Testing olfactory function and mapping the structural olfactory networks in the brain

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This review has been accepted as a thesis together with four previously published papers by Aarhus University the 31st of October 2016 and defended on the 10th of February 2017.

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Dan Med J 2018;65(1):B5432

THE 4 ORIGINAL PAPERS ARE

1. Fjaeldstad A, Kjærgaard T, Van Hartevelt TJ, Møller A, Kringelbach ML, Ovesen T. Olfactory screening: validation of Sniffin' Sticks in Denmark. Clin Otolaryngol. 2015;40, 545-550.
2. Fjaeldstad A, Petersen MA, Ovesen T. Considering chemical resemblance: A possible confounder in olfactory identification tests. Chemosens Percep. 2017;10, 42-48.
3. Fjaeldstad A, Sundbøll J, Niklassen A, Ovesen T. Odour familiarity and identification abilities in adolescents". Chem. Sens. 2017;42 (3): 239-246.
4. Fjaeldstad A, Fernandes HM, van Hartevelt TJ, Gleesborg C, Møller A, Ovesen T, Kringelbach ML. Brain fingerprints of olfaction: a novel structural method for assessing olfactory cortical networks in health and disease. Scientific Reports. 2017;7, 42534

1. INTRODUCTION

1.1 THE EVOLUTION OF THE OLFACTORY SYSTEM

In many ways, the sense of smell can be ascribed as being the primary sense; it is sensed by the first cranial nerve, phylogenetically considered our oldest sense [1], and the first of the sensory systems to embryologically develop in mammals [2]. The chemosensory perception of the world and the subsequent effects on behaviour has an evolutionary trail back to bacteria [3]. However, this ancestry has more relevance for the function of the brain than just evolutionary curiosity; the oldest sense has an exclusive fingerprint of neural pathways, which contributes to the unique role of olfaction in the human brain. While all other senses are connected to the telencephalon via the thalamus, the olfactory input enters its primary cortex without thalamic relay [2,4]. The primary olfactory cortex (OC) is clenched between key cortical areas of limbic and memory processing, to which it is strongly connected via neural pathways (Figure 1.2 and figure 4.3).

In the novel 'Das Parfum', Patrick Süskind depicts an olfactory child prodigy, Jean-Baptiste Grenouille, for whom the sense of smell offers unique perceptions of the world around him [5]. Had his abilities to identify stone, wood, and water come from visual input, he would be regarded as an ordinary boy, but as he is able to distinguish and identify the world around him from the olfactory cues alone, the readers are drawn into a parallel sensory universe. To shift the perceptual weight from vision to smell is counterintuitive, perhaps even animal-like for some. Though supermarkets and expiration dates on milk cartons may have dulled our appreciation of olfaction as a vital tool for procuring food and avoiding decayed foods, the sense of smell still plays a – perhaps more subliminal - role to safeguard our survival. By utilising the strong connections to memory and limbic pathways, babies are from birth using their sense of smell to recognise their mother and are comforted by the flavour of her breast milk [6]. When choosing partners, body odour has an impact on attractiveness and the desire to procreate, which is driven by an olfactory registration of optimal genetic compatibility [7,8]. Furthermore, olfaction is a potent trigger of pleasure [9,10], emotions, and memory [11], and in this way guiding through many aspects of life. Yet, little attention is given to the impact of olfaction.

1.2 FROM CHEMISTRY THROUGH SENSATION TO ACTIVATION OF THE PRIMARY OLFACTORY CORTEX

While other senses have a clearly defined spectrum of sound frequencies or wavelengths of light, the chemical senses – and especially olfaction – have proved difficult to quantify. Since ancient times, there have been attempts to classify odours. One of the first descriptions of odour classification dates back to Theophrastus (371-287 BC), a student of Aristotle, who wrote: "Odours in general, like tastes, are due to mixture; for anything which is uncompounded has no smell, just as it has no taste: therefore simple substances such as water, air and fire; on the other hand earth is the one elementary substance which has a smell, or at least to a greater extent than the others, because it is of a more composite character…" [12]. From this short paragraph, we can appreciate the complexity and challenges of understanding how odours are perceived; in ancient Greece, water, fire, air and earth were perceived as the smallest components, the building blocks for everything, the basic elements. With a modern understanding of chemistry, a major step has been taken towards more accurately describing the constituents of smell.

From describing the volatile chemical constituents of an odour, the next step in the process of understanding olfaction is the sensation and peripheral perception of odours. In this step, volatile odorants enter the nasal cavity, travel to the olfactory cleft, where they bind to olfactory receptors (OR) on the olfactory epithelium. In the aqueous mucus of the epithelium, odorant binding proteins are believed to enhance the binding of hydrophobic odorant to the ORs [13].

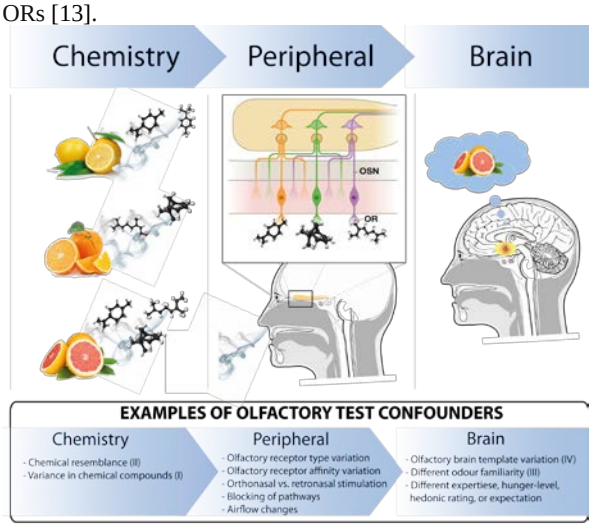

Figure 1.1. Examples of possible confounders in olfactory testing. There are possible confounders in olfactory testing on all levels - from chemistry to peripheral sensation to perception and brain processing -

depending on the design and methods applied. In this dissertation, study I exemplified the variance in chemical compounds, as different cultivars of apples have heterogeneous profiles of volatile odorants. Study II investigated chemical resemblance within the same odour-object category. Study III investigated the role of odour familiarity, while study IV investigated the impact of olfactory brain template variation. OSN: Olfactory sensory neuron. OR: Olfactory receptor.

The ORs were only characterised a few decades ago by the Nobel Prize winning work of Linda Buck and Richard Axel [14]. Although the OR activation mechanism by chemical binding between the odorant and OR is supported among many researchers in the field, there is still an ongoing discussion on this matter, where Luca Turin and colleagues argue for an important role of the vibrational properties of an odorant in the binding to OR [15-17]. Whatever the exact mechanisms of odorant binding may entail, there is an increasing understanding of how the olfactory neurons mature to express only one OR gene [18], how the olfactory placode preserves its stem cells throughout life to replace ORs [2], and how the patterns of OR activation by an odorant can encode the identity of odours [19].

The human receptor repertoire consists of approximately 350-400 active OR types, where most odours activate a certain subset of these ORs in order to create an odour-image [20]. This odour-image is created on the level of the ORs and the olfactory bulb, but is not uniform across individuals; due to genetic polymorphisms alone, the OR alleles differ functionally between individuals with more than 30% [21]. Combined with variations in the expression of active human ORs [22], the sensation and peripheral registration of odour have substantial variation [23]. The expression of certain receptors may also completely change the perception of an odour, which seems to be the case with cilantro (Coriandrum sativum); the expression of the receptor OR6A2 is proposed to be the reason why many people detects a soapy aroma in cilantro due to the overlap of aldehydes in the two odour-images [24,25]. Consequently, if odours have a high chemical resemblance, this can result in overlapping odour-images, which may cause difficulties in discriminating odours even in normosmics (Figure 1.1 and study II).

The route of odour stimulation can also be important, as retronasal and orthonasal stimuli are perceived differently [26]. This duality in the perception of flavour is unique to mammals [27], with the orthonasal function is believed to be optimised for sensing certain qualities of odours from a distance (is there a trace of food or danger in the air?). Once the food has passed the orthonasal odour evaluation, the retronasal odours form an important integrated part of flavour perception and the decision to swallow [28].

Signalling of odorant binding to the OR is conveyed through the cribriform plate by the olfactory sensory neuron (OSN) to the glomeruli, where OSNs with identical ORs converge in glomeruli in the olfactory bulb [29]. Already at the glomerular level, there seems to be differences in the neural architecture; glomeruli that process information from a broadly tuned OR have a higher degree of lateral processing and fewer connections to the OC, compared with glomeruli that process information from ORs that are more finely tuned in their selectivity of compatible odours [30]. These peripheral differences in processing, along with the neurogenesis and plasticity [31], emphasise the roles of ORs and the olfactory bulb as important factors in understanding olfactory processing [32]. However, with the limited spatial resolution of current neuroimaging techniques, the best described finding in humans is that the size of the olfactory bulb often decreases in diseases affecting olfactory function [33]. Although the bulb size can be used as a supportive parameter in the diagnostics of anosmic patients, it is difficult to use this measure to differentiate between etiologies when the mechanisms behind these processes are still unclear [34].

In the glomeruli, the OR neurons synapse with second order neurons, namely mitral cells, periglomerular cells, and tufted cells. While the periglomerular cells have a role in local modulation and output inhibition [35], the axons of the mitral and tufted cells constitute the lateral olfactory tract [36]. The lateral olfactory tract ends at the synapses of the primary olfactory cortex – these synapses are fairly important, as they define what the primary OC is [37]. This direct input from second order neurons to the OC without thalamic relay makes olfaction unique among all senses [4].

The two different types of projection neurons convey different information from the olfactory bulb to the OC: the tufted cells exhibited shorter onset latency across a wide range of odour concentrations, while the mitral cells only responded to stronger odour concentrations; furthermore, the tufted cells were restricted to focal targets in the anterior part of the piriform cortex, while the mitral cells had synapses across the entire primary olfactory cortex [38]. This segregation of afferent information underlines the segregation of olfactory processing in the OC, which is key in defining templates for investigating olfactory processing, as highlighted in study IV of this thesis.

While most data on the molecular level and cellular connections are based on studies in flies, mice, and non-human primates, it is generally believed that these findings are also applicable to humans [39]. However, with recent advances in neuroimaging, an emerging understanding of the complex olfactory processing in humans has been underway for the last few decades. This is described in more detail in chapter 1.5 of this thesis and in study IV [40].

1.3 OLFACTORY IMPORTANCE IN HEALTH

Olfaction has for millennia been vital for survival, as spoiled foods could prove fatal – even after the invention of refrigerators and expiration dates. Orthonasal smell would guide the decision to initiate eating, whereas retronasal olfaction would evaluate the food before ingestion in conjunction with taste, tactile sensation, temperature, and sound [10]. The dependence on olfaction for survival has led to theories that this sense has been one of the most important driving forces for developing the brain [27]. In order to ensure a sensible reaction to an olfactory stimulus, some odour inputs are attributed a positive hedonic valence, such as the scent of the mother for a newborn infant [41,42]. Other odours induce the exact opposite effect, such as an innate stress hormone response for predators [43,44].

With pleasure as a common currency, odours can inflict similar responses in the brain as other fundamental rewards, such as food, sex, and social stimuli [45], as well as more abstract rewards, such as art, money, and music [46]. It is important to emphasise that the perception and processing of food is affected by all senses, though olfaction has been acknowledged as a key contributor (Figure 1.2). Odours are also essential in social communication [47,48], in dietary behaviour [49], and can even influence the choice of partner [7]. Though less obvious than other senses such as vision, hearing, or touch, we are strongly affected by the olfactory cues we perceive. Consequently, the loss of the olfactory sense can lead to a substantial reduction in the quality of life [50], and even increase the risk of depression [51].

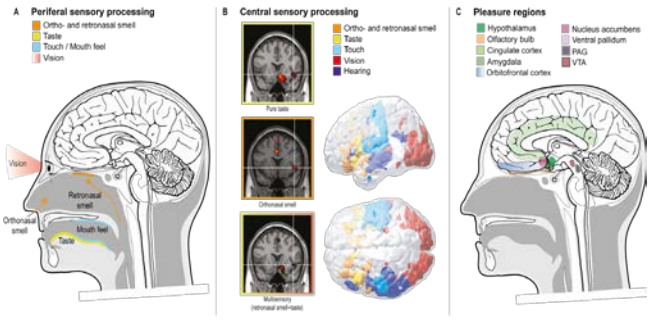

Figure 1.2. Sensory processing and the hedonic regions involved. (A) All senses are used in the evaluation of possible food sources. (B) There is a uniform topology of cortical activation between human subjects across all sensory modalities. (C) There is a large group of hedonic regions and hot spots in the brain, to many of which, olfactory input are potent triggers [10].

1.4 OLFACTORY LOSS IN DISEASE

Among the otherwise healthy general population, between 10-20% suffers from an impaired sense of smell, of which approximately one fifth are anosmic [52-56]. Olfactory assessment is essential for elucidating the degree of olfactory loss and, as such, forms an important part of the otorhinolaryngologic examination, especially *e.g.* in patients undergoing nasal or skull base surgery, in patients with nasal or sinus diseases, and in patients with olfactory loss to evaluate the effects of surgery or medical intervention [57]. In addition to affecting the quality of life, olfactory loss can be a prodromal symptom and potentially an early clinical biomarker of neurologic, psychiatric, and neurodegenerative diseases, such as Alzheimer's and Parkinson's disease [58-60]. It can thus be used to support diagnostics and as a prognostic assessor [61-64].

In an etiological analysis of patients suffering from olfactory disorders in otorhinolaryngological clinics, 72% were due to sinonasal causes (*i.e.* rhinitis or chronic rhinosinositis (with/without nasal polyposis)), 11% were due to post infectious inflammation, 5% were due to head trauma, 1% were congenital, 5% were caused by tumours, toxicity or iatrogenic, while 6% were idiopathic at the time of the visit [65,66]. However, depending on etiology classification, and the selection of patients for a given clinical setting, the distribution of underlying pathologies varies. In a recent large study on the causes of olfactory loss, the etiologies of olfactory loss were registered for 8,615 patients, who presented with the symptom 'olfactory loss' or who underwent olfactory testing as part of their clinical diagnosis [67]. In this study, 35% were due to viral causes, 23% were idiopathic, 19% were sinonasal, 17% were due to head trauma, 2,4% were congenital, 1,8% were neurodegenerative, 1% were due to toxic exposure, and 0,4% were due to tumours or stroke.

Overall, the etiology of olfactory loss can be divided into three main categories: conductive dysfunction, sensorineural dysfunction, and dysfunction of central pathways. However, these studies clearly accentuate the diversity in etiologies, and the demand for improving the diagnostic tests to accurately detect hyposmia or anosmia, and ultimately differentiate between the causes of olfactory deficits.

1.5 TESTING OLFACTORY FUNCTION

Olfactory function can be hard to quantify. The most common feature of olfactory function to be quantified is the ability to identify an odour (odour identification testing), but also other aspects of olfactory function can be tested, such as the ability to discriminate between odours (odour discrimination testing), the concentration required for detecting an odour (odour threshold testing), and odour memory can be measured [54,68] using simple sources of odours.

Olfactory testing is available in several different forms, from tests and examinations relevant for examining underlying processing, pathology or etiologies in research settings, to more clinically applicable tests optimised for patient screening and diagnostics. All tests have their advantages and limitations in terms of practicability, time consumption, cost, and potential gain of information, which are important to take into consideration when planning a diagnostic program.

Apart from the quantifiable measures of olfactory function, some patients suffer from qualitative alterations in olfactory function, such as distorted perception of odours (parosmia) or olfactory perception without stimulus (phantosmia). These qualitative alterations are not normally tested with standard olfactory test-batteries, but can be measured using *e.g.* questionnaires on perception and hedonic yield.

1.5.1 Psychophysical testing

The most widely used olfactory test is the Sniffin' Sticks (Burghart Messtechnik GmbH, Wedel, Germany), which is a psychophysical test for determining olfactory function. This test has been translated and validated in several European countries, including Italy [69], Germany [54], Greece [70], the Netherlands [71], United Kingdom [72], Turkey [73], Poland [74], and Portugal [75]. It is based on felt-tip pen-like devices containing common odours selected specifically to be applicable in the general European population [76]. It is available in two versions: the Sniffin' Sticks identification test (SIT), for a fast screening of olfactory function (12- or 16-item tests), and the Sniffin' Sticks test for evaluating odorant threshold, discrimination and identification (TDI) abilities (112-items test) [77,78]. The main purpose of the SIT is a rapid screening to identify patients who need additional olfactory diagnostic evaluation, while the TDI-test offers a more comprehensive understanding of the severity of olfactory deficits. A major advantage of the Sniffin' Sticks, is the extensive amounts of normative data generated for both normosmics, hyposmics, and anosmics [54,67]. Furthermore, the re-test reliability is also well established, making it a solid tool for reassessing patients after treatment or surgery [79,80].

Another widely used test for olfactory identification is the University of Pennsylvania Smell Identification test (UPSIT), where odorants are microencapsulated on the paper of the test kits [81]. The identification scores of the UPSIT test have been shown to be comparable with the Sniffin' Sticks identification test [82]. The UPSIT does offer the advantage of not requiring a healthcare professional present during testing [1], and by microencapsulating odorants, olfactory testing can reach unprecedented amounts of subjects, exemplified by the National Geographic Smell Survey that collected data from more than a million participants [83]. However, as each test can only be scratched and smelled once, it can quickly become expensive compared to other olfactory tests. Furthermore, to test different components of olfactory abilities in patients can be important for achieving a comprehensive evaluation of the of olfactory function, as some patient groups may suffer from more pronounced losses in certain qualities of their function [84,85].

Several methods have also been made available for retronasal olfactory testing. One example is the candy smell test [86], where the sweet-tasting medium for odour delivery can have an advantage especially in young children, while other tests apply retronasal stimulation by using oral application of grocery store condiments and food items in powder form [87,88].

Numerous other psychophysical tests for testing olfaction have been described [89-91]. Although different test scores varies between etiologies on a large-group level [85], differentiating etiologies of hyposmia or anosmia lies beyond the information acquired from psychophysical testing, independent of which tests are being applied.

1.5.2 Electrophysical olfactory assessment

For most clinical purposes, psychophysical testing is sufficient to assess olfactory function. However, as it requires the ability and will of the patient to cooperate, more objective measures of olfactory function may be needed. This can be made possible by measuring response upon stimulation of the olfactory epithelium with an odour using an olfactometer, ensuring the required temporal accuracy. Olfactory event-related potentials can then be measured at the level of the olfactory epithelium with electro-olfactography

(this requires an electrode in contact with the olfactory epithelium in top of the nasal cavity), or more centrally with electroencephalography (EEG) (with electrodes on the scalp) [33,92,93].

In medico-legal cases where an objective assessment is obligatory, central measurements of olfactory neural activation are useful, which can be obtained in a reproducible manner with EEG at specialised olfactory clinics.

1.5.3 Neuroimaging

Conductive causes of hyposmia/anosmia can sought to be identified with an endoscopic nasal examination, or a Computer Tomography (CT) scan of the sinuses if chronic rhinosinusitis is a differential diagnosis. If the endoscopic nasal examination is normal, yet the olfactory testing reveals a diminished olfactory function, further causal investigation is required. If there are no signs of chronic rhinosinusitis, inflammation, or plausible explanations in the past medical history, a sensorineural etiology should be considered [57]. The quest for sensorineural causes can require an array of different tactics dependent on the suspected cause, which can range from trauma [94] to depression [95], schizophrenia [96], and the aforementioned neurodegenerative diseases. Structural sequences of magnetic resonance imaging (MRI), such as T1 and T2-weighted scans, can potentially disclose tumours [97], as well as changes to the olfactory bulb [33]. Functional magnetic resonance imaging (fMRI) studies can elaborate on activation of primary and secondary olfactory cortices. On a group level, the high spatial resolution of fMRI has provided many insights as to which areas are activated by olfactory stimuli. Nevertheless, the low temporal resolution has left researchers in the dark concerning the temporal sequence of activation cascades.

Magnetoencephalography (MEG) has in recent years been introduced as a scanning modality in olfactory research. MEG data can be used to detect new aspects of odour-induced changes in brain activity [98,99], as it offers a high temporal resolution. This can contribute to new insights on cerebral olfactory torrents of activation on a millisecond scale. The MEG sensors record the magnetic fields produced by any perpendicular electric current, in accordance with Maxwell's equations. However, the magnetic field sensors used in MEG have a limitation in their ability to capture a balanced picture of olfactory brain activation. Firstly, estimating the cerebral current source of the measured magnetic field distribution is driven by *a priori* source assumptions making the analysis of MEG results highly susceptible for interpretation errors (the inverse problem) [100,101]. As the piriform cortices and other olfactory areas of interest are located far from the skull (and magnetic sensors), the cortices are, thus, difficult to detect and differentiate. Secondly, the electric current dipoles of firing neurons must have parallel orientations to give rise to measurable magnetic fields, so the magnetic fields are mostly limited to a layer of pyramidal cells situated perpendicular in the cortical surfaces of the sulci; thus, the secondary olfactory areas includes amygdala, hippocampus and several other deeper cerebral areas [102], which do not produce a uniform magnetic signal ideal for source localisation. Nonetheless, recent advances in pre- and post-processing of MEG data have made it possible to detect activation in these deep structures [103,104]. By conducting experimental and control tasks with identical stimulus parameters in the scanner, an estimated activity of more superficial brain areas can be subtracted in order to detect weak deeper sources [105].

Due to these limitations, multiple repetitions of stimuli is necessary to improve the signal to noise ratio. However, the amount of repetitions is limited by the time-costly odour free phase between odorant stimulation, and the stimulation duration is limited due to irritation of the olfactory epithelium. If a MEG study aims to investigate evoked potentials on a subject level instead of a group level, the requirement for increasing signal to noise ratio is increased even further. Prior studies of single subject level MEG analysis had to use several thousand repetitions of stimuli to improve the signal to noise ratio, even though the cortical area of interest was closer to the magnetic field sensors, compared to the olfactory cortex [106]. As the analysis of MEG signals requires good *a priori* assumptions of activated areas to take the inverse problem into account, a detailed structural knowledge is essential before conducting MEG scans, to ensure the full potential of this olfactory neuroimaging modality. This calls for a highly detailed description of the primary olfactory cortex. However, if used in conjunction with other neuroimaging modalities, MEG can provide valuable information, especially due to its high temporal resolution.

All functional olfactory neuroimaging modalities are dependent on a normal conduction of odorants to the olfactory receptors and a functioning peripheral sensorineural conduction of stimuli. The conduction of odorants can be affected by small changes in the nasal epithelium due to various factors, such as irritants, temperature change, inflammation, a common cold, and the nasal cycle, causing major - and fluctuating - variations of olfactory stimulation and subsequent cortical activation. These functional scanning modalities produce a snapshot of the activation in that exact time and space. This does not necessarily have any consequences at a group-level; however, the low reliability of olfactory activations should prompt major reservations over using fMRI of human olfaction as a diagnostic tool in single subjects [107]. Although fMRI has contributed immensely to the understanding of the functions of the living human brain, increasing concerns have been raised regarding the reliability of this surrogate measure of brain activity [108,109]. Although claims of limitations can be made concerning fMRI (and probably all other neuroimaging modalities, for that matter), functional findings from fMRI studies may be reinforced by other neuroimaging modalities, by adding a necessary dimension of confirmatory analysis or supportive results.

Structural olfactory neuroimaging does not rely on successful olfactory stimulation and activation of relevant brain areas. With structural MRI and diffusion tensor imaging (DTI) scans, it is possible to visualise the volume of primary and secondary olfactory cortices, which can be correlated to psychophysical olfactory testing scores [110]. Segura and colleagues showed that olfactory performance is also correlated with postcentral gyrus cortical thickness, as well as with fractional anisotropy and mean diffusivity levels in the splenium, other parts of the corpus callosum, and the superior longitudinal fasciculi, offering highly intriguing information on cerebral olfactory structures and plasticity. As the function of the brain is constrained by the structural neural scaffolding [111], investigating structural olfactory connections may add valuable information to our understanding of healthy and pathological olfactory patterns.

Olfactory impairment in Parkinson's disease has been linked with white matter abnormalities around the primary olfactory areas [112]. Furthermore, olfactory dysfunction was associated with atrophy in the piriform cortex and OFC, where progression of olfactory dysfunction was significantly correlated with OFC atrophy [113]. Although these previous studies are based on crude changes in voxel-based morphometry, it is a clear indication of a link between olfactory function and structural changes. This calls for a more detailed analysis of structural changes in diseases affecting olfactory processing and function, such as investigating changes in the structural brain connectivity.

The study of structural brain connectivity has given rise to connectomics - the comprehensive mapping of neural connections in the brain [114]. This map uses DTI to measure the diffusion of water molecules constrained by the white-matter fibre tracts (axons), typically on the scale of millimetres [115,116]. The connectivity between brain regions can be reconstructed using methods such as probabilistic tractography, which combines information

from measures of fractional anisotropy, local level of mean diffusivity, radial diffusivity, and axial diffusivity [117-119], offering detailed information of the structural neural networks of the brain.

With the complexity of olfactory sensation and perception and the subtle differences in alterations of olfactory function in numerous diseases, there is a demand for highly accurate testing of olfactory function. This applies for both research on receptor function, psychophysical testing, and neuroimaging studies. This is essential for a thorough understanding of the mechanisms underlying olfactory processing and the establishment of well-characterised olfactory deficits as prodromal signs of disease in the brain.

1.6 OLFACTION RESEARCH IN DENMARK AND FLAVOUR INSTITUTE

In 2013, when the current PhD was initiated, there were no validated Danish olfactory tests, or clinical assessments available for Danish patients with olfactory deficits, and little coordinated effort to change this. Research on olfaction and disease had only started to emerge, as Professor Therese Ovesen had initiated cooperation between olfactory related medical specialities under the name Olfaction Research Centre Aarhus. This led to the initiation of several olfactory research projects within the field of basic medical science. However, the olfactory research in Aarhus really caught momentum following a meeting in Oxford in 2015. With my supervisors, consisting of a clinical professor in otorhinolaryngology, Therese Ovesen, a neuroscientist and PET-specialist, Arne Møller, from Aarhus University, and a professor in neuroscience with great experience in flavour research, Morten Kringelbach, from the University of Oxford, we founded the Flavour Institute and defined its initial goals and tasks. As a result of the strong profiles and work of my supervisors, a list of prominent researchers in the field of Flavour research agreed to join our advisory board. With promising collaborations between Aarhus, Beijing, Dresden, Yale, and Oxford, a line of passionate young flavour-researchers are about to join the Flavour Institute for their PhDs, postdocs, and medical degree research dissertations. Various tools for olfactory testing have already been validated, while more normative data and several validation studies are in progress, creating a solid base for future flavour studies in Aarhus. The state of the art neuroimaging facilities at Aarhus University, combined with the strong analytic capacity at University of Oxford, opens up for endless research possibilities, where neuroimaging modalities such as PET, MEG, MRI, DTI, fMRI and EEG can be combined with behavioural data and other fields of research at Aarhus University and collaborators.

1.7 STRATEGY OF THE PHD PROJECTS

Given the starting point with Danish clinical olfactory research, the first focus of the PhD was to draw attention to olfaction and olfactory deficits among the general practitioners and ENT clinics in Denmark. Therefore, the initial work included a review article on olfaction [57]. As anosmia and hyposmia are fairly common, but often goes unnoticed by physicians in Denmark, this review article was written in Danish with intent of publication in *Ugeskrift for Laeger*, as this would reach a high number of physicians in both the primary and secondary sector in Denmark. However, as this review was not written in English it cannot constitute a formal part of this PhD dissertation. The article was published online by the journal in 2014. The increase in referrals of anosmic and hyposmic patients to the department of otorhinolaryngology at Aarhus University Hospital indicated a raised awareness of olfactory disorders, a focus on diagnostics of anosmia, and an increased interest from patients for participating in research studies on human olfaction.

During the process of writing the review, it came to my attention that the commonly used clinical tool for olfactory screening, the Danish 12-item Sniffin' Sticks identification test, had not been validated before implementation. As this tool is a fundamental part of my PhD project, we immediately initiated a validation study, which identified and corrected four systematic errors in the original test. This study was published in *Clinical Otolaryngology* in January 2015 and the results were subsequently implemented both clinically, and in olfactory and neurologic research projects.

As we identified a common cause of confusion between descriptors of different citrus fruit odour descriptors, we made an additional study to investigate the underlying mechanisms from a chemical perspective. This study was published in *Chemosensory Perception*.

As we observed that children and adolescents had lower identification scores and had difficulties understanding and recognising the descriptors validated test for adults, further studies of the Sniffin' Sticks as a clinical tool for testing olfaction in adolescents were initiated. Apart from modifying and validating the Sniffin' Sticks for clinical use in an adolescent population, the primary aim of this study is to investigate the role of odour familiarity as an underlying mechanism in different olfactory identification scores between adolescents and adults. This study is in review in *Chemical senses*.

Since we are in the fortunate situation of using multiple neuroimaging modalities for our olfactory research, both in Aarhus and Oxford, a promising approach is to combine structural and functional neuroimaging to gain a more comprehensive understanding of olfaction, and perhaps even extend this further to whole-brain computational modelling. The use of multiple neuroimaging modalities highlighted an important issue that we only came to realise when we began looking at different olfaction data; we identified a discrepancy in the brain templates used for functional neuroimaging and those traditionally used for other neuroimaging modalities [120-122]. We analysed the underlying differences in the structural connectivity network of these olfactory cortical templates, and used this method to introduce a new OC parcellation, which combines prior OC templates with information from the structural connectivity profiles. This study is in review in the *Nature* journal, *Scientific Reports.*

Consequently, a main focus of this PhD is to combine information from functional and structural neuroimaging in order to create an olfactory template that can be used across all neuroimaging modalities, with a secondary focus on improving understanding and application of behavioural measures of olfactory testing.

2. HYPOTHESIS AND AIMS

The main aim of this thesis was to develop tools and methods for optimising olfactory testing in both peripheral and central parts of the olfactory system. Initially, the focus was to ensure that the tools for olfactory testing in Denmark were validated for clinical use and comparable with the international literature. This allowed for us to focus on more generalisable aspects of olfactory testing, such as chemical resemblance in descriptors of identification tests, the role of familiarity in the age-related improvements of olfactory identification skills, and lastly to tie bonds between the structural and functional neuroimaging modalities to form a unified tool for investigating central olfactory processing.

2.1 OLFACTORY SCREENING: VALIDATION OF SNIFFIN' STICKS IN DENMARK

2.1.1 Problem definition

Olfactory identification scores are highly dependent on the familiarity of descriptors, which can be affected by factors such as cultural and linguistic differences. Consequently, the original Sniffin' Sticks publications stated that the four descriptors for any given odorant should have a correct identification rate of at least 75% in a normosmic population [76,77]. This requirement was a great

display of foresight, as the familiarity of odorant descriptors around the world has been shown to have a large degree of variation [69,72,73]. By defining both basic requirements for the identification of each odorant and publishing large datasets of normative data [54], a standardisation of the SIT has to a large extend been established across borders and cultures. This is an absolute prerequisite for having a more unified field of international olfactory research, where results and conclusions have greater external validity. However, the Danish Sniffin' Sticks SIT has not been validated.

2.1.2 Hypothesis and aims

The SIT12 has been translated from German to Danish without validation in a Danish population. As these two countries are closely related both linguistically and culturally, we hypothesised there was no significant difference in correct identification rates between odorants, and that these rates were all above 75% in a normosmic Danish population. We aimed to test this hypothesis and had prepared a modification process if it turned out we had to reject the hypothesis. Hereafter, independent of outcome, we would in the end have a validated tool for olfactory testing in Denmark.

2.2 CONSIDERING CHEMICAL RESEMBLANCE: A POSSIBLE CONFOUNDER IN OLFACTORY IDENTIFICATION TESTS

2.2.1 Problem definition

From the validation of the SIT12 (study I) we learned that some closely related descriptors caused confusion among normosmic participants, where 34 % of test participants identified the lemon odorant as grapefruit. The study design of our validation study enabled us to understand the underlying cause of this confusion; the participants primarily identified the citrus fruit odour-object category of the odorant, but subsequently had difficulties differentiating between the two citrus fruit descriptor options. Comparable difficulties with the lemon odorant were found in a British validation study [72] and a Czech study [123]. However, in the British study, the false descriptor causing systematic confusion was changed from one citrus fruit (grapefruit) descriptor to another (orange) without including input from participants or re-validating the test after modification. As such, the methods used in validation studies of SIT have some variation. This may interfere with the generalisability of olfactory research. Validation studies that do not consider confounding factors such as overlapping chemical volatile odour-molecules and, furthermore, do not perform proper validation after modifying descriptors can be problematic.

2.2.2 Hypothesis and aims

We hypothesised that there was an overlap of chemical compounds between the chemical volatile molecules in the Sniffin' Sticks felt-tip pen and several citrus fruit descriptors, including both grapefruit and orange.

As olfactory testing is teeming with potential pitfalls due to the complexity of olfactory sensation and perception (Figure 1.1), we aimed to illustrate how an error in odorant identification could have a possible explanation in the resemblance of the chemical odour-image produced by the odorant and the descriptors available for that odorant in the forced-multiple choice olfactory test. The main aim for the study was to emphasise the importance of re-validation after changes in olfactory tests, exemplified by the risk of identification error potentially due to chemical resemblance.

2.3 ODOUR FAMILIARITY AND IDENTIFICATION ABILITIES IN ADOLESCENTS

2.3.1 Problem definition

Throughout childhood and adolescence there is a gradual increase in self-perceived olfactory significance [124], and current literature indicates that the ability to identify odours also gradually increase throughout this developmental period [54,125]. When tested with standard olfactory tests for adults, some of the odorants were found to have very low identification scores in both children and adolescents, thus, removal of these odorants from the identification test has been proposed for testing this age group [126]. These studies provide important normative data on age-related identification scores for the SIT-16 / SIT-14, which is highly relevant in the clinical setting. The underlying mechanisms for why adolescents are inferior in identification abilities compared with adults are still to a large degree unknown. Several studies have mentioned odour familiarity as a possible cause of the inferior odour identification scores in adolescents [86,125]. However, the notion that odour familiarity should play a key role in the inferior identification abilities has not yet been properly tested.

2.3.2 Hypothesis and aims

We hypothesised that odour familiarity is an important influential factor in the decreased odour identification abilities in adolescents. To test this hypothesis, we designed three sub studies with the following aims:

- Firstly, our aim was to evaluate age related differences in odour familiarity by mapping odour familiarity of adolescents and adults for different categories of odour-objects.
- Secondly, our aim was to create a validated version of SIT-16 for adolescents with familiarity of descriptor odours taken into account.
- Thirdly, our aim was to test if an identification test modified specifically for adolescents would still result in inferior identification scores compared with an adult population.

2.4 BRAIN FINGERPRINTS OF OLFACTION: A NOVEL STRUCTURAL METHOD FOR ASSESSING OLFACTORY CORTICAL NETWORKS IN HEALTH AND DISEASE

2.4.1 Problem definition

With a large heterogeneous group of diseases affecting olfactory function at an early stage of pathology, olfaction has been highlighted as a possible biomarker for early detection of diseases and for understanding neural disease mechanisms [64,127-129]. However, in order for olfaction to function as a biomarker, a better understanding of olfactory processing is needed, in both health and diseases. Deciphering the underlying processing of olfaction has shown to be a quite difficult task, as all levels of olfactory sensing offers several pitfalls in interpretation: the odorant stimuli in itself can cause several problems [130]; odour sensitivity can be influenced by genetics [131], along with smoking habits [132], age [133], culture, and gender [134]; sleepiness and attention during testing can alter the patterns of olfactory cortical activation [135,136]; hedonics can alter olfactory cortical activation [137], however, hedonic responses are highly individual, and may even change due to hunger levels during an experiment [138] (Figure 1.1.).

In spite of the large amount of factors influencing olfactory processing, much has been gained from human neuroimaging studies since the first functional approach to identify of olfactory processing in the early nineties [139]. In this rapidly developing field, different studies - each with unique purposes, scan parameters, and analysis methods – have contributed with small pieces of the puzzle to gain a more comprehensive understanding of olfacto-

ry processing. However, for the conundrum to be solved, it is imperative that all pieces added are parts of the same puzzle – a discussion on the role of the primary olfactory cortex must be based on a common agreement as to how the primary olfactory cortex is defined. Since Zatorre's initial findings [139], several other parcellations of the olfactory cortex have been used. Consequently, Seubert and colleagues defined a template of the olfactory cortex by adding all information from previous functional neuroimaging studies (PET and fMRI) and conducting a statistical activation likelihood estimation to define the common area of activation [120,121,140]. Though this meta-analytic approach does have the advantage of assembling the cumulative data of olfactory cortical activation, the issue of low temporal resolution of fMRI and – especially – PET, makes it impossible to rule out inclusion of secondary and tertiary olfactory areas in the parcellation. In a comparison of this template with other brain parcellation templates, there was a large mismatch in inclusion of olfactory regions, as well as inclusion of non-olfactory regions in this meta-analysis-derived template.

Instead of combining methodologies and neuroimaging modalities, the definition of existing templates for the primary olfactory cortex has so far been constructed using either primarily anatomy [122] or functional measures of brain activation [120].

2.4.2 Hypothesis and aims

We hypothesise that by combining knowledge from functional olfactory activation [120] and anatomical cortical structure [122] with networks of structural connectivity and pre-existing knowledge on key secondary olfactory areas, we can identify an optimised primary olfactory cortical template. Our aims are to analyse the structural connectivity networks in both the functional primary olfactory template [120] and the structural primary olfactory template [122], and to utilise the connectivity profiles to identify an optimised olfactory cortical template.

3. METHODS AND MATERIALS

3.1 OLFACTORY SCREENING: VALIDATION OF SNIFFIN' STICKS IN DENMARK

3.1.1 Participants and ethics

In total, 102 Danes were included in the study between the age of 18 and 50 years with a subjective normal sense of smell. All participants were tested with the Sniffin' Sticks 12-odorant identification test. The first 51 participants were tested with the original odorant descriptors, while the second half were tested with the modified version of descriptors. Furthermore, they underwent a nasal endoscopic evaluation and filled out the following questionnaires: the sinonasal outcome test (SNOT-22) for sinonasal symptoms, the Major Depression Inventory (MDI) for depressive symptoms, and the Mini-Mental State Examination (MMSE) as a screening for cognitive impairment. Prior to filling out the questionnaires, participants were asked if they wished to be informed of any abnormal questionnaires scores. The study was conducted in accordance with the Helsinki Declaration and was approved by the Danish Ethical Committee.

3.1.2 The Sniffin Sticks 12-identification test

The SIT12 is kit of 12 felt-tip-pens containing commonly known odorants. Each odorant is presented with a correct descriptor and three false descriptors in a forced-multiple choice test. It is an olfactory identification test intended for a fast screening of olfactory function. The test is initiated by letting the participant read the descriptors for the odorant, informing the participant that they are allowed to smell the odorant twice if needed, and subsequently removing the cap of the felt-tip pen and presenting the odorant for the participant by placing the pen 1-2 cm under the nostrils for approximately 3 seconds. All answers were registered along with a score of certainty and reasons for any uncertainties in identifying the correct odorant.

3.1.3 Test modification process

The first 51 participants were tested with the SIT-12 version containing a list of descriptors, which had been directly translated from German without prior validation, and used for several years in Danish research and to a limited degree in clinical settings. Participants rated their certainty of each selected descriptor along with familiarity of all descriptors, and a description of any uncertainties in identification process. This was used to identify and modify descriptors, which more than 25% of participants were uncertain or unfamiliar with [77]. The remaining 51 participants were tested with the SIT-12 containing a modified list of descriptors in order to validate the modified test.

3.2 CONSIDERING CHEMICAL RESEMBLANCE: A POSSIBLE CONFOUNDER IN OLFACTORY IDENTIFICATION TESTS

To investigate the possible role of chemical resemblance in olfactory test identification errors, the most common falsely identified odorant in the Danish SIT12 validation study (study I) was chemically analysed. The volatile molecules identified in the lemon odorant were cross-referenced with volatile molecule profiles of other citrus fruits.

3.2.1 Sample preparation and Gas Chromatography-Mass Spectrometry (GC-MS)

The Sniffin' Sticks felt-tip pen contains a cotton tampon, where dye has been replaced with odorant-liquid. Three samples of the odorant were used for analysis: the head of the felt tip pen and two samples of the cotton tampon. For all samples, the volatile molecules were purged from the dynamic headspace (DHS) into a Tenax-trap. The volatile molecules were desorbed and transferred to a gas chromatograph, where hydrogen gas was used to carry the molecules through the heated polar capillary column, causing the molecules to become separated according to their differences in size, adhesion and polarity. At the end of the capillary column, the separated molecules were analysed with a mass spectrometer.

3.2.2 Identification of chemical compounds and their incidence in citrus fruits

Matching the retention index with Kovats retention index databases identified the volatile molecules, which were cross-referenced with the mass spectra of each molecule in the Wiley database. All identified volatile molecules and their synonyms were added to a search in combination with relevant words on citrus fruits (*e.g.* 'citrus', 'orange', 'grapefruit') in Scopus, PubMed, Web of Science, and SciFinder.

Please see the detailed methods in the original paper [141].

3.3 ODOUR FAMILIARITY AND IDENTIFICATION ABILITIES IN ADOLESCENTS

3.3.1 Participants and ethics

A total of 731 participants were included in the three sub studies: 172 adolescents and 238 adults were included in the odour familiarity study, 72 normosmic adolescents were included in the evaluation and modification of the SIT-16, while 167 normosmic adolescents (age 12-18) and 82 normosmic adults (age 19-55) were included in the study on effects of odour familiarity on identification scores. Adolescent participants were recruited through six different schools in Central Denmark Region and Region of

Southern Denmark, where all of the children's custody holders gave written informed consent prior to enrolment. The three sub studies were conducted in accordance with the Helsinki Declaration and were approved by the Danish Ethical Committee.

3.3.2 Procedures

Data on odour familiarity were collected through an online questionnaire service (Survey-Xact.dk, Ramboll Management Consulting A/S, Denmark), where participants rated their familiarity for 125 common odours on a Likert-scale, ranging from 1-5. The following instruction was given to participants: On a scale from 1-5, please rate how familiar you are with the odour. 1) I would not be able to recognise the odour; 2) I do not think I would be able to recognise the odour; 3) Maybe I would be able to recognise the odour; 4) I think I would be able to recognise the odour; 5) I would be able to recognise the odour. The 125 odours were prospectively placed in ten different odour-object categories, but presented for participants in a random order. The ten odour-object categories were: acrid foods, alcohol, candy, environmental, meat/fish, nuts, other foods, spices/seasoning, sweet foods, and vegetables.

These categories were defined to contain all descriptors from the Sniffin' Sticks (as this was needed in sub study 2). Subsequently, further odour descriptors were added in order to create an extensive list of commonly known odours. This process included interviewing managers of three candy stores and two chefs, identifying ingredients from recipes on the website of a popular adolescent magasine (viunge.dk), identifying common spices from the sales statistics of Santa Maria A/S (biggest spice manufacturer in Denmark), and interviewing twelve adolescents on what smells they notice in their everyday lives. The odours were put in odour-object categories according to the definition of the object (*e.g.* botanical definition of vegetables, herbs, and spices) in collaboration with the two chefs. However, odours changed category if more than 2/3 of the interviewed adolescents agreed (*e.g.* that tomato is a vegetable, even though it is botanically a fruit).

Olfactory testing in the second and third sub study was conducted with the SIT-16 and SIT-16jr, following standard testing procedures [76,77,142]. The modification process in the second sub study was conducted with focus on the following: all odour descriptors with a familiarity score of less than 75% were replaced with more familiar descriptors, and odorants with a low familiarity were paired with highly familiar descriptors. Please see the more detailed methods in the original paper [143].

3.4 BRAIN FINGERPRINTS OF OLFACTION: A NOVEL STRUCTURAL METHOD FOR ASSESSING OLFACTORY CORTICAL NETWORKS IN HEALTH AND DISEASE

In this study, we applied a combination of probabilistic tractography and diffusion tensor imaging (DTI) to two different templates of the primary olfactory cortex in order to identify the underlying structural connectivity networks for each template in a group of right-handed normosmic, healthy, young adults (n=16).

3.4.1 Structural connectivity

As in all other cells of the body, neurons and their neural fibres contain water. Without boundaries limiting the permeability, these water molecules would have a continuous random displacement (isotropic diffusion). However, with the influence of cellular microstructures (*i.e.* microtubules, neurofilaments, the myelin sheath, and the membrane of the axon), the mobility of water molecules in the neural tissue are much more likely to diffuse along the direction of white matter tracts than perpendicular to them (anisotropic diffusion) [116]. This basic physical principal is at the core of DTI and tractography, where the orientation of the white matter architecture is measured by identifying pathways of maximum diffusion coherence, voxel by voxel [144]. Fingerprinting of the structural connectivity of different brain regions has been conducted in schizophrenic patients [145] and in chronic pain patients after treatment with deep brain stimulation [146]. These two studies from the research group in Oxford form the basis for the olfactory fingerprinting and have proven the method to be reliable, even on small sample sizes, and extremely promising [147].

3.4.2 Processing pipeline

The first steps of the pipeline were to co-register the acquired MRI-T1-wieghted scans into the geometric MNI space (a standard anatomical geometric brain matrix made by Montreal Neurological Institute (MNI)). Then we applied the MRI-T1 to DTI transformation matrix (native space) in order to be able to apply the AAL template [122] (MNI-space) directly onto the diffusion images (Figure 3.1A). This allowed for gross anatomical visual inspection of the two OC templates and subsequent construction of two parcellations of the OC: a structural parcellation of the OC as defined by the AAL [122] – the structural olfactory cortical network (sOCN) - and a functional parcellation as defined by the functional activation likelihood estimate [120] - the functional olfactory cortical network (fOCN) (Figure 3.1B).

The dual phase encoding directions were compared and a weighted estimation of accuracy likelihood was calculated, resulting in a merged set of DTI data with reduced distortion [148].

Structural connectivity fingerprints were calculated for both templates after correcting for eddy currents and modelling for crossing fibres on voxel level [149] in the FMRIB diffusion toolbox (FMRIB Software Library (FSL), Oxford, version 5.0). This allowed for estimation of an additional fibre direction, apart from the dominant fibre direction of each voxel [150]. The brain boundaries were automatically defined using the brain extraction tool in FSL and checked by visual inspection of all subjects. The connectivity probability of each voxel was estimated using probabilistic tractography. We sampled 5000 streamline fibres per seed voxel and computed the probability of connection to any target voxel in order to calculate a connectivity measure defined as the proportion of fibres from the seed voxel that reaches the target voxel [149,150]. This measure was recalculated on a regional (parcellation) level by computing a voxel-weighted average of connectivity [146], which was applied to connections between all regions and the OC templates, resulting in distinct connectivity matrices for the fOCN and the sOCN (Figure 3.1C).

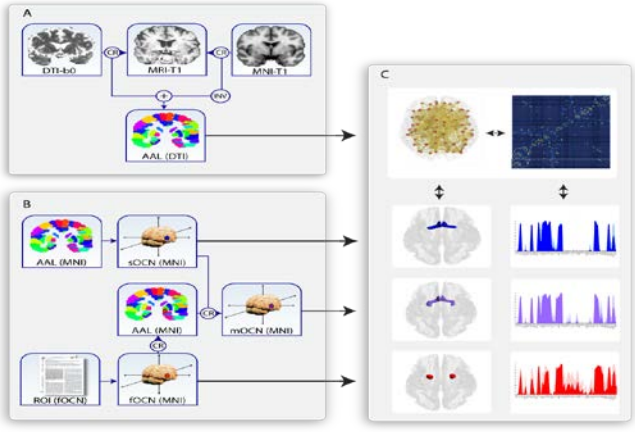

Figure 3.1. Olfactory fingerprint processing pipeline.
(A) With coregistration tools the MNI-coordinates were registered with subject's T1 scans along with the b0 DTI scans. This allowed for cortical parcellation with the AAL template in the subject's DTI scans. (B) Location of olfactory cortical regions of interest were identified [120,122] and added to the AAL parcellation. (C) A structural olfactory fingerprint was calculated for each OCN. Locations of the OCNs are shown in the glass brain and the connectivity profile to other cortical regions are shown in the

graphs (See figure 4.3 for higher resolution of graphs). CR: co-registration; INV: Inversion; +: Merge of images.

3.4.3 Applying cortical restrictions and computing connectivity-based sub-regions

As inclusion of white matter voxels as parcellation seed regions would result in subsequent strong measures of connectivity to both the seed and target regions of this fibre tract, the OCN templates were inspected visually in the MRI-T1 scans (MNI space) of subjects and the standard ICBM152 brain [151]. The templates were also cross referenced with the AAL atlas [122], and the Harvard-Oxford atlas [152]. White matter, CSF, and non-primary olfactory regions [37,122,153,154] were subtracted from the fOCN template, hereby defining the primary OC functional template (fOCN (grey)). The seed voxels in fOCN (grey) and the sOCN that were connected to key secondary olfactory areas were combined into a merged OCN template (mOCN) – creating a novel primary olfactory template. Please see the more detailed methods in the original paper [40].

4. RESULTS

4.1 OLFACTORY SCREENING: VALIDATION OF SNIFFIN' STICKS IN DENMARK

4.1.1 Distribution and causes of descriptor errors

Despite the linguistic and cultural overlap, the translation from the original German descriptors into Danish caused systematic errors and confusion among the Danish participants. Of the 12 Sniffin' Sticks, two odorants (lemon and cinnamon) were accountable for more than 60% of the total amount of errors (Figure 4.1). The participant's scores of certainty and reasons for uncertainties in identifying the correct odorant revealed that the citrus dominance of the lemon odorant caused them to pick randomly between the lemon and grapefruit descriptor, which was described as synthetic by participants. The spicy sweet flavour profile of cinnamon was described as the cause of uncertainty in choosing between the cinnamon and the honey descriptors. Furthermore, two descriptors (curled mint and cloves) had to be changed into more common Danish appellations, as the familiarity of the direct translation was low.

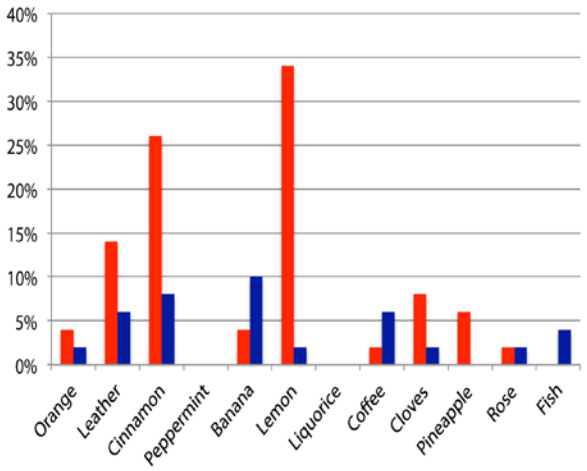

Figure 4.1. Odorant identification errors before (red) and after (blue) modification of the Danish SIT-12.

4.1.2 Modification and validation process

After correcting the unfamiliar descriptors and the two descriptors with overlapping odorant descriptor profiles, the mean identification score improved slightly. More importantly, the modification led to a correct identification rate of ≥75% for all odorants in this normosmic population, with no significant difference in distribution of identification errors (p=0.09). None of the descriptors in the modified version of the SIT12 were rated as unfamiliar to more than 25% of participants.

4.2 CONSIDERING CHEMICAL RESEMBLANCE: A POSSIBLE CONFOUNDER IN OLFACTORY IDENTIFICATION TESTS

4.2.1 Identified volatile molecules and their incidence in the other descriptors

In the chemical analysis, 34 volatile molecules were identified in all three independent samples from the lemon odorant felt-tip pen. Of these, 16 molecules had previously been identified in other citrus fruits, which correlate well with the common initial description of the odorant as 'citrus-like' in study I. The odour references for each volatile compound and the overlapping incidence in other citrus fruits are further described in the original manuscript [143].

4.3 ODOUR FAMILIARITY AND IDENTIFICATION ABILITIES IN ADOLESCENTS

4.3.1 Effect of age on odour familiarity

The familiarity ratings of 125 different common odours revealed a significant difference between adolescents and adults. Adolescents had a lower mean familiarity score (t_{408} = 0.19, p = 0.0051), however, this difference was much more pronounced within the predefined odour-object categories (Table 4.1). See original paper for raw data [143].

When comparing the adult and adolescent scores, both adolescents and adults alike knew the most familiar odours. However, for different odour-object categories the gradients of the curves differed considerably, and for the most unfamiliar odour-object groups in adolescents, an upward tail-effect could be observed, demonstrating a large difference odour familiarity (Figure 4.2).

4.3.2 Effect of odour familiarity on identification abilities

After modifying the SIT-16 to fit the familiarity of adolescents by changing 33 of 64 descriptors, there was no difference in mean adult identification score (14.41 (95%CI: 14.12 – 14.71)) and the adolescent identification score (14.52 (95%CI: 14.33 – 14.72)). There was only a significant difference in the identification rate of a single odorant, cinnamon. Adults were inferior to adolescents in identifying this odorant (p=0.0022), as they incorrectly identified the odour as vanilla (12.2% vs. 3.6% in adolescents) or chocolate (8.5% vs. 2.4% in adolescents).

4.4 BRAIN FINGERPRINTS OF OLFACTION: A NOVEL STRUCTURAL METHOD FOR ASSESSING OLFACTORY CORTICAL NETWORKS IN HEALTH AND DISEASE

4.4.1 Brain areas included in OCN parcellations

The visual inspection of sOCN and fOCN on subject's T1-MRI (MNI-space) scans and the standard ICBM152 brain [151] revealed an overlap of the fOCN with white matter, CSF, and several cortical structures outside the normal anatomical locations of the olfactory cortex [39]. This was confirmed by overlaying the fOCN on two standard parcellation atlases: the AAL template [122] the Harvard-Oxford atlas [152], which was used in the publication defining the fOCN [120] (Table 4.2).

Odour-object category	Odours (n)	Mean familiarity score: Adult	Adolescent	Difference	(95% CI)	p-value
Food related odours						
Candy	12	3.74	3.96	-0.23	(-0.38 - -0.07)	0.0050
Sweet foods	24	3.73	3.72	0.01	(-0.15 - 0.17)	0.8615
Nuts	5	3.68	3.49	0.19	(0.02 - 0.37)	0.0321
Meat/fish	7	4.22	4.17	0.06	(-0.08 - 0.20)	0.4139
Acrid foods	8	4.26	3.84	0.43	(0.29 - 0.56)	<0.0001
Vegetables	17	3.60	3.48	0.12	(-0.04 - 0.28)	0.1507
Spices/ seasoning	20	4.05	3.48	0.57	(0.43 - 0.71)	<0.0001
Other foods	6	3.67	3.57	0.10	(-0.07 - 0.26)	0.2475
Non-food odours						
Alcohol	3	3.90	3.88	0.02	(-0.18 - 0.21)	0.8543
Environmental	23	4.29	3.98	0.31	(0.19 - 0.43)	<0.0001

Table 4.1. Age related familiarity differences of between odour-object categories. Conservative measures with two-tailed t-test were used, t_{408} (Mean Odour-object score was calculated for each participant). Abbreviations: CI, confidence interval.

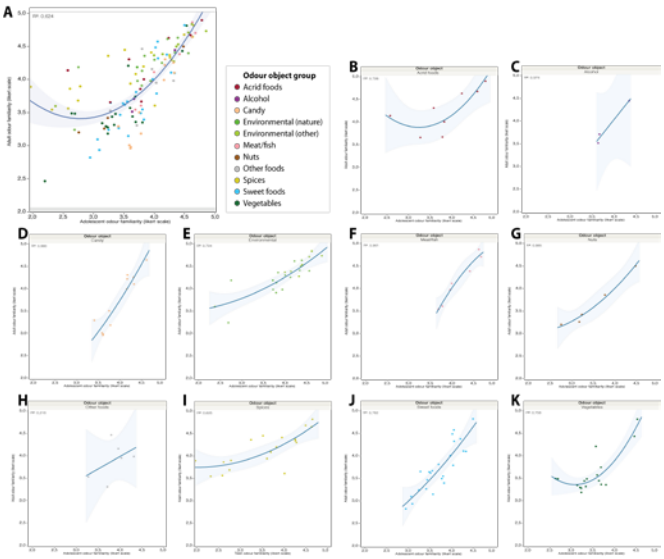

Figure 4.2. Adult and adolescent odour familiarity rating. The graphs of odour familiarity ratings for adolescents and adults show a clustering of the highly familiar odours across all odour-object groups (A), meaning that the high familiarities of these odours are shared across age groups. The slope of the age-related familiarity curve depends on the odour-object group. Odour-object groups: A: All groups. B: Acrid foods. C: Alcohol. D: Candy. E: Environmental. F: Meat/fish. G: Nuts. H: Other foods. I: Spices. J: Sweet foods. K: Vegetables.

Brain region	Right side: AAL	Right side: Harvard Oxford	Left side: AAL	Left side: Harvard Oxford
Primary olfactory areas				
Piriform cortices	7 %	*	8 %	*
Amygdala	27 %	24 %	37 %	45 %
Secondary olfactory areas				
Putamen	17 %	17 %	9 %	6 %
Pallidum	0 %	3 %	0 %	1 %
Parahippocampus	0 %	0 %	7 %	3 %
Hippocampus	<1 %	0 %	6 %	<1 %
Orbitofrontal Cortex	0 %	5 %	0%	0%
Atlas-definition differences				
Un-named grey matter areas	-	~37 %	-	35 %
White matter**	-	~15 %	-	12%
Not contained in atlas**	50 %	-	32 %	-

Table 4.2. Brain areas included in the fOCN. To investigate the degree of overlap with non-primary olfactory cortical regions, the fOCN was added to two different cortical parcellations and compared, the AAL and the Harvard-Oxford atlas. With little variation, both parcellations showed a large overlap with non-primary olfactory cortical areas. *The piriform cortices are not defined in the Harvard-Oxford atlas, but are contained within the un-named grey matter areas. **White matter is not contained in the AAL atlas, in contrast to the Harvard-Oxford atlas.

4.4.2 Connectivity networks of all OCN templates

The probabilistic tractography revealed four unique sets of fingerprints for the four OCNs (sOCN, fOCN, fOCN (grey), and mOCN) (Table 4.3). However, as the original fOCN-templates were overlapping with other cortical regions, these voxels were subtracted from these areas, resulting in a smaller size of these regions in the parcellation (Table 4.2). Thus, the comparison of connectivity was not possible to make with a completely identical parcellation. The normalised non-thresholded structural connectivity measures were used to compute plots of mean connectivity and weighted edges to the centre of gravity to connected cortical regions (Figure 4.3).

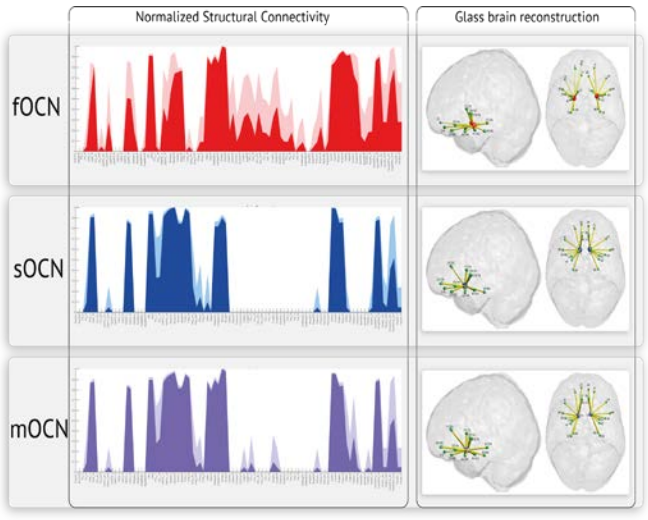

***Figure 4.3.* Normalised structural connectivity fingerprints for the fOCN, sOCN, and mOCN**. The plots represent the normalised mean structural connectivity to all other areas in the brain parcellation (standard deviation represented by lighter colour). The connections are graphically represented as weighted edges from the OCN to the centre of gravity of the connected region. For high-resolution figure, see original publication [40].

Anatomical area	AAL area	fOCN	fOCN (grey)	sOCN	mOCN
Primary olfactory cortical areas					
Piriform cortex (left)	21	100%	100%	*	*
Piriform cortex (right)	22	100%	100%	*	*
Amygdala (left)	41	100%**	100%**	100 %	100%**
Amygdala (right)	42	100%**	100%**	100 %	100%**
Areas with secondary olfactory processing					
Orbitofrontal cortex (left, superior)	5	69%	69%	100 %	100%
Orbitofrontal cortex (right, superior)	6	100%	100%	100 %	100%
Orbitofrontal cortex (left, inferior)	15	69%	69%	100 %	100%
Orbitofrontal cortex (right, inferior)	16	69%	69%	100 %	100%
Orbitofrontal cortex (left, medial)	25	56%	-	100 %	100%
Orbitofrontal cortex (right, medial)	26	-	-	100 %	100%
Gyrus rectus (left)	27	75%	69%	100 %	100%
Gyrus rectus (left)	28	94%	94%	100 %	100%
Insula (left)	29	100%	88%	100 %	100%
Insula (right)	30	100%	100%	100 %	100%
Anterior cingulate cortex (left)	31	-	-	100 %	100%
Anterior cingulate cortex (right)	32	-	-	100 %	100%
Hippocampus (left)	37	100%	100%	-	100%
Hippocampus (right)	38	100%	100%	-	100%
Parahippocampal gyrus (left)	39	100%	100%	100 %	100%
Parahippocampal gyrus (right)	40	100%	100%	100 %	100%
Caudate nucleus (left)	71	100%	100%	100 %	100%
Caudate nucleus (right)	72	100%	100%	100 %	100%
Putamen (left)	73	100%**	100%**	100 %	100%
Putamen (right)	74	100%**	100%**	100 %	100%
Temporal pole (left, superior)	83	100%	100%	100 %	100%
Temporal pole (right, superior)	84	100%	100%	100 %	100%
Temporal pole (left, middle)	87	81%	69%	50%	56%
Temporal pole (right, middle)	88	94%	94%	63%	63%
Areas connected before removal of non-grey matter voxels					
Calcarine fissure (right)	44	50%	-	-	-
Lingual gyrus (right)	48	50%	-	-	-
Occipital lobe (left, middle)	51	50%	-	-	-
Fusiform gyrus (right)	56	56%	-	-	-
Pallidum (left)	73	100%	-	-	-
Pallidum (right)	74	100%	-	-	-
Thalamus (left)	77	94%	-	-	-
Thalamus (right)	78	88%	-	-	-

Table 4.3. **Connectivity from OCN's to other cortical regions.** The strength in connectivity is listed by the percentages of subjects with structural connectivity from the respective OCN to the listed cortical region. The connectivity strengths are ipsilateral. A threshold of 50% of subjects was applied in order to highlight the shared significant connectivity profile.

4.4.3 Connectivity driven sub-parcellation of the mOCN

By merging the sOCN with the primary olfactory grey matter regions of fOCN, the mOCN was created, representing the anatomical and functional derived primary cortex. In order to ensure relevant structural connectivity in the entire mOCN, the mOCN-voxels containing seeds of all fibre connections to secondary olfactory structures (Table 4.3) were identified. These seed voxels were used to construct a sub-parcellation of the mOCN (Figure 4.4).

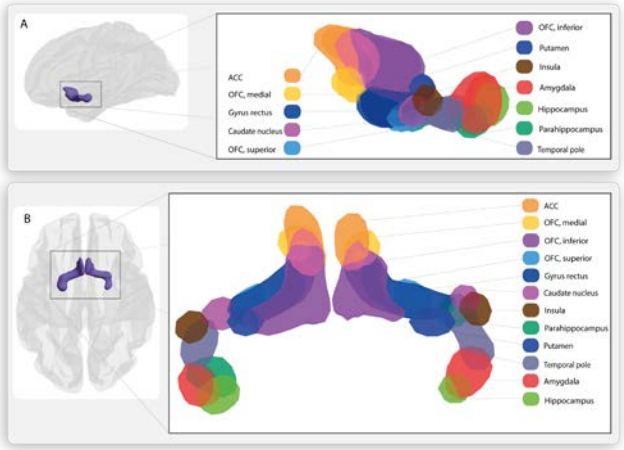

Figure 4.4. **Connectivity-based sub parcellation of the mOCN.** The sub-parcellation was created by identifying seed-voxels for fibre connections to secondary olfactory areas. **A** Lateral view. **B** Inferior view.

5. DISCUSSION AND CONCLUSIONS

5.1 OLFACTORY SCREENING: VALIDATION OF SNIFFIN' STICKS IN DENMARK

We identified a discrepancy between error distributions for identifying the correct descriptor in the 12 odorants. For two odorants, one of the false descriptors had a too high resemblance with the odorant, and had to be changed for the SIT12 to be valid for clinical use in the Danish population.

This study had two implications. Firstly, it created the first validated version of an olfactory test in Danish, which is key for accurately identifying patients in need for further olfactory evaluation and for Danish olfactory research to be comparable with international literature. Secondly, the results display cultural variation in olfactory familiarity and perception, as there was a significant difference between these Danish identification rates for some odorants and the identification rates from just south of the Danish-German border.

A possible underlying mechanism for these dissimilarities may be differences in familiarity of the chemical odour-image for the given correct descriptor. An example of this is described by the Turkish Sniffin' Sticks validation study [73]; the apple odorant, which is based on the odour-image of very sweet apple cultivar, had little resemblance to the apple cultivars commonly consumed in Turkey, but was more often associated to a type of air freshener. This could also be the case in Denmark. From the olfactory test manufacturer's point of view, it can be extremely difficult to create an odorant, which is chemically stable enough to have a long shelf life that at the same time has complete overlap with the expected odour-image of a given descriptor. As odorants were often described as synthetic, factors such as familiarity and resemblance with the other descriptors may play a key role in the identification of a given odorant based on a synthetic odour-image. The chemical resemblance between the correct and false descriptors is described in study II.

5.2 CONSIDERING CHEMICAL RESEMBLANCE: A POSSIBLE CONFOUNDER IN OLFACTORY IDENTIFICATION TESTS

When analysing the volatile molecules in the lemon Sniffin' Sticks odorant (see study I), we identified a chemical profile with high degree of overlap between the correct lemon descriptor and a false citrus fruit descriptor. This may cause substantial overlap in the activated receptors among normosmic participants between the odour-images of the correct and false descriptors (Figure 5.1).

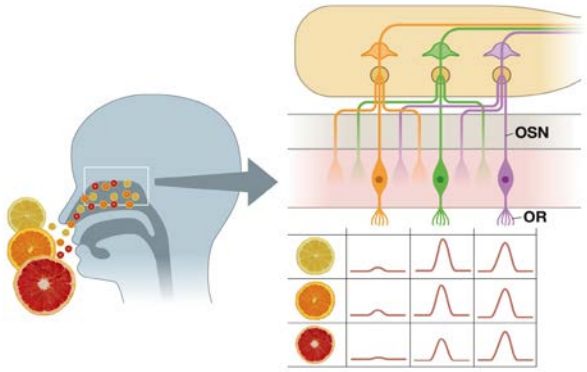

Figure 5.1. Receptor activation of volatile molecules in citrus fruits. Due to the large overlap in volatile chemical compounds of citrus fruits, there is a high degree of overlap in the activated olfactory receptors. This may cause impairment of olfactory identification, which can only be avoided if the odorant is validated with the list of descriptors. OSN: Olfactory Sensory Neuron. OR: Olfactory receptor.

In the creation and modification of an olfactory identification test, it is crucial to avoid systematic errors in the normosmic population. However, this is a fine balance, as too contrasted distractors may enable hyposmics to correctly identify odorants, thus hazing the differentiation between normosmics and hyposmics, while too little contrast between descriptors creates a systematic error, thus hazing the differentiation between normosmics and anosmics [155]. The difficulty of identifying the correct descriptor should therefore be a result of the clinical purpose of the test, and not a result of a lack of consideration in the design of the validation study. Modifying descriptors can be a tedious task if several modification processes are needed before reaching the required identification rate in a new target population [77]. This calls for careful considerations when selecting new descriptors, where both the knowledge on chemical resemblance between descriptors and the familiarity of the odour descriptors in the target population are key components in creating a valid olfactory identification test.

5.3 ODOUR FAMILIARITY AND IDENTIFICATION ABILITIES IN ADOLESCENTS

We identified odour-object dependent differences in odour familiarity between adolescents and adults. While adolescents had higher familiarity scores for candy, adults had higher familiarity scores for spices/seasoning and environmental odours. We applied the adolescent odour familiarity in the modification process of the SIT-16, validated the test in an adolescent group, and subsequently applied the SIT-16jr test to adults and adolescents. With this modified test, adolescents and adults had comparable odour identification scores.

While the findings on odour familiarity may be intuitively logical, this study represents the first data-driven analysis of odour familiarity differences between adolescents and adults. It led to a replacement of 52% of existing descriptors during the modification process of the Danish adult odour identification test. This is comparable with previous validation studies for adapting SIT-16 to a new cultural setting, where it was necessary to change 40-73% of descriptors due to low familiarity ratings [156,157]. As a consequence of differences in experience, knowledge, and perhaps food-habits or time spent on *e.g.* cooking and gardening, it would not be far fetched to claim that there is a cultural difference between adolescents and adults. This is important to take into account when investigating odour identification abilities in children and adolescents in future studies.

These odour-object specific changes in odour familiarity between adolescents and adult could reflect an undergoing learning process: experience with spices-related odours can dawdle until adolescents start spending time on cooking themselves; the low familiarity of environmental odours could to a high degree driven be by the lack of experience and interest in flowers (due to the large difference in lavender and lilac familiarity) and cleaning (due to the large difference in turpentine familiarity); and the differences in familiarity of acrid foods may reflect a low preference for these kinds of food. These differences in familiarity may also simply be a reflection of a lower degree of attention to the odours encountered in everyday life during this period of time, as the personal significance of odours have been shown to increase with age in adolescents [124]. Furthermore, as candy odours were the only odour-object group where the adult population had lower familiarity scores, a link between odour exposure, familiarity, and memory could be hypothesised.

5.4 BRAIN FINGERPRINTS OF OLFACTION: A NOVEL STRUCTURAL METHOD FOR ASSESSING OLFACTORY CORTICAL NETWORKS IN HEALTH AND DISEASE

By using probabilistic tractography, we have developed a method of adding information of underlying structural connectivity to validate existing templates of the olfactory cortex. We have tested two olfactory cortical templates, one defined by anatomical studies [122], and one defined by statistically combining previous functional neuroimaging (fMRI and PET) studies [120]. We found that the template based on functional activation meta-analysis contain white matter, CSF, and non-primary olfactory regions (putamen, parahippocampus, and hippocampus). Furthermore, both templates lacked important structural connections to key secondary olfactory brain regions.

5.4.1 Differences in OCN templates

As the lateral olfactory tract connects to several deep regions of the brain, identification and accurate parcellation of the olfactory cortex is troublesome. There are different templates of the olfactory cortex available, such as the sOCN [122] and fOCN [120], while others define their own template by drawing a region of interest directly in MNI space based on own anatomical experience [158]. Both the sOCN [159-162] and the fOCN [163-165] have been used previously as templates for analysing olfactory cortex activation in neuroimaging studies. With the large differences in these templates for assessing and interpreting olfactory processing, it is difficult – if not directly misleading – to assume that the findings on olfactory processing in one neuroimaging study are transferable to another study if widely different templates for the olfactory cortex is used due to the apparent segregation of function within this cortical area.

5.4.2 Segregation of the primary olfactory cortex

Unlike other sensory modalities, such as vision and hearing, the sense of smell does not seem to have an obvious topographical organisation. However, the piriform cortex seems to be segregated in its processing of olfactory input [102,153,166], which may be the closest approximation of a topographical organisation to date. Where the anterior piriform cortex generates neural patterns of activation even prior to odour stimulation (tightly choreographed with OFC activity) the posterior piriform cortex seems to be more targeted to the odour-stimulation itself [167]. This segregation has

been interpreted as an early sensory representation of predictive modeling, demonstrating the importance of including both the posterior and anterior part of the piriform cortex in an OC parcellation. The sub-parcellation of the mOCN (Figure 4.4) highlights the segregation of the structural connectivity in primary olfactory cortex, and the need for an inclusion of all of these areas in an olfactory cortical template in order to ensure a representative description of olfactory processing.

5.4.3 Clinical implications

As olfactory processing seems to be perturbed in a heterogeneous group of diseases, ranging from neuropsychiatric to neurodegenerative to sino-nasal diseases, there are many implications for this more accurate OCN template.

A reduced activation of hippocampus and amygdala in Parkinson's patients has been demonstrated with functional neuroimaging studies [168]. Furthermore, olfactory impairment in Parkinson's patients is positively correlated with decreased grey matter volumes of piriform cortex and amygdala [113,169], as well as with white matter changes around the OC [112]. The structural olfactory fingerprint can offer a much more detailed insight into these alterations of neural pathways. If combined with other neuroimaging modalities, such as resting state fMRI [170] and MEG [98], and correlated with accurate psychophysical olfactory testing, this could create a strong tool for investigating central olfactory pathology in Parkinson's disease *e.g.* the alterations behind different Braak stages [127]. As such, an OCN template that can be used across neuroimaging modalities – both structural and functional – offers new possibilities in the multimodal investigations on olfactory processing, in both health and disease.

5.5 GENERAL DISCUSSION

5.5.1 Complexity and implications of olfactory testing

The four studies in this thesis highlight the necessity of accuracy in olfactory testing. The validation study of the olfactory identification test emphasises the importance of challenging assumptions of equal odour familiarity across borders. Small cultural differences may have large effects on odour identification abilities, which calls for a modification of the test before clinical implementation. The study on overlapping volatile odours highlights the importance of validating an olfactory test after modification, as descriptors may be perceived analogous due to overlapping odour images in normosmics. The development of odour familiarity seems to follow an exposure-dependent pattern in adolescents, which influences the ability to identify certain groups of odours. The study on structural olfactory connectivity identified significant discrepancies in existing olfactory templates. The highly segregated function and structural connectivity underline the importance of accurately defining the cortical area of primary olfactory processing in neuroimaging studies.

Preexisting knowledge of an odour can have a major impact on interpretation and processing. There seems to be an inherent interdependent relationship between familiarity and memory; a preexisting knowledge of the odour is required for the odour to be familiar; however, if the odour is not familiar, the memory of that odour is less stable [171]. It is unclear which neural mechanisms underlie such development in odour processing. However, though odour familiarity can give a small insight into how the perception of smell changes in different developmental stages, it can also play an important - but more supportive - role as a behavioural measure to improve interpretation of olfactory neuroimaging data (perhaps in conjunction with hedonic odour rating). The developmental differences in olfactory perception can give insights into how the neural processing of olfaction develops, and perhaps offer a deeper understanding of pathological processes when olfactory abilities degenerate in neuropsychiatric and neurodegenerative diseases.

A large structural neuroimaging study found that some of the brain areas containing a mixture of olfactory primary and secondary regions had proportionally larger increases in nodal degree and fibre density from adolescence to adulthood compared to the average developmental changes of the brain [172]. However, as the primary focus of this study is to produce a broader description of developmental changes, the parcellation used was not optimised for analysing specific changes to the olfactory regions of interest. Other studies have investigated the computational processes of the brain by combining the networks of functional connectivity with the structural connectivity of the brain [147]. As such, this suggests the existence of many interesting new methodological approaches to investigating olfactory development, processing and plasticity. With a more accurate template of the olfactory cortex, it is only a matter of applying these existing methods to an olfactory testing paradigm, in order to achieve new insights into olfactory processing. This could serve as a method for establishing an olfactory profile (*e.g.* in different types and phases of dementia, depression or Parkinson's disease), and prove to be a valuable tool in assisting diagnosis. Furthermore, it could potentially be used as a biomarker for disease progression and a surrogate marker for disease modifying drug efficacy [64,173,174]. However, due to the many possible confounders in olfaction, meticulous efforts must be made to control for confounders and effect modulating factors.

5.5.2 Overall conclusion

In the four studies presented in this thesis, there is clear common emphasis on improving olfactory testing. This was accomplished by implementing a validated test in Denmark, investigating some of the key underlying mechanisms and pitfalls in psychophysical testing, and combining anatomical functional neuroimaging knowledge with our own findings of olfactory networks of structural connectivity. By improving the behavioural measures of olfactory testing across cultures, diseases and age groups, and applying multimodal neuroimaging, a better understanding of olfaction shall be achievable. Though much remains to be learned, the work presented in this thesis adds a fraction of fundamental novelty towards a more unified field of neuroimaging research in olfaction.

6. PERSPECTIVES AND FUTURE STUDIES

6.1 POTENTIAL APPLICATION OF FINDINGS IN FUTURE STUDIES

Investigation of changes in structural olfactory connectivity is already underway. In our research group, we have obtained data for applying the olfactory fingerprinting method on schizophrenia patients, and we have currently planned scans on different groups of anosmic patients and hyperosmics individuals. Future MRI-DTI scans of these populations will be used to obtain the structural olfactory fingerprinting. The studies have already paved the way for the implementation of clinical olfactory testing in Denmark, as part of Flavour Institute and the Central Denmark Region. In addition to the studies mentioned above, we plan to initiate a PhD project on olfactory learning and plasticity, which will use the fingerprinting methodology presented in this thesis.

In the near future, we hope to apply the method to several other clinical groups of interest, including depression, phantosmia and neurodegenerative disorders in early and late stages. Combining the structural connectivity measure with a functional network analysis would further strengthen the current knowledge of olfactory processing. This would allow the development of whole-brain computational models, and the incorporation of behavioural measures into the framework, towards a solid characterisation of the dynamical patterns of network integration/segregation linked to olfactory processing. Its application to studies involving olfactory training could provide a powerful tool to investigate neural

plasticity in both health and disease – perhaps even a first step in understanding how to stop or reverse olfactory loss?

6.2 POTENTIAL LIMITATIONS

The studies presented in this thesis reflect only a part of the complexity of olfaction and olfactory testing – from the level of chemical perception, to aspects altering expectations and knowledge, such as cultural, linguistic, and age-related differences in central processing, to the complexity of defining the primary olfactory cortex, thus interpreting neuroimaging studies. Accordingly, the literature upon which our list of secondary olfactory cortices is based may also suffer from incomplete definitions and inaccuracies. However, the structural olfactory fingerprint is based on a dynamic script, where new information can be incorporated into the pipeline, whether this is new knowledge on important functional activation or higher resolution structural neuroimaging techniques.

In a complex system, such as the olfactory system, it is difficult to control for all confounding variables. In the validation study of the Danish SIT12, we included one hundred participants, however, in our collection of normative data for validation of the 32-item extended version of the Sniffin' Sticks, one of the descriptors from the SIT12 seems to be only correctly identified by around 65%, hence not reaching the required 75%. This may be explained by the fact that identification accuracy decreases with longer duration of testing, but may also reflect that a sample size of one hundred subjects may not be enough to ensure generalisability across the entire population.

Even though we found a homogeneous structural olfactory fingerprint in a young population, the shape of the brain can change in relation to disease, age and/or atrophy. It is therefore necessary to include age-matched controls in studies on diseases with olfactory loss, where the pipeline for the OCN analysis needs to be rerun and potentially optimised. As the spatial resolution of neuroimaging increases, it may furthermore be possible to incorporate additional information in the olfactory fingerprint, such as the neural tracts of the mitral and tufted cells from the olfactory bulb in order to optimise the definition of the olfactory cortex even further. However, at present, such would not be possible, attending the very limited structural resolution of DTI sequences.

7. SUMMARY

We perceive the world through our senses. The dependence on these sensory stimuli becomes obvious when we see a visually impaired individual with a guide dog or an individual using sign language. However, individuals with olfactory deficits suffer from a more concealed impairment without any opportunity for diagnostics or treatment in the Danish healthcare system. Around a fifth of the population experience olfactory deficits, of which 1-2% are functionally anosmic. The personal consequences for anosmics can be extensive, lacking not only in hedonic yield related to eating and drinking, but also the socialization during dinners can become niggling. Similarly, social attraction and repulsion can be affected, however, effects linger beyond social consequences and quality of life. An olfactory deficit is a common early symptom in several neurologic, neurodegenerative, and psychiatric diseases. These associations may be due to the central location of the primary olfactory cortex, tugged in and between hedonic hotspots of the brain, and its hard-wired structural connections to key hubs of consciousness and memory in the brain.

A better knowledge of olfactory receptors and odour perception has emerged during the past few decades. This has given rise to a deeper understanding of the etiologies behind olfactory deficits, to the anticipation of utilising the diagnostic potential of olfaction as a prodromal marker of disease, and ultimately to the prospect of improving treatment options for these patients. Much has been accomplished within the field, but much is still beyond our grasp.

In Denmark, the focus on olfaction and olfactory testing has been scarce, at best. The first step of my PhD was therefore a review in Danish, published in the most widely distributed and read Danish journal, "*Ugeskrift for Laeger*", in order to raise awareness on olfactory testing and on a clinical olfactory focus in Central Denmark Region. Secondly, we validated a tool for assessing olfactory function in Danish. The Danish 12-odourant "Sniffin' Sticks" identification test (SIT-12) was modified, validated and published, which allowed us to focus on other aspects of olfactory perception and olfactory testing.

One focus has been to investigate the possible role of overlapping volatile chemical molecules in differentiating closely related descriptors. This study was conducted in order to emphasize the need for a meticulous approach when conducting validation studies of olfactory tests, especially the need for re-validation after a modification process.

Another focus has been on investigating the differences between olfactory identification abilities in adolescents relative to adults. Previous international studies have shown that the identification skills of adolescents are significantly different from their adult compatriots. Earlier validation studies on adolescents have used the adult version of odorant descriptors as the starting point. In our study we examine the role of odour familiarity in the difference between adult and adolescent identification abilities.

The main focus of this PhD has been to develop a method for evaluating central olfactory patency and processing, where individual preferences and sensitivity to specific odours could be removed from the equation, as these parameters has proven troublesome in functional neuroimaging of the olfactory system. We identified that a reference area for investigating primary olfactory processing in neuroimaging included several non-primary structures and lacked the structural neural connections to key secondary olfactory areas. Consequently, we redefined the template for olfactory processing by combining findings from anatomical and functional neuroimaging studies. This has led to the creation of a structural olfactory fingerprint, which is already integrated in six ongoing studies as a tool to investigate pathologic and benign changes in structural olfactory pathways.

8. REFERENCES

1. Philpott CM, Bennett A, Murty GE. A brief history of olfaction and olfactometry. J Laryngol Otol 2008;122:1–6.
2. Schlosser G. Making Senses: Development of Vertebrate Cranial Placodes. Int Rev Cell Molec Biol International review of cell and molecular biology; 2010;283:129–234.
3. Meisel JD, Panda O, Mahanti P, Schroeder FC, et al. Chemosensation of bacterial secondary metabolites modulates neuroendocrine signaling and behavior of C. elegans. Cell Elsevier; 2014;159:267–80.
4. Shepherd GM. Perception without a thalamus how does olfaction do it? Neuron 2005;46:166–8.
5. Süskind P. Das Parfum. Diogenes; 1985. pp. 1–320.
6. Nishitani S, Miyamura T, Tagawa M, Sumi M, et al. The calming effect of a maternal breast milk odor on the human newborn infant. Neuroscience Research 2009;63:66–71.
7. Havlicek J, Roberts SC. MHC-correlated mate choice in humans: A review. Psychoneuroendocrinology 2009;34:497–512.

8. Kromer J, Hummel T, Pietrowski D, Giani AS, et al. Influence of HLA on human partnership and sexual satisfaction. Sci Rep Nature Publishing Group; 2016;6:1–6.

9. Kringelbach ML. Food for thought: hedonic experience beyond homeostasis in the human brain. Neuroscience 2004;126:807–19.

10. Fjaeldstad A, Van Hartevelt TJ, Kringelbach ML. Pleasure of food in the brain. In: Piqueras-Fiszman B, Spence C, editors. Multisensory Flavor Perception 1st ed Elsevier Ltd; 2016. pp. 211–34.

11. Sullivan RM, Wilson DA, Ravel N, Mouly A-M. Olfactory memory networks: from emotional learning to social behaviors. Frontiers in Behavioral Neuroscience Frontiers; 2015;9:36.

12. Forbes RJ. Studies in ancient technology [Internet]. 2nd ed. E J Brill; 1965. pp. 1–110.Available from: https://books.google.dk/books?id=5JAeAAAAIAAJ&printsec=copyright#v=onepage&q&f=false

13. Vogt RG, Prestwich GD, Lerner MR. Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. J Neurobiol Wiley Subscription Services, Inc, A Wiley Company; 1991;22:74–84.

14. Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 1991;65:175–87.

15. Block E, Jang S, Matsunami H, Sekharan S, et al. Implausibility of the vibrational theory of olfaction. Proceedings of the National Academy of Sciences 2015;112:E2766–74.

16. Turin L, Gane S, Georganakis D, Maniati K, et al. Plausibility of the vibrational theory of olfaction. Proc Natl Acad Sci U S A 2015;112:E3154.

17. Reese A, List NH, Kongsted J, Solov'yov IA. How Far Does a Receptor Influence Vibrational Properties of an Odorant? Skoulakis EMC, editor. PLoS ONE Public Library of Science; 2016;11:e0152345–21.

18. Hanchate NK, Kondoh K, Lu Z, Kuang D, et al. Single-cell transcriptomics reveals receptor transformations during olfactory neurogenesis. Science 2015;350:1251–5.

19. Nara K, Saraiva LR, Ye X, Buck LB. A large-scale analysis of odor coding in the olfactory epithelium. J Neurosci 2011;31:9179–91.

20. Shepherd GM. Smell images and the flavour system in the human brain. Nature; 2006;444:316–21.

21. Mainland JD, Keller A, Li YR, Zhou T, et al. The missense of smell: functional variability in the human odorant receptor repertoire. Nat Neurosci 2014;17:114–20.

22. Jiang Y, Matsunami H. Mammalian odorant receptors: functional evolution and variation. Current Opinion in Neurobiology Elsevier Ltd; 2015;34:54–60.

23. McRae JF, Mainland JD, Jaeger SR, Adipietro KA, et al. Genetic variation in the odorant receptor OR2J3 is associated with the ability to detect the 'grassy' smelling odor, cis-3-hexen-1-ol. Chem Senses 2012;37:585–93.

24. Eriksson N, Wu S, Do CB, Kiefer AK, et al. A genetic variant near olfactory receptor genes influences cilantro preference. Flavour BioMed Central; 2012;1:22–9.

25. Mauer L, El-Sohemy A. Prevalence of cilantro (Coriandrum sativum) disliking among different ethnocultural groups. Flavour BioMed Central; 2012;1:8.

26. Small DM, Gerber JC, Mak YE. Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. Neuron 2005;47:593–605.

27. Rowe TB, Shepherd GM. Role of ortho-retronasal olfaction in mammalian cortical evolution. J Comp Neurol 2016;524:471–95.

28. Welge-Lüssen A, Ebnöther M, Wolfensberger M. Swallowing is differentially influenced by retronasal compared with orthonasal stimulation in combination with gustatory stimuli. Chem Senses Oxford University Press; 2009;34:499–502.

29. Mombaerts P, Wang F, Dulac C, Chao SK, et al. Visualizing an olfactory sensory map. Cell 1996;87:675–86.

30. Grabe V, Baschwitz A, Dweck HKM, Lavista-Llanos S, et al. Elucidating the Neuronal Architecture of Olfactory Glomeruli in the Drosophila Antennal Lobe. CellReports ElsevierCompany; 2016;16:3401–13.

31. Liu H, Guthrie KM. Neuronal replacement in the injured olfactory bulb. Experimental Neurology 2011;228:270–82.

32. Weiss T, Sobel N. What's primary about primary olfactory cortex? Nat Neurosci Nature Publishing Group; 2012;15:10–2.

33. Rombaux P, Duprez T, Hummel T. Olfactory bulb volume in the clinical assessment of olfactory dysfunction. Rhinology 2009;47:3–9.

34. Huart C, Rombaux P, Hummel T. Plasticity of the Human Olfactory System: The Olfactory Bulb. Molecules Multidisciplinary Digital Publishing Institute; 2013;18:11586–600.

35. Arruda D, Publio R, Roque AC. The Periglomerular Cell of the Olfactory Bulb and its Role in Controlling Mitral Cell Spiking: A Computational Model. Cymbalyuk G, editor. PLoS ONE Public Library of Science; 2013;8:e56148–14.

36. Mori K, Sakano H. How Is the Olfactory Map Formed and Interpreted in the Mammalian Brain? Annu Rev Neurosci 2011;34:467–99.

37. Carmichael ST, Clugnet MC, Price JL. Central olfactory connections in the macaque monkey. J Comp Neurol 1994;346:403–34.

38. Igarashi KM, Ieki N, An M, Yamaguchi Y, et al. Parallel mitral and tufted cell pathways route distinct odor information to different targets in the olfactory cortex. Journal of Neuroscience 2012;32:7970–85.

39. Gottfried JA, Zald DH. On the scent of human olfactory orbitofrontal cortex: Meta-analysis and comparison to non-human primates. Brain Research Reviews 2005;50:287–304.

40. Fjaeldstad A, Fernandes HM, Van Hartevelt TJ, Gleesborg C, et al. Brain fingerprints of olfaction: a novel structural method for assessing olfactory cortical networks in health and disease. Sci Rep 2017;7:42534.

41. Allam MD-E, Marlier L, Schaal B. Learning at the breast:

Preference formation for an artificial scent and its attraction against the odor of maternal milk. Infant Behavior and Development 2006;29:308–21.

42. Aoyama S, Toshima T, Saito Y, Konishi N, et al. Maternal breast milk odour induces frontal lobe activation in neonates: A NIRS study. Early Human Development Elsevier Ireland Ltd; 2010;86:541–5.

43. Asaba A, Hattori T, Mogi K, Kikusui T. Sexual attractiveness of male chemicals and vocalizations in mice. Front Neurosci Frontiers; 2014;8:231.

44. Kondoh K, Lu Z, Ye X, Olson DP, et al. A specific area of olfactory cortex involved in stress hormone responses to predator odours. Nature Nature Publishing Group; 2016;532:1–15.

45. Kringelbach ML, Berridge KC. The functional neuroanatomy of pleasure and happiness. Discov Med 2010;9:579–87.

46. Berridge KC, Kringelbach ML. Pleasure Systems in the Brain. Neuron Elsevier Inc; 2015;86:1–20.

47. de Groot JHB, Smeets MAM, Kaldewaij A, Duijndam MJA, et al. Chemosignals communicate human emotions. Psychological Science SAGE Publications; 2012;23:1417–24.

48. Gelstein S, Yeshurun Y, Rozenkrantz L, Shushan S, et al. Human tears contain a chemosignal. Science 2011;331:226–30.

49. Aschenbrenner K, Hummel C, Teszmer K, Krone F, et al. The influence of olfactory loss on dietary behaviors. Laryngoscope 2008;118:135–44.

50. Neuland C, Bitter T, Marschner H, Gudziol H, et al. Health-related and specific olfaction-related quality of life in patients with chronic functional anosmia or severe hyposmia. Laryngoscope 2011;121:867–72.

51. Croy I, Nordin S. Olfactory Disorders and Quality of Life--An Updated Review. Chem Senses 2014;39:185–94.

52. Brämerson A, Johansson L, Ek L, Nordin S, et al. Prevalence of olfactory dysfunction: The Skövde population-based study. Laryngoscope John Wiley & Sons, Inc; 2004;114:733–7.

53. Mullol J, Alobid I, Marino-Sanchez F, Quinto L, et al. Furthering the understanding of olfaction, prevalence of loss of smell and risk factors: a population-based survey (OLFACAT study). BMJ Open 2012;2:e001256–6.

54. Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the 'Sniffin' Sticks' including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol 2006;264:237–43.

55. Fark T, Hummel T. Olfactory disorders: distribution according to age and gender in 3,400 patients. Eur Arch Otorhinolaryngol Springer-Verlag; 2013;270:777–9.

56. Vennemann MM, Berger K. The association between smoking and smell and taste impairment in the general population. J Neurol D Steinkopff-Verlag; 2008;255:1121–6.

57. Fjaeldstad A, Clausen CH, Kjærgaard T, Ovesen T. [The forgotten cranial nerve - clinical importance of olfaction]. Ugeskr Laeger 2015;177:265–9.

58. Sohrabi HR, Bates KA, Weinborn MG, Johnston ANB, et al. Olfactory discrimination predicts cognitive decline among community-dwelling older adults. Transl Psychiatry 2012;2:e118.

59. Haehner A, Reichmann H. Olfactory loss in Parkinson's disease. Parkinsons Dis Hindawi Publishing Corporation; 2011;2011:450939–6.

60. Devanand DP, Michaels-Marston KS, Liu X, Pelton GH, et al. Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. Am J Psychiatry 2000;157:1399–405.

61. Mahlknecht P, Iranzo A, Högl B, Frauscher B, et al. Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD. Neurology 2015;84:654–8.

62. Casjens S, Eckert A, Woitalla D, Ellrichmann G, et al. Diagnostic value of the impairment of olfaction in Parkinson's Disease. Hummel T, editor. PLoS ONE 2013;8:e64735.

63. Ponsen MM, Stoffers D, Wolters EC, Booij J, et al. Olfactory testing combined with dopamine transporter imaging as a method to detect prodromal Parkinson's disease. Journal of Neurology, Neurosurgery & Psychiatry BMJ Publishing Group Ltd; 2010;81:396–9.

64. McEvoy LK, Brewer JB. Biomarkers for the clinical evaluation of the cognitively impaired elderly: amyloid is not enough. Imaging Med 2012;4:343–57.

65. Damm M, Temmel A, Welge-Lussen A, Eckel HE, et al. [Olfactory dysfunctions. Epidemiology and therapy in Germany, Austria and Switzerland]. HNO Springer-Verlag; 2004;52:112–20.

66. Fark T, Hummel C, Hähner A, Nin T, et al. Characteristics of taste disorders. Eur Arch Otorhinolaryngol 2013;270:1855–60.

67. Lötsch J, Ultsch A, Hummel T. A Unifying Data-Driven Model of Human Olfactory Pathology Representing Known Etiologies of Dysfunction. Chem Senses 2016;:bjw089–8.

68. Croy I, Zehner C, Larsson M, Zucco GM. Test-Retest Reliability and Validity of the Sniffin' TOM Odor Memory Test. Chem Senses 2015;40:173–9.

69. Eibenstein A, Fioretti AB, Lena C, Rosati N, et al. Olfactory screening test: experience in 102 Italian subjects. Acta Otorhinolaryngol Ital 2005;25:18–22.

70. Konstantinidis I, Printza A, Genetzaki S, Mamali K, et al. Cultural adaptation of an olfactory identification test: the Greek version of Sniffin' Sticks. Rhinology 2008;46:292–6.

71. Boesveldt S, Verbaan D, Knol DL, van Hilten JJ, et al. Odour identification and discrimination in Dutch adults over 45 years. Rhinology 2008;46:131–6.

72. Neumann C, Tsioulos K, Merkonidis C, Salam M, et al. Validation study of the 'Sniffin' Sticks' olfactory test in a British population: a preliminary communication. Clin Otolaryngol Blackwell Publishing Ltd; 2012;37:23–7.

73. Tekeli H, Altundağ A, Salihoğlu M, Cayönü M, et al. The applicability of the 'Sniffin' Sticks' olfactory test in a Turkish population. Med Sci Monit 2013;19:1221–6.

74. Sorokowska A, Hummel T. Polska wersja testu Sniffin' Sticks

– adaptacja i normalizacja. Otolaryngologia Polska 2014;68:308–14.

75. Ribeiro JC, Simões J, Silva F, Silva ED, et al. Cultural Adaptation of the Portuguese Version of the 'Sniffin' Sticks' Smell Test: Reliability, Validity, and Normative Data. Matsunami H, editor. PLoS ONE Public Library of Science; 2016;11:e0148937–12.

76. Kobal G, Hummel T, Sekinger B, Barz S, et al. 'Sniffin' sticks': screening of olfactory performance. Rhinology 1996;34:222–6.

77. Hummel T, Sekinger B, Wolf SR, Pauli E, et al. 'Sniffin' Sticks': Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. Chem Senses Oxford University Press; 1997;22:39–52.

78. Gudziol H, Hummel T. Normative values for the assessment of gustatory function using liquid tastants. Acta Otolaryngol 2007;127:658–61.

79. Gudziol V, L tsch JR, H hner A, Zahnert T, et al. Clinical Significance of Results from Olfactory Testing. Laryngoscope John Wiley & Sons, Inc; 2006;116:1858–63.

80. Haehner A, Mayer AM, Landis BN, Pournaras I, et al. High test-retest reliability of the extended version of the 'Sniffin' Sticks' test. Chem Senses 2009;34:705–11.

81. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. Physiol Behav 1984;32:489–502.

82. Hugh SC, Siu J, Forte V, Campisi P, et al. Olfactory testing in children using objective tools: comparison of Sniffin' Sticks and University of Pennsylvania Smell Identification Test (UPSIT). J Otolaryngol Head Neck Surg BioMed Central Ltd; 2015;44:10.

83. Wysocki CJ, Gilbert AN. National Geographic Smell Survey. Effects of age are heterogenous. Annals of the New York Academy of Sciences 1989;561:12–28.

84. Lötsch J, Reichmann H, Hummel T. Different odor tests contribute differently to the evaluation of olfactory loss. Chem Senses 2008;33:17–21.

85. Whitcroft KL, Cuevas M, Haehner A, Hummel T. Patterns of olfactory impairment reflect underlying disease etiology. Laryngoscope 2016;:1–6.

86. Renner B, Mueller CA, Dreier J, Faulhaber S, et al. The candy smell test: a new test for retronasal olfactory performance. Laryngoscope Wiley Subscription Services, Inc, A Wiley Company; 2009;119:487–95.

87. Croy I, Hoffmann H, Philpott C, Rombaux P, et al. Retronasal testing of olfactory function: an investigation and comparison in seven countries. Eur Arch Otorhinolaryngol 2013;271:1087–95.

88. Heilmann S, Strehle G, Rosenheim K, Damm M. Clinical assessment of retronasal olfactory function. Arch Otolaryngol Head Neck Surg 2002;128:414–8.

89. Doty RL, Smith R, McKeown DA, Raj J. Tests of human olfactory function: principal components analysis suggests that most measure a common source of variance. Percept Psychophys Springer-Verlag; 1994;56:701–7.

90. Doty RL, McKeown DA, Lee WW, Shaman P. A study of the test-retest reliability of ten olfactory tests. Chem Senses 1995;20:645–56.

91. Reden J, Draf C, Frank RA, Hummel T. Comparison of clinical tests of olfactory function. Eur Arch Otorhinolaryngol Springer Berlin Heidelberg; 2015;273:927–31.

92. Rombaux P, Huart C, Mouraux A. Assessment of chemosensory function using electroencephalographic techniques. Rhinology 2012;50:13–21.

93. Lapid H, Hummel T. Recording odor-evoked response potentials at the human olfactory epithelium. Chem Senses Oxford University Press; 2013;38:3–17.

94. Caminiti F, Ciurleo R, De Salvo S, Bramanti P, et al. Post-traumatic olfactory loss: Psychophysical, electrophysiological and neuroradiological findings in three single case studies. Brain Inj 2014;28:1776–80.

95. Croy I, Symmank A, Schellong J, Hummel C, et al. Olfaction as a marker for depression in humans. Journal of Affective Disorders Elsevier; 2014;160:80–6.

96. Turetsky BI, Moberg PJ. An odor-specific threshold deficit implicates abnormal intracellular cyclic AMP signaling in schizophrenia. Am J Psychiatry 2009;166:226–33.

97. Decker JR, Meen EK, Kern RC, Chandra RK. Cost effectiveness of magnetic resonance imaging in the workup of the dysosmia patient. International Forum of Allergy & Rhinology 2012;3:56–61.

98. Boesveldt S, Stam CJ, Knol DL, Verbunt JPA, et al. Advanced time-series analysis of MEG data as a method to explore olfactory function in healthy controls and Parkinson's disease patients. Hum Brain Mapp Wiley Subscription Services, Inc, A Wiley Company; 2009;30:3020–30.

99. Walla P, Hufnagl B, Lehrner J, Mayer D, et al. Evidence of conscious and subconscious olfactory information processing during word encoding: a magnetoencephalographic (MEG) study. Brain Res Cogn Brain Res 2002;14:309–16.

100. Hämäläinen M, Hari R, Ilmoniemi RJ, Knuutila J, et al. Magnetoencephalography—theory, instrumentation, and applications to noninvasive studies of the working human brain. Reviews of Modern Physics American Physical Society; 1993;65:413–97.

101. Miltner W, Braun C, Johnson R, Simpson GV. A test of brain electrical source analysis (BESA): a simulation study. Electroencephalography and Clinical Neurophysiology 1994;91:295–310.

102. Gottfried JA. Central mechanisms of odour object perception. Nat Rev Neurosci 2010;11:628–41.

103. Balderston NL, Schultz DH, Baillet S, Helmstetter FJ. Rapid Amygdala Responses during Trace Fear Conditioning without Awareness. Barnes GR, editor. PLoS ONE 2014;9:e96803–11.

104. Mohseni HR, Kringelbach ML, Woolrich MW, Baker A, et al. Non-Gaussian probabilistic MEG source localisation based on kernel density estimation. NeuroImage The Authors; 2014;87:444–64.

105. Mills T, Lalancette M, Moses SN, Taylor MJ, et al. Techniques for Detection and Localization of Weak Hippocampal and Medial Frontal Sources Using Beamformers in MEG. Brain Topogr 2012;25:248–63.

106. Salvia E, Bestelmeyer PEG, Kotz SA, Rousselet GA, et al. Single-subject analyses of magnetoencephalographic evoked responses to the acoustic properties of affective non-verbal vocalizations. Front Neurosci Frontiers; 2014;8:422.

107. Morrot G, Bonny J-M, Lehallier B, Zanca M. fMRI of human olfaction at the individual level: interindividual variability. J Magn Reson Imaging Wiley Subscription Services, Inc, A Wiley Company; 2013;37:92–100.

108. Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A National Acad Sciences; 2016;113:7900–5.

109. O'Herron P, Chhatbar PY, Levy M, Shen Z, et al. Neural correlates of single-vessel haemodynamic responses in vivo. Nature 2016;534:378–82.

110. Segura B, Baggio HC, Solana E, Palacios EM, et al. Neuroanatomical correlates of olfactory loss in normal aged subjects. Behavioural Brain Research 2013;246:148–53.

111. Hagmann P, Cammoun L, Gigandet X, Meuli R, et al. Mapping the structural core of human cerebral cortex. Friston KJ, editor. PLoS Biol 2008;6:e159.

112. Ibarretxe-Bilbao N, Junqué C, Marti M-J, Valldeoriola F, et al. Olfactory impairment in Parkinson's disease and white matter abnormalities in central olfactory areas: A voxel-based diffusion tensor imaging study. Mov Disord 2010;25:1888–94.

113. Wu X, Yu C, Fan F, Zhang K, et al. Correlation between progressive changes in piriform cortex and olfactory performance in early Parkinson's disease. Eur Neurol 2011;66:98–105.

114. Sporns O, Tononi G, Kötter R. The Human Connectome: A Structural Description of the Human Brain. PLoS Comp Biol 2005;1:e42.

115. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J Magn Reson B 1996;111:209–19.

116. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435–55.

117. Hagmann P, Cammoun L, Gigandet X, Gerhard S, et al. MR connectomics: Principles and challenges. Journal of Neuroscience Methods 2010;194:34–45.

118. Cammoun L, Gigandet X, Meskaldji D, Thiran J-P, et al. Mapping the human connectome at multiple scales with diffusion spectrum MRI. Journal of Neuroscience Methods 2012;203:386–97.

119. Johansen-Berg H, Rushworth MFS. Using diffusion imaging to study human connectional anatomy. Annu Rev Neurosci 2009;32:75–94.

120. Seubert J, Freiherr J, Frasnelli J, Hummel T, et al. Orbitofrontal cortex and olfactory bulb volume predict distinct aspects of olfactory performance in healthy subjects. Cereb Cortex Oxford University Press; 2013;23:2448–56.

121. Seubert J, Freiherr J, Djordjevic J, Lundström JN. Statistical localization of human olfactory cortex. NeuroImage Elsevier Inc; 2013;66:333–42.

122. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, et al. Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage 2002;15:273–89.

123. Martinec Nováková L, Plotěná D, Roberts SC, Havlicek J. Positive relationship between odor identification and affective responses of negatively valenced odors. Front Psychol 2015;6:607.

124. Oleszkiewicz A, Walliczek-Dworschak U, Klötze P, Gerber F, et al. Developmental Changes in Adolescents' Olfactory Performance and Significance of Olfaction. de Castro F, editor. PLoS ONE Public Library of Science; 2016;11:e0157560–9.

125. Sorokowska A, Schriever VA, Gudziol V, Hummel C, et al. Changes of olfactory abilities in relation to age: odor identification in more than 1400 people aged 4 to 80 years. Eur Arch Otorhinolaryngol 2015;272:1937–44.

126. Schriever VA, Mori E, Petters W, Boerner C, et al. The 'Sniff-in' Kids' test--a 14-item odor identification test for children. PLoS ONE 2014;9:e101086.

127. Braak H, Ghebremedhin E, Rüb U, Bratzke H, et al. Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res Springer-Verlag; 2004;318:121–34.

128. Visanji NP, Brooks PL, Hazrati L-N, Lang AE. The prion hypothesis in Parkinson's disease: Braak to the future. Acta Neuropathol Commun 2013;1:2.

129. Hawkes CH, Shephard BC, Daniel SE. Is Parkinson's disease a primary olfactory disorder? Qjm 1999;99:269–80.

130. Auffarth B. Understanding smell—The olfactory stimulus problem. Neuroscience & Biobehavioral Reviews Elsevier Ltd; 2013;37:1667–79.

131. Jaeger SR, McRae JF, Bava CM, Beresford MK, et al. A Mendelian trait for olfactory sensitivity affects odor experience and food selection. Curr Biol 2013;23:1601–5.

132. Frye RE. Dose-Related Effects of Cigarette Smoking on Olfactory Function. JAMA 1990;263:1233.

133. Doty RL, Kamath V. The influences of age on olfaction: a review. Front Psychol 2014;5:20.

134. Ferdenzi C, Roberts SC, Schirmer A, Delplanque S, et al. Variability of Affective Responses to Odors: Culture, Gender, and Olfactory Knowledge. Chem Senses 2013;38:175–86.

135. Ghielmini E, Poryazova R, Baumann CR, Bassetti CL. Sleepiness at the Time of Testing Impairs Olfactory Performance. Eur Neurol 2013;69:58–64.

136. Zelano C, Bensafi M, Porter J, Mainland J, et al. Attentional modulation in human primary olfactory cortex. Nat Neurosci 2004;8:114–20.

137. Anderson AK, Christoff K, Stappen I, Panitz D, et al. Dissociated neural representations of intensity and valence in human olfaction. Nat Neurosci 2003;6:196–202.

138. Kringelbach ML, O'Doherty J, Rolls ET, Andrews C. Activa-

tion of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. Cereb Cortex 2003;13:1064–71.

139. Zatorre RJ, Jones-Gotman M, Evans AC, Meyer E. Functional localization and lateralization of human olfactory cortex. Nature 1992;360:339–40.

140. Turkeltaub PE, Eden GF, Jones KM, Zeffiro TA. Meta-Analysis of the Functional Neuroanatomy of Single-Word Reading: Method and Validation. NeuroImage 2002;16:765–80.

141. Fjaeldstad A, Petersen MA, Ovesen T. Considering Chemical Resemblance: a Possible Confounder in Olfactory Identification Tests. Chem Percept Springer US; 2017;10:42–8.

142. Rumeau C, Nguyen DT, Jankowski R. How to assess olfactory performance with the Sniffin'Sticks test®. European annals of … 2015.

143. Fjaeldstad A, Sundbøll J, Niklassen A, Ovesen T. Odor familiarity and identification abilities in adolescents. Chem Senses 2017;42:239–46.

144. Hagmann P, Jonasson L, Maeder P, Thiran J-P, et al. Understanding Diffusion MR Imaging Techniques: From Scalar Diffusion-weighted Imaging to Diffusion Tensor Imaging and Beyond1. RadioGraphics 2006;26:S205–23.

145. Cabral J, Fernandes HM, Van Hartevelt TJ, James AC, et al. Structural connectivity in schizophrenia and its impact on the dynamics of spontaneous functional networks. Chaos 2013;23:046111.

146. Fernandes HM, Van Hartevelt TJ, Boccard SGJ, Owen SLF, et al. Novel fingerprinting method characterises the necessary and sufficient structural connectivity from deep brain stimulation electrodes for a successful outcome. New J Phys 2015;17:015001.

147. Deco G, Kringelbach ML. Great Expectations: Using Whole-Brain Computational Connectomics for Understanding Neuropsychiatric Disorders. Neuron 2014;84:892–905.

148. Andersson JLR, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. NeuroImage 2003;20:870–88.

149. Behrens TEJ, Woolrich MW, Jenkinson M, Johansen-Berg H, et al. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. 2003;50:1077–88.

150. Behrens TEJ, Berg HJ, Jbabdi S, Rushworth MFS, et al. Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? NeuroImage 2007;34:144–55.

151. Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 1994;18:192–205.

152. Desikan RS, Ségonne F, Fischl B, Quinn BT, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 2006;31:968–80.

153. Gottfried JA, Deichmann R, Winston JS, Dolan RJ. Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. J Neurosci 2002;22:10819–28.

154. Van Hartevelt TJ, Kringelbach ML. The Olfactory System. The Human Nervous System Elsevier; 2012. pp. 1219–38.

155. Gudziol V, Hummel T. The influence of distractors on odor identification. Arch Otolaryngol Head Neck Surg 2009;135:143–5.

156. Shu C-H, Yuan B-C, Lin S-H, Lin C-Z. Cross-cultural application of the 'Sniffin' Sticks' odor identification test. Am J Rhinol 2007;21:570–3.

157. Oleszkiewicz A, Taut M, Sorokowska A, Radwan A, et al. Development of the Arabic version of the 'Sniffin' Sticks' odor identification test. Eur Arch Otorhinolaryngol 2016;273:1179–84.

158. Wang J, Eslinger PJ, Doty RL, Zimmerman EK, et al. Olfactory deficit detected by fMRI in early Alzheimer's disease. Brain Res 2010;1357:184–94.

159. Lötsch J, Walter C, Felden L, Nöth U, et al. The Human Operculo-Insular Cortex Is Pain-Preferentially but Not Pain-Exclusively Activated by Trigeminal and Olfactory Stimuli. Stamatakis EA, editor. PLoS ONE 2012;7:e34798–10.

160. Moessnang C, Pauly K, Kellermann T, Krämer J, et al. The scent of salience — Is there olfactory-trigeminal conditioning in humans? NeuroImage 2013;77:93–104.

161. Milinski M, Croy I, Hummel T, Boehm T. Major histocompatibility complex peptide ligands as olfactory cues in human body odour assessment. Proceedings of the Royal Society B: Biological Sciences 2013;280:20122889–9.

162. Zou L-Q, Yang Z-Y, Wang Y, Lui SSY, et al. What does the nose know? Olfactory function predicts social network size in human. Sci Rep Nature Publishing Group; 2016;6:1–6.

163. Yao L, Pinto JM, Yi X, Li L, et al. Gray Matter Volume Reduction of Olfactory Cortices in Patients With Idiopathic Olfactory Loss. Chem Senses 2014;39:755–60.

164. Cortese BM, McConnell PA, Froeliger B, Leslie K, et al. Burning odor-elicited anxiety in OEF/OIF combat veterans: Inverse relationship to gray matter volume in olfactory cortex. J Psychiatr Res 2015;70:58–66.

165. Sun X, Veldhuizen MG, Babbs AE, Sinha R, et al. Perceptual and Brain Response to Odors Is Associated with Body Mass Index and Postprandial Total Ghrelin Reactivity to a Meal. Chem Senses 2016;41:233–48.

166. Cohen Y, Putrino D, Wilson DA. Dynamic cortical lateralization during olfactory discrimination learning. J Physiol (Lond) 2015;593:1701–14.

167. Zelano C, Mohanty A, Gottfried JA. Olfactory predictive codes and stimulus templates in piriform cortex. Neuron 2011;72:178–87.

168. Westermann B, Wattendorf E, Schwerdtfeger U, Husner A, et al. Functional imaging of the cerebral olfactory system in patients with Parkinson's disease. Journal of Neurology, Neurosurgery & Psychiatry BMJ Publishing Group Ltd; 2008;79:19–24.

169. Wattendorf E, Welge-Lüssen A, Fiedler K, Bilecen D, et al. Olfactory impairment predicts brain atrophy in Parkinson's disease. J Neurosci Society for Neuroscience; 2009;29:15410–3.

170. Su M, Wang S, Fang W, Zhu Y, et al. Alterations in the limbic/paralimbic cortices of Parkinson's disease patients with hyposmia under resting-state functional MRI by regional homogeneity and functional connectivity analysis. Parkinsonism Relat Disord Elsevier Ltd; 2015;21:698–703.

171. Stevenson RJ, Mahmut MK. Familiarity influences odor memory stability. Psychon Bull Rev 2013;20:754–9.

172. Dennis EL, Jahanshad N, McMahon KL, de Zubicaray GI, et al. Development of brain structural connectivity between ages 12 and 30: A 4-Tesla diffusion imaging study in 439 adolescents and adults. NeuroImage 2013;64:671–84.

173. Takeda A, Baba T, Kikuchi A, Hasegawa T, et al. Olfactory dysfunction and dementia in Parkinson's disease. J Parkinsons Dis 2014;4:181–7.

174. Attems J, Walker L, Jellinger KA. Olfactory bulb involvement in neurodegenerative diseases. Acta Neuropathol 2014;127:459–75.