suppressed neurogenesis[87]. Whilst mature ORN loss occurred at approximately 5-7 weeks, reduced odourant sensitivity was seen from week two onwards, suggesting inflammatory cytokine mediated disruption of odourant binding and/or signal transduction. More established inflammation may then lead to a switch in stem cell phenotype, from regenerative to immune[89], potentially reducing the OE's capacity for neurogenesis. This, in addition to other possible mechanisms, is likely responsible for the eventual metaplasia of the OE to squamous and respiratory type epithelium[23,29]. Centrally, several studies have demonstrated reduced OB volume in patients with CRS[91,92], though the effect on structures upstream of the OB has yet to be fully established, as will be discussed later in this thesis.

Furthermore, as mentioned above, OD is most severe in patients with type 2 inflammation, and accordingly has been linked to severe nasal polyposis, aspirin-exacerbated respiratory disease and tissue eosinophilia[108]. The role of type 2 inflammation in the pathogenesis of CRS related OD is being increasingly demonstrated by the introduction of monoclonal antibodies for severe disease. Accordingly, medications such as dupilumab, which inhibits IL-4/13 signalling, has been shown to cause rapid and sustained improvement in subjective and psychophysical olfactory function (for discussion of monoclonal antibodies, see

reference [166]). Given the speed of onset (subjective improvement seen by day 3), one may speculate that IL-4/13 signalling is involved in reversible, cytokine-based OR dysfunction.

1.4.3.2 Post-Infectious Olfactory Dysfunction (PIOD)

The pathogenesis of PIOD has not been fully described. With respect to non-C19 disease, histological evidence suggests damage at the level of the OE, which undergoes remodelling and variable replacement with respiratory or, more rarely, metaplastic squamous epithelium. In the remaining OE, ORN and OR populations are reduced and ORN morphology is altered, with, for example, dendrites that do not reach the mucosal surface[29,167]. As a possible consequence of reduced afferent input, reduced OB volume has been demonstrated in patients with PIOD[168,169]. More direct mechanisms of OB damage through transmission of pathogens via the olfactory nerve have also been proposed, but require further investigation[170,171].

With respect to C19OD, a rapidly evolving research base has demonstrated pathophysiological mechanisms that target the peripheral olfactory system, as well as variable evidence of possible infectious or para-infectious central effects. A single stranded RNA virus, SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) via its glycoprotein spike (S protein), in a process that is facilitated by the priming protein TMPRSS2[172]. In mice, ACE2/TMPRSS2 are expressed by sustentacular cells (OSC) within the OE, and by vascular pericytes within the OB[173,174]. Similar non-neuronal expression has been found in human OE – specifically, OSC, Bowman's gland cells and horizontal basal cells[174]. In human cadaveric work, tissue collected from patients who had died from COVID-19 showed evidence of viral replication within OSC, and viral RNA with the leptomeningeal layers of the OB, but none within either the ORN themselves, or the OB parenchyma[175]. Following SARS-CoV-2 infection in hamsters, Zazhytska *et al.,* demonstrated transcriptional changes and transient depletion of the OSC compartment[176]. Furthermore, they went on to show non-cell autonomous downregulation of OR and OR signalling genes, in both humans and hamsters. This process involved disruption of ORN nuclear architecture, in a way that speculatively may cause delayed reactivation of OR transcription, or

alternatively reactivated transcription of the incorrect OR type (possibly contributing to delayed return of olfactory function after infection or altered odour encoding during restitution of function (causing parosmia), respectively). Other possible mechanisms of either acute/persistent C19OD that have been suggested, to date, include (but are not limited to): OE inflammation with associated secondary effects (e.g. possible inflammation-driven switch in stem cell phenotype[87,89])[176–179]; OE damage secondary to coagulopathic/vascular pathology[180]; transient or persistent changes at the level of the OB[181–183] or upstream central structures[184]. With regards to central structures, it should be noted that whilst SARS-CoV-2 neurotropism has been suggested (and present in animal work for SARS-CoV), this has yet not been confirmed, and it is possible that intracerebral changes seen are due to para-infectious effects or altered peripheral input[184].

1.4.3.3 Post-Traumatic Olfactory Dysfunction (PTOD)

The pathophysiology underlying PTOD may involve a combination of several mechanisms. First, gross nasal or septal fractures, intranasal blood clots or oedema may cause mechanical obstruction of odourant access to the OE. Severe craniofacial injuries may also cause disruption of the neuroepithelium itself, and odourant-OR binding may be affected by changes in olfactory mucus characteristics[185]. Second, more severe coup, contra-coup type injuries or fractures to the midface or anterior skull base may in theory lead to transection of the olfactory fila as they traverse the cribriform plate[186]. This pattern of injury would likely lead to immediate smell loss, and recovery may be limited by scarring and subsequently impaired axonal regeneration/targeting to the OB[187,188]. Finally, intraparenchymal haemorrhages, contusions and subsequent gliosis may cause disruption of central olfactory processing[189,190,191]. Central lesions may take longer to become evident than those secondary to mechanical obstruction, neuroepithelial or nerve lesions. This is in keeping with the observation that some patients experience a delayed onset of olfactory loss after injury. However, PTOD can occur without visible signs of trauma on neuroimaging studies [(189)].

Biopsies of olfactory mucosa from patients with PTOD demonstrate three characteristic histological changes: disruption of OE orientation (where cellular orientation and nuclear location is abnormal), axonal proliferation (abnormal growth of axons within the epithelium and below the basement membrane) and absence of ciliogenesis (dendrites without cilia, and consequently reduced ORs).[29] Furthermore, the degree of OE degeneration appears to be correlated with the severity of injury, and time since injury.[143] These histological changes may be argued to support failed axonogenesis and/or failed axonal targeting to the OB. The latter may be due to injury at the level of the olfactory fila, or potentially secondary to downstream effects from higher central injuries.

1.4.3.4 Peripheral vs. Central Pathobiology

Based on the above, the pathophysiology of PIOD and CRS-related OD appears to originate at the level of the peripheral olfactory system. However, there is also evidence for either subsequent, or potentially simultaneous central pathobiological causes or effects, particularly demonstrated at the level of the OB (see 1.5.3 for further discussion). Similarly, whilst OD due to PTOD, neurodegeneration and aging is strongly associated with central pathology, changes at the peripheral level (e.g. histological changes within the OE[29]) have also been shown. For this reason, the olfactory system as a whole should be taken into account when considering the pathobiology of OD and its assessment.

1.4.4 Epidemiology of Olfactory Dysfunction

Previous epidemiological estimates for the population prevalence of OD have varied widely – from as low as 1.4%[192], to as high as 62.5%[147], depending on sample demographics, assessment method and true population level differences. Generally, estimates tend to be higher where olfaction was tested, rather than self-reported (this will be discussed in more detail in §1.5). Despite this, a recent meta-analysis including 175,073 pre-pandemic subjects demonstrated an overall prevalence of 22.2%, including both hyposmia and anosmia. Approximately 5% of these people are thought to be anosmic, making this form of sensory impairment more common than blindness or profound deafness in the UK[193,194]. Given the large cohort of patients experiencing acute and post-infective C19OD, the true population prevalence is likely dynamically evolving. Nevertheless, using pre-pandemic figures, OD is a highly prevalent sensory impairment affecting at least one in 5 of the general adult population.

1.4.5 Effects of Olfactory Dysfunction

1.4.5.1 Quality of Life

Olfactory dysfunction has both direct and indirect effects, with variable but potentially significant impact on the individual patient and wider society. Broadly speaking, the effects of OD on daily life can be divided into the following main domains: 1. Food and nutrition; 2. Hazard avoidance; 3. Hygiene; 4. Social interaction.

One of the most immediate consequences of OD is reduced enjoyment of food and difficulties in cooking. Orthonasal olfaction facilitates anticipation of food and helps in its preparation – allowing people to identify when food might be burning, or reaching desired stages of preparedness (e.g., being instructed to fry garlic 'until fragrant' or being alerted to food nearing completion by its smell). Retronasal olfaction imparts all the nuances of flavour to food – without which the remaining gustatory dimensions (sweet/salty/sour/bitter/umami) provide a distorted facsimile of the original perceptual experience. In surveys of patients with OD, up to 95%

report issues relating to food/cooking[195]. The consequent effects on appetite and weight appear, however, to vary on an individual basis. Some patients report weight loss (usually due to lack of interest in food), whilst others report no change or weight gain (possibly due to increased reliance on sugar or lack of 'satisfaction' from food eaten leading to increased consumption). In one series, 19% of patients lost weight, and 24% gained weight[196]. In another study, 33-40% of patients ate less/lost weight, whilst 36% ate more/gained weight[195]. The effect of congenital anosmia on these domains, however, does not appear to be as severe. Such patients do not experience a perceptual *change* in their experience of food, and issues tend to be limited to those surrounding cooking. In keeping with this, Croy *et al.,* demonstrated that congenital anosmics do not differ significantly in weight or food preferences compared to age matched controls[197].

Intact olfaction also facilitates hazard avoidance. Related to the above, both ortho- and retronasal olfaction allow identification of spoilt foods or other toxic substances. Patients with OD are also at risk of environmental hazards such as fires, gas leaks or exposure to noxious chemicals. Together, the risk of exposure to environmental hazards causes anxiety and unknown direct exposure to such hazards may cause harm. One series describing 37% of people with OD having experienced at least one olfactory-related hazardous event[198].

Patients with OD are often unable to smell hygiene-related malodours, including those relating to themselves (e.g., body odour/bad breath), their children/dependents (e.g., dirty nappies/unclean clothes) or their homes. This has been shown in several studies to cause anxiety and/or feeling of social stigmatisation, and can be particularly problematic for mothers/primary caregivers[196,199]. Indeed, up to 36% of patients cite this as the most worrying effect of OD[200].

Patients with OD also report issues surrounding social interaction and relationships. This appears to be related in part to social anxiety/stigmatisation caused by real or perceived malodours, as well as perceived belittling or disbelief of their condition, and impaired intimacy with partners. This appears to affect patients with congenital OD as well as acquired OD – one study demonstrated that congenital anosmics have

a lower number of sexual relationships than controls[201]. The role of olfaction in chemosignalling (e.g., mate choice, kin recognition and emotional contagion), and its potential effects on social communication, inclusion and wellbeing are also being increasingly illustrated[202–204].

1.4.5.2 Healthcare Outcomes

Up to 49% of patients with OD experience symptoms of depression[195]. Whilst reduced quality of life inevitably contributes to this, it has been suggested that OD and depression are more integrally linked, with peripheral olfactory input modulating regulation within the limbic system. Indeed, the relationship between depression and olfaction is dose-dependent and reciprocal[205].

As outlined above, OD is an early sign of neurodegenerative disease. OD has also been linked with frailty, possibly at least in part through impact on eating behaviours[206]. Interestingly, OD has also been shown in several studies to increase risk of mortality.[207–211] For example, logistic regression analysis of data from the National Social Life, Health and Aging Project demonstrated that the odds of 5-year mortality in anosmic older adults was over 3 times higher than that of their normosmic peers, after controlling for confounding factors[207].

Together, OD is a marker of important healthcare outcomes. Identification of patients at risk of such outcomes, with subsequent optimisation of their modifiable risk factors could have significant impact on direct and indirect healthcare and societal costs[212,213].

1.4.6 Implications for Clinical Practice and Gaps in Knowledge

Whilst progress is rapid, many questions remain regarding the physiology and pathophysiology of olfaction and its disorders. Precise and reliable prediction of olfactory percept from molecular structure is not yet possible, and the majority of OR remain deorphanised. Outside of the context of syndromic associations or neurodegeneration, genetic testing is not usually helpful in the clinical context.

Histological analysis of the olfactory mucosa is hampered by incomplete knowledge of disease pathogenesis, and current limitations in sampling techniques. Together, further work is needed in these fields to enable meaningful clinical information about olfaction/OD to be derived.

Given the importance of olfaction at the personal and societal levels, and its potential use as a marker of important healthcare outcomes, current approaches to clinical assessment must therefore be optimised, and will be discussed in the following sections.

1.5 Assessment of Olfactory Function

For the purposes of this thesis, the assessment of olfaction and olfactory dysfunction will be divided into four categories, with increased emphasis on the first three, given their clinical utility.

1. Subjective assessment
2. Psychophysical assessment
3. Imaging
4. Electrophysiological assessment

After reviewing how olfaction and OD can be assessed, I will then discuss end-user experience and preferences for olfactory assessment, before summarising my thesis aims and objectives in the next section.

1.5.1 Subjective Assessment

Subjective, self-reported function is an important clinical or research outcome required to determine the personal impact of olfactory function, in health and disease. Broadly speaking, such assessment may target olfaction itself, or the secondary effects of olfactory function or dysfunction, for example on quality of life.

The method by which data is captured varies in formality, standardisation and validation. At one end of this methodological spectrum, unstructured/unguided accounts (for example, as are provided in unprompted social media posts[214]) produce patient/subject driven qualitative data that can be used *prima facie* to describe that person's experience, or which can undergo various degrees of interpretation alongside other similar accounts (using qualitative research techniques) to identify common or important phenomena. Unprompted aspects of the clinical history could be considered in this category, though in practice, the clinical history is guided to varying degrees by the clinician. In an effort to produce data that can easily be compared between subjects or time points, standardised psychometric tools have been produced. Accordingly, these tools transform

perceptual experience into quantitative or qualitative data, which can be used to quantify function/dysfunction or its secondary effects. Such tools vary in complexity from single items targeting specific questions (e.g. 'how well can you smell'), to multiple items covering different conceptual domains (e.g. olfactory function, nutrition, social relationships, quality of life etc).

When such tools are used for the purpose of assessing patient experience of health and disease they are termed 'patient reported outcome measures' (PROMs). Of use in standardised outcomes assessment, PROMs can be used to calculate the 'minimal clinically important difference' (MCID) – the smallest degree of change in an outcome measure that is perceived to be important to the patient, and which may affect their care.[215] The MCID can be calculated for PROMs themselves, through use of another subjective measure of patient experience. Use of the MCID therefore prevents decision making based on outcome differences that may be statistically significant, but clinically meaningless.

Within clinical practice, OD and its effects can be assessed using PROMs that have been expressly developed for this purpose, as part of those assessing wider disease burden (e.g., those targeting CRS) or using non-disease specific tools that provide general measures of quality of life (e.g. the SF-36). The latter allow comparison with other chronic conditions but lack detail that may mean important disease-specific information is lost. This is also true of PROMs targeting particular diseases, in which a limited number of olfactory-specific questions may be included (e.g., only one of the 22-item Sinonasal Outcomes Test ('SNOT-22'), or the 16-item Rhinosinusitis Disability Index). Tools that have been specifically developed to assess OD or its effects include, for example, the Questionnaire of Olfactory Disorders, which measures the impact of OD on daily life (and thereby direct/indirect physical and psychosocial disease effects) and has been widely validated and translated into several languages.

Whilst the usefulness of olfactory-PROMs in capturing personal disease burden and the MCID is well established, there is ongoing debate regarding the diagnostic utility of self-reported olfactory function (i.e., separation of patients into normosmia /hyposmia/anosmia based on their reported olfactory perception). Evolving clinical

opinion can be inferred by the recommendations set out by the EPOS guidelines. In their 2012 iteration, these guidelines stated that '*reduction of smell can be rated by patient subjective report', 'Subjective report of olfaction correlates well with objective tests'* and '*Subjective scores have been found to correlate significantly to objective olfactory threshold and qualitative tests in normal population, rhinosinusitis with and without nasal polyps and other disease conditions.'*[216] In the more recent EPOS-2020 guidelines, an expanded section on olfactory assessment has been included. Therein, the use of 'smell tests' is '*recommended in order to objectively evaluate this disorder'.* However, the continued complexity of opinion was outlined during the steering group Delphi exercise, in which they were 'unclear' or opposed to use of 'smell test(s)' in each of the presented diagnostic/outcomes scenarios.[103]

This early shift in opinion is likely motivated by an increasing body of evidence demonstrating poor diagnostic accuracy of self-report, when compared to more formal psychophysical smell tests. Multiple lines of evidence can be used to illustrate this divergence in assessment techniques. These include: 1. Studies performed in healthy participants; 2. Studies performed in patients; 3. Epidemiological work.

In healthy participants, an early study from Landis *et al.,* demonstrated no significant correlation between subjective olfactory assessment using a single question visual analogue scale (VAS) and psychophysical test score, when subjective testing preceded chemosensory testing (using a composite score of odour threshold, discrimination and identification, see §1.5.2) in testing-naïve participants[217] Rather, subjective assessment of olfactory function correlated significantly with subjective assessment of nasal patency. Similar results have been replicated in the UK: in 2006 Philpott *et al.* demonstrated no significant correlation between odour threshold and subjective assessment of olfactory function using a single item VAS in 186 healthy volunteers[218]. Approaching this problem in a different way, Oleszkiewicz and Hummel interrogated psychophysical test scores in 8,328 people who had self-identified as normosmic (and who had accordingly volunteered as healthy controls for olfactory research). Using a validated test of odour identification, they found that 3.4% of people were unknowingly anosmic (and therefore had no meaningful olfactory perception).[219]

In patient populations, poor correlation between subjective assessment and psychophysical testing has also been shown. In a cohort of patients presenting to clinic with rhinological symptoms (attributable to various underlying aetiologies), only 27.5% were able to accurately categorise their sense of smell according to psychophysical test scores.[220] Similarly, in a cohort of 115 patients with CRS due to undergo functional endoscopic sinus surgery (FESS), 28% were able to correctly categorise their olfactory function.[221] However, in more recent work interrogating a large retrospective dataset (compiled routinely in patients undergoing surgery at a single tertiary centre - total n=6,049, 1227=anosmic, 3113=normosmic), self-reported olfactory function of 'absent' or 'impaired' correctly diagnosed anosmia with a balanced accuracy (mean of sensitivity + specificity) of 79%, and ratings of 'good' or 'excellent' diagnosed normosmia with a balanced accuracy of 64.6%.[222] That being said, this same study found that just under 1/3 of anosmics rated their olfactory function as at least 'average'. Taken together, these studies demonstrate variability in the patient literature. This may reflect differences in self-report accuracy according to clinical scenario where it is likely that those with more sudden and/or profound OD may have greater insight into their condition than those with gradually progressive or less severe impairment.

In epidemiological work, where the division between 'healthy' and 'patient' populations is less clear, estimates of OD prevalence have varied widely with assessment technique and sample population (from 1.4%[192] to 62.5%[147]). In general, self-report produces lower estimates than demonstrated with psychophysical testing. This was confirmed in a recent systematic review and meta-analysis, where prevalence of OD using subjective assessments alone was 9.5%, rising to 21.2 – 35.8% in studies using psychophysical testing methods.[223] Following on from this, some epidemiological work has directly compared these techniques. In a cohort of 1,005 subjects invited for routine health screening in Taiwan, there was no significant correlation between subjective olfactory assessment (Likert-type scale and VAS) and psychophysical testing (odour identification, see §1.5.2).[224] This was true across age groups (range from 18 to 89, divided into groups: 18-35, 36-55, >55 years) and the overall sensitivity of self-assessed OD in identifying chemosensory

test-proven OD was 20%. Similarly low sensitivity rates are replicated in other studies: in a cohort of older adults with normal cognition, sensitivity was 22.7%[225]; overall sensitivity was 19% in another cohort of middle-aged and older adults with 'average' cognitive function[200]; and finally a sensitivity of 21% was demonstrated in another group of middle-aged and older adults with normal cognitive function[226].

Taken together, whilst patients seeking medical care may outperform non-patient populations, generally self-report appears to correlate poorly with psychophysical testing and underestimates OD. It should be noted, however, that most of the literature available uses single-item PROMs, often employing line scales such as the VAS or category, 'Likert-type' scales for this purpose. Expanded olfactory PROMs, in which odour perception is interrogated using multiple items, may more accurately assess function/dysfunction. The Self-Administered Odor Questionnaire (SAOQ), contains 20 such items (frequency of specific odour perception using Likert-type scale), and shows higher psychophysical test (T&T olfactometer) score-anchored performance (sensitivity, specificity, positive and negative predictive values) than single item VAS.[227] However, this tool was designed for use in the Japanese population, and with inclusion of perceived odours such as miso, seaweed, soy sauce and green tea, would require cultural adaptation for use in non-Far Eastern populations. Another multi-item olfactory PROM, developed for use in patients with Parkinson's disease, is the Hyposmia Rating Scale (HRS).[228] This tool includes 6 Likert-type questions on perception of odours in commonly encountered environments, and shows superior correlation with psychophysical testing metrics (odour identification, discrimination and threshold), compared with a single question ('are you experiencing problems with your sense of smell'). Consequently, the HRS had a sensitivity of 70% in diagnosing psychophysical test proven OD, compared with 35% for the single question. However, this tool has yet to be validated in other patient populations, and its general clinical utility is therefore unknown. Use of questionnaires that measure OD-related QOL, such as the QOD and its 'negative statements' variant, have also been shown to correlate with psychophysical test scores in some, but not all patient populations (see ref [229] for review).

1.5.1.1 Implications for Clinical Practice and Gaps in Knowledge

To fully appreciate patient experience, subjective self-report should be undertaken. However, sole use of such measures for diagnostic or outcomes assessment is problematic, for reasons outlined above. Where subjective tools are used clinically to determine olfactory function, multi-item PROMs may provide information that more closely reflects psychophysically determined function. However, even where the best performing tools are used, a significant proportion of patients with OD could be missed. This is particularly important where surgical interventions, with their attendant risks, are considered, and/or during any outcomes assessment.

At present, there is no published information on UK clinical practice with regards to subjective assessment, nor on geographical variations in its use. Therefore, an aim of this thesis is to determine *whether* and *which* PROMs are being used in clinical practice, during the assessment of OD.

1.5.2 Psychophysical Assessment

Psychophysical tests allow for the quantitative assessment of sensory function. Such tests are commonplace in medicine, and frequently used in the assessment of vision, hearing and touch. Perhaps the best-known example in otorhinolaryngology is the measurement of hearing thresholds using the pure tone audiogram. In general, and across sensory systems, psychophysical tests involve presentation of quantifiable and modifiable sensory stimuli to a subject, with subsequent interrogation of their resulting conscious perception. Definitions of normal and abnormal sensory function can then be made by comparing the subject's results with established normative values. When used to test olfactory function, odour stimuli must be provided to a patient, which presents some unique challenges. However, given the issues surrounding subjective reporting of olfactory function, their use allows assessment to be standardised across individuals and time points. Provided the tools used are reliable, have sufficient associated normative data, and are used correctly, diagnoses of normal and abnormal function can be made. This in turn allows for more accurate treatment planning, outcomes assessment, demonstration of disease natural history and determination of malingering. Despite this, psychophysical olfactory tests do have some limitations, particularly when used for clinical purposes, which will be discussed below.

Psychophysical olfactory tests can be generally divided into three categories: 1 – tests of odour threshold; 2 – tests of signal detection; 3 – tests of suprathreshold olfactory function.

1.5.2.1 Tests of Odour Threshold

Odour threshold tests aim to determine the lowest concentration of an odour that is reliably perceived by the subject. They can be divided into those which assess absolute threshold (the lowest concentration of an odour that can reliably be detected) and those which assess differential threshold (the smallest difference in

concentration of an odour that is reliably perceived as stronger/weaker[a])[230]. Differential threshold tests are rarely used clinically and will therefore not be discussed further. Absolute threshold can be subdivided into tests of odour detection threshold (DT) – the lowest concentration of an odourant that can be reliably detected as an unrecognisable smell sensation or 'something' – and tests of odour recognition threshold (RT) – the lowest concentration of an odourant for which the odour *quality* can be reliably recognised[b]. At very low concentrations, odour quality is rarely perceived, and DTs are therefore usually lower than RTs, though this may not always be the case[231]. Care is needed when administering tests of DT to ensure that subjects are reporting their first sensations (i.e., 'something' vs nothing), rather than their first recognition of a 'formed' odour percept (i.e. odour quality), which converts the test to one of RT and will result in falsely inflated, incorrect 'DT'[c]. This confusion is possible where subjects are given a suprathreshold 'example' of the target odour at the start of a testing session. Of the commercially available tests of odour threshold – DT is most common, though RT has notably been used in some large-scale epidemiological work and is more commonly used in Japan[231,232].

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[a] *Differential threshold tests may also be considered as suprathreshold – given that alterations in stimulus strength within the perceptible range are undertaken. The term 'discrimination' can also be used in this context, and care is needed not to confuse this with odour 'quality discrimination' testing, which is more common in clinical practice, and discussed later in this section.*

[b] *The exact recognition task may vary between or within tests – e.g. reporting the odour to be familiar vs. describing the odour vs. correctly identifying the odour. Accordingly, particularly in the case of the latter, RT incorporates aspects of suprathreshold olfactory function – namely odour identification – which will be discussed in following sections.*

[c] ***Important note on terminology*** *– 'low threshold' denotes ability to detect target odour at low concentrations, i.e. high 'sensitivity' to that odour. Conversely, 'high threshold' denotes ability to detect target odour only at high concentrations, i.e. low 'sensitivity' to that odour. Care is needed when discussing DT/RT 'scores' from tests such as the Sniffin' Sticks – in which a higher odour threshold score (with max 16) actually denotes a lower threshold/higher sensitivity.*

The testing paradigm used to determine absolute odour threshold (DT or RT) is generally one of two types: the ascending method of limits (AML) or the single staircase (SnS). Whilst other methods, such as the 'method of constant stimuli'[d] are used in research, they are infrequently used in clinical settings due to the associated long testing times. The AML involves sequential presentation of an odourant from low to high concentration at pre-defined dilution steps. The transition point at which the subject can detect the odour stimulus (the 'limen') is the odour threshold (either DT or RT depending on task). As this procedure involves only crossing the limen once, it is necessary to perform repeat trials to obtain reliable threshold estimates. The SnS procedure (or 'initially ascending SnS' procedure) also involves presentation of an odour from low to high concentration at pre-defined dilution steps. However, on reaching the limen, several 'staircase reversals' are undertaken, whereby a weaker concentration is presented when the previous stimulus has been perceived, and a stronger concentration when the previous stimulus has not been perceived. The threshold is then calculated as the mean of a specified number of staircase reversal points (see Figure 1-1 for example marking sheet from the Sniffin' Sticks test, which uses an SnS procedure). In this way, the limen is 'passed' several times, increasing the reliability of the procedure, without requiring its complete repetition. This puts the SnS at an advantage over the AML, and consequently, the SnS is used in most clinical odour threshold tests. Nevertheless, the SnS is itself time consuming, which is a disadvantage in clinical practice.

[d] *In the method of constant stimuli a range of pre-defined odour concentrations are presented in a random order, with the DT determined from the fitted stimulus-response function. A large number of trials is required, making this test prohibitively long in most clinical settings. However, it is considered a gold standard in classical psychophysics*[230].

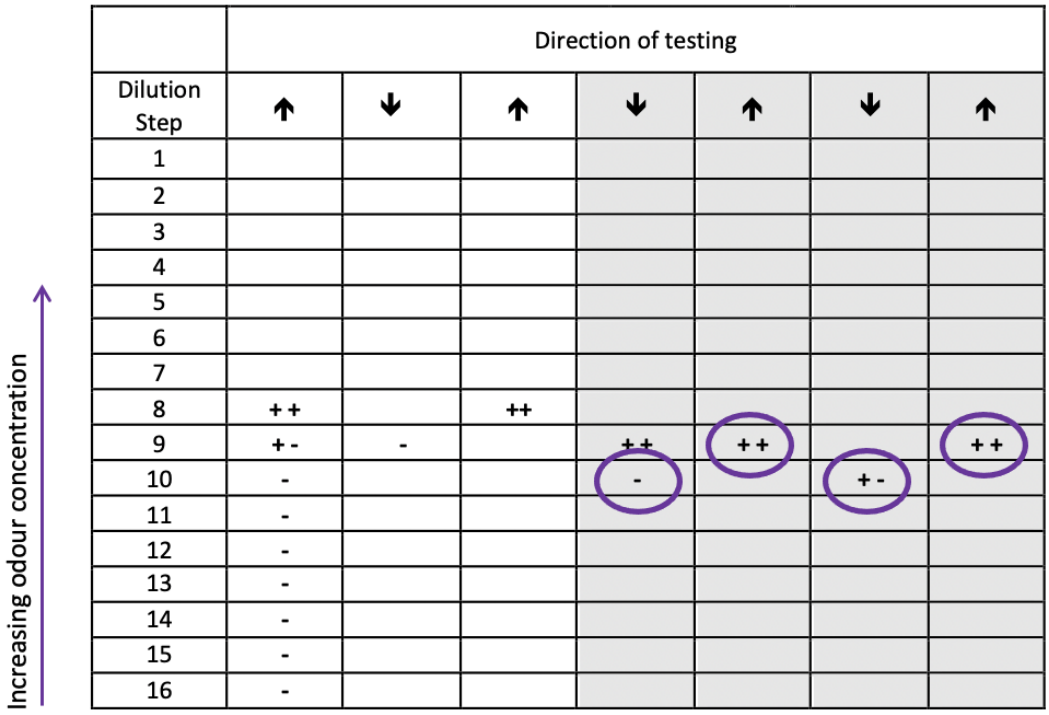

Figure 1-1: Demonstration of scoring in SnS procedure, in this case from the Sniffin' Sticks threshold test. Here, the odour detection threshold is the mean of the last four staircase turning points (circled) = 9.5.

Another important aspect of the testing paradigm is use of 'forced choice' – that is, the presentation of the target stimulus (odour + dilutant) as well as one or more non-odour containing distractors (usually dilutant alone). The subject is then asked to choose which of the presented options contains the odour stimulus – and importantly *must* choose one, even if they are unable to perceive which contains the odour. This procedure helps to control for differing criterion effects – i.e. decision making liberalism/conservatism – between different subjects and different time points, which may otherwise confound the obtained response[230]. This is particularly important for DT, in which very faint sensations are tested; in such scenarios, subjects with more conservative decision-making criteria may be less confident to report a percept than those with more liberal decision criteria (seeFigure 1-2). The forced choice paradigm therefore effectively controls for such criterion effects. Furthermore, the forced choice paradigm also allows for the detection of malingering; even in a patient with complete anosmia, they should obtain a certain

number of correct responses by chance (e.g., 1/3 where one target and 2 distractors are provided). In subjects scoring 0, or significantly less than would be expected by chance, the possibility of malingering should be considered.

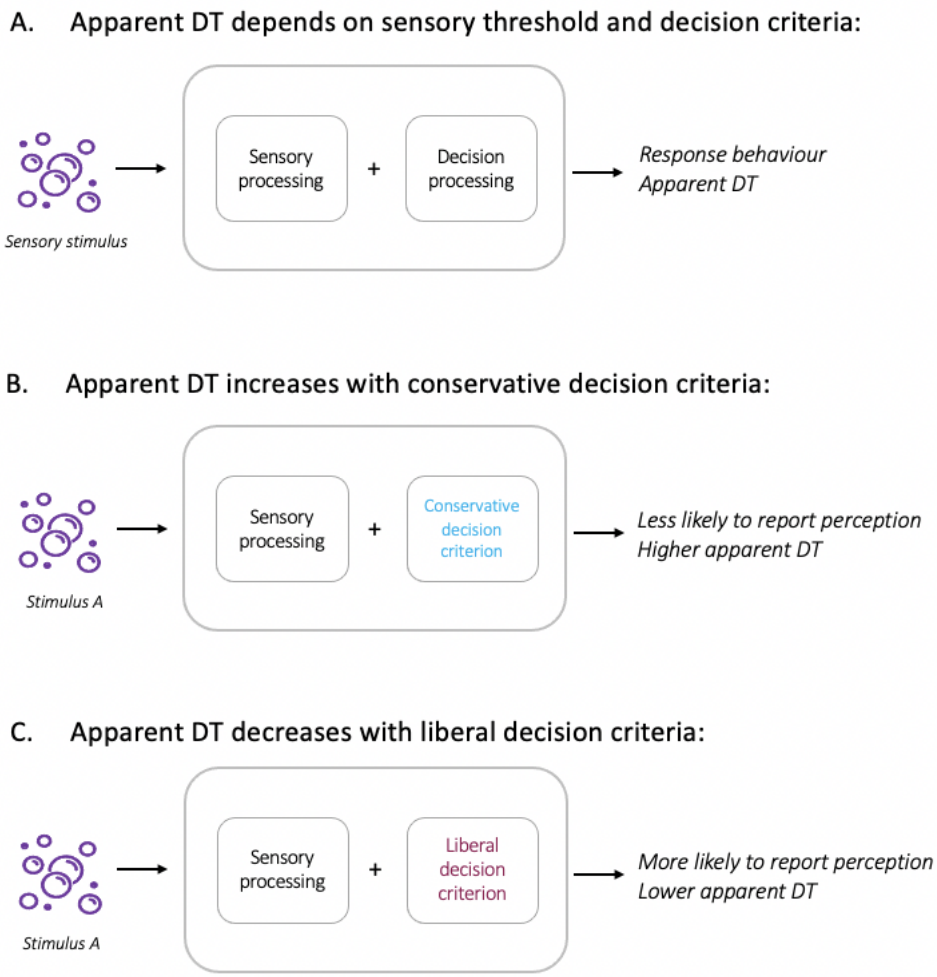

Figure 1-2: Effect of varying decision criteria on apparent detection threshold test score.

The reliability of an odour threshold test is dependent not only on administration paradigm but also on several other factors related to the use of odour stimuli generally, and particularly at very low concentrations. These include dilution method (though this should be fixed and regulated for most commercially available tests), method of odour presentation (e.g. distance from nose, duration of presentation and odour container type), sniff volume, ambient environment (including background odour/ventilation and noise) and shelf life (including evaporation and

potential contamination of target odours which may affect strength or quality)[233,234]. In clinical practice, individual test kits may be used for a high number of patients, used and stored in non-dedicated clinical environments, and administered by clinical staff with minimal training in psychophysical tests. Together, these factors may reduce the reliability of odour threshold tests in clinical environments, with potential impact on diagnosis, treatment planning and outcomes assessment. High levels of awareness amongst clinicians and active strategies to mitigate these issues are required.

Another potential limitation to clinical odour threshold testing is the small number of odours typically interrogated. For example, the commonly used, commercially available Sniffin' Sticks odour threshold test, as well as the more recently developed, commercially available Snap & Sniff odour threshold test both test DT using serial dilutions of a single molecule – phenyl ethyl alcohol (PEA) or n-butanol, and PEA respectively[235,236]. Assessment of only one odour may cause loss of clinical information (due to the comparatively limited range of OR being tested) as well as potential confounding from specific anosmias and genetic variation in OR expression across the population[85,237–239]. Indeed, work has demonstrated that subjects are able to detect odours at lower concentrations, and more reliably when mixtures are used, compared with single molecules[240]. Newer tests have been developed that assess DT using odour mixtures, however, they are not yet available commercially[241].

Despite these potential issues, a significant advantage of DT is that this test – unlike other suprathreshold tests such as odour identification – does not require the subject to be familiar with the target odour. Accordingly, this reduces the cultural specificity of the test, and potential requirement for cross-cultural adaptation.

1.5.2.2 Tests of Signal Detection

Tests of signal detection also aim to determine a subject's sensitivity to odours, however, they do so in a conceptually different way than classical threshold tests. Signal detection theory (SDT) assumes that there is no absolute detection threshold – rather that sensory perception is a continuous stochastic process within the brain,

that can be represented by two Gaussian distributions: 1. noise; 2. noise + signal[242,243]. Noise originates from various sources including, but not limited to – *subject factors* that affect the response criterion, including attention, varying background physiological processes and neuronal firing – *signal factors* including stimulus fidelity (which may be important when considering variations in odour presentation, e.g. odour container, sniff volume, duration of presentation)[230]. Experimentally, a subject is randomly presented with either a low concentration odour (which is often titrated to each subject prior to testing), or blank stimulus, and must report yes or no to presence of an odour. After a sufficient number of trials, a quantitative estimation of both the odour sensitivity (termed 'd) as well as the subjects response criterion (termed ß) can be calculated from the hits (proportion of stimuli perceived when stimulus present) and false alarms (proportion of stimuli incorrectly perceived when stimuli not present) in conjunction with normal probability tables.

The number of trial repetitions needed to produce reliable measures of 'd and ß in olfaction is unclear. In classical SDT work, several hundred trial repetitions are commonly used, which is difficult in olfaction and particularly so for clinical practice. Whilst a small number of studies have used significantly reduced trial numbers[244,245], the effect of such practice on the stability or reliability of the obtained estimates is not fully known. As such, SDT to determine odour sensitivity is less common than classical DT assessment in olfactory research, and I am not aware of any commercially available tests or tests that have been developed 'in house' for use in clinical practice.

1.5.2.3 Tests of Suprathreshold Olfactory Function

Tests of suprathreshold olfactory function do not aim to determine baseline sensitivity to an odour, but rather some other facet of perception. Accordingly, odours of sufficient concentration for detection under conditions of normosmia are used (i.e., 'suprathreshold'). Some common types of suprathreshold olfactory tests include: 1. Identification; 2. Discrimination; 3. Suprathreshold intensity; 4. Hedonic tone/valence; 5. Memory. However, it should be noted that odour identification is

arguably the most common olfactory test, across categories of psychophysics, and particularly within clinical practice. Odour discrimination is also commonly used, and for this reason, the following discussion will focus primarily on these two subtypes.

Odour Identification

Odour identification involves presentation of an odour to a subject, who is then asked to identify the smell. The task therefore involves both recognition of odour quality and recall/communication of its correct identity. Identification test paradigms may either be cued or uncued. Uncued odour identification (i.e. being asked to name the presented odour without prompts) is difficult – even in conditions of normosmia[246–248]. Accordingly, verbal and/or visual cues (representing both target and distractors) are used in most tests[249]. These can either be presented by the examiner (e.g. the examiner asks, 'does this smell like an orange' – hence provides one cue, which may be the correct target or an incorrect distractor) or presented to the subject as a list of words and/or pictures (including target and distractors, see Figure 1-3 for example). Where cues are used, the relative ease or difficulty of the task can be manipulated by choice of distractors. For example, taking vanilla as the target stimulus, the identification task would be easier with non-congruent distractors (e.g., gasoline, smoke, spoiled milk, fish etc.) compared with congruent distractors (e.g. cinnamon, chocolate, cloves, coconut etc). The use of forced-choice paradigms in odour identification, though common, is not universal. For example, the commercially available 'Quick Smell Identification Test' is a 3-item screening test, in which cues are provided for the target odour, 3 distractors, as well as a 'none/other' answer category[250]. Non-forced choice paradigms, as described above, cannot control for criterion effects, nor expose malingering. However, in short screening tests – where abnormal results often require referral for full testing, this may not affect eventual outcome.

For a subject to correctly identify an odour, they must have previously been exposed to that odour, and learnt its identity. Therefore, tests of odour identification may be culturally dependent[251]. For example, the original versions of the Sniffin' Sticks odour

identification test and SIT contain 'sauerkraut' and 'wintergreen' respectively – neither of which are common odours outside of Germany or the USA. Beyond major cultural differences in likelihood of odour exposure, more subtle differences may occur between people with differing levels of education or socio-economic status[252].

Image Redacted

Figure 1-3: Example visual/verbal cues for a 4-alternate forced choice odour identification task (e.g., Sniffin' Sticks). Here, the subject is asked to choose which of the presented cues they believe the presented odour to represent.

Odour Quality Discrimination

Though the term 'discrimination' in psychophysics can be used to describe the differential threshold – as described above – it is more commonly used to describe odour quality discrimination (with the latter definition being used throughout the remainder of this thesis). These tests can be divided into several subtypes depending on the task paradigm, but commonly share the requirement that the subject is able to smell and make same/different judgements about the quality of two or more suprathreshold odours. Perhaps the most common paradigm is the suprathreshold triangle test[230] – as employed by the Sniffin' Sticks discrimination test – in which a subject is presented with three odours in random order (two of which are the same, and one of which is different), and asked to select which of the three is the 'odd one out' (the odour DT component of the Sniffin' Sticks can also be thought of as a triangle test with one odour and two blank distractors). Other, less frequently used paradigms include presentation of two odourants, which may either be the same or different, with the subject being asked to respond accordingly, or matching/grouping of odours based on their similarities or differences. As these tests do not require recognition and identification of odours used, they are less influenced by cultural differences. However, in practice, semantic labelling of an odour likely conveys a strategic advantage used by many subjects, which converts the test from one of odour quality discrimination to semantic label discrimination.

Again, as for the other test types, forced choice paradigms help to control for criterion effects and may reveal malingering. Other considerations that should be borne in mind include the order of presentation in triangle test paradigms, where primacy or recency of the 'different' odour (i.e. whether it is presented first or last – resulting in two 'same' odours occurring in succession) may be associated with better performance[253].

Other Suprathreshold Tests

The suprathreshold intensity of an odour changes as a function of its concentration, and is related to the total number, and firing rate, of recruited neurons[254]. In OD, where some of this neuronal population is dysfunctional, there is an associated decrease in suprathreshold intensity, as well as expected increases in odour threshold. However, it should be noted that perceived odour intensity is also related to other suprathreshold attributes such as quality or hedonic tone: for example, a very unpleasant and highly disliked odour may be rated as 'more intense' than a more qualitatively and emotionally neutral odour of the same strength. Likely related to this, as well as possible redundancy in the olfactory system at suprathreshold levels, intensity ratings are less sensitive to OD than other psychophysical tests[255], and are therefore of limited value as an isolated test of olfactory function. Suprathreshold intensity can be assessed using a number of 'scaling' measures – including categorical (e.g., Likert-type) and line scales (e.g. visual analogue) or magnitude estimation procedures (in which the intensity ratings of stimuli in a presented set are anchored to each other – usually indicated by relative spacing between representative numbers or distance). Of note, these scaling procedures do not use distractors and therefore cannot employ forced choice paradigms. Therefore, criterion effects cannot be controlled.

Hedonic tone or valence describes the perceived pleasantness or unpleasantness of a stimulus, and is also related to the concentration of an odour, though more variably than suprathreshold intensity[256]. It can be measured using suprathreshold scaling measures as described above. The hedonic tone of an odour may significantly

change during qualitative OD, however, the quantitative measurement of such changes for diagnostic purposes is in its infancy. A test based on the identification component of the Sniffin' Sticks was recently developed and validated in normosmic subjects[257]. However, further testing has demonstrated poor sensitivity (ranging between 9% and 26% for the different test indices) for the diagnosis of parosmia[258].

Tests of odour memory aim to determine whether a subject can recognise a target odour or set of odours from distractors after a specified period of time. The utility of such tests in identifying olfactory-specific deficits is confounded by the tendency of subjects to apply semantic labels to the target odours, with later recall being for this label, rather than their recalled olfactory perception. Use of unfamiliar odours and distractors may reduce the efficiency of semantic labelling, but it is difficult to completely control for this strategy.

1.5.2.4 Orthonasal and Retronasal Testing

Most psychophysical tools have been developed to test orthonasal olfactory function. However, in situations where it is not possible to separate retronasal OD from gustatory dysfunction through clinical history (e.g. in cases of COVID-19 related OD[259]), or where comparison of ortho- and retronasal olfactory function is required, retronasal olfactory tests are needed.

The basic psychophysical principles underlying ortho- and retronasal olfactory testing are the same. However, retronasal testing is associated with unique challenges in terms of stimulant delivery. Existing, ecological approaches involve presentation of flavoured powders (e.g. spices or pulverised foods)[260], solutions[261], freeze-dried gels[262] or candy[263] into the subject's oral cavity (usually placed by the examiner onto the tongue). In order to control for the potentially confounding effects of associated gustatory stimulation, 'tasteless' powders and specialised retronasal odour delivery devices have recently been developed[264–266]. Whilst the majority of these test odour identification, it is also possible to test retronasal odour threshold, though these methods are less well established[265,267]. Other, less ecological approaches to retronasal olfactory testing include the intravenous

Alinamin test – in which thiamine propyl disulfide (Alinamin) is injected intravenously and undergoes respiratory excretion, with subsequent odour perception (and its perceptual latency) used as markers for retronasal olfactory function[268,269]. However, due to the invasive nature of this test, and some degree of controversy regarding its mechanism (namely regarding whether odourants are delivered via expired respiratory gases or through capillary diffusion within the OE[270]), this test is rarely used outside of its developing country, Japan.

1.5.2.5 Other Factors that Affect Results

Other factors that have been shown to affect psychophysical test scores include biological sex (with women generally outperforming men)[271], smoking[272] and hunger/satiety – though there is mixed evidence for the effect of the latter two[273,274].

Another important phenomenon to take into consideration is the diminution in perceptual response to prolonged odour exposure caused by adaptation and habituation. Adaptation describes physiological changes that can be demonstrated in peripheral and/or central neural networks (e.g. calcium mediated negative feedback following OR activation), whilst habituation describes alterations in behavioural response[275,276]. Psychophysical testing procedures should aim to reduce these effects by limiting the duration of exposure to strong odourants, as well as overall testing duration, and using as large an odour set as possible (in light of cross-adaptation/habituation). Threshold testing generally follows an 'ascending' procedure (from low to high concentration) to mitigate the potential effects of adaptation/habituation.

Test experience/naivety and longer-term repeat odour exposure may also affect obtained results, where generally practice improves performance (possibly through improved understanding of test goals, learning of identification cues, or increases in odour sensitivity caused by repeat exposure – the latter of which may be considered a form of olfactory training)[85,277]. As outlined above, test environment is also

important – particularly background noise and ambient odours in the testing of odour threshold[234].

Finally, psychophysical olfactory testing may be undertaken bi- or mono/unirhinally (i.e., testing both or one nostril respectively). Demonstrated asymmetry in monorhinal test may be helpful in diagnosing some pathologies – such as unilateral sinonasal neoplasia[278,279] or cognitive impairment[280]. Furthermore, some evidence suggests that asymmetry in monorhinal test scores may occur prior to later development of bilateral OD[281]. Clinically, however, birhinal testing is most common[166] – results represent the better of the two sides, are less susceptible to the impact of the nasal cycle, and best reflect the patient's subjective experience[217,282].

1.5.2.6 Existing Psychophysical Olfactory Tests

Many different psychophysical tools have been described in the literature: 101 individual tools were reviewed in the recently published International Consensus Statement on Allergy and Rhinology: Olfaction ('ICAR:O')[229]. These can be grouped according to underlying psychophysical task (e.g., DT, identification, valence etc), commercial availability or speed of testing. Regarding speed of testing, excluding odour threshold tests, generally those which assess fewer odours are faster to administer, but less reliable. Consequently, tools with very few odours (e.g., 3 or 4) are suggested for screening purposes and require follow up testing with more comprehensive tests in the event of abnormal results. In addition to number and range of odours, test reliability is also affected by administration paradigm, as outlined in the preceding sections. Despite this, only 52 of the aforementioned 101 tools reported their associated test-retest reliability coefficients, and of those that did, some were as low as 0.33 in adults (T&T olfactometer, individual RT for undecalactone)[283], or 0.20 in children (NIH toolbox, identification, Spanish version)[284]. Accordingly, high levels of care are required when selecting tools for use in clinical practice, and when comparing existing results obtained from different tests.

Two commonly used psychophysical tools with large associated normative data sets, and good test-retest reliability are the Smell Identification Test ('SIT-40' or 'SIT',

previously the University of Pennsylvania Smell Identification Test or 'UPSIT') and the Sniffin' Sticks. The SIT-40 is a self-administered odour identification test, which comprises 40 microencapsulated odours (4-alternate, cued forced choice). Patients can be classified as normosmic, 'microsmic' (equivalent of hyposmic – subdivided into mild, moderate and severe microsmia) and anosmic, with a test-retest reliability of 0.9[283]. Whilst normative data is available for this test, at time of writing[e] there was no formally defined minimum clinically important difference – making it difficult to determine clinically meaningful change in olfactory function. The Sniffin Sticks tool comprises three separate tests – odour DT (3-alternate forced choice, initially ascending SnS procedure), discrimination (3-alternate forced choice) and identification (4-alternate, cued forced choice) – which can be used individually or together. The composite 'TDI' score has a test-retest reliability of 0.72 for the original version, and 0.93 for an extended version (in which identification and discrimination tasks use 32 instead of 16 odours)[235,285]. Extensive normative data is available to define normosmia, hyposmia and anosmia[286]. Each of target odours/distractors is presented to the subject by an examiner, using an odorised felt tip pen. This test cannot therefore be self-administered, making it more resource heavy for clinical use. Minimum clinically important differences have, however, been defined for each of the test subcomponents, as well as the composite TDI score[287].

1.5.2.7 Equivalence of Different Psychophysical Olfactory Tests

Differences in individual test features and associated reliability aside, the equivalence of different olfactory psychophysical *tasks* (e.g., identification, DT, valence etc.) is controversial. However, given the large number of different tests described, and their heterogeneity of use within the literature (for example, see Table 1-2), it is important to determine whether results obtained are generalisable. This is particularly important for clinical practice, where treatment regimens – and potentially the provision of surgery, with its associated risks – may be affected.

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[e] *The MCID for the SIT/UPSIT (4 points) has only recently been formally established by Jay Piccirillo's group in 2024*[701].

Study	*Year*	*Sample Number*	*Olfactory Test*
Lund *et al.* [288]	1991	50	Identification, Threshold
Lund *et al.* [289]	1994	650	None
Delank *et al.* [221]	1998	115	Discrimination, Threshold
Blomqvist *et al.* [290]	2001	32	Identification, Threshold
Perry *et al.* [291]	2003	178	None
Jankowski *et al.* [292]	2003	24	None
Ragab *et al.* [293]	2004	90	None
Rowe-Jones *et al.* [294]	2005	109	Threshold
Bonfils, *et al.* [295]	2007	194	None
Minovi, *et al.* [296]	2008	64	Identification
Pade *et al.* [297]	2008	206	Identification
Federspil *et al.* [298]	2008	52	Threshold, discrimination, identification
Ehnhage, *et al.* [299]	2009	52	Threshold
Danielides *et al.* [300]	2009	116	Threshold, discrimination, identification
Litvack, *et al.* [301]	2009	111	Identification
Olsson, *et al.* [302]	2010	158	Threshold
Ehnhage, *et al.* [303]	2012	51	Threshold
Schriever, *et al.* [304]	2013	113	Identification
Baradaranfar, *et al.* [305]	2013	60	Identification, Threshold
Saedi, *et al.* [306]	2013	89	Identification
Andrews *et al.* [307]	2016	113	Identification

Table 1-2: Non-exhaustive list of studies assessing olfactory function after functional endoscopic sinus surgery between 1991 and 2016. There is little consistency in the use of psychophysical testing.

An early study by Doty and colleagues aimed to investigate generalisation of results from different olfactory tests through use of principal component analysis.[308] Olfactory assessment using nine different tests (assessing odour threshold, discrimination, identification, memory, supra-threshold intensity and valence) was performed in 97 healthy subjects. Emergent principal components demonstrated a common source of variance for all tests, except for suprathreshold ratings of odour intensity and valence. This was taken to suggest that results from these tests (except

the latter two) represent a common underlying process and can therefore be used interchangeably.

Whilst all psychophysical tests require the basic mechanisms of OR/OSN activation, with subsequent activation of at least some structures within the primary olfactory network – and whilst it is clear that suprathreshold olfactory tasks would not be possible without the ability to detect an odour – it is plausible that different psychophysical tasks differentially recruit higher neurocognitive processes, at least to some degree. Given that OD pathophysiology may in turn differentially affect peripheral and/or central processing, it is further plausible that some psychophysical tasks may better reflect certain aetiologies than others. For example, a patient with parosmia may have relatively unimpaired odour DT, but significantly impaired odour identification. This argument is supported by three parallel lines of evidence.

First, lesion and neuroimaging studies have demonstrated task-location specificity. Early work from Jones-Gotman and Zatorre investigated psychophysical olfactory function in patients who had undergone selective cerebral excisions[162]. They found deficits in odour identification, but not in odour threshold scores. Patients with AIDS Dementia Complex similarly have impaired odour identification but preserved odour threshold scores[309]. Neuroimaging studies have also demonstrated neural dissociation between different psychophysical aspects of olfaction. For example, the anterior piriform cortex (PC) is responsive to odour structure (i.e. the presence of the stimulus) but not quality, whilst the posterior PC is response to quality but not structure[310]. Similarly the amygdala is responsive to odour intensity but not valence, whilst the converse is true of the orbitofrontal cortex[311].

Second, Hedner and colleagues investigated potential associations between psychometric measures of neurocognitive function and olfactory psychophysical test scores (specifically odour threshold, discrimination and identification components of the Sniffin' Sticks) in healthy participants[312]. Whilst significant correlation was demonstrated between odour threshold and some individual cognitive measures (e.g., general knowledge and vocabulary), these were not controlled for age/sex.

Using hierarchical regression analysis, and controlling for age and sex, they found that executive function [f] and semantic memory [g] significantly contributed to performance in odour discrimination and identification, but not odour threshold. Therefore, the authors concluded that odour threshold is '*primarily driven by low-level perceptual functions'* whilst discrimination and identification as '*higher olfactory functions'* require greater cognitive input.

Third, analysis of large-scale psychophysical datasets has demonstrated variation in the pattern of obtained test scores according to aetiology of OD. In work I undertook in collaboration with Prof Hummel's group in Germany, analysis of composite Sniffin' Sticks results from 1,226 patients demonstrated relatively reduced odour threshold in those with sinonasal disease, compared with suprathreshold tasks (discrimination/identification) in those with Parkinson's disease[313]. Furthermore, data driven analysis using unsupervised machine learning, showed that patterns of Sniffin' Sticks subtest results could be clustered according to the relationship between odour threshold and suprathreshold tasks[314], thereby conceptually differentiating these two categories.

Taken together, it has been suggested that suprathreshold olfactory tasks may be more sensitive to central pathology, whilst odour threshold may be more sensitive to pathology of the peripheral olfactory system. Resultant patterns in subtest scores could therefore add clinical information during initial ± outcomes assessment. Accordingly, the 2017 and 2023 versions of the Position Paper on Olfactory Dysfunction (guidelines on the diagnosis and management of OD, of which I am an author) both recommend use of odour threshold as well as a suprathreshold tests of olfactory function, where possible, in both clinical and research settings. However, the ICAR:O guidelines did not clearly recommend use of odour threshold in clinical

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[f] *Executive function describes higher order neurocognitive processes (including cognitive flexibility, inhibitory control and working memory) that regulate goal directed behaviour.*[702]

[g] *Semantic memory is a type of declarative memory that encodes general knowledge (including facts, meanings and concepts), that is not related to personal experience.*[702]

testing. This difference appears to be based on the common source of variance demonstrated in the above-described principal component analysis, as well as the potential issues surrounding reliability of odour threshold testing in clinical environments, as outlined above.

1.5.2.8 Use of Psychophysical Tools for Clinical Diagnosis of OD and Outcomes Assessment

Irrespective of specific test type used, psychophysical tests employed clinically should be reliable (with known test-retest reliability) and their scores only be used to define olfactory impairment and improvement in conjunction with normative data. Hyposmia is usually defined using the 10th percentile of normal test scores gathered from a population of healthy young subjects[235,315]. Anosmia, however, is defined on the basis of the empirical distribution of scores obtained by anosmic people[316,317]. For tests with sufficient normative data, age-related normative values may be published, though diagnoses of impairment are generally made with reference to young healthy adults[166]. Reporting improvement or deterioration in test scores should be related to clinically significant change for the test in question (the minimal clinically important difference).

1.5.2.9 Implications for Clinical Practice and Gaps in Knowledge

Psychophysical tools allow for necessary standardisation in the assessment of olfactory function. However, the large number of different tools described and lack of unified guidance regarding their use introduces potential for significant heterogeneity in assessment practice, the implications of which are not fully known. To my knowledge, the last available UK clinician reported data on psychophysical test use was from 2009, at which point *most clinicians did not use any form of testing in their practice*[318]. Therefore, an aim of this thesis is to determine *whether* and *which* psychophysical tools are currently being used by clinicians in the UK during their assessment of olfactory function and dysfunction OD. I additionally aim to gather international data, and a secondary aim is to explore any potential

geographical variations in practice. In an effort to inform potential future service reform, another secondary aim is to explore potential barriers to such psychophysical testing.

1.5.3 Imaging

Medical imaging is ubiquitous in modern healthcare. At its core, such imaging aims to identify reliable, radiologically derived features that accurately map to some aspect of function or disease. Broadly speaking, two main approaches have been used to study olfaction and its associated diseases – structural imaging (of both peripheral and central olfactory systems) and functional neuroimaging, though use of the latter is largely limited to research settings. Accordingly, I will first discuss structural imaging and its current/potential future use in the clinical assessment of olfaction. In the sections that follow, I will then discuss functional imaging, and its relation to clinical assessment and the aims of this thesis.

1.5.3.1 Structural Imaging

Structural imaging is a process through which anatomical representations of the body are produced, based on some interaction between an energy source and the tissue target. Olfactory imaging may be focussed on the peripheral (i.e., all craniofacial structures up to but not including the OB) or central olfactory system (i.e. all intracranial structures involved in olfaction). The aim of such imaging may be to delineate anatomy, including normal and abnormal features, characterise pathology or derive some proxy measure of function/dysfunction. The latter may encompass a number of approaches, ranging from radiological scoring systems used as a proxy for disease burden (e.g., Lund Mackay score[h]), to the correlation of behavioural assessment outcomes with some quantified measure of neuroanatomy – that is, the establishment of *neuroanatomical correlates* of olfaction.

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[h] *The Lund-Mackay score is a CT based score of CRS disease burden in which each sinus group (frontal, maxillary, anterior ethmoid, posterior ethmoid, sphenoid, ostiomeatal complex) is assigned a value between 0 and 2 depending on the degree of opacification (0 = none, 1 = partial, 2 = complete). The ostiomeatal complex is scored as 0 (not obstruction) or 2 (obstructed). Each side is scored separately and the maximum possible score is 24.*[289]

The peripheral olfactory system is commonly imaged using computed tomography (CT) and/or magnetic resonance imaging (MRI), depending on suspected pathology. Within the context of sinonasal inflammatory disease, existing guidelines describe appropriate use of imaging – which, in the absence of suspected complications, usually involves CT of the paranasal sinuses[103,319,320]. Scores of inflammatory disease burden such as the Lund-Mackay score have been shown to correlate with olfactory psychophysical scores, though often with low to moderate strength (e.g. one study demonstrated correlations of r = -0.27 to -0.30 in patients with CRSwNP and CRSsNP respectively[321]). Scores targeting opacification of the olfactory cleft have also been shown to correlate with psychophysical results[321–324]. Indeed, this association may be stronger than correlations with Lund-Mackay score, though possibly only in patients with polyps[325]. It should be noted, however, that sinonasal inflammation is a common incidental finding in asymptomatic patients, with mucosal thickening demonstrated in up to 38% of patients in some series[326,327], and mean 'incidental' Lund-Mackay scores as high as 4.3[328]. Similarly, Loftus and colleagues demonstrated a mean olfactory cleft opacification score of 47.1% in their population of 30 non-CRS controls. This degree of 'incidental' opacification was significantly less than patients with CRSwNP (77.6%, P<0.001), but very similar to that seen in patients with CRSsNP (49.5%, P=0.46). Given the dynamic nature of mucosal inflammation (particularly relevant within the small anatomical confines of the olfactory cleft), and associated potential effects on inter- and intraindividual variability, further work is needed to characterise the utility of sinonasal/OC scores in the clinical assessment of olfaction.

Looking beyond sinonasal inflammation, craniofacial imaging may also be of use in cases of PTOD, iatrogenic OD or idiopathic phantosmia – where craniofacial injury or endogenous sinonasal odour source (for example fungal ball/sinusitis) may be delineated, respectively.

Due to its superior visualisation of soft tissue, MRI is generally preferred for investigation of intracranial structures and pathology related to OD. At present, MRI brain is most frequently used for two main purposes: 1. OB identification/characterisation; 2. Identification of pathology affecting the central olfactory networks. Individually or together, interrogation of these targets may

provide diagnostic +/- prognostic information (e.g., through identification of aplasia, atrophy, gliosis or neoplasia in olfactory regions) in various suspected underlying pathologies, including PTOD, idiopathic OD, neurodegeneration, and congenital OD. However, when I began this PhD, no clear guidelines were available for imaging outside of the context of sinonasal OD. Moreover, there is debate in the literature about the diagnostic yield and thereby cost-effectiveness of MRI brain for OD. An early study from Powell and colleagues investigated imaging outcomes from 100 consecutive patients undergoing MRI for non-CRS related OD. They demonstrated abnormalities in 19% of scans, with 7% containing findings related to OD (though clinical management was only affected in one patient)[329]. Similarly, in Hoekman's cohort of 130 patients with idiopathic OD, 6 were found to have abnormal MRI brain findings, with one case reported as causative[330]. A diagnostic yield of 4.9% was demonstrated in another study of 122 patients with idiopathic OD – all of whom had intracranial neoplasms. These USA-based authors performed a cost-effectiveness analysis and concluded that the associated medical malpractice costs were sufficient to warrant MRI scanning in this patient group[331]. However, another economic analysis (that took malpractice as well as wider societal costs into account) concluded that routine scanning in OD was not justified in idiopathic OD[332]. To my knowledge no UK cost-based analyses have been performed. This, combined with lack of evidence-based guidelines or adequate representative data in other aetiologies of OD, means that scanning decisions are likely to vary considerably between different regions, institutions, and individual clinicians.

However, at present, there is little available data on current clinical imaging practice in the assessment of olfaction. Though a recent retrospective review of MRI use in a UK tertiary smell and taste clinic demonstrated a scan rate of 42% across 409 patients of different aetiological subtype, this represents a select group of patients attending a specialist centre[333]. The last information regarding *general* UK practice is from two separate surveys in 2007[334] and 2009[318], neither of which included more than a single question on 'investigations' in OD. Moreover, none of these studies attempted to explore the specific clinical goals motivating clinicians to request imaging.

An aim of this thesis is therefore to capture current clinical imaging practice – including which scans are being undertaken, and why – with a particular emphasis on MRI scanning of the central olfactory networks, given the controversy regarding its use.

Furthermore, following on from issues surrounding subjective and psychophysical measurement described in the preceding sections, another major aim of this thesis is to explore new ways in which olfaction could be assessed. Given the ubiquity of imaging in most modern healthcare systems, the non-invasive, non-ionising nature of MRI scanning, and its established – though controversial – use as an investigation in the assessment of OD, I aim to focus on the use of MRI brain in the assessment of olfaction, and in particular, the establishment of clinically relevant neuroanatomical correlates of olfactory dysfunction. The establishment of such correlates could inform future development of personalised olfactory biomarkers. In theory, use of such biomarkers (either alone or as part of a wider clinical scoring system) could provide:

1. More 'objective' assessment of OD that is not subject to the limitations of current subjective or psychophysical testing practice
2. Personalised prognostic information in patients with OD
3. 'Pre-clinical' identification of those at risk of developing OD, or diseases in which OD are an early feature (e.g., neurodegenerative conditions such as Alzheimer's Disease)
4. More precise targeting for future invasive therapies, such as putative olfactory implants

In the following section, I will therefore describe the use of structural imaging in the assessment of olfaction and OD in more detail – focussing on the use of imaging to establish potential neuroanatomical correlates of olfactory behaviour and disease. To date, the olfactory bulb has received attention with regards to its role and clinical utility as a neuroanatomical correlate of olfaction. However, few higher order regions have been investigated. I will outline the current evidence base and provide justification for the further investigation of higher order regions.

1.5.3.1.1 Olfactory Bulb

The olfactory bulbs are small bilateral structures within the anterior cranial fossa. As the only known recipients of OSN axons in humans, they have traditionally been considered obligatory first relays in the primary olfactory network, and are now known to facilitate integration of peripheral and central olfactory-related signals[73]. Early human cadaveric studies demonstrated variation in OB size, thought to be caused by differences in ORN population, with consequent effect on glomerular size/number[335]. Significantly reduced OB size was documented in bulbs with no apparent OSN synaptic input, and the degree of OB atrophy was shown to increase with age[335,336]. In animals, prolonged olfactory deprivation leads to marked OB atrophy, caused by reductions in various cell populations, depending on duration of deprivation and timing relative to developmental stage[337,338]. However, such atrophy is reversible, with increased neuronal/glial populations and corresponding OB size following restoration of olfactory input in rats[339]. Regarding neuronal changes, 'structural plasticity' within the OB has been attributed to two neurobiological processes: 1. interneuron replacement (periglomerular and granular cells) due to migration of neuroblasts along the rostral migratory system; 2. synaptogenesis within glomeruli due to OSN axonogenesis[169,340].

Observations such as these prompted work investigating whether meaningful information about human olfactory function can be derived from macroscopic features of OB structure. That is, whether the OB serves as a neuroanatomical correlate of olfactory function/dysfunction, and whether meaningful clinical information can be gained from their radiological assessment. Following initial technical challenges (due to their position immediately dorsal to the cribriform plate and underlying air-filled nasal cavity), today, the OBs can be adequately imaged using high resolution MRI. Despite their small size, the OB are anatomically distinct regions with (in most cases) clearly defined borders (that are particularly well contrasted against the bright surrounding CSF on T2-weighted MR images) – allowing their relatively easy morphological characterisation using *manual segmentation* techniques. Though inherently 'observer-dependent' (with particular risk for measurement error at the most rostral/caudal ends of the OB[341]), early

studies using such techniques were able to demonstrate good measures of intra-/interrater reliability and phantom-anchored *in vivo* accuracy, when performed by an experienced researcher[342]. Morphological characteristics that have received attention include OB *volume* and, more recently, OB *shape*.

Thought to reflect both proper development and subsequent atrophy, multiple studies have investigated the association between OB volume (OBV) and olfactory function, in patient and healthy participant populations. In the latter group, positive correlation between OBV and psychophysical test scores have been demonstrated in some, but not all studies. In early work from Yousem and colleagues, OB + tract volume was calculated using T1-weighted MR images, and no correlation between these derived volumes and odour identification score (SIT-40) could be demonstrated in healthy participants (aged 22-78), whilst significant inter-individual variation in volume was found[343]. In their later study of 125 healthy controls, Bushhüter *et al.,* demonstrated statistically significant positive correlations between OBV and Sniffin' Stick odour threshold (left OBV only), odour identification (right/left OBV) and composite threshold/discrimination/identification (TDI) score (right/left OBV). However, it should be noted that the strength of the correlations demonstrated were weak to moderate, ranging from values of 0.19 (left OBV/odour threshold) to 0.48 (right OBV/odour identification) (Pearson's *r*). Moreover, when controlled for age, only the resultant partial correlation between right sided OBV and odour identification remained statistically significant, and was weak in strength (r = 0.23, P=0.014). Again, as demonstrated in earlier work, and possibly reducing the strength of structure-function correlations observed, there were high levels of interindividual variation in OBV (with values ranging from 37 to 98mm^3 for left OBV, and 41 to 97mm^3 for right OBV). Nevertheless, age and sex specific normative data for OB hypoplasia were derived from the study population (based on the distribution of OBV observed, and not related to psychophysical olfactory function), which have been widely used in subsequent work.

In patients, early studies from several groups demonstrated either absent, or hypoplastic OBs in congenital anosmics[344–346]. Subsequent work expanded on these findings, demonstrating reduced OBVs in patients with acquired OD secondary to a

variety of pathologies, including PIOD[168,347–349], PTOD[347,349,350], IOD[351], and more variably in sinonasal[352] and neurodegenerative diseases[353,354]. Accordingly, it was argued that reduced OBV reflects OD. Whilst there were some specific limitations in early studies – for example, involving use of low-resolution MRI, unclear procedures regarding correction for potential confounders (e.g., age or total intracranial volume), or small participant numbers – the major limitation of all such studies is their cross-sectional design, with which it is not possible to determine causation.

To address the issue of causation, several approaches surrounding the principle of dose-response have been used[355]. First, some studies have demonstrated that patients with more severe OD have smaller OBVs, either through demonstration of statistically significant positive correlation between OBV and psychophysical test scores (as variably shown in healthy control populations, above), or through demonstration of lower group-wise mean OBV in subjects with worse, compared with better olfactory function. This has been shown in patients with PIOD[168,348] and PTOD[168]. However, not all studies have replicated such association – for example, in a cohort of patients with OD of mixed cause, Goektas *et al.,* were unable to demonstrate significant correlation between OBV and psychophysical test score[356]. In line with this variability, a recent systematic review of structural MR imaging in olfaction found significant correlation between OBV and psychophysical scores in seven of the eleven studies interrogated[357]. Second, statistically significant negative correlations between OBV and disease duration have been demonstrated in some, but not all studies. For example, in patients with PIOD, Rombaux and colleagues were able to demonstrate moderately strong negative correlation between left and right OBV and duration of OD (r = -0.57, r = -0.59, both $p<0.05$, respectively) in 26 patients. However, Mueller and colleagues were unable to demonstrate statistically significant correlation between OBV and disease duration in their mixed cohort of patients with PIOD (n=22) and PTOD (n=9)[168]. Other studies have also failed to demonstrate significant correlation between disease duration and OBV[356,358].

Together with evidence from control populations, these studies strengthen the association between OBV and olfaction in health and disease. However, there is variability in the literature and, moreover, they remain correlative. Longitudinal

studies provide better evidence of causation, as they allow for characterisation of pre-intervention olfactory function, application of a temporally defined and standardised intervention across participants and subsequent measurement of resultant olfactory function, ideally in comparison to a control group[359]. The biological plausibility for such potential changes in human OBV is provided by animal work, as described above.

A limited amount of longitudinal data is available demonstrating changing OBV in association with changing olfactory function, i.e., 'structural plasticity'. In their study of 20 patients with OD of mixed cause, Haehner and colleagues investigated change in OBV after a mean follow up interval of 15 months. Whilst there were both increases and decreases in OBV, they demonstrated statistically significant positive correlation between change in odour threshold (but not identification/discrimination) score and change in OBV in patients who were initially hyposmic (n=13) but not in those who were initially anosmic[360]. A later study from the same group investigated change in OBV and psychophysical test score in patients undergoing surgical treatment for CRS (n=19), compared with healthy controls (n=18)[92]. Three months after surgery, mean OBV and psychophysical test scores had increased in the patient group, and the authors demonstrated significant positive correlation between change in OBV and change in odour threshold (but not identification/discrimination).

Taken together, OBV does appear to reflect olfactory function and dysfunction. However, the use of the OBV in clinical practice is limited by high levels of inter-individual variability – as outlined by Mueller *et al.,* normosmic subjects may have OBV within the hypoplastic range, and similarly patients with OD may have OBV within the normal range[168]. Consequently, OBV may be more useful as an intraindividual measure – perhaps tracking olfactory function over time or providing prognostic information. In line with this, work in patients with PIOD and PTOD has demonstrated significant positive correlation between change in OBV and change in psychophysical test score, with greater improvement in those with initially larger OBV, and no improvement in those with a total OBV of ≤40mm^3.[361] Alternatively,

OBV could be combined with other radiological ± clinical information to form more sensitive/specific scoring systems.

Another feature of OB morphometry – OB shape – has also recently been proposed as a potential proxy measure for olfactory function/dysfunction. Though identification of shape is inherently subjective, and thereby prone to measurement error without appropriate education/experience, it can be done more quickly and simply, without use of specialised software. Using cross-sectional data, two separate groups have proposed classification systems for OB shape – where, for example, patients with OD are more commonly seen to have irregular, or 'scattered' configurations[362,363]. Again, however, high levels of interindividual variation are present in these systems, and – to my knowledge – no longitudinal data is yet available.

Finally, when considering the clinical utility of OB morphometry it is interesting to note that several recent studies have demonstrated human olfactory perception in the absence of radiologically demonstrable OB[364–366]. Using extensive psychophysical testing as well as multimodal (structural and olfactory functional MRI), Weiss and colleagues described intact olfactory perception in two left-handed women without apparent OBs. Through subsequent interrogation of publicly available databases (the Human Connectome Project/NIH toolbox results) the authors concluded that ~0.6% of women, and ~4% of left-handed women, had olfactory perception in the absence of radiologically demonstrable OBs. Assuming these women do not have OBs which are merely too small to be imaged using current techniques, the anatomical and physiological processes allowing them to smell are unknown. Where such findings were upheld, for example from histological evidence, this may accordingly serve to illustrate the extreme plasticity of the human olfactory system.

Given the above, it follows that potential neuroanatomical correlates of olfactory function and dysfunction upstream to the OB should be considered.

1.5.3.1.2 Higher Order Structures

Comparatively few structures upstream to the OB have been investigated, possibly reflecting limitations of the techniques used. Despite good early measures of reliability/accuracy, manual segmentation is operator dependent, usually requires assessment by more than one experienced researcher or radiologist, is time consuming, and perhaps most importantly, is best applied to anatomically unambiguous brain regions (such as the OB). These limitations also apply to other operator-dependent methods, such as subjective categorisation of morphological abnormalities, as are used in some radiological scoring systems.

An alternative approach is to analyse structural images using *computational morphometry* techniques that allow for the investigation of volume and shape. Common techniques include voxel-based morphometry (VBM), deformation-based morphometry (DBM), surface-based morphometry (SBM) (all of which use high resolution T1-weighted MRI) as well as diffusion tensor imaging (DTI) and diffusion spectrum imaging (DSI) (which use diffusion weighted MRI). In general, these techniques require pre-processing steps that allow for different brains to be globally aligned into the same stereotactic space, whilst preserving local, imaging-based tissue characteristics. By matching global, but preserving local brain tissue composition, VBM, for example, allows for the interrogation of grey and white matter volume (or density/concentration, depending on pre-processing procedures) in specified locations. Statistical inferences on structure or shape can then be drawn, either within or between subjects. In addition to being less operator-dependent – and so more 'objective' – a major advantage of these techniques over manual morphometry is that they allow for differentiation of tissue throughout the brain, and are therefore not limited to anatomically distinct areas (such as the OB or hippocampus, for example) – though it should be noted that the OBs cannot be segmented in this way due to their size and position in relation to the cribriform plate and underlying air-filled nasal cavity. Consequently, such 'whole-brain (WB) analysis' allows comparisons between groups without defining regions of interest *a priori.* Having said this, specific, pre-defined anatomical regions can also be investigated, where there are strong *a priori* hypotheses ('region of interest (ROI)

analysis'). Whilst these techniques do have potential limitations (e.g. the problem of multiple comparisons and the balancing of type I/II error – important when allocating significance thresholds), previous work has shown good correlation with manual morphometry and/or post-mortem histology, in areas including subcortical brain regions, thereby supporting their biological validity[367–371]. [For more details regarding VBM and SBM techniques, see §3.3.3]. Accordingly, computational morphometry has been used to delineate neuroanatomy in health and disease, and some attempts have been made to link such anatomy with quantified measures of function.

In normosmic subjects, a relatively small number of studies have investigated the relationship between psychophysical olfactory function and brain structure. Early work from Franselli and colleagues demonstrated significant correlation between Sniffin' Sticks test scores and cortical thickness within areas of the primary (including piriform cortex, though only in women) and secondary olfactory networks (including right insula, right OFC) as well as areas not classically associated with olfaction (including right dorsal postcentral gyrus – suggested to be involved with sniffing), in 46 young subjects (mean age 24)[372]. The correlations observed were positive – indicating that 'more is better' – a thicker cortex is associated with better olfactory function. Their results were predominantly right sided, and some were affected by sex (e.g., piriform cortex). Another, larger study attempted to link grey matter volume (GMV) with Sniffin' Sticks scores in a young cohort of 90 normosmic subjects (mean age 35)[373]. Accordingly, Seubert and colleagues demonstrated positive correlations between composite TDI score and GMV within the right OFC, as well as the OB (as assessed using manual planimetry).

A limited number of studies have also attempted to link olfactory expertise with morphological features, in a way analogous to previous work in experts with enhanced auditory or visuomotor skills, e.g. musicians/athletes[374–376]. Delon-Martin *et al.,* compared GMV in perfumers ('old experts'), perfumery students ('young experts') and naïve age/sex-matched controls ('old controls', 'young controls') (n=14, 13, 13, 8 respectively)[377]. Comparing all experts with all controls, they demonstrated increased GMV within the bilateral OFC (gyrus rectus and medial orbital gyrus), left

anterior cingulate and left caudate, though there was variation in this pattern with age. Furthermore, positive correlation was demonstrated between age (used as a proxy for duration of olfactory expertise) and GMV within the left OFC of 'old experts', whilst it was negatively correlated in 'old controls'. Similar positive and negative correlation was demonstrated within the anterior piriform cortex, though this only reached statistical significance in the control group. Together, the authors suggest that olfactory expertise increases GMV, and thereby counteracts the effect of aging in these regions. Of note, the reverse condition of decreased GMV in experts was not reported, and importantly, psychophysical olfactory function was not tested in either expert or control groups. Looking at a similar, but different group of chemosensory experts, Banks and colleagues investigated potential structural and functional differences between master sommeliers and normosmic, naïve controls (n=13 in each group)[378]. They demonstrated increased GMV within the bilateral entorhinal cortex (cluster extending into left hippocampus) and right insula in sommeliers compared with controls. They further demonstrated significant positive correlation between cortical thickness in the right entorhinal cortex and duration of experience within the sommelier group (and interestingly, a negative correlation between GMV and experience in the right insula, though this failed to reach statistical significance). Decreased GMV in sommeliers compared to controls was not reported. When interpreting these results, however, it should be noted that there was a significant difference in age between groups, with sommeliers being older than controls (mean ages 44 and 34 years respectively). Furthermore, the difference in odour identification score (SIT-40) between groups, though statistically significant, was small (36.8 and 34.2 out of 40 in sommeliers and controls respectively). Given that the MCID of the SIT-40 is usually taken as 4[379,380], group level differences in this study are likely to be related to higher-order olfactory functions. In line with this, studies relating to expertise, rather than more basic psychophysical-anchored olfactory function, are likely limited in their direct clinical application.

In patient populations, VBM has been used to link altered GMV with various sensory deficits, compared to healthy controls. This includes adults with hearing loss[381], unilateral vestibular deafferentation[382] or blindness[383,384]. Early work in olfaction

aimed to characterise morphological brain differences in patients with acquired OD of varying severity (rather than according to aetiology of OD) compared to normosmic controls. In separate studies, Bitter and colleagues investigated such differences in patients with anosmia and hyposmia. In anosmic patients (mixed aetiology including PIOD, PTOD, IOD), they demonstrated multiple areas of GMV reduction compared to age matched controls (n=17 each group, mean age patients 49, controls 40 (ns))[385]. Using a whole brain analysis (P<0.001, uncorrected for multiple comparison), small areas identified included the piriform cortex, insula, OFC, hippocampus and parahippocampus (see Table 1-3 for full results). Larger areas were demonstrated within the cerebellum, dorsolateral prefrontal cortex, superior occipital gyrus, nucleus accumbens, superior subcallosal gyrus, and medial prefrontal cortex (including anterior and middle cingulate cortices) (with the medial prefrontal cortex cluster reaching significance at a conservative threshold corrected for multiple comparisons, $P<0.001_{FWE}$). No areas of increased GMV in patients were demonstrated. It should be noted, however, that the patient cohort included those with PTOD, which may confound morphological results due to injury-related structural changes that are not related to olfaction. Accordingly, the authors undertook a separate subgroup analysis during which such patients were excluded. Results of this subgroup analysis (n=12) were reported as '*similar…especially within the medial prefrontal cortex and subcallosal gyrus/nucleus accumbens [the largest clusters demonstrated across the whole group]*', though full results were not reported. In another study, this group investigated structural change in hyposmic patients of mixed cause (SND, PTOD, PIOD) compared with controls (n=23, 43 respectively, P<0.001)[386]. Referring back to their study in anosmics (with which no direct statistical comparisons were made), areas of reduced GMV common to both hyposmics and anosmics included the anterior cingulate, insula, OFC, piriform cortex, fusiform gyrus, precuneus and cerebellum. The area of GMV reduction in the region of the medial prefrontal cortex (including the anterior cingulate) was less extensive in hyposmics – interpreted by the authors as evidence of '*strong correlation'* between olfactory function and GMV in this area. A study from another group also assessed morphological change in anosmic patients of mixed cause (PIOD, PTOD, IOD)[387]. Peng and colleagues demonstrated reduced GMV (but no increased GMV)

within areas of the primary and secondary olfactory networks, including the piriform cortex, anterior cingulate cortex, OFC and insula (see Table 1-3). They further demonstrated differences in white matter volume that positively correlated with GMV change. No subgroup analysis was provided in which PTOD patients were excluded, though only 2 such patients were included in this study.

Later work aimed to characterise morphological differences according to aetiology of OD. Accordingly, altered GMV has been demonstrated in patients with congenital OD, as well as acquired forms of impairment including PIOD, IOD, PTOD and neurodegenerative conditions. Whilst care is needed when comparing such studies, due to differences in neuroimaging outcome measures (e.g. cortical thickness vs GMV vs total ROI volume), differences in statistical approach (e.g. significance thresholding, whole brain vs ROI analysis) and, importantly sample characteristics (particularly important when considering PTOD and OD related to neurodegenerative diseases – both of which may demonstrate direct, confounding, non-OD related structural change), several regions appear to be more commonly associated with psychophysical olfactory function/dysfunction than others. These include the PC, OFC, insula, ACC and pHPC. With the exception of congenital OD, in which both increases and decreases in morphological measures have been demonstrated, these studies have generally linked OD with reductions in morphological measures, e.g., reduced GMV (see Table 1-3).

	Bitter et al., 2010	***Bitter et al., 2010***	***Peng et al., 2013***	***Frasnelli et al., 2013***	***Yao et al., 2014***	***Han et al., 2017***	***Yao et al., 2018***	***Han et al., 2018***
	Mixed, anosmia	**Mixed, hyposmia**	**Mixed**	**Congenital**	**IOD**	***SND***	**PIOD**	**PTOD**
Piriform Cortex	R	R	R	L	L			R/L
Entorhinal cortex				L				
Insular Cortex	R	R/L	R		R/L	R		L
OFC	R	R	R/L		R/L	R	R	R/L
Gyrus Rectus						R		R/L
Middle Frontal Gyrus			R/L	*R*				
Superior Frontal Gyrus	R/L		R/L					R
Dorsolateral Prefrontal Gyrus	R/L							
Anterior Cingulate	R/L*	R	R/L		R/L			R
Middle Cingulate	R/L*							L
HPC	R							
Parahippocampus / Fusiform	R	R/L	R/L		R/L			R/L
Thalamus						L		L
Cerebellum	R/L	L	R/L					L
Inferior Temporal Gyrus	L		R/L					L
Middle Temporal Gyrus	L	R	R/L					
Superior Temporal Gyrus	R		R/L					
Nucleus Accumbens / Subcallosal	R/L		R					
Superior Occipital Gyrus	R							
Middle Occipital Gyrus	L		R/L					
Supramarginal Gyrus	R		R/L					
Precuneus	R/L	R/L	R					
Lingual Gyrus	R							R/L
Other:	Inferior Parietal (L) Precentral Gyrus (L)			*Superior Parietal (R/L)*				Caudate (L) Calcarine (R/L) *Angular Gyrus (R)*

Table 1-3: Summary of early VBM studies in patients with OD (excluding neurodegenerative conditions). Results in *italic* indicate GM density/volume *increases* in patients compared to controls (patients > controls). All other non-italic results indicate GM density/volume patients < controls. Asterix indicates regions included in medial prefrontal cortex cluster described by Bitter and colleagues.

However, as was observed for the OB, the computational morphometry literature described is cross-sectional and therefore, strictly speaking, provides evidence of correlation, not causation[355]. Accordingly, for example, reduced GMV in the OFC of patients with olfactory impairment may be: 1. causing OD; 2. predisposing to OD; 3. compensating for OD; 4. incidentally related to OD. This is of particular importance in higher order olfactory regions. Unlike the OB, which has direct links to the peripheral olfactory organ and is thought to be specific to olfactory processing, higher order olfactory regions are multi-functional – that is, they are not specific to olfaction and may be involved in multiple processes spanning different sensory, affective or neurocognitive domains. For this reason, the 'causation gap' is particularly important when considering such structures as neuroanatomical correlates of olfactory function/dysfunction. Without evidence for causation, the clinical use of such regions – for example as future personalised biomarkers, or even eventual targets of interventional therapies (e.g., a putative olfactory implant) – may at best be ineffective and at worst, potentially harmful.

Again, several approaches have been used to investigate causation in higher order structures. First, as for the OB, a limited amount of evidence has been provided using the principle of dose-response. As described in healthy populations, significant positive correlation has been demonstrated between psychophysical olfactory function and morphological measures within the piriform cortex, insula and OFC[372,373]. In patients, increased 'GMV atrophy' has been demonstrated in those with OD of longer duration. Peng and colleagues demonstrated more extensive reduction in GMV in patients with a duration of OD longer than 1 year[387]. Though full quantitative results are not reported, 'dose-respondent' regions with greater GMV loss appeared to include the anterior cingulate, middle temporal and superior frontal gyri. Similarly, in their study of anosmics of mixed cause, Bitter and colleagues reported 'stronger atrophy' in patients with OD for more than 2 years[385]. Whilst again, full quantitative results were not reported, one area in which this was demonstrated was the piriform cortex, where lower GMV was seen with longer duration. In addition to the principle of dose-response, Bitter *et al.,* also used

another approach to investigate the functional significance of their GMV alterations: olfactory functional MRI. Accordingly, odour-induced functional activity was demonstrated in 20 normosmic participants, with areas of activation spatially overlapping GMV reduction within the medial prefrontal cortex, piriform cortex and dorsolateral prefrontal cortex. Of note, however, this was a separate cohort of controls (n=20) than was used in their structural work, and no functional imaging was performed in their patient group.

Whilst these studies strengthen the association between GMV and olfaction, both 'dose-response' and cross-sectional functional imaging (which will be discussed in more detail in the following section) remain correlative measures – and whilst their additive and/or overlapping results help build the case for these regions as neuroanatomical correlates of olfaction, again, as for the OB, longitudinal work provides better evidence of causation. However, when I embarked on this PhD, no longitudinal studies investigating change in GMV in response to changing olfactory function had been published.

In addition to the demonstrated structural plasticity of the OB[92], biological plausibility for higher order experience-dependent structural change (in areas that are not directly connected to the periphery, as in the case of the OB) can be found in the wider neuroimaging literature. 'Functional plasticity' in the adult human brain is well established, allowing for adaptation to internal and external environmental stimuli leading to behavioural changes such as learning[388–390]. This process is underpinned by microscopic structural changes (for example in dendritic arborisation) and modifications in chemical and electrical synaptic transmission itself (through processes such as long-term potentiation and depression – collectively 'synaptic plasticity')[391]. *In vivo* demonstration of macroscopic, experience-based, higher order 'structural plasticity' is less well established, but has been possible in recent years, due to the advances in neuroimaging acquisition and analysis described above. One early line of research was in subjects learning to juggle, in whom significant alterations of grey matter density within the occipitoparietal region (involved in visuomotor coordination), as well as the underlying white matter, were seen[376,392,393]. Furthermore, evidence for potential microstructural mechanisms of

such change, in known regions of the central olfactory network, have also been demonstrated in animals. For example, rats that have undergone a course of odour discrimination training have been shown to have increased dendritic spine density amongst the pyramidal neurons of their PCs [394]. Furthermore, neurogenesis has been suggested to occur in the PC and amygdala, as well as the OB [13,395–397].

1.5.3.2 Implications for Clinical Practice and Gaps in Knowledge

A central aim of this thesis is therefore to prospectively investigate whether putative higher order olfactory regions undergo structural plasticity, in association with alterations in psychophysical olfactory function, using computational morphometry techniques. The demonstration of such plasticity would provide higher strength of evidence for the implicated regions as neuroanatomical correlates of OD, particularly important when considering their future clinical use.

1.5.3.3 Functional Imaging

Functional neuroimaging is an umbrella term for techniques that primarily assess brain function, rather than structure. These methods vary in their temporal and spatial resolution and include functional MRI (fMRI), positron emission tomography (PET), electroencephalography (EEG), magnetoencephalography, functional ultrasound imaging, functional near-infrared spectroscopy and single-photon emission computed tomography. Human olfaction is most commonly studied using fMRI – a non-invasive technique that produces proxy measures of neuronal activity (the Blood-Oxygen-Level Dependent, 'BOLD' contrast) based on changes in local blood oxygenation levels, caused by neurovascular coupling (for further information see §3.3.3.4). fMRI has been used in olfactory research for the *in vivo* demonstration of brain regions and/or networks involved in central olfactory processing, and to a more limited extent to investigate potential differences in such processing between patient and healthy control populations.

In healthy control participants, functional activity has been demonstrated in response to various experimental paradigms, including passive and active odour-smelling and odour-free sniffing. Such work has helped to delineate structures of the primary and secondary olfactory networks, as well as identifying the possible involvement of additional regions that had not previously been considered part of the olfactory networks, such as the cerebellum. Meta-analytic methods such as Activation Likelihood Estimation (ALE – a method in which whole brain statistical maps of voxel-wise activation likelihood are created using data from published studies) have been used in an effort both to pool data, and to investigate whether methodological differences in experimental paradigms significantly affect results. Accordingly, when addressing the condition 'odour vs [no-odour] baseline', Seubert and colleagues demonstrated high probability of activation within several regions, including the piriform cortex, insula, OFC and anterior cingulate gyrus. Several of these regions (e.g., piriform cortex, OFC and insula) as well as other areas (e.g. amygdala, hippocampus, nucleus accumbens) were differentially affected by experimental paradigm (e.g. cued/uncued, sniffing/passive smelling) and sex.

A more limited number of studies have been performed in patient populations. An early fMRI study from Levy and colleagues demonstrated reduced odour-induced activations in prespecified ROIs (including the orbitofrontal cortex, cingulate, piriform and entorhinal cortex, hippocampus and amygdala) in eight patients with non-neurodegeneration related hyposmia, compared to normosmic controls.[398] However, this work came before the widespread use of voxel-based analysis software such as Statistical Parametric Mapping (SPM) and instead relied on limited visual analysis of pixel-wise activation. Odour delivery was additionally achieved manually, without the use of an olfactometer, making stimulus presentation timing variable, and the patient cohort was heterogenous, consisting of PTOD, sinonasal dysfunction, congenital dysfunction (without specification regarding whether this was sporadic or syndromic) and carcinoma-related dysfunction. Accordingly, these results should be viewed with caution. As mentioned above, Bitter and colleagues used olfactory fMRI to provide evidence for the functional significance of structural alterations in GMV they had identified in their anosmic cohort[399]. However, they did

not perform functional imaging in their patient population, but rather in a separate healthy control group. The insight this provides into the functional implications of the structural alterations seen is therefore limited. More recently, Pellegrino and colleagues assessed odour induced fMRI activation in a cohort of 11 patients with hyposmia of mixed cause (PIOD, idiopathic and PTOD), compared to normosmic controls.[400] They demonstrated similar, but reduced levels of activation in hyposmics (as assessed through apparent gross, non-statistical comparison of peak and cluster level results) within the left orbitofrontal cortex, insula and parahippocampus. Voxel-wise statistics between groups showed significantly greater activation in the left anterior cingulate and right orbitofrontal cortex in normosmics vs hyposmics, and significantly greater activation in the parahippocampus, anterior cingulate and precuneus in hyposmics vs normosmics. Again, however, this study has limitations: in addition to their cohort being heterogenous in underlying disease aetiology, their patient groups consisted of only women, whilst their control group was mixed sex. In patients with PTOD, Han and colleagues demonstrated reduced odour-induced BOLD activity within the 'primary olfactory cortex' (comprising the PC and part of the amygdala) and insula, compared with healthy controls[401]. Of note, again, care should be taken when generalising results from patients with PTOD, in whom traumatic brain lesions may theoretically cause altered central processing independent of olfactory dysfunction.

Whilst olfactory fMRI has therefore helped to delineate central olfactory processing and provided some limited understanding of its potential alterations in disease, the technique has several limitations which mean that it cannot currently be used for the clinical assessment of olfaction. First, where experimental paradigms require delivery of odourants to subjects undergoing MR scanning, specialist equipment (including an MR compatible olfactometer and suitable waveguide allowing access between control/scanner rooms) and expertise (including complicated acquisition and analysis) is needed, which may be prohibitive in clinical settings. Second, and perhaps most importantly, there is a high degree of intra- and interindividual variability in BOLD signal – which has been shown for general, as well as olfactory specific activations. Whilst group level analyses mitigate these issues, this inherent

variability currently precludes meaningful olfactory assessment on an individual level. Recently, Yunpeng and colleagues attempted to differentiate between patients with olfactory dysfunction (IOD and congenital loss, mean TDI score of 11.9 ± 4.1) and normosmic subjects (mean TDI score 35.23 ± 3.7) using fMRI. Whilst they demonstrated increased BOLD activation in normosmics within two of their three interrogated ROIs (primary olfactory cortex – an area encompassing PC and amygdala, OFC and insula) at the group level, individually derived BOLD signal indices (% change) could not be used to separate patients from controls for any of the areas studied. Furthermore, they were unable to demonstrate significant correlation between psychophysical test score (TDI) or three different individually-based measures of BOLD activity (% change in BOLD, cluster size or peak-z score)[402]. Accordingly, structural markers of OD are a more immediately attractive clinical target than their functional counterpart, particularly in patient populations who may undergo structural imaging as part of their routine diagnostic workup.

Nevertheless, parallel, group-level fMRI could be used to help establish the functional significance of putative structural plasticity. In this way, establishment of 'functionally significant' structural plasticity would provide further evidence for the role of target regions as neuroanatomical correlates of OD. However, to my knowledge, at time of writing there were no such multimodal longitudinal studies.

Therefore, one of my further aims in this thesis is to investigate whether any structural plasticity seen in association with alterations in psychophysical olfactory function is accompanied by changes in olfactory induced BOLD activation, i.e., 'functional plasticity'.

1.5.4 Electrophysiological Assessment

Electrophysiological assessment of olfactory function can be performed using electro-olfactography (EOG) or chemosensory electroencephalography (EEG).[403–407] EOG involves the recording of generator potential via an electrode in contact with the OE. Chemosensory EEG allows for direct recording of intracranial neuronal response to olfactory or trigeminal stimuli. Both EEG and EOG can help to differentiate between normosmia, hyposmia and anosmia, but should be used in combination with clinical and psychophysical assessment, due to the presence of false positive and false negative results.[408]

As EEG and EOG are both event related, delivery of a known concentration of odourant must be precisely controlled using an olfactometer. State of the art air-dilution olfactometers allow for delivery of odourant embedded in a constant airflow, with fast rise times (c. 15ms) and short, precise stimulus presentation times (c.200ms). The airflow within which the odourant is embedded is humidified and warmed at high flow rates, thereby preventing inadvertent thermo-mechanical trigeminal activation. Accurate control of odourant delivery in this way, allows for event related analysis. However, the use of air-dilution olfactometers such as these, and therefore the performance of event related olfactory work, is limited to a few centres worldwide due to the high associated costs of obtaining and installing such equipment. It is unlikely that these techniques will be used in clinical practice in the near future, and for this reason I will not be addressing chemosensory electrophysiology further in my research.

1.6 End-User Experience and Preferences for Olfactory Assessment

NHS England describes 'patient experience' as one of three statutory domains required for quality healthcare (in addition to 'clinical effectiveness' and 'safety')[409,410]. Internationally, patient experience is also recognised as an integral facet of quality, by the World Health Organisation [https://www.who.int/health-topics/quality-of-care#tab=tab_1], the US-based Institute of Medicine [https://www.ahrq.gov/patient-safety/quality-resources/tools/chtoolbx/understand/index.html], and the Council of the European Union[411], amongst others. Patients with higher levels of satisfaction appear to have better outcomes, with meta-analytic work demonstrating positive associations between measures of patient experience and clinical effectiveness, as well as patient safety[412].

In 2011, the NHS National Quality Board produced the 'NHS Patient Experience Framework' – a working definition of patient experience, produced to facilitate its measurement across integrated NHS services in the UK. The latter framework outlined eight key areas of patient experience, several of which are relevant to assessment of olfactory function/dysfunction in the UK, including '*information, communication, and education on clinical status, progress [and] prognosis'* and '*access to care'*. It therefore follows, that without access to accurate and reliable assessment of olfactory function or dysfunction, it may not be possible to achieve good outcomes in these areas. Furthermore, awareness of '*quality of life issues*' as well as '*emotional support'* were also outlined in this framework, which – given the documented impact of OD on both quality of life and mental health (see §1.4.5)– are also relevant in its assessment.

A limited number of previous studies have broadly addressed patient experience of olfactory healthcare. Generally, this work has demonstrated poor levels of satisfaction across various domains of care[413–416]. Furthermore, whilst recent qualitative research has outlined the importance of appropriate olfactory diagnosis

and supporting explanations in the patient narrative[415], to my knowledge, no previous work has specifically addressed 'end-user' (patient and healthcare seeking adult) experience of, and preferences for, the *assessment* of olfaction.

To this end, an aim of my thesis is to capture end-user experience of, and preferences for, olfactory assessment using a mixed quantitative, and semi-qualitative approach. Through capturing such data, I hope to gain insight into areas of practice that are working well, and areas that could be modified or improved, as well as preferences for 'ideal' assessment. In doing so, I hope to better understand the quality of care being provided to patients today, and to make constructive suggestions for change. Finally, capturing patient experience provides some degree of comparative 'real-world' evidence for the clinician-reported data that I also aim to obtain in this thesis.

2 Thesis Aims and Objectives

Olfactory dysfunction is a relatively unique sensory impairment: though we know it is highly prevalent, and that it has significant impact at both personal and societal levels, there appears to be wide heterogeneity in both *how* and importantly, *whether* it is assessed clinically. This is not the case in other sensory impairments – generally, if a patient attends their doctor complaining of visual disturbance or hearing impairment, they will eventually have their eyes or ears tested, and usually in a manner that is standardised between healthcare providers.

Whilst the current state of practice is unknown, the last available (though now historical) UK data suggests that clinicians typically don't test the sense of smell. Given the poor correlation between subjective patient report, and more objective methods, such practice is problematic, potentially leading to incorrect diagnoses and treatment plans, inaccurate outcomes assessment, and limited insight for patients into their condition. When olfaction is assessed, there is little consensus on how this should be done: different psychophysical tools assess different aspects of olfaction, with controversy over whether their results are equivalent. Furthermore, to my knowledge, no previous work has specifically explored patient experience and preferences for olfactory assessment. Finally, the use of neuroanatomical correlates of OD could potentially offer 'objective' diagnostic ± prognostic information to compliment clinical assessment techniques. Whilst the OB can be assessed in this way, whether such assessment is performed during routine clinical practice is unknown. Moreover, areas upstream of the OB have not been fully explored for this purpose.

With these issues and opportunities in mind, the main aims and objectives of my thesis are described in two halves, Themes A and B, below.

Theme A: Current Practice in the Assessment of Olfaction – Clinician and End-User Perspective

The first broad aim of my thesis is to characterise current practice in the clinical assessment of olfaction. I aim to interrogate clinical practice through two routes: clinicians and 'end-users' (patients and healthcare seeking adults). In doing so, I also aim to determine whether end-users are satisfied with the olfactory assessments they have received, and their preferences for such assessment.

My **key objectives** were:

1. In **Chapter 4**, to capture current UK clinician-reported practice in the assessment of olfaction, with a particular emphasis on psychophysical smell testing, PROMs and imaging, using a cross-sectional non-probability sampling technique, through anonymous online survey. My secondary objectives were to explore barriers to psychophysical smell testing and to describe current international practice, outlining potential geographical variations therein.
2. In **Chapter 5**, to capture end-user experience of, and preferences for the assessment of olfaction, using cross-sectional non-probability sampling with a patient co-produced mixed quantitative and semi-qualitative anonymous online survey. My secondary objective was to use responses to the above survey as 'real-world' data on clinical assessment, for comparison with my results from chapter 4.

Theme B: Neuroanatomical Correlates of Olfactory Dysfunction

The second broad aim of my thesis is to explore new ways in which olfaction could be assessed. The OB is currently considered a neuroanatomical correlate of olfaction – that is, its structure has been linked to olfactory function. Looking beyond the OB, reduced grey matter volume has been demonstrated in the central olfactory networks of patients with OD, compared to healthy controls. However, the associated literature base for these findings is cross-sectional, so providing limited

evidence for causation. This is particularly important upstream to the OB, as these regions are multi-functional (i.e., involved in more than one sensory, affective, or neurocognitive process). Longitudinal studies provide better evidence of causation. Therefore, in Theme B, I aimed to examine the role of higher order olfactory regions as potential neuroanatomical correlates of olfactory dysfunction, through prospectively interrogating the effect of clinically relevant changing olfactory function on these structures.

My **key objectives** were:

1. In **Chapter 6**, to provide proof of principle for change in macroscopic grey matter volume ('structural plasticity') within regions of the central olfactory networks, upstream of the OB, following FESS for CRS using a prospective computational morphometry neuroimaging approach.
2. In **Chapter 7**, to expand and extend on this work by investigating the functional significance of this structural plasticity through use of a prospective multimodal neuroimaging approach, including computational morphometry and olfactory functional MRI, again in patients undergoing FESS for CRS, compared with non-CRS controls.
3. In **Chapter 8,** to extend on this work by exploring whether regions that underwent 'functionally significant structural plasticity' in chapter 7 represent aetiology/treatment specific neuroanatomical correlates of OD, using a prospective multimodal neuroimaging approach, in patients with non-CRS OD undergoing functional septorhinoplasty (fSRP), compared with normosmic controls.

3 General Methods

3.1 Statement of Contribution

All the work described in this chapter was performed by me, unless specifically stated otherwise. Some of the content of this chapter was used in the methodology sections of the published manuscripts from experimental chapters 4 – 8. The authorship contributions for those manuscripts can be found in the relevant chapters.

3.2 Theme A: Current Practice in the Assessment of Olfaction – Clinician and End-User Perspective

The following sections describe my clinician and end-user survey work, found in experimental chapters 4 and 5.

3.2.1 Clinician Survey

Literature Review

I performed a systematic literature review for relevant guidelines and original research published until Feb 2021. Databases interrogated included Medline (PubMed inception – current), Embase (Jan 1958 – current), Google Scholar (limited to first 1000 results), as well as the preprint servers MedRxiv and BioRxiv. I constructed search terms using Boolean operators, truncation and MeSH mapping, as required. I additionally hand-searched the reference lists of key publications and citing literature.

Survey Development

As there was no appropriate existing survey tool, I created an anonymous online questionnaire de novo. My target population was ENT surgeons who assess olfaction. To further assist in development of my questionnaire, I assembled a panel of UK-based clinicians who provided feedback during three iterative rounds of item generation and reduction, with initial content being generated through systematic guideline/supporting literature review, as above. This panel comprised both consultant ENT surgeons considered to be experts in olfaction (defined as ≥ 5 publications in olfaction, excluding CRS) and consultant ENT surgeons considered to be representative of non-expert respondents (<5 publications in olfaction, excluding CRS). I additionally included a non-ENT clinician who provided feedback on general questionnaire flow and technical ease of use on various devices. The inclusion of clinicians with varying interest in olfaction during development and piloting allowed better approximation of respondents within the target population, and allowed determination of both face and content validity[i, 417]. To prevent dominance by expert members, feedback was collected and discussed anonymously during all rounds. Several aspects of this process was in keeping with a modified Delphi process (systematic literature review, iterative rounds of content review until consensus, anonymity of feedback)[418]. However, unlike the standard RAND/UCLA Delphi process, in which the review process is concluded once a pre-determined consensus threshold is reached (typically 70-80% agreement) I ended the review process after three rounds[419]. Additionally, I asked for specific, free-text feedback on each individual question. Survey piloting was performed simultaneously during iterative rounds of panel review. Characteristics of the questionnaire panel are provided in Table 3-1. See appendix 10.1 for panel feedback questionnaire. Further details regarding item generation/reduction can be found in §4.4

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[i] *Face validity requires a process of review by experts and sample participants and is obtained when these individuals believe the questionnaire measures what it is intended to measure. Content validity requires a process of review by experts – who must ensure that the final survey assesses all relevant aspects of the topic in question.*

Member	Group	Publications in Olfaction	Hospital	Time since CCT
Professor of Rhinology and Olfactology	Expert	>30	Tertiary referral	≥ 10 years
Professor of Rhinology	Expert	>10	Tertiary referral	≥ 10 years
Associate Professor of Rhinology	Expert	7	Tertiary referral	≥ 10 years
Consultant Rhinologist	Expert	7	Tertiary referral	< 10 years
Consultant Rhinologist	Non-expert	3	Tertiary referral	≥ 10 years
Consultant ENT surgeon	Non-expert	0	District General	≥ 10 years
Consultant ENT surgeon	Non-expert	0	District General	< 10 years
Orthopaedic StR	Non-ENT	0	District General	N/A

Table 3-1: Characteristics of UK questionnaire development panel. CCT = 'Certificate of Completion of Training', awarded at end of higher surgical training.

For my international survey, content validity was further established through review and piloting by an international panel of experts. For this purpose, I assembled 10 members of the Clinical Olfactory Working Group (COWoG - an international association of ENT surgeons that is supported by the European Rhinologic Society, and in collaboration with EUFOREA, The Olfaction and Gustation Working Group of the German ENT society, the Swiss Society for Oto-Rhino-Laryngology and the IAR, and of which I am a member). Again, questionnaire piloting was performed simultaneously with iterative rounds of panel review.

Questions stems were focused on a single domain, and were carefully worded so that they were unambiguous, unbiased and easy to understand[420]. In general, language was tailored to the target population. Additional education or clarification was provided where the question stem addressed a concept that might not be

familiar to the respondent. For example, whilst general ENT consultants would be expected to know the term 'olfaction' they may not be familiar with the terms 'orthonasal olfaction' or 'retronasal olfaction'. These were therefore clarified within the questionnaire.

I used a 'multiple-screens' format (multiple linked pages), as this has been shown to maximise survey efficiency, and in an attempt to make the survey 'user-friendly' and reduce human error in data entry[421]. For clarity, each page contained questions relating to a single assessment domain (with the exception of the optional 'more detailed' questions, where multiple domains were addressed on one page). Branching logic was also employed – meaning that respondents only answered questions that were relevant to them, and were given the option to skip the optional, 'more detailed' questions. Various question formats were used, including open and closed constructs, the latter including nominal (e.g., multiple tick boxes) or ordinal (e.g. 'Likert' scales) formats. Nominal response lists were constructed to be as exclusive and exhaustive as possible. Open 'free-text' responses were used either to expand on closed questions (where further elaboration or new issues were sought, or where nominal response lists were non-exhaustive) or to allow further comments at the end of a question domain/survey as a whole. Invitation of free text comments has previously been suggested to empower respondents[422] and potentially increase response rates[423]. Where possible, 'radio buttons' were used – in which the respondent must choose only one response from a nominal or ordinal list (when selected, these automatically deselect all other options in the list). This answer format has been shown to reduce missing data compared with text boxes [421]. Item non-response was reduced by making key questions 'required' (that is, the respondent could not progress through the survey until an answer had been provided).

To prevent 'multiple participation' (i.e., the ability for a participant to undertake the survey multiple times), 'sign-in' was required prior to survey completion on one of the electronic survey platforms used (Google Forms). During piloting it was felt that this would/may cause: 1. additional time for survey completion; 2. respondent concerns regarding data anonymity; 3. perceived increased complication of

participation. Furthermore, it was collectively agreed that clinicians were unlikely to complete the survey more than once, due to the length of the questionnaire and high daily workload of the typical clinician. Accordingly, 'sign-in' was not used.

As response rates are known to increase through use of incentives[424], on completion, respondents were offered copies of the Position Paper on Olfactory Dysfunction (PPOD)[425] and British Rhinological Society Consensus Guidelines on Management of New Onset Anosmia in the COVID Pandemic [426]. I gained appropriate permissions for this from Rhinology journal and the British Rhinological Society.

Test-retest reliability was determined in a subset of 2 members of the development panel. Following an interval of 2 weeks, excluding free text answers, there was 100% agreement between scores at time points.

Survey Distribution

I chose to distribute my survey through ENT-UK, which is the professional membership body for ENT surgeons and allied specialties (e.g., audiologists) in the United Kingdom. They represent the broadest membership of ENT surgeons in the country and are the only unified source of access to both general and subspecialist consultants. They additionally provide a distribution service for questionnaires that have been reviewed and approved by their in-house ENT-UK survey guardian, ensuring the quality of distributed work and thereby aiming to maintain good response rates. As membership lists are not made available to researchers for data protection purposes, random sampling of consultant ENT members is not possible. Instead, invitations to participate are distributed by ENT-UK to their entire membership via email. This form of distribution is therefore cross-sectional non-probability sampling [417].

International distribution was facilitated by collaborating members of COWoG. Where possible, distribution through professional societies was undertaken (cross-sectional non-probability sampling). Where necessary, however, other distribution

methods were also pursued on a pragmatic basis, in order to increase survey coverage.

Survey distribution in the UK was performed during the COVID-19 pandemic. Accordingly, concurrent use of in-person or postal distribution was not pursued in the UK.

Ethical Considerations

As a combined service evaluation/audit, the questionnaire was not classified as research according to NHS Health Research Authority guidance (see: http://www.hra-decisiontools.org.uk/research/). Exempt status was confirmed by Prof Michael Heinrich, Joint Chair of the UCL Research Ethics Committee. Audit registration was approved for national/international distribution by the Royal National ENT & Eastman Dental Hospitals (University College London Hospitals NHS Foundation Trust). Where required, further local permissions were obtained by distributing COWoG members. As all data was anonymous and 'non-sensitive', there were no data protection issues of note. However, a 'Microsoft Forms' version of the questionnaire was provided to COWoG where necessary for international GDPR compliance, as well as the default 'Google Forms' version. Within the UK, the survey was distributed as a 'Survey Monkey' questionnaire, as required by ENT-UK. Cross-platform surveys were identical.

Statistical Analysis

Sample Size

As this was a service evaluation with no minimum significant difference/effect size of interest for the main outcome measures, a formal power calculation, as used in hypothesis testing/comparison of effect size, was not performed.

However, other methods can be used to produce minimum sample size estimates for survey methodologies. Cochran's sample size formula for infinite populations can be

used to produce a sample size estimate (where either means or proportions are being assessed), for a given confidence interval and margin of error, when the population in question is large/infinite[427]. A subsequent 'finite population correction' can be used when the total population in question is known[427]. For the resultant sample size estimation to be valid, however, Cochran's sample size formula requires that the sampling method applied is random[427,428]. As I was unable to perform random sampling, I performed the following calculations for exploratory purposes only, in relation to my primary aim (capturing current UK practice):

Cochran's sample size calculation for infinite populations:

$$n_0 = \frac{Z^2 p(1-p)}{E^2}$$

Where:

n_0 = initial sample size prior to correction for finite population

Z = z-value reflecting confidence interval (here 1.96 for 95% confidence)

p = estimated proportion of population with attribute of interest (e.g., performs psychophysical testing) (here taken as 0.5 for maximum variability)

E = margin of error (here taken as 0.1 for 10%)

Using this equation my exploratory sample size estimate for an infinite population would be:

$$n_0 = \frac{(1.96^2)(0.5)(1-0.5)}{0.1^2} = \frac{(3.8416)(0.5)(0.5)}{0.01} = \frac{0.9604}{0.01} = 96.4$$

Correction for finite populations:

$$n_{adj} = \frac{n_0}{1 + \frac{n_0 - 1}{N}}$$

Where:

n_{adj} = adjusted sample size

n_0 = initial sample size prior to correction

N = total population size*

*Regarding total population size – the ENT UK membership at time of distribution was slightly higher than outlined in their 2020-21 annual report[429], at 2,165 (personal communication). This number included allied specialties and honorary members. The true UK ENT workforce at that time was smaller than this, most likely between 1,300 and 1,400 (including 'specialty and associate specialist' doctors and trainees), based on contemporaneously available data from 2018, and more recent data from 2022-23[430–432]. As my sampling method was not random, however, I chose to use 2,165 to produce a more conservative sample size estimate.

Using this finite population correction, my adjusted sample size estimate would be:

$$n_{adj} = \frac{n_0}{1 + \frac{n_0 - 1}{N}} = \frac{96.4}{1 + \frac{96.4 - 1}{2165}} = \frac{96.4}{1.044} = 92.3$$

Accordingly, where I achieve a UK sample size of ≥92 people, my results would be statistically valid with a 95% confidence interval, and a ±10% margin of error, under conditions of random sampling. However, bearing in mind the above caveat regarding the assumption of random sampling, I used a minimum sample size of 92 as an exploratory guide, and caution will be required when interpreting the validity of my results.

I did not calculate exploratory minimum sample size numbers for each additional international country surveyed, as description of potential geographical variation in clinical practice was a secondary aim.

Qualitative Data

I analysed the qualitative data obtained from my questionnaire using qualitative content analysis[433]. During the preparation phase, I read through the data several times. As the majority of questions required a relatively limited range of answers [e.g., where the question was intended to act as an extension to a closed question (e.g. 'other – please specify') or where the question was a substitution for a closed question (e.g. 'which [PROMS] do you use?')], little transformation of the data was required and 'manifest' content [j] was analysed. Given the lack of previous information on this topic, an inductive approach [k] to coding was employed. Frequency of coded content occurrence was recorded and used as a proxy for significance to the overall responding sample. Where more qualitatively rich text was provided [e.g. for open ended questions such as 'How has the COVID-19 pandemic changed your practice in the assessment of patients with OD?', and 'Is there anything else you would like to say about the clinical assessment of olfaction / OD' (placed at end of survey to enable respondents to convey any information not captured elsewhere)], coded content was additionally grouped into a limited number of non-mutually exclusive higher order subheadings, if possible.

[j] *Manifest content describes explicit meaning. Latent describes underlying meaning.*[453,703]

[k] *Qualitative analysis can be performed using an inductive or deductive approach. During the inductive approach categories and concepts are derived from the data (i.e. 'data-driven'). This is used in studies where there is little previous knowledge and the researcher is not trying to test a pre-existing theory. Deductive approaches involve organisation of data into pre-existing categories and concepts, which have been derived from previous work (i.e. theory-driven)*[433,453].

3.2.2 End-User Survey

Literature Review

As for my clinician survey, I performed a systematic literature review for relevant research published until Dec 2021. Databases interrogated included Medline (PubMed inception – current), Embase (Jan 1958 – current), Google Scholar (limited to first 1000 results), as well as the preprint servers MedRxiv and BioRxiv. I constructed search terms using Boolean operators, truncation and MeSH mapping, as required. I additionally hand-searched the reference lists of key publications and citing literature.

Survey Development

As for my clinician survey, there was no appropriate existing survey tool, and therefore I created an anonymous online survey *de novo.* My target population was 'healthcare seeking adults', i.e., those who had undergone, or might in the future undergo, olfactory assessment by a healthcare professional. I chose to use a co-production model for the development of my end-user survey. Patients, families and carers ('end-users') are key stakeholders in both healthcare and healthcare-related research. They provide insight into relevant issues surrounding the diagnosis and management of disease, as well as the delivery of care – so highlighting issues that may otherwise remain unknown to clinicians and researchers[434]. In addition to practical benefits, such as comprehensive coverage of salient research questions and increased recruitment and retainment in research studies, there are additional ethical benefits to creating a patient-centred approach[435]. Together, this has been reflected in an increasing body of co-produced research, and requirements for patient and participant involvement set by many research and funding bodies[436]. However, at time of survey planning, whist a limited amount of research had been performed looking at patient experience of olfactory healthcare, to my knowledge there were no *co-produced* studies assessing end-user experience of olfactory assessment.

I chose to use co-production for both the development and distribution of the survey. For this, I partnered with Christine Kelly (CK), a UK-based patient with OD, and founder of the olfaction patient charity 'AbScent' (at time of writing, one of two charities for people with OD in the UK). Through her role both as a patient, patient advocate, and founder of AbScent, CK has been involved in several previous research studies[437–441]. She therefore has unique insight into the personal role of the patient, the collective needs of patient groups, and the interaction between patient and researchers. Given this unique role, I chose to use a 'key informant' co-production model, comprising several iterative and recursive rounds of item generation and reduction in collaboration with CK – see §5.4.1 for more details. Piloting was performed both within the item generation/reduction process ('expert'), and with a further panel of 5 test participants ('non-expert'). Accordingly, both face and content validity were established.

Questions were worded so that they were unambiguous, unbiased and easy to understand [420]. Full descriptions of olfactory deficits were provided in lay terms, e.g., for retronasal olfaction the following descriptions were provided*: 'I have problems tasting flavours [without flavour, which relies on the sense of smell, food would only taste sweet, salty, sour, bitter or savoury]'* and '*for example, being able to tell that you are drinking orange juice (without looking at it). If you lose or partially lose this ability, you may only be able to taste your food as sweet, salty, bitter, sour or savoury. [Please note that the experience of 'flavour' is dependent on the sense of smell]*'. As for my clinician survey, 'multiple-screens' and branching logic were employed, to increase survey efficiency and ensure that respondents did not answer questions that were not relevant to them (e.g., respondents who had not seen a healthcare professional or did not have OD only answered questions on 'preferences for assessment', and not those pertaining to their experiences of olfactory assessment)[421]. Various question formats were used, including open and closed constructs, the latter including nominal (e.g., multiple tick boxes) or ordinal (e.g. 'Likert' scales) formats. Whilst the majority of the data collected was quantitative, two free text (long answer) questions were included to gather semi-qualitative data surrounding respondent experience. Whilst in-person data collection techniques

such as interview/focus groups may have facilitated gathering of more in-depth qualitative data (allowing researcher – participant interaction in a qualitative setting), this was not possible due to the intercurrent COVID-19 pandemic.

Survey Distribution

I chose to use social media recruitment for my end-user survey. The use of social media in healthcare, and healthcare research is a rapidly emerging field[442]. In 2021 (time of survey dissemination planning) an estimated 61.8% of the global population, and 98% of the UK population used the internet; social networks were the most frequently accessed website/app type, with an estimated 57.6% of the global population using such content for an average of 2hrs and 27 minutes per day – higher than average time spent reading press media (online/print), at 2hr and 5 minutes.[443] The most frequently accessed social media platform at that time was Facebook, with approximately 2.9 billion users globally, and more than 50 million UK users throughout 2021 (>75% of the UK population[444])[445]. Traditionally, social media was thought to preferentially target younger and more affluent people[446]. However, recent evidence has demonstrated increased use in older and more socioeconomically varied populations. For example, in 2019, 68% of American 50-64 year olds, and 46% of ≥65s used Facebook[447], and in 2018, 78% of Canadians aged ≥55 with access to the internet used Facebook[448]. Furthermore, work from 2016 found that 71.9% of homeless young people regularly used social media, most commonly Facebook[449]. Accordingly, social media platforms, and particularly Facebook, offer attractive tools through which to potentially access many people, of diverse background, who may otherwise be hard to reach.

In line with this, previous work investigating participant recruitment through social media has demonstrated good results. In a recent review of mental health research, recruitment through Facebook demonstrated equivalent or better results (regarding number and final cost of enrolled participants) than traditional methods (e.g. postal) in 68.3% of studies[450]. Furthermore, such recruitment appears to produce representative or partially representative samples – a recent study found that

participants from a sample recruited through Facebook were representative of the target population in 8 out of 13 studied characteristics[448]. Within otorhinolaryngology, Facebook has been used to recruit patients with various conditions, including idiopathic subglottic stenosis, tinnitus and allergic rhinitis[451]. The ease of access afforded through social media recruitment was particularly important to my work, given the intercurrent pandemic and its pragmatic implications on other forms of recruitment. I therefore elected to recruit participants for my end-user survey using social media. Furthermore, at time of distribution, AbScent's main patient forum was hosted by Facebook – with its collective membership at peak being in excess of 30,000 people. Membership was 'closed' (i.e., requiring administrator approval or invitation to join) and content was moderated in collaboration with a scientific advisory panel, to ensure that only evidence-based information was promoted. Accordingly, the AbScent Facebook channel became an established method for survey dissemination during the pandemic – with the charity distributing surveys from more than 30 different institutions during this time (personal communication with CK). The AbScent Facebook group therefore provided an efficient, tested route through which to access my target audience, and I chose to use this method for participant recruitment/survey distribution – which provided cross-sectional, non-probability sampling[417].

Again, as for my clinician survey, given the intercurrent limitations of the pandemic, in-person or postal distribution methods were not pursued.

Test-retest reliability was determined in a subset of 2 pilot participants, and (excluding qualitative/free text answers), as for my clinician survey there was 100% agreement in scores with a 2-week interval.

Ethical Considerations

Ethical approval was obtained from the UCL Research Ethics Committee (REC Approval ID Number: 20479/001). All data collected were anonymous and non-sensitive – no IP or email addresses were collected, and respondents were asked not

to provide any identifiable information regarding themselves or their healthcare provider(s). Informed written consent was obtained from all respondents. Those who did not give consent, or those who were less than 18 years were unable to access to the questionnaire.

Statistical Analysis

Sample Size

As for my clinician survey, there was no minimum significant difference/effect size of interest available for my survey questions. Therefore, a formal power calculation, as used for hypothesis testing/comparison of effect size, was not performed.

However, for exploratory purposes, I again calculated a minimum sample size estimate using Cochran's sample size formulae[427,428]. Again, this was exploratory, given that my distribution technique violated of the assumption of random sampling.

I chose to use the same confidence interval, margin of error and estimated population proportion, of 95% (giving a z-score of 1.96), 10% and 0.5 respectively. Therefore, the initial uncorrected sample size estimate was as for my clinician survey, $n_0 = 96.4$.

As a reminder, the correction for finite populations is:

$$n_{adj} = \frac{n_0}{1 + \frac{n_0 - 1}{N}}$$

Where:

n_{adj} = adjusted sample size

n_0 = initial sample size prior to correction

N = total population size*

*Estimation of total population for my end-user survey was complicated by my use of social media for distribution (whereby people within a Facebook group would only see the survey advertisement if they were online during the recruitment period). The

total number of Facebook group members may thereby grossly overestimate the number of potential respondents who saw the advertisement, and unfortunately page traffic information was not available. However, in an effort to produce as conservative a sample size estimate as possible, I elected to use the total Facebook group number at that time, of 34,536 (as of 4.3.22), during my sample size calculations. I did not, however, use this for calculation of response rate, given the above complexities.

Accordingly, my adjusted sample size estimate was:

$$n_{adj} = \frac{n_0}{1 + \frac{n_0 - 1}{N}} = \frac{96.4}{1 + \frac{96.4 - 1}{34536}} = \frac{96.4}{1.00276} = 96.1$$

Accordingly, where I achieve a sample size of ≥96 people, my results would be statistically valid with a 95% confidence interval, and a ±10% margin of error, under conditions of random sampling. Again, however, given my violation of the latter assumption, I used a minimum sample size of 96 as an exploratory guide. Caution will be required when interpreting the validity of my results.

Qualitative Analysis

Qualitative data were analysed using thematic analysis. I chose thematic analysis instead of qualitative content analysis in this chapter, as I was more interested in the underlying and emergent themes within the data, and less concerned regarding frequency of their occurrence[452]. I used a six-step approach, as described by Clarke and Braun [453]: 1. *Data familiarisation* – I actively read all data several times; 2. *Initial code generation* – I coded both manifest and latent content manually using an inductive framework (see footnotes j and k). Patterns and relationships between codes were noted for later theme identification; 3. *Theme identification* – I then analysed coded/collated data to identify overarching themes using an inductive, inclusive and recursive approach; 4. *Theme review* – I then reviewed data for

commonality and coherence to their assigned theme, ensuring no undue overlap into another theme. Where overlap did occur, or where themes were too small/large, I modified them as necessary. A similar analytical process was then applied at the higher theme level, with respect to the dataset as a whole; 5. *Theme definition/naming* – I then defined and delineated individual theme scope, identified major and minor subthemes and selected representative data extracts; 6. *Report production* – I made use of data extracts and supportive narrative, data visualisation and finally, I framed findings within the wider research landscape.

3.3 Theme B: Neuroanatomical Correlates of Olfactory Dysfunction

The following sections describe my prospective neuroimaging work, found in experimental chapters 6 – 8.

3.3.1 Participants

Patients

Adult patients (≥18 years) were recruited from the University Hospital Carl Gustav Carus Dresden (Dresden, Germany) and the Royal National ENT Hospital (formally the Royal National Throat Nose and Ear Hospital) for experimental chapters 6 – 7, and 8 respectively. As these were prospective observational (cohort) studies, patients were only recruited from those who had already been listed for surgery (FESS or fSRP) for established clinical indications, and according to contemporaneous guidelines.

For my studies involving CRS and FESS (experimental chapters 6 and 7), I aimed to recruit a sample of participants with OD at the group level. As OD is a cardinal symptom of CRS, with high associated prevalence, I consecutively recruited eligible

patients from those awaiting surgery. For my final study in patients undergoing fSRP (experimental chapter 8), as my primary objective was to determine whether the regions that underwent 'functionally significant structural plasticity' in chapter 7 represented aetiology/treatment specific neuroanatomical correlates of OD, I aimed to recruit a sample of participants with non-CRS OD at the individual level. Accordingly, I recruited a non-consecutive, pragmatic sample of patients with non-CRS OD from those awaiting fSRP.

Controls

Control groups were recruited for experimental chapters 7 and 8. In the former, this consisted of a convenience sample of age and sex matched healthy volunteers who were free from CRS or other known causes of olfactory dysfunction (see §7.4.1 for further details). This was a prospective control group, and as for patients, I undertook their assessment pre- and post-operatively.

In experimental chapter 8, in an attempt to further isolate psychophysical olfactory function as the variable of interest, age and sex matched normosmic controls were taken from the same population of patients awaiting functional septorhinoplasty for nasal obstruction (see §8.4.1 for further details). This was a cross-sectional control group and assessment was therefore only undertaken pre-operatively.

Additional Recruitment Criteria

Detailed inclusion and exclusion criteria can be found in the respective experimental chapters, but broadly, any patients or controls with additional co-morbidities that could cause OD were excluded, as were those who were unable to undergo MRI scanning or were not available for follow up.

As there is mixed evidence for the effect of smoking on olfactory function (see ref [119] for discussion), participants were not excluded based on smoking status, though they were asked to refrain from smoking for 1 hour prior to scanning. Participants were not excluded based on BMI, except where this precluded MRI scanning.

Participants were assessed for handedness using a modified version of the Edinburgh Handedness Inventory[454]. However, as handedness does not appear to affect passive olfactory processing[455], and this was pragmatic clinical sampling, otherwise eligible participants were not excluded based on handedness alone.

3.3.2 Clinical and Psychophysical Outcome Measures

All clinical and psychophysical assessment was performed by me in the UK (chapter 8), and with the assistance of JF and JN in Germany (chapters 6 and 7). Please see experimental chapters for full authorship contributions.

Clinical Assessment

All participants underwent thorough clinical history and examination (see experimental chapters for details) prior to psychophysical and other clinical outcome measurement. Those with current or recent UTRI, or subjective flare of AR (within 3 weeks) were either excluded or deferred for reassessment at a later date. All recruitment was completed prior to the onset of the COVID-19 pandemic. Therefore, SARS-CoV-2 testing was not undertaken.

Endoscopic examination was performed using a rigid 0°, 4mm Hopkins Rod nasendoscope (Karl Storz), using a standardised three pass technique, without use of topical decongestant/anaesthesia (decontamination was performed in line with local hospital protocol). Endoscopic findings in patients with CRS were assessed using the Lund-Kennedy (LK) scoring system[456]. The system allocates points unilaterally for polyps, oedema and discharge (+ scarring and crusting post-operatively), with total score being the total of left and right sides (see appendix 10.5). The test-retest reliability is good and there is moderate inter-rater reliability (intraclass correlation coefficients of 0.83 and 0.51 respectively)[457]. I am experienced in use of the LK system through my clinical training and accordingly provided training to JF and JN who assisted with data collection in Germany. Following training, a test of 3

participants was undertaken in which my score was compared to those of JF/JN – and throughout JF and JN achieved LK scores ±1 point of my own.

Allergic rhinitis status was established though clinical history and skin prick testing (performed as part of standard clinical workup).

Patient Reported Outcome Measures

I used patient reported outcome measures (PROMS) in experimental chapters 6—8. In my CRS studies, I used the Sinonasal Outcome Test (SNOT) – a disease-specific score that assesses health and health related quality of life, including 'physical', 'functional' and 'emotional' domains [458,459]. Specifically, I used the SNOT-20 German Adapted Version, which was validated for use in the German population and (unlike the original SNOT-20) includes a specific question on subjective olfactory function [Q10, 'decreased smell', see appendix 10.5][460,461]. At time of planning/data collection, the later version of the SNOT questionnaire, the SNOT-22[459], was not yet validated in the German population. However, one of the main differences of interest between the original SNOT-20 and later SNOT-22 was inclusion of a question on smell/taste (as well as nasal obstruction).

In my cohort of participants with nasal obstruction, I used three different PROMs. First, the SNOT-23, which is a derivative of the CRS-specific SNOT-22 tool that has been adapted and validated for use in patients with nasal obstruction undergoing functional septorhinoplasty surgery[462,463]. As for the SNOT-22, this tool contains one question on subjective olfaction – though of note this question does not differentiate between smell and taste [Q21, 'sense of taste and smell', see appendix 10.5]. Accordingly, a single item Visual Analogue Scale (VAS) for olfaction was also used ['problems with sense of smell', 0=none, 10=very bad, see appendix 10.5]. This was provided on paper, with a total length of 10cm, and participants were asked to mark on the line the severity of their symptom. I chose to use a VAS as these are simple to construct and use, and because continuous psychometric measures have been shown previously to have greater reliability and validity than discrete measures[464]. Use of VAS was additionally recommended in contemporaneously

available guidelines[425]. Finally, I used the Nasal Obstruction Symptom Evaluation (NOSE) in my cohort of participants with nasal obstruction undergoing functional septorhinoplasty. NOSE is a validated tool that assesses burden of disease in nasal obstruction, which directly interrogates nasal patency, as well as sleep and obstruction during exercise (see appendix 10.5)[465].

All PROMS were undertaken in both patient and, where applicable, control groups.

Psychophysical Olfactory Function

Psychophysical olfactory function was tested in line with contemporaneously available guidelines. Accordingly, for experimental chapters 6—8, I tested psychophysical olfactory function using the Sniffin' Sticks (SS) test battery (original version[I], Burghart Messtechnik). As described in part during §1.5.2.6, the SS comprises three separate subcomponents which test DT (using either phenyl ethyl alcohol or N-butanol as target odour), identification and discrimination[235]. The composite 'TDI' score is the sum of the individual T, D and I score. Test-retest reliability has been established for each subcomponent separately (correlation coefficients: T – ranging from 0.61 up to 0.85[466]; I – 0.73[235]; D – 0.54[235]) as well as for the composite TDI (correlation coefficient 0.72[235]). Extensive normative data exists – including age and sex specific data (largest series to date total N = 9,139[286]), and unlike some other available tests at time of study planning, minimum clinically important differences had been established for each of the individual subcomponents (T/D/I) as well as the composite TDI score[287]. The SS have also been validated for use in both German and UK populations[286,467,468].

[I] *The original version of the SS contains 16 items (triplets or individual pens, see main body of text for details) within each subcomponent (T/D/I). An extended version of the SS has been described with a higher number of test items within the discrimination and identification subcomponents (32 items each), however, this was not commercially available at time of study planning. Further, as testing times were high in some participants during pilot testing, I felt that the increase in participant burden that this extended test would involve was unacceptable in my clinical cohort.*

I was trained in administration of the SS test by TH, who was part of the team who developed the test[235], and then gave appropriate training to JN and JF. I performed all psychophysical assessment in the UK myself. In Germany, I was assisted by JN and JF.

All psychophysical testing was performed in a quiet, well-ventilated room. I began testing with the odour threshold subcomponent to avoid issues surrounding habituation/adaptation following prolonged suprathreshold odour exposure. The SS threshold test assesses absolute, detection threshold using a 16-step three-alternate forced choice single staircase paradigm (see §1.5.2 for more details on absolute detection threshold, single staircase and forced choice paradigms in psychophysical testing). The SS are felt tip like pens (approximate length 14cm, inner diameter 1.3cm) with the central tampon containing 4ml of odourant, odourant diluted in propylene glycol or propylene glycol alone. Each staircase step comprises presentation of three pens, one of which is the target odour-containing pen, and the other two of which contain dilutant (propylene glycol) only. The order of target pen presentation with each triplet was pseudorandomised. Immediately after triplet presentation, participants were asked to choose which pen contained an odour, using a forced choice paradigm. Each staircase step (triplet of pens) contains a target pen of different concentration – comprising a geometric series of 16 concentrations, with dilution ratio of 1:2 from a stock 4% solution (prepared by Burghart, Messtechnik). Testing begins with the lowest target pen concentration, and successively stronger concentration triplets are presented, until the participant correctly identifies the same odour concentration twice in a row. At this point the staircase is reversed, and triplets with successively weaker target pen concentrations were presented. The staircase is reversed again when the participant is unable to correctly identify the concentration being tested. This process continues until seven staircase reversals have occurred, and the participants' threshold score (ranging 1—16) is the mean of the last four reversals.

The discrimination subcomponent of the SS test assesses suprathreshold odour quality discrimination (not differential threshold, see §1.5.2 for more details). As for odour threshold, the test comprises 16 sets of odour triplets. However, each pen

within the triplet contains an odour – at a concentration shown to be suprathreshold in a normosmic population[235]. Two of these odours are identical, and one is different (the 'target'). The three pens are again presented to the participant in a pseudorandomised order, and immediately following presentation, they are asked to choose which of the three is different to the other two (the 'odd one out'). Again, a forced choice paradigm is used. This is repeated for all 16 pen triplets (for full list of odours used in the SS discrimination test, see [235]). Each target pen correctly identified is given a score of 1, therefore possible total discrimination score ranged from 0—16.

The odour identification subcomponent of the SS test assesses suprathreshold, cued-odour identification. It comprises 16 individual pens, each containing a different odourant of suprathreshold concentration (for full list of odours used in the SS identification test, again see [235]). Following presentation, the participant is asked to choose which of the standardised visual/written descriptors (for example, see Figure 1-3) they believed the target odourant represented. Again, a forced choice paradigm was used. Each target correctly identified was given a score of 1, therefore possible total discrimination score ranged from 0—16.

During testing (T/D/I), I removed the cap of each pen, and placed the pen's tampon approximately 2cm from each nostril. Stimulus presentation was for approximately 3 seconds. The interstimulus interval between each pen within a triplet was approximately 3s. The interstimulus interval between triplets of pens, or between the individual pens within the identification task was approximately 20s. Participants are blindfolded for the threshold and discrimination testing components (but not for identification), not allowed the touch the pens, and only allowed to smell each pen once. Handwashing was performed prior to psychophysical testing using a fragrance-free soap (no alcohol gels were used). No contact is made with the SS tampon during testing (either by subject or examiner), and previous work has demonstrated no bacterial contamination if the test is conducted in this way[235]. As all recruitment was completed before the onset of the COVID-19 pandemic, no additional infection control measures were taken. The SS were stored carefully (all lids correctly in place), within a temperature-controlled environment (chemosensory lab, Germany

or research equipment storage room, RNTNE Hospital or Lysholm Department of Neuroradiology). Each test set was used within its recommended shelf life, after which they were replaced.

Pilot testing in a cohort of ten clinical patients was performed. Completion of the composite SS test (T, D and I), took between 16 and 55 minutes. Longer testing times were anecdotally associated with higher participant burden. As I was working with prospective clinical cohorts, and because my primary aim was to investigate neuroanatomical correlates of OD, I elected to perform all psychophysical testing birhinally. Furthermore, birhinal olfactory function arguably best represents the patient or participants' lived olfactory experience. Finally, in order to further reduce participant burden, in my work with patients with CRS undergoing FESS (chapters 6 and 7), I elected to use only the T and I components of the SS, as the link between olfactory impairment and improvement is well established for this condition and treatment, respectively. More specifically, I chose to use threshold and identification as previous work has suggested these may best represent peripheral and central olfactory function, respectively[152]. This approach was in keeping with guidance from the Position Paper on Olfactory Dysfunction[425]. In my chapter 8 work, in patients undergoing functional septorhinoplasty, in which recruitment of patients with established OD (hyposmia/anosmia) + nasal obstruction was required, and as the effects of septorhinoplasty on olfaction are less well studied (and in this case, where my secondary aim was to explore mechanism by which this procedure could affect olfactory function), I elected to perform full TDI testing.

Cut off scores for diagnosis of hyposmia and anosmia using the composite SS TDI score (possible range 1-48) have been previously established, based on extensive normative data. Whilst age and sex specific normative data is available, diagnoses are made in reference to a young, healthy population (age 21-30)[286]. Accordingly, normosmia was attributed where TDI was ≥30.75, hyposmia where TDI is >16, but <30.75, and anosmia ≤16. In my work with CRS patients, in which I used the T and I subcomponents of the SS test, normosmia was attributed where T ≥5.75 and I ≥11[286]. The minimal clinically important difference (MCID) for T, D and I are ≥2.5 points, ≥3points and ≥3 points respectively, and ≥5.5 points for composite TDI[287].

Peak Nasal Inspiratory Flow

I used peak nasal inspiratory flow (PNIF) as an estimate of nasal patency in my experimental chapters 6—8. PNIF measures maximal inspiratory flow through the nose, and thereby differs from anterior rhinomanometry, which measures transnasal pressure and airflow (∴ nasal resistance) during quiet respiration[469]. PNIF therefore measures turbulent flow, and is dependent on participant effort, as well as factors that affect total lung capacity (e.g., height, lung disease). However, it has been shown to have equivalent sensitivity and specificity to anterior rhinomanometry for the diagnosis of nasal obstruction in healthy participants and patients of varying aetiology[470,471], and has been significantly correlated with clinical signs of inflammatory disease in patients with rhinitis[472], as well as subjective nasal obstruction in patients with mixed chronic nasal pathology[473]. Furthermore, PNIF has been shown to correlate significantly with SNOT-22 and NOSE scores in patients with CRS[474]. Normative PNIF values have been established (unobstructed ≥120L/min), as has the MCID (≥20L/min), and good test retest reliability has been established (intraclass correlation coefficient 0.92)[470,475]. Finally, it is quick and easy to perform, can be used uni- or birhinally, and adds minimal participant burden[471,476]. It is also of interest that previous work using computational fluid dynamics demonstrated that airflow through, and odourant delivery to, the olfactory cleft is not well reflected by rhinomanometry metrics[47]. For these reasons, and because my primary aim was to investigate neuroanatomical correlates of OD, I elected to use PNIF as my measure of nasal airflow.

I used the residual volume method[472,477] to measure PNIF, using a modified Youlten peak flow meter [Clement Clark International, UK, measurements between 30 and 370L/min]. In brief, participants were asked to inhale maximally through their nose, using a standardised procedure (sitting upright, mouth closed, mask held firmly in place but without distortion of nasal anatomy). The best of three measurements was taken, in keeping with established practice[477]. Equipment was disinfected (2% chlorhexidine/70% alcohol device wipes, Clinell, GAMA Healthcare) and dried between participants. In my CRS work (experimental chapters 6 and 7), this was performed birhinally. In my patients with nasal obstruction undergoing functional

septorhinoplasty, this was performed bi- and unirhinally. For the unirhinal measurement, testing was repeated with occlusion of right and then left nostril (order of first test side pseudorandomised between participants) using adhesive tape (Microfoam©, 3M), as previously described[476]. As for birhinal measurement, the best of three attempts was used for each unirhinal side.

Radiological Disease Burden Scoring

The Lund-Mackay CRS staging system was originally described to include symptom scores, radiological and endoscopic findings[478]. However, the symptomatic and endoscopic aspects are rarely used, and the radiological system is now known as the 'Lund-Mackay' score[479] (see footnote h, §1.5.3.1 for description of score). Whilst the LM score was developed, and is most commonly performed using CT images, previous work has demonstrated high levels of correlation between CT and MRI derived scores[480]. Mean 'normal' score in patients without CRS has been previously demonstrated as 4.3[328]. I calculated MRI-based LM scores in my experimental chapter 8, in which my aim was to exclude CRS-related OD (see chapter for more details).

3.3.3 Imaging

3.3.3.1 MRI Safety Checks

Prior to scanning, all participants underwent standard MRI safety checks, in line with local protocol at the Lysholm Department of Neuroradiology and the Radiology Department of the University Hospital Carl Gustav Carus. Checks were completed with the participant by the allocated radiographer for the scanning session. Broadly, these questions cover presence of implanted medical devices, intracranial or cardiovascular metal (including clips, shunts or clips), other metal including piercings and tattoos, recent surgery or metallic dental implants, pregnancy and presence of anything else that could move or heat during scanning (e.g., hearing aids/dentures/coloured contact lenses). Medical conditions such as those which could affect breathing/lying flat in the scanner were also assessed.

Participants were observed throughout scanning by me (+ LM in the UK), JF or JN and the allocated radiographer. Participants were given alarm buttons, and could communicate with us via the speaker system, allowing scanning to be paused if required.

3.3.3.2 Imaging Acquisition Protocols

Imaging acquisition was performed using a 3-T scanner (experimental chapters 6-7, Germany: Verio, Siemens, Erlangen, Germany; experimental chapter 8, UK: 3-T MAGNETOM Prisma, Siemens, Erlangen, Germany). Detailed structural (T1 and T2-weighted) and functional (T2*) imaging acquisition protocols were developed with CR and TH in Germany, and LM and TH in the UK, and can be found in their respective experimental chapters. I converted raw DICOM images obtained from the scanners into Neuroimaging Informatics Technology Initiative (NIfTI) format for subsequent analysis, using MRIConvert (version 2.1, Lewis Center for Neuroimaging, University of Oregon, USA).

3.3.3.3 Structural Neuroimaging

I used T1-based computational morphometry techniques for analysis of brain structure upstream of the OB. Specifically, I analysed differences in GM volume between and within subjects using voxel-based morphometry (experimental chapters 6 – 8). I additionally used a volume-based approach to analyse cortical thickness (experimental chapters 7 and 8). Finally, due to limitations in their segmentation using the above approaches, I used standard, manual morphometry of T2-weighted images for the analysis of OB volume, in my experimental chapter 6.

Imaging Analysis – VBM

I performed all VBM analysis. Supervision was provided by PH, LM and TH. Throughout my analysis, I used the CAT12 toolbox (available from http://dbm.neuro.uni-jena.de/vbm/) implemented in SPM12 (Wellcome Centre of Imaging Neuroscience, UCL, London, UK) and MATLAB (The MathWorks, Natick, MA, USA). Specific details can be found in experimental chapters 6-8. In the following section, I will provide a basic theoretical overview of the steps performed therein.

As introduced in §1.5.3.1.2, voxel-based morphometry uses high resolution T1-weighted images to compare local tissue between brains that have been globally aligned into the same stereotactic space. Statistical inferences can then be drawn, either within or between subjects, on a voxel-wise basis. Both grey and white matter (WM) can be assessed using VBM, though due to limited signal intensity variation, VBM has limited sensitivity to detect WM variation. My decision to focus on GM volume in this thesis was partially influenced by this, but largely due to the majority of cross-sectional VBM work in patients with OD being focussed on grey matter.

Pre-processing

Within the CAT12/SPM framework, images are initially subjected to a number of pre-processing steps, in order to globally align but locally preserve tissue density/volume for later statistical inferences. Broadly, these include tissue classification (segmentation), spatial normalisation and smoothing, which will be discussed in more detail below.

- *Segmentation:* Tissue classification or 'segmentation' describes partitioning of an image into different tissue types (e.g., GM, WM, CSF). This can be achieved using voxel-wise signal intensity, and some prior knowledge of the signal intensity distribution of different tissue types[481]. Accordingly, tissue probability maps, which encode anatomical variability, can be used to provide prior information to guide voxel-wise tissue segmentation. Of note,

voxel-wise signal intensity can be affected by inhomogeneities[m] in the scanner's magnetic field – which particularly affects high field (1.5T+) scanners. Such inhomogeneities can lead to confounding, systematic differences in signal strength. Therefore, prior to, or as part of tissue segmentation, correction of field inhomogeneities is required through a process often termed 'bias correction'. However, signal intensity distributions for different tissue types overlap even after bias correction. This is in part due to the potential presence of different tissue types within the same voxel (which is particularly an issue at the junction between tissue types, or where WM crosses GM), termed 'partial volume effect'. The CAT12 toolbox automatically models for partial volume effect, which can also be addressed using masking thresholds (that exclude voxels with low-tissue probability values)[482].

- *Spatial Normalization:* In addition to segmentation of brains into their respective tissue types, these brains, or their tissue-specific 'segments' (GM, WM etc.), must also be spatially aligned, or registered, into a standardised space that facilitates voxel-wise, anatomical comparisons. This must be done in a way that provides global alignment, whilst preserving local variability, to facilitate comparison of structural differences. This can be achieved through a combination of linear and non-linear transformations or 'normalization'. Linear normalization[n] transforms each voxel within the image in the same way and therefore corrects for inter-individual differences in global brain size and shape, allowing for registration of images to a template space. Non-

[m] *Inhomogeneities can be caused by issues with the magnet (static magnetic field inhomogeneities), temporal instability (in magnetic field due to environmental factors or software instability) or differential magnetic susceptibility between tissues (susceptibility-induced inhomogeneities).*[704]

[n] *This can be achieved in SPM through the 12-parameter affine transformation – a linear transformation that combines translation and rotation (in x, y and z-axis, the 'rigid body transformation') as well as scaling and shearing (also in x, y and z-axis).*

linear normalisation (or 'warping') allows for differential transformations locally within a brain, and therefore correct for inter-individual differences in size, shape and position. The spatial transformations undertaken produce a 'deformation field', which maps how structures were altered when matching brains (either two brains together, or brain to template). The amount by which individual voxels have changed in volume can be derived from these deformation fields as the Jacobian determinants[o]. The Jacobian determinant can then be used to correct for volume changes in GM segments that occurred during the normalization process. The resultant 'modulated' GM volume therefore reflects the original GM volume prior to spatial normalization, allowing for analysis of variation in local regional GM volume between difference brains, or across time points. Of note, total intracranial volume must additionally be corrected for, prior to statistical comparison. SPM and the CAT12 toolbox register images to the International Consortium of Brain Mapping (ICBM) 2009c Nonlinear Asymmetric space (MNI152NLin2009cAsym), commonly referred to as Montreal Neurological Institute (MNI) space – a standardized coordinate space which allows for comparison of results across different studies.

- *Spatial Smoothing:* Spatial smoothing describes the process in which an image is convolved with a three-dimensional Gaussian kernel[p]. Accordingly, each smoothed voxel represents the average of its signal and the weighted mean signal from neighbouring voxels. Smoothing blurs images and thereby achieves several purposes. First, it compensates for residual interindividual

[o] *In essence, the Jacobian determinant is a scaling factor between different coordinate spaces. Accordingly, it is used to describe voxel-wise expansion/contraction that has occurred during the transformations undertaken for normalization.*

[p] *A kernel is a convolution matrix that is used to modify each voxel within an image, so producing a new output image.*

variation in local anatomy after normalisation. Second, smoothing reduces the effect of noise, by making the analysis less sensitive to effects smaller than the size of the smoothing kernel used (where very small effects are more likely to be due to noise). Third, using a Gaussian kernel makes imaging data more normally distributed, so making use of parametric statistical tests valid.

In practice, SPM and CAT12 integrate several of the above processes. For example, following application of a denoising filter, CAT12 uses SPM's 'unified segmentation' – an integrated generative model that combines image registration (and thereby elements of normalisation as described above), tissue classification and bias correction. The CAT12 toolbox performs additional steps as part of its default pipeline, including correction for the effects of white matter hyperintensities and differential effects of myelination on GM. Further steps specific to longitudinal pre-processing are described in experimental chapters 6-8. An overview of my longitudinal pre-processing pipeline is shown in Figure 3-1.

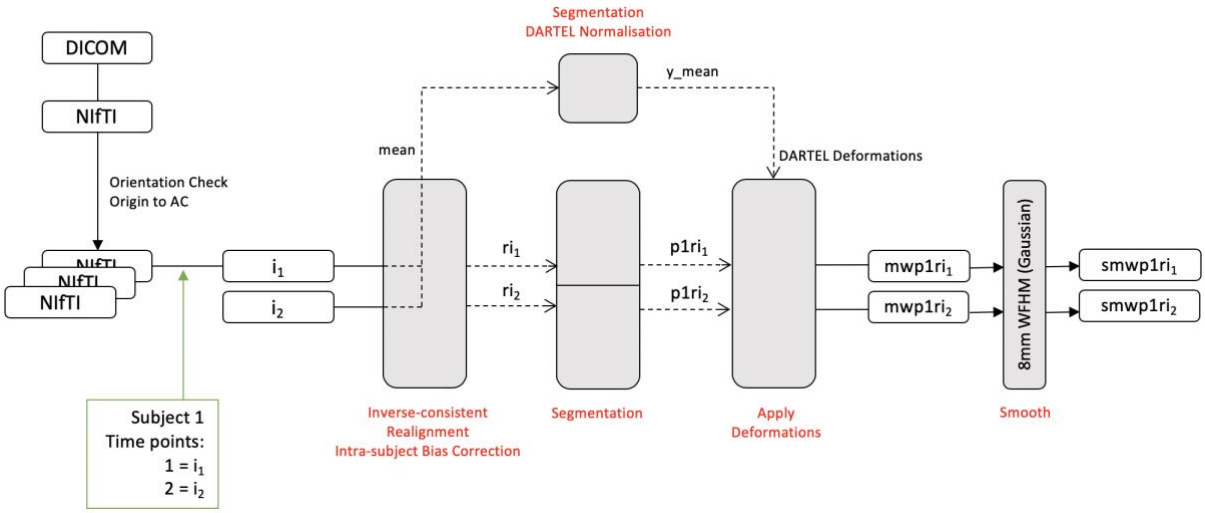

Figure 3-1: Longitudinal pre-processing pipeline used during VBM analysis in chapters 6 - 8.

Statistical Analysis

The above pre-processing steps produce smoothed, normalised tissue segments, which can then be subjected to group level statistical inferences. Detailed information on my statistical analysis can be found in experimental chapters 6 - 8. In the following sections I will again provide a basic theoretical overview of this process, including a brief discussion of multiple comparisons and results thresholding.

I used the General Linear Model (GLM), implemented in SPM, as the basis of my VBM analysis. This models voxel-wise tissue volume (or density, depending on pre-processing steps) throughout the brain, thereby allowing identification of GM regions that are significantly related to the effect of interest[483]. The GLM can be expressed in matrix notation as follows:

$$Y = X(\beta) + e$$

Here the dependent variable, *Y*, is a vector of observed voxel intensities (and thereby GM volume) taken from the normalised, smoothed GM segments across all subjects. The independent variable is the design matrix, *X*, which contains vectors of all regressors of interest (e.g., group, time) and no interest/nuisance (e.g., sex, age, total intracranial volume). The resultant 'beta weights', represented by the vector *ß*, are the parameter estimates for each regressor, which quantify the relative contribution of each to the observed data. Finally, the vector *e*, represents the residual error between observed and predicted data. Using the GLM, a test statistic (e.g., T/F test) can be created for each voxel within the brain (a 'mass univariate approach') across subjects, which results in the creation of a 'statistical parametric map' (SPM, from which the software derives its name). Significant regions can then be identified in relation to the effect of interest, and the set statistical significance threshold.

Significance Thresholding and Multiple Comparisons

Significance thresholding in neuroimaging must balance the risk of incurring type I errors (false positives) against type II errors (false negatives). The risk of type I error in any mass-variate analysis approach is high, due to the large number of statistical tests performed, and associated problem of multiple comparisons. This is particularly problematic for whole-brain analyses. Various strategies to mitigate this risk have been established and include limitation of test volume and thereby number of statistical tests performed (e.g., using region of interest analyses[484]), or use of the false discovery rate (FDR) or family-wise error (FWE) corrections for multiple comparisons[485]. The most conservative of these is the FWE correction, which can be applied automatically in SPM, and is based on random field theory. Whilst results surviving FWE correction are theoretically the most statistically robust, given its conservative nature, there is increased risk of type II error, and acceptance of the null hypothesis when it is false[486–488]. This is particularly problematic for fields such as olfactory fMRI, where the signal to noise ratio is thought to be less robust than other fields, such as vision[489,490]. Potentially, this may also be an issue in olfactory VBM, as the primary and secondary olfactory networks are spatially widespread[32], such that changes seen may be granular and dispersed. As a general approach, I have used more conservative thresholding (e.g., FWE correction) for whole brain analyses, in which the risk of false positives is highest. For areas in which I have a particular *a priori* interest, and in which I therefore have lower tolerance for type II errors, I have used a combined approach, whereby I limited the number of statistical tests performed to voxels within specific regions of interest (region of interest analysis with 'small volume adjustment')[377,484,491,492], and used more lenient thresholds for the results therein (see experimental chapters 6 – 8 for more details).

Statistical significance can be further attributed at the voxel level, the cluster level (a cluster being a group of contiguous voxels that are related *en masse* to an effect of interest, and therefore to each other), or combined voxel/cluster level ('intensity/cluster thresholding'). Significance thresholding at the voxel level utilises the test statistic peak magnitude for the voxel in question, whilst cluster-based thresholding involves defining statistically significant clusters based on the number

of neighbouring voxels whose individual test statistics reach a set minimum threshold (thereby requiring definition of a voxel-level 'primary threshold' and a cluster-level 'extent threshold')[488]. Whilst cluster-based thresholding is one of the most common approaches in neuroimaging, and has been used previously in VBM[493–496], it can be problematic for the latter purpose, as a cluster's spatial extent depends on the underlying smoothness of the data – which is not uniform ('non-stationary') throughout the brain (with, for example, areas of the cortex being smoother than subcortical areas, where clusters are more likely to be large and therefore reach the set extent threshold)[483,497]. For this reason, I used voxel-based inferences throughout my VBM work, as has been previously recommended[483]. For more details, see experimental chapters 6 – 8.

Imaging Analysis – Cortical Thickness

I performed all cortical thickness (CTh) analysis, with supervision from LM and TH. I analysed CTh using CAT12, implemented in SPM12. Again, detailed procedures can be found in experimental chapters 7-8, and the following provides a basic theoretical overview of this analysis.

Surface based morphometry (SBM) is another macroscopic computational morphometry technique that, unlike VBM – which focuses on both cortical and subcortical structures – focuses on cortical morphological characteristics only, including volume, thickness, area, and gyrification. Unlike VBM, which uses a voxel-based approach (and thereby divides the brain into a 3D grid), SBM techniques produce stereotactically aligned, surface based 'meshes'[q] that represent cortical tissue boundaries, and upon which later, vertex-wise statistical analyses are based. CTh is one of the most frequently used SBM measures, and represents the distance between the GM 'outer surface' (GM/CSF boundary or pial surface) and 'inner

[q] *A 'mesh' refers to the tessellated cortical surface – a representation of the brain's cortex as a mesh of interconnected triangle faces and vertices.*

surface' (GM/WM boundary) [498]. CTh has been used as a biomarker for normal development [499], aging [500] and various pathological states [501,502].

In brief, CAT12 automatically performs two initial pre-processing steps for SBM: surface creation and surface registration. Surface creation involves a 'projection-based thickness approach'. Here estimation of initial cortical thickness (WM to pial boundary) and initial central surface (mid-way between WM and pial surfaces) are performed in a combined step (with concurrent automatic quality improvement steps). Subsequently, correction of topological defects and surface refinement are performed prior to creation of pial and WM surface meshes. Similar to VBM, spatial smoothing is performed after registration. Subsequent calculation of CTh involves repeat measurement of the WM to pial distance, across a large number of surface points.

Statistical Analysis

As for VBM I used a GLM approach implemented within SPM. SBM in CAT12 can also be considered a mass univariate technique, as statistical tests are similarly applied to vertices throughout the brain. Therefore, similar principles apply with regards to multiple comparisons as described in the above section on VBM. More detail on my CTh analyses can be found in experimental chapters 7 and 8.

Imaging Analysis – Manual Morphometry

Analysis of OB volume was performed in experimental chapter 6. MPRAGE sequences produce poor images of the OB resulting in their limited segmentation. Therefore, voxel based morphometric analysis of T1 images is not the ideal method by which to determine volume changes in these structures[399]. OB images were instead obtained using a focused acquisition paradigm. JF performed manual morphometry of the OB, using AMIRA 3D software (Visage Imaging, Carlsbad, Ca, USA) (see §6.4.4 for details). Volumetric analysis using manual planimetric contouring in this way has been shown to be reliable and accurate[342]. I then used the

derived volumes for subsequent analysis (all of which was performed by myself). Data were first tested for normality, and parametric or non-parametric tests used accordingly, using GraphPad Prism (GraphPad Software, LaJolla, CA) and SPSS (Statistical Package for the Social Sciences, Version 24.0, SPSS Inc. Chicago, IL, USA).

3.3.3.4 Functional Imaging

I used concurrent prospective olfactory fMRI to investigate the functional significance of putative structural plasticity during my experimental chapters 7 and 8. In the following sections I will provide a brief theoretical overview of fMRI, followed by my experimental methodology and analysis approach.

Functional MRI produces surrogate representations of neuronal activity through detection of the Blood Oxygen Level Dependent (BOLD) signal. The BOLD signal takes advantage of the differential magnetic properties of haemoglobin depending on its oxygen binding state: oxyhaemoglobin is weakly diamagnetic, whereas deoxyhaemoglobin is strongly paramagnetic. The T2*-weighted MRI signal is affected by local inhomogeneities of the magnetic field. Due to its strong paramagnetism, deoxyhaemoglobin causes local field inhomogeneities and thereby T2* signal loss. Neuronal activity affects the relative concentrations of oxy- and deoxyhaemoglobin due to changes in oxygen consumption and delivery. Active neurons use oxygen during aerobic respiration for ATP production, which is subsequently used for a variety of cellular processes, such as restoration/maintenance of chemical concentration gradients, or the production and vesicular packaging of neurotransmitters. Therefore, when a local area of neurons undergoes increased activity, following an initial period of oxyhaemoglobin depletion (due to initial consumption of local oxygen), neurovascular coupling results in increased blood flow and subsequent blood volume to that region, and so increased delivery of oxyhaemoglobin (termed the haemodynamic response). This increased oxyhaemoglobin delivery causes 'wash out' of deoxyhaemoglobin and consequently, reduced T2* signal loss, which is detected as 'increased' BOLD signal. During fMRI scanning, BOLD signal is prospectively measured across time, during which basal neuronal activity ('resting state' fMRI) or neuronal activity in response to

a task or stimulus ('task-based' fMRI) is measured. Generally, sequential sets of images across the whole brain are collected at each time point – with each set of axial slices collected at these different times points called a 'volume', collectively the 'time series'. In task-based olfactory fMRI, odour(s) are presented to a subject undergoing scanning, and BOLD signal is concurrently recorded throughout the collected time series.[485,503–505]

In the following sections I will describe my experimental paradigm, the equipment I used to deliver odours to participants undergoing scanning, and choice of odourants.

fMRI Study Design

Functional MRI experimental paradigms can be broadly divided into resting state (without stimulus/task) and task based (with stimulus/task) designs, with the latter being further divided into block and event-based designs. Block designs typically involve presentation of stimuli (either continuously or repeatedly with short interstimulus intervals) or performance of tasks during discrete 'on blocks', followed by stimulus/task-free 'off blocks' (or alternatively presentation of an alternative stimulus/task). On and off blocks are alternated in this way repeatedly throughout the duration of scanning with that stimulus/task (the 'functional run'). Subsequently, the average BOLD signal between on and off blocks is compared, where this represents the summated average haemodynamic response during each condition (on vs off). This type of design contrasts with event-related fMRI, where stimuli/tasks are presented as discrete events at any point (often randomly) throughout the functional run. Subsequently the BOLD signal associated with each event (and thereby individual associated haemodynamic response) is modelled. Block designs generally have greater signal to noise ratios than event related designs (as the HRF is theoretically allowed to reach maximum amplitude during on blocks, and then return to baseline during off blocks) and therefore have higher statistical power than event-related designs. This is important in olfactory fMRI, as BOLD activation is less robust than in other sensory stimuli such as vision or audition[489,490]. Furthermore, compared with block designs, olfactory event-related paradigms require faster

stimulus rise and fall times, and thereby high flow olfactometers with thermostabilisation. Accordingly, block designs are well established in olfactory fMRI.

Whilst there are some disadvantages to block designs in olfactory fMRI such as adaptation/habituation[r] or prediction effect, as my primary aim was to determine any, potentially subtle change in general olfactory function over time, in a limited clinical sample of participants, I chose to maximise my statistical power and thereby used a block fMRI design for experimental chapters 7 and 8. More details can be found in the respective experimental chapters. More detail regarding adaptation/habituation can be found in my pilot work below.

Olfactometer

Delivery of odourants to participants undergoing MRI scanning poses technical challenges. Odours must be delivered to the subject, whilst in the scanner, in a reliable and temporally precise way. Accordingly, odour delivery using an olfactometer is required – though given the relatively slow time course of the haemodynamic response, odours can be delivered with temporally less precise olfactometers than required for electrophysiological techniques. Furthermore, in part due to these lower flow rates, thermostabilisation (warming) is not required. However, ferromagnetic equipment cannot be introduced within MRI shield room, necessitating specialised olfactometers and/or waveguides through which odour delivery systems can be passed. To that end, I elected to use a portable

[r] *The terms adaptation and habituation are used variably in the literature. 'Habituation' is often used to describe reduced behavioural responsiveness to a presented odour. However, it has also been used to specifically describe changes in central cognitive processing leading to this behavioural change. Conversely, adaptation is often used to describe changes at the receptor level (e.g. negative feedback cascades affecting cyclic nucleotide gated cation channels), but some authors also use the term to describe changes in central cognitive processing. Typically, adaptation occurs more quickly (milliseconds vs seconds-minutes) and is shorter lived than habituation. See aslo §1.5.2.5.*

olfactometer, as described and produced by Sommer *et al.,* that has previously been validated for use in olfactory fMRI[506]. This was kindly provided by TH in Germany, and Professor Barry Smith, Institute of Philosophy, School of Advanced Studies, in the UK.

This olfactometer comprises three basic components: 1. Airflow unit; 2. Odourant unit; 3. Delivery unit.

Airflow Unit: This unit comprises the air inlet, control and distribution components of the olfactometer. The olfactometer does not provide its own airflow. Therefore, in both experimental chapters 7 and 8, in Germany and London respectively, clean, dry air was obtained from the MRI shield room via the hospital grade variable area flowmeter, available for patient care. I confirmed the accuracy of the shield room flowmeter with a second, handheld flowmeter (Cole Palmer) at the start of each scanning session. Upon entry into the olfactometer, air is directed into the 'airflow valve', which is a three-way pneumatic switching valve. When this valve is 'off' air exits through an 'exhaust' port, which maintains steady airflow. When this valve is 'on' airflow is directed through the 'normally open' port, which is split into four hoses, to allow testing of maximum 3 odourants and a control, non-odour. Each of these four hoses are also controlled by a three-way pneumatic switching valve ('odourant valves'). All valves are 24V solenoid controlled and are activated either electronically or manually via rocker switches housed on the front 'Manual Airflow Control Panel'. Valve activation status is indicated by LEDs on the latter panel. The airflow reaching the delivery unit is always equal to the airflow entering the device, irrespective of the number of valves simultaneously open, as it uses a continuous airflow design. This component of the olfactometer is non-modifiable.

Odourant Unit: The odourant unit comprises four 100ml capacity, 29/32 standard taper joint gas washing bottles (neoLab, Heidelberg, Germany) into which 50ml liquid odourants or control fluid (distilled water, Darrant Chemicals, UK) was placed.

Air passes into the gas washing bottle and through frit cartridges[s] contained therein. Frit cartridges cause bubbling of the airflow through the liquid, thereby increasing the surface area for contact between air and odourant, and maximising odourisation of the resultant humidified air. This component of the olfactometer is modifiable.

Delivery Unit: Odourised or control air then exits the gas washing bottles and is delivered to the subject within the MRI scanner. This is achieved through a combination of silicone (CamLab, Cambridge UK) and PTFE tubing (Teflon© 4mm ID, SourcingMap, UK). This component of the olfactometer is modifiable.

The olfactometer is controlled using a bespoke open-source C++ based program (available from: http://github.com/sommeru/riech-o-mat). For my purposes this was implemented in Ubuntu (an open-source operating system based on Linux, available from: https://ubuntu.com/) on an Asus EeePC netbook (Taipei, Taiwan). The program uses a 5-digit binary code, with 1 = valve open, and 0 = valve closed, with leading digit representing airflow valve, and subsequent digits odourant valves. The olfactometer delivers trapezoid shaped pulses of airflow to the subject, with rise/fall times after valve switching of approximately 120ms – with stability of the stimulus being dependent on consistency of the supply airflow. During development testing, the olfactometer airflow was shown by Sommer *et al.,* to be constant over a period of one hour at 2 ± 0.1L/min, and validated for use in block-design fMRI experiments.[506]

I undertook pre-pilot testing using the above olfactometer in both the UK (Institute of Philosophy Lab, School of Advanced Studies, and Lysholm Department of Neuroradiology, UCLH, London) and Germany (Interdisciplinary Smell and Taste Lab, and Department of Radiology, Universitätsklinikum Carl Gustav Carus Dresden). In all locations, airflow was available via a variable area flowmeter. Following pre-pilot testing, I modified the default odourant and delivery units as follows. Gas washing bottles were not housed within the olfactometer but within the MRI shield room.

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[s] *Frit cartridges or 'sintered discs' comprise finely porous glass structures made by sintering glass participles into a solid but porous component.*

Accordingly, the distance between gas washing bottle and subject was reduced, so reducing the dead space for odourant travel. The olfactometer was connected to gas washing bottles using silicone tubing (3mm ID tubing connecting to olfactometer system connector, followed by 4 to 8mm adaptor, then 6.5mm ID tubing with non-return valve, prior to attachment to gas washing bottles, all CamLab (Cambridge, UK), item nos: 1150012, 1136772, 1150013, 1136641), with sufficient length for tubing to reach from the MRI control room, through the waveguide, into the MRI shield room and up to a designated area next to the scanner, in both Germany and the UK. The delivery unit comprised silicone and PTFE tubing connected using Y adaptors, of sufficient length to reach from the gas washing bottles to the subject whilst in the MRI scanner. PTFE was used for all parts of the delivery system in which there was varying odourant/control flow to minimise absorption of confounding odour. The distal most end PTFE segment and a bespoke PTFE birhinal nasal delivery segment was changed between each patient for infection control purposes. I primed the olfactometer prior to each use, without which there was a delay of first odour condition onset. See Figure 3-2 for diagram of olfactometer setup.

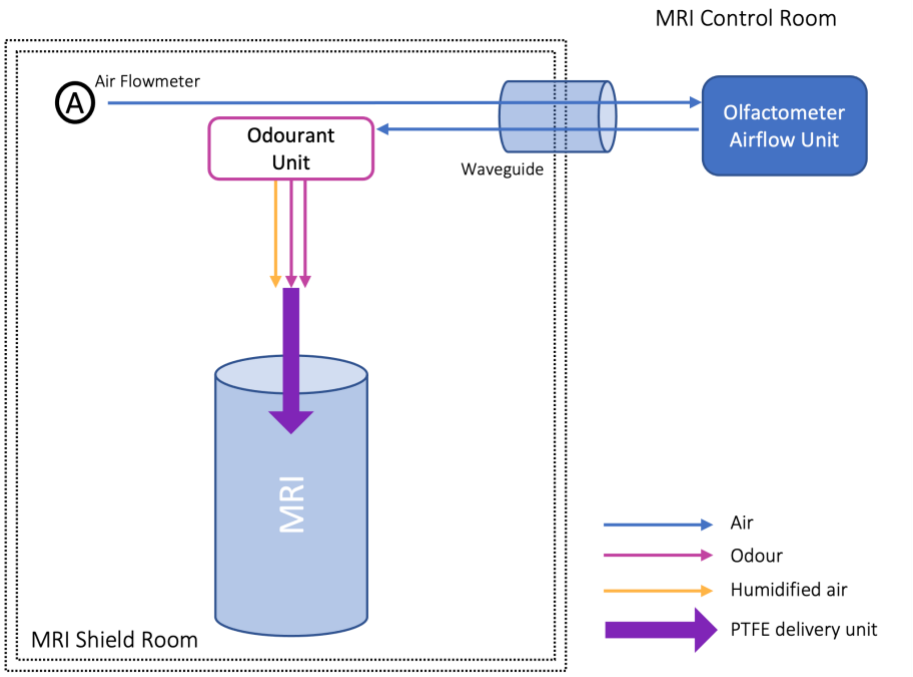


Figure 3-2: Olfactometer setup.

Odourants

I chose one food odour and one non-food odour, to cover potential differences in processing within the secondary olfactory network (in particular the insula, which is known as a key node within the flavour network[507]), and to mitigate any potential effects of differential hunger/satiety in participants[273,508]. Odours were initially screened by myself, JF, JN and TH during pre-pilot testing, and cis-3-hexan-1-ol (smell of cut grass: Germany - neat, Fluka Chemicals, Gillingham, UK; UK - Firmenich, Middlesex, UK) and banana aroma (Germany - neat, Frey+Lau, Henstedt- Ulzburg; UK - neat, Dale Air, Rochdale, UK) were chosen as they were isointense, with banana being relatively more pleasant than cis-3-hexanol. I additionally chose one single-molecule odour and one aroma (a multi-molecular mixture), in case of differential central activation, and because odour mixtures have previously been shown to elicit more reliable odour threshold scores (DT) across time[240]. Neither odour had a strong trigeminal component, which was confirmed in pilot testing with low flow rates (see below). Given the duration of scanning required per odour (see experimental paradigm), only two odours were used. This is in keeping with previous studies[400,509,510].

Pilot Testing

Following adaptation of the olfactometer/delivery system as above, and using the final scanning olfactometer setup, I undertook pilot testing in the UK (Institute of Philosophy Lab, School of Advanced Studies) and Germany (Interdisciplinary Smell and Taste Lab, Dresden). I gathered a convenience pilot sample of 10 participants in the UK and 8 in Germany.

Flow rates were limited to 1l/min, as participants were able to perceive thermomechanical stimulation at 2l/min and higher. However, at this flow rate, there was no significant change in thermomechanical sensation between the on/off conditions, in keeping with minimal trigeminal activation.

I used an uncued passive block design – that is, patients were not alerted at the start of 'on blocks', and they were not allocated specific tasks to perform during stimulus presentation. They were, however, asked to rate odour intensity and hedonic valence at the end of the functional run for each odour, so encouraging the participant to attend to the presented stimulus[400,509].

Sniffing is thought to activate a central olfactory sensorimotor network (for example, involving areas of the cerebellum but also potentially parts of the primary olfactory network[511]), that can be stimulated by airflow in the absence of odours. Differential rates of sniffing are therefore a potential source of confounding during uncued passive block designs. One early strategy developed to mitigate the effect of differential sniffing on olfactory BOLD activation was velopharyngeal closure[490]. However, during pilot testing I found that this was a difficult technique for participants to learn, and for me to confirm their proper technique. Moreover, in my pilot subjects who were able to perform velopharyngeal closure efficiently, they reported reduced subjective intensity, delays in perception of stimulus on-/offset (possibly as this technique was developed for use in olfactory EEG[31] – where higher flow rate olfactometers are used) and found it difficult to sustain the technique for the duration of the functional run. As my pilot participants were a group of healthy volunteers, I anticipated these difficulties could be further exaggerated in my clinical cohort. In particular, reduced intensity in a cohort of patients with hyposmia/anosmia could render the odours subthreshold differentially, depending on individual ability to correctly perform velopharyngeal closure (sniff volume can affect detection threshold, see §1.5.2.1). Furthermore, previous meta-analytic work has demonstrated stronger activations in areas of the central olfactory networks, including the OFC, when studies employing natural breathing were compared with velopharyngeal closure[31]. Together, for these reasons I elected not to use velopharyngeal closure as part of my fMRI protocol. Instead, I asked participants to breath regularly throughout the functional run, with their mouth slightly open. I found during pilot testing that this reduced irregular sniffing behaviour was more acceptable to participants and did not affect their subjective odour perception like

velopharyngeal closure. Use of 'natural breathing' (without velopharyngeal closure) is well established in the neuroimaging literature[400,509,512,513].

Previous work has demonstrated habituation in odour-induced BOLD response within areas of the primary olfactory network at ≥30 seconds[514]. Other work has demonstrated differential responses throughout the primary and secondary olfactory networks, that are more pronounced after 60 seconds, but which can occur in some structures as soon as 15 seconds[515]. Accordingly, I piloted block durations between 15 and 30 seconds. In Germany, my olfactometer set up required a longer time period for odour washout than in the UK. As this could potentially delay theoretical return of the BOLD signal to baseline, and confound comparison of on and off conditions, I elected to increase the duration of off blocks compared to on blocks in experimental chapter 7. Here, I used on blocks of 15s followed by off blocks of 30s. In the UK, where subjective washout during off blocks was faster, I used on and off blocks of 20s each.

Within each on block, there was less subjective decrease in olfactory perception (due to adaptation/habituation) using pulsed odour presentation within a stream of humified air, compared with continuous odour presentation. There was also better washout during off blocks using pulsed odour presentation. Similar block durations and pulsed odour presentation during on blocks have been used successfully in the olfactory fMRI literature[400,509]. Please see experimental chapters 7 and 8 for more details.

Within the MRI shield room, soft padding was used to reduce intra-scan head movement. Participants were additionally asked to reduce any voluntary movement as much as possible (including swallowing) during functional runs.

Further results from pilot testing can be found in appendix 10.6.

Pre-Scan Screening and Instructions

As mentioned above, all participants were asked prior to assessment whether they had a recent URTI, flare of allergic rhinitis or any other acute changes in their sense

of smell of note ± deferral where appropriate. MRI safety checks were undertaken as outlined in §3.3.3.1.

In an attempt to control the potential effect of differential levels of participant satiety on functional imaging results[508], patients were asked to eat normally on the day of scanning until one hour prior to their session during which they were nil by mouth except water.

Breathing instructions were given as outlined in the above section. Participants were given ear plugs to reduce MRI noise during scanning (which has now been shown to affect olfactory processing[516]).

Imaging Analysis

I performed all fMRI analysis. Supervision was provided by PH, LM and TH. Throughout my analysis, I used SPM12 (Wellcome Centre of Imaging Neuroscience, UCL, London, UK) implemented in MATLAB (The MathWorks, Natick, MA, USA).

Pre-processing

As described above, fMRI produces four dimensional images that capture fluctuations in BOLD signal across time. These images must undergo pre-processing steps that reduce noise, allow for anatomical localisation of BOLD signal, and subsequent statistical analysis of this signal in relation to the experimental paradigm across different brains or time points.

Several of the pre-processing steps required for fMRI are the same as those described in VBM. These include segmentation of T1 images into different tissue types, spatial normalisation to standardised template space and smoothing of both T1/T2* images. In addition to these steps, functional images must be corrected for movement artefact across time, and functional/anatomical images must be co-registered. Once pre-processing has been completed, the smoothed, normalised functional images can be subjected to statistical analysis.

Tissue segmentation, normalisation and smoothing were described in detail above. Correction of movement artefact, and co-registration of functional/anatomical images are further discussed below:

- *Motion Correction:* A key assumption of fMRI analysis is that interrogated voxels are from the same anatomical location within the brain. However, given that fMRI volumes are collected across time, movement within the MRI scanner poses a considerable source of confounding variance or noise. In order to reduce the relative effect of movement during an obtained time series, functional images are realigned. This can be performed in SPM using rigid body transformations (a 6-parameter linear transformation described in footnote n), in which each image within the time series is moved[t] to a reference image (either a specified image, e.g., the first or the mean image). As previously described, linear transformations do not change brain shape, only size and position. However, alterations in brain shape are possible (e.g., due to rapid motion between the acquisition of slices within a volume, imaging artefacts such as field inhomogeneities or growth/atrophy in longitudinal data) and these can be corrected using non-linear transformations (often called 'unwarping' in SPM fMRI pre-processing). The end result of these processes are functional images that are spatially aligned within the time series.

- *Co-Registration:* Co-registration aligns structural and functional images, allowing anatomical localisation of BOLD signal within subjects, and facilitating more accurate spatial normalisation of functional data. This

[t] *This process involves initial iterative estimation of optimal transformation parameters according to an 'objective function' (e.g. mean square difference), between the two images in question. Once they have been established, they are used to re-sample the images that are being moved to the reference image, in SPM using B-spline interpolation (which uses a series of basis functions).*

process is similar to realignment in motion correction but uses cross-modality images. For this reason, the objective function used to estimate transformation parameters varies from that used during realignment.

For further details regarding pre-processing of my fMRI data, please see experimental chapters 7 and 8.

Statistical Analysis

Once fMRI data has undergone pre-processing, the resultant normalised smoothed functional images can be subjected to statistical analysis. This analysis occurs in two stages: first level, within subject analysis and second level, between subject or 'group-level' analysis. In essence, these levels model: how the BOLD signal varies at each voxel across time within a single subject; how these estimated effects vary between participants. I used the General Linear Model (GLM) for each of these two levels, implemented in SPM. Detailed information regarding my statistical analysis can be found in experimental chapters 7 and 8. In the following sections I will provide a basic theoretical overview of first and second level analyses.

During first level analysis, a design matrix is defined according to the experimental paradigm and convolved with the haemodynamic response function (in my case, the canonical HRF), to produce estimates of the expected BOLD signal for each condition (e.g., on and off blocks). These estimates (the experimental conditions, or 'test regressors'), as well as other confounding or nuisance variables (e.g., sex, age), are then defined as the independent variables within the GLM [as outlined during the previous section on VBM: $Y = X(\beta) + e$], with the dependent variable being the voxel-wise time series data obtained from the pre-processed functional images. The first level design is identical for all subjects. Accordingly, a test statistic (e.g., T or F test) is created for each voxel within the brain at each time point (again, a mass univariate approach), which results in the creation of a statistical parametric map. Such maps are created for each subject, and each effect of interest ('contrast'), which comprises first level analysis.[483,485,517–519]

Second level analysis allows hypothesis testing at the group level. SPM uses a hierarchical statistical framework that accounts for both within subject variability (at the first level) and between-subject variability (at the second level). This two-stage 'summary statistic approach' approximates a 'mixed/random effects' analysis, and thereby allows population level inferences to be derived. Again, a GLM is used (see above equation), where *Y* = the individual contrast images derived from first level analysis, *X* = second level design matrix, *ß* is the estimated BOLD signal for each condition and *e* is the residual error. Again, test statistics (e.g., T/F tests) are then produced on a voxel-wise basis, to produce the second level statistical parametric map. Inferences can then be drawn in relation to the population and hypothesis in question.[485,520]

Significance Thresholding and Multiple Comparisons

Similar to VBM, test statistics are calculated for each voxel. Again, therefore, strategies for multiple comparison correction must be used. However, as discussed in the VBM section, it is important to balance the risk of type I error with that of incurring a type II error – which is particularly important in olfactory fMRI given potentially poor signal to noise ratio[489,490]. In general, my approach to significance thresholding for fMRI was the same as for VBM (please see previous section for discussion). Whilst spatial non-stationarity may still occur in fMRI analysis, I elected to use additional cluster level inference (specifically, intensity/cluster thresholding) as a further strategy to balance the risks of type I and type II error[486], in my exploratory analysis where I was using a more lenient voxel-level significance threshold. Please see experimental chapters 7 and 8 for more details.

3.3.4 Sample Size

In classical hypothesis testing, statistical power (the probability of rejecting the null hypothesis when it is false) is defined according to: 1. effect size (and its variance); 2. alpha value; 3. sample size. Determination of sample size required to achieve a pre-specified statistical power (e.g., 80%), therefore requires some pre-existing

knowledge of effect size. In neuroimaging studies, effect size is the percent signal change between experimental and control conditions. Given the mass univariate approach used in SPM, an effect size can be defined for each individual voxel (e.g., ~120,000 voxels for a whole brain analysis), or a mean effect size across a pre-defined cluster of voxels. Furthermore, the variance of the effect size at each voxel/cluster is required – including both intra- and inter-subject variability (which is very difficult to predict *a priori*). With this in mind, most approaches to power calculations in neuroimaging require pilot data[521] or reliance on simulated data[522], neither of which was available for my first experimental neuroimaging chapter, 6. For my experimental chapters 7 and 8, as I was performing multimodal neuroimaging studies, estimated effect size and its variance at each voxel/cluster of voxels would be required for each modality type (VBM/fMRI/CTh), with arbitrary prioritisation of one modality in determining sample size.

For these reasons, I estimated minimum required participant number from the available literature[400,523–525], as is common practice in the neuroimaging literature[526]. From the limited *longitudinal* olfactory neuroimaging literature available at the time (though these studies focused on fMRI only), a minimum sample size of 7 patients was set[524,527]. My final sample size took into account the pragmatic limitations of prospective clinical cohorts, in the pre-pandemic population.

3.3.5 Ethical Considerations

As my neuroimaging studies involved patients, I gained ethical approval from relevant healthcare authority. In Germany (for experimental chapters 6 and 7), my work was approved by the Ethics Committee of the Faculty of Medicine Carl Gustav Carus University Hospital, Technische Universität Dresden, Germany (EK number 56022016). In the UK I obtained NHS ethical approval (REC ref 14/SC/1180). Across locations, all work was conducted in accordance with the Declaration of Helsinki. All patients and controls provided full informed written consent prior to participation. Patients were free to withdraw from the studies at any point, without otherwise influencing their clinical care.

4 Clinical Assessment of Olfaction: UK and International Clinician-Reported Practice

4.1 Summary

Accurate olfactory assessment is necessary for good clinical and research practice but is highly dependent on the assessment technique used. Current practice with regard to UK/international clinical assessment is unknown. In this chapter I describe results of an anonymous online survey that I produced and distributed with the aid of a panel of UK/international rhinologists and experts in olfaction. My primary aim was to delineate current UK clinical practice, with particular emphasis on psychophysical smell testing, patient reported outcome measures and imaging. My secondary aims were to explore potential barriers to psychophysical testing and to describe geographical variations in practice. Responses were received from 465 clinicians (217 from UK, 17 countries total). Country-specific response rate varied, with the lowest rate being obtained from Japan (1.4%) and highest from Greece (72.5%). Subgroup analysis according to subspeciality training in rhinology ('rhinologists' and 'non-rhinologists') was performed, with geographical comparisons only made according to subgroup. Most UK clinicians do not perform psychophysical smell testing during any of the presented clinical scenarios - though rhinologists did so more often than non-rhinologists. The most frequent barriers to testing related to service provision (e.g., time/funding limitations). Whilst there was variability in practice, in general, international respondents performed psychophysical testing more frequently than those from the UK. Approximately 3/4 of all respondents said they would like to receive training in psychophysical smell testing. Patient reported outcome measures were infrequently used in the UK/internationally. More UK respondents performed diagnostic MRI scanning than international respondents. My results provide the most comprehensive picture of current clinical practice to date, and a detailed database for use in future research and service planning. Finally, I suggest recommendations to improve practice, including increased education and

funding for psychophysical smell testing. I hope this will promote accurate and reliable olfactory assessment, as is the accepted standard in other sensory systems.

4.2 Statement of Contribution

This chapter, including its summary, has been adapted from my published paper: **Whitcroft KL, Alobid I, Altundag A, Andrews P, Carrie S, Fahmy M, Fjaeldstad AW, Gane S, Hopkins C, Hsieh JW, Huart C, Hummel T, Konstantinidis I, Landis BN, Mori E, Mullol J, Philpott C, Poulios A, Vodička J, Ward VM. International clinical assessment of smell: An international, cross-sectional survey of current practice in the assessment of olfaction. *Clinical Otolaryngology*. 2024; 49(2): 220–234. doi: 10.1111/coa.14123. PMID: 38153760.** For the purposes of my thesis, sections have been expanded or reduced and language/style modified as necessary.

With input from PA, SC, SG, CH, CP, AP and VW, I wrote the survey. IA, AA, AWF, JWH, CH, TH, IK, BNL, EM, JM and JV reviewed the survey for international suitability and distributed in their respective countries of origin. MF assisted with administration. I analysed the data and interpreted the results. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

4.3 Introduction

Appropriate assessment of olfactory function is paramount for good clinical and research practice – enabling accurate diagnosis, therapeutic decision making and outcomes assessment. However, the current state of UK and international practice with regards to clinical assessment is largely unknown. To my knowledge, the last available data from the UK was obtained in 2009 [318]. Since this time, comprehensive international guidelines on the assessment and management of OD have been published[100,146,229,425]. In particular, these guidelines recommend use of psychophysical testing, given evidence that subjective assessment correlates poorly with more objective chemosensory tests (see §1.5.1 and §1.5.2)[528]. However,

variability in guidance remains – for example, regarding which aspects of olfaction to test using psychophysics. Moreover, adherence to such guidelines is unknown. Additionally, there have been no prior attempts to characterise geographical variations in practice, nor barriers to psychophysical smell testing.

I therefore performed the International Clinical Assessment of Smell (ICAS) Survey – the first comprehensive cross-sectional survey of UK and international clinical practice amongst ENT surgeons in the assessment of OD, with reference to the only available international guidelines at time of survey - the Position Paper on Olfactory Dysfunction (PPOD)[425].

My primary aim was to describe current UK practice in the assessment of olfaction, with particular focus on psychophysical smell testing, but also with reference to subjective assessment and imaging. My secondary aims were to describe international clinical practice in order to explore potential geographical variations in such practice, and to delineate current barriers to smell testing.

4.4 Methods

4.4.1 Survey development and distribution

My target population was ENT surgeons who assess olfaction. As there was no appropriate existing survey tool, I created an anonymous online questionnaire de novo, with the help of a UK-based 'development panel', whom I assembled. Please see §3.2.1 for details.

Survey item generation was performed in two steps: 1 – guideline/supporting literature review and identification of assessment domains of interest; 2 – simultaneous item generation/reduction. Step 2, as well as survey piloting, was performed during three iterative rounds of panel review.

Three main assessment domains were identified: A – psychophysical assessment (and barriers thereof); B – subjective assessment (patient reported outcome measures, 'PROMS'); C – imaging (MRI/other). As psychophysical testing was my primary interest, this domain contained the greatest number and most detailed

questions (covering frequency of psychophysical testing during diagnostic scenarios, medical and surgical outcomes assessment, and assessment of potential treatment complications). To address my secondary aim (barriers to smell testing) – question stems covering potential 'barrier domains' (funding, knowledge and lack of perceived importance) were included, embedded within the relevant assessment domain section. Questions referred to routine practice – except for a separate stem on the effects of the pandemic. During piloting, several topics were deemed highly specialist (e.g., assessment of retronasal olfaction or use of psychophysical tests for the assessment of qualitative OD), or were not directly related to the target domains. Rather than removing these 'more detailed' questions entirely, they were made optional (skippable using branching logic). In this way, such questions could be viewed as purposive sampling of those with greater interest in olfaction. For more information on survey construction, see §3.2.1.

Following development, the survey was approved for distribution by the ENT-UK survey guardian and distributed electronically between May-June 2021 (cross-sectional non-probability sampling[417]). Reminder rounds were sent in an attempt to reduce survey non-response but read-receipts were not available. All data was collected anonymously – respondent IP and email addresses were not captured. In light of the intercurrent pandemic, in-person/postal methods of survey distribution were not pursued.

Prior to international distribution, the survey underwent further review and piloting amongst an international panel of experts in olfaction, whom I assembled from members of COWoG (see §3.2.1 for details). Again, questionnaire piloting was performed simultaneously with iterative rounds of panel review. Minor changes were made for international audiences (e.g., insurance) if needed, but no changes were made to existing questions that would prevent UK/international comparison. The survey was written in English. Distribution was facilitated by local panel members with circulation via professional society mailing lists where possible. Again, all data was collected anonymously – respondent IP and email addresses were not captured. International distribution took place between September 2021 – January 2022.

The final UK and international questionnaires can be found in the appendices 10.2 and 10.3, respectively.

4.4.2 Ethical considerations

As outlined in §3.2.1, this combined service evaluation/audit was not classified as research by the NHS Health Research Authority. Instead, audit registration for national/international distribution was approved by the Royal National ENT & Eastman Dental Hospitals (University College London Hospitals NHS Foundation Trust). Data collected were anonymous, non-sensitive and voluntary. See §3.2.1 for more details.

4.4.3 Statistical analysis

An exploratory UK sample size of 92 was used as a minimum guide. See §3.2.1 for details. Quantitative data were analysed using GraphPad Prism (GraphPad Software, LaJolla, CA). Data were assessed for normality and parametric or nonparametric tests used as appropriate. If response rates to individual questions were lower than total respondents (due to dropout/branching logic), this is stated. Missing data were excluded from statistical analysis. Proportions are given for total *respondent number* or total *response number*, where answers were non-mutually exclusive. Subgroup analysis was performed for 'UK' and 'international' responses and comparison between cohorts was performed for the main assessment domains. Results are reported in line with the CROSS guidelines.

I analysed my qualitative data using qualitative content analysis. Please see §3.2.1 for further details.

Figures were prepared using GraphPad Prism (GraphPad Software, LaJolla, CA), Microsoft PowerPoint (Microsoft Corporation, Redmond, WA) and MapChart (CC BY-SA 4.0, available from: https://www.mapchart.net/world.html).

4.5 Results

4.5.1 Sample Population

Results were obtained from 465 respondents, of whom 217 were from the UK. The geographical distribution of remaining 'international' respondents (17 countries in total) is shown in Figure 4-1. Country-specific response rate varied from 1.4% to 72.5%, with lower rates being obtained where distribution was to mailing lists including multiple specialties/subspecialties (see Table 4-1).

Within the UK, 90.8% of respondents saw patients with OD and 20.5% were 'rhinologists'/had subspecialty training in rhinology. 53.9% worked in a district general hospital (DGH), 36.4% in a tertiary referral hospital (TRH) and the remainder another setting ('other', 9.7%) (165 respondents). Internationally, 96.4% of respondents saw patients with OD and 40.7% had subspecialty training in rhinology (significantly higher than in the UK, $\chi^2_{(1)}=17.5$, $P<0.0001$). Most international respondents worked in a TRH (47.6% of respondents, significantly higher than in the UK at 36.4%, $\chi^2_{(1)}=5.08$, $P=0.024$), followed by DGHs (27.4%), private clinics (23.0%) or 'other' (2.0%).

In order to inform later subgroup analysis, I was interested in differences in practice between rhinologists/non-rhinologists, and clinicians working in TRHs/DGHs. Therefore, the frequency of psychophysical testing during the initial assessment of OD (including: OD as a presenting or isolated symptom; OD in association with another presenting symptom) was used as a test question. Similar patterns of results were obtained for rhinologist/TRH-respondents and non-rhinologists/DGH-respondents (see appendix 0). Accordingly, direct statistical comparison of these groups was undertaken. In both the UK and internationally, there were no statistically significant differences in proportions of respondents across all frequencies of testing in rhinologists vs TRH respondents or in non-rhinologists vs DGH respondents [OD as a presenting or isolated symptom: UK – rhinologists vs TRH respondents ($\chi^2_{(4)}=1.6$, $P=0.81$), non-rhinologists vs DGH respondents ($\chi^2_{(4)}=0.6$, $P=0.97$); International – rhinologists vs TRH respondents ($\chi^2_{(4)}=4.5$, $P=0.34$), non-rhinologists vs DGH respondents ($\chi^2_{(4)}=4.3$, $P=0.36$)] [OD in association with another

presenting symptom: UK – rhinologists vs TRH respondents ($\chi^2_{(4)}$=1.1, *P*=0.9), non-rhinologists vs DGH respondents ($\chi^2_{(4)}$=0.8, *P*=0.94); International – rhinologists vs TRH respondents ($\chi^2_{(4)}$=5.9, *P*=0.21), non-rhinologists vs DGH respondents ($\chi^2_{(4)}$=2.2, *P*=0.70)]. It is therefore likely that rhinologists/TRH respondents, and non-rhinologists/DGH respondents either have similar testing practices and/or are overlapping subgroup samples, and further subgroup analysis was undertaken for rhinologists vs non-rhinologists only.

As distribution method, response rates and proportion of 'rhinologists' varied geographically, direct country comparison was not performed, due to probable differences in selection bias. Instead, subgroup analysis according to subspeciality training in rhinology ('rhinologists' and 'non-rhinologists') was performed, with geographical comparisons only made according to subgroup.

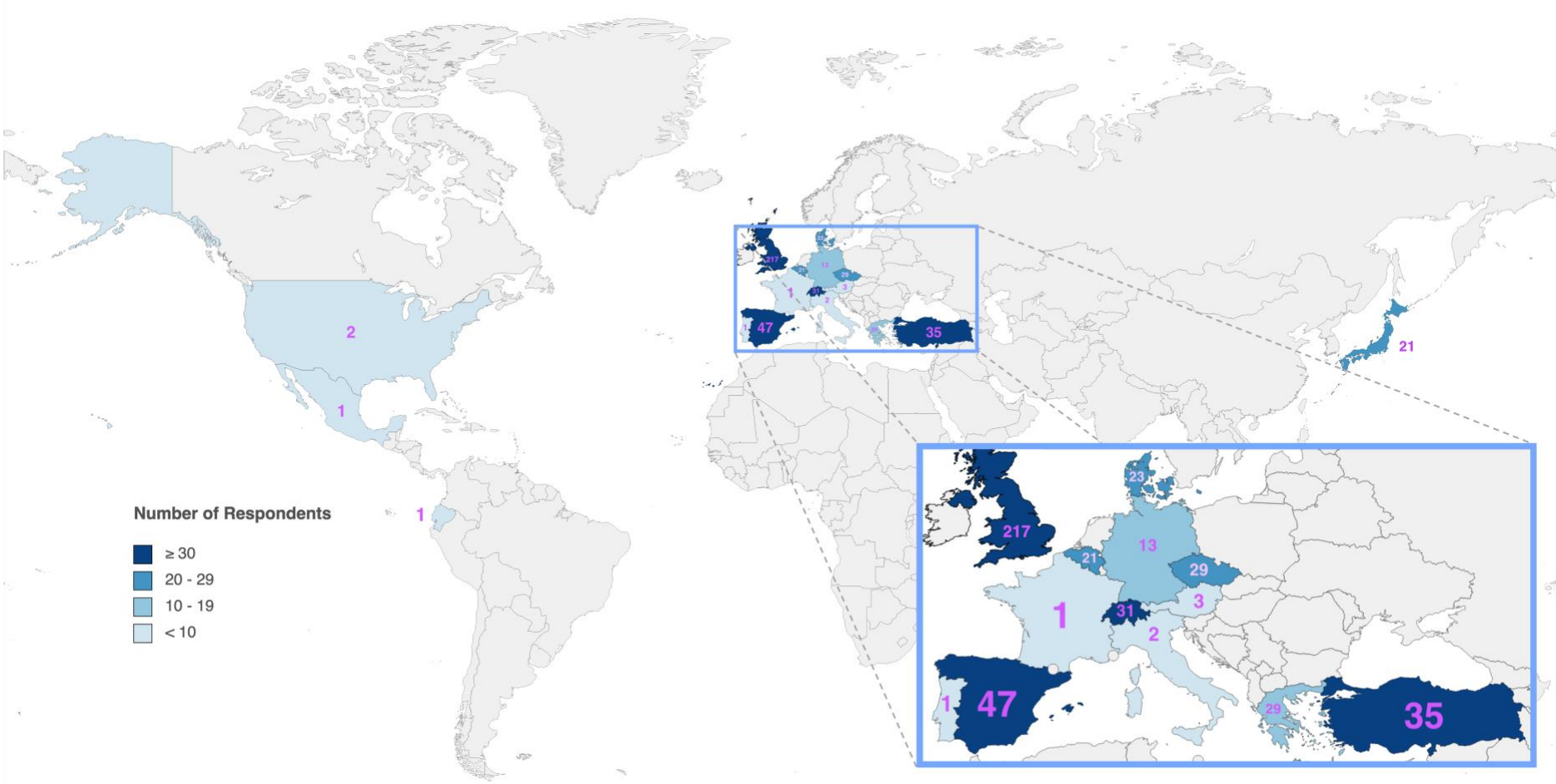


Figure 4-1: Geographical Distribution of International Respondents. Initial graphic created with MapChart.

Country	Response Rate (%)	Surveys Distributed	Distribution method
Greece	72.5	40	Regional/local mailing list
Belgium	70.0*[a]	30*	Regional/local mailing list
Switzerland	66.0	47	National mailing list (rhinology)
Turkey	42.7	82	Regional/local mailing list
Denmark	15.3*[b^]	150*	Conference, regional/local mailing list
UK	10.0[†]	2165	National mailing list
Czech Republic	6.9[†]	420	National mailing list
Germany	4.3[†]	300	National mailing list
Spain	1.6[†]	3000	National mailing list
Japan	1.4[†]	1513	National mailing list

Table 4-1: Response rates and distribution methods. * = approximate response rate [a – survey distribution delegated to nominated clinician within specified healthcare centres (snowball sampling); b – distribution at conference involving word of mouth (convenience/snowball sampling)]. [†] = mailing list including multiple subspecialities / specialities (including allied specialties). ^ = distribution included international recipients beyond country of origin.

4.5.2 Psychophysical Testing

Within the UK, across all respondents, and within the rhinologist and non-rhinologist subgroups, the largest proportion of clinicians 'never' performed psychophysical testing in any of the clinical scenarios presented (covering diagnostics, outcomes and complications assessment). Looking into variations in testing practice across these scenarios, there was no statistically significant difference in the proportion of respondents performing smell testing (across all frequencies) when comparing 'diagnosis—OD as a presenting or isolated symptom' with 'diagnosis—OD in association with another presenting symptom' ($\chi^2_{(4)}$=4.2, *P*=0.37), 'outcomes—before medical intervention' ($\chi^2_{(4)}$=8.1, *P*=0.09), 'outcomes—after medical intervention' ($\chi^2_{(4)}$=2.5, *P*=0.64), 'outcomes—before surgical intervention' ($\chi^2_{(4)}$=2.2, *P*=0.71), or 'outcomes—after surgical intervention' ($\chi^2_{(4)}$=4.9, *P*=0.3). There was, however, a statistically significant difference in smell testing when comparing

'diagnosis—OD as a presenting or isolated symptom' and 'complications—pre-op[21]' ($\chi^2_{(4)}$=33.2, *P*<0.0001), as well as 'complications—post-op' ($\chi^2_{(4)}$=33.5, *P*<0.0001). Therefore, it would appear that UK smell testing practice is similar across diagnostic and outcomes assessment scenarios, but that practice varies between these scenarios and complications monitoring, with higher proportions of respondents 'never' performing smell testing in the latter. Comparing rhinologist to non-rhinologist subgroups a statistically significantly higher proportion of rhinologists 'always' or 'most of the time' performed testing during the initial assessment of OD as a presenting/isolated symptom, before/after surgical intervention, and before/after surgical intervention that could cause OD as a complication (for full results see Table 4-2).

For international respondents, there generally appeared to be greater variation in practice across assessment scenarios, and greater proportions of clinicians performing psychophysical testing, particularly within the rhinologist subgroup. Looking into variations in testing practice across these scenarios, there was no statistically significant difference in the proportion of respondents performing smell testing (across all frequencies) when comparing 'diagnosis—OD as a presenting or isolated symptom' with 'outcomes—before medical intervention' ($\chi^2_{(4)}$=3.8, *P*=0.44), or 'outcomes—before surgical intervention' ($\chi^2_{(4)}$=4.1, *P*=0.4). There was, however, a statistically significant difference in smell testing when comparing 'diagnosis—OD as a presenting or isolated symptom' and 'diagnosis—OD in association with another presenting symptom' ($\chi^2_{(4)}$=46.8, *P*<0.0001), 'outcomes—after medical intervention' ($\chi^2_{(4)}$=24.4, *P*<0.0001), 'outcomes—after surgical intervention' ($\chi^2_{(4)}$=31.4, *P*<0.0001), 'complications—pre-op' ($\chi^2_{(4)}$=60.3, *P*<0.0001) and 'complications—post-op' ($\chi^2_{(4)}$=103.7, *P*<0.0001). In general, smell testing appears to be performed more frequently during the initial diagnosis of OD as a presenting or isolated symptom, or prior to interventions for OD. When comparing rhinologist to non-rhinologist subgroups, statistically significantly higher proportions of rhinologists 'always', 'most

[21] *'Complications – pre/post op'…these questions interrogated testing practice before and after surgical procedures that could cause OD as a complication. See appendices 10.3 and 10.3.*

of the time' or 'sometimes' performed psychophysical testing, across all of the clinical scenarios presented (see Table 4-2).

Directly comparing UK and international responses, in both rhinologist/non-rhinologist subgroups, where statistically significant differences in proportions of testing were found, it was more frequently performed internationally (see Table 4-2). Figure 4-2 compares UK/international psychophysical test use during the initial assessment of OD. Figure 4-3 shows country-specific diagnostic practice in rhinologist/non-rhinologist subgroups.

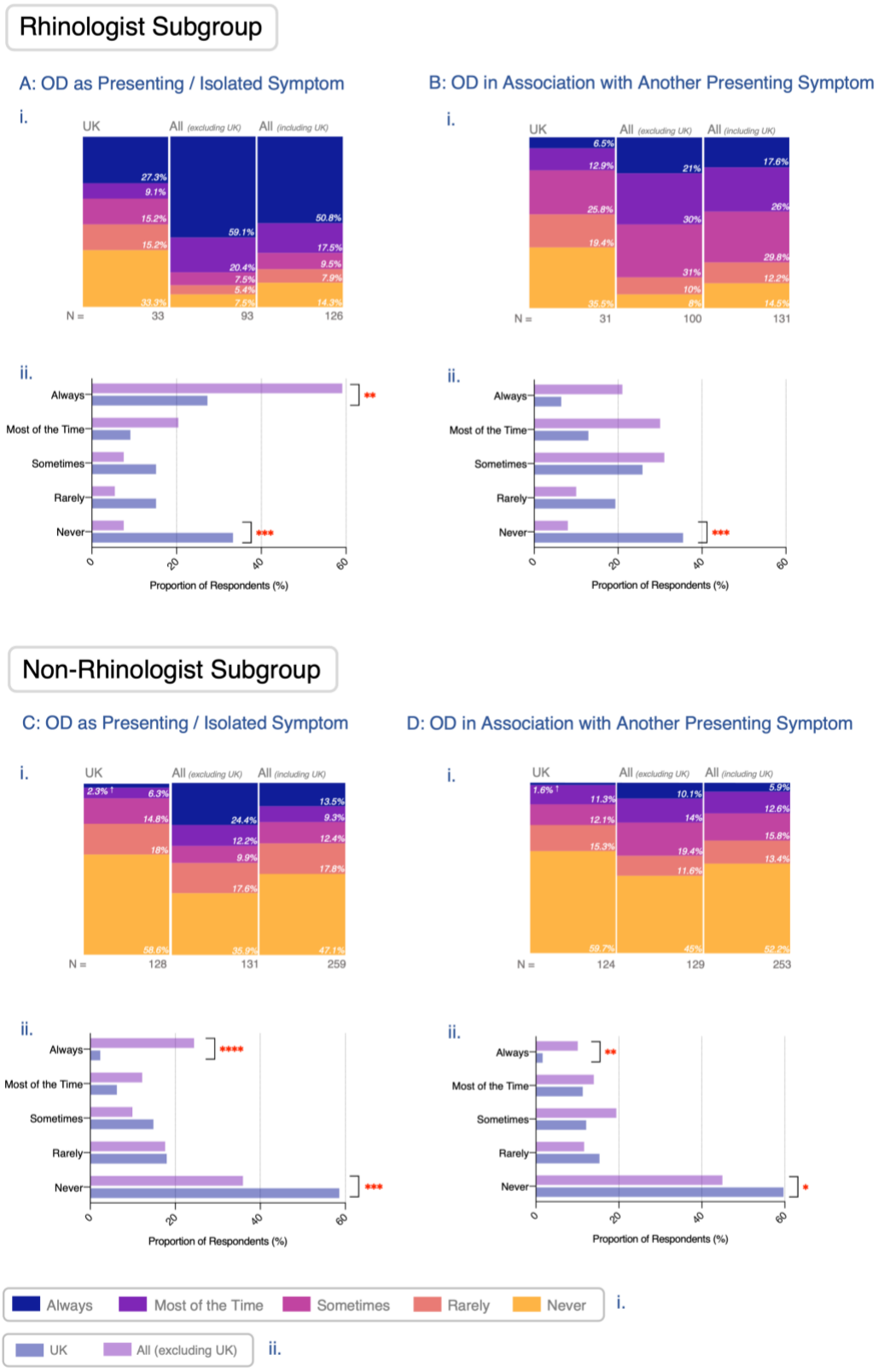


Figure 4-2: UK and international smell testing during initial assessment of olfactory dysfunction (OD). (i): Percent stacked column charts showing distribution of testing frequencies in the UK, all (excluding UK) and all (including UK) in rhinologist and non-rhinologist subgroups, for OD as a presenting/isolated symptom (A/C) or OD in association with another presenting symptom (B/D). (ii): Bar charts comparing distribution of testing frequencies between UK and all (excluding UK), for OD as a presenting/isolated symptom (A/C) or OD in association with another presenting symptom (B/D). Asterisks indicate statistically significant results—* $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$. Note- percentages shown rounded to one decimal place.

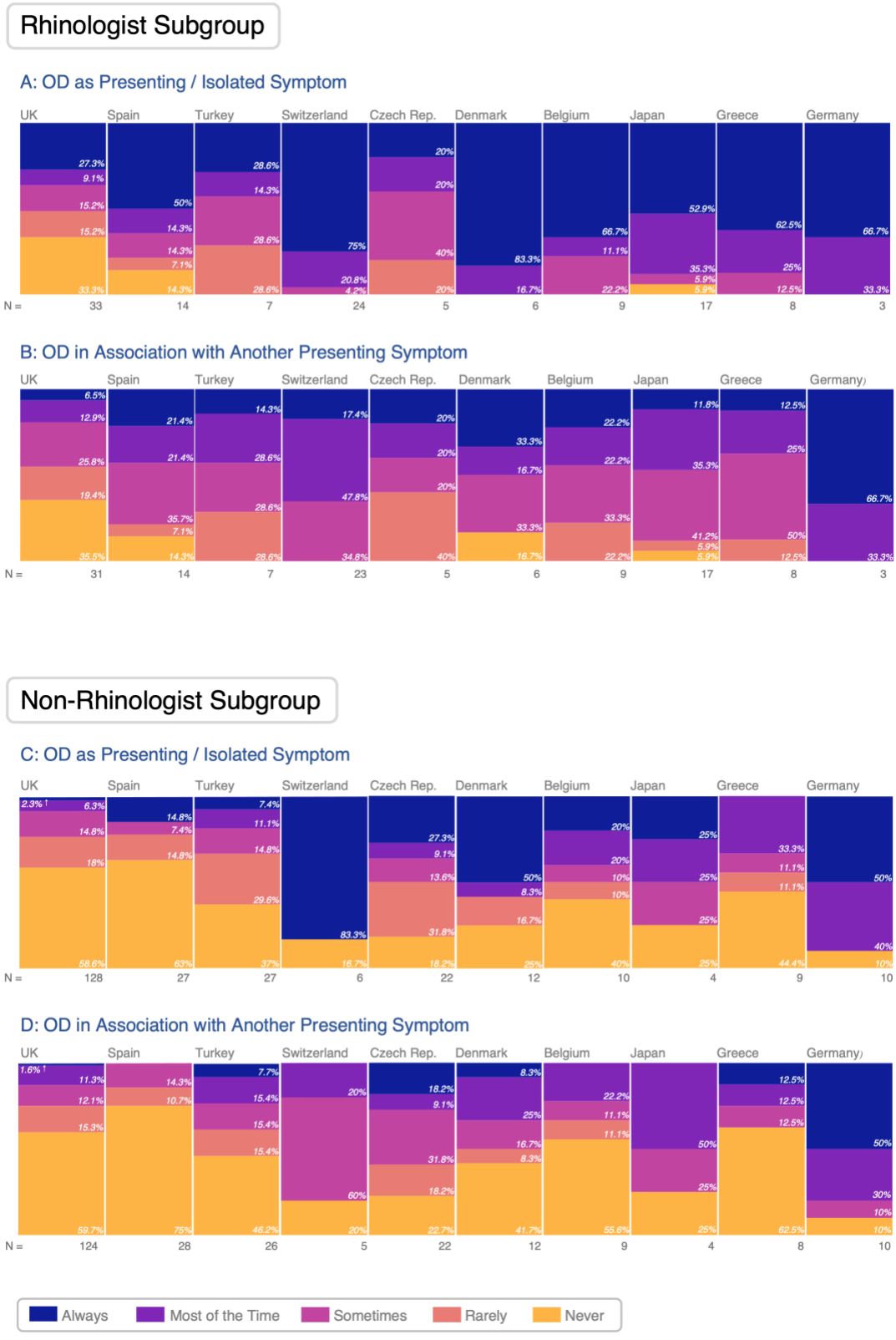


Figure 4-3: UK and international smell testing during initial assessment of olfactory dysfunction (OD). Percent stacked column charts showing distribution of testing frequencies in all countries with total respondents $n \geq 10$ (from left to right in order of descending total (rhinologist + non-rhinologist) participant number), in rhinologist and non-rhinologist subgroups, for OD as a presenting/isolated symptom (A/C) or OD in association with another presenting symptom (B/D). Note- percentages shown rounded to one decimal place.

In both the UK and internationally, the most common type of test used was odour identification, followed by discrimination and threshold (UK - 27.3%, 12.9% and 6.7% of responses respectively; international – 38.4%, 21.6% and 21.4% respectively). The most common specific type of test was the 'Smell Identification Test' in the UK, the 'Sniffin' Sticks' internationally.

Barriers to routine psychophysical testing, as well as maximum acceptable duration of smell testing are shown in Figure 4-4.

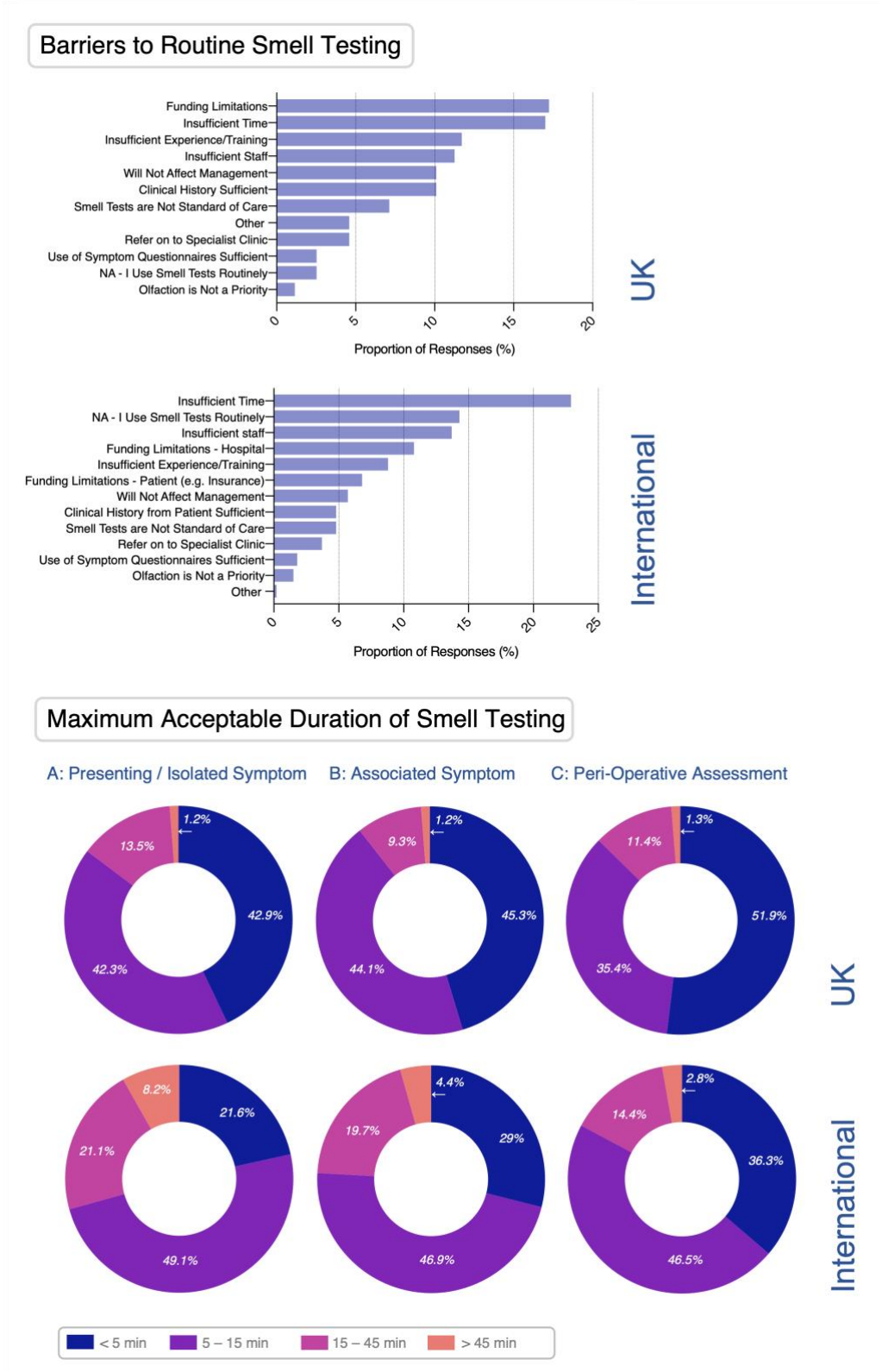


Figure 4-4: UK and international barriers to routine psychophysical testing (bar chart, top - note N = total non-mutually exclusive responses) and maximum acceptable testing time (donut chart, bottom: for the assessment of (A) OD as a presenting/isolated symptom, (B) OD in association with another presenting symptom, (C) during the perioperative assessment of olfaction for a surgical intervention that could cause OD as a complication). Note- percentages shown rounded to one decimal place.

Approximately 1 in 5 of both UK and international respondents had no knowledge/experience of psychophysical smell tests (21.8% and 18.6% respectively). Across both cohorts, the most frequent source of knowledge was clinical experience, followed by self-directed study, post-graduate training, courses, medical school and 'other' (UK – 36.1%, 29.9%, 17.0%, 7.2%, 7.2% and 2.9% respectively, of 194 total responses; International - 46.3%, 20.5%, 14.0%, 11.8%, 5.6% and 1.7% respectively of 322 total responses). In the UK, just over one quarter of respondents (26.7% of 165) were not aware of any guidelines for the diagnosis and management of OD. More respondents were aware of the BRS Consensus Guidelines on 'Management of new onset anosmia the COVID-19 Pandemic' [529] than the Position Paper on Olfactory Dysfunction [425] (93 and 48 responses out of 197 total, respectively). Internationally, 33.5% of the 248 respondents were not aware of any guidelines. Of those who were, the most commonly known was the Position Paper on Olfactory Dysfunction [425] (32.8% of 387 responses), followed by the BRS Consensus Guidelines on 'Management of new onset anosmia in the COVID-19 pandemic'[529] (19.6% of responses). Most respondents (UK – 77.6%, international – 63.3%) said they would like to receive training in use of psychophysical tests.

Psychophysical Assessment

Initial Diagnosis

		UK				*International*				*UK vs International*	
OD as presenting or isolated symptom		***All (n=164)***	***Rhinologist (n=33)***	***Non-Rhinologists (n=128)***	***Rhinologists vs Non-Rhinologists***	***All (n=233)***	***Rhinologist (n=93)***	***Non-Rhinologists (n=131)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	5.5%	27.3%	2.3%	$\chi^2_{(1)}$=23.6 *P*<0.0001	39.5%	59.1%	24.4%	$\chi^2_{(1)}$=27.6 P<0.0001	$\chi^2_{(1)}$=9.9 *P*=0.002	$\chi^2_{(1)}$=27.0 *P*<0.0001
	Most of the time	7.3%	9.1%	6.3%	ns	15.9%	20.4%	12.2%	ns	ns	ns
	Sometimes	14.6%	15.2%	14.8%	ns	8.6%	7.5%	9.9%	ns	ns	ns
	Rarely	17.7%	15.2%	18.0%	ns	12.9%	5.4%	17.6%	$\chi^2_{(1)}$=7.4 P=0.007	ns	ns
	Never	54.9%	33.3%	58.6%	$\chi^2_{(1)}$=6.7 *P*=0.01	23.2%	7.5%	35.9%	$\chi^2_{(1)}$=23.9 P<0.0001	$\chi^2_{(1)}$=13.3 *P*=0.0003	$\chi^2_{(1)}$=13.4 *P*=0.0003
OD in association with another presenting symptom		***All (n=160)***	***Rhinologist (n=31)***	***Non-Rhinologists (n=124)***	***Rhinologists vs Non-Rhinologists***	***All (n=229)***	***Rhinologist (n=100)***	***Non-Rhinologists (n=129)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	2.5%	6.5%	1.6%	ns	14.9%	21.0%	10.1%	$\chi^2_{(1)}$=5.3, P=0.02	ns	$\chi^2_{(1)}$=8.1 *P*=0.004
	Most of the time	12.5%	12.9%	11.3%	ns	21.0%	30.0%	14.0%	$\chi^2_{(1)}$=8.6 P=0.003	ns	ns
	Sometimes	13.1%	25.8%	12.1%	ns	24.5%	31.0%	19.4%	$\chi^2_{(1)}$=4.1 P=0.04	ns	ns
	Rarely	16.3%	19.4%	15.3%	ns	10.9%	10.0%	11.6%	ns	ns	ns
	Never	55.6%	35.5%	59.7%	$\chi^2_{(1)}$=5.7 *P*=0.02	28.8%	8.0%	45.0%	$\chi^2_{(1)}$=37.5 P<0.0001	$\chi^2_{(1)}$=14.4 *P*=0.0001	$\chi^2_{(1)}$=5.5 *P*=0.02

Table continued overleaf…

Outcomes Assessment

		UK				*International*				*UK vs International*	
Before medical intervention (where OD most troublesome symptom)		***All (n=159)***	***Rhinologist (n=27)***	***Non-Rhinologists (n=131)***	***Rhinologists vs Non-Rhinologists***	***All (n=219)***	***Rhinologist (n=100)***	***Non-Rhinologists (n=119)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	6.3%	7.4%	4.6%	ns	33.3%	47.0%	21.8%	$\chi^2_{(1)}$=15.5 *P*<0.0001	$\chi^2_{(1)}$=14.1 *P*=0.0002	$\chi^2_{(1)}$=16.7 *P*<0.0001
	Most of the time	10.7%	14.8%	9.9%		19.2%	25.0%	14.3%	$\chi^2_{(1)}$=4.02 *P*=0.045	ns	ns
	Sometimes	5.7%	3.7%	6.1%		12.3%	15.0%	10.1%	ns	ns	ns
	Rarely	16.4%	18.5%	16.0%		11.0%	6.0%	15.1%	$\chi^2_{(1)}$=4.6 *P*=0.03	$\chi^2_{(1)}$=4.2 *P*=0.04	ns
	Never	61.0%	55.6%	63.4%		24.2%	7.0%	38.7%	$\chi^2_{(1)}$=29.7 *P*<0.0001	$\chi^2_{(1)}$=35.0 *P*<0.0001	$\chi^2_{(1)}$=15.2 *P*<0.0001
After medical intervention (where OD most troublesome symptom)		***All (n=156)***	***Rhinologist (n=27)***	***Non-Rhinologists (n=128)***	***Rhinologists vs Non-Rhinologists***	***All (n=215)***	***Rhinologist (n=99)***	***Non-Rhinologists (n=116)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	5.1%	11.1%	3.9%	ns	21.4%	28.3%	15.5%	$\chi^2_{(1)}$=5.2 *P*=0.02	ns	$\chi^2_{(1)}$=9.6 *P*=0.002
	Most of the time	7.7%	3.7%	8.6%		20.5%	31.3%	11.2%	$\chi^2_{(1)}$=13.3 *P*=0.0003	$\chi^2_{(1)}$=8.5 *P*=0.004	ns
	Sometimes	9.0%	3.7%	8.6%		20.5%	23.2%	18.1%	ns	$\chi^2_{(1)}$=5.3 *P*=0.02	$\chi^2_{(1)}$=4.8 *P*=0.03
	Rarely	18.6%	25.9%	17.2%		14.4%	11.1%	17.2%	ns	ns	ns
	Never	59.6%	55.6%	61.7%		23.3%	6.1%	37.9%	$\chi^2_{(1)}$=30.4 *P*<0.0001	$\chi^2_{(1)}$=22.1 *P*<0.0001	$\chi^2_{(1)}$=13.8 *P*=0.0002

Table continued overleaf…

Before surgical intervention (where OD most troublesome symptom)		***All (n=159)***	***Rhinologist (n=31)***	***Non-Rhinologists (n=128))***	***Rhinologists vs Non-Rhinologists***	***All (n=168)***	***Rhinologist (n=81)***	***Non-Rhinologists (n=84)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	3.8%	6.5%	1.6%	ns	30.4%	40.7%	21.4%	$\chi^2_{(1)}$=7.2 *P*=0.007	$\chi^2_{(1)}$=12.3 *P*=0.0005	$\chi^2_{(1)}$=23.4 *P*<0.0001
	Most of the time	5.7%	16.1%	3.9%	$\chi^2_{(1)}$=6.3 P=0.01	20.8%	29.6%	9.5%	$\chi^2_{(1)}$=10.7 *P*=0.001	ns	ns
	Sometimes	11.3%	16.1%	11.7%	ns	9.5%	11.1%	8.3%	ns	ns	ns
	Rarely	17.6%	22.6%	16.4%	ns	14.9%	11.1%	19.0%	ns	ns	ns
	Never	61.6%	38.7%	66.4%	$\chi^2_{(1)}$=8.05 P=0.005	24.4%	7.4%	41.7%	$\chi^2_{(1)}$=25.9 *P*<0.0001	$\chi^2_{(1)}$=16.3 *P*<0.0001	$\chi^2_{(1)}$=12.6 *P*=0.0004
After surgical intervention (where OD most troublesome symptom)		***All (n=158)***	***Rhinologist (n=31)***	***Non-Rhinologists (n=127)***	***Rhinologists vs Non-Rhinologists***	***All (n=159)***	***Rhinologist (n=85)***	***Non-Rhinologists (n=74)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	1.9%	6.45%	0.8%	$\chi^2_{(1)}$=4.3 P=0.04	20.0%	25.9%	12.2%	$\chi^2_{(1)}$=4.8 *P*=0.03	$\chi^2_{(1)}$=5.2 *P*=0.02	$\chi^2_{(1)}$=12.8 *P*=0.0003
	Most of the time	5.7%	9.68%	5.5%	ns	14.2%	21.2%	5.4%	$\chi^2_{(1)}$=8.3, *P*=0.004	ns	ns
	Sometimes	10.8%	19.35%	8.7%	ns	25.2%	31.8%	21.6%	ns	ns	$\chi^2_{(1)}$=6.8 *P*=0.009
	Rarely	19.6%	25.81%	18.1%	ns	20.0%	11.8%	28.4%	$\chi^2_{(1)}$=7.0 *P*=0.008	ns	ns
	Never	62.0%	38.71%	66.9%	$\chi^2_{(1)}$=8.4 P=0.004	20.6%	9.4%	32.4%	$\chi^2_{(1)}$=13.0 *P*=0.0003	$\chi^2_{(1)}$=13.7 *P*=0.0002	$\chi^2_{(1)}$=22.4 *P*<0.0001

Table continued overleaf…

Perioperative Testing in Normosmics (Olfactory Complications)											
		UK				*International*				*UK vs International*	
Before surgical intervention that could cause OD as a complication (pre-op)		***All (n=161)***	***Rhinologist (n=33)***	***Non-Rhinologists (n=128)***	***Rhinologists vs Non-Rhinologists***	***All (n=213)***	***Rhinologist (n=96)***	***Non-Rhinologists (n=116)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	1.2%	3.0%	0.8%	ns	13.6%	16.7%	11.2%	ns	$\chi^2_{(1)}$=4.0 *P*=0.046	$\chi^2_{(1)}$=12.2 *P*=0.0005
	Most of the time	1.2%	9.1%	0.8%	$\chi^2_{(1)}$=7.5 P=0.006	10.8%	18.8%	3.4%	$\chi^2_{(1)}$=13.2 *P*=0.0003	ns	ns
	Sometimes	3.7%	9.1%	2.3%	ns	5.2%	6.3%	4.3%	ns	ns	Ns
	Rarely	11.2%	15.2%	10.9%	Ns	16.4%	24.0%	10.3%	$\chi^2_{(1)}$=7.1 *P*=0.008	ns	ns
	Never	82.6%	63.6%	85.2%	$\chi^2_{(1)}$=7.8 P=0.005	54.0%	34.4%	70.7%	$\chi^2_{(1)}$=27.9 *P*<0.0001	$\chi^2_{(1)}$=8.6 *P*=0.003	$\chi^2_{(1)}$=7.5 *P*=0.006
After surgical intervention that could cause OD as a complication (post-op)		***All (n=160)***	***Rhinologist (n=31)***	***Non-Rhinologists (n=127)***	***Rhinologists vs Non-Rhinologists***	***All (n=204)***	***Rhinologist (n=92)***	***Non-Rhinologists (n=112)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	0.6%	0.0%	0.8%	ns	4.4%	7.6%	1.8%	$\chi^2_{(1)}$=4.2 *P*=0.04	ns	ns
	Most of the time	1.3%	9.7%	0.8%	$\chi^2_{(1)}$=8.0 P=0.005	5.4%	8.7%	2.7%	ns	ns	ns
	Sometimes	3.1%	6.5%	2.4%	ns	14.2%	18.5%	10.7%	ns	ns	$\chi^2_{(1)}$=7.1 *P*=0.008
	Rarely	14.4%	22.6%	11.8%	ns	25.0%	34.8%	17.0%	$\chi^2_{(1)}$=8.6 *P*=0.003	ns	ns
	Never	80.6%	61.3%	84.3%	$\chi^2_{(1)}$=8.1 P=0.004	51.0%	30.4%	67.9%	$\chi^2_{(1)}$=28.3 *P*<0.0001	$\chi^2_{(1)}$=9.4 *P*=0.002	$\chi^2_{(1)}$=8.9 *P*=0.003

Table 4-2: UK and international results for psychophysical assessment. Please note - where individual question response rates were less than the total, this was due to either branching logic or dropout. Test statistic (parametric or non-parametric as appropriate) and associated *P* value given for statistically significant results (where *P*<0.05). Statistical significance tested between groups as per headings (UK rhinologists vs non-rhinologists; international rhinologists vs non-rhinologists; UK vs international rhinologists; UK vs international non-rhinologists). Note- percentages shown rounded to one decimal place. Abbreviations: ns, non-statistically significant.

4.5.3 Imaging

The highest proportions of UK respondents, overall and within rhinologist/non-rhinologist subgroups, performed diagnostic MRI scanning ‘always’ or ‘most of the time’. Indeed, 36.4% of rhinologists ‘always’ scanned. Internationally, the highest proportion of respondents ‘sometimes’ scanned, followed by ‘most of the time’. There was no significant difference in frequencies of scanning between rhinologist or non-rhinologist subgroups, in either the UK or internationally. Comparing UK and international practice, in both rhinologist/non-rhinologist subgroups, a significantly higher proportion of UK respondents ‘always’ scanned. In the non-rhinologist subgroup, significantly more international respondents ‘sometimes’ or ‘rarely’ scanned (see Table 4-3 and Figure 4-5).

UK Respondents were asked to describe when they would perform an MRI for olfactory dysfunction. Content from 87 respondents’ free text entries included the following in order of frequency: normal endoscopy/not CRS (25 responses); unclear cause/idiopathic (18 responses); suspicion of tumour (intracranial or intranasal) (17 responses); neurological symptoms/signs (14 responses); severe quantitative OD (9 responses); not COVID-19 related (6 responses); head trauma (6 responses); suspicion of congenital OD (5 responses); presence of qualitative OD (parosmia or phantosmia) (4 responses); persistence/long-term (4 responses); refractive to treatment (4 responses); acute onset (2 responses); based on CT findings (2 responses); progressive (1 response); suspicion of endocrine pathology (1 response); non-traumatic cause (1 response). Internationally, content from 136 respondents’ free text entries included the following in order of frequency: unclear cause/idiopathic (51 responses); suspicion of tumour (intracranial or intranasal) (35 responses); head trauma (19 responses); neurological symptoms/signs (16 responses); normal endoscopy/not CRS (16 responses); suspicion of congenital OD (10 responses); routine practice (10 responses); persistence/long term (9); not related to URTI (6 responses); not related to COVID-19 (5 responses); refractive to treatment (4 responses); acute onset (4 responses); suspected PIOD (3 responses); parosmia (2 responses); severe quantitative OD (2 responses); anxious patient (2 responses); medico-legal cases (2 responses); progressive (1 response); normal CT (1

response); suspected complications of CRS (1 response); other nasal symptoms (1 response); suspicion of sarcoidosis (1 response); OD in association with taste dysfunction (1 response); for volumetric assessment of OB (1 response).

Respondents were also asked to describe their aim in performing MRI scanning from a list of non-mutually exclusive options. In both the UK and internationally, the most frequently chosen option was 'exclude neoplasm', followed by 'exclude non-neoplastic structural abnormality upstream of the olfactory bulbs' and 'assess olfactory bulbs (gross – present/absent)' (UK – 39.5%, 24.6% and 24.1% respectively of 382 total responses; International - 34.0%, 23.3% and 21.9% respectively of 580 total responses). More international respondents performed volumetric assessment than in the UK (16.4% vs 8.4% of total responses respectively). CT of the paranasal sinuses was the most frequent 'other' scan used by respondents for the assessment of OD as a presenting or isolated symptom.

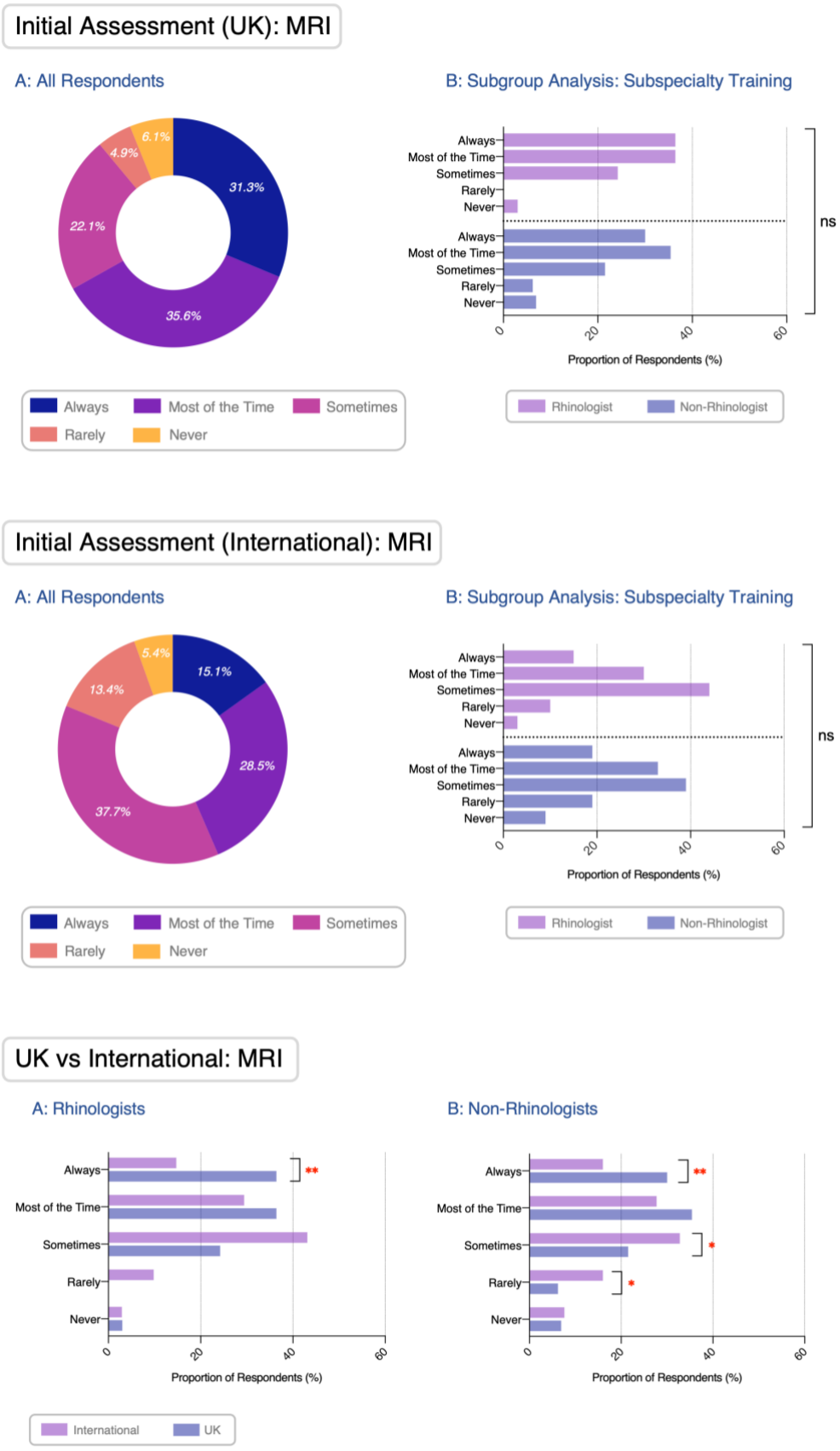


Figure 4-5: UK (top), international (middle) and UK vs international (bottom) MRI use during initial assessment of OD. Donut charts show frequency of scanning across all respondents, bar charts show frequency of scanning in rhinologist vs non-rhinologist groups (top, middle) or in international vs UK rhinologists (bottom, A) and non-rhinologists (bottom, B). Asterisks indicate statistically significant results—* $p < .05$, ** $p < .01$.

Imaging											
		UK				*International*				*UK vs International*	
MRI brain/olfactory tract during the initial assessment of OD as a presenting/isolated symptom		***All (n=163)***	***Rhinologist (n=33)***	***Non-Rhinologists (n=130)***	***Rhinologists vs Non-Rhinologists***	***All (n=239)***	***Rhinologist (n=102)***	***Non-Rhinologists (n=119)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	31.3%	36.4%	30.0%	ns	15.1%	14.7%	16.0%	ns	$\chi^2_{(1)}$=7.3 *P*=0.007	$\chi^2_{(1)}$=6.8 *P*=0.009
	Most of the time	35.6%	36.4%	35.4%		28.5%	29.4%	27.7%		ns	ns
	Sometimes	22.1%	24.2%	21.5%		37.7%	43.1%	32.8%		ns	$\chi^2_{(1)}$=4.0 *P*=0.046
	Rarely	4.9%	0.0%	6.2%		13.4%	9.8%	16.0%		ns	$\chi^2_{(1)}$=6.2 *P*=0.01
	Never	6.1%	3.0%	6.9%		5.4%	2.9%	7.6%		ns	ns

Table 4-3: UK and international results for imaging. Please note - where individual question response rates were less than the total, this was due to either branching logic or dropout. Test statistic (parametric or non-parametric as appropriate) and associated *P* value given for statistically significant results (where *P*<0.05). NS = non-statistically significant. Statistical significance tested between groups as per headings (UK rhinologists vs non-rhinologists; international rhinologists vs non-rhinologists; UK vs international rhinologists; UK vs international non-rhinologists). Note- percentages shown rounded to one decimal place. Abbreviations: ns, non-statistically significant.

4.5.4 PROMs

Across all clinicians, rhinologist and non-rhinologist subgroups, the highest proportion of respondents 'never' used PROMs during their initial assessment of OD, both in the UK and internationally (see Table 4-4 and Figure 4-6). However, practice varied between rhinologists/non-rhinologists, with the former group using PROMS more frequently. In both the UK and internationally, the 'SNOT-22' questionnaire was the most frequently used PROM.

Regarding specific PROM use, during free text responses, 'SNOT-22' was the most frequently reported in the UK (26 responses) and internationally (44 responses, including 'SNOT' [sic] and 'SNOT-20 German adapted version'). In the UK, other reported PROMS were as follows: 'Questionnaire of Olfactory Disorders' (5 responses), 'SNOT-23' (2 responses), 'NOSE' (2 responses), the short version of the 'Questionnaire of Olfactory Disorders-Negative Statements' (1 response), and the Modified Monell-Jefferson Taste and Smell Questionnaire (1 response). Only one UK respondent reported using VAS or a dedicated measure of depression (Becks Depression Inventory). Internationally, VAS were the second most frequently used PROM (17 responses). The 'Questionnaire of Olfactory Disorders', quality of life questionnaires [NOS] and nationally specific questionnaires (e.g., the 2003 Japanese Rhinologic Society 20-item questionnaire) were the next most frequently used (7, 5 and 5 responses respectively). There were 23 other PROMs used internationally, all of which received 1—3 responses, including measures of cognition (e.g., Mini Mental State Examination), depression (e.g. Beck's Depression Inventory, Major Depression Inventory) and qualitative olfactory dysfunction [NOS].

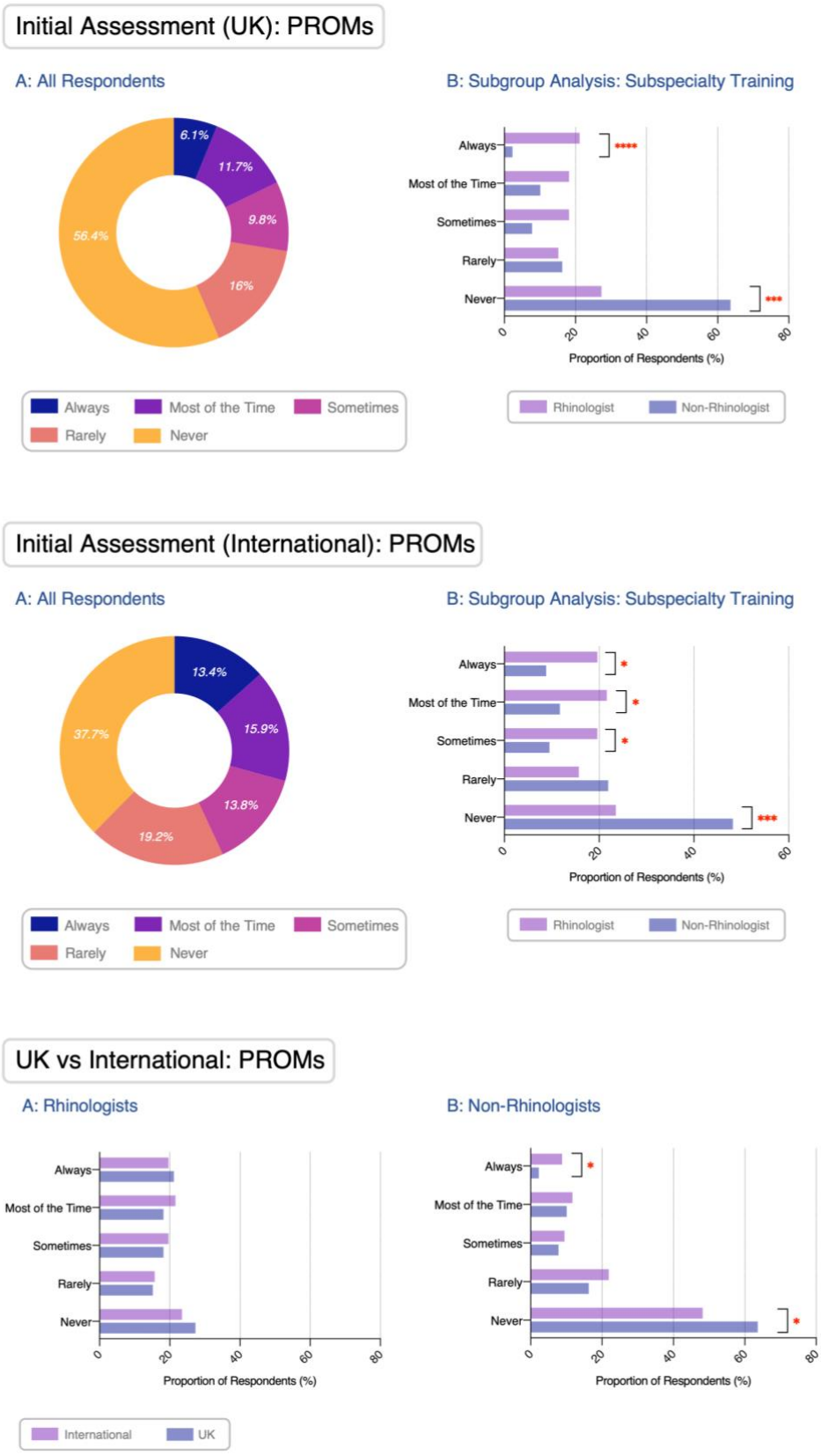


Figure 4-6: UK (top), international (middle) and UK vs international (bottom) PROM use during initial assessment of OD. Donut charts show frequency of testing across all respondents, bar charts show frequency of testing in rhinologist vs non-rhinologist groups (top, middle) or in international vs UK rhinologists (bottom, A) and non-rhinologists (bottom, B). Please note varying *x* axis scale in bar charts. Asterisks indicate statistically significant results—* $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.

Subjective Assessment											
		UK				*International*				*UK vs International*	
Use of PROMs during assessment of OD (isolated/presenting or associated symptom)		***All (n=163)***	***Rhinologist (n=33)***	***Non-Rhinologists (n=129)***	***Rhinologists vs Non-Rhinologists***	***All (n=239)***	***Rhinologist (n=102)***	***Non-Rhinologists (n=137)***	***Rhinologists vs Non-Rhinologists***	***Rhinologists***	***Non-Rhinologists***
	Always	6.1%	21.2%	2.3%	$\chi^2_{(1)}$=16.2 *P*<0.0001	13.4%	19.6%	8.8%	$\chi^2_{(1)}$=5.9 *P*=0.02	ns	$\chi^2_{(1)}$=5.2 *P*=0.02
	Most of the time	11.7%	18.2%	10.1%	ns	15.9%	21.6%	11.7%	$\chi^2_{(1)}$=4.3 *P*=0.04		ns
	Sometimes	9.8%	18.2%	7.8%	ns	13.8%	19.6%	9.5%	$\chi^2_{(1)}$=6.4 *P*=0.01		ns
	Rarely	16.0%	15.2%	16.3%	ns	19.2%	15.7%	21.9%	ns		ns
	Never	56.4%	27.3%	63.6%	$\chi^2_{(1)}$=14.1 *P*=0.0002	37.7%	23.5%	48.2%	$\chi^2_{(1)}$=15.1 *P*=0.0001		$\chi^2_{(1)}$=6.4 *P*=0.01

Table 4-4: UK and international results for subjective assessment. Please note - where individual question response rates were less than the total, this was due to either branching logic or dropout. Test statistic (parametric or non-parametric as appropriate) and associated P value given for statistically significant results (where P<0.05). NS = non-statistically significant. Statistical significance tested between groups as per headings (UK rhinologists vs non-rhinologists; international rhinologists vs non-rhinologists; UK vs international rhinologists; UK vs international non-rhinologists). Figures (proportions/test statistics) shown to one decimal place, p values shown to one significant figure.

4.5.5 Further Optional Questions

4.5.5.1 Retronasal Smell Tests

Seventy-eight UK respondents answered the optional question on use of retronasal smell tests. Of these, only two used such tests – one the 'Candy Smell Test' and one flavour powders.

One hundred twenty-five international respondents answered this question. Of these, 20% used such tests. The most common specific type of retronasal test used was the 'Retronasal Olfaction Test' or flavour powders (14 responses), followed by intravenous olfactory tests (6 responses), the Candy Smell Test (2 responses) and home-made tests (2 responses). Though taste tests were not specifically questioned, 2 respondents reported using taste strips.

4.5.5.2 Assessment of Qualitative OD

Eighty-two UK respondents answered the optional question on assessment of qualitative OD. From a list of non-mutually exclusive options, the most commonly used method was clinical history (66.1% of 124 total responses), followed by symptom questionnaires (17.7% of responses) and quantitative smell tests (e.g., Sniffin' Sticks/UPSIT) (13.7% of responses). Only one respondent said they used a specific qualitative smell test such as the Sniffin' Sticks parosmia test ('SSParoT').

One hundred twenty-six international respondents answered this question. The most common assessment method was clinical history (49.8% of 235 total responses), followed by quantitative smell tests (25.5% of responses) and symptom questionnaires (20.9% of responses). A total of 8 respondents reported using smell tests specific for qualitative OD (e.g., 'SSParoT') (3.4% of responses) and one respondent reported using application of local anaesthetic in the OC.

4.5.5.3 Assessment of Issues Related to OD

Of the 74 UK respondents who reported their assessment and referral practice with regard to problems associated with OD, memory/neurological issues were the most

commonly assessed (47.3%) or referred on (55.4%), followed by mental health issues (31.1% assessed, 37.8% referred on). An equal number of respondents would assess for nutritional and endocrine issues (both 20.3%), but more respondents would refer on for the latter (35.1% endocrine, 24.3% nutrition).

Internationally, of the 66 respondents who reported their practice, as in the UK, memory/neurological issues were the most commonly assessed (33.7% of 98 total responses) or referred on (29.1%), followed by assessment of mental health issues (assessed – 28.6%; referred on – 25.5%). Slightly more respondents reported assessing for nutritional issues than endocrine issues (19.4% and 18.4% respectively), though this pattern was reversed for referral practices (19.5% and 25.9% respectively).

4.5.5.4 Effect of COVID-19 Pandemic

Within the UK, prior to the COVID-19 pandemic, the mean number of patients seen with OD per month was 4.5 (SD 6.4, range 0–35, 75 respondents). At the time of questionnaire completion, the mean number of patients seen per month had statistically significantly increased to 6.4 (SD 6.9, range 0-30; W=938, P<0.0001). One respondent commented that they were no longer seeing patients due to shielding and two commented that they were not seeing 'routine' patients (including those with OD) due to pandemic backlog. Internationally, the mean number of patients seen with OD per month prior to the pandemic was 13.6 (SD 21.6, range 0–120, 120 respondents). At the time of questionnaire completion, the mean number of patients seen per month had statistically significantly increased to 18.1 (SD 24.1, range 0-120; W=3330, P<0.0001).

Prior to the pandemic, the four most common aetiologies of OD seen in the UK were: OD associated with inflammatory sinonasal disease (28.6% of 269 total responses); PIOD (23.4%); idiopathic OD (19.3%) and PTOD (11.2%). At time of questionnaire completion, the four most common aetiologies of OD seen in the UK were: OD associated with inflammatory sinonasal disease (25.1% of 263 total responses); COVID-19 related OD (20.5%); PIOD (19.8%) and idiopathic (16.4%).

Amongst international respondents, the same patterns of results were demonstrated. Prior to the pandemic, the four most common aetiologies of OD seen internationally were: OD associated with inflammatory sinonasal disease (26.1% of 440 total responses); PIOD (25.0%); idiopathic OD (17.1%) and PTOD (16.1%). At time of questionnaire completion, the four most common aetiologies of OD seen internationally were: OD associated with inflammatory sinonasal disease (24.6% of 447 total responses); COVID-19 related OD (23.5%); PIOD (19.7%) and idiopathic (11.2%).

When asked how the COVID-19 pandemic had changed their practice, 36 UK respondents provided free text comments (respondents were instructed to 'leave blank' if it had not affected their practice). Though respondents were specifically asked about their practice 'in the assessment of OD', answers covered all aspects of care during the pandemic and were analysed accordingly. Content could be arranged into the following, non-mutually exclusive subheadings: 1. infection control (patient/clinician); 2. workload (clinician); 3. attitudes and diagnostic assumptions (patient/clinician); 4. information and knowledge (patient/clinician); 5. treatment. Subheadings 1 and 3 were the most frequently occurring, followed by subheadings 2, 4 and 5. With regard to infection control (subheading 1), comments mainly focused on examination, including nasendoscopy, which is an aerosol generating procedure. There was concern over increased procedural difficulty, and risk to clinician health: *'Rigid nasal endoscopy used to be routine for all patients with OD. Now, I only do it when it's absolutely necessary due to the risk of contracting COVID', 'I try to avoid endoscopy if possible', 'Routine clinical examination [is] hampered'.* Respondents also commented on reduced frequency of smell testing in relation to infection control ('*Also, I perform smell tests much less often'*) and use of telemedicine to perform 'virtual' clinics. As could be expected, respondents reported increased OD workload (subheading 2) due to the pandemic ('*more patients with olfactory problems', 'created additional long COVID anosmia clinics'*). However, reduced ability to see 'non-urgent' OD cases due to pandemic backlog was also reported. Respondents also described apparent diagnostic assumptions (subheading 3), on both the part of the clinician and patient, and their effects on practice: '*We see*

them later, because many don't think it's anything other than covid', 'If Covid related probably do not investigate further following normal examination (endoscopy)', 'Confirmed covid cases less likely to be investigated with imaging', 'far [fewer] scans if very much covid related'. However, several respondents said that they were now more thorough in their clinical assessment ('*It has totally changed my assessment of OD patients, I am now obliged to do [as] detailed evaluation as diagnostic test availability will allow*', '*Much more detailed history including parosmia / phantosmia questions'*). This may be due to changing attitudes towards OD in clinicians, or increased expectations in a more educated patient cohort (subheading 3). However, further data is needed for clarification and expansion of this point. Comments were made by respondents regarding the mode of delivery of information (subheading 4) to patients (*'Increased use of online resources suggested to patients including fifthsense.org and abscent.org', 'use of patient information sheets'*), as well as the need to gather more information ('*Made me consider setting up a proper clinical study'*). Finally, several respondents also described the effect of the pandemic on their treatment practice (subheading 5): '*Recommend smell training more - less sure what to do with steroids as [they were] always the treatment that seemed to have [the] most evidence to be effective', 'I now robustly recommend smell retraining [sic]'*.

Sixty-two international respondents provided free text comments on how the COVID-19 pandemic had changed their practice (again, respondents were instructed to 'leave blank' if it had not affected their practice). As in the UK, content covered all aspects of olfactory care, and the majority could be organised into subheadings 1 – 5. For example, respondents described less frequent nasendoscopy, increased use of personal protective equipment and reduced or altered psychophysical smell testing in response to infection control issues (subheading 1): '*Less endoscopes [sic]', 'Wear the N95 mask to see and test patients', 'Decrease in olfactory testing due to pandemic', 'all smell tests are performed at home (UPSIT)', 'Use of screening tests for early assessment and full test 2 weeks later'*. However, some respondents described increased use of smell tests in response to the pandemic: '*Before we didn't perform the sniffin test [sic]', 'I have now olfactory test [sic]', 'I am more prone to smell*

testing in post-COVID patients'. This increase in testing may have been due to increased demand (due both to increased number of patients with OD, and due to increased patient awareness/expectations) as well as increased awareness/education on the part of the clinician (subheadings 2, 3 and 4): 'More patients, more worried about it [sic]', '*There was a surge in postviral cases with delays in consultations especially for smell testing – we hired / trained more staff to smell test patients', 'The patients' own interest has required increasing my level of knowledge and proposing specific personnel to perform olfactometric tests [sic]', 'I have now olfactory test and I have learned much more of olfaction [sic]', 'It boosted our knowledge, our research, our use of sniffin tests'.* The use of online resources was less commonly cited amongst international respondents, though increased research was evident: '*We have now many patients coming from all the central Italy who come to our center to have OD assessment and participation in studies for olfactory rehabilitation [sic]*'. As in the UK, investigations were modified according to actual/likely COVID-19 status – in particular, scanning in suspected C19OD was less common (subheading 3): '*Previously MRI was performed in all patients (including post-infectious). Now, I rarely ask MRI for post infectious (Covid 19 or not)', 'In COVID-19 related I do not perform MRI scan'*. Internationally, antibody testing was also cited, which was not mentioned in the UK. Subheading 2 occurred most frequently followed by subheading 1, and 4. The remaining subheadings (3 and 5) were least frequent.

4.5.6 'Any Other Comments'

Sixteen UK respondents provided comments at the end of the questionnaire when asked 'is there anything else you would like to say about the clinical assessment of olfaction / OD'. Categorisation of comments into subheadings was difficult due to the small number of responses, and of those received, many did not specifically address the clinical assessment of olfaction/OD. With this in mind, comments were addressed more generally.

Two UK respondents described poor training (‘*ENT doctors definitely need more training in this matter’, ‘This is something that is frequently glossed over in medical school and I had no formal training about it as a postgraduate trainee’*) and three described need for official guidance (‘*It’s clearly now a massive problem and a national standard work up should be produced by ENT-UK / BRS ideally and widely disseminated so we are all doing the same /right thing* [sic]’). Several comments were left which implied a negative or dismissive view towards the assessment or treatment of patients with OD. In one of the longest comments provided (88 words), one respondent said that they did not see an ‘*obvious reason to assess for [OD] as management decisions rarely affected by anything other than history’*. Another respondent said, ‘*Those who have always had OD prior to Covid 19 & were not bothered, are now trudging into ENT clinics hoping there is now a cure/treatment [sic]’*. However, these were countered by several more positive comments, such as: *‘I am glad attention is being focussed on this important aspect of quality of life. At last*!’, ‘*Agree it is poorly served’*, ‘*To encourage proper detailed history taking and referral to a specialist rhinology clinic for assessment’.*

Sixteen international respondents provided free text comments at the end of the questionnaire. Two respondents commented that ‘simple’ smell tests were needed. A further two respondents commented that standard protocols were needed – one saying ‘*world standard of olfactory test is needed’*. One respondent emphasised the need for funding and one the need for reimbursement for testing. Again, as in the UK, due to the small number of comments made, content was not organised into subheadings.

4.6 Discussion

4.6.1 Key Findings

To my knowledge this is the first detailed international survey of clinical practice in the assessment of olfaction. Responses were received from 465 clinicians, with the largest cohort originating from the UK, and country-specific response rate ranging from 1.4 to 72.5%. Most UK clinicians do not use psychophysical smell testing during their assessment of patients with OD, though rhinologists do so more frequently than non-rhinologists. There was more variability in international practice, and comparing by subspecialty training, where statistically significant differences in practice existed, testing was more common outside of the UK. The most common barriers to psychophysical testing were funding/time limitations. PROMs were infrequently used in the UK or internationally, though nearly a third of UK clinicians 'always' perform MRI scanning during the initial assessment of patients with OD.

4.6.2 Current State of Practice

As outlined in §1.5 assessment of OD can be performed using approaches ranging from subjective report to functional neuroimaging and electrophysiology. Subjective report can be captured through clinical history, anchored scales/questions, or more formally using validated PROMs. These methods are important for understanding patient experience and calculating the minimal clinically important difference. However, subjective assessment has been shown to correlate poorly with more 'objective' chemosensory testing, in both patient and healthy populations [217,218,220,221]. Commonly used, validated psychophysical tests, such as the SIT or Sniffin' Sticks, arguably represent the gold standard of clinical assessment[528]. Therefore, in the first (PPOD[425]), and subsequent sets of international guidelines[146,229], a key recommendation is that subjective report should not be performed in isolation, but rather combined with psychophysical smell testing. This mirrors the standard of care that is expected during the assessment of hearing or visual impairment.

Within the UK, most clinicians do not perform psychophysical testing in any of the clinical scenarios presented, including diagnosis, outcomes assessment or surgical complications monitoring. Indeed, for the initial assessment of OD as a presenting or isolated symptom, across all UK respondents, only 5.5% routinely tested, whilst 72.6% 'rarely' or 'never' tested. Comparing rhinologists with non-rhinologists, as could be expected, there was higher uptake in the former group, particularly at the extremes of testing frequency. The most common barriers to routine smell testing in the UK related to service provision (insufficient funding/time/staff) and lack of experience/training. It therefore follows that most respondents felt testing should take <5 minutes, irrespective of clinical scenario. Interestingly, despite poor rates of psychophysical testing, PROMs were not used consistently in the UK.

Whilst comparisons between UK and international cohorts should be interpreted with care (see limitations section), in general, there were higher levels of psychophysical testing amongst international respondents, across all clinical scenarios. The most common barrier to routine psychophysical testing amongst international clinicians was 'insufficient time', though other issues surrounding service provision (including insufficient staff/hospital-related funding) were also common. Despite this, international respondents were more tolerant towards longer smell tests, with most choosing 5-15 minutes as maximum acceptable testing time. As in the UK, PROMs were not consistently used – though they were used more frequently in the rhinologist subgroup.

Interestingly, in both the UK and international cohorts, 'refer on to specialist clinic' was an infrequent reason for not performing routine psychophysical testing. Furthermore, in both cohorts, approximately 3/4 said they would like to receive training in psychophysical testing.

Regarding imaging, a large proportion of UK-respondents 'always' performed MRI scanning of the brain/olfactory tract during the initial assessment of OD (31.3% of all respondents, 36.4% of rhinologists). Compared with the UK, international respondents performed MRI scanning less frequently and with more variability – with 'sometimes' being the most frequently chosen response. MRI can be used to provide diagnostic (through identification of structural abnormalities, e.g., neoplasia

or OB hypo-/aplasia) and/or prognostic information (e.g., volumetric assessment of the OB). Whilst lack of hypothetical-aetiology information and relative subjectivity of the Likert-frequency terms used (particularly 'most of the time'/'sometimes'/'rarely') somewhat limits interpretation of my data, it is likely that the cohort of clinicians who 'always' scan contains two subgroups – those who perform MRI scanning to obtain prognostic information, and those who scan for more indiscriminate diagnostic purposes (supported by 'exclusion of neoplasm' being the most frequently chosen aim of scanning overall). In the latter subgroup, a more tailored approach could be encouraged through increased psychophysical testing, education and more comprehensive imaging guidelines[333,425]. Such an approach could enable more cost-effective healthcare and limit patient burden, including associated indirect healthcare costs. Savings made could, in theory, be directed towards providing more funding for psychophysical smell testing. At the time of survey, no imaging guidelines were available, however, the 2023 update to the PPOD now provides expert-agreed recommendations on scanning practice for different suspected aetiologies of OD[166]. Future work should aim to interrogate imaging practice in more detail, through prospective auditing of aetiology-specific scanning practice and subsequent diagnostic/prognostic outcome yield. Ultimately, the establishment of evidence-based imaging practice amongst all clinicians is needed to ensure that patients receive access to appropriate investigations as standard. Finally, the clinical utility of MRI scans should be maximised as far as possible. To this end, I will investigate potential neuroanatomical correlates of OD, upstream of the OB, in the second half of this thesis (Theme B).

4.6.3 Comparisons with Other Studies

The last available UK data on clinical practice in the assessment of olfaction is almost two decades old. In 2007, McNeill and colleagues published results from a UK survey of clinical practice on the assessment and management of olfactory dysfunction [334]. Information was gathered from 266 otolaryngologists, of whom 97.4% evaluated patients with OD. Of these only 5.4% routinely used psychophysical smell tests, whilst 39.8% used them less frequently than this. Most clinicians, however, did not

use any form of smell test (54.8%). Similar results were obtained during a 2009 survey of British ENT consultants by Williams and colleagues: of 256 responding clinicians, 37% stated that they would perform a formal smell test when assessing a hypothetical patient with PIOD (∴ 63% did not test) [318]. Regarding imaging, 36.6% and 29% of respondents in these respective surveys performed MRIs – also comparable to my 31.3% (across all respondents) who 'always' performed MRI.

Accordingly, there appears to have been little progress in UK-based assessment practice since this time. However, these surveys were limited in scope: each contained less than four basic questions on use of specific psychophysical tools and radiological investigations, but no further information on assessment was gathered. For example, general and specific situations in which smell tests would be performed (e.g., during diagnosis, outcomes and complications assessment), use of PROMs or rationale for radiological investigations were not addressed. I have now significantly expanded on these early findings, providing the most comprehensive available insights into practice in different clinical scenarios, in different subspecialty groups, as well as barriers to psychophysical testing. To my knowledge, no other unified international surveys have been conducted.

4.6.4 Study Limitations and Future Work

The major limitations to this study were: 1. variable distribution method/response rate; 2. comparison of different healthcare systems; 3. language barriers; 4. intercurrent pandemic.

Whilst survey distribution was conducted via national mailing lists/professional societies where possible, alternative methods were necessary in four countries. Response rates varied with distribution method and were lower where mailing lists targeted multiple subspecialties (including clinicians who do not see patients with OD). This, in addition to geographical differences in the proportion of respondents with subspeciality training in rhinology, prevented direct country comparisons, due to probable differences in selection bias. To mitigate these effects, as well as inherent differences in healthcare systems, only subgroup comparison according to

subspecialty training in rhinology was performed. Whilst I recognise such training may itself vary geographically, I would argue that appropriate olfactory assessment should be standard of care, and both known/available to all 'rhinologists' across healthcare systems. However, to further mitigate the effects of selection bias and improve the validity and generalisability of the results obtained, future studies should aim to use probability (random) sampling with a standardised distribution method across geographical boundaries. Within the UK, whilst my total sample size was almost 2.5 x my minimum sample size of 92, this minimum value was an exploratory guide, given that my non-random sampling technique violated the assumptions for the sample size formulae used (see §3.2.1). Use of random sampling (with sufficiently high response rate) would allow for valid calculations of minimum sample size, and greater confidence in the results obtained. That being said, even in the context of random sampling, voluntary survey response rates in clinicians are often low (mean 15.7% in recent review of oral and maxillofacial surgery questionnaires[530]) – future work should therefore additionally consider strategies to increase responses, e.g. financial incentives[424]. Finally, due to software limitations, I was unable to gather information on unique site visitor/completion rate. As survey drop-out may represent a source of selection bias (with those completing the survey having greater interest in olfaction compared with those who didn't), future work should aim to collect this information, which could in turn be used to determine systematic differences between 'drop-out' and 'non-drop-out' respondent groups and ultimately better inform the generalisability of gathered data.

Unfortunately, funding was not available to facilitate translation of the survey. This may have excluded clinicians with limited English literacy skills. Future work should provide standardised translation to the local language of the target country.

This survey was distributed during the COVID-19 pandemic. Whilst it was targeted at routine practice, I recognise that it was undertaken at a uniquely challenging time, that may limit the generalisability of my results. This survey should therefore be repeated in future, non-pandemic circumstances.

Finally, I used a UK-based panel of both olfactory experts and non-experts to assist in the development of my questionnaire. Whilst I do not consider this to be a limitation

of my study, the design of future questionnaires on this and allied topics may benefit from multiple stakeholder involvement, including end-users, clinicians, nurses/auxiliary clinical staff and policy makers.

4.6.5 Conclusion

To my knowledge, this is the most detailed description of current clinical practice in the assessment of OD to date. I have outlined various areas in which such practice falls short of the standards outlined by contemporaneously available guidelines.

Accordingly, I suggest the following key recommendations:

- Increased education in olfaction and appropriate psychophysical testing at under-/postgraduate level.
- Increased publicity for existing/future guidelines on the assessment of olfaction, including key documents being available via open access and distributed via national/international societies/other mailing lists.
- Increased funding for provision of psychophysical testing – covering provision of tests, staff and clinic time.
- Clear referral pathways to specialist clinics where full assessment is not locally available.
- Future psychophysical tests should be efficient but clinically informative. Use of novel technologies (e.g., automation) to reduce clinical testing burden should be explored.
- Future surveys may benefit from multi-stakeholder involvement at the design stage and should be distributed using randomised sampling in a way that is standardised across geographical boundaries, with appropriate translation to the local language.

Much of the above would be better introduced and sustained through the engagement of national and international societies and/or clinical and research

collaboratives, both those that are specifically dedicated to olfaction, and those that serve rhinology and ENT more generally.

I hope that these recommendations will help to shape future practice, and rightfully promote accurate and reliable olfactory assessment, as is the accepted standard in other sensory systems.

5 Clinical Assessment of Olfaction: End-User Experience and Preferences

5.1 Summary

Accurate assessment of olfaction is an important first step in the healthcare narrative, affording insights that validate and legitimise patient experience. Whilst previous work has demonstrated dissatisfaction amongst patients in their olfactory healthcare encounters, only very limited previous work has specifically investigated patient experience of olfactory assessment, and none has investigated patient preferences for such assessment. In this chapter, I describe the results of a patient-co-produced survey that was distributed internationally via social media to healthcare seeking adults. My primary aim was to capture end-user (patient and healthcare seeking adults) experience of, and preferences for the assessment of olfaction. Specifically, I aimed to describe: 1. how patients are being assessed clinically; 2. how satisfied patients are with their assessment, and factors that affect this; 3. preferences for assessment in healthcare seeking adults. My secondary aim was to gather 'real-world' data for comparison with clinician-reported results from chapter 4. Quantitative and semi-qualitative data on experience and preferences for olfactory assessment were gathered and analysed using standard quantitative techniques and thematic analysis respectively. Five hundred seventy-six people responded, most of whom were female, affected by COVID-19 and from the USA/UK (with the remaining respondents being distributed across a further 31 countries). Just over half of respondents had been assessed by a healthcare professional – with GP/family doctor being the most commonly consulted, followed by ENT surgeons. People with subjective anosmia were more likely to be seen by an ENT surgeon. Only 15.6% and 16.9% of respondents (across specialties and geographical locations) had undergone systematic assessment with psychophysical smell tests or PROMs, respectively. In respondents who had been assessed by ENT (across geographical locations), these figures were 24.6% and 26.3%, respectively. Across all respondents,

the highest proportion had not been referred for imaging. However, amongst those who had been seen by ENT in the UK, the highest proportion had been referred for an MRI. Mean satisfaction was higher in those seen by ENT, and those who had undergone more thorough assessment, particularly imaging. Interestingly, respondents prioritise orthonasal odour identification over other forms of smell test. Unfortunately, many felt that healthcare professionals (across different specialties) were dismissive towards OD and lacked appropriate knowledge of both its pathophysiology and effects. My results provide the first in-depth analysis of end-user experience and preferences for olfactory assessment. Accordingly, I build on my recommendations for change in the previous chapter and propose simple steps that can be taken to improve olfactory assessment from the end-user perspective.

5.2 Statement of Contribution

This chapter has been adapted from my published paper: **Whitcroft KL, Kelly C, Andrews P. Patient Experience and Preferences for the Assessment of Olfaction: The Patient International Clinical Assessment of Smell Survey. *ORL* (2024) 86 (1): 16–31. doi: 10.1159/000535794. PMID: 38266502.** For the purposes of my thesis, sections have been expanded or reduced and language/style modified as necessary.

CK and I co-produced the survey. I analysed the data and interpreted the results. I wrote the manuscript. CK and PA critically appraised the resultant manuscript for intellectual content and approved its final version.

5.3 Introduction

Prior to the COVID-19 pandemic, olfactory dysfunction (OD) was thought to affect just over one fifth of the general adult population[223]. SARS-CoV-2 has significantly added to this number, though it is unclear how many patients will go on to develop long-term, PIOD. With roles in environmental navigation and hazard avoidance, social communication and nutrition, the personal impact of OD on physical and mental health is significant[199]. Indeed, approximately 1/3 of patients with OD

experience symptoms of depression[205]. Consequently, OD carries large direct and indirect healthcare and wider societal costs[212,213].

As outlined previously, the accuracy of olfactory assessment varies according to technique used, and in my previous chapter, I demonstrated marked heterogeneity in clinician reported assessment practice. Accurate assessment of olfactory function and dysfunction is important, not just for good clinical and research care, but also as an important first step in the patient narrative. In their 2021 study, Burges Watson and colleagues concluded that appropriate diagnosis and supporting explanations 'validated, legitimised and normalised people's experiences' of OD [415].

Patient experience is one of three UK statutory domains for quality healthcare (in addition to safety and clinical effectiveness)[409], with access to accurate and reliable olfactory assessment being relevant to several key areas within the 'NHS Patient Experience Framework'[531]. Whilst some previous studies have explored general experience of and barriers to good olfactory care[414,532], only very limited previous work from the Netherlands has specifically addressed patient experience of olfactory assessment, and none preferences for such assessment[416]. Furthermore, to my knowledge no previous work in this area has been patient co-produced. I therefore aimed to expand on my clinician-based work by addressing the assessment of olfaction through the lens of the healthcare seeking adult. More specifically, using a patient co-produced survey, my primary aims were to describe: 1. how patients are being assessed clinically; 2. how satisfied patients are with their assessment, and factors that affect this; 3. preferences for assessment in healthcare seeking adults. My secondary aim was to capture 'real world' data on the clinical assessment of olfaction for comparison with my clinician-reported data collected in chapter 4.

5.4 Methods

5.4.1 Survey development and distribution

I wrote a patient co-produced anonymous questionnaire for distribution online. My target population was olfactory 'healthcare seeking' adults (≥18 years) – i.e., those who had undergone, or might in the future undergo, olfactory assessment by a healthcare professional. As I was interested in the views of people *seeking* healthcare, formal diagnosis of OD was not required as part of the eligibility criteria; data from respondents who had not been seen by a healthcare professional or who did not have OD were only collected regarding my third primary aim, 'preferences for assessment' (see information regarding branching logic,§0). Respondents assessed at any care level were included (primary/secondary/tertiary care), as were those assessed by different specialties (e.g, ENT/neurology – though subgroup analysis for the ENT subgroup was planned in order to compare my results with my chapter 4 work[533]). Item generation and reduction was undertaken using a recursive, two-step process: 1. literature review – including my chapter 4 results, contemporaneously available guidelines[146,425,534] and other relevant literature; 2. key informant co-production – CK is an 'expert patient', founder of the OD charity AbScent and a moderator for its online support groups. Simultaneous item generation and reduction was performed during initial discussions, which were informed by the above literature, between myself and CK. I then wrote the questionnaire. This was followed by detailed draft questionnaire review by CK and subsequent item modification as appropriate. The final questionnaire covered the following domains:

- Respondent's self-reported olfactory function and demographics
- Assessment
    - Details including psychophysical tests, PROMs and imaging
    - Experience
    - Preferences

Further details on survey construction can be found in §0. Piloting was performed during iterative rounds of feedback with CK, as well as with a further sample of 5 pilot participants.

Dissemination of the survey was undertaken in collaboration with my co-producing colleagues, the AbScent charity [https://abscent.org/] via their main social media platform (see §0 for details). Accordingly, online distribution was performed via a post on the AbScent Facebook Support Group – which was in place between 2nd – 8th Feb 2022 (though the survey remained open to responses until June 2022). No page traffic information was available. Given the intercurrent limitations of the pandemic, in-person distribution methods were not pursued.

The full questionnaire can be found in appendix 10.4.

5.4.2 Ethical considerations

Ethical approval was obtained from UCL (REC Approval ID Number: 20479/001). Informed, written consent was obtained from all respondents. See §0 for more details.

5.4.3 Statistical analysis

An exploratory UK sample size of 96 was used as a minimum guide. See §0 for details. Quantitative data were analysed using GraphPad Prism (GraphPad Software, LaJolla, CA). Data were assessed for normality and parametric or nonparametric tests used. If response rates to individual questions were lower than total respondents (due to dropout/branching logic), this is stated. Proportions are given for total respondent number or total response number, where answers were non-mutually exclusive. Missing data were excluded from statistical analysis. Results are given as mean/median (± standard deviation).

I analysed my qualitative data using thematic analysis. Please see §0 for further details.

Figures were prepared using GraphPad Prism (GraphPad Software, LaJolla, CA), Microsoft PowerPoint (Microsoft Corporation, Redmond, WA) and MapChart (CC BY-SA 4.0, available from: https://www.mapchart.net/world.html).

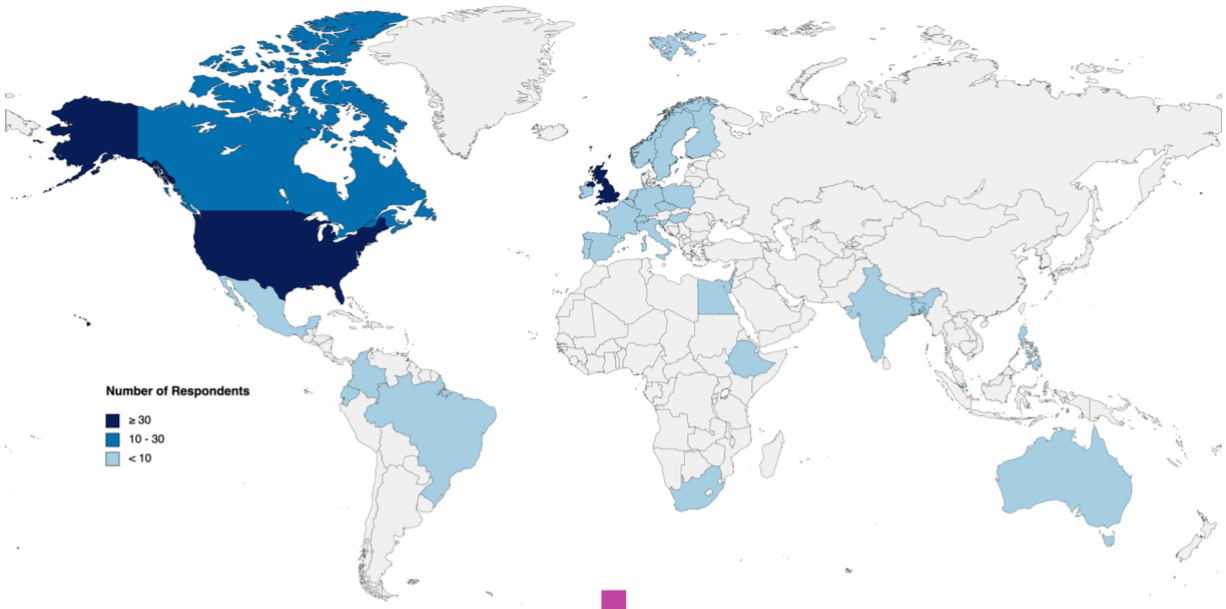
Results are reported in line with CROSS guidelines[535]. Whilst lay terms were used in the questionnaire, only their clinical/operational correlates are reported in the results.

5.5 Results

5.5.1 Sample Population

Data was gathered from 576 respondents. Given the nature of distribution, it was not possible to determine how many people had seen the survey invitation, preventing calculation of response rate. 47.1% of respondents were from the USA and 38.9% from the UK. The remaining respondents were widely distributed across a further 31 countries, all of which contributed less than 1% of the total cohort, except Canada, which contributed 2.8% (see Figure 5-1). Accordingly, analysis was performed across the entire cohort and UK/USA subgroups. Subgroup analysis was also performed separately for those who were assessed by ENT across the whole cohort and within the UK/USA. Mean age across all respondents was 46 (12) years and the majority were female (88.5%). See

Table 5-1 for full demographics.



Region									
Americas		Africa		Europe		Asia		Oceania	
Country	*Respondents*	*Country*	*Respondents*	*Country*	*Respondents*	*Country*	*Respondents*	*Country*	*Respondents*
USA	271	S Africa	3	UK	224	Israel	3	Australia	3
Canada	16	Ethiopia	1	Sweden	5	Lebanon	2		
Brazil	2	Egypt	1	France	4	India	2		
Mexico	2			Spain	4	Singapore	2		
Colombia	1			Switzerland	4	Bangladesh	1		
Ecuador	1			Germany	3	Philippines	1		
				Ireland	3				
				The Netherlands	3				
				Portugal	3				
				Italy	2				
				Poland	2				
				Norway	2				
				Belgium	1				
				Czech Republic	1				
				Finland	1				
				Hungary	1				
				Slovenia	1				

Figure 5-1: Geographical distribution of respondents

5.5.2 Self-Reported OD Type, Type of and Access to Healthcare Assessment

Across the whole cohort, in response to the question 'do you have a problem with your sense of smell', 561 answered 'yes', eight 'maybe' and seven 'no'. The majority of respondents stated that their problem was related to COVID-19 ('yes'/'maybe' – 492 ('C19OD'), 'no' – 77). The types of self-reported OD overall, and within subgroups with or without COVID-19 are shown in Figure 2.

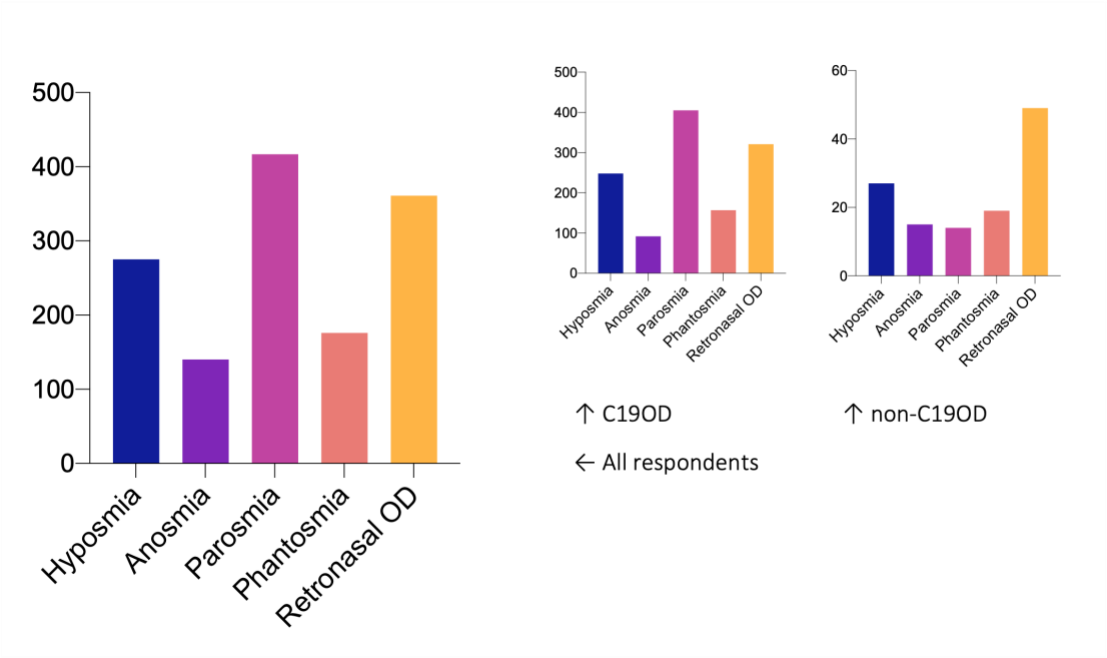


Figure 5-2: Self-reported OD type across all respondents (left) and those with C19OD (top left) and non-C19OD (top right). Please note that options were not mutually exclusive, and different y axis scale.

Across all respondents, there was a statistically significantly higher proportion of hyposmia and parosmia in the C19OD group than the non-C19OD group (hyposmia: 50.4% vs 35.1% respectively, χ^2=6.3, *P*=0.012; parosmia: 82.3% vs 18.2% respectively, χ^2=141.1, *P*<0.0001). Conversely, there was a statistically significant lower proportion of respondents reporting anosmia in the C19OD group than the non-C19OD group (18.7% vs 67.5% respectively, χ^2=84, *P*<0.0001). There was no statistically significant difference in proportion of phantosmia or retronasal OD according to C19OD status.

Just over half of all respondents reporting OD/possible OD had seen a healthcare professional (55.2%). A significantly higher proportion of USA respondents had been assessed than UK respondents (61.5% vs 47.3%, χ^2=9.9, *P*=0.002). Across the whole cohort and within UK and USA subgroups, respondents had most commonly seen their GP/family doctor, followed by an ENT surgeon (seeTable 5-1). Within both the UK and USA subgroups, a significantly higher proportion of patients with C19OD had not been assessed by a healthcare professional, than those with non-C19OD (UK: 58.4% vs 18.8% respectively, χ^2=17.3, *P*<0.0001; USA: 43.5% vs 3.0% respectively, Fisher's Exact, *P*<0.0001).

Across all respondents, 40.8% of respondents had not seen a 'specialist' (e.g., ENT/neurologist). A higher proportion of respondents in the UK had not been assessed by a specialist, compared with the USA (48.6% vs 38.6% respectively), though this difference in proportions did not reach statistical significance (χ^2=2.6, *P*=0.1). Overall, 21.6% of respondents reported problems in accessing specialist care, with 'my GP/family doctor did not want to refer me' being the most commonly selected reason (15.0% of all respondents, 28.6% of UK respondents and 7.8% of USA respondents). Common 'other' reasons for difficulty in accessing specialist care included long waiting list times, particularly within the UK.

Across all respondents, significantly fewer patients were assessed by ENT surgeons when parosmia was reported, than when it was not (24.3% vs 46.5% respectively, χ^2=26.5, *P*<0.0001). Conversely, significantly more patients who reported anosmia were assessed by ENT (50.0% vs 23.8% respectively, χ^2=34.9, *P*<0.0001). Given the significantly varying proportions of parosmia/anosmia according to C19OD status (outlined above), further analysis was performed for C19OD +/- subgroups. The statistically significant relationship between parosmia reporting and assessment by ENT disappeared when C19OD and non-C19OD subgroups were analysed separately, indicating that C19OD status confounded these results. However, in both C19OD and non-C19OD groups, the proportion of patients assessed by ENT was significantly higher when anosmia was reported than when it was not (C19OD - 33.7% vs 22.5% respectively, χ^2=5.1, *P*=0.025; non-C19OD - 78.9% vs 52% respectively, χ^2=5.8,

P=0.02). There were no significant differences in proportions of respondents assessed by ENT according to presence of hyposmia, phantosmia or retronasal OD.

Demographics

Question	Answer *(Operational correlate, where relevant)*	*All (n=576)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=224)*	*UK ENT (n=49)*	*USA (n=271)*	*USA ENT (n=98)*
Do you have a problem with your sense of smell?	***Yes***	561 97.40%	175 100%	137 99%	218 97.30%	49 100%	270 99.60%	98 100%
	No	7 1.20%	-	0	2 1.00%	-	1 0.40%	-
	Maybe	8 1.40%	-	2 1.40%	4 1.80%	-	0	-
What is the problem with your sense of smell? * †	***Hyposmia***	274 48.20%	79 45.10%	64 46.04%	100 45.05%	15 30.60%	132 48.90%	49 50.00%
	Anosmia	140 24.60%	72 41.10%	29 20.86%	55 24.77%	24 49.00%	72 26.70%	38 38.80%
	Parosmia	417 73.30%	102 58.30%	115 82.73%	158 71.17%	22 44.90%	206 76.30%	64 65.30%
	Phantosmia	176 30.90%	50 28.60%	48 34.53%	60 27.03%	9 18.40%	93 34.40%	32 32.70%
	Retronasal OD	361 63.40%	114 65.10%	95 68.35%	142 63.96%	33 67.30%	181 67.00%	64 65.30%
	Other	22 3.90%	1 0.60%	0 0%%	5 2.25%	1 2.00%	6 2.20%	0 0%

Table continued overleaf…

Question	Answer *(Operational correlate, where relevant)*	*All (n=576)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=224)*	*UK ENT (n=49)*	*USA (n=271)*	*USA ENT (n=98)*
Is this problem related to COVID-19? ^ ***N (%)***	***Yes***	454 79.80%	105 60.00%	118 84.89%	175 78.80%	23 46.90%	218 80.70%	65 66.30%
	No	77 13.50%	54 30.90%	14 10.07%	32 14.40%	21 42.90%	33 12.20%	25 25.50%
	Maybe	38 6.70%	16 9.10%	7 5.04%	15 6.80%	5 10.20%	19 7.00%	8 8.20%
How old are you? (years)	***Mean (SD)***	46 (12)	48 (14)	45 (11)	47 (12)	50 (15)	47 (12)	48 (13)
Do you identify as? ***N (%)***	***Female***	510 88.50%	144 82.30%	131 94.24%	197 87.90%	38 77.60%	248 91.50%	85 86.70%
	Male	61 10.60%	29 16.60%	8 5.76%	26 11.60%	11 22.40%	22 8.10%	12 12.20%
	Prefer not to say	3 0.50%	2 1.10%	0 0.00%	1 0.40%	0 0.00%	1 0.40%	1 1.00%
	Other	2 0.30%	0 0	0 0	0 0	0 0	0 0	0 0

Table continued overleaf…

Assessment Details

Question	Answer *(Operational correlate, where relevant)*	*All (n=569)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=222)*	*UK ENT (n=49)*	*USA (n=270)*	*USA ENT (n=98)*
Have you seen a healthcare professional about your sense of smell? ^ ***N (%)***	***Yes***	314 55.20%	175 100.00%	139 100.00%	105 47.30%	49 100.00%	166 61.50%	98 100.00%
	No	255 44.80%	-	-	117 52.70%	-	104 38.50%	-
What kind of healthcare professional(s) have you seen about your sense of smell? * ‡ ***N (%)***	***GP/family Dr***	249 79.30%	-	129 92.81%	91 86.70%	-	125 75.30%	-
	ENT	175 55.70%	175 100.00%	-	49 46.70%	49 100.00%	98 59.00%	98 100.00%
	Neurologist	36 11.50%	-	8 5.76%	6 5.70%	-	19 11.40%	-
	Specialist Nurse	13 4.10%	-	4 2.88%	6 5.70%	-	6 3.60%	-
	Physician Associate/Assistant	15 4.80%	-	8 5.76%	1 1.00%	-	12 7.20%	-
	Other	33 10.50%	-	14 10.07%	4 3.80%	-	15 9.00%	-
	No specialist seen	128 40.8%	-	127 91.37%	51 48.60%	-	64 38.60%	-

Table continued overleaf…

Question	Answer *(Operational correlate, where relevant)*	*All (n=569)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=222)*	*UK ENT (n=49)*	*USA (n=270)*	*USA ENT (n=98)*
Did you have any problems accessing specialist care (e.g. ENT/Neurologist)? * ‡ ***N (%)***	***N/A***	61 19.40%	-	-	15 14.30%	-	38 22.90%	-
	No	166 52.90%	-	-	38 36.20%	-	97 58.40%	-
	Yes - my GP/family doctor did not want to refer me	47 15.00%	-	-	30 28.60%	-	13 7.80%	-
	Yes - my GP/family doctor was unable to refer me	13 4.10%	-	-	9 8.60%	-	3 1.80%	-
	Yes – problems due to insurance coverage	8 2.50%	-	-	0 0.00%	-	6 3.60%	-
	Other	34 10.80%	-	-	16 15.20%	-	9 5.40%	-
During your assessment with a healthcare professional, did they use a 'smell test'? ‡ ***N (%)***	***Yes***	49 15.60%	43 24.60%	6 4.30%	11 10.50%	10 20.40%	30 18.10%	26 26.50%
	No	265 84.40%	132 75.40%	134 96.40%	94 89.50%	39 79.60%	136 81.90%	72 73.50%
	Not sure	0	0	0	0	0	0	0

Table continued overleaf…

Question	Answer *(Operational correlate, where relevant)*	*All (n=576)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=224)*	*UK ENT (n=49)*	*USA (n=271)*	*USA ENT (n=98)*
If you took a 'smell test', what were you asked to do? * ¤ ***N (%)***	***Identification***	47 95.92%	41 95.35%	5 100.00%	11 100.00%	10 100.00%	26 86.67%	23 88.46%
	Discrimination	7 14.29%	6 13.95%	1 20.00%	1 9.09%	1 10.00%	4 13.33%	3 11.54%
	Threshold	6 12.24%	5 11.63%	1 20.00%	1 9.09%	1 10.00%	2 6.67%	1 3.85%
	Retronasal identification	8 16.33%	6 13.95%	2 20.00%	1 9.09%	1 10.00%	3 10.00%	2 7.69%
	Hedonics	3 6.12%	2 4.65%	1 20.00%	0 0.00%	0 0.00%	3 10.00%	2 7.69%
If you took a smell test roughly how long did it take?	***Mean in min (SD)***	20.7 (17.3)	22.6 (20.0)	13.3 (17.1)	21.9 (11.7)	24.0 (10.0)	24.3 (23.0)	25.4 (23.6)
Did you complete any questionnaires about your symptoms? ‡ ***N (%)***	***Yes***	53 16.90%	46 26.30%	7 5.04%	8 7.60%	8 16.30%	37 22.30%	30 30.60%
	No	261 83.10%	129 73.70%	132 94.96%	97 92.40%	41 83.70%	129 77.70%	68 69.40%

Table continued overleaf…

Question	Answer *(Operational correlate, where relevant)*	*All (n=576)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=224)*	*UK ENT (n=49)*	*USA (n=271)*	*USA ENT (n=98)*
Were you referred for a scan?* ‡ ***N (%)***	***Yes – MRI***	**69** 22.00%	**61** 34.90%	**9** 6.47%	**27** 25.70%	**20** 40.80%	**33** 19.90%	**28** 28.60%
	Yes – CT	53 16.90%	46 26.30%	8 5.76%	18 17.10%	11 22.40%	31 18.70%	27 27.60%
	Yes – not sure type	9 2.90%	8 4.60%	0 0.00%	0 0.00%	0 0.00%	8 4.80%	7 7.10%
	No	192 61.10%	72 41.10%	121 87.05%	63 60.00%	13 26.50%	101 60.80%	43 43.90%
	Other	5 1.60%	2 1.10%	2 1.44%	0 0.00%	0 0.00%	1 0.60%	0 0.00%
How satisfied were you with your assessment?	***Mean (SD)***	2.15 (1.26)	2.45 (1.33)	1.78 (1.1)	2.08 (1.21)	2.51 (1.36)	2.13 (1.27)	2.34 (1.29)
Did you have to pay for your assessment? (i.e., it was not provided by the NHS or covered by your insurance) ‡ ***N (%)***	***Yes***	75 23.90%	59 33.70%	15 10.79%	16 15.20%	14 28.60%	68 41.00%	48 49.00%
	No	208 66.20%	99 56.60%	109 78.42%	85 81.00%	34 69.40%	98 59.00%	50 51.00%
	Other	31 9.90%	17 9.70%	15 10.79%	4 3.80%	1 2.00%	0 0.00%	0.00%

Table continued overleaf…

Assessment Preferences

Question	Answer *(Operational correlate, where relevant)*	*All (n=576)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=224)*	*UK ENT (n=49)*	*USA (n=271)*	*USA ENT (n=98)*
For a problem with my sense of smell, I would prefer to be assessed by: *N (%)*	***GP/family doctor or other non-specialist***	55 9.50%	7 4.00%	18 12.95%	16 7.10%	1 2.00%	32 11.80%	5 5.10%
	Specialist (e.g. ENT/Neurologist)	497 86.30%	161 92.00%	116 83.45%	200 89.30%	47 95.90%	228 84.10%	88 89.80%
	Other	24 4.20%	7 4.00%	5 3.60%	8 3.60%	1 2.00%	11 4.10%	5 5.10%
How much time are you willing to spend taking a 'smell test' as part of an assessment of your sense of smell? *N (%)*	***< 5 min***	19 3.30%	2 1.10%	3 2.16%	6 2.70%	1 2.00%	12 4.40%	1 1.00%
	5 – 15 min	105 18.20%	28 16.00%	27 19.42%	26 11.60%	3 6.10%	60 22.10%	19 19.40%
	15 – 45 min	156 27.10%	49 28.00%	36 25.90%	55 24.60%	11 22.40%	83 30.60%	32 32.70%
	> 45 min	227 39.40%	67 38.30%	58 41.73%	97 43.30%	20 40.80%	99 36.50%	38 38.80%
	Other	69 12.00%	29 16.60%	15 10.79%	40 17.90%	14 28.60%	17 6.30%	8 8.20%

Table continued overleaf…

Question	Answer *(Operational correlate, where relevant)*	*All (n=576)*	*All ENT (n=175)*	*All non-ENT (n=139)*	*UK (n=224)*	*UK ENT (n=49)*	*USA (n=271)*	*USA ENT (n=98)*
Are you willing to travel outside of your local area for specialist smell assessment? *N (%)*	***Yes***	389	134	97	169	43	161	69
		67.50%	76.60%	69.78%	75.40%	87.80%	59.40%	70.40%
	No	58	14	12	13	2	37	9
		10.10%	8.00%	8.63%	5.80%	4.10%	13.70%	9.20%
	Maybe	129	27	30	42	4	73	20
		22.40%	15.40%	21.58%	18.80%	8.20%	26.90%	20.40%

Table 5-1: Demographics, assessment details and assessment preferences in following respondent groups (in order of columns from left to right): all; all seen by ENT; all seen by non-ENT providers; all UK respondents; all UK respondents seen by ENT; all USA respondents; all USA respondents seen by ENT. *Answers are not mutually exclusive, † % = proportion of respondents reporting symptom with respect to all those answering "yes"/"maybe" to "Do you have a problem with your sense of smell." ^ Total *n* excludes those who answered "no" to "Do you have a problem with your sense of smell." ‡ % = proportion of respondents with respect to all those who had seen a healthcare professional. ¤ % = proportion of respondents with respect to all those who took a smell test.

5.5.3 Smell tests

Overall, 15.6% of respondents who had been assessed by a healthcare professional had undergone psychophysical smell testing. A significantly higher proportion of respondents who had been assessed by an ENT surgeon underwent smell testing, compared to non-ENT healthcare professionals (24.6% vs 4.3% respectively, χ^2=26.3, *P*<0.0001). There was a higher proportion of smell testing amongst respondents who had seen an ENT surgeon in the USA than the UK (26.5% vs 20.4%), though this difference did not reach statistical significance. The most common type of smell test used, across all respondents and in each subgroup, was odour identification, and the least common was hedonic valence (see Table 5-1). Across all respondents the mean time taken for smell testing was 20.7 (17.3) minutes [22.6 (20.0) in ENT surgeons, 13.3 (17.1) in non-ENT healthcare professionals].

Respondents were asked to rank common clinically tested aspects of smell in order of importance to them. Across the whole cohort, rankings were as follows (with associated operational correlate, where relevant):

1. Odour identification (orthonasal odour identification)
2. Flavour identification (retronasal odour identification)
3. Odour detection (odour detection threshold)
4. Odour discrimination (odour quality discrimination)
5. Odour pleasantness/unpleasantness (hedonic valence)

5.5.4 Patient Reported Outcome Measures (PROMs)

Overall, 16.9% of respondents who had been assessed by a healthcare professional completed questionnaires about their symptoms (PROMs). As for smell testing, a significantly higher proportion of respondents who had been assessed by an ENT surgeon completed PROMs, compared to non-ENT healthcare professionals (26.3% vs 5.0% respectively, χ^2=24.9, *P*<0.0001). Again, there was a higher proportion of PROM use amongst respondents who had seen an ENT surgeon in the USA than the UK (30.6% vs 16.3%), though this difference did not reach statistical significance (see Table 5-1).

5.5.5 Imaging

Overall, 61.1% of respondents who had undergone assessment by a healthcare professional had *not* been referred[22] for imaging of any kind. Significantly fewer patients were referred for imaging by non-ENT healthcare providers than ENT surgeons (proportion of respondents *not* referred, ENT vs non-ENT, 41.1% vs 87.1%, χ^2=68.9, *P*<0.0001). Comparing practice amongst ENT surgeons, significantly fewer respondents were referred for imaging within the USA than UK (proportion of respondents *not* referred, UK vs USA, 26.5% vs 43.9% respectively, χ^2=68.9, *P*<0.0001). Indeed, within the UK, the highest proportion of patients had been referred for an MRI scan (40.8%). Of those who were referred for imaging, across all respondents and in each subgroup, MRI was the most common type of scan performed (though this was a small margin over CT in the USA, see Table 5-1).

5.5.6 Satisfaction

Of those respondents who had undergone assessment by a healthcare professional, overall mean satisfaction (where 1 = least satisfied and 5 = most satisfied) was 2.15 (1.26). Mean satisfaction was significantly higher in respondents seen by an ENT surgeon, than those who were seen by another type of non-ENT healthcare provider (2.45 (1.33) vs 1.78 (1.1), *U*=8684, *P*<0.0001). Within the ENT subgroup, mean satisfaction was higher in those respondents who had been referred for imaging (any kind) and in those who had completed PROMs (imaging (referred vs not) – 2.65 (1.38) vs 2.16 (1.21), *U*=2999, *P*=0.02; PROMs (completed vs not) – 2.80 (1.41) vs 2.32 (1.29), *U*=2383, *P*=0.041). Whilst mean satisfaction was higher in respondents who had undergone smell testing than those who had not (2.58 (1.38) vs 2.40 (1.32) respectively), this did not reach statistical significance. In the non-ENT assessed subgroup, mean satisfaction was significantly higher in those respondents who had undergone smell testing and in those who had been referred for imaging (smell

[22] *Please note - referral practice was interrogated as I was interested in clinical practice, rather than downstream/logistical barriers to scanning after point of referral.*

testing (performed vs not) – 3.33 (1.03) vs 1.71 (1.0), *U*=101.5, *P*=0.0004; imaging (referred vs not) – 2.31 (1.30) vs 1.71 (1.0), *U*=701, *P*=0.041). Again, whilst mean satisfaction was higher in those who underwent PROM testing than those who did not (3 (2) vs 1.7 (1.0) respectively), this did not reach statistical significance. Further information regarding number of patients undergoing specific test combinations, and associated mean satisfaction levels, can be found in Table 5-2.

Specific Test Combinations (N, *mean satisfaction)*

	All (n=314)	***All ENT (n=175)***	***All non-ENT (n=139)***	***UK (n=105)***	***UK ENT (n=49)***	***USA (n=166)***	***USA ENT (n=98)***
None	159 *1.75 ± 0.98*	44 *2.09 ± 1.14*	115 *1.62 ± 0.88*	63 *1.73 ± 0.95*	13 *2.08 ± 1.04*	75 *1.67 ± 0.94*	23 *1.91 ± 1.10*
Smell Test Only	10 *2.2 ± 1.03*	7 *1.86 ± 0.90*	3 *3.0 ± 1.0*	1 *2.0 ± 0*	1 *2.0 ± 0*	7 *2.29 ± 1.11*	5 *2.0 ± 1.0*
PROM Only	14 *2.07 ± 1.38*	10 *1.90 ±1.20*	4 *2.50 ± 1.92*	0 -	0 -	12 *2.08 ± 1.44*	8 *1.88 ± 1.25*
Scan Only	79 *2.46 ± 1.34*	67 *2.52 ± 1.36*	12 *2.08 ± 1.17*	29 *2.62 ± 1.43*	24 *2.75 ± 1.51*	40 *2.33 ± 1.25*	34 *2.32 ± 1.22*
Smell Test + PROM	12 *2.92 ± 1.62*	11 *2.73 ± 1.56*	1 *5.0 ± 0*	1 *1.0 ± 0*	1 *1.0 ± 0*	8 *3.38 ± 1.60*	7 *3.14 ± 1.57*
Smell Test + Scan	13 *2.39 ± 1.39*	11 *2.27 ± 1.49*	2 *3.0 ± 0*	4 *2.0 ± 1.16*	3 *1.67 ± 1.16*	7 *2.29 ± 1.38*	6 *2.17 ± 1.47*
PROM + Scan	13 *3.31 ± 1.49*	11 *3.36 ± 1.36*	2 *3.0 ± 2.83*	2 *3.50 ± 0.71*	2 *3.50 ± 0.71*	9 *3.0 ± 1.66*	7 *3.0 ± 1.53*
Smell Test + PROM + Scan	14 *3.07 ± 1.27*	14 *3.07 ± 1.27*	0 -	5 *3.0 ± 1.41*	5 *3.0 ± 1.41*	8 *3.13 ± 1.36*	8 *3.13 ± 1.36*

Table 5-2: Detailed breakdown of respondent number and associated satisfaction (mean ± SD) for different specific test combinations.

To gain greater insight into patient experience, respondents were asked two semi-qualitative free text questions: 1 – 'During your assessment, what was done well?'; 2 – 'During your assessment, what could have been done better'. Across the whole cohort of those who had been assessed, all but 8 respondents provided free text

answers to question 1, totalling 4,555 words of free text, and all but 18 respondents answered question 2, totalling 5,090 words.

Whilst the questions were specifically aimed at assessment, many respondents provided information regarding their wider olfactory healthcare journey, e.g., treatment and follow up. This information was additionally analysed to produce themes in relation to general olfactory healthcare, not limited to assessment. Furthermore, there was significant overlap in positive/negative experiences in both question 1 and 2 (e.g., 'nothing'/'not much' was stated by 65 respondents in response to question 1). Consequently, emergent themes were stratified independent to question. The main themes identified were 'knowledge', 'attitudes', 'rigour' and 'healthcare systems'. Various major and minor sub-themes were additionally identified, with some interaction between these. These, as well as representative data extracts can be found in Figure 5-3.

Theme	Major Sub-Theme	Minor Sub-Theme	Example Text - questions [1] & [2]
Knowledge	Provider's knowledge	• Anatomy + physiology of smell • Pathophysiology (quantitative + qualitative OD) • Collateral effects (psychosocial + physical) • Resultant preference for specialists	*'anatomy was explained' [1]* *'GP had no knowledge or interest in issue' [2]* *'all the ENTs I have seen so far (at least 3) kept saying that they didn't know much about smell disorders' [1]* *'I was the educator. She knew nothing really about it' [1]* *'They need to be more educated in the condition' [2]* *'Doctor doesn't know how to treat parosmia or cause of it' [1]* *'the doctor sounded like he had no idea what he was talking about' [1]* *'I felt I knew more about smell loss than both of them [ENT]' [1]* *'My doctor said she hadn't heard of such a thing. No more was said about it.' [1] '* *I received very little information or answers. I had to research everything myself' [2]* *'ENT explained various reasons for loss of smell' [1]* *'Not one of my physicians offered any patient handout information' [2]* *'Dr told me about smell training and handed me a printout on how to build a kit' [1]* *'Some expertise in this field' [2]* *'Encourage more research' [2]*
	Information Sharing	• Explanations • Joint decision making • Dissemination (written info/signposting to online resources) • Balance in Dr-patient relationship	
	Unknowns	• Research • Preference for specialist	
Attitudes	Professional-ism	• Communication skills • Avoiding comparison with other conditions	*'ENT was so rude, his comment was well Mrs soandso [sic], you have no smell but you don't have cancer!' [1]* *'GP thinks I'm not a priority as I'm not (in his view) at risk of dying from my condition' [1]* *'ENT…reassured me they believed me about my parosmia' [1]* *'First of all, I was listened to and believed' [1]* *'hospital…laughed…said I was lucky not to be able to smell what they could' [2]* *'Don't treat me like I don't matter because I have to live with this every second of every single day' [2]* *'I wish she had referred me to an ENT or given me info or even just ACTED LIKE SHE CARED!!! [sic]' [2]*
	Compassion	• Time • Interest/active listening • Reassurance/validation of concerns	
Rigour	Assessment	• History – OD and collateral effects • Examination, including FNE • Chemosensory testing ◦ Explanation of procedure, including 'forced choice' ◦ Test for qualitative OD ◦ Improved testing (particularly re qualitative) • Investigations ◦ Bloods ◦ Imaging • Discussion of results	*'I was diagnosed' [1]* *'Proper tests were done to try and assess the problem' [1]* *'ENT specialist took time to take detailed history' [1]* *'None of them asked how this was affecting my life' [2]* *'I was given a thorough exam (throat, nose, nose scoped, lots of questions asked, smell test) ' [1]* *'Full examination of my sinuses, smell test, lengthy discussion and education session' [1]* *'I was very satisfied with thoroughness of exam' [1]* *'They looked for anything fixable which they did not find – this is the right process ruling out other causes' [1]* *'Could have done more specific tests' [2]* *'standardized test to compare future visits with to compare improvement or not [sic]' [2]* *'smell test. Thorough analysis of smells that are abnormal' [2]* *'Smell test to assess and would allow awareness of changing symptoms' [2]* *'more accurate smell test [hyposmia/parosmia]' [2]* *'I feel smell tests don't really work [parosmia]' [2]* *'all tests were done for confirmation (MRI) and a very detailed letter issued to my GP. Small things but it made me feel believed as to how severe things had got and that someone cared' [1]* *'looked at other causes of smell loss' [1]* *'The health provider documented my loss of taste and smell as a health issue and possible permanent disability in my health record so that other clinicians and my insurance company are aware that I may need future treatments, testing' [1]* *'I had to insist that my symptoms were documented' [1]* *'talked through available evidence for treatments' [1]*
	Diagnosis	• Consideration of differentials • Documentation	
	Management	• Willingness to consider different treatment options • Address OD and collateral effects • Re-assess after intervention	
Healthcare Systems	Access	• Access to specialist care ◦ Secondary/tertiary referrals ◦ Allied specialists for collateral effects of OD • Access to follow up care • Face to face assessment • Timely	*'Referred to a specialist' [2]* *'I would have loved to be send [sic] to a specific unit to start smell training with the doctor's support. I felt abandoned' [2]* *'He could have given me a list of places that worked with loss of smell/taste' [2]* *'A quicker appointment. Not cancelling the appointment…then cancelling the follow up appointment' [2]* *'Nutritionist gave advice on food selection' [1]* *'Feedback and further consultation on possible options' [2]* *'Once the MRI result came back as clear, I was discharged from the ENT specialist without any opportunity to discuss the problems which I continue to live with and without signposting to other support' [2]* *'waste of my time and money' [1]* *'he did put a scope up my nose, but never told me it was a scope….so that was a nice bill' [1]*
	Affordability	• Specialist care • Travel	

Figure 5-3: Thematic analysis results showing theme, major and minor sub-themes, and exemplar text for question 1 ('what was done well') and 2 ('what could have been done better'). FNE = flexible nasendoscopy.

5.5.7 Preferences

Across the whole cohort, and in each subgroup, most respondents stated that they would prefer to be assessed by a specialist. Of the 'other' preferences entered in the free text, multiple respondents answered that they would prefer to be seen by 'anyone with the appropriate knowledge'. Across the whole cohort/in each subgroup, most respondents stated they were willing to travel to receive specialist smell assessment. See Table 5-1 for details.

Again, across the whole cohort, and in each subgroup, when asked 'how much time are you willing to spend taking a smell test as part of an assessment of your sense of smell', most respondents stated that they would spend >45 minutes. Of those who chose 'other', respondents most frequently stated that they were willing to spend 'any' amount of time being tested, often additionally qualifying this statement according to whether the smell test would be of benefit to their assessment.

5.6 Discussion

5.6.1 Key Findings

To my knowledge, this is the first co-produced survey to specifically address experience of, as well as preferences for, the assessment of olfaction in end-users. Responses were gathered from 576 participants across 33 countries, with the USA and UK representing the largest cohorts. The majority of respondents were female and reported COVID-19 related OD. Just over 40% of respondents had not been seen by a specialist (ENT/neurologist).

My key and/or novel findings were as follows:

- People reporting anosmia are more likely to be seen by an ENT surgeon.
- Evidence-guided assessment with psychophysical smell testing or PROMs is infrequently performed.
- People prioritise orthonasal odour identification over other forms of smell test.
- Most people are willing to travel and spend any amount of time necessary to undergo smell testing.
- Many people felt that healthcare professionals were dismissive and lacked appropriate knowledge of OD (including pathophysiology and effects).

5.6.2 Quantitative Results

As outlined previously in this thesis, the accuracy of olfactory assessment varies according to technique used[528]. Consequently, guidelines have been published, outlining good clinical and research practice[119,146,229,425,534]. In particular, unstructured subjective reporting without concurrent psychophysical smell testing is discouraged. However, I demonstrated poor adherence to such guidelines in my last chapter. Results from my clinician survey demonstrated that 72.6% of UK ENT surgeons 'never' or 'rarely' performed smell testing during the initial assessment of OD as a presenting or isolated symptom (see §4.5.2). Similar results were found across different diagnostic, outcomes and complications monitoring scenarios. My

present work corroborates these findings, having demonstrated poor rates of psychophysical testing amongst patients who had been assessed by a healthcare professional: across all geographical locations, 15.6% of all respondents and 24.6% of those assessed by an ENT surgeon; within the UK, 10.5% of all respondents and 20.4% of those assessed by an ENT surgeon (∴ 79.6% seen by UK ENT were not tested)[23].

During my clinician survey, limitations around service provision (including lack of funding/staff/time) were the major reported barriers to routine psychophysical testing. Whilst I did not directly explore perceived barriers to testing in this study, two themes identified during my qualitative analysis were 'attitudes' and 'healthcare systems'. These will be discussed in more detail below, but briefly, many respondents felt that their condition was not prioritised, due both to the attitudes of individual clinicians and limitations of current healthcare systems.

Importantly, to my knowledge, no previous work has investigated which of the commonly testable aspects of smell are most important to end-users. Accordingly, I demonstrated that orthonasal odour identification was felt to be most important, following by retronasal odour (flavour) identification and odour threshold. Odour discrimination and hedonic valence were felt to be least important. This was interesting, particularly as there was a high proportion of reported parosmia in the responding cohort. These novel results may help guide future psychophysical test development and service planning – particularly for clinical settings in which time and resources are limited.

I also found poor uptake of structured symptom questionnaires (PROMs) – which were used in 16.9% of all respondents, 26.3% of those assessed by an ENT surgeon (across geographical locations), and 16.3% of those assessed by an ENT surgeon in the UK (∴ 83.7% seen by UK ENT were not tested). Again, this was in line with my

[23] *Please note that only descriptive results are provided for comparison between clinician-reported and end-user reported data. I did not perform any statistical comparisons between these data sets due their multiple inherent differences making such statistics inappropriate.*

clinician-reported practice, where 72.4% of UK ENT surgeons reported 'never' or 'rarely' using PROMs (see §4.5.4). My current findings therefore confirm poor adherence to published guidelines that discourage use of unstructured subjective reporting. With regard to imaging, as could be expected, rates of referral were higher amongst those respondents assessed by ENT. Though single-centre recommendations for MRI scanning have been made[333], thorough imaging recommendations in contemporaneously available guidelines at time of survey were lacking[229,425]. However, the recently updated Position Paper on Olfactory Dysfunction: 2023 now contains a comprehensive set of expert-agreed imaging recommendations. Whilst this represents a useful educational resource and service planning framework, as discussed in §4.6.2, further prospective audit of imaging practice and associated outcomes is needed for the development robust, evidence-based imaging guidelines.

Overall assessment satisfaction was moderate at 2.15 out of 5 (where 5 = highest satisfaction), and significantly higher in those who had been assessed by ENT than another type of non-ENT healthcare professional (2.45 vs 1.78 respectively). Mean satisfaction was significantly higher in respondents who had undergone imaging, in both ENT/non-ENT subgroups. In those assessed by ENT, mean satisfaction was also higher if PROMs and smell testing had been used, though this only reached statistical significance in the former. In non-ENT assessed respondents, mean satisfaction was also higher in those who underwent PROMs/smell testing, though in this case, only the latter reached significance. In both ENT and non-ENT subgroups, it is possible that small *N* number may have affected the results obtained, and with larger cohorts, increased satisfaction may have reached significance for both smell testing and PROMs. Nevertheless, it would appear that, in general, satisfaction levels are higher where more thorough assessment is undertaken. This finding is in line with the results of my qualitative analysis, where an important emergent theme was 'rigour'. However, it is important to bear in mind, especially with regard to imaging, that such investigations may not be clinically appropriate in all cases. Accordingly, patients should be educated during their consultations as to why investigations are

or are not required in their case, with reference made to relevant guidelines where appropriate.

It should be noted that systematic differences between healthcare systems may have differentially affected the results obtained. Resource poor counties may accordingly be less able to provide, for example, access to smell testing or imaging, and insurance-based systems such as the USA may vary with individual respondent. I have attempted to address this through subgroup analysis, but care should be taken when comparing across geographical cohorts. Further, as discussed in the limitations below, representation for non-UK/USA respondents was limited, and future work should aim to increase the number of responses from across a wider international network.

5.6.3 Qualitative Results

In response to the questions [during your assessment] 'what was done well'/'what could have been done better', answers were provided covering all aspects of respondents' healthcare journeys. Emergent themes included 'knowledge', 'attitudes', 'rigour' and 'healthcare systems'.

As could be expected, patients appear to prefer assessment by a clinician with good knowledge of their condition, who empathises with and validates their concerns both with respect to direct, and indirect effects of OD. Dissemination of available knowledge through referral to online information/support groups and take-home written information was also valued. Importantly, however, many respondents described lack of both general and specific knowledge in their assessing healthcare professional, and perceived lack of empathy and validation was widespread ('*I felt I was a problem patient'; 'Nothing was done well as I was not believed. I was told it was in my imagination, so no smell test was offered'; 'My doctor dismissed my concerns and symptoms completely'; 'Drs [sic] were clueless. No compassion. No empathy. No advice. No answers.')*.

As was additionally evidenced through mean satisfaction ratings, rigorous assessment is preferred, including full history and examination, smell testing,

relevant investigations and referral for specialist/allied specialist assessment where required. Interestingly, thorough and transparent discussions about available treatments and their likelihood for success were often prioritised independent to actual positive treatment outcome.

Concerns surrounding healthcare system limitations included lack of appropriate referrals, long waiting lists and lack of follow up assessment/care. Patients who were told to 'wait and see' and discharged from care felt abandoned and lacked insight into the subsequent progress of their condition that would otherwise have been afforded by repeat assessment. Many also felt that research was important, focussing both on pathophysiology and possible treatments. When asked 'what was done well' one respondent summarised many of the above points: '*First of all, I was listened to and believed. I was given a thorough exam (throat, nose, nose scoped, lots of questions asked, smell test). I also had a 3 month follow up with the same ENT and was retested. He didn't know much about parosmia (my primary care doc, while sympathetic and curious knew practically nothing about it) but was happy to learn from me…. A real key is validation for patients.'* When asked 'what could have been done better', another respondent said: *'Try to understand the real impact onto our psychological and physical wellbeing and show a genuine interest in finding out more about smell disorders and possible studies and therapies. To this day we still get fobbed off by saying "we don't know enough about these problems" and all we get offered is steroids and nasal washes'.* Another respondent said: '*Time, compassion, recommendations, research.'*

Whilst most patients valued the insights afforded by smell testing, it was interesting to note that several felt such tests needed improvement. One respondent felt that use of identification without threshold minimised the severity of her condition '*I think more weight should have been given to the fact that the experience was much less intense than it should have been'.* This observation is in keeping with current guidance, which states that ideally threshold should be tested as well as identification/discrimination. However, as was demonstrated here, and in my clinician survey – if performed, testing most commonly focuses on odour identification alone. Given this practice, it was, however, reassuring that most

respondents felt odour identification was the most important aspect of olfaction that is commonly tested clinically. That being said, retronasal odour identification – which was rated as the second most important aspect of olfaction – is infrequently performed in clinical practice (see §4.5.5.1).

Of note, it also became apparent that the supporting information/explanations given during psychophysical testing may be insufficient – for example, the 'forced choice' paradigm, which is required by most tests, appears to cause confusion/concern. This could be easily mitigated with education from the healthcare provider/test administrator and/or provision of standardised written information prior to testing.

With regard to type of OD, the limitations of smell testing were most frequently raised by patients with parosmia. At present, psychophysical testing cannot be used to diagnose qualitative OD. Instead, parosmia and phantosmia are currently diagnosed by clinical history and can be quantified using validated questionnaires [528]. The place of psychophysical testing in qualitative OD is, however, an area of current research. For example, an adaptation of the Sniffin' Sticks test (the SSparoT) was recently developed specifically for the purpose of identifying parosmia [257]. Whilst this was successfully validated in 162 normosmic subjects, subsequent work using the short version of the SSparoT in patients with post-infectious OD demonstrated poor sensitivity rates (29% and 6% for the different test metrics)[258]. Further research into how qualitative OD can be tested using psychophysics is therefore required. This is particularly important given the wide-ranging physical and psychosocial impact of qualitative OD, which has been shown to affect eating, nutrition, weight, relationships, and psychological well-being[415]. Accordingly, patients with qualitative OD appear to be more significantly impacted than those with quantitative dysfunction – which has been shown through lower quality of life scores, higher depression scores, and reduced coping ability[536,537]. Furthermore, given the frequency of parosmia as part of C19OD, qualitative OD is likely to represent a significant burden at the societal level until a time at which C19 prevalence decreases.

Respondents were generally willing to travel for specialist care, and to spend as much time as necessary for thorough smell testing. With regard to referral practice, I

highlighted in my clinician survey that ENT surgeons infrequently cited 'refer on to specialist clinic' as reason for not performing routine psychophysical testing. Combined with my patient driven data, this confirms that improved referral pathways are required, where thorough local assessment is unavailable – be this from primary to secondary, or from secondary to tertiary care levels. Receiving specialist clinics should be equipped to perform comprehensive chemosensory testing in line with current guidelines – that is, including psychophysical olfactory and screening gustatory testing. Ideally, such clinics should be multi-disciplinary, including as a minimum psychological and nutritionist support, and with established links to other relevant specialities (e.g., neurology/paediatrics/endocrinology) as well as patient support networks. In this way, such clinics should provide relevant assessment for OD as well as its collateral effects. Assessment for common psychological sequelae may identify co-morbidities such as anxiety/depression, which, if left undiagnosed, may otherwise cause reduced quality of life and increased long-term healthcare needs.

5.6.4 Comparisons with other studies

My results are in keeping with international patient-reported data on olfactory assessment. In 2015, Boesveldt and colleagues reported results from their survey of 83 Dutch patients. They found that 56% of their cohort had not undergone chemosensory testing during their assessment and 17% were very unsatisfied with the care they had received[416]. Looking into patient experience in more detail, in their UK-based qualitative study, Erskine and colleagues described 'negative or unhelpful' interactions with healthcare professionals, as well as lack of empathy[413]. Burges Watson and colleagues described the experience of patients with C19-related qualitative OD, and again highlighted the importance of clinicians' knowledge and attitudes, including empathy for patients who may otherwise feel 'abandoned'[415]. These authors further commented that '*[accurate diagnosis]and explanations validated, legitimised and normalised people's experiences'*. Ball *et al.,* recently investigated UK barriers to effective olfactory healthcare and again found that many patients felt their condition had not been 'recognised' by their healthcare provider

and/or had experienced difficulty in obtaining referrals to secondary/tertiary care[414]. Whilst such findings are generally in keeping with my qualitative results, none of these studies addressed patient experience or preferences for olfactory assessment in detail. Indeed, the James Lind Alliance recently proposed ten UK research priorities in smell and taste, one of which was, '*how can the testing and investigations into smell/taste disorders be improved'*[1]*.* My data is a first step towards addressing this question in more detail.

5.6.5 Limitations and Future Work

The main limitations to this work were: 1. online survey methodology; 2. cohort demographics; 3. intercurrent pandemic.

There are several limitations inherent to the online survey methodology used, including selection bias, recall bias and 'multiple participation' effect. My method of survey distribution meant only patients who accessed the AbScent Facebook group during the 'live period' were invited to participate. This introduces a selection bias towards those who were frequently active on this platform, potentially overrepresenting younger people in need of more support (due either to their condition, or the level of support available to them elsewhere). Despite this, the resultant cohort did sample my target population - people who have/are likely to display healthcare seeking behaviour. Furthermore, my sample size was large – at 576, this was 6 x my minimum exploratory sample size of 96. Indeed, using Cochran's sample size formulae (see §0 for details), with all other factors held constant, a sample of this size would reduce my margin of error to 4.03%, had my sampling technique been random. Together, whilst my large sample size helps to reduce the effects of non-random sampling/selection bias, my results still should be interpreted with a degree of caution, when attempting to generalise to the true patient population. Future work should aim to utilise random sampling of patients undergoing assessment at primary/secondary/tertiary care levels, thereby mitigating selection bias associated with social media recruitment, and producing more representative samples. Prospective survey at time of assessment would also

mitigate the effects of recall bias, which may differentially affect respondents' ability to accurately report events. Questions such as, *'If you took a 'smell test', roughly how long did it take?*', may have been particularly affected by recall bias, meaning interpretation of such results should be undertaken with caution. Finally, though obtained data was hand searched for irregularities or repetition, the survey was 'open' and 'multiple participation' (i.e., the ability for a participant to undertake the survey multiple times) was theoretically possible. Any future surveys should aim to prevent multiple participation through use of appropriate software, whilst maintaining respondent anonymity.

Due to the membership demographics of the charity, most respondents were from the UK and USA. Whilst responses were also received from 31 other countries, these represented less than 3% of the total cohort, meaning that the insight afforded into 'international practice' was limited. To thoroughly assess practice at the international level, and to compare such practice between regions, future work should include standardised probabilistic sampling across different geographical regions – with target regional respondent numbers being adjusted according to population size. The survey should also be translated into the local language of the respondents.

As outlined in the general methods (§3.2.2), the use of social media in healthcare and healthcare research is a rapidly emerging field. At the time of survey dissemination planning, 98% of the UK population used the internet[443], and >75% used Facebook[444]. Furthermore, increasing numbers of older adults, as well as economically diverse populations have been shown to use social media regularly, including 71% of homeless young people[449]. Given the intercurrent limitations of the pandemic, online survey distribution through the AbScent Facebook group offered a theoretically equitable strategy for reaching a large target population. That being said, those without access to the internet, for example due to economic or health-related reasons such as visual impairment, were excluded. This may have been reflected in the above geographical variation in survey uptake – with more responses from more economically developed countries. Furthermore, the majority of respondents were women. This may reflect the increased propensity of women to

seek help, and/or the increased frequency of OD in women[425]. However, it also reflects lack of diversity in my responding cohort. Finally, I only included limited demographic questions (including age, gender and country of residence), as I did not want to increase the length of the survey or ask questions that were potentially less relevant to my outcomes of interest. In doing so, it was not possible to fully characterise the level of diversity achieved in my cohort. Future prospective work – as described above – should aim to include full equality, diversity and inclusivity principles (EDI) throughout the research process.

It should be noted that this research was conducted during the unique pressures of the pandemic. Factors such as long waiting lists for 'routine' clinical work and infection control may have negatively confounded my findings. Accordingly, I demonstrated that (in the UK/USA) significantly fewer patients with C19OD had been assessed by a healthcare professional than those without C19OD. For this reason, future patient surveys should ideally be performed at a time when the pandemic is no longer causing significant impact on healthcare provision. However, it is interesting to note that my findings (for example with regards to the proportion of respondents who had undergone smell testing) were in keeping with my clinician survey – in which doctors were asked to respond in relation to their *routine (non-pandemic) practice* [533], as well as earlier UK based clinician surveys, performed prior to the onset of the pandemic (see[318,334]). Finally, it is also worth considering that whilst the proportion of patients with C19OD may decrease in the future, post-infectious OD has, and will likely continue to represent one of the main underlying aetiologies of OD. Therefore, the high proportion of C19OD in my current sample, in itself, should not be viewed as a limitation, and conceptually differentiated from the effect of the pandemic on healthcare provision.

I used 'key informant' interview as my chosen method of survey co-production. This is an established practice in which small groups or individuals with 'unique knowledge' are used for 'exploring fields without much pre-existing information'[420]. In this respect, CK is the ideal 'expert patient'. However, her unique insights likely set her apart from the typical healthcare seeking respondent. In particular, her previous exposure to research could have made her more familiar with and therefore

accepting of complex methodologies, influenced her priorities through frequent contact with other stakeholders (e.g. doctors, researchers/scientists, policy makers), or caused other unknown biases in her approach. Furthermore, whilst CK had high levels of contact with other end-users (through her role as founder of AbScent), using her as my sole 'key informant', I accordingly limited the breadth of 'lived' OD experience to which I had access during early phases of survey development. Balanced against the unique insights she afforded, I do not consider use of CK as an expert patient/key informant to be a major limitation. Moreover, I additionally included non-expert participants during survey piloting in order to establish face validity (see §3.2.2). However, future co-produced work would benefit from the additional use of focus groups during survey development to better represent the target audience. Including more end-users in this way would also allow more careful adherence to EDI principles during the co-production process.

Finally, though not a limitation, it should be noted that this survey specifically aimed to capture the experience and preferences of *patients* and/or *healthcare seeking adults*. Particularly with respect to my qualitative results, it is therefore not possible to investigate the underlying root cause for some of the views held using the current approach. For example, where respondents felt their healthcare provider was 'dismissive', multiple underlying factors may have contributed to this perception, including, but not limited to, workplace pressures (time/staff/equipment limitations), clinical prioritisation, training/educational background, or communication skills. Whilst I addressed barriers to smell testing in my clinician-based survey, future work should aim to combine ongoing audit of clinical practice/outcomes with patient satisfaction. In this way, integrated stakeholder and outcomes analysis may lead to better, more effective healthcare strategies.

5.6.6 Conclusions

In this co-produced study, I investigated for the first time, experiences of and preferences for olfactory assessment, through the lens of patients and healthcare seeking adults. I hope that my novel results will be of benefit, particularly with

regard to future service provision planning, funding allocation, and chemosensory test development. I also hope that my findings will improve olfactory care by better aligning clinical and patient priorities.

Based on these findings, I propose the following practical recommendations for change:

- Increased clinician education regarding:
    - Olfaction
        - Physiology/pathophysiology
        - Collateral effects of OD, including psychosocial
        - Current guidelines for the assessment of OD, including but not limited to:
            - Principles and logistics of psychophysical testing
            - Use of PROMs
            - Appropriate use of imaging
    - Communication skills
        - Supported by standardised written information for patients
        - Need for patient education regarding requirements for specific investigations (particularly imaging)
- Establishment of local specialist centres/'hubs' where full chemosensory testing can be performed, and to which clear referral pathways exist
    - Ideally, such clinics should be multidisciplinary with psychological/nutritionist input as a minimum. Where this is not possible, clear referral pathways to allied specialists should be established.
- Funding
    - For clinician education
    - For the establishment of specialist hubs
    - For ongoing research and its dissemination

6 Structural Plasticity of the Central Olfactory Networks Upstream of the OB: Proof of Principle

6.1 Summary

Reduced GM volume has been demonstrated in patients with OD. Such regions may therefore represent neuroanatomical correlates of OD – areas in which dysfunction can be related to structure. However, the studies from which these observations were made were cross-sectional, and so provide evidence of association, not causation. The longitudinal demonstration of treatment-dependent structural alterations, 'structural plasticity', would provide better evidence for causation. Whilst the OB has been shown to undergo such change, to my knowledge, structural plasticity of regions upstream of the OB has not yet been demonstrated. Disorders of the peripheral olfactory system, such as chronic rhinosinusitis (CRS), provide an ideal model to study GM structural plasticity, given that patients may experience long periods of olfactory impairment, followed by near complete recovery with treatment. I therefore performed an exploratory prospective longitudinal study in patients undergoing surgical treatment for CRS. I used voxel-based morphometry (VBM) to investigate GM volume change in 12 patients (M:F = 7:5; 47.2 ± 14.9 years), 3 months post-op. There was a significant improvement in olfactory function according to birhinal psychophysical testing. I performed a voxel-wise region of interest analysis. I found significantly increased post-operative GM volumes within the primary (piriform cortex, amygdala) and secondary (orbitofrontal cortex, caudate nucleus, hippocampal–parahippocampal complex and bilateral temporal poles) olfactory networks, and decreased GM volumes within the secondary network only (caudate nucleus and temporal pole, hippocampal–parahippocampal complex). As a control measure, I assessed GM change within the primary sensory cortices for vision, somatosensation and audition, (V1, S1 and A1), where there were no

suprathreshold voxels. To my knowledge, this is the first study to provide proof of principle for clinically-relevant GM structural plasticity within the primary and secondary olfactory cortices, in association with improved olfaction.

6.2 Statement of Contribution

This chapter has been adapted from my published paper: **Whitcroft KL, Fischer J, Han P, Raue C, Bensafi M, Gudziol V, Andrews P, Hummel T (2018) Structural Plasticity of the Primary and Secondary Olfactory cortices: Increased Gray Matter Volume Following Surgical Treatment for Chronic Rhinosinusitis. Neuroscience 395:22–34.** For the purposes of my thesis, sections have been expanded or reduced and language/style modified as necessary.

PA, TH and I planned the study. JF and I gathered all data – JF provided English/German translation where necessary. CR provided administrative scanning support including MRI physics input. JF performed manual segmentation of the OB. I analysed all remaining data and interpreted the results. PH and TH provided analysis advice, and TH analysis overview. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

6.3 Introduction

Plasticity of the adult mammalian brain is well established[388–390], allowing for adaptation to internal and external environmental stimuli that leads to behavioural changes such as learning. In recent years, *in vivo* neuroimaging studies have demonstrated macroscopic anatomical differences in functionally relevant brain regions, between and within human subjects of varying experience. For example, Draganski and colleagues showed transient increases in grey matter volume within brain regions involved with complex visual motion, after 3 months of daily juggling practice in prior novices[376]. It has been suggested that such differences represent the anatomical counterpart to functional plasticity.

Similar to training programmes, it follows that long-term sensory deficits may cause experience-dependent structural plasticity. Indeed, functionally relevant grey and/or white matter volume reduction has been demonstrated in adults with hearing loss[381], unilateral vestibular deafferentation[382] or blindness[383,384]. The olfactory system is less well studied than other sensory modalities, however, it provides a good neurobiological model to investigate structural plasticity, given that patients with peripheral olfactory dysfunction may have long periods of deficit followed by near complete recovery after treatment. Such plasticity has been suggested within the olfactory bulb (OB), where manual segmentation techniques have been used to demonstrate reduced volume in patients with olfactory impairment, as compared to controls[168,538,539]. Moreover, OB volume has been shown to increase following treatment for olfactory impairment [92].

Grey matter structural plasticity upstream of the OB, in other areas of the primary and secondary olfactory cortices, has not yet been established. Whilst cross-sectional voxel based morphometry (VBM) studies have demonstrated upstream grey and white matter volume reduction in association with olfactory loss[385,493,540], determination of causality in such cases is not possible. In order to investigate sensory-dependent plasticity in these areas, longitudinal studies are needed[359]. Demonstration of structural plasticity is a necessary first step in establishing clinically relevant neuroanatomical correlates of olfactory function/dysfunction, that may one day be useful as personalised biomarkers.

Chronic rhinosinusitis (CRS) is an umbrella term used to describe long-term inflammatory conditions of the nose and paranasal sinuses. It is common, affecting 10.9% of the European population[104], and can lead to olfactory dysfunction in 61-95% of patients[109,114]. Treatment is initially conservative, with surgery reserved for medically refractive cases [101]. Previous studies have shown that olfactory function improves after treatment, including surgery, as assessed using psychophysical tools[298,300,541]. Treatment of chronic rhinosinusitis provides a particularly attractive neurobiological model to investigate structural plasticity of the central olfactory system: CRS is a common condition that affects olfactory function, and which can be successfully treated with surgery – a temporally defined intervention that can be

relatively well stereotyped across patients. Whilst OB volume has been investigated in this cohort[92], longitudinal assessment of upstream grey matter structural plasticity has not yet been performed.

The aim of this study was therefore as follows: to determine whether change in olfactory function after surgical treatment for CRS is accompanied by structural changes in olfactory brain regions, using VBM and manual planimetry. I hypothesize that improved olfactory function will result in increased GM volumes within putative olfactory eloquent regions.

6.4 Methods

6.4.1 Experimental Setting and Design

I performed a collaborative, longitudinal prospective study with the Interdisciplinary Smell and Taste Clinic, part of the University Hospital Carl Gustav Carus Dresden, Germany. Consecutive adult patients (>18years) with chronic rhinosinusitis (with or without nasal polyps) awaiting functional endoscopic sinus surgery at the Department of Otorhinolaryngology were considered for recruitment. Prior to inclusion in the study, patients were carefully screened for eligibility. Eligible patients had CRS with or without nasal polyposis and had undergone a period of conservative medical treatment according to the contemporaneously available European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS-12)[101], but were medically refractive. Patients with neurological, psychiatric or other systemic conditions affecting olfaction were excluded. Finally, only those patients who were available for follow up assessment at three months were included. See §3.3.1 for more details.

Clinical assessment, olfactory testing and magnetic resonance scanning was performed preoperatively and again at 3 months post-operatively. Clinical assessment included patient history and validated questionnaires (SNOT-20 German Adapted Version[460]), rigid nasal endoscopy (with findings rated according to Lund Kennedy scoring system[456]) and peak nasal inspiratory flow rate[473,542]. Disease duration was obtained through patient report, as per normal clinical practice. Olfactory testing was conducted in accordance with the Position Paper on Olfactory

Dysfunction[543], and therefore included odour threshold and identification subcomponents of the Sniffin' Sticks (SS), which I tested birhinally (for detailed description of testing procedure please see §3.3.2). Threshold and identification were chosen as previous work has suggested these best represent peripheral and central olfactory function, respectively[152]. Given that my cohort included elderly patients, the discrimination component of the Sniffin' Sticks test battery was not undertaken to reduce participant burden. As I aimed to assess olfactory function in a way that was functionally relevant to patients, and in order to further reduce participant burden in my clinical sample, I performed birhinal psychophysical testing only. Normosmia was attributed where T≥ 5.75 and I≥ 11[286]. Clinically significant improvement in SS scores were assumed as follows: threshold ≥2.5 points, identification ≥3 points[287].

6.4.2 Imaging Acquisition

Whole brain magnetic resonance imaging was performed using a 3-T scanner (Verio, Siemens, Erlangen, Germany) with 8-channel phased-array head coil. Axial T1-weighted images were acquired using a 3-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence. The following parameters were used: repetition time (TR), 1890 ms; echo time (TE), 3.24 ms; inversion time (TI), 1100 ms; field of view (FOV), 280 mm; voxel size, 0.73 × 0.73 × 1 mm; and flip angle, 15° (in total, 176 contiguous slices). OB images were obtained using a focused acquisition paradigm: T2-weighted fast spin-echo images (0.5 × 0.5 × 1.2 mm, no interslice gap) were acquired of the anterior and middle cranial fossae.

6.4.3 Imaging Analysis: Voxel-Based Morphometry

I performed voxel based morphometry using the CAT12 toolbox (available from http://dbm.neuro.uni-jena.de/vbm/) implemented in SPM12 (Wellcome Centre of Imaging Neuroscience, Institute of Neurology, UCL, London, UK; http://www.fil.ion.ucl.ac.uk/spm) and MATLAB (The MathWorks, Natick, MA, USA). T1 images were first visually inspected for obvious motion artefact and appropriate

orientation according to SPM priors. These images were then segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF), using the longitudinal segmentation tool. This process involves an initial intra-subject inverse-consistent (symmetrical) spatial realignment with bias correction between the pre-operative and post-operative images. In addition to segmentation of images from each time point, a mean image across time points is produced. Estimated spatial normalisation parameters were then calculated for the segmented mean image, using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL)[483]. The resultant DARTEL deformations are then applied to the segmented images at each time point, prior to their modulation. Images were then smoothed using a Gaussian kernel (full-width at half-maximum, 8 mm). I performed automated data quality checks as per the CAT12 toolbox. Voxel-wise differences in GM between pre-operative and post-operative scans were assessed using a flexible factorial model, with the between subject factor = subject (1 level: patients) and the within subject factor = time (2 levels: first scan, second scan). In order to control for the effect of total intracranial volume ['TIV', summated GM, WM and CSF volume[483]] and age, these were set as 'nuisance covariates' during model specification. I then performed T tests for significant increases and decreases in GM volume between visits. An absolute threshold masking value of 0.2 was applied to avoid possible edge effects between different tissue types[377,540].

As I was interested in the GM volume within brain regions known to be relevant to olfaction and have strong a priori hypotheses regarding these olfaction-relevant areas, I performed a region of interest (ROI) analysis. Defined ROIs included bilateral areas of the primary and secondary olfactory cortices, as recently defined in a merged functional and structural olfactory network map[32]: primary [piriform cortex (PC), amygdala], secondary [orbitofrontal cortex (OFC), caudate nucleus, anterior cingulate cortex (ACC), insula, putamen, pallidum, hippocampal-parahippocampal complex, thalamus and temporal poles]. The OFC ROI was constructed as per Kahnt *et al.,* 2012 and therefore included the bilateral AAL regions of: superior, middle, inferior and medial orbital gyri as well as the rectal gyri[544]. Functional endoscopic sinus surgery is a rhinological procedure that is thought to improve olfactory

function in CRS patients[541], but should not produce changes in other sensory systems. Therefore, as a control measure, voxel-wise GM volume was also assessed within the primary somatosensory, auditory and visual cortices (S1, A1 and V1 respectively). I constructed ROIs within the WFU_PickAtlas software (available from: http://fmri.wfubmc.edu/software/pickatlas), based on the Automated Anatomic Labeling (AAL) atlas[545] for olfactory regions and Brodmann Areas for S1 (3, 1, 2), A1 (41, 42) and V1 (17), based on the Talairach Atlas[546]. Significant voxels are reported in relation to the Montreal Neurological Institute (MNI) coordinate space. In addition to limiting my total number of statistical tests through conducting a ROI analysis (small volume correction implemented through ROI function in WFU_PickAtlas), in order to further reduce my risk of Type I error, I additionally used a Bonferroni correction ('BC'): my α level (0.05) was divided by the number of ROIs used (14), giving a significance threshold of $p<0.0036_{BC}$[377,492]. In order to avoid issues surrounding non-stationarity in voxel based volumetric analysis[483], I did not use any cluster-based inferences (e.g. cluster size threshold), and report only voxel based results.

I was interested in whether increases in GM volume observed after surgery were related to other factors, for example, disease duration, endoscopic scores, psychophysical test scores or OB volume (calculated through manual planimetry). In order to determine whether change in GM after surgery was correlated with other factors, GM volume (beta weights) pre- and post- surgery were extracted from significant voxel clusters using the MarsBaR toolbox for SPM (available from: http://marsbar.sourceforge.net/)[373]. Only significant GM within the a priori ROIs were extracted for correlation, that is, regression analysis with target variables for later correlation were not used to define clusters, so ensuring variable independence and avoiding circular analysis[547,548].

I prepared images using the Xjview toolbox for SPM (available from: http://www.alivelearn.net/xjview/) and Microsoft PowerPoint (Microsoft Corporation, Redmond, WA).

For more details on scanning procedures and VBM analysis, see §3.3.3.

6.4.4 Imaging Analysis: Manual Planimetry

Following acquisition, JF used manual planimetry (segmentation) to determine OB volume using AMIRA 3D software (Visage Imaging, Carlsbad, CA, USA). During this process, the surface area of each OB slice was first calculated (mm^2). The surface area of each slice was then summated and multiplied by slice thickness, to give OB volume in mm3. The rostral demarcation of the OB was taken as the point of sudden diameter increase, as has been previously described[343]. All subsequent calculations involving OBV were performed by myself.

6.4.5 Statistical Analysis

Outside of the SPM platform, I performed supporting statistical analysis using SPSS (Statistical Package for the Social Sciences, Version 24.0, SPSS Inc. Chicago, IL, USA) and GraphPad Prism (version 6, GraphPad Software, LaJolla, USA). Statistical significance was attributed where $p<0.05$, unless stated otherwise. Data was assessed for normality and parametric or non-parametric tests used as appropriate. Unless otherwise specified, data is given as mean (SD).

I prepared images for inclusion in the manuscript using the Xjview toolbox for SPM (available from: http://www.alivelearn.net/xjview/) and Microsoft PowerPoint (Microsoft Corporation, Redmond, WA).

6.4.6 Ethical Considerations

I obtained appropriate ethical approval from the Ethics Committee of the Faculty of Medicine Carl Gustav Carus University Hospital, Dresden, Germany (EK number 56022016), and patients gave written, informed consent. Please see §3.3.5 for detailed information.

6.4.7 Funding

Funding was kindly provided for scanning from Prof Hummel [source of funding – Deutsche Forschungsgemeinschaft (DFG HU441/18-1)].

6.5 Results

6.5.1 Demographics, Behavioural and Clinical Scores

Twelve patients were included [M:F 7:5; mean age 47.2 (14.9) years]. Self-reported mean duration of disease was 12.5 (15.5) years, with a range of four months to 57 years. Diagnoses and details of surgery are shown in Table 6-1. Psychophysical test scores, clinical examination findings and questionnaire scores are shown in Table 6-2.

There was a statistically significant improvement in olfactory threshold, identification and composite threshold + identification scores at 3 months post-operatively; group T and I scores fell below normosmia preoperatively but improved to normosmic postoperatively. The group mean improvement in threshold score additionally reached clinical significance (as defined by an increase of ≥2.5 points), whilst that for identification fell just short of clinical significance (≥3 points).

Furthermore, there was a statistically significant improvement in both overall SNOT-20 GAV score, as well as for the olfaction specific question (Q10). There were also significant improvement in Lund-Kennedy score, however the increase in peak nasal inspiratory flow rate (PNIF), which is an indicator of nasal patency, did not reach statistical significance (possibly due to variation in individual patient effort[542]). See Table 6-2.

All patients were treated post-operatively with intranasal corticosteroid spray and saline irrigation.

Patient	Surgery Type	Findings	Procedure
1	Revision	Bilateral nasal polyposis, DNS	Septoplasty Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
2	Primary	Bilateral nasal polyposis, DNS	Septoplasty Bilateral polypectomy/FESS (maxillary, ethmoid and frontal sinuses)
3	Revision	Right sided nasal polyposis	Right sided polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
4	Primary	Mucosal oedema, isolated antrochoanal polyp	Antrochoanal polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
5	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
6	Primary	Mucosal oedema, septal perforation	Bilateral FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
7	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
8	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
9	Primary	Bilateral nasal polyposis, DNS	Septoplasty Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
10	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
11	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
12	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and sinuses)

Table 6-1: Main findings and surgical procedure for each of the study patients. FESS = functional endoscopic sinus surgery; DNS = deviated nasal septum.

	Pre-operative	Post-operative
"Sniffin' Sticks" Score		
Threshold (T)	5.1 (3.6)	7.7 (3.3)*
Identification (I)	9.0 (4.1)	11.6 (1.9)*
T+I	14.1 (7.5)	19.2 (4.5)*
Lund-Kennedy Score		
Bilateral	8.6 (3.7)	5.7 (3.4)*
Right	4.2 (1.9)	3.1 (1.7)*
Left	4.4 (2.0)	2.6 (1.9)*
SNOT-20 GAV Score	30.1 (11.9)	11.8 (11.9)*
SNOT-20 (Q10 Score)	2.8 (1.8)	1.4 (1.9)*
PNIF	110.8 (45.6)	132.5 (38.6)

Table 6-2**:** Psychophysical olfactory test, clinical examination and questionnaire test scores. * Indicates statistically significant result (P<0.05). Results shown as mean (SD).

6.5.2 Grey Matter Volume: Voxel-Based Morphometry

Controlling for age and TIV, there were significant increases in GM volume within the pre-specified ROIs after surgery. The significant voxels were in areas of the primary and secondary olfactory networks, including the left PC, right amygdala, right OFC, right caudate nucleus, bilateral temporal poles and right hippocampal-parahippocampal complex (Table 6-3 and Figure 6-1). There were no suprathreshold voxels within the other olfactory ROIs. Furthermore, there were no areas of significant GM volume increase within the control ROIs: S1, A1 or V1.

		MNI Coordinates			
Region	***Side***	***X***	***Y***	***Z***	***T Score***
Piriform Cortex	L	-21	8	-16	3.96
Amygdala	R	20	-2	-12	4.09
Orbitofrontal Cortex	R	28	22	-21	4.40
Caudate Nucleus	R	9	18	-3	3.92
HPC - pHPC complex	R	15	-4	-24	7.46
Temporal Pole	R	62	6	-4	3.98
	R	21	10	-40	3.87
	R	36	24	-28	3.74
	R	60	6	-22	3.50
	L	-36	9	-27	4.90
	L	-22	6	-26	4.12
S1	*No Suprathreshold Results*				
A1	*No Suprathreshold Results*				
V1	*No Suprathreshold Results*				

Table 6-3: Increases in GM volume within a priori ROIs. Results threshold $p<0.0036_{BC}$. Results controlled for age and TIV. Coordinates are expressed in MNI space.

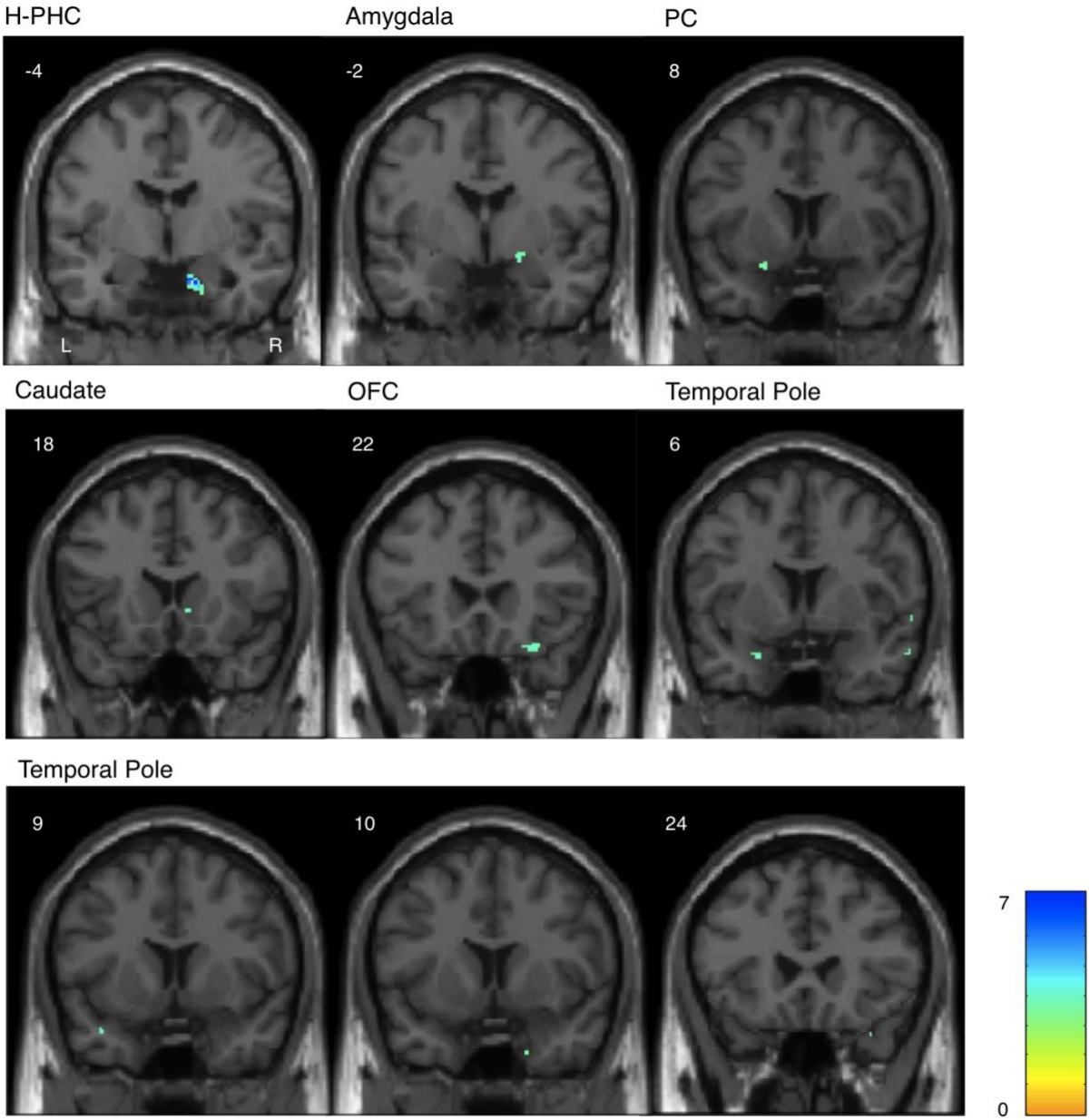


Figure 6-1: Coronal sections showing significant voxels from ROI analysis in primary and secondary olfactory regions (T score scale to right). Threshold set to *P*<0.0036corr. Abbreviations: H-PC, hippocampal–parahippocampal complex; PC, piriform cortex; OFC, orbitofrontal cortex. Y coordinate shown in top left corner, expressed in MNI space. Side according to neurological convention, as shown in top left panel.

Again, controlling for age and TIV, there was significant reduction in GM volume after surgery within the left caudate and temporal poles, and bilateral hippocampal + parahippocampal complex, (see Table 6-4). There were no other significant voxels within the pre-specified ROIs.

		MNI Coordinates			
Region	***Side***	***X***	***Y***	***Z***	***T Score***
Caudate Nucleus	L	-10	10	6	4.07
Temporal Pole	L	-32	14	-40	3.71
HPC - pHPC Complex	R	32	-26	-15	4.10
	L	-16	-20	-18	4.30

Table 6-4: GM volume reduction within pre-specified ROIs. Results threshold p<0.0036BC. Results controlled for age and TIV. Coordinates are expressed in MNI space.

In order to determine whether increases in GM volume seen after surgery are related to other factors, I used extracted GM volumes from significant clusters within the *a priori* ROIs, to perform correlation with self-reported duration, Lund-Kennedy score and psychophysical olfactory scores (SS). Accordingly, I found no significant correlation between Lund-Kennedy score (pre-/post-operative and change in score after surgery) or disease duration and GM volume (pre-/post-/change in volume after surgery) within the significant clusters. With regards to psychophysical olfactory scores, I found no significant correlations between pre-operative SS score or change in SS score and GM volumes. There were, however, significant correlations found between post-operative SS scores and several GM clusters, as shown in Table 6-5.

		Significant Correlations		
			GM Volume	
	Region	***Pre-op***	***Post-op***	***Change***
Post-op Identification	Amygdala *(20, -2, -12)*	-	r=0.62 *P*=0.032	r=0.59 *P*=0.046
	Piriform Cortex *(-21, 8, -16)*	-	-	r=0.64, *P*=0.026
	Temporal Poles *(62, 6, -4)*	-	-	r=0.63 *P*=0.030
	Temporal Poles *(21, 10, -40)*	-	-	r=0.64 *P*=0.028
Post-op Threshold	Amygdala *(20, -2, -12)*	-	-	r=0.60 *P*=0.038
	Orbitofrontal cortex *(28, 22, -21)*	-	r=0.60 *P*=0.041	-
Post-op Threshold + Identification	Amygdala *(20, -2, -12)*	-	r=0.59 *P*=0.046	r=0.69 *P*=0.014
	Temporal Poles *(21, 10, -40)*	-	-	r=0.62 *P*=0.035

Table 6-5: Statistically significant correlations between SS scores and GM volume (controlled for age and TIV). Pearson's or Spearman's correlation coefficients (r) and associated *P* values are shown.

6.5.3 OB Volume: Manual planimetry

There was an increase in mean left, right and combined left + right OB volume after surgery however, this did not reach statistical significance (see Table 6-6).

	OB Volume	
	Pre-op	***Post-op***
Left	41.2 (13.9)	46.5 (12.4)
Right	39.6 (14.3)	46.6 (15.7)
Combined L+R	80.8 (26.2)	93.1 (26.8)

Table 6-6: OB volume in mm3, results shown as mean (SD).

There were no significant correlations between left, right or L+R OB volume and age, disease duration or SS test scores. This was true for pre-/post-/change in OB volume and age/duration, as well as pre-/post-/change in SS.

As I was interested in whether OB structural plasticity was related to GM plasticity, I performed correlation analysis between change in OB volume and change in GM volume (GM extracted volumes from a priori ROIs as described). Accordingly, I found a statistically significant, positive correlation between change in right OB volume and change in GM volume within the significant OFC cluster (28, 22, -21) [Spearman's r=0.69, *P*=0.017, see Figure 6-2]. I did not find statistically significant correlations between change in OB volume and change in GM volume in any of the other ROIs.

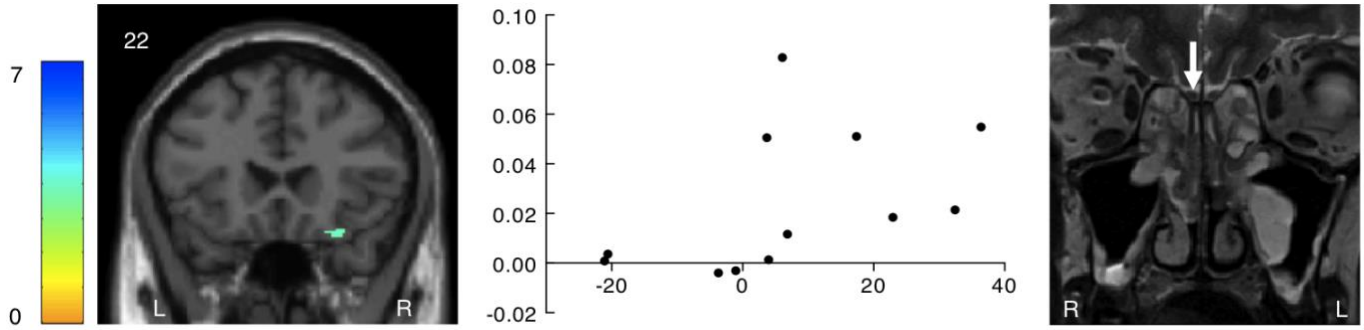


Figure 6-2: Scatterplot showing significant correlation (Spearman's r= 0.69, *P*= 0.017) between change in right OB volume (x-axis, mm3) and change in GM volume (y-axis, arbitrary units) within the significant OFC cluster (28, 22, -21). Left coronal T1 section = OFC cluster (y coordinate shown in top left corner, scale bar showing T score to left of image), right coronal T2 image = preoperative bilateral OB in a patient with visible nasal polyps (white arrow showing right OB – please note neurological siding convention in left coronal T1 image, and radiological siding convention in right coronal T2 image, as marked). Abbreviations: OB, olfactory bulb; OFC, orbitofrontal cortex.

6.6 Discussion

6.6.1 Key Findings

To my knowledge, this is the first work to demonstrate change in GM volume, in association with improved olfactory function, in areas of the primary and secondary olfactory cortices upstream of the OB. Specifically, I found significant increase in GM volume within the piriform cortex, amygdala, orbitofrontal cortex, caudate nucleus, temporal poles and hippocampal-parahippocampal complex, at 3 months post functional endoscopic sinus surgery for CRS. Areas of GM volume reduction were

found in the caudate nucleus, temporal pole and hippocampus-parahippocampus. No such changes were observed in the control regions (S1/A1/V1). These findings were accompanied by statistically significant increases in psychophysical test scores, as well as patient reported outcome measures. However, direct correlations between behavioural and GM measures were inconsistent.

This work provides proof of principle for clinically-relevant structural plasticity within the central olfactory networks. Whilst increases in GM volume were demonstrated within several regions, in keeping with my original hypothesis, areas of decreased GM volume were also demonstrated. The functional significance of these changes requires further study.

6.6.2 Anatomical Regions that Underwent Change in GM Volume

I demonstrated significant increase in GM volume within the piriform cortex. As the principal recipient of afferent input from the OB, the PC is a key area of central olfactory processing, and considered a major component of the primary olfactory network, as evidenced through functional imaging studies.[73] The role of the PC appears to include valence encoding[310,549], attentional modulation[550], as well as odour recognition and memory.[551,552] Regarding the potential link between PC structure and function, previous cross-sectional studies have shown reduced GM volume within the PC of patients with olfactory dysfunction of varying cause. Bitter and colleagues demonstrated GM reductions in the PC of patients with acquired anosmia of mixed cause (including sinonasal, post-infectious and post-traumatic dysfunction), compared with healthy controls.[385] This same group also demonstrated PC GM volume loss in patients with hyposmia, again of mixed cause.[493] Reduced GM volume within the PC has also been shown in patients with idiopathic olfactory loss.[496] My data adds to this literature by providing, to my knowledge, the first longitudinal evidence for clinically-relevant change in GM volume within the PC in association with improved olfactory function.

I additionally demonstrated increased GM volume within the amygdala, which is also considered part of the primary olfactory network. Functional imaging has

demonstrated involvement in odour intensity and hedonic encoding[311], as well as processing of emotionally relevant olfactory stimuli.[553] In patients with moderately advanced Parkinson's disease, amygdala volume has been shown to correlate with olfactory function, where lower psychophysical test scores can be used to predict lower GM volume.[554] However, at time of writing, to my knowledge altered GM amygdala volume had not been demonstrated in cross-sectional studies of patients with non-neurodegeneration related OD. Therefore, in light of my initial longitudinal results, this region deserves further study.

Finally, I demonstrated increased GM volume within the OFC. The OFC receives cortico-cortical input from the piriform cortex and is a well described area of secondary odour processing, shown in functional neuroimaging studies to be important for multimodal sensory integration[555], as well as affective- and experience-dependent odour percept encoding.[311,556,557] Localised structural lesions of the right OFC have been shown to impair conscious odour processing, despite evidence of residual odour-induced behavioural, autonomic and neuroimaging responses.[558] Positive correlation between OFC GM cortical thickness and psychophysical olfactory test scores has been previously demonstrated in healthy control participants.[372,373] Cross-sectional studies have demonstrated reduced OFC GM volume in patients with OD[399,493,495,559]. OFC volume is also increased in perfumers, which is attributed to olfactory experience-dependent structural reorganisation.[377] Again, my longitudinal data adds to this cross-sectional work, and provides proof of principle for clinically-relevant structural plasticity within this region, and thereby stronger evidence for a link between structure and function.

Within the temporal poles, caudate nucleus and hippocampal-parahippocampal complex, the changes seen were more complex, with both increases and decreases in GM volume. The *temporal poles* are known to receive connections from the primary and secondary olfactory networks (including the amygdala and orbitofrontal cortex) and have been linked to olfaction in both lesion[560–562] and functional neuroimaging studies.[563] It is thought that the temporal poles act to assign emotional valence to highly processed sensory stimuli[564], and may also be involved in odour memory[561]. The *caudate nucleus* is involved in associative learning [565] and

reward processing [566], and has recently been linked to correct odour recognition[567]. Finally, the *hippocampus* has been demonstrated in primate studies to receive disynaptic connections from the OB [30], and is frequently activated during cross-sectional olfactory functional neuroimaging.[31] In turn, the hippocampus is densely connected to the *parahippocampus*, which is also involved in olfactory processing, as evidenced by structural and functional studies.[32] Together, the hippocampal-parahippocampal complex is important for odour discrimination learning and recognition memory, and with its close connections to the amygdala, emotionally toned and odour-evoked autobiographical memory.[567–573] Parahippocampal GM volume reduction has been demonstrated by Yao *et al.* in their cohort of patients with idiopathic olfactory dysfunction.[496] Bitter and colleagues also demonstrated hippocampal and parahippocampal GM volume reductions in their cohort of anosmic patients.[385] Though speculative, the increases and decreases in GM volume within these regions may indicate particularly dynamic, clinically-relevant structural plasticity.

6.6.3 Functional Implications of Changing GM Volume

Together, these results demonstrate that areas of the primary and secondary olfactory networks underwent change in VBM-derived GM volume in association with improved olfactory function, following surgery for CRS. Whilst this provides a higher level of evidence for the link between structure and form in these regions, and thereby their role as neuroanatomical correlates of OD, in an attempt to further isolate the role of changing olfactory function in driving these changes, I performed a comparative ROI analysis within the primary visual, auditory and somatosensory cortices: V1, A1 and S1. Accordingly, I found no significant GM volume change in these areas. This is in keeping with my hypothesis that FESS leads to GM structural reorganisation within olfactory eloquent brain regions, due to improvement in peripheral olfactory function. However, this approach is discussed in 'study limitations' below.

To further investigate the role of olfaction as causative variable of interest, I performed correlation analysis between extracted GM volume (beta weights) and psychophysical test scores. I found moderately large significant positive correlations between post-operative threshold, identification and composite threshold + identification scores and either post-operative GM volume or change in GM volume within the significant clusters of the piriform cortex, amygdala, orbitofrontal cortex and temporal poles. Such results help to confirm the functional relevance of these regions during recovery of olfactory function. I did not, however, find significant correlations between pre-op SS scores and pre-op GM volume in significant clusters, nor, importantly, between change in SS scores and change in GM volume in significant clusters, as might have been expected. This may have been due to small sample size, or, in the case of pre-op correlations, due to limitation of GM volumes tested to those clusters that showed significant change after surgery (an approach that was adopted to investigate factors that were relevant to structural plasticity).

Whilst the above adds some strength to the functional significance of the changes seen, to better investigate the role of changing olfaction in these alterations, and thereby further strengthen the potential link between function and structure, I plan to perform concurrent olfactory fMRI in my next experimental chapter.

6.6.4 OB Volume and Correlation with GM Volume

Whilst left, right and combined left + right OB volume increased after surgery, this did not reach statistical significance.

The OB is thought to be a highly plastic structure, in which volume reflects olfactory function. Such plasticity is underpinned by studies demonstrating new axonal connections from the regenerating sensory neurons of the olfactory neuroepithelium.[13] The OB may also be a target for new interneurons produced in the subventricular zone, though the persistence of such neurogenesis into adulthood in humans is debated.[340,574] Accordingly, reduced OB volumes have been demonstrated using structural neuroimaging in patients with olfactory dysfunction of varying cause.[397,575] Furthermore, previous work has demonstrated a significant

increase in mean OB volume in 19 patients who had undergone surgical treatment for CRS with nasal polyps.[92] However, other studies have failed to demonstrate reduced OB volume in CRS patients compared to controls (see §1.5.3.1.1 for detailed discussion).[91,540] Such discrepancies may reflect heterogeneity in underlying aetiological subtype of CRS in the patients studied (for example the presence of an allergic component), or may be related to the inherent fluctuations in olfactory function characteristic of this condition.[152] With regard to the latter, patient groups in whom reduced OB volume is best demonstrated do not typically experience fluctuations in olfactory function (e.g. post-infectious, post-traumatic or neurodegeneration related dysfunction). The association between olfactory function and OB volume in CRS therefore requires further investigation, though it may be that such function is not well reflected by this structure, in this patient population. Such inadequacies are further suggested by the lack of significant correlation I demonstrated between SS test scores and OB volume. However, my sample size was small, meaning such conclusions should be made with caution.

To my knowledge, this is the first study to investigate correlation between OB and olfactory-GM plasticity. There was a moderately large significant positive correlation between change in right-sided OB volume and change in GM volume within the significant OFC cluster (28, 22, -21). One may speculate that greater peripheral olfactory input to the right OB after surgery leads to its increased size, which in turn leads to increased input and consequent structural plasticity within the upstream OFC. However, such speculation again is made with caution, given that I did not demonstrate significant correlation between OB volume and SS test scores and that the accuracy with which the OB reflects olfactory function in CRS is not fully established.

Given the inconsistent results I demonstrated in this cohort, and as my primary aim in this thesis is to study upstream central structures, I elected not to focus on the OB in my further chapters.

6.6.5 Limitations and Future Work

As this was an initial exploratory study, I chose to balance the risks of incurring type I and type II errors in my neuroimaging analysis through use of region of interest/small volume correction, as well as Bonferroni correction of my set alpha level. Whilst both of these approaches have been previously described[377,484,491,492], other, more conservative approaches (e.g., use of the FWE correction for multiple comparisons) would give better assurance against false positives. Similarly, in my GM-behavioural correlation analyses, I used an uncorrected alpha level (P<0.05). A more conservative approach could have involved Bonferroni correction of my alpha level for the number of statistical tests performed. Again, as this was an exploratory study, I do not feel it significantly affects the conclusions drawn, however, I aim to use more conservative statistical approaches in my next chapter.

My findings are also limited by lack of a prospective control group. I attempted to compensate for this through use of GM volume analysis within the primary somatosensory, auditory and visual cortices. However, this method of control analysis does not account for potential inherent fluctuations in GM structure that could be present within the olfactory networks, but not in V1, S1 and A1. Furthermore, many of the results I demonstrated were within areas of the secondary olfactory network, and though speculative, structural stability/fluctuations within primary and secondary sensory networks may not be equivalent.

Therefore, having established proof of principle for GM structural plasticity in the central olfactory networks upstream of the OB, to confirm and further explore my findings, I will perform a further longitudinal study with the benefit of a parallel, prospective control group, as described in the next chapter. Moreover, whilst these changes were seen following treatment for OD, with associated improvements in psychophysical test scores, to better characterise the functional relevance of the structural plasticity observed, I will perform a multimodal study, with olfactory fMRI in addition to structural morphometry.

6.6.6 Conclusions

To my knowledge, this study provides the first evidence for higher order structural plasticity within the primary and secondary olfactory networks, in association with improved psychophysical test scores, after FESS for CRS. Whilst this longitudinal evidence provides initial proof of principle for clinically-relevant structural plasticity, the functional significance of these changes requires further investigation. My future work, as described above, will aim to confirm and expand on these results by use of multimodal structural and functional neuroimaging in both patients and healthy controls.

7 What is the Functional Significance of the Structural Plasticity Observed?

7.1 Summary

In chapter six, I provided proof of principle for treatment-dependent structural plasticity within regions of the primary and secondary olfactory networks, strengthening the potential link between structure and function in these regions. However, the functional significance of such change requires further investigation, particularly where these regions may be considered as personalised biomarkers in future. I therefore performed a longitudinal multimodal neuroimaging study investigating structural (indicated by change in GM volume or cortical thickness) and functional (indicated by increase in olfactory BOLD signal) plasticity in 24 patients undergoing surgical treatment for chronic rhinosinusitis, compared with 17 healthy controls. Comparing patients with controls, I demonstrated significant group x time interactions within regions of the primary and secondary olfactory networks three months after surgery. In an effort to isolate changing olfactory function as the variable of interest, I further compared patients who had experienced clinically significant improvement in olfactory function vs. those who had not and found significant group x time interactions within areas of the secondary olfactory network (ACC, HPC, OFC, pHPC and TP). Within group analysis in patients who had clinically improved demonstrated significant increases in GM volume in the HPC and pHPC, and significant decreases in GM volume within the ACC, HPC, OFC, TP and insula. Finally, I demonstrated increased BOLD signal within the ACC, OFC, TP and insula – interestingly all areas in which there were decreases in GM volume. To my knowledge, this is the first study to demonstrate structural and functional plasticity of the central olfactory networks, thereby helping to confirm these areas as neuroanatomical correlates of olfactory dysfunction.

7.2 Statement of Contribution

This chapter has been adapted from my published paper: **Whitcroft KL, Noltus J, Andrews P, Hummel T. Sinonasal surgery alters brain structure and function: Neuroanatomical correlates of olfactory dysfunction. Journal of Neuroscience Research. 2021 Sep;99(9):2156-2171.** For the purposes of my thesis, sections have been expanded or reduced and language/style modified as necessary.

PA, TH, JN and I planned the study. JN and I gathered all data – JN provided English – German translation where necessary. I analysed all data and interpreted the results. TH provided analysis advice. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

7.3 Introduction

Structural differences in olfactory eloquent regions may represent the neuroanatomical correlates of olfactory dysfunction. Such differences have been demonstrated in the OB, and upstream regions of the central olfactory networks[399,493,540,559,576]. However, the majority of this evidence is cross-sectional, thereby strictly providing evidence for correlation with OD, not causation[359]. This is particularly important in multi-functional, higher order regions, where neuroanatomical differences observed in OD may be: 1. caused by OD; 2. predisposing to OD; 3. compensating for OD; 4. incidentally associated with OD. Longitudinal studies provide better evidence for causation, which is needed when considering future use of such regions as clinical biomarkers of OD.

In my last chapter, I provided proof of principle for structural plasticity of the central olfactory networks, upstream of the OB. Since undertaking this work, two further studies have been performed that also address GM structural plasticity of the central olfactory network – both interrogating the effects of olfactory training on GM volume - one in patients and one in normosmic participants[577,578]. Whilst these studies provide further evidence that structures within the central olfactory network can undergo plastic change, what they – and the work in my last chapter – fail to provide, is evidence for the functional significance of such change.

I therefore aimed to build on this work by performing, to my knowledge, the first prospective multimodal neuroimaging study to assess for functional, as well as structural plasticity, in patients undergoing FESS for CRS. Accordingly, I performed voxel-based morphometry, analysis of cortical thickness (CTh) and olfactory functional MRI in patients with CRS, before and after FESS, as well as in a matched longitudinal healthy control group. In doing so, I hoped to further characterise the extent and nature of structural plasticity within the primary and secondary olfactory networks and the functional significance of any such change. I hypothesised that improved olfactory function will be associated with altered GM volume and/or CTh within structures of the primary and/or secondary olfactory networks, and that structural changes will be accompanied by increased functional activity. Identification of such areas will ultimately help to confirm their role as neuroanatomical correlates of OD.

7.4 Methods

7.4.1 Experimental Setting and Design

I performed a prospective longitudinal study in adult patients (≥18 years) with chronic rhinosinusitis (CRS) and healthy adult controls (≥18 years), in collaboration with the Interdisciplinary Smell and Taste Clinic, University Hospital Carl Gustav Carus Dresden, Germany. Patients were diagnosed with CRS with or without nasal polyposis and were consecutively recruited from those awaiting FESS at the University Hospital Carl Gustav Carus. All patients had been diagnosed and undergone initial medical treatment according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2012[319]. I excluded patients with neurological (including head injury), psychiatric or other conditions affecting olfactory function, as well as those who were not available for follow-up testing post-operatively, or those who had contra-indications to MRI scanning. The final patient cohort builds on my initial pilot group of 12 patients from the previous chapter. I recruited a convenience sample of control participants (≥18 years) from the local population, who were age and sex matched with the patient cohort. Only controls who were free from sinonasal pathology, as well as neurological (including head injury), psychiatric and other conditions that affect olfaction were included. As for the patient cohort, only those available for follow up assessment at 3 months, and those without contraindications to MRI scanning were included. For the cohort of controls undergoing functional imaging, only those who were normosmic at baseline, and with stable olfactory function across the two assessment sessions were included in the final analysis. See §3.3.1 for more details.

All patients and controls underwent clinical assessment, psychophysical olfactory testing and neuroimaging (see §3.3.2 and §3.3.3 for more details). This was performed at baseline (visit 1), and again at 3 months post-operatively or equivalent for controls (visit 2). Prior to assessment/scanning, all participants were screened for recent URTI or flare of AR (within 3 weeks). Clinical assessment included thorough medical history taking, as well as completion of the 'SNOT20' (GAV)[460]. Clinical examination included three-pass rigid nasendoscopy (with findings rated according

to the validated Lund-Kennedy scoring system[456]) as well as peak nasal inspiratory flow rate[473,579]. Psychophysical olfactory testing was performed using the 'threshold' (T) and 'identification' (I) components of the validated Sniffin' Sticks tool (see §3.3.2 for more details)[152]. Normosmia was attributed where T≥ 5.75 and I≥ 11[286]. The minimum clinically important difference for T and I are ≥ 2.5 points and ≥ 3 points respectively[287]. Clinically significant increase in composite TI score was therefore taken as ≥ 5.5 points.

7.4.2 Functional MRI Paradigm

All control participants, and patient 13 onwards underwent functional, in addition to structural imaging. I used two odourants for functional imaging (one per functional run): banana (neat, aroma, Frey+Lau, Henstedt-Ulzburg, Germany) and cis-3-Hexan-1-ol (neat, single molecule with smell of cut grass, Fluka Chemicals, Gillingham, UK). During each run, a single odourant was presented birhinally in a block design. During 'on' blocks, odours were delivered in 1-second pulses, embedded in 1L/min clean humidified air, with a 2-second interstimulus interval. During 'off' blocks, clean humidified air only was delivered. Odourants were delivered to participants via Teflon® nasal cannulae (4mm internal diameter) and through use of a computer controlled olfactometer[506] (see §3.3.3.4 for more details). On blocks were of duration 15s (6 volumes) and off blocks were of duration 30s (12 volumes). There were 9 on and 9 off blocks, totalling 170 volumes (including 8 initial dummy volumes). Each participant therefore underwent two functional runs per scanning session, with order of first odour pseudorandomised and counter-balanced across participants. At the end of each functional run, participants were asked to rate odour intensity (0-10, 10 = strongest) and hedonic valence (-5 to + 5, +5 = most pleasant). See Figure 7-1 for schematic diagram of experimental paradigm.

All subjects underwent on the day screening, including for MRI safety and acute change in olfactory function, and were given standard pre-scan instructions (see §3.3.3.1 and §3.3.3.4 for more details).

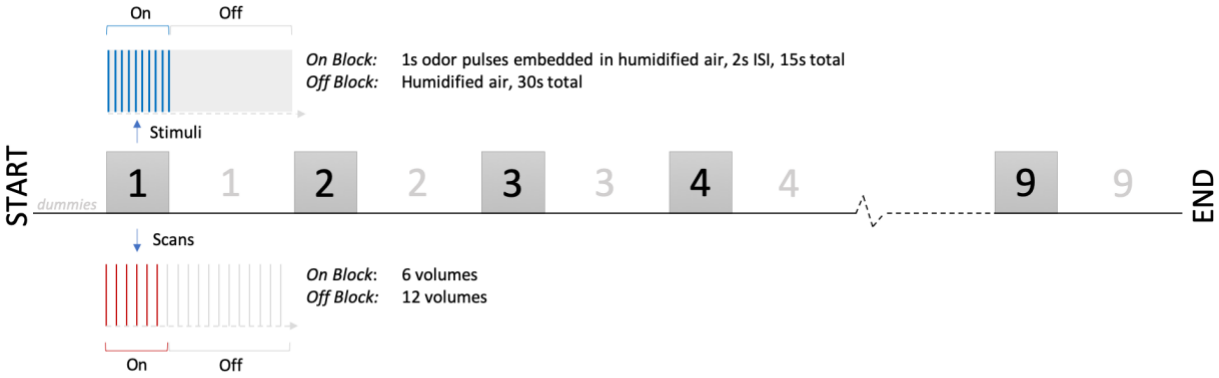


Figure 7-1: Schematic diagram of fMRI paradigm

7.4.3 Imaging Acquisition

Whole brain MRI was performed using a 3-T scanner (Verio, Siemens, Erlangen, Germany) with 8-channel phased-array head coil. Axial T1-weighted images were acquired using a 3-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence. The following parameters were used: repetition time (TR), 1890 ms; echo time (TE), 3.24 ms; inversion time (TI), 1100 ms; field of view (FOV), 280 mm; voxel size, 0.73 × 0.73 × 1 mm; and flip angle, 15° (in total, 176 contiguous slices). Functional data were collected using a 2D GE-EPI sequence, TR 2500ms, TE 22ms, FA 90°, voxel size 3*3*3mm.

7.4.4 Imaging Analysis: Voxel Based Morphometry

I performed voxel-based morphometry using the CAT12 toolbox (available from http://dbm.neuro.uni-jena.de/vbm/) implemented in SPM12 (Wellcome Centre of Imaging Neuroscience, UCL, London, UK) and MATLAB (The MathWorks, Natick, MA, USA). Pre-processing steps were undertaken as in the last chapter (see §3.3.3.3 and §6.4.3).

I compared change in GM volume between patients and controls using a flexible factorial model with the between subject factor = group (2 levels: patient, control) and the within subject factor = time (2 levels: first scan, second scan). An F test for

significant interaction between group and time was performed, controlling for total intracranial volume ['TIV', summated GM, WM and CSF volume [483]], age, sex and duration of condition. To account for inter-individual variability, and in an attempt to separate the effects of changing olfaction from those of surgery, I further compared change in GM volume between patients who had experienced a clinically significant improvement in olfactory function (increase in composite TI ≥ 5.5 points) with those patients whose scores were worse or unchanged after surgery. Again, this was performed using a flexible factorial model with the between subject factor = group (2 levels: patient_improved, patient_not improved) and the within subject factor = time (2 levels: first scan, second scan). An F test for significant group x time interaction was performed, controlling for TIV, age, sex and duration of condition. A within group comparison to determine GM volume change after surgery in patients who had clinically improved was also performed using a flexible factorial model, with the between subject factor = subject (1 level: patients) and the within subject factor = time (2 levels: first scan, second scan), controlling for TIV. T tests for significant increase and decrease in GM volume between visits were performed. An absolute threshold masking value of 0.1 was applied to avoid possible edge effects between different tissue types [377,385,540].

Finally, in order to further investigate potential associations between change in psychophysical score and change in GM volume, I extracted beta weights from clusters of significant GM volume change demonstrated during the above within group analysis. As I did not use psychophysical scores to identify these clusters, circular analysis was avoided. Extracted beta weight values were used to test for significant correlation between change in GM volume (ΔGM volume = second scan – first scan) and change in psychophysical score (ΔT/I/TI = post-op score – pre-op score). Results were thresholded using a *P* value that was Bonferroni corrected for multiple comparisons.

As I was interested in the GM volume within brain regions known to be relevant to olfaction and have strong *a priori* hypotheses regarding these olfaction-relevant areas, I performed a region of interest (ROI) analysis, in addition to whole brain analysis. Defined ROIs included bilateral areas of the primary and secondary

olfactory cortices as defined by Gottfried and Fjaeldstad *et al.,* [32,580]. ROIs from the primary olfactory network included the 'olfactory cortex' (or piriform cortex, PC), amygdala and entorhinal cortex. ROIs from the secondary olfactory network included the orbitofrontal cortex (OFC), caudate nucleus, anterior cingulate cortex (ACC), insula, putamen, pallidum, hippocampus (HPC), parahippocampus, thalamus and temporal poles (TP). ROIs were constructed within the WFU_PickAtlas software (available from: http://fmri.wfubmc.edu/software/pickatlas), based on the Automated Anatomical Labeling (AAL) atlas[545] for all regions except for the entorhinal cortex, which was constructed using Brodmann Areas 28 + 34, based on the Talairach Atlas[546]. I chose to differentiate the entorhinal cortex as an independent ROI from the parahippocampus, as this is anatomically known to receive direct input from the OB and therefore forms part of the primary olfactory network. The OFC ROI was constructed as per Kahnt *et al.,* 2012 and therefore included the bilateral AAL regions of: superior, middle, inferior and medial orbital gyri as well as the rectal gyri [544]. All whole brain analyses were corrected for multiple comparisons at the family wise error level ($P<0.05_{FWE}$). For the *a priori* ROI analysis, small volume corrections were implemented through the 'ROI' function in WFU_PickAtlas and results were further corrected for multiple comparisons at the FWE level ($P<0.05$), or at a more lenient uncorrected threshold of $P<0.001$, where no or limited results survived correction for multiple comparisons. In order to avoid issues surrounding non-stationarity in voxel-based volumetric analysis [497], I report only voxel-based results (see §3.3.3.4 for more details).

I prepared images for inclusion in the manuscript using the Xjview toolbox for SPM (available from: http://www.alivelearn.net/xjview/) and Microsoft PowerPoint (Microsoft Corporation, Redmond, WA).

7.4.5 Imaging Analysis: Cortical Thickness (CTh)

I analysed CTh using CAT12, implemented in SPM12. Patient and control T1 weighted images were initially segmented using the surface and thickness estimation writing options. As longitudinal segmentation was performed, as in the VBM pipeline, in

addition to segmentation of images from each time point, a mean image across time points was produced. Estimated spatial normalisation parameters were calculated for the segmented mean image and applied to the first and second images. Resultant surface data from both the right and left hemispheres were then smoothed using a 15mm FWHM kernel. For more information regarding CTh pre-processing in CAT112, see §3.3.3.3.

As for VBM, I compared change in CTh between groups (patient vs control and patients_improved vs patients_not improved) and within groups (patient_improved) using flexible factorial models. As for the VBM analysis F and T tests were used, with results corrected for sex, age and duration of condition (but not TIV, which unlike VBM is not required[482]). All CTh analyses were performed at the whole brain level, with results thresholded at $P<0.05_{FWE}$.

7.4.6 Imaging Analysis: Functional MRI

I analysed functional data using SPM12. Anatomical T1-weighted images were inspected and reoriented according to SPM priors during VBM analysis. I also visually inspected functional images for correct orientation according to SPM priors. Pre-processing involved initial realignment and unwarping of functional images followed by segmentation of T1-weighted images according to SPM tissue probability maps. Co-registration of functional and anatomical images was then performed, as well as normalisation to MNI space. Finally, data were smoothed using an 8mm FWHM kernel. I then performed a first level analysis in which the condition 'odour > baseline' was modelled for each subject, using the canonical HRF. Resultant contrast images were then subjected to a second level random-effects analysis. Second level between and within group analyses were performed using flexible factorial models as for structural analyses. Whole brain analyses were corrected for multiple comparisons at $P<0.05_{FWE}$. *A priori* ROI analysis (with small volume correction) was conducted as per structural work, with results thresholded at $P<0.05_{FWE}$, or a more lenient $P<0.001_{uncorr}$. As part of an exploratory analysis, I was interested in functional change within *a priori* ROIs that had demonstrated significant structural results.

Accordingly, I additionally performed small volume corrected ROI analysis in these areas using a Bonferroni corrected p value: $P<0.05$/[number of significant ROI]. Unlike in my VBM analysis, where I only used voxel-based inference (due to non-stationarity[483]), I chose to use intensity/cluster thresholding as an additional correction for multiple comparisons at these lenient thresholds, and only report clusters of ≥10 voxels. Again, results of uncorrected analyses are only reported where either no or limited results survived after correction for multiple comparisons. See §3.3.3.4 for more details.

7.4.7 Statistical Analysis

I performed supporting statistical analysis using GraphPad Prism (version 6, GraphPad Software, LaJolla, USA), with parametric or non- parametric tests as appropriate. Unless specified otherwise, statistical significance was attributed where $P<0.05$ and data are given as mean (SD) for parametric data or median for non-parametric data.

7.4.8 Ethical Considerations

This study was approved by the Ethics Committee of the Faculty of Medicine Carl Gustav Carus University Hospital, Dresden, Germany (EK number 56022016), and was conducted in accordance with the Declaration of Helsinki. All patients and controls provided full informed written consent prior to participation. Please see §3.3.5 for detailed information.

7.4.9 Funding

Funding was kindly provided for scanning from Prof Hummel of the Interdisciplinary Smell and Taste Clinic, Dresden [Deutsche Forschungsgemeinschaft (DFG HU441/18-1)].

7.5 Results

7.5.1 Demographics, Behavioural and Clinical Scores

7.5.1.1 Structural Cohort (All Participants)

T1 weighted images were available from 25 patients and 17 controls. One patient was excluded from further analysis due to cerebral atrophy, leaving 24 in total. There was no significant difference between groups in age (median age patients 47 (range 27-74), controls 44 (range 27-69), n=24:17, U=167, P=0.47) or sex (M:F = 15:9 patients, 11:6 controls, Fisher's exact test, P=0.99). The mean duration of CRS was 8 years (range 7 months to 57 years). Diagnoses and details of surgery can be found in Table 7-1. All patients were treated post-operatively with intranasal corticosteroid spray and saline irrigation.

Patient	Surgery	Findings	Procedure
1	Revision	Bilateral nasal polyposis, DNS	Septoplasty, bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
2	Primary	Bilateral nasal polyposis, DNS	Septoplasty, bilateral polypectomy/FESS (maxillary, ethmoid and frontal sinuses)
3	Revision	Right sided nasal polyposis	Right sided polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
4	Primary	Mucosal oedema, isolated antrochoanal polyp	Antrochoanal polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
5	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
6	Primary	Mucosal oedema, septal perforation	Bilateral FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
7	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
8	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
9	Primary	Bilateral nasal polyposis, DNS	Septoplasty, bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
10	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
11	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
12	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and sinuses)
13	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
14	Primary	Bilateral mucosal oedema	Bilateral FESS (maxillary and ethmoid sinuses)
15	Primary	Bilateral nasal polyposis, left concha bullosa, DNS	Septoplasty, resection of concha bullosa, bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid sinuses)
16	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
17	Revision	Left sided nasal polyposis	Left sided polypectomy/FESS (frontal)
18	Primary	Bilateral nasal polyposis, DNS	Septoplasty, bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
19	Primary	Bilateral nasal polyposis, DNS	Septoplasty, bilateral polypectomy/FESS (maxillary, ethmoid and frontal sinuses)
20	Primary	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
21	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
22	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid and sphenoid sinuses)
23	Revision	Bilateral nasal polyposis	Bilateral polypectomy/FESS (maxillary, ethmoid, sphenoid and frontal sinuses)
24	Primary	Bilateral nasal polyposis, right concha bullosa, DNS	Septoplasty, resection of concha bullosa, bilateral polypectomy/FESS (maxillary, ethmoid, frontal sinuses)

Table 7-1: Surgical Findings and Procedure. Main findings and surgical procedure for each of the study patients. FESS = functional endoscopic sinus surgery; DNS = deviated nasal septum.

Average threshold (T), identification (I) and composite threshold + identification (TI) scores were statistically significantly higher in the control group than the patient group at visit 1 and 2. In the patient group, there were statistically significant improvements in T, I and composite TI scores after surgery: group T and I scores fell below normosmia preoperatively but improved to normosmic levels postoperatively. Test scores reached clinically significant improvement after surgery in 10 patients for T ($\geq$ 2.5), 8 patients for I ($\geq$ 3) and 8 patients for composite TI ($\geq$ 5.5).

PNIF was significantly higher in controls than patients at visit 1 but not visit 2. Within the patient group, there was a statistically significant improvement in mean PNIF after surgery, which reached clinical significance in 15 patients ($\geq$20L/min). SNOT20 score was significantly higher in patients than controls at visit 1 and visit 2, and there was a statistically significant reduction in SNOT20 score in patients after surgery. Similarly, Lund-Kennedy (LK) score was significantly higher in patients than controls both at visit 1 and 2. Within the patient group, there was a statistically significant reduction in LK score after surgery.

Full clinical and behavioural data are shown in Table 7-2.

All Participants

	Visit 1			Visit 2			
Score	**Patients (n=24)**	**Controls (n=17)**	**Patient vs Controls**	**Patients (n=24)**	**Controls (n=17)**	**Patients vs Controls**	**Patients: (visit 1) vs (visit 2)**
T	4.5†	8.0†	*U*=121.5, *P*=0.028	7.6 (3.6)	9.6 (2.3)	t_{39}=2.158, *P*=0.037	*W*=170, *P*=0.008
I	10.0†1	14.0†1	*U*=64, *P*<0.0001	12.0†	15.0†	*U*=105, *P*=0.007	t_{23}=3.532, *P*=0.002
TI	13.63†	22.50†	*U*=104.5, *P*=0.007	20.38†	24.00†	*U*=111.5, *P*=0.013	t_{23}=3.845, *P*=0.001
SNOT20	34.3 (11.6)	5.7 (5.2)	t_{39}=9.22, *P*<0.0001	13.5†1	4.0†1	*U*=106.5, *P*=0.009	t_{23}=8.629, *P*<0.0001
PNIF	107.9 (46.0)	140.9 (43.3)	t_{39}=2.320, *P*=0.026	131.3 (45.8)	132.6 (45.3)	t_{39}=0.0967, *P*=0.923	t_{23}=2.759, *P*=0.011
LK	5.5†	0.0†	*U*=29, *P*<0.0001	3.5†	0.0†	*U*=72.5, *P*<0.0001	*W*=-144, *P*=0.005

fMRI Subgroup

	Visit 1			Visit 2			
Score	**Patients (n=12)**	**Controls (n=12)**	**Patient vs Controls**	**Patients (n=12)**	**Controls (n=12)**	**Patients vs Controls**	**Patients: (visit 1) vs (visit 2)**
T	5.375†1	8.250†1	*U*=40.5, *P*=0.070	7.27 (4.1)	9.73 (2.1)	t_{22}=1.842, *P*=0.079	*W*=35, *P*=0.1289
I	9.75 (4.1)	14.08 (0.9)	t_{22}=3.582, *P*=0.002	11.9 (2.7)	14.2 (1.6)	t_{22}=2.481, *P*=0.021	t_{11}=2.469, *P*=0.0312
TI	15.23 (7.8)	22.9 (3.0)	t_{22}=3.206, *P*=0.004	19.2 (6.3)	23.9 (2.8)	t_{22}=2.355, *P*=0.028	t_{11}=2.498, *P*=0.0296
SNOT20	38.4 (10.9)	5.6 (5.7)	t_{22}=9.242, *P*<0.0001	20.9 (13.3)	5.5 (5.4)	t_{22}=3.726, *P*=0.001	t_{11}=5.183, *P*=0.0003
PNIF	90.0†	143.0†	*U*=36, *P*=0.036	130 (53.7)	143.8 (45.7)	t_{22}=0.6751, *P*=0.507	*W*=46, *P*=0.0410
LK	2.5†	0.0†	*U*=15, *P*=0.000	0.0†	0.0†	*U*=52, *P*=0.093	*W*=-55, *P*=0.0020

Table 7-2: Clinical and behavioural scores in patients and controls shown as mean (SD) or †median values for visit 1 and visit 2, in all participants and fMRI subgroup. †1 Patient group data parametric, hence paired t test between visits.

7.5.1.2 fMRI Cohort

Functional data was available for a subset of 12 patients and 12 controls. There was no significant difference in age (mean age patients 50 (12) (range 32 – 74), controls 45 (14) (range 27-69), n=12:12, t_{22}=0.9227, P=0.3662) or sex (M:F = 8:4 patients, 8:4 controls, Fisher's exact test, P>0.99) between groups. Mean duration of CRS was 5 years (range 1 to 17). The patient fMRI group T and I scores again fell below normosmia preoperatively but improved to normosmic levels postoperatively. Improvement in psychophysical test score reached clinical significance in 5 patients for T (≥ 2.5) 4 patients for I (≥ 3) and 3 patients for composite TI (≥5.5). There were statistically significant improvements in PNIF, SNOT20 and LK scores in the fMRI patient group after surgery. See Table 7-2 for full results.

7.5.2 Voxel Based Morphometry

7.5.2.1 Patient vs Controls

For the interaction between group (patients vs controls) and time (first vs second scan), two adjacent clusters within the left OFC survived at P<0.05$_{FWE}$, during *a priori* ROI analysis. At P<0.001$_{uncorr}$ significant clusters were also demonstrated within the right entorhinal cortex and right PC (see Table 7-3). No voxels survived thresholding (P<0.05$_{FWE}$) at the whole brain level.

			MNI Coordinates				
Threshold	***ROI***	***Side***	***X***	***Y***	***Z***	***Z Score***	***F Score***
$P<0.05_{FWE}$	OFC	L	-3	36	-27	4.25	25.67
		L	-3	33	-27	4.17	24.59
$P<0.001_{uncorr}$	Entorhinal cortex	R	15	-2	-26	3.43	15.81
	PC	R	2	14	-8	3.25	14.09

Table 7-3: Voxels of significant interaction between group (patients vs controls) and time (first vs second scan) for GM volume during a priori ROI analysis.

7.5.2.2 Clinically Improved vs Not Clinically Improved Patients

Eight patients experienced a clinically significant improvement in composite TI score and seven had scores that were worse or unchanged after surgery. Comparing these groups (patients_improved vs patients_not improved) mitigates the potentially confounding effect of surgical stress, which may have led to non-olfactory related changes in the patient group, compared to the non-surgical normosmic control group. Accordingly, there was a cluster of significant interaction between time and group within the right ACC that survived thresholding at $P<0.05_{FWE,}$ during *a priori* ROI analysis. At $P<0.001_{uncorr}$, additional clusters were demonstrated within the right HPC, bilateral OFC, left parahippocampus and left TP (see Table 7-4). No voxels survived thresholding at the whole brain level ($P<0.05_{FWE}$).

			MNI Coordinates				
Threshold	***ROI***	***Side***	***X***	***Y***	***Z***	***Z Score***	***F Score***
$P<0.05_{FWE}$	ACC	R	8	32	24	4.24	51.49
$P<0.001_{uncorr}$	HPC	R	36	-14	-21	3.37	23.80
	OFC	L	-26	51	-4	3.71	32.13
		R	2	34	-15	3.28	22.08
		R	26	57	-4	3.27	21.82
	Parahippocampus	L	-22	-30	-18	3.21	20.75
		L	-24	-28	-20	3.12	19.13
	TP	L	-45	15	-36	3.27	21.83
		L	-46	6	-12	3.16	19.9

Table 7-4: Voxels of significant interaction between group (patient_improved vs patient_not improved) and time (first vs second scan) for GM volume during a priori ROI analysis.

7.5.2.3 Change in GM Volume After Surgery – Clinically Improved Patient Group

Within the group of patients with clinically improved composite TI score after surgery there were areas of both increased and decreased GM volume during *a priori* ROI analysis. At $P<0.05_{FWE}$, a cluster of significant increase in GM volume was found within the right HPC. At $P<0.001_{uncorr}$, there was an additional cluster of increased GM volume within the right parahippocampus, and several clusters of decreased GM volume within the right ACC, bilateral HPC, insula, OFC and left TP (see Table 7-5, Figure 7-2 and Figure 7-3). No results survived thresholding at the whole brain level ($P<0.05_{FWE}$).

			MNI Coordinates			
Threshold	***ROI***	***Side***	***X***	***Y***	***Z***	***T Score***
			Increased GM Volume			
$P<0.05_{FWE}$	HPC	R	34	-15	-20	9.43
$P<0.001_{uncorr}$	Parahippocampus	R	30	-20	-26	7.73
			Decreased GM Volume			
$P<0.001_{uncorr}$	ACC	R	6	32	24	8.75
	HPC	L	-27	-38	-2	8.0
		L	-34	-33	-8	5.78
		R	32	-40	-2	4.81
	Insula	R	38	16	6	7.46
		R	46	-10	0	5.55
		R	42	-16	-4	5.35
		L	-39	0	3	5.11
	OFC	R	48	24	-6	9.6
		R	14	39	-4	7.71
		R	52	34	-6	6.68
		R	48	45	-4	6.22
		L	-10	44	-6	5.68
		L	-26	52	-4	5.64
		Midline	0	45	-14	5.1
		L	-2	45	-20	5.05
	TP	L	-44	16	-18	6.56

Table 7-5: Voxels of significant GM volume change after surgery within the clinically improved patient cohort.

7.5.2.4 Correlation Between Change in GM Volume and Change in Psychophysical Score

Within the subgroup of patients who clinically improved after surgery, there were no correlations between Δ GM volume (from 19 clusters of significant GM change as outlined in Table 7-5) and Δ psychophysical score that were statistically significant at the specified results threshold of $P<0.0026$ [Bonferroni corrected $P<0.05/19$].

7.5.3 Cortical Thickness

No voxels survived thresholding ($P<0.05_{FWE}$) during between (patients vs controls; patients_improved vs patients_not improved) or within group (patients_improved) analyses at the whole brain level.

7.5.4 Functional MRI

Descriptive statistics for the perceived intensity and hedonic valence of the odours grass and banana in patients and controls are provided in Table 7-6. Odours were isointense in patient and control groups; functional analysis of the conditions banana and grass were pooled.

Patients

	Visit 1			Visit 2		
	Banana	**Grass**	**Banana vs Grass**	**Banana**	**Grass**	**Banana vs Grass**
Intensity	3.5†	2.5†	*W*=-8.0, *P*=0.578	8.5†	7.5†	*W*=-16.0, *P*=0.125
Valence	0.50 (1.88)	1.083 (2.021)	t_{11}=0.8449, *P*=0.416	1.33 (3.06)	0.92 (3.70)	t_{11}=0.2987, *P*=0.771

Controls

	Visit 1			Visit 2		
	Banana	**Grass**	**Banana vs Grass**	**Banana**	**Grass**	**Banana vs Grass**
Intensity	7.0†	9.0†	*W*=15.0, *P*=0.328	7.17 (1.95)	7.67 (1.56)	t_{11}=0.7479, *P*=0.470
Valence	1.923 (2.53)	-1.54 (2.76)	t_{11}=4.099, *P*=0.002	0.77 (2.89)	0.077 (2.75)	t_{11}=1.091, *P*=0.297

Table 7-6: Intensity and hedonic ratings of fMRI odours, in patients and controls, shown as mean (SD) or †median values for visit 1 and visit 2.

No significant group by time interaction was found when comparing change in patient and control functional activity for any odour (grass/banana) during whole brain or ROI analysis, either at $P<0.05_{FWE}$ or $P<0.001_{uncorr}$. As significant structural results were demonstrated in 8 ROIs (ACC, entorhinal cortex, HPC, insula, OFC, PC, parahippocampus and TP), these areas were further interrogated for significant functional interaction with results thresholded at $P<0.003125$ [Bonferroni corrected $P< 0.05/(8 \times 2_{Right + Left})$]. At this more lenient threshold there was one small area of significant time by group interaction in the right parahippocampus. However, this cluster did not survive correction by the cluster criterion of ≥10 voxels.

Within group analysis was not limited to patients who had experienced clinical improvement in composite TI score after surgery (≥5.5) due to small n number (n=3). Therefore, within group analysis was performed across all patients in the subgroup (n=12). As I was particularly interested in the functional significance of structural changes demonstrated, I limited my within-group *a priori* ROI analysis to the 8 regions outlined above. At $P<0.003125$ and ≥10 voxels, there were significant increases in functional activity after surgery within the left ACC, bilateral insula, bilateral OFC and right TP (see Table 7-7, Figure 7-2 and Figure 7-3). The bilateral clusters within the OFC additionally reached significance at $P<0.05_{FWE}$. There were no significant clusters of increased functional activity after surgery that survived thresholding at $P<0.05_{FWE}$ during whole brain analysis.

			MNI Coordinates				
Threshold	***ROI***	***Side***	***X***	***Y***	***Z***	***T Score***	***k***
$P<0.05_{FWE}$	OFC	R	40	28	-2	5.61	2
		L	-30	24	-12	5.14	3
$P<0.003125_{BC}$	ACC	L	-8	34	24	4.26	45
≥10 voxels	Insula	R	40	26	-2	4.52	100
		L	-34	24	2	4.31	230
		R	38	16	-14	3.81	43
		R	50	0	-2	3.32	11
	OFC	R	40	28	-2	5.61	37
		L	-30	24	-12	5.14	132
	TP	R	46	12	-18	3.42	10

Table 7-7: Clusters of significant increase in BOLD signal in patients after surgery. K = size of cluster in voxels.

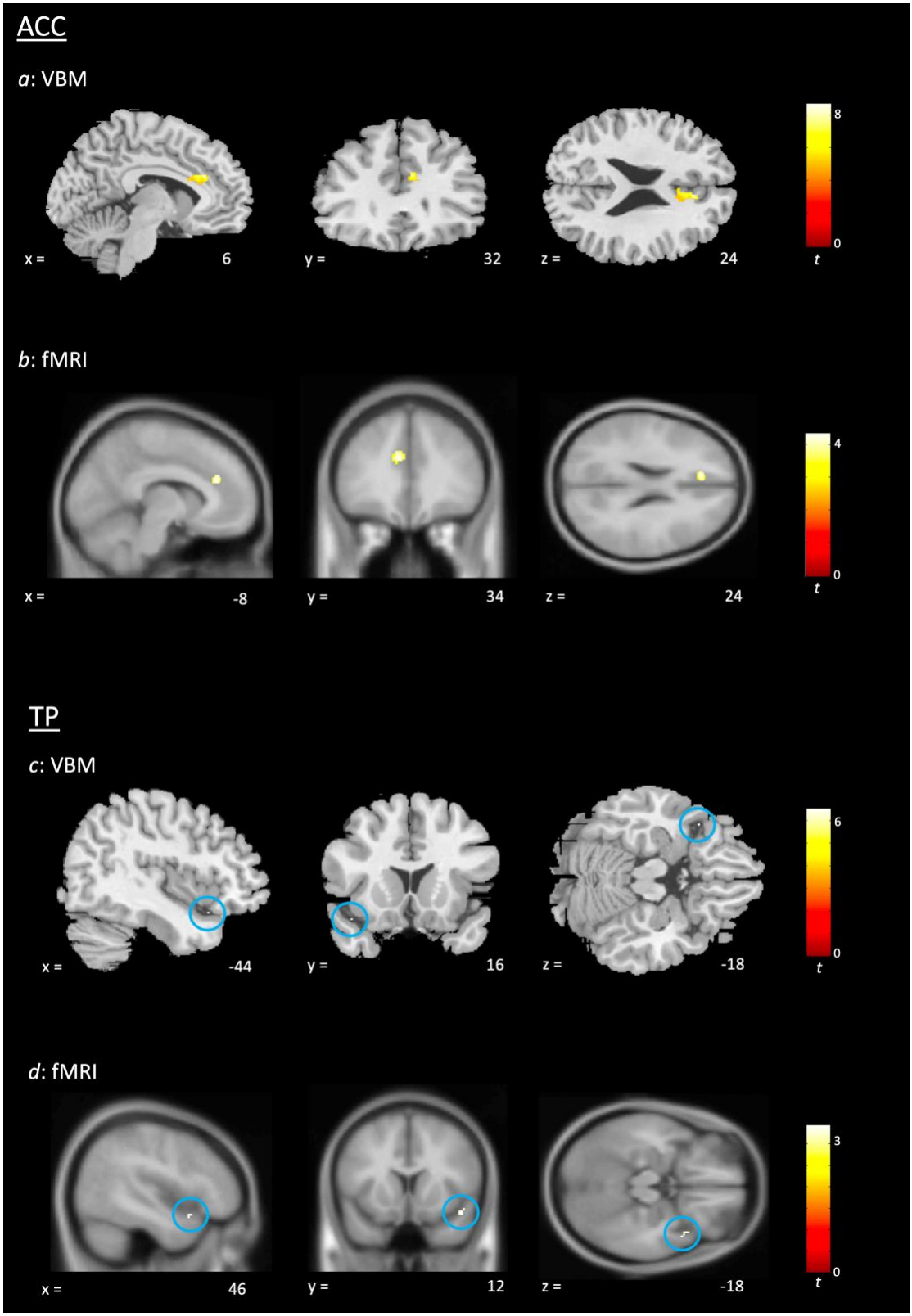


Figure 7-2: Structural and functional MRI results for ACC and TP. To help differentiate between imaging modalities, VBM results are shown using the ch2bet stripped skull brain template and fMRI results are shown using the avg152T1 brain template. Small clusters have been circled: blue circles highlight a single significant cluster. All coordinates are in MNI space. Color bars show associated peak T score (please note that the maximum integer labeled does not reach top of color bar range). Subsections: (a) VBM results for decreased GM volume after surgery within the ACC, in improved patient group ($p < 0.001$uncorr); (b) fMRI results for increased BOLD signal after surgery within the ACC, in patient group ($p < 0.003125$BC, ≥10 voxels); (c) VBM results for decreased GM volume after surgery within the TP, in improved patient group ($p < 0.001$uncorr); (d) fMRI results for increased BOLD signal after surgery within the TP, in patient group ($p < 0.003125$BC, ≥10 voxels). Abbreviations: ACC, anterior cingulate cortex; TP, temporal pole(s).

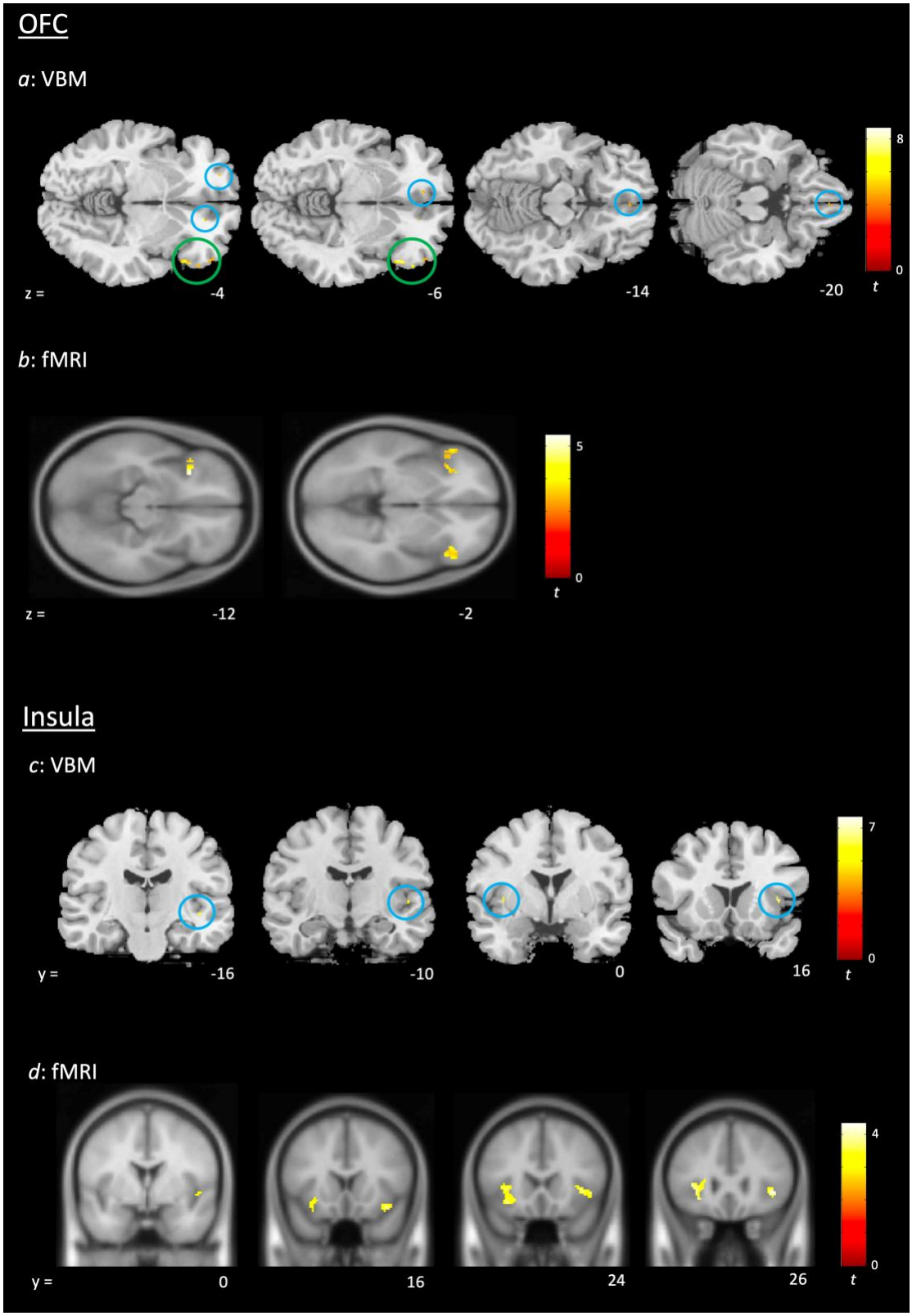


Figure 7-3: Structural and functional MRI results for OFC and Insula. To help differentiate between imaging modalities, VBM results are shown using the ch2bet stripped skull brain template and fMRI results are shown using the avg152T1 brain template. Small clusters have been circled: blue circles highlight a single significant cluster; green circles highlight multiple significant clusters. All coordinates are in MNI space. Color bars show associated peak T score (please note that the maximum integer labeled does not reach top of color bar range). For ease of display— axial sections only shown for OFC results and coronal sections only shown for results within the insula. Subsections: (a) VBM results for decreased GM volume after surgery within the OFC, in improved patient group ($p < 0.001$uncorr); (b) fMRI results for increased BOLD signal after surgery within the OFC, in patient group ($p < 0.003125$BC, ≥10 voxels); (c) VBM results for decreased GM volume after surgery within the insula, in improved patient group ($p < 0.001$uncorr); (d) fMRI results for increased BOLD signal after surgery within the insula, in patient group ($p < 0.003125$BC, ≥10 voxels). Abbreviations: OFC, orbitofrontal cortex.

7.6 Discussion

7.6.1 Key Findings

To my knowledge, this is the first prospective study to demonstrate structural as well as functional plasticity of the central olfactory networks in relation to improved olfactory function. I used four separate approaches to identify *a priori* regions of interest in which change in GM volume was related to olfactory improvement. *First*, I compared change in GM volume between patients and controls and demonstrated significant group x time interaction within areas of the primary (PC, entorhinal cortex) and secondary olfactory network (OFC). *Second*, I compared change in GM volume between patients who had clinically significant improvements in psychophysical (TI) scores after surgery with those who did not, and found significant group x time interaction within areas of the secondary olfactory network, (ACC, HPC, OFC, parahippocampus and TP). I also performed a within group analysis in clinically improved patients and demonstrated significant changes in GM volume within areas of the secondary olfactory network (ACC, HPC, insula, OFC, parahippocampus and TP). *Third*, I tested for correlation between Δ GM volume and Δ psychophysical test score in clusters of significant structural change after surgery, though no results survived correction for multiple comparisons. *Fourth*, I interrogated the regions identified in steps 1-3 for increased functional activity within my fMRI subgroup and demonstrated increases in BOLD signal after surgery within four regions: ACC, insula, OFC and TP. Of interest, in patients who had a clinically significant improvement in TI score after surgery, there were significant reductions in GM volume within these four areas. I did not demonstrate any significant alterations in CTh after surgery at the given results threshold.

7.6.2 Plasticity of the Olfactory Networks

My most interesting results were found within the OFC, ACC, insula and TP, where both structural and functional plasticity were observed. The OFC, ACC and insula are well recognised components of the secondary olfactory network. Accordingly, high probabilities for olfactory activation have been demonstrated in these regions during

meta-analysis of functional neuroimaging studies[31]. More specifically, and as described in the last chapter, the OFC is thought to be involved in experience- and affect-dependent odour percept encoding[311,556,557], multimodal sensory integration[555], perceptual decision making[581] and conscious odour perception[558]. The ACC is thought to have a role in hedonic odour processing[556], odour recognition memory[582] and a potential role in modulation of olfactory attention[583], whilst the insula receives olfactory, gustatory and trigeminal information and is thought to act as a multimodal chemosensory convergence zone[507] involved in flavour perception. The temporal poles receive connections from regions within the primary and secondary olfactory networks, including the amygdala, OFC and insula, and though less well established, have been linked to olfaction in functional neuroimaging[563] and lesion studies[560–562]. In particular, the temporal poles assign emotional valence to sensory stimuli[564] and may be involved in odour memory [561].

Monosynaptic connections between the ACC and primary olfactory network (specifically the anterior olfactory nucleus (AON)) have been demonstrated in primates[583]. Given that the AON has bilateral feedforward connections with structures of the primary olfactory network and feedback connections with the olfactory bulbs, as well as extensive connections with both the posterior OFC and anterior insula[30,583,584], this emphasises the potentially important functional link between these structures, and may underlie the pattern of results I demonstrated, where structural and functional plasticity appeared to be most robust within the ACC, insula and OFC. The functional link between these regions has also been highlighted by time course series in which temporally overlapping activation of these structures occurs[515,581].

It is of particular interest that the increased functional activity I demonstrated within the OFC, ACC, insula and TP was accompanied by areas of decreased, rather than increased GM volume. As outlined in §1.5.3.1.2, previous cross-sectional morphological work has demonstrated significant positive correlation between psychophysical test scores and both GM volume[373] and CTh[372] within the right OFC of healthy participants. Increased GM volume has also been demonstrated within the bilateral OFC of perfumers (considered to be olfactory experts) compared with non-

expert controls[377]. Similarly, increased GM volume has been demonstrated within the right insula of master sommeliers (considered to be olfactory experts)[378], or left insula of 'super-smellers'[585], compared to controls. Furthermore, cross-sectional VBM studies have demonstrated GM volume loss within the OFC, ACC and insula in patients with olfactory dysfunction of various aetiologies[386,387,399,496,586–588]. Though lesions of the TP are observed in post-traumatic OD[588], to my knowledge, no previous cross-sectional studies have reported reduced GM volume or CTh of the TP in other patient populations.

Morphometric studies of the OFC, ACC and insula (less so for the TP) would therefore appear to indicate that increased function is related to increased GM volume or CTh. This contrasts with my results, where I demonstrated reduced GM volume in association with increased functional activity in these regions. However, as these studies were cross-sectional, when considering this apparent divergence, it is also worth looking at newly emergent longitudinal work.

Two recent prospective studies have assessed change in GM volume and/or CT after a period of olfactory training (OT), either in patients with post-infectious olfactory dysfunction[578], or in healthy controls[577], at 12 and 6 weeks respectively. Gellrich and colleagues demonstrated a small area of increased GM volume within the right OFC during subgroup analysis of PIOD patients who had clinically improved after 12 weeks of training. On whole group analysis, this OFC cluster was not present, though there was significantly increased GM volume within the hippocampus and parahippocampus. The authors did not demonstrate significant GM volume change within the insula, ACC or TP. Al Ain and colleagues demonstrated increased GM volume within the left OFC and right TP during within group (post-OT –pre-OT) but not between group (group (OT/control) x time interaction) comparisons. Whole brain and region of interest analysis, however, demonstrated no significant correlations between change in GM volume/CTh or change in psychophysical test score in this study. Neither Gellrich *et al.,* nor Al Ain *et al.,* reported results of analysis for decreased GM volume/CTh.

Comparison of these results with my own is, however, limited by different approaches to analysis, patient populations and treatment interventions. Olfactory

training has been shown to improve odour identification more than odour threshold[589], the latter of which has been argued to better represent the peripheral olfactory system[313], which is targeted by functional endoscopic sinus surgery. Whilst the exact mechanisms remain to be elucidated, it is possible that OT could improve olfactory function in a mechanistically different way than surgery. One could speculate that sinonasal surgery, by targeting the peripheral olfactory organ and consequently increasing afferent input to the central system, results in a bottom-up process that improves the efficiency of existing networks, unlike OT, which may involve more top-down processes. Increasing the efficiency of existing networks could conceptually involve pruning of redundant synapses (+/- other unknown cellular changes, e.g., glial) and consequently reduced GM volume. Similar arguments have been made for reduced GM volume and CTh in areas such as the OFC and OC in healthy controls, compared to patients with isolated congenital anosmia[590]. Top down processes such as OT, or other 'learning', may conversely involve mechanisms such as axonal remodelling, synaptogenesis or dendritic spine growth[394,591], neurogenesis [[13,395–397,592] but also see e.g. [593]] or glial alterations that result in increased GM volume/CT – as seen in OT or in subjects with high levels of 'olfactory expertise' [for further discussion, please see §9.2.3]. Such speculative differences in short-term structural plasticity do not theoretically preclude GM atrophy following prolonged reduced afferent input or other central dysfunction associated with disease states, as seen in cross-sectional patient studies.

That being said, similar to Gellrich *et al.,* within the group of patients who had clinically improved, I also demonstrated increased GM volume within the parahippocampus, and both increased and decreased GM volume within the hippocampus. Further, I found significant time x group interactions within both these regions when comparing patients who had clinically improved to those who hadn't. Comparing BOLD signal change in patients versus normosmic controls, whilst a small cluster of significant time x group interaction was demonstrated in the right parahippocampus, this did not survive the cluster criterion of 10 voxels. Furthermore, during within group analyses, I was not able to demonstrate significant

increase in BOLD signal within these regions. This will be discussed in more detail in the general discussion (see §9.2).

Taken together, the relationship between neuroanatomical structure and function is likely complex. It is possible that different regions undergo different types of structural alteration following treatment for OD, potentially driven by different underlying physiological/pathophysiological processes (see §9.2 for further discussion) that may be related to the type of OD or type of treatment. To this end, in my next chapter I aim to expand on my work here by performing a further multimodal neuroimaging study in patients with non-CRS OD, undergoing functional septorhinoplasty – a surgical procedure that primarily aims to improve nasal airflow, and which has been shown to improve olfactory function.

Despite these complexities, I suggest that change in GM volume and/or CTh, independent of directionality, indicates structural plasticity where association with change in olfactory function can be demonstrated. Accordingly, my longitudinal results help to confirm the role of the OFC, ACC, insula and TP as neuroanatomical correlates of OD – building on the existing cross-sectional literature linking structure to function in these regions.

7.6.3 Study Limitations and Future Work

Given the observed inter-individual variation in improvement in olfactory function after surgery, and in order to investigate the effects of changing olfaction – separated from the potentially confounding effects of surgical stress – I subdivided my patient cohort for part of my structural analysis into those who had experienced clinically significant improvement, and those who had not. Therefore, the participant numbers within my subgroup analyses were small. Whilst statistically significant results within the context of a small sample may actually represent a larger effect size than the same results seen in a larger sample (see §8.6.4 for further discussion), smaller samples are more prone to sampling variation. At present, it is unknown whether there is significant inter-individual variability in structural and/or functional plasticity (though see ref[364] and associated discussion in §1.5.3.1.2). Future

longitudinal studies may wish to focus exclusively on patients, recruiting a larger initial cohort, which should allow subsequent analysis according to clinical improvement with larger subgroups (and mitigate the confounding effect of surgical stress). Furthermore, as olfactory fMRI was only performed in a subset of 12 patients, it was not possible to subdivide this group according to clinically significant improvement. Future work should therefore use a larger fMRI group – ideally comprising the entire structural cohort.

Finally, the temporal course of structural changes following alteration in sensory input may be neither linear, nor contemporaneous with fMRI demonstrable functional plasticity. For this reason, it would be of interest to perform a prolonged longitudinal study, with an increased number of time points after surgery (falling both earlier and later than in my current work), to determine whether an initial reduction in GM volume is followed by a subsequent increase, or whether changes in BOLD signal may precede or lag some of the structural changes seen – for example within the hippocampus and parahippocampus. Given the duration required to perform clinical longitudinal studies (in which 'real-world' surgery occurs for patients across an unpredictable time period, meaning their recruitment and follow up times are staggered, significantly prolonging the overall study duration), however, this is beyond the scope of my PhD.

7.6.4 Conclusions

To my knowledge, this is the first study to demonstrate structural as well as functional plasticity in association with improved olfactory function. In particular, I demonstrated 'functionally significant' structural plasticity within the ACC, insula, OFC and TP, after surgical treatment for CRS. This multimodal longitudinal evidence provides a stronger link between structure and form in these regions, helping to confirm their role as clinically relevant neuroanatomical correlates of OD. In my next chapter, I aim to determine whether the changes seen were aetiology/treatment specific.

8 Is the Observed Functionally Significant Structural Plasticity Treatment/Aetiology Specific?

8.1 Summary

In my previous chapter, I demonstrated 'functionally significant structural plasticity' within the central olfactory networks, in association with improved olfaction after surgical treatment of chronic rhinosinusitis (CRS). To confirm and expand on these findings, and in particular to determine whether they are aetiology and/or treatment specific, the primary aim of this study was to determine whether these same regions undergo functionally significant structural plasticity following functional septorhinoplasty (fSRP), in patients with non-CRS olfactory dysfunction (OD), of mixed cause. fSRP has previously been shown to improve olfactory function, and the secondary aim of this study was to provide initial insights into possible mechanisms by which fSRP affects olfaction. I performed a prospective, multimodal neuroimaging study in participants undergoing fSRP, including patients with non-CRS OD of mixed cause, as well as normosmic controls. Participants underwent psychophysical olfactory testing, assessment of nasal airway, structural and functional neuroimaging. This was performed pre- (n=10) and postoperatively (n=9) in patients, and preoperatively in controls (n=10). There was a statistically and clinically significant improvement in mean psychophysical olfactory scores after surgery. This was associated with structural and functional plasticity within areas of the central olfactory network (ACC, OFC, insula and TP). Improved psychophysical scores were significantly correlated with change in bilateral measures of nasal airflow, not measures of airflow symmetry, suggesting that improved overall airflow was more important than correction of septal deviation. This work provides further evidence for the role of these regions as neuroanatomical correlates of general OD.

8.2 Statement of Contribution

This chapter has been adapted from my published paper: **Whitcroft KL, Mancini L, Yousry T, Hummel T, Andrews P (2023) Functional septorhinoplasty alters brain structure and function: neuroanatomical correlates of olfactory dysfunction. Front Allergy: 4:1079945.** For the purposes of my thesis, sections have been expanded or reduced and language/style modified as necessary.

PA, TH, LM, TY and I planned the study. LM provided MRI physics input (imaging acquisition). LM and I gathered all data. I analysed all data and interpreted the results. LM provided analysis advice. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

8.3 Introduction

In chapter 7, I demonstrated functionally relevant structural plasticity within the ACC, insula, OFC and TPs, in association with improved olfaction, after FESS for CRS[594]. This longitudinal data provides better strength of evidence[359] for these regions as neuroanatomical correlates of OD than previous cross-sectional work[399,493,540,559,576]. Unexpectedly, however, I demonstrated decreased GM volume in these regions – a finding that had not been reported elsewhere in the small amount of emergent longitudinal literature at the time[577,578]. However, these studies were not in patients with CRS, and had interrogated structural changes following olfactory training, not FESS. To both confirm and expand on these findings, and in particular to determine whether the changes demonstrated in these structures are aetiology and treatment specific, the primary aim of the present study was to characterise structural and functional plasticity of these regions in response to functional septorhinoplasty in patients with non-CRS OD.

Functional septorhinoplasty (fSRP) is a surgical procedure that aims to improve bilateral nasal airflow, and has previously been shown to improve olfaction, though the relevant evidence base is limited by methodological inconsistencies[595]. Given the

paucity of currently available treatment options for OD, my secondary aim was to gather pilot data investigating the potential mechanism by which fSRP improves olfaction, using subjective, psychophysical, and for the first time, more 'objective' structural and functional neuroimaging measures.

I therefore performed a prospective, multimodal neuroimaging study (VBM, analysis of cortical thickness and olfactory fMRI) in patients with non-CRS OD undergoing fSRP, compared with a normosmic preoperative surgical control group. I hypothesised that improved olfactory function will be accompanied by increased BOLD signal and structural change within the ACC, insula, OFC and TP. Where my results replicate those demonstrated in my CRS cohort, this will help to confirm these regions as clinically-relevant neuroanatomical correlates of general olfactory dysfunction, independent of aetiology/treatment.

8.4 Methods

8.4.1 Experimental setting and design

I performed a prospective cohort study based at the Royal National ENT Hospital (formally the Royal National Throat Nose and Ear Hospital), and the Lysholm Department of Neuroradiology, the National Hospital for Neurology & Neurosurgery, both part of UCLH. Adult patients (≥18 years) with OD undergoing fSRP to improve nasal airflow were included. Patients were only eligible for the study if they both had established OD *and* required fSRP, limiting the available (pre-pandemic) patient population. As my primary aim was to investigate the neuroanatomical correlates of OD, patients with nasal obstruction alone were not included, given the variability in olfactory function within this cohort[297,596]. Therefore, a pragmatic study sample was used and patients with nasal obstruction + OD (defined as TDI score of <30.75 – see below) of mixed, non-CRS aetiology were included. CRS was excluded by PA based on the contemporaneously available EPOS 2012 guidelines[319], including clinical history, examination findings (with 3-pass endoscopy), and imaging. Patients with allergic rhinitis (diagnosed based on clinical history and skin prick testing for common aeroallergens) were only included where their OD persisted despite full medical management according to the EPOS 2012 guidelines [319]. Olfactory dysfunction was defined according to contemporaneously available guidelines[425]. However, of note, the term 'idiopathic' was used in patients with nasal obstruction ± allergic rhinitis, but no other clinically identifiable cause of OD. Patients with OD due to head injury or suspected/confirmed neurodegenerative disease were excluded, due to potential baseline structural brain alterations. No patients underwent olfactory training before or during the study period. I additionally excluded patients who were not available for follow-up testing, or those who had contra-indications to MRI scanning. Normosmic control participants were taken from the same population of patients with nasal obstruction awaiting fSRP in an effort to ensure the groups were otherwise comparable, reduce the effect of confounding factors, and better target olfactory function as the target variable of interest. Controls were age/sex matched, with other exclusion criteria as per patients.

All participants underwent clinical assessment, psychophysical testing and neuroimaging (see §3.3.2 for more details). In patients, this was performed at baseline (visit 1), and again at 4 months post-operatively (visit 2, see Figure 8-1). Controls were assessed at baseline only. Clinical assessment included thorough medical history taking and completion of 'SNOT23'[463], 'NOSE' score[465] and VAS[579]. Clinical examination included three-pass rigid nasendoscopy and PNIF. In the patient cohort unilateral PNIF measurements were collected at visit 1 and 2 in order to determine change in symmetry of nasal airflow after surgery. These were used to calculate two scores, with the aim of directly measuring the functional significance of septal deviation [where '*R*' and '*L*' = unilateral PNIF flow rate for right and left side. PNIF values of <30L/min assigned 0]: 1. The absolute difference in airflow between right and left nostrils in L/min ('*AD*'); 2. Airflow symmetry ('*AS*') – where 0 denotes equal airflow between right and left sides, and values closer to 0 indicate greater symmetry.

$$AD = |R - L|$$

$$AS = |\frac{R}{R + L} - 0.5|$$

Psychophysical olfactory testing was performed using the birhinal Sniffin' Sticks tool (full TDI, see §3.3.2 for more details)[152]. Normosmia was attributed where TDI was ≥ 30.75, hyposmia where TDI is >16, but <30.75, and functional anosmia ≤16[286]. The MCID for T, D and I are ≥2.5 points and ≥3 points respectively and TDI ≥5.5 points[287].

MRI scans were also used to calculate Lund-Mackay (LM) scores for both patient and control groups. Mean 'normal' LM score in patients without CRS has been previously demonstrated as 4.3[328]. See §3.3.2 for more details.

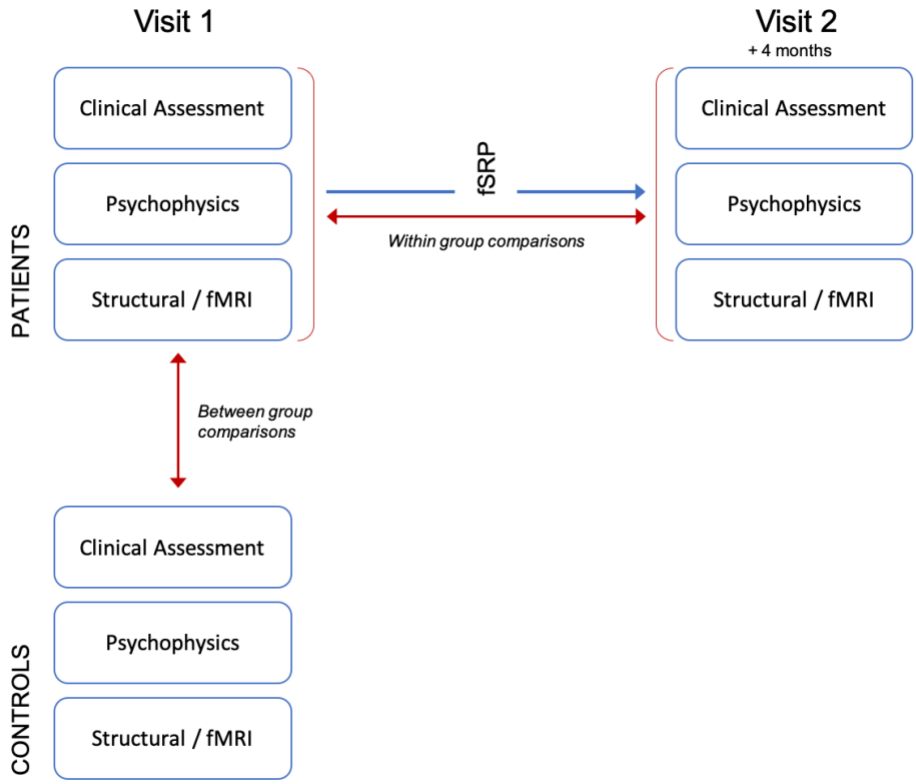


Figure 8-1: Experimental design.

All patients underwent fSRP by PA using a standardised external approach, aiming to maximise symmetrical, bilateral nasal airflow. This involved three main stages: 1 – septoplasty with nasal bone realignment, increasing airway symmetry; 2 – internal nasal valve augmentation using spreader grafts (autologous cartilage), increasing width of nasal airway; 3 – external valve augmentation using columellar strut (autologous cartilage), increasing height of nasal airway. Figure 8-2 illustrates standardised placement of spreader grafts and columellar struts.

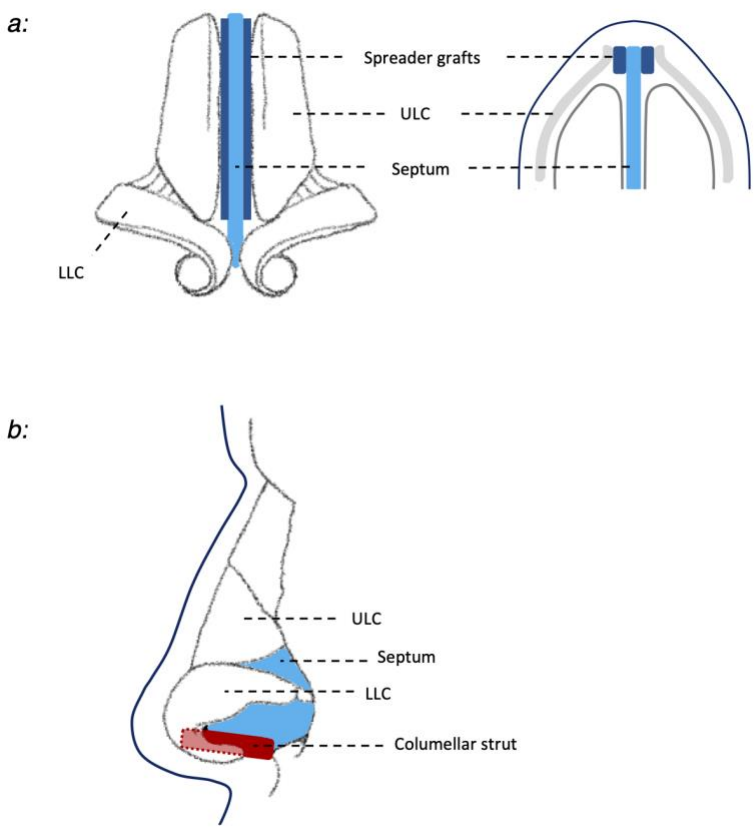


Figure 8-2: Diagram showing: (A) standardised placement of spreader grafts between septum and upper lateral cartilage; (B) standardised placement of columellar strut between medial crura of lower lateral cartilage. ULC, upper lateral cartilage; LLC, lower lateral cartilage.

8.4.2 Functional MRI Paradigm

All participants underwent olfactory functional MRI, in addition to structural imaging. Two odourants were used for functional imaging (one per functional run): banana (neat, aroma, Dale Air, Rochdale, UK) and cis-3-hexenol (neat, Firmenich, Middlesex, UK). During each run, a single odourant was presented birhinally in a block design. During 'on' blocks, odours were delivered in 1-second pulses, embedded in 1L/min clean humidified air, with a 2-second interstimulus interval. During 'off' blocks, clean humidified air only was delivered. Odourants were delivered to participants via Teflon® nasal cannulae (4mm internal diameter) and through use of a computer controlled olfactometer[506]. Due to low flow rates (which do not produce perceptible thermo-mechanical trigeminal activation), warming was

not required. On and off blocks were of duration 20s. There were 9 on and 9 off blocks, with 233 volumes in total. Each participant underwent two functional runs per scanning session, with order of first odour pseudorandomised and counter-balanced across participants. At the end of each functional run, participants were asked to rate odour intensity (0-10, 10 = strongest) and hedonic valence (-5 to + 5, +5 = most pleasant). See Figure 8-3 for schematic diagram of experimental paradigm.

Pre-scan preparation was performed as standard (see §3.3.3.1 and §3.3.3.4 for more details).

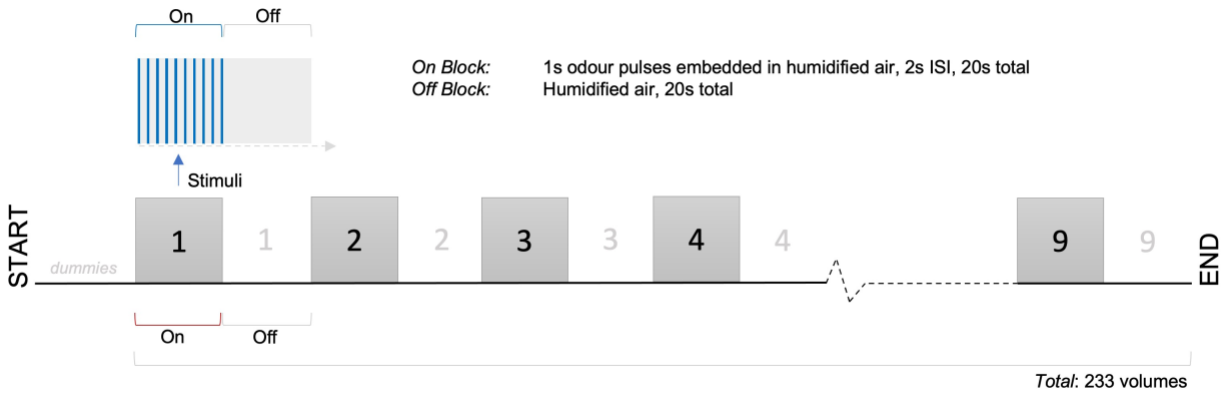


Figure 8-3: fMRI experimental paradigm.

8.4.3 Imaging Acquisition

Whole brain MRI was performed using a 3-T scanner (MAGNETOM Prisma, Siemens, Erlangen, Germany) with 64-channel head coil. Sagittal T1-weighted images were acquired using a 3-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence. The following parameters were used: repetition time (TR), 2000ms; echo time (TE), 1.96ms; inversion time (TI), 880ms; field of view (FOV), 282x282 mm^2; matrix size, 256x256; one slab, 208 slices per slab; voxel size, 1.1×1.1×1.1mm^3; and flip angle, 8°. Functional data were collected using a 2D GE-EPI

sequence, TR 1550ms, TE 26ms, FOV 200mm, FA 75°, voxel size 2.5×2.5×2.5mm (in total, 50 slices).

8.4.4 Imaging Analysis: Voxel-Based Morphometry

I performed voxel-based morphometry using the CAT12 toolbox (available from http://dbm.neuro.uni-jena.de/vbm/) implemented in SPM12 (Wellcome Centre of Imaging Neuroscience, UCL, London, UK) and MATLAB (The MathWorks, Natick, MA, USA). Pre-processing steps were undertaken as in chapters 6 and 7.

I compared differences in GM volume between patients and controls using a two sample T test, controlling for total intracranial volume ['TIV', summated GM, WM and CSF volume [483]], age and sex. I also performed a within group comparison to determine GM volume change after surgery in patients, using a flexible factorial model at the second level, with the between subject factor = subject (1 level: patients) and the within subject factor = time (2 levels: first scan, second scan), controlling for TIV. T tests for significant increase and decrease in GM volume between visits were performed. An absolute threshold masking value of 0.1 was applied to avoid possible edge effects between different tissue types [377,385,540].

In order to further investigate potential associations between change in psychophysical score and change in GM volume, beta weights were extracted from clusters of significant GM volume change demonstrated during the above within group analysis. As psychophysical scores were not used to identify these clusters, circular analysis was avoided. Extracted beta weight values were used to test for significant correlation between change in GM volume (ΔGM volume = second scan – first scan) and change in psychophysical score (ΔT/I/TI = post-op score – pre-op score). Results were thresholded using a *P* value that was Bonferroni corrected for multiple comparisons.

As I was particularly interested in plastic change within the areas identified in my previous chapter – the ACC, insula, OFC and TP – I performed a region of interest (ROI) analysis, in addition to whole brain analysis. The *a priori* ROIs were constructed within the WFU_PickAtlas software (available from:

http://fmri.wfubmc.edu/software/pickatlas), based on the Automated Anatomical Labeling (AAL) atlas[545]. The OFC ROI was constructed as per Kahnt *et al.,* and described in the last chapter[544]. All whole brain analyses were corrected for multiple comparisons at the family wise error level ($P<0.05_{FWE}$). For the *a priori* ROI analysis, small volume corrections were implemented through the 'ROI' function in WFU_PickAtlas and results were further corrected for multiple comparisons at the FWE level ($P<0.05_{FWE}$), or at an uncorrected threshold of $P<0.001_{uncorr}$. For exploratory purposes, I additionally used a more lenient Bonferroni corrected *P* value: $P=0.05/[\text{number of ROI} \times 2_{Right+Left}]=0.05/8=0.00625$. In order to avoid issues surrounding non-stationarity in voxel based volumetric analysis[483,497] I report only voxel based results.

8.4.5 Imaging Analysis: Cortical Thickness

I additionally analysed cortical thickness using CAT12. Patient and control T1 weighted images were pre-processed as in the last chapter. Change in CTh was compared between groups (patient vs control) and within groups (patient preoperative vs postoperative), as for VBM analysis. All CTh analyses were performed at the whole brain level, with results thresholded at $P<0.05_{FWE}$.

8.4.6 Imaging Analysis: Functional MRI

Again, I analysed functional data using SPM12. Pre-processing of images was performed as in the last chapter. I then performed a first level analysis in which the condition 'odour > baseline' was modelled for each subject, using the canonical HRF. Resultant contrast images were then subjected to a second level random-effects analysis. Second level between and within group analyses were performed as for structural analyses. I additionally performed a second level regression analysis in order to test for positive correlations between psychophysical test score and BOLD signal, across all scans, correcting for age, sex and group (patient_visit 1, patient_visit 2, control). Whole brain analyses were corrected for multiple comparisons at $P<0.05_{FWE}$. *A priori* ROI analysis (with small volume correction) was

conducted as per structural work, with results thresholded at $P<0.05_{FWE}$, $P<0.001_{uncorr}$ or the exploratory $P<0.00625$. I additionally used cluster-based inference for the latter two lenient thresholds and only report clusters of ≥10 voxels.

I prepared images using the Xjview toolbox for SPM (available from: http://www.alivelearn.net/xjview/) and Microsoft PowerPoint (Microsoft Corporation, Redmond, WA).

8.4.7 Statistical Analysis

I performed supporting data analysis using GraphPad Prism (version 6, GraphPad Software, LaJolla, USA). Unless specified otherwise, statistical significance was attributed where $P<0.05$ and data are given as mean (SD) for parametric data or median for non-parametric.

8.4.8 Ethical considerations

This study received NHS ethical approval (REC ref 14/SC/1180) and was conducted in accordance with the Declaration of Helsinki. All participants provided full informed written consent prior to participation. See 3.3.5 for more details.

8.4.9 Funding

Funding for scanning was kindly provided by the Lysholm Department of Neuroradiology Clinical Research Management Group.

8.5 Results

8.5.1 Demographics, behavioural and clinical scores

Twenty participants were initially recruited: ten patients with nasal obstruction + OD and ten controls with nasal obstruction alone. One patient was lost to follow up (PIOD). There was no statistically significant difference in age (mean age patients 35.8 (12.3), controls 38.1 (13.0), Fisher's Exact *P*>0.99), sex (M:F patients 8:2, controls 8.2, Fisher's Exact *P*>0.99) or proportion of subjects with allergic rhinitis (patients 5, controls 4, Fisher's Exact *P*>0.99) between patients and controls. The mean duration of OD in the patient group was 6 (3) years. Clinical information regarding septal deformity, allergic rhinitis status and surgical procedure is provided in Table 8-1. T1-weighted images were available from all participants. Functional images from one patient (preoperative visit) and one control were excluded from analysis due to breath holding/movement artefact. Full behavioural and clinical scores are shown in Table 8-2. There were no reported surgical complications or known requirements for revision within the patient group.

	OD				
Patient	**Aetiology**	**Severity**	**AR Status**	**Deformity**	**Procedure**
1	IOD	Anosmic	Positive	DNS L, III	Septoplasty, NB realignment, spreader grafts, columellar strut
2	IOD	Hyposmic	Positive	DNS L, II/III	Septoplasty, NB realignment, spreader grafts, columellar strut
3	IOD	Hyposmic	Negative	DNS L, I/II DNS R, III	Septoplasty, NB realignment, spreader grafts, columellar strut
4	PIOD	Hyposmic	Negative	DNS L, I/II/III	Septoplasty, NB realignment, spreader grafts, columellar strut
5	PIOD	Hyposmic	Negative	DNS L, III	Septoplasty, NB realignment, spreader grafts, columellar strut
6	IOD	Hyposmic	Positive	DNS L, I/II DNS R, III/IV	Septoplasty, NB realignment, spreader grafts, columellar strut
7	IOD	Anosmic	Positive	DNS L, III/IV	Septoplasty, NB realignment, spreader grafts
8	IOD	Anosmic	Negative	DNS L, I/II DNS R, III	Septoplasty, NB realignment, spreader grafts, columellar strut
9	IOD	Hyposmic	Positive	DNS R, II/III	Septoplasty, NB realignment, spreader grafts, columellar strut

Table 8-1: Clinical deformity and surgical procedure. DNS = deviated nasal septum with Cottle area, L=left, R=right, IOD = idiopathic olfactory dysfunction, PIOD = post-infectious olfactory dysfunction, NB = nasal bones

Psychophysical Olfactory Scores

	Patients vs Controls (visit 1)			Patients: Pre- vs Postoperative (visit 1 vs visit 2)		
	Patients	**Controls**	**Patient vs Controls**	**Preoperative**	**Postoperative**	**Pre vs Postoperative**
	n=10	**n=10**		**n=9**	**n=9**	
T	3.3 (2.01)	8.5 (2.08)	t_{18}=5.88, *P*<0.0001*	3.3 (2.13)	5.08 (2.96)	t_8=2.04, *P*=0.076
D	7.30 (3.34)	12.4 (1.65)	t_{18}=4.34, *P*=0.0004*	7.22 (3.53)	9.44 (2.87)	t_8=1.66, *P*=0.14
I	7.0 (4.08)	13.0 (1.16)	t_{18}=4.47, *P*=0.0003*	7.0 (4.33)	9.44 (3.61)	t_8=2.35, *P*=0.047*
TDI	17.60 (8.20)	33.93 (1.83)	t_{18}=6.14, *P*<0.001*	17.47 (8.69)	23.97 (8.55)[c]	t_8=2.55, *P*=0.034*

Clinical Scores

	Patients vs Controls (visit 1)			Patients: Pre- vs Postoperative (visit 1 vs visit 2)		
	Patients	**Controls**	**Patient vs Controls**	**Preoperative**	**Postoperative**	**Pre vs Postoperative**
	n=10	**n=10**		**n=9**	**n=9**	
SNOT-23	52.4 (22.74)	42.3 (25.02)	t_{18}=0.85, *P*=0.41	50.13 (25.2)	20.75 (17.50)	t_8=2.99, *P*=0.02*
SNOT-23: Olfaction	3.6 (1.27)	2.3 (1.83)	t_{18}=1.85, *P*=0.08	4†	2.65 (2.0)	*W*=-4.0, *P*=0.50
VAS: Olfaction	8.45 (1.12)	4.13 (2.54)	t_{18}=4.93, *P*=0.0001*	8.3 (1.1)	5.25 (3.8)	t_8=2.70, *P*=0.031*
NOSE	59.5 (31.3)	68 (22.5)	t_{18}=0.74, *P*=0.47	58.1 (33.2)	31.3 (29.7)	t_8=1.81, *P*=0.11
LM	2† (mean 2.7 (2.71))	1† (mean 2.9 (3.04))	*U*=45.5, *P*=0.75	1† (mean 3.22 (3.03))	1† (mean 2.89 (3.14))	*W*=-2, *P*=0.75
PNIF (Bilateral)	94.0 (44.4)	99.0 (46.77)	t_{18}=0.25, *P*=0.81	92.2 (46.7)	102.8 (34.8)	t_8=0.60, *P*=0.56

Nasal Airflow Symmetry Scores

	Patients vs Controls (visit 1)			Patients: Pre- vs Postoperative (visit 1 vs visit 2)		
	Patients	**Controls**	**Patient vs Controls**	**Preoperative**	**Postoperative**	**Pre vs Postoperative**
	n=10	**n=10**		**n=9**	**n=9**	
AD	-	-	-	45.6 (40.3)	18.9 (15.4)[a]	t_8=1.87, *P*=0.099
AS	-	-	-	0.17† [mean 0.26 (0.23)]	0.06† [mean 0.11 (0.15)][b]	*W*=-22, *P*=0.15

Table 8-2: Group average nasal airflow symmetry, psychophysical and clinical scores in patients and controls, shown as mean (SD) or †median. a/b = at individual level, improvement seen in 5 patients. c= at individual level, clinically significant improvement seen 5 patients. Statistically significant results denoted by *.

At visit 1, there was a statistically and clinically significant difference in TDI score between patient and control groups, with the group mean falling within the hyposmic range for patients. After surgery, there was a statistically and clinically significant increase in group mean TDI in patients, with clinically significant improvements in TDI in five individuals (see Table 8-2). There was no significant difference in mean LM score post-operatively. At the individual level, LM score was unchanged in six patients, decreased in two and increased in one. There was no significant difference in change in TDI score post-operatively comparing patients with AR, vs patients without AR [7.75 (5.16), vs 4.94 (10.71) respectively, *U*=8, *P*=0.73].

There were no statistically significant correlations between psychophysical test scores (T/D/I/TDI) and LM score. There were no statistically significant correlations between change in *AD* or change in *AS* and change in psychophysical test score (T/D/I/TDI) after surgery. There was, however, a significant positive correlation between ΔT and ΔPNIF (bilateral) (r=0.68, *P*=0.04) (see Figure 8-4).

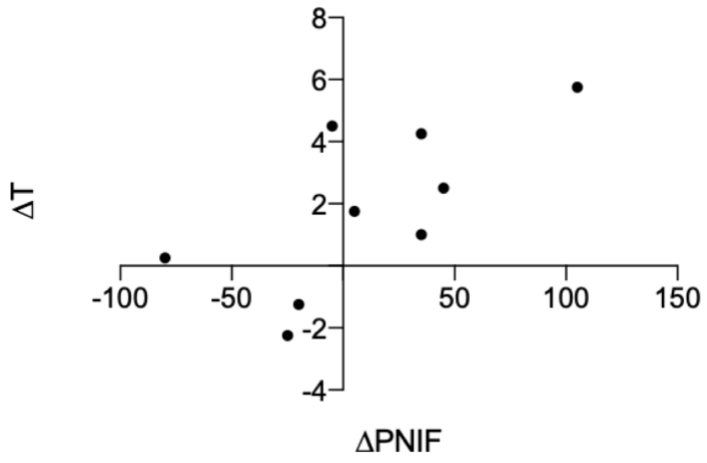


Figure 8-4: Significant positive correlation between change in PNIF and change in T score after surgery, r = 0.68, P= 0.04

8.5.2 Voxel Based Morphometry

8.5.2.1 Patients vs Controls

During *a priori* ROI analysis, I found small areas of decreased GM volume within the bilateral OFC, but more widespread areas of increased GM volume within each of the interrogated regions, in patients compared to controls (see Table 8-3 and Figure 8-5). No voxels survived thresholding at the whole brain level.

			MNI Coordinates			
Threshold	***ROI***	***Side***	***X***	***Y***	***Z***	***T Score***
		Patient < Control				
***P*<0.00625**	OFC	L	-36	22	-20	2.99
		R	45	42	-21	2.87
		Patient > Control				
***P*<0.001**	ACC	R	12	52	12	4.61
	Insula	L	-38	-3	-14	4.01
		L	-38	-4	3	3.75
		L	-36	-3	0	3.74
***P*<0.00625**	Insula†	R	44	0	-8	3.35
	OFC	L	-14	14	-14	3.50
		L	-4	27	-26	3.08
		L	-54	27	-9	3.01
		R	20	12	-14	2.99
		L	-39	32	-12	2.89
	TP	L	-40	2	-14	3.33

Table 8-3: Results of VBM ROI analysis in patients vs controls. Voxels of significant GM volume difference between patients and controls (visit 1). Patients < controls indicates area of decreased GM volume in patients. Patients > controls indicates areas of increased GM volume in patients. †Results reported to demonstrate bilaterality at this lenient threshold—as left sided results significant at P < 0.001, only right sided results shown.

8.5.2.2 Change in GM Volume After Surgery

During my within group longitudinal analysis, potentially in line with my preoperative between group findings, I demonstrated more widespread areas of decreased GM volume than increased GM volume after surgery, within the *a priori* ROIs (see Table 8-4 and Figure 8-6). Again, no voxels survived thresholding at the whole brain level.

			MNI Coordinates			
Threshold	**ROI**	**Side**	**X**	**Y**	**Z**	**T Score**
		Increased GM Volume				
***P*<0.00625**	OFC	R	36	30	-20	3.58
		Decreased GM Volume				
***P*<0.001**	ACC	L	-15	48	-2	4.71
	Insula	L	-33	12	-12	7.27
		L	-38	-15	4	4.69
		L	-34	-3	14	4.65
	OFC	L	-45	39	-8	7.24
		L	-4	54	-12	5.20
		L	-39	51	-4	4.99
		R	6	57	-15	4.70
***P*<0.00625**	Insula[†]	R	39	-8	8	4.04
		R	42	-2	-14	3.44
	TP	L	-44	8	-14	4.30
		L	-54	6	-8	3.61
		L	-24	2	-36	3.58
		R	60	14	-15	3.52
		L	-54	3	0	3.50
		L	-40	2	-14	3.36
		R	44	21	-38	3.31
		R	62	3	2	3.31

Table 8-4: Voxels of significant change in GM volume after surgery from ROI analysis within patient group (visit 1 vs 2). Results are only shown at more lenient thresholds where none survive at $P<0.05_{FWE}$ or P<0.001 as applicable ([†]results reported to demonstrate bilaterality at this lenient threshold – as left sided results significant at P<0.001, only right sided results shown).

8.5.2.3 Correlation Between Change in Psychophysical Score and Change in GM Volume

There were no correlations between ΔGM volume (from 19 clusters of significant GM change as outlined in Table 8-4) and Δpsychophysical score that were statistically significant at the specified results threshold of $P<0.0026$ [Bonferroni corrected $P<0.05/19$].

8.5.3 Cortical Thickness

No results survived thresholding during between (preoperative patient vs control) or within (patient group: before vs after surgery) group analyses at the whole brain level ($P<0.05_{FWE}$).

8.5.4 Functional MRI

Perceived intensity and hedonic valence for the two odour stimuli did not differ significantly within the patient group, at visit 1 or 2 (see Table 8-5). Similarly, there was no significant difference in perceived intensity or hedonic valence for the two odours within the control group. Further analysis of the conditions 'banana' and 'grass' were pooled.

	Patients								**Controls**		
	Visit 1			**Visit 2**			**Visit 1 vs Visit 2**		**Visit 1**		
	Banana	**Grass**	**Banana vs Grass**	**Banana**	**Grass**	**Banana vs Grass**	**Banana**	**Grass**	**Banana**	**Grass**	**Banana vs Grass**
Intensity	3.44 (3.17)	2.94 (2.78)	t_8=0.55, P=0.60	4.44 (3.47)	4.94 (3.21)	t_8=1.029, P=0.33	t_8=1.50, P=0.17	t_8=2.77, P=0.024*	6.5 (0.87)	6.39 (2.15)	t_8=0.22, P=0.83
Valence	1.06 (1.84)	0.0†	W=-11, P=0.28	1.50 (2.21)	0.11 (2.32)	t_8=1.17, P=0.27	t_8=0.50, P=0.63	t_8=0.45, P=0.67	3.11 (2.32)	0.67 (2.73)	t_8=1.80., P=0.11

Table 8-5: Intensity and hedonic ratings (valence) of fMRI odours, shown as mean (SD) or †median values.
*Indicates statistically significant result.

8.5.4.1 Patients vs Controls

Increased functional activity was demonstrated within the ACC of controls, compared with patients, during *a priori* ROI analysis (cluster peak originating to right but extending across midline) (see Table 8-6 (A) and Figure 8-5). No voxels survived thresholding at the whole brain level.

8.5.4.2 Change in Functional Activity After Surgery

During *a priori* ROI analysis, there were clusters of increased BOLD signal after surgery that survived thresholding at $P<0.05_{FWE}$. These were more extensive or bilateral at the lenient thresholds (see Table 8-6 (B) and Figure 8-6). During whole brain analysis, there was a small cluster of significantly increased BOLD signal that survived thresholding at $P<0.05_{FWE,}$ within the left planum polare.

8.5.4.3 Correlation Between Psychophysical Score and BOLD Signal

At the exploratory threshold, I found clusters of significant positive correlation between BOLD signal and composite TDI score as well as individual T and D scores within the right insula (see Table 8-7). There were additionally clusters of significant positive correlation between T score and BOLD signal within the right OFC and ACC, though only the former survived thresholding at the cluster criterion. Of note, clusters within the OFC and insula closely neighbour clusters of increased BOLD signal after surgery.

A: Patients vs Controls (visit 1)

ROI Analysis

			MNI Coordinates				
Threshold	**ROI**	**Side**	**X**	**Y**	**Z**	**T Score**	**k**
***P*<0.00625, 10 voxels**	ACC	R[†]	4	36	22	3.22	78

Whole Brain Analysis

No suprathreshold voxels

B: Patients: Pre- vs Postoperative (visit 1 vs visit 2)

ROI Analysis

Threshold	**ROI**	**Side**	**X**	**Y**	**Z**	**T Score**	**k**
***P*<0.05$_{FWE}$**	ACC	R	4	6	28	4.98	3
	Insula	R	40	22	-6	4.58	1
	OFC	R	22	40	-14	5.05	1
		L	-24	40	-12	5.04	1
***P*<0.001, ≥10 voxels**	ACC	R	4	6	28	4.98	13
	Insula	R	40	22	-6	4.58	12
	OFC	R	22	40	-14	5.05	42
		L	-24	40	-12	5.04	14
***P*<0.00625, ≥10 voxels**	ACC	R	4	6	28	4.98	36
	Insula	R	40	22	-6	4.58	32
	OFC	R	22	40	-14	5.05	57
		L	-24	40	-12	5.04	36
		L	-30	28	-14	4.40	15
		R	42	28	-6	3.82	22
	TP	L	-46	6	-16	3.76	54

Whole Brain Analysis

Threshold	**Region**	**Side**	**X**	**Y**	**Z**	**T Score**	**k**
***P*<0.05$_{FWE}$**	Planum Polare	L	-44	-4	-24	6.73	3

Table 8-6: fMRI results. A) Clusters of increased BOLD signal in controls, compared with patients (visit 1) ([†]cluster crosses midline) B) Clusters of increased BOLD signal after surgery within the patient group (visit 1 vs 2) [for A and B significant results shown at each threshold level in order to demonstrate corresponding cluster size]

Correlation: Psychophysical Score ∝ BOLD

ROI Analysis

Threshold	Psychophysical Score	ROI	*Side*	*X*	*Y*	*Z*	*T Score*	*k*
***P*<0.00625, ≥10 voxels**	T	OFC	R	22	44	-14	3.48	20
		Insula	R	40	20	6	3.49	129
	D	Insula	R	42	18	-6	2.90	36
	TDI	Insula	R	36	18	4	3.14	89

Whole Brain Analysis

No suprathreshold voxels

Table 8-7: Clusters of significant positive correlation between psychophysical test score and BOLD signal.

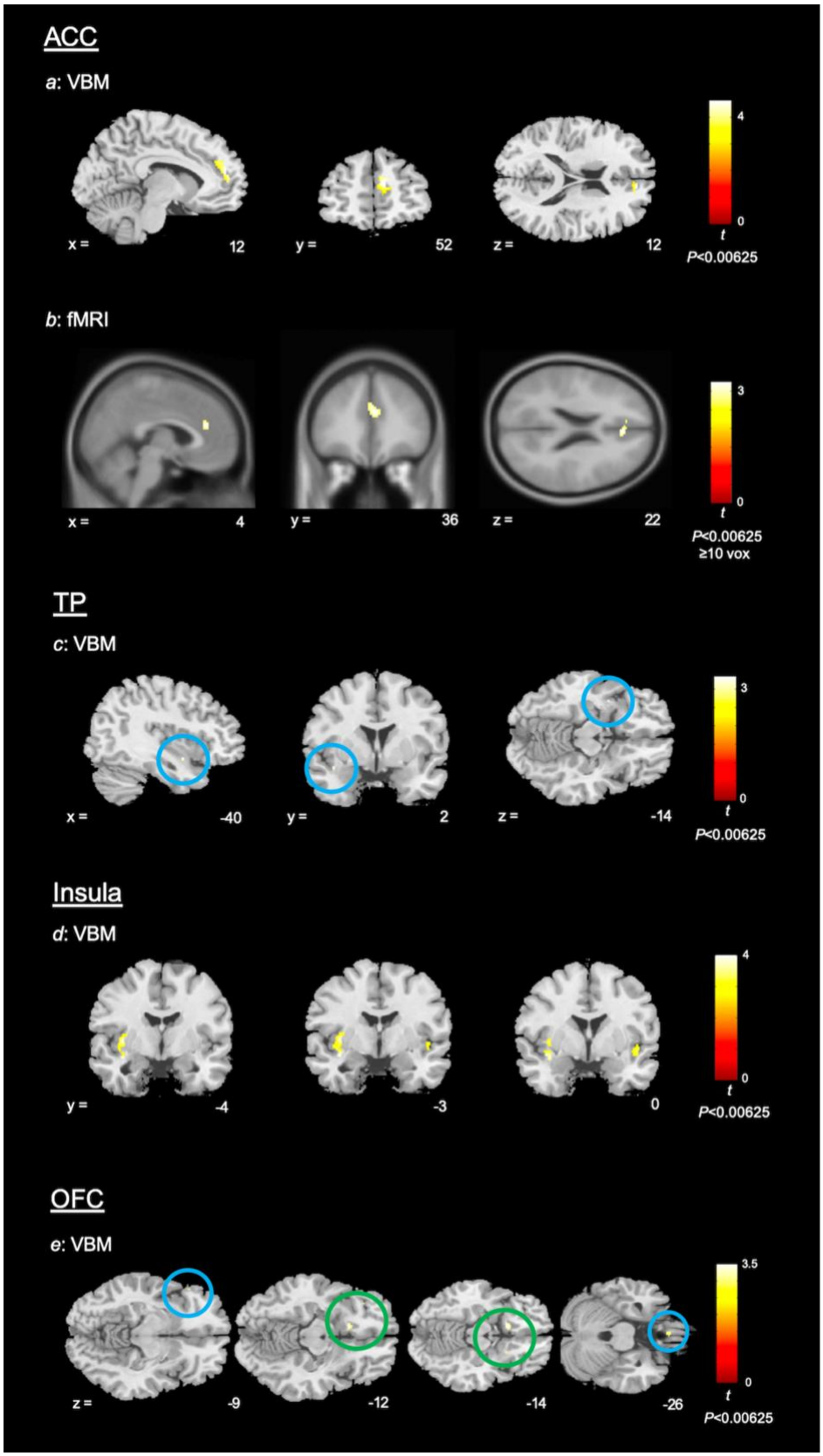


Figure 8-5: Neuroimaging results for patients vs controls (visit 1). To help differentiate between imaging modalities, VBM results are shown using the ch2bet stripped skull brain template and fMRI results are shown using the avg152T1 brain template. All coordinates are in MNI space. Colour bars show associated peak T score (please note that the maximum integer labelled may not reach top of colour bar range). For ease of display—axial sections only shown for OFC results and coronal sections only shown for results within the insula. Subsections: (a) VBM results for increased GM volume within the ACC of patients compared with controls ($P < 0.00625$); (b) fMRI results for increased BOLD signal with the ACC of controls compared with patients ($P < 0.00625$, ≥10 voxels); (c–e) VBM results for increased GM volume in patients compared with controls, within the TP, insula and OFC ($P < 0.00625$). Small clusters have been circled: blue circles highlight a single significant cluster; green circles highlight multiple significant clusters. Abbreviations: ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; TP, temporal pole(s).

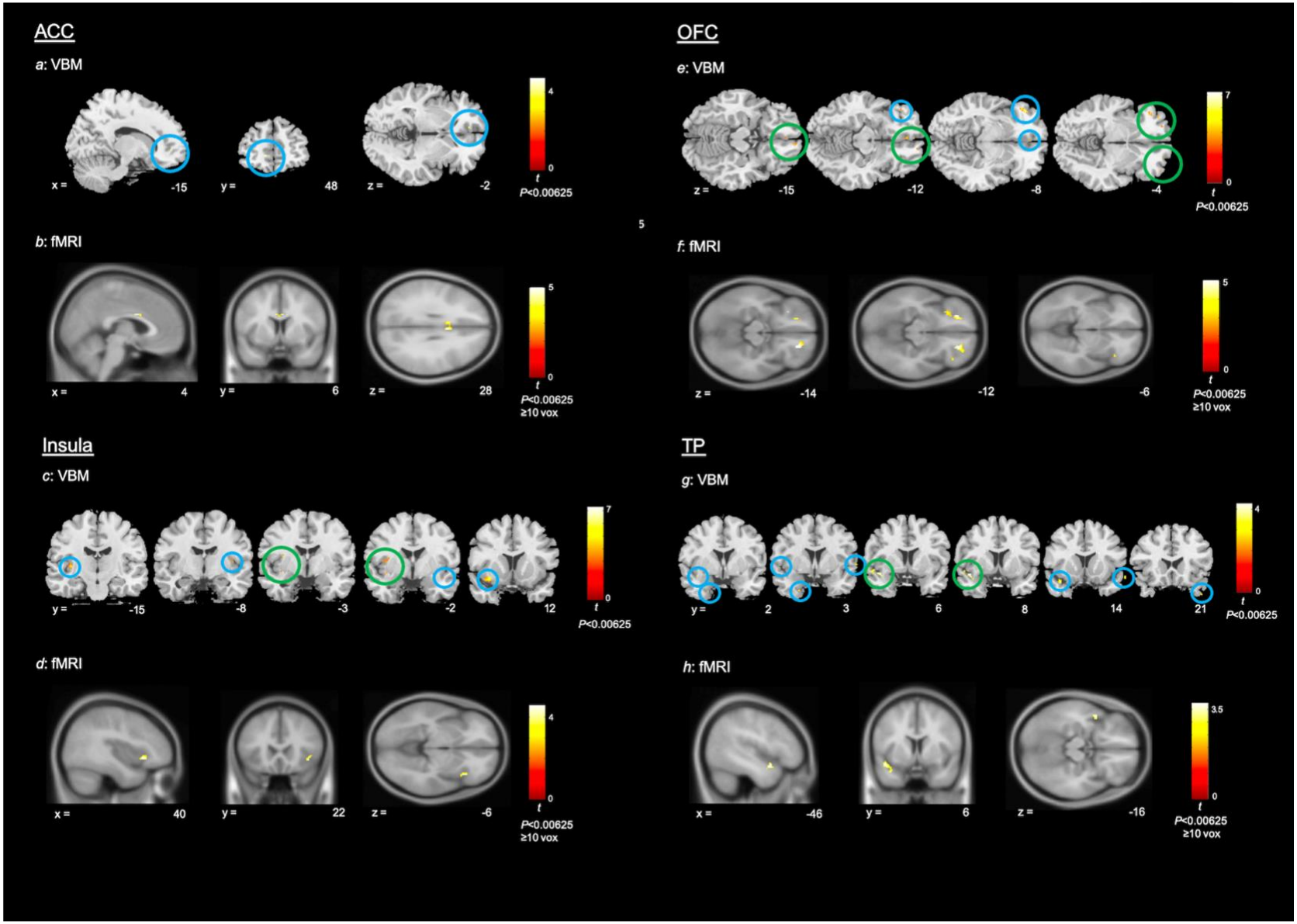


Figure 8-6: Neuroimaging results for patients pre- vs postoperative (visit 1 vs. 2): Structural and functional MRI results for decrease in GM volume and increase in BOLD signal after surgery, in the patient group. For ease of display—axial sections only shown for OFC results and coronal sections only shown for VBM results within the insula and TP. Subsections: (a) VBM results for decreased GM volume within the ACC of patients after surgery ($P < 0.00625$); (b) fMRI results for increased BOLD signal within the ACC of patients after surgery ($P < 0.00625$, ≥10 voxels); (c–h) VBM results for decreased GM volume and fMRI results for increased BOLD signal within the insula, OFC and TP of patients after surgery. Blue circles highlight a single significant cluster; green circles highlight multiple significant clusters. Abbreviations: ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; TP, temporal pole(s).

8.6 Discussion

8.6.1 Key results

To my knowledge, this is the first prospective study to demonstrate structural and functional plasticity in association with improved olfactory function following fSRP, in patients with non-CRS OD of mixed cause. In each of the *a priori* ROIs I demonstrated significant change in GM volume as well as increase in BOLD signal after surgery. Across modalities, results within the ACC and insula were most statistically robust, followed by the OFC and finally the TP. With regards to structural plasticity – I demonstrated reduced GM volume within each of the *a priori* ROIs, as well as a small area of increased GM volume within the OFC. Possibly related to this, comparison of GM volume demonstrated areas of reduced GM volume within the OFC but more widespread areas of increased GM volume within each of the four ROIs, in preoperative patients compared with controls. Finally, there was a small cluster of increased BOLD signal after surgery that survived thresholding during whole brain analysis ($P<0.05_{FWE}$) within the left planum polare.

8.6.2 Neuroanatomical correlates of olfactory dysfunction

The ACC, insula and OFC are well established nodes within the secondary olfactory network and are frequently activated during functional neuroimaging studies [31] [see previous chapters §6.6 and §7.6 for detailed discussion]. Human tractography studies have demonstrated direct connections between the insula and ACC, the insula and OFC, as well as between the ACC and OFC[584,597]. The functional importance of this anatomical connectivity is highlighted by time series in which activations of the ACC, OFC and insula temporally overlap[515,581]. More generally, the anterior insula and dorsal ACC are important nodes within the salience network: a bilateral system that integrates emotional and interoceptive input with external sensory information and which interacts with other neurocognitive networks such as the central executive network and default-mode network[598,599]. It is worth noting that the ventromedial prefrontal cortex (whose boundaries, depending on definition, either overlap or are synonymous with the OFC) is a key node within the default

mode network[600,601]. These structural and functional interconnections may underlie the pattern of results I demonstrated in my previous chapters – in which I demonstrated functionally significant structural plasticity within the ACC, insula and OFC [594] – and which I have replicated in the current study.

I demonstrated less statistically robust results for structural and functional plasticity within the TP. However, I additionally demonstrated increased BOLD signal after surgery within the structurally adjacent left planum polare, which survived thresholding at the whole brain level. Though less well established, the TP is a component of the secondary olfactory network and more generally involved in multimodal sensory integration, particularly in the context of social cognition [602]. In humans, anatomical connections between the TP and the insula, OFC and ACC have been demonstrated[584,603]. Part of the superior temporal gyrus (STG), the planum polare neighbours the temporal pole and has known anatomical connections with the insula[584]. The STG is also known to have connections with the OFC[597], making these regions highly interconnected. Similar to the TP, the STG is thought to be involved in the hedonic processing of olfactory stimuli[604] and more generally in contextual integration[605]. It has also been suggested that the left planum polare and TP may be part of a joint network (which also includes the insula and OFC) that guides behaviour in response to salient olfactory stimuli[606]. My observed increase in BOLD signal after surgery within the left planum polare may therefore be related to the other changes I observed within structures of the salience network. However, this remains speculative at present.

Of interest, I replicated the direction of structural plasticity observed in my previous chapter: improved olfactory function (as evidence by increased olfactory BOLD signal and improved psychophysical test scores) appears to be associated with reductions in GM volume in these regions. This is in contrast to results demonstrated in patients undergoing olfactory training, where GM volume appears to increase in association with improved olfactory function[577,578]. I previously hypothesised that mechanistic differences may underlie the differences seen: olfactory training may involve some degree of top-down learning processes leading to increased GM volume, whilst surgery – through modification of the peripheral olfactory apparatus and thereby

increased peripheral input – may involve a bottom-up process leading to reduced GM volume, possibly through improved network efficiency and associated synaptic pruning or other microanatomical changes. In theory, such differences in short-term structural plasticity do not preclude GM atrophy following prolonged reduced afferent input or other central olfactory dysfunction.

This theory for mechanistically divergent improvement in olfactory function is supported by observations that olfactory training improves odour identification more than odour threshold[589], the latter of which is thought to better reflect peripheral olfactory apparatus function[313], as targeted by surgery. Accordingly, it is interesting to note that in my current cohort, clusters of increased BOLD signal after surgery were spatially aligned with clusters of significant positive correlation between BOLD signal and threshold (T) score within the OFC and insula. Taken together, where reduced GM volume and increased BOLD signal are speculated to reflect better network efficiency (caused by increased peripheral sensory input), the anatomical regions involved may implicate changes within networks that modulate attention to olfactory stimuli. In line with this, patients are thought to spend more time attending to odours than healthy controls[607]. However, this remains highly speculative and requires investigation with future longitudinal work.

Finally, as outlined in the previous chapter, the differences in directionality in GM volume I demonstrated (when compared both with longitudinal olfactory training work and cross sectional disease state VBM -see[594]) may also be due to a non-linear time course in structural plasticity or, more simply, due to sampling variation. However, I would suggest, given my replicated demonstration of functionally relevant structural plasticity within the ACC, OFC, insula and TP, that these regions are neuroanatomical correlates of OD, independent of directionality of GM volume change. Moreover, as I replicated these results in patients with OD of mixed cause undergoing fSRP, the plasticity demonstrated appears to be related to general, rather than disease-specific OD or treatment-specific change in olfaction.

8.6.3 Novel fSRP Mechanistic Insights

Previous evidence for improved olfaction in functional septorhinoplasty is limited by methodological inconsistencies. A meta-analysis by Pfaff and colleagues demonstrated overall improvement in olfactory function, but studies varied in terms of procedure (functional vs aesthetic), baseline olfactory function, and outcome measures used[595]. Comparison of different outcome measures is particularly problematic: as described previously, subjective and psychophysical measures are known to correlate poorly, in both patient and healthy participant cohorts [217,218,528]. To my knowledge, this is the first study to investigate the effect of fSRP on olfactory function using multicomponent psychophysical and patient-reported measures of olfaction, as well as a novel and objective outcome measure – structural and functional plasticity. Accordingly, statistically significant group level improvements in composite TDI and identification score were demonstrated after surgery. At the individual patient level, clinically significant improvement in TDI score was demonstrated in 5 out of 9 patients. Statistically significant improvements in SNOT-23, and VAS-olfaction scores were also demonstrated at the group level. However, no statistically significant improvements in threshold, identification, or SNOT-23 (olfaction score) were seen. Furthermore, whilst there were improvements post-operatively in NOSE, AD, AS score and PNIF, these did not reach statistical significance. Whilst this work provides some initial pilot evidence, my primary aim was not to investigate the utility of fSRP as a treatment for OD. Therefore, future work is needed for this purpose, with a sample size sufficiently powered according to the MCID for TDI score and prospective control groups. Moreover, it was not possible within the confines of my current study design to determine whether the improvements in olfactory function demonstrated were due to correction of nasal obstruction alone, or whether there could be some superadded effect of improved airflow on PIOD, IOD or AR. To investigate this, in addition to appropriately powered sample sizes, future work may benefit from use of multiple patient [nasal obstruction alone, OD (PIOD or IOD) alone, nasal obstruction + OD] and prospective control groups [non-surgical and ideally sham surgical]. Subjects should undergo unirhinal psychophysical testing and more detailed analysis of nasal aerodynamics,

ideally including a technique that facilitates analysis of airflow to the olfactory cleft. In practice, use of an approach similar to my chapter seven work may be beneficial – in which patients with improved olfactory function post-operatively are compared with those without improved function. Associations between olfactory change and nasal airflow measures could then be assessed. Histological analysis of OE would be of use, if possible, as would analysis of nasal and olfactory mucus. Finally, such studies should either exclude patients with AR, or include sufficiently powered AR and non-AR groups to enable their meaningful comparison.

Whilst the primary aim of this work was not to investigate the efficacy of fSRP as a treatment for OD, my results do provide some initial insights into potential mechanisms for improvement in olfactory function following fSRP. Of interest, I demonstrated significant positive correlation between change in threshold score after surgery and change in bilateral PNIF score. However, I was unable to demonstrate significant positive correlations between change in *AD* or *AS* score and change in psychophysical test scores. It would therefore appear that changes in overall airflow were more physiologically important with respect to olfaction than improved nasal airflow symmetry, following fSRP in this cohort. Previous work using computational fluid dynamics has demonstrated that airflow to the olfactory cleft region is critically affected by anatomical alterations within the olfactory cleft itself, and importantly, the internal nasal valve ('INV') region[47]. As augmentation of the bilateral INV was performed in my study as standard, one may speculate that resultant changes in nasal airflow facilitated odourant access to the olfactory cleft, which was better reflected by changes in bilateral PNIF than measures of airflow symmetry. Increasing odourant access to the OC may improve olfaction in the short-term by increased odourant-olfactory receptor binding and long-term by a putative bottom-up plasticity process induced by improved peripheral input. The latter may be reflected in my neuroimaging findings, where I demonstrated spatial alignment between clusters of increased BOLD signal after surgery and clusters of significant positive correlation between BOLD signal and T score within the OFC and insula.

Whilst my findings require replication in a larger cohort, I would suggest that augmentation of the bilateral INV may be beneficial to olfaction and that future

research should aim to investigate this further, in patients both with and without significant septal deviations. Finally, my results may explain the mixed evidence for improved olfaction after septoplasty[304], which preferentially corrects symmetry rather than overall nasal airflow.

8.6.4 Study Limitations

Three limitations of this work in relation to my primary aims are: 1— small sample size; 2 – lack of prospective control arm; 3 – mixed aetiology of OD.

My study sample size was determined in relation to my primary neuroimaging aim, with minimum participant number determined from existing literature [400,523,524], and available pre-pandemic, clinical population [see 3.3.4 for further discussion]. Whilst my final sample size was comparatively small, I was able to demonstrate significant results using established methods to control for false positives, indicating my respective effect sizes may in fact be larger than if the same were demonstrated with a lager sample[608]. Whilst a larger sample size may have revealed further significant results and potentially reduced sampling variation, lack thereof does not invalidate the current findings of this pilot study. However, future work should aim to incorporate larger participant numbers. In particular, this would enable subgroup analysis according to clinical improvement in psychophysical test score, as was performed in my previous chapter.

Lack of prospective control arm is a major limitation of the current study. However, my previous work demonstrated these neuroanatomical regions to undergo functionally significant structural plasticity in comparison with a prospective control group [594]. Furthermore, there is precedent for this study design in the neuroimaging literature[524]. However, future studies should incorporate a prospective control arm where possible. Alternatively, a larger cohort of patients would have enabled subgroup comparison between those who had experienced clinically significant improvement in psychophysical olfactory test scores compared with those who had not, in doing so mitigating the potentially confounding effect of surgical stress when comparing surgical patients to healthy, non-surgical controls.

Another potential limitation was use of a mixed OD aetiology cohort. As a clinical study with a pragmatic study sample, it was not possible to recruit eligible patients from only one underlying aetiology of OD due to the small available (pre-pandemic) patient population. However, there is extensive precedent for use of mixed aetiology cohorts in the olfactory neuroimaging literature[425]. Moreover, as my primary aim was to determine whether functionally significant structural plasticity occurs following treatment of general, rather than aetiology-specific OD, a mixed aetiology study sample was felt to be appropriate. Furthermore, due to the pragmatic study sample, it was not possible to exclude participants based on allergic rhinitis (AR) status. In light of this, participants were carefully screened (clinical history, endoscopy and imaging findings), to exclude CRS, in line with current guidelines[103,319]. To further mitigate the potential effects of AR, and other potentially unknown confounding factors, my control group was taken from a cohort of normosmic patients also awaiting functional septorhinoplasty. Accordingly, there was no significant difference in the proportion of participants with AR in the patient vs control group (see table 8.1). However, larger future studies should aim to exclude patients with AR, or to include sufficiently high sample sizes to allowed appropriately powered subgroup analysis according to AR-status.

Regarding my secondary aims, despite being intuitive and conceptually simple, my nasal airway measures (AD and AS score) were not validated prior to use. In further research where the primary aim is to investigate the utility of fSRP as a treatment for OD, these scores should be validated against a 'gold standard' measure of nasal airflow, as well as subjective patient scores of nasal obstruction.

8.6.5 Conclusion

To my knowledge, this is the first prospective study to demonstrate structural and functional plasticity in association with improved olfactory function following fSRP, in patients with non-CRS OD of mixed cause. Combined with my previous work, this longitudinal evidence supports the role of the ACC, insula, OFC, and TP as neuroanatomical correlates of general, rather than disease specific, OD. These

regions are good targets for future investigation into their utility as personalised biomarkers of OD.

9 General Discussion

In the following chapter, I will summarise my main findings for Themes A and B before providing further discussion of these results in the context of the wider and newly emergent literature and their implications for the clinical assessment of human olfaction.

9.1 Theme A

9.1.1 Summary of Theme A

When I began this PhD, the current state of UK practice regarding the assessment of olfaction was unknown. Given the widely divergent range of techniques available, their associated varying reliability, and lack of unified guidance for their use, the overarching aim of my thesis' Theme A was to explore the current assessment of olfaction, through both clinician and patient perspectives.

In **chapter four** I performed a cross-sectional survey of ENT surgeons who assess olfaction. My primary aim was to determine UK practice with regard to psychophysical smell testing, patient reported outcome measures and imaging. My secondary aim was to outline geographical variations in practice and barriers to psychophysical testing. Responses were received from 465 clinicians, with the majority from the UK (217 from UK, 17 countries total). Most UK respondents do not perform psychophysical testing during any of the presented clinical scenarios. Whilst there was more variability in practice, international respondents tended to perform psychophysical testing more frequently. Where significant differences between rhinologist/non-rhinologist subgroups were found, testing was more frequently performed in the former, irrespective of geographical location. In both the UK/internationally, pressures associated with service provision (e.g., funding/time limitations) were the most frequent barriers to such testing. Most UK/international respondents said they would like to receive further education in the use of chemosensory tests. Despite lack of routine psychophysical testing, PROMs were

infrequently used, both in the UK/internationally. A relatively high proportion of UK respondents performed MRI scanning during their initial assessment of OD, usually for diagnostic purposes. International respondents scanned with more variability. My data comprises the most comprehensive description of current practice in the assessment of OD to date. Furthermore, to my knowledge, this is the first international survey of practice, which provides initial insights into geographical variations in practice.

In **chapter five** I performed a cross-sectional, co-produced survey of patients and healthcare seeking adults. My primary aims were to describe: 1. how patients are being assessed clinically; 2. how satisfied patients are with their assessment, and factors that affect this; 3. preferences for assessment in healthcare seeking adults. My secondary aim was to capture 'real world' data on clinical practice to compare with my clinician-reported data. Responses were received from 576 people, from 33 countries – though the majority were from the USA and UK. Most respondents were female and affected by COVID-19. Just over half of respondents had been assessed by a healthcare professional. Of those who had, across all respondents less than one fifth had undergone psychophysical smell testing. Similarly, less than one fifth had completed a questionnaire about their symptoms. Within the UK subgroup, these figures were slightly lower. Across all respondents, the highest proportion had *not* been referred for imaging. However, amongst those who had been seen by ENT in the UK, the highest proportion had been referred for an MRI. Mean satisfaction rates were higher in respondents who had been seen by ENT and in those who had undergone more thorough assessment, particularly imaging. Patients and healthcare seeking adults prioritise orthonasal odour identification, prefer assessment by a specialist, and are willing to travel for such specialist assessment. Unfortunately, many respondents were unhappy with their care – and felt that healthcare professions (across specialties) were both dismissive towards OD and lacked appropriate knowledge of its causes and effects. To my knowledge, these results provide the first in-depth analysis of end-user experience and preferences for olfactory assessment.

In the following sections I will provide further analysis of my key results, integrating my clinician and end-user reported data, and highlighting potential limitations in this approach. I will additionally expand on my discussion regarding barriers to psychophysical testing and focus on areas for further research and potential change.

9.1.2 Psychophysical Testing

9.1.2.1 Current State of Practice

My clinician-reported data confirms poor uptake of psychophysical smell testing in the UK. Amongst ENT surgeons, comparison with previously published data demonstrates little progress in almost 20 years. Indeed, where McNeill and colleagues demonstrated in 2007 that only 5.4% of clinicians performed smell tests routinely, and 54.8% never did so[334], I demonstrated strikingly similar results – 5.5% of my respondents performed such tests routinely and 54.9% never tested (during initial diagnostic assessment, across all respondents). Whilst care is required when comparing across geographical cohorts (see §4.6.4), international respondents appear to perform smell testing more regularly than in the UK – with 39.5% of all respondents routinely testing during initial assessment. To my knowledge, my data is the first to interrogate use of psychophysical testing in different clinical scenarios, where there was little deviation in practice across those presented. Whilst lack of testing in patients presenting with OD as a presenting or isolated symptom is most concerning, it was also of interest that the majority of both UK and international respondents 'rarely' or 'never' tested olfaction in normosmic patients undergoing surgical procedures in which OD is a potential complication (UK – pre-op 93.8%, post-op 95%; International – pre-op 70.4%, post-op 76%). Using the analogy of ear surgery in patients who had not undergone a hearing test, or eye surgery in a patient who had not undergone acuity/visual field testing, this highlights the disparity between olfactory assessment and the accepted standard in other sensory systems.

My end-user survey provided corroborating 'real world' evidence for the poor uptake of psychophysical smell testing by clinicians in the UK. As outlined previously, across all UK respondents, only 10.5% of patients underwent smell testing during

their consultation. Within the subgroup of patients who had been seen by an ENT surgeon in the UK, 79.6% did not undergo testing – similar to the 72.6% of UK ENT respondents who 'never' or 'rarely' tested in my clinician survey. However, within my group of patients who had seen an ENT surgeon outside of the UK, 73.8% of patients (including those in the USA) and 75.0% (excluding those in the USA) did *not* undergo smell testing (see supplemental results, appendix 10.8). These figures are higher than those obtained in my clinician survey – where, across all respondents, 36.1% 'never' or 'rarely' tested. The discrepancy between these figures is likely due to a combination of factors.

First, it should be noted that the geographical distribution of 'international' end-user and clinician respondents was poorly matched: excluding the UK, the majority of end-user respondents were from the USA, with the remaining being evenly divided between European (49.4%) and non-European (50.6%) countries (including economically diverse locations, e.g. Ethiopia, Bangladesh, Lebanon, Ecuador, Mexico, India, Canada, Norway and Singapore); the majority of non-UK clinician respondents were from European countries, with further significant subgroups from the Middle or Far East (Turkey and Japan), and only two respondents from the USA. Geographical differences in healthcare funding (with a higher representation of patients from lower income countries in my end-user data) and potential geographical differences in COVID-19 prevalence and impact (also potentially reflected in my end-user data) may have contributed to the discrepancy in results seen. Second, the proportion of ENT surgeons with subspecialty training in rhinology within my end-user data is unknown. Accordingly, it is possible that my 'international' cohort of patients were seen by a higher proportion of non-rhinologists. This would be more in line with my clinician figures for testing in my international, non-rhinologists subgroup – where 53.5% of clinicians never/rarely tested for OD as an isolated or presenting symptom. Third, selection bias may have differentially affected my results, with more motivated/interested ENT clinicians preferentially responding, and less satisfied patients, or those patients in need of more support, preferentially responding. This may have resulted in over- and underestimations in reported testing rates respectively, and may have particularly

affected my 'international' clinician-reported data due to variation in survey distribution method, including use of regional/local mailing lists, and large differences in response rates (ranging from 1.4% response rate in Japan, where the survey was sent out to 1,513 individuals on a national mailing list, to 72.5% in Greece, where the survey was sent out to 40 individuals on a regional/local mailing list). Whilst comparison of practice according to subspecialty training in rhinology helped to mitigate the effect of differential selection bias across geographical cohorts within my clinician work, lack of subspecialty information in my end-user work precludes such subspecialty-specific comparison between patient and clinician data. Furthermore, whilst my minimum sample sizes were surpassed in both clinician and end-user surveys, this was an exploratory target as my sampling method in both groups was not randomised. To confirm international practice, as discussed in chapters 4 and 5, future surveys of clinicians and/or patients should utilise randomised sampling with a standardised distribution method across difference geographical regions, as well as appropriate survey translation.

9.1.2.2 Barriers to Psychophysics Use – Funding Limitations

The most frequent barriers to routine smell testing in the UK were funding limitations and insufficient time. Internationally, insufficient time, staff and funding were most common. As funding models vary considerably between different healthcare systems, I will limit the following discussion to the UK, though underlying principles may be transferable across geographical boundaries.

In England, funding for healthcare services is determined according to the NHS Payment Scheme (which replaces the previous 'Payment by Results' system)[609]. This is a process through which clinical commissioners pay healthcare providers for each patient treated or seen, including secondary care outpatient attendances and inpatient episodes. Outpatient appointments are charged at a set rate for new and follow up patients, as well as for multi-disciplinary appointments. This means that, for example, patients attending with hearing loss who undergo audiological assessment with a pure tone audiogram are charged at a higher rate as this is a multi-professional outpatient episode. However, for smell tests that require

administration, this is done by nursing or healthcare support staff assigned to a clinic (if not by the clinician themselves) and these episodes therefore do not qualify as multi-professional. Furthermore, the system by which payments are made per outpatient episode (with higher payments for new patients) means that smell and taste clinics are likely to attract less funding overall, as fewer patients are seen compared with general clinics due to the time necessary to undertake chemosensory testing, and examination requirements (with current recommendations including full head and neck exam with nasendoscopy for all patients[166,229,425]). Finally, unlike procedures such as diagnostic blood tests or nasendoscopy, there is no clinical code for administration of smell tests in the current NHS manual for operative/procedural coding – the National Clinical Coding Standards OPCS-4 (2023). As such, there is no recognised national tariff for chemosensory tests, nor the necessary staff to administer such tests. Accordingly, staff, time and funding for smell testing must be allocated from existing outpatient or inpatient resources.

Funding constraints have previously been highlighted as problematic in specialist smell and taste clinics[106,610]. Consequently, some specialist clinics have limited the availability of testing to new patients[106]. Whilst increased funding has been made available through COVID-19 related resources (e.g., 'long-COVID clinics'), the amount specifically allocated to OD, the duration for which this will be available, and the ultimate effect on clinical practice is as yet unknown.

Smell tests themselves vary in price and burden of administration. Time and staffing can be reduced by use of tools that are self-administered and self-scored. However, at present, only a limited number of such tests are available commercially. Amongst them are short screening tests such as the 4 and 8-item 'NHANES Pocket Smell Test', which tests odour identification using 4- and 8-item target odours, with a 4-alternate forced choice paradigm, at a cost of $9.95 per single use test (correct at time of writing). A range of other screening tests for odour identification are commercially available, some of which, though not self-administered/scored, use very few target odours meaning their test time and associated burden of administration/scoring is minimal. For example, the 'Q-SIT' and 'Q-Sticks' both test just three odours and take only a few minutes to administer/score, and are relatively cheap to purchase.

However, given the limited range of target stimuli, these tests are only able to separate patients with normosmia from those with abnormal function, and those who achieve intermediate scores require further testing. Of the full olfactory tests that are commercially available, the SIT/UPSIT and Sniffin Sticks are the most frequently used across both clinical and research capacities. Regarding clinical practice, both have advantages and disadvantages. The SIT/UPSIT is a 40-item 'scratch and sniff' tool that can be self-administered, but only tests odour identification – a disadvantage considering the suggested association between threshold and peripheral olfactory function, and the cultural specificity of identification tests (see §1.5.2). The Sniffin Sticks test odour threshold, as well as suprathreshold identification and discrimination, but require administration by an investigator/clinical staff, which, in my experience, can take in excess of 45 minutes (particularly in older patients) and may therefore be prohibitive in busy clinical settings. Self-administered odour threshold tests would circumvent these issues. However, to my knowledge, the only self-administered odour threshold test commercially available at time of writing is the 'Self-Administered Computerized Olfactory Testing System (SCOTS)'[611]. This is a fully automated test in which phenyl ethyl alcohol threshold is ascertained using a patient controlled 2-alternate forced choice staircase paradigm. Though convenient, at $59,500.00 (6-month shelf-life after which refill required - $850.00, correct at time of writing, see https://sensonics.com), this system is likely too expensive for all but well-funded specialist centres with a high patient throughput. A number of new tests have been described in the literature that address some of the limitations of currently available tools. These include, for example, the 'SMELL-R/SMELL-S', a self-administered, non-semantic test of odour detection threshold and odour 'resolution' (a variant of odour discrimination in which 'overlap' in molecular mixtures is tested)[241]. In addition to being self-administered, this test has several other advantages: being non-semantic and using unfamiliar odour mixtures reduces bias from previous olfactory and cultural experience; use of odour mixtures mitigates the effect of specific anosmias and variations in sensitivity to specific single molecules, as seen in normosmic individuals. Other self-administered odour threshold tests have also been developed – such as the 'Adaptive Olfactory Measure of Threshold (ArOMa-T)', which uses an

adaptive Bayesian algorithm to test single-molecule odour threshold using a disposable odour delivery card[612]. At present, neither the SMELL-S/R or ArOMa-T are commercially available. It has yet to be shown, however, whether olfactory tests that do not interrogate odour identification may be less sensitive to cognitive decline as seen in aging and neurodegeneration. Furthermore, as demonstrated in my end-user survey, patients and healthcare seeking adults prioritise tests of orthonasal odour identification. The ideal theoretical test for *current* clinical practice (with associated funding limitations) would therefore be one that combines self-administered odour threshold and identification.

Given the current limitations to commercially available psychophysical smell tests, and until adequate funding is available, a practical solution for clinical practice might be for patients to initially undergo local testing at primary/secondary care using a self-administered or short administered screening test. Those who are identified to have abnormal or indeterminate scores could then be referred onto a specialist chemosensory clinic where full testing would be undertaken. My data demonstrated theoretical stakeholder support for referral networks such as these: most UK ENT surgeons favour tests of <5 minutes; most patients favour specialist assessment, are willing to travel outside of their local area for such assessment and are willing to undergo testing longer than 15 minutes. My qualitative end-user data also provides support for specialist referral networks – indeed, 'Healthcare Systems' was an overarching emergent theme – with current limitations in terms of access to appropriate care (including specialist care and follow up) and affordability being major subthemes. In line with this, when asked what could have been done better during their assessment, comments such as the following were common: '*referred to a specialist*', '*he could have given me a list of places that worked with loss of smell/taste*', '*I would have loved to be send [sic] to a specific unit to start smell training with the doctor's support. I felt abandoned*.' Affordability was more of a concern in the USA/other locations with insurance/patient funded healthcare models – with patients appearing to be most displeased when they had paid for a consultation that they perceived to be inadequate in terms of diagnosis or management.

However, despite the above, my data indicates that 'referral on to specialist clinics' is an infrequently cited reason for *not* performing routine psychophysical smell testing. At the time of survey distribution, only 8 smell and taste clinics were listed on the UK-based patient charity 'Fifth Sense' website's specialist clinic information page[613]. Given that ~2.8 million ENT outpatient appointments occur annually in the UK[614], and ~22% of the general population was thought to experience OD prior to the pandemic[223], it is unlikely that current specialist networks are sufficient to support onward referral of all patients in need of smell testing. Introduction of locally available self-administered and/or screening tests may reduce the number of required referrals, however, more specialist clinics are needed, with clear and well publicised referral pathways. Finally, interrogation of primary care practice, and analysis of primary to secondary care referral pathways is also required.

9.1.2.3 Barriers to Use – Knowledge

Despite the COVID-19-related increase in awareness of OD, 'insufficient experience/training' was the third most common reason for not performing psychophysical testing routinely in the UK, and fourth most common internationally (excluding 'NA – I use smell tests routinely). Furthermore, approximately 1 in 5 UK and international respondents had 'no knowledge or experience in use of smell tests'. Under- and postgraduate training programmes vary considerably between different countries, and for this reason, my discussion will again be largely focussed on the UK – though with transferrable overarching principles.

Higher surgical training in the UK is guided by the Intercollegiate Surgical Curriculum Programme (ISCP) curricula. Within the Otolaryngology curriculum, specific, but limited references are made to OD. In the current (2017) version, 'Disorders of the sense of smell' are listed as a 'key topic', and surgeons are expected to 'be competent in the management of' such disorders by completion of training. Similar references to OD are also made in the previous available curricula (2010 and 2007; available from https://www.iscp.ac.uk/curriculum/surgical/surgical_syllabus_list.aspx).

Furthermore, in order to complete clinical training in England (and receive the

Certification of Completion of Training, 'CCT'), ENT surgeons are required to pass the Intercollegiate Specialty Examination in Otolaryngology (FRCS). The current exam syllabus (JCIE syllabus, 2016 version) includes a subsection on 'Disorders of Olfaction'. This requires knowledge of 'the scientific basis for the assessment of olfactory dysfunction' and 'the commonly used tests of olfaction'. Candidates must also be 'competent at performing a formal assessment of olfaction using appropriate validated assessment techniques'. One would therefore expect some familiarity with psychophysical smell tests in newly qualified consultants. Whether theoretical knowledge, potentially in the absence of substantive clinical experience, is sufficient to drive clinical practice in olfactory assessment is, however, unclear. This is particularly relevant given the small number of smell and taste clinics in the UK, which significantly limits the opportunity for exposure to formal psychophysical testing practices during training. Lack of sufficient coverage during under- and postgraduate training was highlighted in my clinician-reported data – only 7.2% and 17% of UK respondents had gained knowledge of smell tests from medical school or post-graduate training respectively. This was further evidenced by a small number of free text comments: '*This is something that is frequently glossed over in medical school and I had no formal training about it as a postgraduate trainee', 'ENT doctors need definitely more training in this matter', 'poorly taught, overwhelming demand now in view of covid [sic]', 'Only ever came across smell testing for exam purposes, never used it.'* Finally, the need for further education was reflected in more than three quarters of UK respondents saying that they would like training in use of psychophysical smell tests. Similar to the UK, only 11.8% and 20.5% of non-UK respondents had gained knowledge of smell tests from medical school or post-graduate training respectively, 63.3% said they would like to receive further education.

After completion of training, clinical practice is further augmented by local, regional and national policy, protocols and guidelines. Clinical guidelines, which are generally produced from the contemporaneous research base and expert consensus, are arguably most influential in setting standards of care, being used to shape subsequent clinical protocols and funding policy[615]. The 'European Position Paper on

Rhinosinusitis and Nasal Polyps (EPOS)' provides guidance on the diagnosis and management of CRS, of which OD is a cardinal symptom. To my knowledge, these were the first set of international guidelines to explicitly discuss the diagnosis of OD, though admittedly as a symptom associated with CRS, not an isolated presenting complaint. The 2012 version of the EPOS guidelines stated that 'subjective report of olfaction correlates well with objective tests' and provided no clear recommendations on use of psychophysical tests. As a highly-cited clinical resource (5793 Google Scholar listed citations at time of writing), and given the frequency of CRS related OD[104,616], it is possible that these guidelines may have influenced the poor uptake of psychophysical smell testing in the UK and elsewhere. The recently updated 'EPOS-2020' (with 2675 citations at time of writing) considerably expands on its discussion of olfactory assessment, but still does not provide clear advice on psychophysical testing with 'unclear' or 'negative' outcomes of steering-group Delphi processes addressing psychophysical testing in different clinical scenarios [103]. Other international CRS guidelines available at the time of survey included the 2016[320] and 2021[617] versions of the 'International Consensus Statement on Allergy and Rhinology: Rhinosinusitis (ICAR: RS)'. Whilst both versions of this document discuss olfaction as an outcome in research relating to CRS, neither explicitly discusses the tools used for its measurement, including psychophysical smell tests. Again, these are well cited resources – with 921 and 433 citations at time of writing, respectively. Accordingly, the existing CRS literature may have negatively influenced adoption of psychophysical smell testing in clinical practice.

At the time of survey distribution, to my knowledge, the only available set of international guidelines specifically addressing the diagnosis and management of OD was the 2017 'Position Paper on Olfactory Dysfunction'. I wrote this document in collaboration with Prof Thomas Hummel (and with input from a further 37 international co-authors), which described the contemporaneous research base and provided expert-agreed recommendations relating to both olfactory assessment, and management of OD. Relating to assessment, the following recommendations were agreed:

i. ‘In patients reporting dysfunction, olfactory assessment should be undertaken in order to fully determine disease burden and clinical impact of interventions.’
ii. ‘Subjective olfactory assessment should not be undertaken in isolation, given its poor accuracy.’
iii. ‘Psychophysical assessment tools used in clinical and research settings should include tests of odour threshold, and/or one of odour identification or discrimination. Ideally, however, testing should include two or three of these subcomponents.’
iv. ‘Psychophysical assessment tools should be reliable and validated for the target population.’

Since publication of the original PPOD, we released an updated version in 2023 [166]. In addition to this, another set of international guidelines were released in 2022 – the ‘International Consensus Statement on Allergy and Rhinology: Olfaction’ (ICAR: O)[229]. This comprehensive document reviewed the current research base and provided expert agreed recommendations, where there was sufficient available evidence. Similar to both PPOD versions, this document recommends use of validated psychophysical smell tests, though arguably favours use of odour identification tests over odour threshold, potentially highlighting continued controversy in clinical/research practice. However, neither the ICAR:O nor the PPOD-2023 were available at time of survey distribution.

The only UK-specific guidelines available at time of survey were produced by the British Rhinological Society (BRS) and ENT-UK in 2020 (BRS Consensus Guidelines on ‘Management of new onset anosmia the COVID-19 Pandemic’)[426]. These guidelines, again of which I am a co-author, cover diagnosis and management of new onset OD but do not make reference to psychophysical smell testing, either for patients with or without suspected COVID-19. They were produced, however, for use specifically within the pandemic, with a view to reducing unnecessary face-to-face patient contact and potential viral spread. However, given the increased awareness of OD

during this time, it is possible that these guidelines could also drive poor psychophysical testing outside of the context of the pandemic.

My clinician data confirmed poor knowledge of olfactory guidelines – with 26.7% of UK and 33.5% of international clinicians being unaware of any. Within the UK, almost twice as many respondents were aware of the BRS pandemic guidelines than the PPOD – 47.2% and 24.4% of respondents respectively. Outside of the UK this situation was reversed – with more respondents being aware of the PPOD than the BRS guidelines (32.8% And 19.6% respectively). This pattern of knowledge may reflect the higher uptake of psychophysical testing in my international clinician cohort. Finally, a number of clinicians both in the UK and internationally stated that they did not perform psychophysical testing routinely as 'clinical history sufficient' (10.1% of UK total non-mutually exclusive responses, 4.8% internationally), 'will not affect management' (10.1% UK, 5.7% internationally) and 'smell tests are not standard of care' (7.1% UK, 4.8% internationally). These responses reflect poor knowledge of available guidelines, in which use of validated psychophysical smell tests is required for accurate diagnosis and subsequent development of appropriate treatment regimen.

My qualitative patient data corroborates poor levels of knowledge 'in the field' – indeed 'Provider's Knowledge' was a major subtheme in the overarching emergent 'Knowledge' theme. When asked to describe what was done well or what could have been done better during their assessment by a healthcare professional, comments such '*they need to be more educated in the condition'* and '*Drs [sic] were clueless'*, were common, and targeted across different levels of care. Comments relating to ENT surgeons included: '*all the ENTs I have seen so far (at least 3) kept saying that they didn't know much about smell disorders'*, '*I felt I knew more about smell loss than both of them [ENT]*'. Synthesising my qualitative data, patients appear to prefer assessment by a healthcare professional who has knowledge of the anatomy and physiology of smell, the pathophysiology of quantitative and qualitative OD and the collateral physical and psychosocial effects of OD. Consequently, there was a preference amongst responders for specialist care.

To address issues surrounding provider knowledge, both of olfaction and its disorders (including physical and psychosocial sequelae), and more specifically regarding psychophysical smell tests – including their underlying principles and practical implementation – increased education should be provided at under and postgraduate levels. Particularly regarding the latter, for ENT surgeons, this should ideally include a minimum number of attendances at a specialist smell and taste clinics as part of their higher surgical training and CCST requirements – where practical knowledge of chemosensory smell tests could be obtained. Given the limited number of smell and taste clinics in the UK, this training requirement is integrally linked with requirements for increased funding to extend specialist clinic networks. In turn, increased exposure to and education in olfaction and its disorders may help to recruit future olfactory specialists, who would then feed back into and sustain these specialist networks.

Finally, increased awareness of existing and future olfactory guidelines is needed. Endorsement and dissemination of such guidelines by national societies and publicity at national and international meetings would help to facilitate this goal.

9.1.2.4 Barriers to Use – Perceived Lack of Importance

To my knowledge, there is no previous evidence specifically addressing the perceived importance of olfaction amongst UK/international ENT surgeons. Therefore, though limited, my clinician data provides the first direct insight into the attitudes of ENT surgeons towards olfaction. In theory, where clinicians do not prioritise OD, this may disincentivise them to undertake thorough olfactory assessment (including smell tests), or to overcome external barriers preventing them from doing so (for example lack of funding). During my question on barriers to routine psychophysical testing, a non-mutually exclusive option 'olfaction is not a priority' was available. In the UK this option was selected least frequently – at only 1.1% of total responses (though it was possible for it not to be selected at all). Internationally, this option was also least frequently selected, after 'other', at 1.5% of total responses. Some limited evidence of positivity towards olfaction and OD was also seen in free text answers to 'any other comments' at the end of the survey (*'I*

am glad attention is being focussed on this important aspect of quality of life. At last!'), though some more negative comments were also left ('*Those who have always had OD prior to Covid 19 & were not bothered, are now trudging into ENT clinics hoping there is now a cure/treatment [sic]'*). Together, it would appear that, though not universal, the majority of my sampled ENT surgeons did not display overtly negative attitudes towards OD.

Unfortunately, however, this was not well reflected in my end-user data. Addressing qualitative data from my question '[during your assessment] what was done well/could have been done better', an overarching theme was 'Attitudes', with major sub-themes 'Professionalism' and 'Compassion'. Many patients reported encounters in which they felt they were not listened to or believed, whilst others reported belittling comparisons with other conditions, and/or poor communication skills. Though many of these comments were targeted towards primary care or non-ENT secondary care (e.g., '*GP thinks I'm not a priority as I'm not (in his view) at risk of dying from my condition'* and *'I wish she…even just ACTED LIKE SHE CARED!!! [sic]*'), others were targeted towards ENT surgeons. In response to 'what could have been done better' the following comments were left within the subgroup of patients who had been seen by ENT: '*empathy'* (lack of empathy was a frequent response); *'ENT was so rude, his comment was well MRs so and so [sic], you have no smell but you don't have cancer!'*; *'The ENT could have listened to me more and not assume that he knew everything'*; *'they could not tell me anything about it. I do not feel they took it really seriously'*; *'Been [sic] taken seriously….not made to believe it was all in my head'*; *'Compassion, empathy. Don't treat me like I don't matter because I have to live with this every single second of every single day.'* Whilst there were also multiple positive comments (e.g., '*ENT specialist took time to take detailed history and reassured me they believed me about the parosmia'*) the volume of negative comments regarding encounters with ENT surgeons raises concern, and is in keeping with the existing literature on patient experience of olfactory healthcare.

Previous qualitative analysis of online patient support group posts has demonstrated insufficient support from healthcare professionals, including lack of interest, empathy and understanding[415,438]. Similarly, free text data from patients responding

to a questionnaire distributed by a UK-based olfaction charity ('Fifth Sense'), demonstrated poorly perceived clinician attitudes[414]. Quotations from free text comments included: *'…even ENT specialists do not see this problem which truly affects your quality of life as even [sic] a problem*'; '*…it's minimized by people and professionals who think it must be nice not to smell kids' dirty nappies'*; '*The attitude is almost 'Well, at least you are not deaf or blind*." Another, pre-pandemic UK-based survey undertaken by Fifth Sense also highlighted a lack of engagement from medical professionals [195]. Quotations from free text comments included: '*One of the most depressing issues is the lack of concern by the medical profession… If I had lost my hearing, sight, a limb or had been disfigured more help would have been given*.'; '*the doctor…actually told me that I should consider myself lucky it wasn't my sight. It's over 9 years later and I am still very angry at the medical response*.'

Patient experience is one of three statutory domains required for quality healthcare in the UK, as described by NHS England[409]. Internationally, other organisations including the US-based Institute of Medicine, and the WHO, also describe the importance of patient experience in quality healthcare. My end-user data describing poor levels of professionalism and compassion are concerning, and likely to unnecessarily impact on patient experience. Looking more widely at healthcare across different disciplines, recent meta-analytic work demonstrated that 'empathic care' (a term which overlaps with '*compassion'* and where '*clinical empathy'* involves understanding/expression of understanding and consequent therapeutically helpful action) was associated with improved patient satisfaction, and other outcomes – ranging from medication adherence to survival[618]. Empathic care is likely to be particularly important in OD, where possible sequelae include reduced quality of life and mental ill health[199], and where prognosis is often poor or unclear[166,229]. Specific training programmes for empathic care have been described[619,620], and it would be of interest to trial such novel interventions in olfactory care.

The mismatch between my end-user results (which are supported by the existing literature) and clinician results regarding attitudes may, as described in the previous section, be in part due to differences in selection bias – with more positive/interested clinicians and less satisfied patients preferentially responding.

Therefore, it would be of interest to repeat both surveys outside of the context of the pandemic, using probabilistic/random sampling and possibly some form of monetary incentive to increase response rates. These approaches should theoretically reduce the impact of the pandemic (which may have caused increased interest in clinicians, and variable barriers to care in patients), and selection bias, and in doing so, may reveal slightly less divergent results. However, survey data will continue to be at risk of other bias – including, for example potential acquiescence/agreement bias in clinicians – and despite incentives, there is no guarantee that high response rates can be achieved. For these reasons, the best quality data would be obtained from integrated prospective clinical audit, randomly selected clinician interviews ± mandatory survey with probabilistic sampling, and multiple domain patient outcome measurement (including clinical and qualitative, experiential outcomes). To achieve such goals, adequate funding and standardised coverage of multiple geographic locations would be required.

Until a time at which such research can be undertaken, the true scale of the problem regarding attitudes towards olfaction will remain unclear. However, it would appear that at least some ENT surgeons involved in olfactory care would benefit from further education on the direct and secondary physical and psychosocial impact of OD. Such education alone may be sufficient for many clinicians, whilst for others, additional training to improve their communication skills and their provision of empathic care may be required.

9.1.3 Subjective Olfactory Assessment – PROMs

To my knowledge, my clinician survey was the first to explore use of PROMs in the clinical assessment of OD. As described in chapter 4, Across all UK respondents, 6.1% ‘always’ used PROMs during their initial assessment of OD (as an isolated/presenting or associated symptom), whilst 72.4% ‘never/rarely’ did so. In my international cohort, across all respondents, 13.4% ‘always’ and 56.9% ‘never/rarely’ used PROMs.

My clinician-reported data was relatively well reflected by my end-user data, in both the UK as well as internationally. In the UK, 83.7% of patients who saw an ENT

surgeon did not complete any PROMs. Internationally, in my subgroup that included respondents from the USA, 69.8% of patients seen by ENT did not complete PROMs, and in my subgroup of patients that excluded those from the USA (which was a better comparator to my clinician data, in which there were a low number of clinicians from the USA), 71.4% did not complete PROMs (see supplemental results, appendix 10.8). Together, it is apparent that most ENT surgeons – both within and outside of the UK – do not perform any form of formal PROMs testing.

Lack of formal PROM use is particularly problematic when combined with poor rates of psychophysical smell testing, as particularly demonstrated within the UK. One may extrapolate from this data that subjective olfactory function, as well as direct and indirect burden of OD, is largely being captured through unstructured patient report (i.e., patient history). With regard to olfactory function, this is problematic for reasons already discussed, affecting both diagnostic accuracy and subsequent treatment regimen choice, as well as intervention and complication outcomes measurement. Regarding direct and indirect disease burden, lack of PROMs again means that appropriate onward referrals cannot be made (e.g., in cases of depression or cognitive impairment), and the effect of interventions on these domains cannot be well quantified.

Where PROMs were being used, the most frequent specific tool – both in the UK and internationally – was the SNOT-22 or one of its variants (e.g., the SNOT-23 or German Adapted Version of the SNOT-20). Whilst the SNOT-22 is a well-validated and reliable measure of disease burden in CRS, it contains just one question on 'decreased sense of smell/taste'. It is not intended to be used as a tool for diagnosis of OD (particularly given the lack of attempted discrimination between smell/taste in the non-German version), and the isolated use of the chemosensory question has not been validated for diagnostic or outcomes assessment[621,622]. Furthermore, previous work has demonstrated relatively poor psychometric performance of the chemosensory question compared with other items within the tool (possibly due to this lack of differentiation between smell and taste)[623,624]. Despite this, the SNOT-22 and its variants are well known, commonly available, relatively quick to administer and may already be undertaken routinely in the care of many CRS patients.

Consequently, the different SNOT tools have been used frequently in olfactory CRS research (for good review of CRS-olfaction literature see ICAR:O, ref [229]), and this appears to be reflected in clinical practice. However, given the limitations described, the results of SNOT-22 or its individual chemosensory question, should be interpreted with caution. As a single-item alternative to the chemosensory SNOT question, a VAS or Likert type question with appropriate anchor statements, could provide more targeted information on specific aspects of olfactory function (including separation of smell and taste) or direct/indirect effects of OD. Though individual VAS/Likert questions are usually unvalidated, such an approach is likely to be more reliable and produce data more amenable to comparison between people/time points than unstructured history alone[119]. Ideally, however, and especially in clinical practices where psychophysical testing is not undertaken, validated multi-item PROMs should be used for the assessment of olfactory function. Tools such as the Self-Administered Odor Questionnaire (SAOQ)[227] or Hyposmia Rating Scale (HRS)[228] have moderately strong correlation with psychophysical test scores, though as outlined in the introduction, require cultural adaptation and further validation before use in non-Japanese/PD populations respectively.

Whilst I did not explore specific barriers to PROM use, extrapolating from my psychophysical clinician-reported data, insufficient time, staff and funding are likely to have negatively impacted. This fits with my anecdotal experience of working in the NHS, where staffing limitations (either in number or continuity of nursing/auxiliary staff supporting a particular clinic) and time pressures represent significant obstacles to the introduction of consistent PROM use. Unlike smell testing, improved uptake of PROMs is theoretically possible without significant and widespread funding – especially where innovative technologies are used. For example, computerised adaptive testing is a method that uses information response theory to build algorithms which allow patients to undertake a personalised subset of questions within a particular PROM, producing scores that are in keeping with the full tool[625,626]. Such approaches could significantly reduce time required for questionnaire completion, associated respondent burden/fatigue and streamline clinic efficiency, particularly where combined with automated distribution, e.g., via

QR codes placed in the clinic waiting rooms/on patient appointment letters. Resultant data could be anonymised and uploaded to a cloud storage system for future audit/research purposes (with appropriate permissions and patient consent).

9.1.4 Imaging

As for psychophysics, the last available data regarding clinical imaging practice amongst UK ENT surgeons is from McNeill *et al.*, and Williams *et al*. In their single question on 'investigations' in OD, 36.6% of McNeill's respondents stated that they would perform MRI[334]. In Williams and colleagues' clinical vignette of PIOD, 29% of respondents stated that they would perform MRI. Information regarding anatomical target or purposes of scanning was not provided during either of these surveys. Again, there has been little change in practice since this time, with my clinician reported data demonstrating a similar proportion of UK respondents (31.3%) 'always' performing MRI scanning during the initial assessment of OD. Internationally, fewer clinicians always scanned (15.1%). Interestingly, there was no statistically significant difference in scanning practice between rhinologists and non-rhinologists in either the UK or internationally.

I also collected data on frequency and type of scanning in my end-user survey. Amongst the subgroup of patients who had been seen by an ENT surgeon in the UK, respondents were most frequently referred for an MRI, followed by no scan and then CT (40.8%, 26.5% and 22.4% of all respondents, respectively). Patients seen by ENT surgeons internationally were most frequently *not referred* for a scan, followed by MRI and CT (subgroup including USA – 45.2%, 29.4% and 25.4% respectively; subgroup excluding USA – 50.0%, 32.1% and 17.9% respectively, see supplemental results, appendix 10.8). Comparison of patient and clinician data on scanning is more complex than comparison of smell testing or PROM use – there are few situations in which a patient complaining of OD would not be appropriate for psychophysics/PROMs, whilst clinical presentation and patient specific factors (e.g., clinical presentation and signs, contraindications to MRI etc.) create a more nuanced picture with regards to scanning. This, combined with the systematic differences

between my clinician and end-user data discussed above, means comparison of these data sets should be done with caution. Nevertheless, the pattern of results seen in my end-user data was similar to that seen in my clinician data – MRI scanning is undertaken in a significant proportion of UK patients and is less common internationally.

I used two approaches to determine MRI scanning target/intent in my clinician survey. First, free text responses were sought, in an effort to produce exploratory data that was not guided or limited by the question stem. Second, respondents were asked what their aim was in scanning from a set of non-mutually exclusive options. The resultant data together suggests that most clinicians perform scanning for diagnostic purposes, in particular for the exclusion of neoplastic disease, and in cases with normal endoscopic findings or unclear/idiopathic cause. Volumetric assessment of the OB, which can be used for both diagnostic and prognostic purposes, attracted a small number of responses, though slightly more internationally than in the UK (16.4% and 8.4% of total responses, respectively).

Together, my data indicates that nearly a third of UK ENT surgeons always perform MRI scanning in the initial assessment of patients OD as a presenting/isolated complaint, the majority of whom appear to be doing so for diagnostic purposes. Given the poor uptake of routine smell testing or PROMs in the UK (5.5% and 6.1%, respectively), and poor correlation between subjective report and psychophysical smell testing, it is likely that some of these patients are being scanned in the absence of test-proven OD. This is of particular interest given the debated diagnostic yield and cost-effectiveness of MRI scanning. As described in §1.5.3.1 several studies have been performed in which institution-specific case series have been interrogated for diagnostic yield, ± subsequent economic analysis and resultant imaging recommendations. Looking at this literature in more detail, it is unsurprising given my data, that several of these studies are based on patients who had not undergone psychophysical smell testing. Specifically, Powell and colleagues identified findings related to OD in 7 (though clinical management was only affect in one patient) of 100 consecutive MRIs in non-CRS related OD[329]. The authors concluded that MRI scanning *'may not be necessary in most patients with olfactory dysfunction',* and

'*imaging adds little to the patient history and clinical examination findings'*. However, none of the patients in this series underwent psychophysical testing. Similarly, Decker and colleagues reported results from their series of 122 patients with IOD, demonstrating a diagnostic yield of 4.9%[627]. In this case, the authors concluded based on a cost-effectiveness analysis (that included data on USA-based medical malpractice settlements) that scanning *should* be performed. Again, however, their patients had not undergone psychophysical testing. Shortly later, Rudmik and colleagues performed a modelling-based cost-effectiveness evaluation of MRI in IOD[332]. This group concluded that routine MRI was *not* cost-effective. However, data for this study was only obtained from two prior studies – one from Hoekman *et al.*, in which psychophysical testing was performed using (using the SIT)[330], and the other from Decker *et al.,* in which testing was not performed, as described above. In theory, lack of psychophysical testing could confound the results derived from these studies. As described previously, further prospective audit of MRI diagnostic yield in OD (with associated psychophysical testing) is required to better inform future, evidence-based guidance.

Finally, it was of interest that, across all patients seen by ENT, as well as all UK patients seen by ENT, satisfaction rates were highest in those who were referred for imaging + tested using PROMs (see Table 5-2, §5.5.6). More generally, patients appeared to prefer more thorough investigation, though smell testing did not appear to increase satisfaction rates as much as imaging. Of note, however, my data on satisfaction rates in specific test combinations was limited by low *N* numbers, given the overall poor uptake of smell testing/PROMs. Future audit of patient outcomes should include satisfaction rates to further investigate these trends. As outlined previously, increased patient education regarding necessity for scanning may also be required.

Given the high rates of scanning in current UK practice, and until further evidence-based guidance on imaging in relation to OD can be undertaken, steps should be taken to maximise clinical yield from those scans undertaken. At present, few clinicians perform volumetric assessment of the OB. It would be of interest to perform UK-level consultation with radiologists to determine whether manual or

automatic planimetry or in future more advanced methods, such as VBM, could be integrated into clinical care. These considerations are also important for the establishment of clinically relevant neuroanatomical markers of olfaction/OD and their use as future personalised biomarkers, which I explored in Theme B of this thesis.

9.1.5 Conclusions and Future Work

My thesis contains the most comprehensive available description of current practice in the assessment of olfaction, and the first in-depth analysis of end-user experience and preferences for assessment. I hope these results will serve as a critical resource for future research and service planning.

Expanding on the work contained within my thesis, I have initiated or collaborated in several other projects. These include:

1. Survey of clinical practice in North America (Canada + USA) (ongoing).
2. Survey of clinical practice in paediatric ENT surgeons (ongoing).
3. Development of new patient co-produced smell test, the Novel Olfactory Sorting Task (completed)[628].
4. Development of a new validated PROM, the 'Smell-Qx' Questionnaire (completed)[629].
5. Early-stage project looking at use of computerised adaptive testing in olfactory, and more widely, ENT care (ongoing).

9.2 Theme B

9.2.1 Summary of Theme B

My findings from Theme A demonstrated a poor standard of care in the clinical assessment of olfaction – structured subjective and psychophysical assessment are not performed routinely, and patients are often dissatisfied with their care. However, and particularly in the UK, MRI brain is frequently performed during the investigation of OD, and imaging is associated with increased patient satisfaction. These findings complement the underlying motivation of my thesis' 'Theme B' – to explore new ways in which olfaction could be assessed clinically, and particularly, the establishment of clinically relevant neuroanatomical markers of OD – upstream of the OB.

In **chapter six**, I provided proof of principle for structural plasticity of the primary and secondary olfactory networks, in association with improved olfactory function in patients undergoing FESS for CRS. The changes demonstrated were bidirectional – with GM volume increases and decreases in different areas of the primary and secondary olfactory networks.

In **chapter seven** I expanded on these structural findings and explored their functional significance. Accordingly, I prospectively studied CRS patients undergoing FESS using a larger patient cohort, with a parallel healthy control group and multimodal structural and olfactory functional imaging. Comparing GM volume change between patients and controls, there were significant time x group voxels within areas of the primary and secondary olfactory networks, including the PC, OFC and entorhinal cortex. Comparing structural change between patients who experienced clinically significant improvement in psychophysical-based olfactory function after surgery to those who didn't, there were significant time x group voxels within areas of the secondary olfactory network, including the ACC, HPC, pHPC, OFC and TP. Within the group of patients in whom there had been clinically significant improvement in olfactory function after surgery, there were GM volume increases within the HPC and pHPC, and GM volume decreases in the ACC, HPC, insula, OFC and TP. Across the whole subgroup who underwent olfactory fMRI, there were

significant increases in BOLD signal within the ACC, OFC, insula and TP – all regions in which there were decreases in GM volume within the group of patients who had clinically improved.

In **chapter eight**, I aimed to determine whether the functionally significant GM changes I had demonstrated were aetiology and treatment specific. I therefore performed a multimodal prospective neuroimaging study in patients with non-CRS OD undergoing functional septorhinoplasty. In this smaller group, I replicated my findings – demonstrating increased GM volume after surgery within the OFC, and decreased GM volume within the ACC, OFC, insula and TP. Increased BOLD signal was demonstrated in all of these regions after surgery, and interestingly, I was able to demonstrate significant positive correlation between psychophysical odour threshold score and BOLD signal within the OFC and insula that were spatially aligned with clusters of increased BOLD signal after surgery.

Together, these studies demonstrate functionally significant structural plasticity within the central olfactory networks after surgical treatment in acquired OD. My results therefore provide a higher level of evidence for the link between structure and olfactory function in these regions, as well as their potential role as clinically relevant neuroanatomical markers of OD. However, unlike previous cross-sectional data, in which patients with OD demonstrated predominately reduced GM volumes compared with controls, my data appears to reflect a more nuanced picture, in which both increases, and importantly, functionally significant decreases in GM volume may occur in association with improved olfaction.

Whilst detailed discussion was provided in the preceding chapters, in the following section I will provide a further analysis of my overall results in the context of the wider neuroimaging literature, and some more recent key developments in olfactory neuroimaging. I will then discuss more general points regarding my neurobiological models and approaches to analysis.

9.2.2 The Relationship Between Brain Structure and Function

The early cross-sectional literature linking neuroanatomical variation with olfactory function demonstrated relatively widespread results throughout the primary, or more commonly, secondary olfactory networks. Furthermore, these studies demonstrated predominantly positive associations – that is, 'less is less' and consequently 'more is more'. This has been shown in studies of healthy controls – in which positive correlations between putative olfactory eloquent regions and psychophysical test scores have been demonstrated, as well as in cohorts of patients with acquired OD – where decreased GM volume or density has been demonstrated compared with controls (see §1.5.3.1.2 for detailed discussion). This early work did not commonly demonstrate or report the reverse conditions – negative correlations between psychophysical test scores, or increased GM volume in patients compared with controls. However, as discussed in the introduction, cross-sectional studies provide evidence of correlation, not causation. This is particularly problematic in the study of multi-functional brain regions, where structural differences between groups may be caused by, predisposing to, compensating for, or incidentally related to the behavioural measure in question (e.g., olfactory function or dysfunction). Accordingly, there has been increased focus within the neuroscience community towards alternative methods that provide stronger evidence of causation[359,630].

Longitudinal studies provide such evidence. In addition to the work within this PhD, a small number of other longitudinal studies have now been published in healthy participants and patients, with varying results. As described previously, Al Ain and colleagues investigated the effects of olfactory training (OT) on healthy subjects, compared to matched controls undergoing no training, or 'visual control training'. They demonstrated significant group x time interactions for cortical thickness within areas including the superior temporal gyrus and inferior frontal gyrus – the latter of which also showed increased thickness during within group comparisons of those undergoing OT. The authors additionally demonstrated increased GM volume within the OFC and TP (as well as several other regions) during within group comparison (post – pre-OT), though these clusters were not present compared to controls (group x time interaction). Of note, neither the reverse conditions of decreased cortical

thickness/GM volume were reported, nor were patients stratified according to presence or degree of olfactory improvement. Several studies have also been performed in patient groups. Also described previously, Gellrich and colleagues prospectively investigated change in GM volume after OT in patients with PIOD[578]. Across their whole cohort (which included patients who had/had not achieved clinically significant improvement in their psychophysical test scores), the authors demonstrated increased GM volume within several regions, including the HPC/pHPC (as well as the thalamus and cerebellum). During subgroup analysis of their patients who had achieved clinically significant improvement in psychophysical test scores, they additionally demonstrated increased GM volume within the OFC. Whilst Gellrich *et al.,* did not have a prospective control group, cross-sectional comparisons between pre-OT patients and a healthy control group demonstrated reduced GM volume in the bilateral HPC/pHPC in patients. The reverse condition of reduced GM volume was not reported for any of these analyses. In another more recent study of OT in patients with idiopathic OD from the same group, Han and colleagues reported slightly more complex results[631]. Comparing pre-OT patients to a healthy control group, *increased* GM volume was demonstrated within the OFC of *patients*, and there were no significant clusters of reduced GM in patients compared to controls. Within group comparisons of pre- and post-OT GM volume within the patient group revealed both increases and decreases in GM volume (increased GM – multiple including OFC/gyrus rectus, thalamus, cerebellum, superior frontal cortex etc; decreased GM – frontal cortex, cerebellum, angular gyrus etc). Together, despite potential mechanistic differences in intervention [see §7.6 for discussion of speculative 'bottom up' vs 'top down' differences in olfactory interventions], there is some degree of spatial overlap between these studies and my own work within the OFC, HPC, pHPC and TP. Furthermore, it was interesting to note the bidirectional changes in GM volume within the latter study.

Also focusing on patients, another longitudinal study was performed recently that was similar to my work. Güllmar and colleagues investigated the effect of improved olfaction after FESS for CRS, on brain structure – but importantly focussed on white matter change, rather than GM volume or cortical thickness[632]. Whilst they did not

have a longitudinal normosmic control group, again, similar to part of my chapter 7 work, the authors divided their prospective cohort into those who had improved and those who had not improved based on psychophysical testing, making subsequent comparisons between these two groups using diffusion tensor imaging (DTI). Accordingly, they demonstrated group differences in subcortical white matter below the lateral orbital sulcus (OFC), the pHPC and the inferior temporal sulcus (which begins near the TP). They additionally demonstrated significant correlation between DTI parameters and TDI score in the ACC and amygdala. Whilst I was unable to replicate the structural changes demonstrated within the amygdala in my chapter 6 work, otherwise there was good regional overlap between this work, and the results of my chapter 7 group x time results, where I compared patients who had improved on psychophysical testing to those who had not (with significant results within the ACC, HPC, pHPC, OFC and TP). Whilst our studies were investigating different aspects of brain structure, previous work has demonstrated correlation between DTI based parameters and grey matter volume[633]. Combined with my functional results, this strengthens the evidence for these regions as neuroanatomical correlates of OD.

Whilst my, and the above work, focussed on structural plasticity following improvements in olfactory function through targeted intervention, the recent COVID-19 pandemic has enabled opportunistic prospective study of patients affected by SARS-CoV-2. One such large-scale study was recently published using UK Biobank data (a large longitudinal multimodal neuroimaging database of older adults – aged 51-81 years). Accordingly, Douaud and colleagues investigated the effect of SARS-CoV-2 infection on brain morphometry in 401 subjects who had tested positive for COVID-19 between their two pre-arranged scans, compared to 384 controls who had not knowingly been infected. Through analysis of this data, they demonstrated greater reduction in cortical thickness and reduced 'tissue contrast' (similar to GM density) in the OFC and pHPC of patients compared to controls. They also found greater changes in markers of tissue damage in brain regions connected functionally to the PC, particularly the ACC, OFC and insula. Whilst these results are based on a very large longitudinal dataset – this study did not include any form of olfactory testing. The relationship between such changes and olfactory function are therefore

strictly speculative, and difficult to disentangle from potential non-olfactory effects of SARS-CoV-2. However, it is interesting to note the high degree of spatial overlap in neuroanatomical regions affected in a large cohort of SARS-CoV-2 infected subjects, with my prospective multimodal work: in areas where Douaud found *decreased* cortical thickness after infection, I found *increased or bidirectional* GM volume change after treatment (pHPC – though note I demonstrated no significant increase in BOLD signal – and OFC, respectively) and in areas where Douaud found markers of increased tissue damage after infection, I found increased BOLD signal after treatment, as well as reduced GM volume (ACC, OFC and insula). Complications due to lack of psychophysical olfactory testing in Douaud's work aside, this anatomical overlap again strengthens the evidence for these regions as neuroanatomical correlates of OD.

Of note, whilst I did not demonstrate increased BOLD signal within the pHPC or HPC – I did demonstrate significant group x time interaction when comparing GM volume in patients who had improved olfactory function after FESS, compared with those who did not, as well as significant increase in GM volume after surgery within the improved group (which was statistically significant at a conservative threshold – $P<0.05_{FWE}$ – within the HPC). Given that other prospective work demonstrated structural plasticity in these regions, they merit further investigation for associated functional plasticity. If such work replicates my null findings – one could potentially speculate that increased GM volume within the pHPC/HPC supports olfactory processing that is poorly captured by block fMRI paradigms in which the conditions 'odour present' and 'odour absent/baseline' are compared. Given that the pHPC and HPC have been linked with higher order olfactory processing – such as odour discrimination learning, and various aspects of odour-associated memory, including odour-evoked autobiographical memory[568,582] – it follows that activation in these regions may not be consistently demonstrated using simple odour detection paradigms. Indeed, during their ALE-based metanalysis, Seubert and colleagues did not demonstrate significant ALE maxima within either of these regions for peak activations in odour vs baseline contrasts[31].

Focussing on the direction of structural change, in addition to the longitudinal work from Han *et al.,* described above, other advanced (though cross-sectional) neuroimaging methods have provided increasing evidence for the complexity of relationship between brain structure and olfactory function. Accordingly, Iravani and colleagues recently investigated the effect of acquired OD on brain morphometry, using a multivariate technique for pattern analysis of voxel-wise morphometry (support vector machine with searchlight procedure – a form of supervised machine learning used to produce classification accuracy maps for differentiating patients from controls based on brain morphometry). In patients, they demonstrated *increased* GM volume in areas of the primary and secondary olfactory networks, including the PC, TP and inferior frontal gyrus, compared with controls. They additionally found areas of reduced GM volume within the angular gyrus of patients, compared to controls. Furthermore, of interest, the authors demonstrated *negative* correlations between psychophysical test score (TDI) and GM volume within the PC and TP – the latter of which aligns well with my demonstration of increased BOLD activity in association with decreased GM volume within the TP. Finally, support for the complexity of relationship between form and function can also be found looking to the congenital and early-loss sensory deficit literature – where both increases and decreases in measures of grey and white matter have been demonstrated in patients with various sensory impairments. For example, early work from Noppeney and colleagues demonstrated both areas of decreased GM volume as well as 'compensatory' increases in white matter volume, in early-blind subjects[634]. Similarly, Leporé and colleagues demonstrated bidirectional volume changes in both early and late-onset blindness, with stronger effects seen in the former group[635]. Though congenital OD is relatively rare, and the resultant literature base therefore small, looking upstream of the OB (which is hypo- or aplastic in these cohorts) work from Franselli and colleagues demonstrated increased GM density within piriform and entorhinal cortices of patients, compared to controls, as well as increased cortical thickness in the PC, and bidirectional changes in cortical thickness of the OFC[590]. In a recent series of studies from Peter and colleagues, the authors first demonstrated, and then later replicated, areas of increased GM volume and cortical thickness within the OFC, but not PC, of congenital anosmics compared to matched

normosmic controls. Taken together, though care is needed when comparing populations with congenital vs acquired OD, these findings better reflect my bidirectional results than the early 'more is more' literature base. Indeed, it appears that some patients with OD may have areas of greater GM volume within functionally relevant areas, and GM volume may decrease in these areas in association with improved olfactory function. The underlying mechanisms leading to such changes, however, remain unknown[389]. In addition to my relevant discussions in chapters 6 through 8, I will provide a further overview of neurobiologically plausible microstructural mechanisms for such change in the next section with a particular emphasis on macroscopic GM volume as demonstrated by VBM.

In conclusion, the emergent literature base provides supporting evidence for the neuroanatomical regions demonstrated in my work – including the ACC, OFC, insula and TP, as well as the pHPC and HPC. Moreover, my work helps to establish the clinical relevance of these regions, making them good future targets for potential investigation as personalised biomarkers of OD, though the increasingly apparent complexity of relationship between structure and function raises new challenges for this goal.

9.2.3 What Causes Changes in GM Volume Demonstrated by VBM

VBM at its core, is a method of comparing T1 signal strength between different brains (from either different people or different time points), that have undergone global alignment, tissue specific segmentation and anatomical parcellation. Multiple changes may lead to differing tissue relaxation properties and consequent changes in voxel-wise T1 signal, and consequently grey or white matter volume/density, together interpreted as 'structural plasticity'. These include differences in cortical thickness or folding as well as microscopic changes, which may theoretically affect neuronal, glial, vascular, glymphatic or extracellular compartments. Regarding neuronal/glial populations – putative changes may affect the proportion of relative cell types, total cell number/density, cell size, shape (e.g. changes in dendritic spines, synaptic changes, or axonal remodelling) or myelination[591]. Vascular changes may

involve angiogenesis, endothelial cell proliferation or changes in vessel permeability with secondary effects on the extracellular and/or glymphatic compartments[636,637].

Whilst the relative contributions of these potential changes to VBM-derived alterations in GM volume are as yet unknown, early animal work has demonstrated rapid microscopic changes in response to altered sensory input. Trachtenberg and colleagues showed dynamic changes in pyramidal cell dendritic spine populations over the course of days within the barrel cortex (part of the somatosensory cortex) of mice that had undergone whisker modifications[638]. The authors linked such changes with synaptic formation/elimination and suggested this underpins the synaptic plasticity that facilitates experience-based alterations in neural networks. Within an olfactory context, increased apical dendritic spine density has also been demonstrated in pyramidal cells within the PC of rats that had undergone a three day course of odour discrimination[394]. Again, the authors linked these changes with alterations in synaptic numbers. Slower processes, including putative adult neurogenesis have also been suggested in areas such as the OB, PC and amygdala[13,395–397]. However, whilst this provides insight into processes that may underlie experience-dependent structural plasticity, none of this early work attempted to investigate how such microscopic changes relate to macroscopic MRI signal.

Addressing this, a small number of recent studies have attempted to compare MRI findings (either *in* or *ex vivo*) with associated measures of tissue composition. Tissue composition may be assessed using advanced *in vivo* imaging techniques (e.g. two-photon microscopy - a type of nonlinear optical microscopy that allows for relatively deep tissue penetration and detection of fluorescent probes in living animals[639]) – or using post-mortem histological analysis. In non-olfactory work, Keifer and colleagues demonstrated significant positive correlation between *ex vivo* 'VBM signal' and dendritic spine density, but not cortical thickness, within the auditory cortices of mice that had undergone auditory fear conditioning[640]. Similarly, Lerch and colleagues demonstrated increased *ex vivo* hippocampal volumes in mice that underwent a spatial learning paradigm, which in turn correlated with a marker for neuronal process remodelling (but not markers of neurogenesis, neuronal/glial cell

number or size)[641]. Using *in vivo* MRI and two-photon imaging (with nuclear florescent probes), Asan and colleagues recently investigated the cellular counterparts to age-related VBM-based GM change in mice. Using a prospective design, the authors found GM volume was associated with multiple different aspects of tissue composition, including overall cortical volume, cell density, nearest neighbour distance and mean nucleus volume, with 'fraction of largest nuclei' the strongest predictor of GM volume. Interestingly, larger average nucleus volumes were associated with smaller GM volumes. Given that neurons generally have larger nuclei than glia, the authors speculate that GM volume may be affected by the cell-type composition of the imaged tissue. Extrapolating from this, tissue with a higher *glia to neuron* ratio could theoretically demonstrate higher GM volumes, and tissue with a higher *neuron to glia* ratio, smaller GM volumes.

How this early evidence relates to the structural changes demonstrated in my work remains speculative. In areas where I found increased GM volume (e.g. the HPC/pHPC), it is possible that this may have reflected increased dendritic spine density, or possibly longer term neurogenesis – as has been suggested to occur within the HPC (see [592,642–645], but also [646]). Whilst decreased GM volume in the context of aging or disease is usually interpreted as neuronal atrophy, in the context of my short-term work, where there were associated increases in BOLD signal, this is unlikely. Speculative microscopic changes underlying decreases in GM volume associated with increased functional activity could include increased synaptic pruning (and consequent reduction in dendritic spine density) associated with greater network efficiency. As discussed in §7.6, this theory has been used to explain greater cortical thickness in putative olfactory regions of patients with congenital anosmia. Alternatively, decreased GM volume associated with increased functional activity could be related to changes in relative cellular populations – with greater neuron to glia ratio, as suggested by Asan *et al.,*[647] – potentially related to inflammation, and which will be discussed in more detail in the following section. Overall, further animal and human work is required to determine the mechanisms underlying both increased and decreased GM volume, which may be multiple and overlapping.

9.2.4 Chronic Rhinosinusitis as a Neurobiological Model

As outlined in §1.4.2.1, chronic rhinosinusitis is a common sinonasal inflammatory condition that causes OD in a high proportion of patients. Furthermore, CRS-related OD frequently improves with treatment, which is not the case in many other pathologies. Therefore, CRS provides a good neurobiological model to investigate potential plasticity of the central olfactory networks.

Interestingly, it has recently been suggested that CRS may affect neurocognitive function. Work from Soler and colleagues demonstrated significantly worse scores of subjective cognition and reaction times in patients with CRS (with/without polyps) compared to non-CRS controls[648]. This same group later went on to demonstrate reduced processing speeds and worse scores of selective attention in patients with CRS, compared to controls, as well as improvement in subjective cognitive function after medical or surgical treatment[649,650]. In line with this, previous work based on population-based sampling in Taiwan demonstrated a significantly higher prevalence of prior CRS diagnosis in subjects with dementia compared to those without[651]. Recent work from South Korea also found a significant association between presence of CRS and mild cognitive impairment (MCI) at baseline, as well as greater deterioration in cognitive scores (MMSE, mean inter-test interval 41.8 months) within their subgroup group of patients with dementia + CRS, compared to patients with dementia alone[652]. It should be noted, however, that diagnosis of CRS in this paper was made retrospectively using MRI-based Lund-Mackay scores rather than clinical information. Moreover, olfaction was either not assessed, or associations with olfaction were not reported in any of these studies.

The link between cognitive dysfunction and OD in the context of aging and neurodegeneration is well known[653]. In line with this, later work has demonstrated significant associations between CRS related OD and cognitive function: in 2023 Chang and colleagues found significantly lower cognitive scores (Montreal cognitive assessment, MoCA) and a higher proportion of patients with MCI in CRS patients with OD compared to CRS patients without OD. Furthermore, after adjusting for patient demographics, OD was the only surviving significant risk factor for MCI in CRS patients.

The pathophysiological mechanisms linking CRS and CRS-related OD with cognitive dysfunction are unknown. Poor sleep, snoring, depression, cardiovascular and cerebrovascular diseases have all been suggested as possible links[654–656]. An association between peripheral inflammation and neurocognitive dysfunction has been increasingly suggested in recent years in patients both with and without established neurodegenerative disease. In patients with AD, Holmes *et al.,* demonstrated that episodes of acute systemic inflammation were associated with increased serum TNF-α and a two-fold increase in cognitive decline rate over the next six months[657]. Conversely, patients with low serum TNF-α throughout the study period did not experience cognitive decline. Similarly, Ide and colleagues found that baseline periodontitis was associated with a six-fold increase in cognitive decline over the following six months, independent of baseline cognition, in patients with AD[658]. Dysregulation of various other inflammatory mediators, both serum and CSF, have also been linked with MCI and AD[659]. In older adults without known neurodegenerative disease, increased serum inflammatory markers (Il-1 and TNF-α) have been associated with increased risk of developing AD[660]. Furthermore, links between peripheral immune dysregulation and the presence, severity, or progression of several other neurological or psychiatric conditions, that are also linked with OD, has also been shown. These include Parkinson's disease, depression, multiple sclerosis, systemic lupus erythematosus and schizophrenia[661]. Though the underlying mechanisms linking peripheral inflammation, olfaction and neurocognitive dysfunction are still an area of active research, it has been suggested that peripheral inflammation could in turn lead to a neuroinflammatory response, through various mechanisms, including but not limited to activation of microglia, and subsequent microgliosis[662]. Where such inflammation affects regions of the central olfactory networks, this could theoretically cause structural alterations, and in turn be reflected as OD. Interestingly, the areas that underwent change in my studies (the HPC, pHPC, ACC, insula, OFC and TP) are regions thought to be affected by neurodegeneration-related and/or peripheral inflammation[663–669].

Though entirely speculative, it is interesting to consider my results in this wider context. Whilst patients with known neurological conditions were excluded, with the

above in mind, it is possible that patients with CRS may demonstrate structural or functional brain alterations, compared with non-CRS controls, that may potentially be either related to, or independent of OD. In theory, the latter could confound my patient to healthy control comparisons in chapter seven. However, to better isolate olfaction as the target variable of interest, I went on to compare subgroups of CRS patients who had experienced a clinically significant improvement in olfactory function after surgery, with those who had not. In this way, any potentially confounding, non-olfactory effects of CRS were reduced. That being said, given that the pathogenesis of CRS is inflammatory, it is possible that those patients with significantly improved olfactory function after surgery may have experienced a larger reduction in associated peripheral (± speculative central) inflammation than patients who did not experience improved olfactory function. To further ensure that the structural results I demonstrated were related to changing olfaction, I performed olfactory functional imaging and demonstrated significant increase in BOLD signal after surgery within the ACC, OFC, insula and TP – where GM volume reductions were observed – but not in the HPC or pHPC – where increases in GM volume were seen. As discussed above, lack of increased BOLD signal in the latter two regions requires replication, and where null results were again found, this could potentially be attributable to the block fMRI paradigm used. This is of particular interest given that hippocampal volume has previously been shown to correlate inversely with peripheral markers of inflammation, such as Il-6, in middle aged adults[668]. Increasing HPC GM volume, as seen in my results, could therefore theoretically be in keeping with reducing levels of inflammation with treatment ± neuronal process remodelling as previously outlined. Furthermore, as described above, Asan and colleagues[647] suggested that decreases in VBM derived GM volume could be related to changes in relative cellular populations – specifically greater neuron to glia ratio. Whilst highly speculative, it is interesting to consider whether the GM volume reductions seen in the ACC, OFC, insula and TP – that were associated with increased BOLD signal – could be due to some treatment related change in neuroinflammatory status, for example by decreasing microgliosis with treatment. Potentially in line with this, Geo *et al.,* demonstrated decreasing microglial populations within the facial nucleus

during clinical recovery after facial nerve axotomy[663]. These reductions were due to apoptosis or cell migration and allowed restoration of the microglial steady state.

These theories require further investigation – and whilst this was not a primary aim of my PhD, if such work was able to demonstrate a neuroinflammatory response to sinonasal inflammation, reflected by OD and reversible with treatment of peripheral disease, this would be an exciting area of future study.

9.2.5 Non-CRS Patient Group

In chapter 8, I went on to investigate whether the functionally significant structural plasticity I demonstrated in my CRS patients could be replicated in patients with non-CRS OD undergoing fSRP. I did this to determine whether the significant changes seen were aetiology/treatment dependent. At time of recruitment (which was pre-pandemic), the available population of patients with OD who required fSRP was small. For this reason, it was necessary to recruit a pragmatic sample of patients with OD of mixed cause.

The use of mixed aetiology cohorts in chemosensory research, though not ideal, is not uncommon. Several previous structural neuroimaging studies have used patients with mixed aetiologies (see §1.5.3.1.2), some early studies even including patients with PTOD – which could theoretically cause baseline, non-OD related structural abnormalities. For this reason, PTOD patients, as well as those with neurological or neurodegenerative, as well as known psychiatric illnesses were excluded. However, for my purposes, given that I was interested in whether the changes I demonstrated in my chapter seven work was unique to CRS/FESS, use of an otherwise mixed, non-CRS OD patient sample fit my thesis aims.

Careful exclusion of CRS was important, as previous work has suggested a potential link between nasal septal deviation (NSD) and sinonasal inflammation, possibly through obstruction of the ostiomeatal complex[670]. However, the existing literature base is variable, with either conflicting results or modest effect sizes. Several radiological studies have failed to demonstrate significant associations between sinonasal inflammation and anatomical variants, including NSD. Shpilberg and

colleagues found no statistically significant difference in the prevalence of NSD comparing CRS patients with 'minimal' sinonasal inflammation (defined as <1mm 'mucosal thickening with no obstruction of the sinus drainage passages') vs those with 'significant disease' (subjects with more than 'minimal' disease as described above)[671]. Similarly, Jones and colleagues demonstrated no significant difference in prevalence of NSD when comparing 100 CT scans of patients with CRS, compared with 100 scans from non-CRS controls. In 2010, Orlandi published a systematic review analysing the association between NSD and sinonasal inflammation[672]. From 13 eligible studies, he demonstrated a significant, dose dependent association. However, the included studies were poorly powered, there was insufficient differentiation of acute, recurrent acute and chronic rhinosinusitis, and the resultant effect size was modest (OR 1.47). More recently, data from the Korean National Health and Nutritional Examination Survey (years 2008-2012) was analysed to address the prevalence and risk factors for allergic rhinitis, CRS and NSD[673]. A large number of adult subjects (28,912) underwent interview and nasendoscopy, with CRS diagnosed in line with the EPOS-2012 guidelines, and NSD based on endoscopy findings. The prevalence of NSD + CRSsNP was higher than NSD + CRSwNP, and after adjusting for relevant risk factors, NSD increased the risk of CRSsNP but not CRSwNP. However, the effect size was small (OR 1.16).

I excluded CRS in line with contemporaneously available (EPOS-2012) guidelines. Accordingly, each included patient was assessed, both by myself and Peter Andrews, Professor of Rhinology at the Royal National ENT Hospital (formerly Royal National Throat Nose and Ear Hospital). Assessment included clinical history, full examination (including three-pass nasendoscopy) and imaging (with standardised reporting from a consultant radiologist). Included patients therefore did not have clinically or radiologically evident CRS at time of diagnosis or research assessment.

I additionally calculated Lund-Mackay (LM) scores based on my MRI scan findings, as a radiological indicator of potential sinonasal inflammation. Pre-operatively, there was no significant difference in mean LM score between patients and controls (2.7 vs 2.9 respectively). Furthermore, whilst there was a slight decrease in score within the patient group post-operatively, this did not reach statistical significance (pre-op 3.22,

post-op 2.89) [though note – to my knowledge – there is no published MCID for LM score in relation to other validated measures of sinonasal inflammation]. Moreover, derived scores were in line with incidental LM scores in non-CRS populations. Early work from Ashraf and Bhattacharyya demonstrated a mean LM score of 4.26 in patients *without* a clinical diagnosis of CRS, who had undergone scanning of the internal auditor meati, orbits or pituitary[328]. More recent meta-analytic work incorporating data from 16,966 asymptomatic patients demonstrated a slightly lower mean incidental LM score of 2.24, though just under 15% of these subjects were additionally found to have an LM of 4 or greater[674]. These data therefore support lack of significant sinonasal inflammation at time of scanning, at the group level.

At the individual level, LM score was unchanged after surgery in six of my subjects, decreased in two subjects, and increased in one. As mentioned, I am not aware of a published MCID for the LM score, and therefore the implication of these individual level changes on potential degree of sinonasal inflammation is unknown. It is worth noting, however, that these individual level changes did not correspond consistently with changes in psychophysical test score - in the two patients in whom LM decreased, TDI increased in one and decreased in the other – and in the one patient in whom LM increased, TDI also increased. These results are in line with my lack of significant correlation between LM score and psychophysical test scores (T/D/I/TDI). Therefore, whilst changing LM score in these individual patients may have indicated some degree of change in underlying radiologically evident sinonasal inflammation, the implications of such changes in relation to presence of clinically significant inflammation, and its potential impact on olfaction, remains unclear. In light of these complexities at the individual level, future work in patients with NSD should aim to use additional measures to quantify potential sinonasal inflammation.

Subjects with AR were not excluded for pragmatic reasons (due to limitation in available sample size). As there can be significant overlap in symptomatic presentation between AR and CRS, again, careful exclusion of the latter was necessary. This was initially based on clinical history and examination as above. However, given the potential association of the two, AR patients additionally

underwent a period of treatment for CRS in line with EPOS-2012 guidelines, and were only included where their OD persisted despite this. AR itself is associated with OD, though the prevalence of OD in rhinitis varies considerably in the available literature – possibly due to inadvertent inclusion of patients with CRS into some studies. In a systematic review of 36 studies, the prevalence of OD in AR was most commonly between 20 to 40%, and the severity of OD in AR was generally less than in other conditions, with anosmia being infrequent[113]. In a large population-based sample from Sweden (n=10,670), the prevalence of OD in subjects with AR was similar to that in the 'healthy' population (13.1 and 10.9% respectively)[675]. However, it should be noted that both categorisation into AR/non-AR groups, and olfactory function, were self-reported in this study. As my primary aim was to investigate whether the structural/functional plasticity I demonstrated in chapter 7 was specific to CRS/FESS, and thereby could be replicated following another treatment approach for general, rather than aetiology-specific OD, the inclusion of patients with AR fit within these aims. However, given that the underlying pathophysiology of AR involves inflammation – which may either cause physical obstruction of odourants to the OC[676], or potentially inflammatory changes within the OE itself[113,676–678] – division between AR and CRS, and their potential impact on olfaction, is more difficult at the pathophysiological level. With regard to my cohort, whilst the mean improvement in TDI score post-operatively was slightly higher in my AR group vs my non-AR group (7.75 vs 4.94), this did not reach statistical or clinical significance. Furthermore, only one of the patients in whom there had been a change in LM score post-operatively had AR. Despite this, it would be interesting in future work to recruit sufficient patient numbers to adequately power subgroup analysis according to AR status. Further combining this with measures of peripheral ± central inflammation could shed light into whether my replicated changes in chapter 8 were driven by potential alterations in AR status, with speculative upstream neuroinflammatory effects. Where future work aimed to determine whether the functionally significant structural plasticity I demonstrated was driven by *any* changes in peripheral olfactory system inflammation, rather than specifically CRS, AR should be excluded. However, exclusion of patients with any inflammation at the peripheral olfactory

system may be challenging given increasing evidence for its role in the pathophysiology of OD at this level[89].

9.2.6 Surgery as an Intervention for OD

Using surgery as a model intervention for OD has several advantages. First, it provides a temporally defined treatment, which is not dependent on patient compliance – for example as with olfactory training or regular medication use. Second, surgical procedures can be relatively well stereotyped across patients – and particularly in the case of CRS, patients undergo a standardised, evidence-based regimen of medical treatment – both before and after their procedure[103,319,617]. Finally, for elective procedures such as FESS or septorhinoplasty, patients must be physiologically healthy enough to undergo a general anaesthetic – separating them from those whose olfactory function may not improve due to other confounding health issues.

It has, however, been previously suggested that surgical procedures themselves may carry significant placebo/nocebo responses, confounding comparisons to non-surgical controls[679,680]. Whilst previous meta-analytic work has demonstrated overall positive effects of surgery vs sham, the demonstrated effect size was modest, and varied according to procedure studied[681]. Indeed, surgery and invasive interventions related to pain or weight loss demonstrated no significant benefit compared with sham alone. These confounding non-specific or 'placebo' effects were speculated to be attributable to natural disease history (with possible symptomatic 'regression to the mean') or response to the 'ritual' of medical intervention (including hospital-like settings, meetings with authoritative providers, repeated suggestions regarding outcomes etc), with the latter likely impacting on the proposed link between 'behaviour, brain and bodily responses'[681,682]. Related to the latter, the emotional impact of surgery may be of particular interest in olfactory outcomes, given the significant overlap between olfactory and limbic system networks, and close link between olfaction and depression as well as general quality of life[199].

More specifically, the potential effect of surgical stress and general anaesthesia (GA) on brain structure and function should be also considered. Negative neurocognitive effects following surgery/GA have been demonstrated at extremes of age. In young children, and very young non-human animal models, repeat or prolonged exposure to GA has been associated with adverse long-term neurocognitive function, as well as alterations in brain morphometry (including the orbitofrontal cortex and hippocampus)[683,684]. In older adults, post-operative neurocognitive function may be affected acutely (as in post-operative delirium), or more subtly over a longer time frame (as seen in post-operative cognitive dysfunction)[685]. At both extremes of age, the relative contributions of anaesthetic agents, surgical stress and patient specific vulnerabilities are not fully clear, nor likely to be independent. General anaesthesia causes reduction in overall cerebral metabolic rate, and variable changes in cerebral blood flow, dependent on the agent used[686]. Furthermore, some anaesthetic agents have demonstrated neurotoxicity in animal models[687]. Surgery itself produces an acute inflammatory state, which could potentially lead to conditions of neuroinflammation[688]. Finally, early neurological development or undiagnosed neurodegeneration, as well as other medical comorbidities (often for which the patient is undergoing surgery or receiving a GA) may render patients differentially vulnerable to physiological stress, causing adverse subsequent brain changes in some, but not others[689]. In line with these complexities, recent interrogation of a large longitudinal aging cohort (Mayo Clinic Study of Aging) demonstrated greater cortical thinning in regions commonly associated with neurodegeneration in older adults who had been exposed to surgery with GA[690], surgery with regional anaesthetic[691], or critical care admissions[692], compared with those who had not. However, to my knowledge, similar effects in younger adults – or at shorter durations post procedure – have not been reported.

The specific effects of surgery/GA on olfaction have been addressed in a small number of studies. Two recent RCTs assessed the effects of different anaesthetic agents on olfaction. Sari and colleagues demonstrated transient impairment of

odour identification[x] at 3 hours post-operatively in microlaryngoscopy patients receiving sevoflurane but not desflurane[693]. Subsequent testing at one week demonstrated improvement in 'memory/identification' performance back to pre-operative baseline. Odour detection threshold was not affected by either volatile anaesthetic. In another RCT – in which patients were randomized to receive regional anaesthesia, volatile GA with sevoflurane, volatile GA with isoflurane, or total intravenous anaesthesia with propofol – early (3hrs post-operative), transient deficits in odour identification (measured using the SIT-40) but not odour threshold (in house 2-alternate forced choice n-butyl alcohol test – of note, all patients achieved the same sore pre- and post-operatively raising questions about ceiling effect and the discriminatory value of this test), were again demonstrated in the sevoflurane group[694]. In a more recent study, sevoflurane associated odour identification deficits (tested using the 12-item Sniffin Sticks odour identification test) was shown to be associated with poor pre-operative cognitive function[695]. This was demonstrated at between 16 and 26 hours post-operatively. In, to my knowledge, the longest pre-/post-operative testing interval was demonstrated by Hernandez *at al.,* who reported Sniffin Sticks TDI results on average 6.1 days apart (with pre-op testing done the night before/day of surgery)[696]. No significant change in subjective or psychophysical test scores was demonstrated. The majority of patients in this study received GA (though type of anaesthetic used was not reported), though a small subgroup received regional anaesthesia (including spinal or nerve blocks). Subgroup analysis according to anaesthetic type (GA/regional/local) was not reported. To my knowledge, no long-term studies of olfactory function after surgery/GA have been performed.

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[x] *Please note – the authors report their findings as odour 'memory' rather than identification. However, on careful reading of the manuscript – it would appear that odour 'memory' was measured using the Connecticut Chemosensory Clinical Research Center (CCCRC) olfactory test – which measures monorhinal odour identification and DT. No clear modifications to the test were reported to facilitate measurement of odour memory, rather than odour identification.*

Despite lack of specific olfactory or more general morphological brain data in the general adult population, it is not unreasonable to speculate that surgery/GA *could* have a confounding effect in the comparisons of surgical and non-surgical control cohorts.

Consequently, I attempted to mitigate these effects in chapter 7, by comparing subgroups of patients who had experienced a clinically significant improvement in olfactory function after surgery, with those who had not. In this way, both target/control groups had undergone similar surgical procedures, so controlling for the potentially unknown effects of surgery/GA, and better isolating change in olfactory function as the target variable of interest. Interestingly, the significant time x group interaction that had been present when comparing patients with healthy, non-surgical controls within areas of the primary olfactory network (OC and entorhinal cortex), was no longer significant. Assuming these findings survived replication, future work should aim to determine whether sinonasal surgery could affect the primary olfactory network, in a way that is not reflected by changes in psychophysical olfactory function.

The primary aim of my thesis' Theme B was to investigate the neuroanatomical correlates of OD, by interrogating the effects of improving olfactory function. Whilst I used rhinosurgical procedures as my model interventions, the precise mechanism by which the surgical interventions used caused improvement in olfactory function was *not* a primary focus. That being said – it is generally accepted that FESS improves olfactory function through a combination of mechanisms, including – facilitation of airflow to the OE through removal of physically obstructive polyps, a reduction in overall inflammatory load and facilitation post-operatively of medication delivery (e.g. intranasal steroids, saline irrigation)[301]. The role of functional septorhinoplasty in augmenting olfaction is less well established. Whist I provided some initial insights into possible mechanisms [with significant correlation between change in psychophysical olfactory function (DT) and bilateral PNIF, but not measures of nasal airway symmetry – see §8.6.3 for further discussion], these require further study. Use of complimentary techniques such as computational fluid dynamics, may help to further investigate the role of anatomical modification of the nose on olfaction[47].

This of particular importance, given the previously demonstrated lack of significant correlation between airflow within the olfactory cleft, and measures of total nasal airflow[47].

9.2.7 Conclusions and Future work

In Theme B, I first provided proof of principle for structural plasticity within the olfactory networks, and then provided evidence for the functional significance of such change, in relation to improving olfactory function. Accordingly, my results demonstrate more robust evidence for these regions as neuroanatomical correlates of OD than previous cross-sectional data, at the group level. The clinical utility of these regions at the individual level, for example as potential biomarkers of OD, requires further investigation. This is made more challenging by the emergent complexity of relationship between structure and form – whilst the direct association between GM volume and function ('more is more') demonstrated in early cross-sectional work appears to hold for some regions (HPC, pHPC), inverse relationships were demonstrated in other areas (ACC, insula, OFC, TP), the underlying reasons for which are speculative, but which could involve differential effects of underlying processes such as neuroinflammation.

In addition to the further work described in the previous sections of this general discussion, and my individual experimental chapters 6 – 8, I suggest two additional inter-related lines of research following on from Theme B of this PhD. First, the potential role of the above regions as clinically relevant biomarkers of OD should be tested at the individual level through development of predictive models and subsequent validation in independent data sets. Models using combinations of different regions, including the OB, could also be explored. Furthermore, exploration of multimodal models, integrating structural with psychophysical function would be of interest, particularly where neuroimaging data could be used to improve the utility of short or screening psychophysical smell tests, that are easier to perform in a busy clinical setting.

Second, underlying mechanisms behind the demonstrated variability in GM volume change should be explored. Given my, and other recently emergent neuroimaging and basic science results, it would be particularly interesting to explore the role of neuroinflammation in such changes. For example, future longitudinal neuroimaging studies in patients with CRS could aim to quantify systemic (including type 2 vs. non-type 2 inflammation), nasal and central (e.g., CSF) inflammation, as well as comprehensive measures of cognition and their relation to olfaction. Brain microstructural changes and their association with both inflammation, as well as their effects on VBM-derived GM volume, should also ideally be studied in animal models of CRS and OD.

Future work may benefit from use of artificial intelligence (AI), including natural language processing, deep learning techniques and AI-enhanced search engines. Whilst these were not available to me during my research, their recent emergence – when used ethically and appropriately – may facilitate more efficient workstreams[697–699]. For example, the AI-driven search engine 'Dimensions.AI' (available from: https://app.dimensions.ai/discover/publication) uses natural language processing to allow comprehensive integrated analysis of information from multiple data sources – including but not limited to publications, patents, grants, policy documents and trials. Use of such tools may result in faster, more comprehensive, and complete literature searches. Use of AI in other areas (for example in generation of code or manuscript writing), would require careful supervision and appropriate citation – the latter to ensure that ethical standards were upheld. The potential negative impacts of routine AI use – for example on the development of necessary skills in young researchers, lack of reproducibility or the known environmental cost – should also be carefully considered when planning fair and sustainable future work[699].

9.3 General Conclusions

'From the sublime nostalgia of the madeleine to the mundanity of a midweek dinner, smell gives flavour to food, emotion to memories, and connects us to each other.'[700]

I hope my work helps to move clinical practice forward, motivating clinicians and researchers to progress towards accurate assessment of olfactory dysfunction, in patient-focussed and novel ways.

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10 Appendices

10.1 Development Panel Feedback Questionnaire

Panel Feedback Questionnaire

General Comments

1. The length of the survey - excluding the additional optional questions - was:

   Mark only one oval.

   - OK
   - Too long
   - Too short
   - Other: _____

2. The length of the survey - including the additional optional questions - was:

   Mark only one oval.

   - OK
   - Too long
   - Too short
   - Other: _____

3. The instructions were easy to understand:

   Mark only one oval.

   - Yes
   - No
   - Other: _____

4. The questions were easy to understand:

   Mark only one oval.

   - Yes
   - No
   - Other: _____

5. The questions were unambiguous:

   Mark only one oval.

   - Yes
   - No
   - Other: _____

6. Was there anything included that you think was unnecessary?

7. Was there anything left out that you think should have been included?

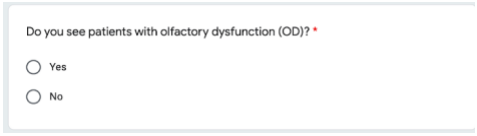
8. Any other general comments?

Feedback on specific questions

If you have any feedback on specific questions or instructions (as shown in screenshots), please enter it below. If you don't have any specific feedback, please skip this section.

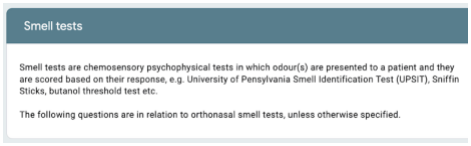
9. Question 1 [NB - if 'No' is selected, this will take the respondent to question 11]

   Please enter any feedback below.



10. Information box on smell tests

    Please enter any feedback below.



11. Question 2

Please enter any feedback below.

Do you perform smell tests during your initial assessment of olfactory dysfunction (OD):

	OD as presenting or isolated symptom	OD in association with another presenting symptom
Always	☐	☐
Sometimes	☐	☐
Never	☐	☐

12. Question 3

Please enter any feedback below.

For a procedure that may affect olfaction (e.g. FESS/ESS) do you perform smell tests peri-operatively:

	Pre-op	Post-op
Always	☐	☐
Sometimes	☐	☐
Never	☐	☐

13. Question 4

Please enter any feedback below.

What kind of smell test(s) do you use? *

Please tick all that apply:

- ☐ Identification test (patient asked to identify an odour, often from a list of options e.g. Smell Identification test/'UPSIT', 'Sniffin Sticks' identification test)
- ☐ Discrimination test (patient asked to discriminate between different odours e.g. 'Sniffin Sticks' discrimination test)
- ☐ Threshold test (lowest concentration of odour a patient is able to smell e.g. 'Smell Threshold Test', 'Sniffin Sticks' threshold test)
- ☐ None
- ☐ Other:

14. Question 5

Please enter any feedback below.

If you DO NOT use smell tests, why? *

Please tick all that apply:

- ☐ NA - I use smell tests
- ☐ Refer on to specialist clinic
- ☐ Insufficient time
- ☐ Insufficient staff
- ☐ Insufficient experience/training
- ☐ Funding limitations
- ☐ Clinical history from patient sufficient
- ☐ Use of symptom questionnaires sufficient
- ☐ Smell tests are not standard of care
- ☐ Will not affect management
- ☐ Olfaction is not a priority
- ☐ Other:

15. Question 6

Please enter any feedback below.

Imaging

Do you perform MRI brain/olfactory tract in your assessment of OD as a presenting or isolated symptom?: *

- ◯ Always
- ◯ Sometimes
- ◯ Never

16. Question 7

Please enter any feedback below.

If you answered 'sometimes' above, please describe when you would perform this investigation:

Your answer

17. Question 8

Please enter any feedback below.

Your aims in performing MRI brain/olfactory tract are to:

Please tick all that apply:

- ☐ Assess olfactory bulbs (gross - without volume measurement)
- ☐ Assess olfactory bulbs (with volume measurement)
- ☐ Exclude neoplasm
- ☐ Exclude non-neoplastic structural abnormality upstream of the olfactory bulbs
- ☐ NA - I don't perform this investigation
- ☐ Other:

18. Question 9

Please enter any feedback below.

Patient reported outcome measures

Do you use patient reported outcome measures / symptom questionnaires in your assessment of OD? *

Where OD is either presenting / isolated symptom, or in association with another presenting symptom.

- ◯ Always
- ◯ Sometimes
- ◯ Never

19. Question 10

Please enter any feedback below.

If you answered 'always' or 'sometimes', which ones do you use?

Your answer

20. Question 11

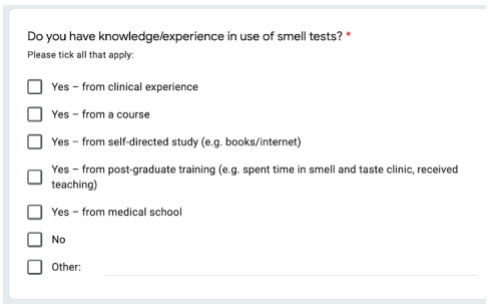
Please enter any feedback below.

How much time would you be willing to spend performing smell test(s) for the initial assessment of:

	OD as presenting or isolated symptom	OD in association with another presenting symptom	Peri-operative assessment of olfaction
< 5 minutes	☐	☐	☐
5 - 15 minutes	☐	☐	☐
15 - 45 minutes	☐	☐	☐
> 45 minutes	☐	☐	☐

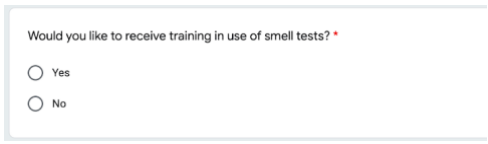
21. Question 12

Please enter any feedback below.



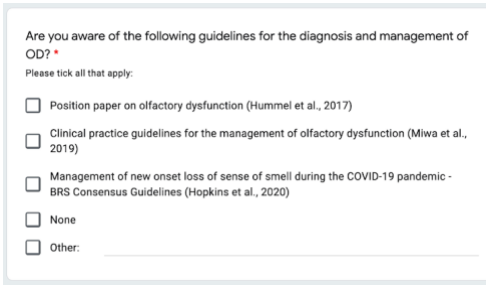
22. Question 13

Please enter any feedback below.



23. Question 14

Please enter any feedback below.

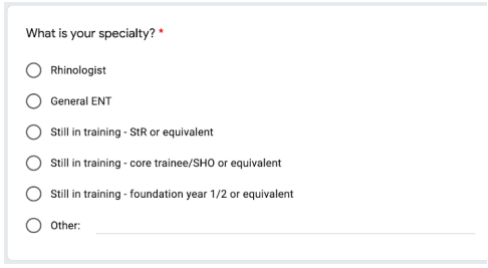


24. Question 15

Please enter any feedback below.

25. Question 16

Please enter any feedback below.



26. Question 17

Please enter any feedback below.

Do you have time to answer a few more detailed questions?

*If you do not see patients with OD, please choose 'No (take me to end of survey)'

- ◯ Yes
- ◯ No (take me to end of survey)

27. Question 18

Please enter any feedback below.

Do you perform ORTHONASAL smell tests for suspected/confirmed:

Please tick all that apply.

	Congenital olfactory dysfunction	Malignancy (sinonasal/anterior skull base)	Medico-legal cases (any cause)	Neurodegenerative conditions	Post-infectious olfactory dysfunction (including post-viral)	F tra olf dys
Diagnosis	☐	☐	☐	☐	☐	
Follow-up	☐	☐	☐	☐	☐	

28. Question 19

Please enter any feedback below.

Which ORTHONASAL smell test(s) do you use?

e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin Sticks, Nez du Vin etc.

Your answer

29. Question 20

Please enter any feedback below.

Do you perform RETRONASAL smell tests for suspected/confirmed:

Please tick all that apply.

	Congenital olfactory dysfunction	Malignancy (sinonasal/anterior skull base)	Medico-legal cases (any cause)	Neurodegenerative conditions	Post-infectious olfactory dysfunction (including post-viral)	F tra olf dysf
Diagnosis	☐	☐	☐	☐	☐	
Follow-up	☐	☐	☐	☐	☐	

30. Question 21

Please enter any feedback below.

Which RETRONASAL smell test(s) do you use?

e.g. Retronasal Olfaction Test, Candy Smell Test etc.

Your answer

31. Question 22

Please enter any feedback below.

Where do you see patients with olfactory dysfunction as a presenting or isolated symptom?

Tick all that apply.

- [ ] Dedicated smell +/- taste clinic
- [ ] General rhinology clinic
- [ ] Allergy or medical rhinology clinic
- [ ] General ENT clinic
- [ ] Private clinic
- [ ] Other:

32. Question 23

Please enter any feedback below.

BEFORE the COVID-19 pandemic, how many patients with olfactory dysfunction did you see in an average month?

Your answer

33. Question 24

Please enter any feedback below.

BEFORE the COVID-19 pandemic, what causes of olfactory dysfunction did you see most commonly?

Please tick one only.

- ◯ Congenital
- ◯ Idiopathic
- ◯ Neurodegenerative
- ◯ Post-infectious (including post-viral)
- ◯ Post-traumatic (e.g. head injury)
- ◯ Sinonasal - inflammatory (e.g. CRS)
- ◯ Sinonasal - malignancy
- ◯ Sinonasal - structural (e.g. deviated nasal septum)
- ◯ Other:

34. Question 25

Please enter any feedback below.

How many patients with olfactory dysfunction do you CURRENTLY see in an average month?

Your answer

35. Question 26

Please enter any feedback below.

What causes of olfactory dysfunction do you see most commonly NOW?

Please tick one only.

- ◯ Congenital
- ◯ COVID-19 related
- ◯ Idiopathic
- ◯ Neurodegenerative
- ◯ Post-infectious (including non-COVID 19 post-viral)
- ◯ Post-traumatic (e.g. head injury)
- ◯ Sinonasal - inflammatory (e.g. CRS)
- ◯ Sinonasal - malignancy
- ◯ Sinonasal - structural (e.g. deviated nasal septum)
- ◯ Other:

36. Question 27

Please enter any feedback below.

How has the COVID-19 pandemic changed your practice in the assessment of patients with olfactory dysfunction?

If the pandemic has not affected your practice, please leave blank.

Your answer

About you

37. On average, how many patients with OD (as a presenting or isolated symptom) do you see per month?

38. Where do you see patients with olfactory dysfunction as a presenting or isolated symptom?

Please tick all that apply.

Tick all that apply.

- ☐ Dedicated smell +/- taste clinic
- ☐ General rhinology clinic
- ☐ Allergy or medical rhinology clinic
- ☐ General ENT clinic
- ☐ Private clinic
- ☐ Other:

Thank you

Thank you for your input. If you have any questions, please contact me on katherine.whitcroft.15@ucl.ac.uk

Thanks again!

Katie

10.2 UK Clinician Questionnaire

UK Clinician Questionnaire

* Indicates required question

1. Do you see patients with olfactory dysfunction (OD)? *

   This includes OD as an isolated or presenting symptom, or in association with another symptom (e.g. chronic rhinosinusitis)

   Mark only one oval.

   - ◯ Yes
   - ◯ No *Skip to section 6 ()*

Smell tests

Smell tests are chemosensory psychophysical tests in which odour(s) are presented to a patient and they are scored based on their response, e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin' Sticks, butanol threshold test etc.

The following questions are in relation to orthonasal* smell tests, unless otherwise specified.

*Orthonasal = odour flows from the nostrils to the olfactory cleft, as in sniffing.

2. Do you perform smell tests during your initial assessment of patients with olfactory dysfunction (OD):

   Please answer in relation to your routine practice, not as part of a clinical trial or other special circumstance.

   Tick all that apply.

	OD as presenting or isolated symptom	OD in association with another presenting symptom
Always	☐	☐
Most of the time	☐	☐
Sometimes	☐	☐
Rarely	☐	☐
Never	☐	☐

3. Do you perform smell tests before / after treating a patient in whom OD is the most bothersome symptom?

   Please answer in relation to your routine practice, not as part of a clinical trial or other special circumstance.

   Tick all that apply.

	Medical intervention: BEFORE	Medical intervention: AFTER	Surgical intervention: BEFORE	Surgical intervention: AFTER
Always	☐	☐	☐	☐
Most of the time	☐	☐	☐	☐
Sometimes	☐	☐	☐	☐
Rarely	☐	☐	☐	☐
Never	☐	☐	☐	☐

4. In patients with normal olfaction, do you perform perioperative smell tests for surgical procedures that could cause OD as a complication?

   Please answer in relation to your routine practice, not as part of a clinical trial or other special circumstance.

   Tick all that apply.

	Pre-op	Post-op
Always	☐	☐
Most of the time	☐	☐
Sometimes	☐	☐
Rarely	☐	☐
Never	☐	☐

5. What kind of smell test(s) do you use? *

   Please tick all that apply:

   Tick all that apply.

   - ☐ Identification test (patient asked to identify an odour, often from a list of options e.g. Smell Identification test/'UPSIT', 'Sniffin Sticks' identification test)
   - ☐ Discrimination test (patient asked to discriminate between different odours e.g. 'Sniffin Sticks' discrimination test)
   - ☐ Threshold test (lowest concentration of odour a patient is able to smell e.g. 'Smell Threshold Test', 'Sniffin Sticks' threshold test)
   - ☐ None
   - ☐ Other: _____

6. If applicable / known, which specific smell test(s) do you use?

   e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin' Sticks, Nez du Vin etc.

   _____

7. What stops you from using smell tests in your routine clinical practice? *

   Please tick all that apply:

   Tick all that apply.

   - ☐ NA - I use smell tests routinely
   - ☐ Refer on to specialist clinic
   - ☐ Insufficient time
   - ☐ Insufficient staff
   - ☐ Insufficient experience/training
   - ☐ Funding limitations
   - ☐ Clinical history from patient sufficient
   - ☐ Use of symptom questionnaires sufficient
   - ☐ Smell tests are not standard of care
   - ☐ Will not affect management
   - ☐ Olfaction is not a priority
   - ☐ Other: _____

Imaging

8. Do you perform MRI brain / olfactory tract in your assessment of OD as a presenting or isolated symptom?: *

   Mark only one oval.

   - ◯ Always
   - ◯ Most of the time
   - ◯ Sometimes
   - ◯ Rarely
   - ◯ Never

9. Please describe when you would perform this investigation:

10. Your aims in performing MRI brain / olfactory tract are to: *

Please tick all that apply.

Tick all that apply.

- [ ] Assess olfactory bulbs (gross - e.g. present / absent)
- [ ] Assess olfactory bulbs (with volume measurement)
- [ ] Exclude neoplasm
- [ ] Exclude non-neoplastic structural abnormality upstream of the olfactory bulbs
- [ ] NA - I don't perform this investigation
- [ ] Other: _____

11. Do you perform any other scans for OD as a presenting or isolated symptom, and if so, which?

Patient reported outcome measures

12. Do you use patient reported outcome measures / symptom questionnaires in your assessment of OD? *

Where OD is either presenting / isolated symptom, or in association with another presenting symptom.

Mark only one oval.

- ( ) Always
- ( ) Most of the time
- ( ) Sometimes
- ( ) Rarely
- ( ) Never

13. Which ones do you use?

Skip to question 14

Smell tests are chemosensory psychophysical tests in which odour(s) are presented to a patient and they are scored based on their response, e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin' Sticks, butanol threshold test etc.

The following questions are in relation to orthonasal* smell tests, unless otherwise specified.

*Orthonasal = odour flows from the nostrils to the olfactory cleft, as in sniffing.

14. What is the maximum duration of smell testing you would consider acceptable for the initial assessment of:

Please answer in relation to your own practice/clinic, without COVID-19-related restrictions.

Tick all that apply.

	OD as presenting or isolated symptom	OD in association with another presenting symptom	Peri-operative assessment of olfaction
< 5 minutes	☐	☐	☐
5 - 15 minutes	☐	☐	☐
15 - 45 minutes	☐	☐	☐
> 45 minutes	☐	☐	☐

15. Do you have knowledge / experience in use of smell tests? *

Please tick all that apply:

Tick all that apply.

- [ ] Yes – from clinical experience
- [ ] Yes – from a course
- [ ] Yes – from self-directed study (e.g. books/internet)
- [ ] Yes – from post-graduate training (e.g. spent time in smell and taste clinic, received teaching)
- [ ] Yes – from medical school
- [ ] No
- [ ] Other: _____

16. Would you like to receive training in use of smell tests? *

Mark only one oval.

- ( ) Yes
- ( ) No
- ( ) Other: _____

17. Are you aware of the following guidelines for the diagnosis and management of OD? *

Please tick all that apply:

Tick all that apply.

- [ ] Position paper on olfactory dysfunction (Hummel et al., 2017, Rhinology)
- [ ] Clinical practice guidelines for the management of olfactory dysfunction (Miwa et al., 2019, Auris Nasus Larynx)
- [ ] Management of new onset loss of sense of smell during the COVID-19 pandemic - BRS Consensus Guidelines (Hopkins et al., 2020, Clinical Otolaryngology)
- [ ] Clinical Olfactory Working Group consensus statement on the treatment of postinfectious olfactory dysfunction (Addison et al., 2021, J Allergy Clin Immunol)
- [ ] Systemic corticosteroids in coronavirus disease 2019 (COVID-19)-related smell dysfunction: an international view (Huart et al., 2021, IFAR)
- [ ] None
- [ ] Other: _____

18. Where do you work? *

Mark only one oval.

- ( ) A district general hospital
- ( ) A tertiary referral hospital
- ( ) Other: _____

19. What best describes you? *

Mark only one oval.

- ( ) Consultant ENT surgeon - general
- ( ) Consultant ENT surgeon - subspeciality rhinology
- ( ) Consultant ENT surgeon - other subspecialty
- ( ) Non-consultant career grade surgeon
- ( ) Still in training - StR or equivalent
- ( ) Still in training - core trainee/SHO or equivalent
- ( ) Still in training - foundation year 1/2 or equivalent
- ( ) Other: _____

20. Do you have time to answer a few more detailed questions?

*If you do not see patients with OD, please choose 'No (take me to end of survey)'

Mark only one oval.

- ( ) Yes
- ( ) No (take me to end of survey) *Skip to section 11 ()*

Please note:

Orthonasal = odour flows from the nostrils to the olfactory cleft, as in sniffing.

Retronasal = odour flows to the olfactory cleft from the oral cavity/oropharynx during eating/swallowing (forms basis of flavour perception).

21. Do you assess / refer for problems related to OD?

Please tick all that apply / leave blank if appropriate.

Tick all that apply.

	Mental health	Nutrition / diet	Memory / neurology	Endocrine	Other
Assess	☐	☐	☐	☐	☐
Refer on as needed	☐	☐	☐	☐	☐

22. How do you diagnose qualitative olfactory dysfunction (parosmia / phantosmia)?

Please tick all that apply.

Tick all that apply.

- ☐ Clinical history
- ☐ Symptom questionnaire
- ☐ Smell test - generic, e.g. UPSIT / Sniffin' Sticks
- ☐ Smell test - specific for qualitative OD, e.g. Sniffin' sticks parosmia test ('SSParoT')
- ☐ Other: _____

23. Do you perform ORTHONASAL smell tests* for suspected/confirmed:

Please tick all that apply / leave blank if appropriate. *e.g. UPSIT, Sniffin' Sticks, Nez du Vin

Tick all that apply.

	Congenital olfactory dysfunction	Malignancy (sinonasal/ anterior skull base)	Medico-legal cases (any cause)	Neurodegenerative conditions	Post-infectious olfactory dysfunction (including post-viral)	tr o dys
Diagnosis	☐	☐	☐	☐	☐	
Follow-up	☐	☐	☐	☐	☐	

24. Do you perform RETRONASAL smell tests?:

Mark only one oval.

- ( ) Yes
- ( ) No

25. If you answered 'yes' to the above question, please describe your practice:

For example, who do you test and what test do you use (e.g. Retronasal Olfaction Test, Candy Smell Test etc.)?

_____

_____

_____

_____

_____

26. Where do you see patients with olfactory dysfunction as a presenting or isolated symptom?

Tick all that apply.

Tick all that apply.

- ☐ Dedicated smell +/- taste clinic
- ☐ General rhinology clinic
- ☐ Allergy or medical rhinology clinic
- ☐ General ENT clinic
- ☐ Private clinic
- ☐ Other: _____

27. BEFORE the COVID-19 pandemic, how many patients with olfactory dysfunction did you see in an average month?

_____

28. BEFORE the COVID-19 pandemic, what were the top 4 causes of olfactory dysfunction you saw most commonly?

Please tick top four.

Tick all that apply.

- ☐ Congenital
- ☐ Idiopathic
- ☐ Neurodegenerative
- ☐ Post-infectious (including post-viral)
- ☐ Post-traumatic (e.g. head injury)
- ☐ Sinonasal - inflammatory (e.g. CRS)
- ☐ Sinonasal - malignancy
- ☐ Sinonasal - structural (e.g. deviated nasal septum)
- ☐ Other: _____

29. How many patients with olfactory dysfunction do you CURRENTLY see in an average month?

_____

30. What top 4 causes of olfactory dysfunction do you see most commonly NOW?

Please tick top four.

Tick all that apply.

- ☐ Congenital
- ☐ COVID-19 related
- ☐ Idiopathic
- ☐ Neurodegenerative
- ☐ Post-infectious (including non-COVID 19 post-viral)
- ☐ Post-traumatic (e.g. head injury)
- ☐ Sinonasal - inflammatory (e.g. CRS)
- ☐ Sinonasal - malignancy
- ☐ Sinonasal - structural (e.g. deviated nasal septum)
- ☐ Other: _____

31. How has the COVID-19 pandemic changed your practice in the assessment of patients with OD?
If the pandemic has not affected your practice, please leave blank.

Anything else?

32. Is there anything else you would like to say about the clinical assessment of olfaction / OD?

10.3 International Clinician Questionnaire

International Clinician Questionnaire

* Indicates required question

1. Do you see patients with olfactory dysfunction (OD)? *

   This includes OD as an isolated or presenting symptom, or in association with another symptom (for example, chronic rhinosinusitis)

   Mark only one oval.

   - ◯ Yes (daily)
   - ◯ Yes (weekly)
   - ◯ Yes (monthly)
   - ◯ Yes (less than once per month)
   - ◯ No *Skip to section 6 ()*

Smell tests

Smell tests are chemosensory psychophysical tests in which odour(s) are presented to a patient and they are scored based on their response, e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin' Sticks, butanol threshold test etc.

The following questions are in relation to orthonasal* smell tests, unless otherwise specified.

*Orthonasal = odour flows from the nostrils to the olfactory cleft, as in sniffing.

2. Do you perform smell tests during your initial assessment of patients with olfactory dysfunction (OD):

   Please answer in relation to your routine practice, not as part of a clinical trial or other special circumstance.

   Tick all that apply.

	OD as presenting or isolated symptom	OD in association with another presenting symptom
Always	☐	☐
Most of the time	☐	☐
Sometimes	☐	☐
Rarely	☐	☐
Never	☐	☐

3. Do you perform smell tests before / after treating a patient in whom OD is the most bothersome symptom?

   Please answer in relation to your routine practice, not as part of a clinical trial or other special circumstance.

   Tick all that apply.

	Medical intervention: BEFORE	Medical intervention: AFTER	Surgical intervention: BEFORE	Surgical intervention: AFTER
Always	☐	☐	☐	☐
Most of the time	☐	☐	☐	☐
Sometimes	☐	☐	☐	☐
Rarely	☐	☐	☐	☐
Never	☐	☐	☐	☐
N/A - I do not perform this type of intervention	☐	☐	☐	☐

4. In patients with normal olfaction, do you perform perioperative smell tests for surgical procedures that could cause OD as a complication?

   Please answer in relation to your routine practice, not as part of a clinical trial or other special circumstance.

   Tick all that apply.

	Pre-op	Post-op
Always	☐	☐
Most of the time	☐	☐
Sometimes	☐	☐
Rarely	☐	☐
Never	☐	☐
N/A - I am not involved in surgical care	☐	☐

5. What kind of smell test(s) do you use? *

   Please tick all that apply:

   Tick all that apply.

   - ☐ Identification test (patient asked to identify an odour, often from a list of options e.g. Smell Identification test/'UPSIT', 'Sniffin Sticks' identification test)
   - ☐ Discrimination test (patient asked to discriminate between different odours e.g. 'Sniffin Sticks' discrimination test)
   - ☐ Threshold test (lowest concentration of odour a patient is able to smell e.g. 'Smell Threshold Test', 'Sniffin Sticks' threshold test, T&T olfactometer))
   - ☐ None
   - ☐ Other: _____

6. If applicable / known, which specific smell test(s) do you use?

e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin' Sticks, Nez du Vin etc.

7. What stops you from using smell tests in your routine clinical practice? *

Please tick all that apply:

Tick all that apply.

- [ ] NA - I use smell tests routinely
- [ ] Refer on to specialist clinic
- [ ] Insufficient time
- [ ] Insufficient staff
- [ ] Insufficient experience/training
- [ ] Funding limitations (from your hospital/institution)
- [ ] Funding limitations (from the patient, including testing not being covered by insurance)
- [ ] Clinical history from patient sufficient
- [ ] Use of symptom questionnaires sufficient
- [ ] Smell tests are not standard of care
- [ ] Will not affect management
- [ ] Olfaction is not a priority
- [ ] Other: _____

Imaging

8. Do you perform MRI brain / olfactory tract in your assessment of OD as a presenting or isolated symptom?: *

Mark only one oval.

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

9. Please describe when you would perform this investigation:

10. Your aims in performing MRI brain / olfactory tract are to: *

Please tick all that apply.

Tick all that apply.

- [ ] Assess olfactory bulbs (gross - e.g. present / absent)
- [ ] Assess olfactory bulbs (with volume measurement)
- [ ] Exclude neoplasm
- [ ] Exclude non-neoplastic structural abnormality upstream of the olfactory bulbs
- [ ] NA - I don't perform this investigation
- [ ] Other: _____

11. Do you perform any other scans for OD as a presenting or isolated symptom, and if so, which?

Patient reported outcome measures

12. Do you use patient reported outcome measures / symptom questionnaires in your assessment of OD? *

Where OD is either presenting / isolated symptom, or in association with another presenting symptom.

Mark only one oval.

- ◯ Always
- ◯ Most of the time
- ◯ Sometimes
- ◯ Rarely
- ◯ Never

13. Which ones do you use?

Skip to question 14

Smell tests are chemosensory psychophysical tests in which odour(s) are presented to a patient and they are scored based on their response, e.g. University of Pennsylvania Smell Identification Test (UPSIT), Sniffin' Sticks, butanol threshold test etc.

The following questions are in relation to orthonasal* smell tests, unless otherwise specified.

*Orthonasal = odour flows from the nostrils to the olfactory cleft, as in sniffing.

14. What is the maximum duration of smell testing you would consider acceptable for the initial assessment of:

Please answer in relation to your own practice/clinic, without COVID-19-related restrictions. *If you DON'T see patients with olfactory dysfunction (OD) - please answer regarding what you believe would be acceptable if you DID. **Note this question refers to smell testing time only, e.g. UPSIT/Sniffin Sticks/T&T olfactometer testing time.

Tick all that apply.

	OD as presenting or isolated symptom	OD in association with another presenting symptom	Peri-operative assessment of olfaction
< 5 minutes	☐	☐	☐
5 - 15 minutes	☐	☐	☐
15 - 45 minutes	☐	☐	☐
> 45 minutes	☐	☐	☐

15. Do you have knowledge / experience in use of smell tests? *

Please tick all that apply:

Tick all that apply.

- [ ] Yes – from clinical experience
- [ ] Yes – from a course
- [ ] Yes – from self-directed study (e.g. books/internet)
- [ ] Yes – from post-graduate training (e.g. spent time in smell and taste clinic, received teaching)
- [ ] Yes – from medical school
- [ ] No
- [ ] Other: _____

16. Would you like to receive training in use of smell tests? *

Mark only one oval.

- Yes
- No
- Other: _____

17. Are you aware of the following guidelines for the diagnosis and management of OD? *

Please tick all that apply:

Tick all that apply.

- Position paper on olfactory dysfunction (Hummel et al., 2017, Rhinology)
- Clinical practice guidelines for the management of olfactory dysfunction (Miwa et al., 2019, Auris Nasus Larynx)
- Management of new onset loss of sense of smell during the COVID-19 pandemic - BRS Consensus Guidelines (Hopkins et al., 2020, Clinical Otolaryngology)
- Clinical Olfactory Working Group consensus statement on the treatment of postinfectious olfactory dysfunction (Addison et al., 2021, J Allergy Clin Immunol)
- Systemic corticosteroids in coronavirus disease 2019 (COVID-19)-related smell dysfunction: an international view (Huart et al., 2021, IFAR)
- None
- Other: _____

18. Where do you work? *

Mark only one oval.

- A district general hospital
- A tertiary referral hospital
- Private ENT clinic or equivalent
- Other: _____

19. In what country do you work? *

_____

20. What is your speciality? *

Mark only one oval.

- ENT
- Neurology
- Allergy/Medical Rhinology
- Other: _____

21. What best describes you? *

Mark only one oval.

- Completed training (e.g. consultant/attending ENT) – including subspecialty training (e.g. fellowship) in rhinology
- Completed training (e.g. consultant/attending ENT) – including subspecialty training (e.g. fellowship) in another ENT subspecialty
- Completed training (e.g. consultant/attending ENT) – no subspecialty training
- Still training – early years (e.g. foundation doctor/SHO/early years residency)
- Still training – later years (e.g. StR/ later years residency/fellow)
- Other: _____

22. Do you have time to answer a few more detailed questions?

*If you do not see patients with OD, please choose 'No (take me to end of survey)'

Mark only one oval.

- Yes
- No (take me to end of survey) *Skip to section 11 ()*

Please note:

Orthonasal = odour flows from the nostrils to the olfactory cleft, as in sniffing.

Retronasal = odour flows to the olfactory cleft from the oral cavity/oropharynx during eating/swallowing (forms basis of flavour perception).

23. Do you assess / refer for problems related to OD?

Please tick all that apply / leave blank if appropriate.

Tick all that apply.

	Mental health	Nutrition / diet	Memory / neurology	Endocrine	Other
Assess	☐	☐	☐	☐	☐
Refer on as needed	☐	☐	☐	☐	☐

24. How do you diagnose qualitative olfactory dysfunction (parosmia / phantosmia)?

Please tick all that apply.

Tick all that apply.

- Clinical history
- Symptom questionnaire
- Smell test - generic, e.g. UPSIT / Sniffin' Sticks
- Smell test - specific for qualitative OD, e.g. Sniffin' sticks parosmia test ('SSParoT')
- Other: _____

25. Do you perform ORTHONASAL smell tests* for suspected/confirmed:

Please tick all that apply / leave blank if appropriate. *e.g. UPSIT, Sniffin' Sticks, Nez du Vin

Tick all that apply.

	Congenital olfactory dysfunction	Malignancy (sinonasal/ anterior skull base)	Medico-legal cases (any cause)	Neurodegenerative conditions	Post-infectious olfactory dysfunction (including post-viral)	tra o dys
Diagnosis	☐	☐	☐	☐	☐	
Follow-up	☐	☐	☐	☐	☐	

26. Do you perform RETRONASAL smell tests?:

Mark only one oval.

- Yes
- No

27. If you answered 'yes' to the above question, please describe your practice:

For example, who do you test and what test do you use (e.g. Retronasal Olfaction Test, Candy Smell Test etc.)?

_____

_____

_____

_____

_____

28. Where do you see patients with olfactory dysfunction as a presenting or isolated symptom?

Tick all that apply.

Tick all that apply.

- [ ] Dedicated smell +/- taste clinic
- [ ] General rhinology clinic
- [ ] Allergy or medical rhinology clinic
- [ ] General ENT clinic
- [ ] Private clinic
- [ ] Other: _____

29. BEFORE the COVID-19 pandemic, how many patients with olfactory dysfunction did you see in an average month?

_____

30. BEFORE the COVID-19 pandemic, what were the top 4 causes of olfactory dysfunction you saw most commonly?

Please tick top four.

Tick all that apply.

- [ ] Congenital
- [ ] Idiopathic
- [ ] Neurodegenerative
- [ ] Post-infectious (including post-viral)
- [ ] Post-traumatic (e.g. head injury)
- [ ] Sinonasal - inKammatory (e.g. CRS)
- [ ] Sinonasal - malignancy
- [ ] Sinonasal - structural (e.g. deviated nasal septum)
- [ ] Other: _____

31. How many patients with olfactory dysfunction do you CURRENTLY see in an average month?

_____

32. What top 4 causes of olfactory dysfunction do you see most commonly NOW?

Please tick top four.

Tick all that apply.

- [ ] Congenital
- [ ] COVID-19 related
- [ ] Idiopathic
- [ ] Neurodegenerative
- [ ] Post-infectious (non-COVID 19 post-viral)
- [ ] Post-traumatic (e.g. head injury)
- [ ] Sinonasal - inKammatory (e.g. CRS)
- [ ] Sinonasal - malignancy
- [ ] Sinonasal - structural (e.g. deviated nasal septum)
- [ ] Other: _____

33. How has the COVID-19 pandemic changed your practice in the assessment of patients with OD?

If the pandemic has not affected your practice, please leave blank.

_____

_____

_____

_____

_____

Anything else?

34. Is there anything else you would like to say about the clinical assessment of olfaction / OD?

_____

_____

_____

_____

_____

10.4 End-User Questionnaire

UCL

Assessing Your Sense of Smell

Welcome!

We think progress can only be made when doctors and scientists **work together with patients and members of the public**. Therefore, we would like to invite you to participate in this research project - which we hope will help to shape future clinical practice and research about the sense of smell.

Before the pandemic, approximately 1/5 of the general population had an impaired sense of smell. Because of COVID-19, more than 110 million people are thought to have experienced some degree of smell loss. Importantly, we know that there is variation in the accuracy and reliability of measures used to assess the sense of smell, and that some patients with impaired smell are not satisfied with their initial healthcare encounters.

We would like to address these issues. This voluntary survey will therefore cover:

- What commonly tested aspects of smell are most important to you
- If you've had your sense of smell assessed, and by whom
- How your sense of smell was assessed
- Whether you were satisfied with this assessment
- Whether there is anything that could have been done differently

It will only take a few minutes to complete and is entirely anonymous - we will NOT ask for any information that would allow you to be identified, or for any contact details. This work is part of the doctoral studies of Dr Katherine Whitcroft - an ENT surgeon with a research interest in the sense of smell.

If you are aged 18+ and are interested in taking part, please click next to view the Participant Information.

UCL Research Ethics Committee Approval ID Number: 20479/001
Title of Study: Assessment of Olfactory Function [Assessing Your Sense of Smell]
Department: Ear Institute
Name and Contact Details of the Researcher(s): Dr K Whitcroft: katherine.whitcroft.15@ucl.ac.uk
Name and Contact Details of the Principal Researcher: Dr P Andrews: peterandrews@nhs.net

1. Invitation
Thank you for considering taking part in this research project. Before you decide whether to take part, it is important that you understand why this research is being done, and what participation will involve for you. Please take time to read the following information carefully and discuss it with others if you wish. If there is anything that is not clear, or you would like more information, please do ask us using the above contact details. Please print this webpage if you would like a copy

2. What is the project's purpose?
Before the pandemic, approximately 20% of the general adult population had an impaired sense of smell. As impaired smell is now known to commonly occur due to COVID-19, it is likely that many more people will have experienced this condition, some of whom will experience long-term impairment. Impaired smell can cause issues surrounding food enjoyment, social communication (for example, recognising the smell of loved ones) and perception of environmental hazards such as smoke/gas. Because of this, some people with impaired smell can become depressed. It is therefore important that the sense of smell is accurately assessed, so that impairment can be properly identified, and appropriate support given. It is also important that we assess smell in a way that is meaningful to people – especially as previous research has shown that some patients are dissatisfied with their initial healthcare encounters. Therefore, the aim of this research is to find out what aspects of smell are most important to people, how people are currently having their sense of smell assessed in medical settings, and what their preferences for assessment are. This work is part of the doctoral studies of Dr Katherine Whitcroft - an ENT surgeon with a research interest in the sense of smell.

3. Why have I been chosen?
Anyone with a normal, or an impaired sense of smell, is invited to participate. There is no upper age limit, but we are only able to collect answers from people aged 18 or over. You will be asked to confirm that you are 18 or over at the start of the survey.

4. What will happen to me if I take part?
You will be asked to complete an anonymous, online survey. You will be asked to confirm that you are 18 or over at the start of the survey, that you have read this information sheet and that you consent to participate. The survey should take no longer than 5 minutes to complete. We will not ask for your email address or any other information which might allow us to contact you after you have submitted your answers.

5. Do I have to take part?
No, this research is voluntary and whether or not you take part is your decision. If you do decide to participate, you may withdraw at any time by simply closing your browser window.

However, once you have submitted your responses, because they are anonymous, we will not be able to identify them meaning that we will be unable to delete them for you at a later date. It is therefore important that if you decide you no longer want to take part, that you do not submit your answers (by pressing 'done' or 'submit' on the last page of the survey).

Participant Information (page 2 of 3)

6. What are the possible disadvantages and risks of taking part?
We hope that you will find participating in this research a positive experience. However, it is possible that thinking about losing your sense of smell, or negative healthcare experiences you may have had, may be distressing. If this is the case, you are free to stop taking the survey at any point (though we would ask you not to submit your answers at the end of the survey). If you do become distressed about a negative healthcare experience in the UK, you may find it helpful to speak to the Patient Advice and Liaison Service (https://www.nhs.uk/nhs-services/hospitals/what-is-pals-patient-advice-and-liaison-service/). If you live outside of the UK, please see your local government website for advice on how to raise concerns. If you would like more information or support about smell impairment, there are charities and support groups that may be of help, for example, 'AbScent' (https://abscent.org/).

7. What are the possible benefits of taking part?
By participating in this research, we hope that it will enable you to have your voice heard and to help improve future medical care/research. There will not, however, be any other direct benefits for you if you take part.

8. What if something goes wrong?
We hope that nothing will go wrong. If it does, and you would like to discuss this or make a complaint, please contact the principal researcher: peterandrews@nhs.net. If you would like to escalate higher than the principal researcher, you may contact the Chair of the UCL Research Ethics Committee – ethics@ucl.ac.uk.

9. Will my taking part in this project be kept confidential?
All of the information we be collecting in this research is anonymous. We would ask that you do not enter any information into free text boxes that could be used to identify you or your healthcare service/provider. We will not be collecting email or IP addresses. All anonymous data collected will be kept strictly confidential. You will not be able to be identified in any reports or publications that are produced as a result of this research.

10. Limits to confidentiality
Confidentiality will be respected subject to legal constraints and professional guidelines.

11. What will happen to the results of the research project?
We aim to publish and present the results of this research at medical conferences, in medical journals and to patient groups. This data will additionally be used by K Whitcroft as part of her doctoral studies, and deleted upon it's completion. If you would like access to the final results, please email katherine.whitcroft.15@ucl.ac.uk.

12. Local Data Protection Privacy Notice
Notice:
The controller for this project will be University College London (UCL). The UCL Data Protection Officer provides oversight of UCL activities involving the processing of personal data, and can be contacted at data-protection@ucl.ac.uk

This 'local' privacy notice sets out the information that applies to this particular study. Further information on how UCL uses participant information can be found in our 'general' privacy notice:
For participants in health and care research studies, click here: https://www.ucl.ac.uk/legal-services/privacy/ucl-general-privacy-notice-participants-and-researchers-health-and-care-research-studies
The information that is required to be provided to participants under data protection legislation (GDPR and DPA 2018) is provided across both the 'local' and 'general' privacy notices.

The categories of personal data used will be as follows: None
The lawful basis that would be used to process your personal data will be: N/A – no personal data will be collected.
If you are concerned about how your anonymised data is being processed, or if you would like to contact us about your rights, please contact the UCL data protection officer, Alex Potts, in the first instance at data-protection@ucl.ac.uk.

Participant Information (page 3 of 3)

13. Contact for further information
If you would like any further information, please contact katherine.whitcroft.15@ucl.ac.uk or peterandrews@nhs.net

Thank you for reading this information sheet and for considering taking part in this research study. If you would like to keep a copy of this information, please save or print the relevant webpage.

If you are happy to proceed, please click 'next'.

1. I am 18 years or older *

- ◯ Yes
- ◯ No

2. I have read and understood the participant information and consent to participate in this study *

- ◯ Yes
- ◯ No

3. Aspects of smell that are important to you:

Please **rank** the following 5 aspects of smell **in order of their importance to you**, where the option at the top is most important to you, and the option at the bottom is least important.

Rank the options by dragging and dropping them into the desired order, or clicking the up/down arrows at the right hand side of the option boxes (if using a mobile/cell phone - please scroll to see the options at the far right or left of the screen). *

Being able to **correctly IDENTIFY smells:** *For example, being able to tell that an orange is an orange, or recognise the smell of a family member. If you lose or partially lose this ability, whilst you may still be able to smell 'something' when sniffing a previously familiar odour, you may no longer be able to identify it.*

Being able to **TELL DIFFERENT smells APART**, without necessarily being able to identify or recognise them: *For example, being able to tell that an orange and a banana smell different to each other, regardless of whether you know it's an orange and a banana. If you lose or partially lose this ability, smells may be difficult to tell apart - potentially all smelling the same or not smelling like much at all.*

Being able to **DETECT smells**, without necessarily being able to identify or recognise them: *For example, being able to smell something that other people might be able to smell in the environment (like the smell of coffee or a bakery), regardless of whether you recognise or can identify it. When you lose or partially lose this ability, you may not be able to smell the odour at all, or you may need to be much closer to the source than other people, in order to smell it.*

Being able to correctly **IDENTIFY flavours**: *For example, being able to tell that you are drinking orange juice (without looking at it). If you lose or partially lose this ability, you may only be able to taste your food as sweet, salty, bitter, sour or savoury. [Please note that the experience of 'flavour' is dependent on the sense of smell.]*

Being able to **judge whether smells are PLEASANT or UNPLEASANT:** *For example, experiencing the smell of something you normally enjoy the smell of, as pleasant. If you lose this ability, you may find that things which previously smelt unpleasant to you (e.g. a dirty nappy/diaper), no longer smell bad, or that things you used to enjoy the smell of (e.g. coffee or fruit), have now become unpleasant.*

About your sense of smell:

4. Do you have a problem with your sense of smell? *

- ◯ Yes
- ◯ No
- ◯ Maybe

5. What is the problem with your sense of smell?

Please tick all of the options that apply to you. *

- ☐ My sense of smell is reduced
- ☐ My sense of smell is gone
- ☐ Smells are distorted (parosmia)
- ☐ I can smell odours that aren't there (phantosmia)
- ☐ I have problems tasting flavours *[without flavour, which relies on the sense of smell, food would only taste sweet, salty, sour, bitter or savoury]*
- ☐ Other

6. Is this problem related to COVID-19? *

- ◯ Yes
- ◯ No
- ◯ Maybe

7. Have you seen a healthcare professional about your sense of smell? *

- ◯ Yes
- ◯ No

Your assessment:

8. What kind of healthcare professional(s) have you seen about your sense of smell?

Please tick all of the options that apply to you. *

- ☐ General practitioner (GP) / family doctor
- ☐ ENT (Ear Nose and Throat surgeon/doctor - also known as an Otolaryngologist or Rhinologist)
- ☐ Neurologist
- ☐ Specialist Nurse
- ☐ Physician Assistant/Associate
- ☐ None
- ☐ Other

9. Did you have any problems accessing specialist care (e.g. ENT/neurologist)? *

- [ ] N/A - I have not tried to access specialist care
- [ ] No - I did not have any problems accessing specialist care
- [ ] Yes - my GP/family doctor did not want to refer me
- [ ] Yes - my GP/family doctor was unable to refer me
- [ ] Yes - problems due to insurance coverage
- [ ] Other

10. During your assessment with a healthcare professional, did they use a 'smell test'?

If you have been seen multiple healthcare professionals, please answer in relation to your most detailed assessment.

A 'smell test' involves smelling an odour or group of odours and then answering a question about it/them. These tests can be self administered (e.g. a scratch and sniff test) or administered by healthcare professionals. You may be given a score at the end of the test, which is used for diagnosis of normal or impaired smell. *

- ◯ Yes
- ◯ No
- ◯ Not sure

11. If you took a 'smell test', what were you asked to do?

Please tick all of the options that apply to you. If you didn't take a smell test, please leave blank.

- [ ] Identify an odour (possibly from a list of options)
- [ ] Pick the 'odd one out', or which odour was different from the others
- [ ] Detect the weakest concentration of an odour you can smell (you may have been asked either when you could smell an odour, or which of several options had a smell)
- [ ] Identify a flavour
- [ ] Decide how much you liked or disliked an odour
- [ ] Other

12. If you took a 'smell test', roughly how long did it take?

If you didn't take a smell test, please leave blank.

13. Did you complete any questionnaires about your symptoms? *

- ◯ Yes
- ◯ No

14. Were you referred for a scan? *

- [ ] Yes - MRI scan *[This scan uses strong magnets. The scanner is tunnel shaped.]*
- [ ] Yes - CT *[Similar to an x-ray, this scan uses radiation. The scanner is doughnut shaped.]*
- [ ] Yes - not sure what kind of scan it was
- [ ] No
- [ ] Other

15. How satisfied were you with your assessment?

1 = not at all satisfied
5 = completely satisfied *

1	2	3	4	5

16. During your assessment, what was done well?

Please don't enter any details that could allow identification of you or your healthcare provider. *

17. During your assessment, what could have been done better?

Please don't enter any details that could allow identification of you or your healthcare provider. *

18. Did you have to pay for your assessment?

i.e. it was not provided by the NHS or covered by your insurance *

- ◯ Yes
- ◯ No
- ◯ Other

Preferences for assessment:

If you DO have a problem with your sense of smell - please answer the following questions for how you would ideally like to be/have been assessed, not how you have been assessed already.

If you DON'T have a problem with your sense of smell - please answer the following questions for how you think you would ideally like to be assessed if you developed this problem in the future.

19. For a problem with my sense of smell, I would prefer to be assessed by: *

- ◯ My own GP / family doctor or other non-specialist
- ◯ A specialist (e.g. ENT/neurologist)
- ◯ Other

20. How much time are you willing to spend taking a 'smell test' as part of an assessment of your sense of smell?

A 'smell test' involves smelling an odour or group of odours and then answering a question about it/them. These tests can be self administered (e.g. a scratch and sniff test) or administered by healthcare professionals. You may be given a score at the end of the test, which is used for diagnosis of normal or impaired smell. *

- ◯ Less than 5 minutes
- ◯ 5 to 15 minutes
- ◯ 15 to 45 minutes
- ◯ More than 45 minutes
- ◯ Other

21. Are you willing to travel outside of your local area for specialist smell assessment? *

- ◯ Yes
- ◯ No
- ◯ Maybe

About you

22. What is your current country of residence? *

23. How old are you? *

24. Do you identify as: *

- ◯ Female
- ◯ Male
- ◯ Prefer not to say
- ◯ Other

Thank you!

You have reached the end of the survey.

10.5 Clinical Scores / PROMs

Lund-Kennedy Score:

	Right	Left
Oedema ***0*** *- absent* ***1*** *- mild* ***2*** *- severe*		
Polyps ***0*** *- absent* ***1*** *- middle meatus only* ***2*** *- beyond middle meatus*		
Discharge ***0*** *- absent* ***1*** *- serous* ***2*** *- purulent*		
Crusting (post op) ***0*** *- absent* ***1*** *- mild* ***2*** *- severe*		
Scarring (post op) ***0*** *- absent* ***1*** *- mild* ***2*** *- severe*		
TOTALS:		

SNOT-20 GAV:

Um beurteilen zu können, wie stark die einzelnen Symptome ausgeprägt sind, kreuzen Sie bitte bei jeder einzelnen Frage die entsprechende Ziffer an.		Kein Problem	Sehr geringes Problem	Kleines Problem	Mittelgradiges Problem	Hochgradiges Problem	Schlechter kann es nicht mehr werden
Einzelfragen							
1	Nasenatmungsbehinderung	0	1	2	3	4	5
2	Niesreiz	0	1	2	3	4	5
3	Ständiges Naselaufen	0	1	2	3	4	5
4	Sekretfluss in den Rachen	0	1	2	3	4	5
5	Dickes, schleimiges Nasensekret	0	1	2	3	4	5
6	Räusperzwang, trockener Hals	0	1	2	3	4	5
7	Husten	0	1	2	3	4	5
8	Druckgefühl auf den Ohren	0	1	2	3	4	5
9	Ohrenschmerz	0	1	2	3	4	5
10	Riechminderung	0	1	2	3	4	5
11	Schwindelgefühl	0	1	2	3	4	5
12	Gesichtsschmerz, Druckgefühl im Gesicht	0	1	2	3	4	5
13	Probleme beim Einschlafen	0	1	2	3	4	5
14	Nächtliches Aufwachen	0	1	2	3	4	5
15	Tagesmüdigkeit	0	1	2	3	4	5
16	Verminderte Leistungsfähigkeit	0	1	2	3	4	5
17	Konzentrationsschwäche	0	1	2	3	4	5
18	Frustrationen/ Rastlosigkeit/ Reizbarkeit	0	1	2	3	4	5
19	Traurigkeit	0	1	2	3	4	5
20	Nebenhöhlenbeschwerden sind mir peinlich	0	1	2	3	4	5

SNOT-23:

Modified sino-nasal outcome test

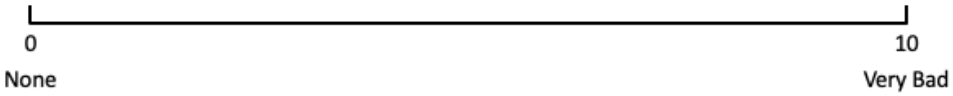
Below you will find a list of symptoms and social/emotional consequences of your nasal disorder.
We would like to know more about these problems and would appreciate you answering the following questions to the best of your ability.
There are no right or wrong answers, and only you can provide us with this information.
Please rate your problems, as they have been over the last two weeks.
Considering how severe the problem is when you experience it and how frequently it happens.
Please rate each item below on how 'bad' it is by circling the number that corresponds with how you feel.

	No problem	**Very mild problem**	**Mild or slight problem**	**Moderate problem**	**Severe problem**	**Problem as bad as it can be**
1. Need to blow nose	0	1	2	3	4	5
2. Sneezing	0	1	2	3	4	5
3. Runny nose	0	1	2	3	4	5
4. Cough	0	1	2	3	4	5
5. Post nasal discharge (dripping at the back of your nose)	0	1	2	3	4	5
6. Thick nasal discharge	0	1	2	3	4	5
7. Ear fullness	0	1	2	3	4	5
8. Dizziness	0	1	2	3	4	5
9. Ear pain/pressure	0	1	2	3	4	5
10. Facial pain/pressure	0	1	2	3	4	5
11. Difficulty falling asleep	0	1	2	3	4	5
12. Waking up at night	0	1	2	3	4	5
13. Lack of a good nights sleep	0	1	2	3	4	5
14. Waking up tired	0	1	2	3	4	5
15. Fatigue during the day	0	1	2	3	4	5
16. Reduced productivity	0	1	2	3	4	5
17. Reduced concentration	0	1	2	3	4	5
18. Frustrated /restless /irritable	0	1	2	3	4	5
19. Sad	0	1	2	3	4	5
20. Embarrassed	0	1	2	3	4	5
21. Sense of taste and smell	0	1	2	3	4	5
22. Blockage / congestion of nose	0	1	2	3	4	5
23. Concern with shape of nose	0	1	2	3	4	5
TOTALS						
					Grand TOTAL:	

VAS:

Please mark on the line from 0 – 10 with an X to show how severe your nasal symptoms have been recently.

Problems with Sense of Smell:



NOSE:

Nasal Obstruction Symptom Evaluation Over the past one month, how much of a problem were the following conditions for you?					
	Not a problem	Very mild problem	Moderate problem	Fairly bad problem	Severe problem
Nasal congestion or stuffiness	0	1	2	3	4
Nasal blockage or obstruction	0	1	2	3	4
Trouble breathing through my nose	0	1	2	3	4
Trouble sleeping	0	1	2	3	4
Unable to get enough air through my nose during exercise	0	1	2	3	4
				TOTAL (x 5):	

10.6 Neuroimaging Pilot Results

UK Pilot Data:

			Banana		Cis-3-hex	
Age	TDI	Gender 1=F	Intensity	Valence	Intensity	Valence
64	29	1	5	0	6	-1
62	31.5		3	2	4	2
31	35.5		7.5	0	7	-1
58	38.5		5	4	6	-1
28	39.25	1	6	0	7	-1
29	39.5	1	6	1	6	-1
31	34.5	1	4.5	1.5	6	-1
32	39.5	1	6	2	4	-1
26	32.5		3	2	4	1

Mean age = 39 (± 15) years

Mean TDI = 35.4 (± 3.7)

Mean intensity banana = 5.3 (± 1.5), median 5.5

Mean intensity cis-3-hex = 5.6 (±1.7), median 6

No significant difference in intensity between odours (W=16, P=0.44)

Mean valence banana = 1.5 (± 1.3), median 1.8

Mean valence cis-3-hex = -0.5 (±1.1), median -1

Significant difference in valence between odours (W=-45, P=0.004)

Germany Pilot Data:

			Banana		Cis-3-hex	
Age	TDI	Gender 1=F	Intensity	Valence	Intensity	Valence
32	37	1	7	1	7	-4
26	38.25		10	-4	9	-5
25	38.25	1	7	2	8	0
29	34	1	9	2	7	1
25	27	1	8	2	9	3
32	34.5	1	8	1	8	-1
27	37		7	2	5	0
25	37.5		7	2	7	0

Mean age = 27 (± 3) years

Mean TDI = 35.4 (± 3.8)

Mean intensity banana = 7.8 (± 1.1), median 7.5

Mean intensity cis-3-hex = 7.5 (± 1.3), median 7.5

No significant difference in intensity between odours (*W*=-15, *P*=0.06)

Mean valence banana = 1.0 (± 2.1), median 2.0

Mean valence cis-3-hex = -0.75 (± 2.6), median 0.0

Significant difference in valence between odours (*W*=-32, *P*=0.03)

10.7 Clinician Survey Supplementary Results

Subgroup analysis according to hospital type was performed. During the initial assessment of OD as a presenting or isolated symptom, a statistically significant higher proportion of respondents working in tertiary referral hospitals (TRH) 'always' used smell tests than in district general hospitals (DGH) (17.3% of 52 respondents in TRHs, 3.7% of 82 respondents in DGHs; $\chi^2_{(1)}$=7.3, *P*=0.007). The difference in proportions between hospital types was not significant for any other frequency of testing ('most of the time', $\chi^2_{(1)}$=0.13, *P*=0.72; 'sometimes', $\chi^2_{(1)}$=0.28, *P*=0.6; 'rarely', $\chi^2_{(1)}$=0.19, *P*=0.66; 'never', $\chi^2_{(1)}$=3.1, *P*=0.08). With regards to the assessment of OD in association with another presenting symptom, a statistically significant higher proportion of respondents working in TRHs 'sometimes' used smell tests than in DGHs (23.5% of 51 respondents in TRHs, 8.6% of 81 respondents in DGH; $\chi^2_{(1)}$=5.7, *P*=0.02). Again, the difference in proportions between hospital types was not significant for any other frequency of testing ('always', $\chi^2_{(1)}$=0.23, *P*=0.64; 'most of the time', $\chi^2_{(1)}$=0.30, *P*=0.6; 'rarely', $\chi^2_{(1)}$=0.13, *P*=0.72), though the difference in proportion of respondents 'never' performing smell testing almost reached significance, and was higher in DGHs (43.1% in TRH, 60.5% in DGH; $\chi^2_{(1)}$=3.4, *P*=0.052).

Similar results were obtained during subgroup analysis according to subspecialist training in rhinology ('rhinologists' vs 'non-rhinologists'). A statistically significant higher proportion of rhinologists 'always' performed smell testing during the initial assessment of OD as a presenting or isolated symptom than non-rhinologists (27.3% of 33 rhinologists, 2.3% of 128 non-rhinologists; $\chi^2_{(1)}$=23.6, *P*<0.0001). Furthermore, a statistically significant higher proportion of non-rhinologists 'never' performed smell testing compared with rhinologists (33.3% of rhinologists, 58.6% of non-rhinologists; $\chi^2_{(1)}$=6.7, *P*=0.01). There was no statistically significant difference in proportions of rhinologists vs non-rhinologists for the remaining frequencies of smell testing ('most of the time, $\chi^2_{(1)}$=0.33, *P*=0.56; 'sometimes, $\chi^2_{(1)}$=0.002, *P*=0.97; 'rarely', $\chi^2_{(1)}$=0.15, *P*=0.7). When assessing patients with OD in association with another presenting symptom, a significantly higher proportion of non-rhinologists

'never' performed smell testing compared with rhinologists (35.5% of 31 rhinologists, 59.7% of 124 non-rhinologists; $\chi^2_{(1)}$=5.7, *P*=0.02). There was no significant difference between rhinologists and non-rhinologists for the remaining frequencies of testing ('always', $\chi^2_{(1)}$=2.3, *P*=0.13; 'most of the time, $\chi^2_{(1)}$=0.06, *P*=0.8; 'sometimes, $\chi^2_{(1)}$=3.7, *P*=0.06; 'rarely', $\chi^2_{(1)}$=0.3, *P*=0.6). See Figure below.

Given the similar pattern of results obtained for the above subgroup analyses, direct comparison of rhinologists vs TRH and non-rhinologists vs DGHs was undertaken. For OD as a presenting or isolated symptom, there was no difference in proportions of respondents across all frequencies in rhinologists vs TRH respondents ($\chi^2_{(4)}$=1.6, *P*=0.81) or in non-rhinologists vs DGH respondents ($\chi^2_{(4)}$=0.6, *P*=0.97). Similarly, for OD in association with another presenting symptom, there was no significant difference in rhinologists vs TRH respondents ($\chi^2_{(4)}$=1.1, *P*=0.9) or in non-rhinologists vs DGH respondents ($\chi^2_{(4)}$=0.8, *P*=0.94). It is therefore likely that rhinologists/TRH respondents, and non-rhinologists/DGH respondents either have similar testing practices and/or are overlapping subgroup samples, in line with more specialist care being provided in tertiary referral hospitals. Subgroup analysis will therefore only be made for rhinologists vs. non-rhinologists for the remainder of UK data.

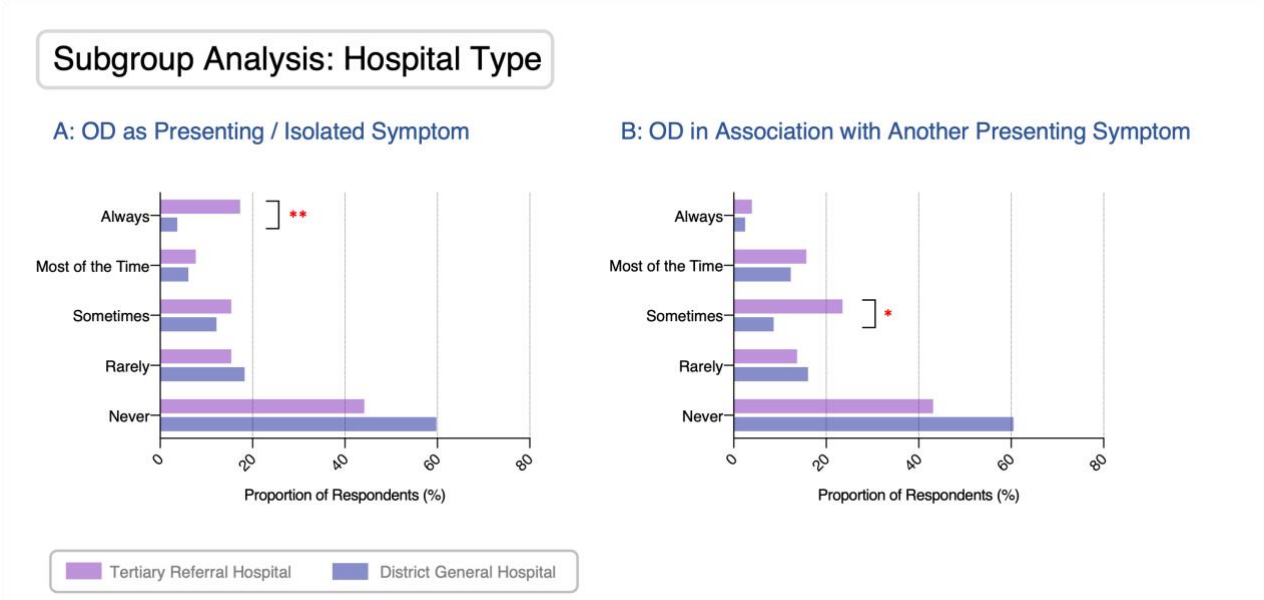


Figure: Smell Testing During Initial Assessment - Subgroup Analysis by Hospital Type

10.8 End-User Survey Supplementary Results

Assessment										
Question	Answer	All (n=569)	All ENT (n=175)	All non-ENT (n=139)	UK (n=222)	UK ENT (n=49)	USA (n=270)	USA ENT (n=98)	***All non-UK ENT (including USA) (n=126)***	***All non-UK ENT (excluding USA) (n=28)***
During your assessment with a healthcare professional, did they use a 'smell test'? ‡	***Yes***	49 15.60%	43 24.60%	6 4.30%	11 10.50%	10 20.40%	30 18.10%	26 26.50%	***33*** ***26.20%***	***7*** ***25.0%***
	No	265 84.40%	132 75.40%	134 96.40%	94 89.50%	39 79.60%	136 81.90%	72 73.50%	***93*** ***73.81%***	***21*** ***75%***
	Not sure	0	0	0	0	0	0	0	***0***	***0***
Did you complete any questionnaires about your symptoms? ‡	***Yes***	53 16.90%	46 26.30%	7 5.04%	8 7.60%	8 16.30%	37 22.30%	30 30.60%	***38*** ***30.2%***	***8*** ***28.6%***
	No	261 83.10%	129 73.70%	132 94.96%	97 92.40%	41 83.70%	129 77.70%	68 69.40%	***88*** ***69.8%***	***20*** ***71.4%***
Were you referred for a scan?* ‡	***Yes – MRI***	69 22.00%	61 34.90%	9 6.47%	27 25.70%	20 40.80%	33 19.90%	28 28.60%	***37*** ***29.4%***	***9*** ***32.1%***
	Yes – CT	53 16.90%	46 26.30%	8 5.76%	18 17.10%	11 22.40%	31 18.70%	27 27.60%	***32*** ***25.4%***	***5*** ***17.9%***
	Yes – not sure type	9 2.90%	8 4.60%	0 0.00%	0 0.00%	0 0.00%	8 4.80%	7 7.10%	***8*** ***6.4%***	***1*** ***3.6%***
	No	192 61.10%	72 41.10%	121 87.05%	63 60.00%	13 26.50%	101 60.80%	43 43.90%	***57*** ***45.2%***	***14*** ***50.0%***
	Other	5 1.60%	2 1.10%	2 1.44%	0 0.00%	0 0.00%	1 0.60%	0 0.00%	***0*** ***0.00%***	***0*** ***0.00%***

Supplementary Table 1: Additional results from end-user survey shown in ***bold italic*** (right most two columns).

10.9 UCL Research Paper Declaration Form

UCL Research Paper Declaration Form

referencing the doctoral candidate's own published work(s)

Please use this form to declare if parts of your thesis are already available in another format, e.g. if data, text, or figures:

- *have been uploaded to a preprint server*
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1. **For a research manuscript that has already been published** (if not yet published, please skip to section 2)

a) **What is the title of the manuscript?**

1. Olfactory Function and Dysfunction. In: Cummings Otolaryngology, Head and Neck Surgery 7th Ed
2. Structural Plasticity of the Primary and Secondary Olfactory cortices: Increased Gray Matter Volume Following Surgical Treatment for Chronic Rhinosinusitis
3. Sinonasal surgery alters brain structure and function: Neuroanatomical correlates of olfactory dysfunction
4. Functional septorhinoplasty alters brain structure and function: neuroanatomical correlates of olfactory dysfunction.
5. Patient Experience and Preferences for the Assessment of Olfaction: The Patient International Clinical Assessment of Smell
6. International clinical assessment of smell: An international, cross-sectional survey of current practice in the assessment of olfaction.

b) **Please include a link to or doi for the work**

1. ISBN: 9780323611794, https://shop.elsevier.com/books/cummings-otolaryngology/flint/978-0-323-61179-4
2. 10.1016/j.neuroscience.2018.10.011
3. 10.1002/jnr.24897
4. 10.3389/falgy.2023.1079945
5. 10.1159/000535794
6. 10.1111/coa.14123

c) **Where was the work published?**

1. Cummings Otolaryngology, Head and Neck Surgery, 7th Ed.
2. Neuroscience
3. Journal of Neuroscience Research
4. Frontiers in Allergy
5. ORL
6. Clinical Otolaryngology

d) **Who published the work?** (e.g. OUP)

1. Elsevier

2. Elsevier
3. Wiley
4. Frontiers
5. Karger
6. Wiley

e) **When was the work published?**

1. 2020
2. 2018
3. 2021
4. 2023
5. 2024
6. 2024

f) **List the manuscript's authors in the order they appear on the publication**

1. Whitcroft KL, Hummel T
2. Whitcroft KL, Fischer J, Han P, Raue C, Bensafi M, Gudziol V, Andrews P, Hummel T
3. Whitcroft KL, Noltus J, Andrews P, Hummel T.
4. Whitcroft KL, Mancini L, Yousry T, Hummel T, Andrews P
5. Whitcroft KL, Kelly C, Andrews P.
6. Whitcroft KL, Alobid I, Altundag A, Andrews P, Carrie S, Fahmy M, Fjaeldstad AW, Gane S, Hopkins C, Hsieh JW, Huart C, Hummel T, Konstantinidis I, Landis BN, Mori E, Mullol J, Philpott C, Poulios A, Vodička J, Ward VM.

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3. For multi-authored work, please give a statement of contribution covering all authors (if single-author, please skip to section 4)

1. For this book chapter, I performed the literature review, synthesised relevant information and wrote the manuscript. TH critically appraised the resultant manuscript for intellectual content and approved its final version. For the purposes of this thesis, sections have been expanded, reduced or moved, where relevant.

2. PA, TH and I planned the study. JF and I gathered all data – JF provided English/German translation where necessary. CH provided administrative scanning support including MRI physics input. JF performed manual segmentation of the OB. I analysed all remaining data and interpreted the results. PH and TH provided analysis advice, and TH analysis overview. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

3. PA, TH, JN and I planned the study. JN and I gathered all data – JN provided English – German translation where necessary. I analysed all data and interpreted the results. TH provided analysis advice. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

4. PA, TH, LM, TY and I planned the study. LM provided MRI physics input (imaging acquisition). LM and I gathered all data. I analysed all data and interpreted the results. LM provided analysis advice. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

5. CK and I co-produced the survey. I analysed the data and interpreted the results. I wrote the manuscript. CK and PA critically appraised the resultant manuscript for intellectual content and approved its final version.

6. With input from PA, SC, SG, CH, CP, AP and VW, I wrote the survey. IA, AA, AWF, JWH, CH, TH, IK, BNL, EM, JM and JV reviewed the survey for international suitability and distributed in their respective countries of origin. MF assisted with administration. I analysed the data and interpreted the results. I wrote the manuscript. All authors critically appraised the resultant manuscript for intellectual content and approved its final version.

4. In which chapter(s) of your thesis can this material be found?

1. Chapter 1

2. Chapter 6
3. Chapter 7
4. Chapter 8
5. Chapter 5
6. Chapter 4

5. e-Signatures confirming that the information above is accurate (this form should be co-signed by the supervisor/ senior author unless this is not appropriate, e.g. if the paper was a single-author work)

Candidate

K Whitcroft

Date:

30.5.25

Supervisor/ Senior Author (where appropriate)

1-3 Thomas Hummel

4-6 Peter Andrews

Date

30.5.25