The Correlation between Olfactory Function, Olfactory Bulb Volume, and Olfactory Sulcus Depth in Healthy Subjects

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Master thesis

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CONTENTS

ACKNOWLEDGEMENTS

The research and the work for this master thesis was carried out at The Smell and Taste Clinic, Carl Gustav Carus University Hospital, TU Dresden, Germany.

First, we would like to thank our supervisor Dr. odont. Preet Bano Singh for giving us the opportunity to travel and visit the most acknowledged smell and taste clinic in Europe. You have been a source of great knowledge and inspiration to us. Thank you for guiding us and introducing us to this field of research.

We would also like to thank Prof. Dr. med. Thomas Hummel for welcoming us to his lab with open arms and showing us how research is done on a high level. We feel privileged to have been tutored by one of the greatest scientist in this field.

At last a huge thank you to our dear lab colleagues at the time, Dr. Çağdaş Güdücü, Dr. Annachiara Cavazzana, Dr. Pengfei Han, Dr. Ania Oleszkiewicz, Dr. Antti Knaapila and Charlotte Enger, for sharing their knowledge with us and helping us on our way. We have great many memories from the fun times we had together while staying in Dresden.

ABBREVIATIONS

OB – Olfactory bulb

OS – Olfactory sulcus

OE – Olfactory epithelium

HBPT – Heart beat perception task

TDI – Threshold discrimination identification (olfactory function score)

PHQ – Patient health questionnaire

PPTE – Plane of the posterior tangent through the eyeball

NORWEGIAN ABSTRACT

Introduksjon: Forstyrrelser i luktesansen er veldig vanlig i den generelle befolkningen. Det har blitt rapportert at over 50% av befolkningen over 65 år har forstyrrelser i luktesansen [1]. Luktesansen er også en viktig sanseevne for inntak av et sunt og friskt kosthold. Studier har vist at 50% av pasienter med forstyrret luktesans rapporterer misnøye ved inntak av mat og bruker en større mengde av blant annet sukker i kostholdet [2]. Høyt sukkerinntak kan lede til økt kariesforekomst og andre systemiske sykdommer. Endringer i hjernen i luktesenteret har vært foreslått som mulig årsak til luktforstyrrelser [3]. Likevel vet man lite om sammenhengene mellom luktekolben (olfactory bulbus, OB), sulcus olfactorius (olfactory sulcus, OS) og luktesansen.

Aim: Vi ville undersøke om det var en sammenheng mellom friske menneskers evne til å oppfatte lukt, volumet på deres bulbus olfactorius (OB) og dybden av deres sulcus olfactorius (OS).

Materialer og metoder: Følgende data ble innsamlet fra 31 friske deltagere: Spørreskjema for generell anamnese. Herunder alder, kjønn, psykisk og fysisk helse. Deltakernes evne til å oppfatte lukt ble testet ved bruk av "Sniffin' sticks" systemet. Deres subjektive mening hva gjelder ulike odørs intensitet (intensitetsskår), evnen til å skille mellom ulike odører, evnen til å identifisere ulike odører samt hvor mye odør som skulle til før de klarte å oppfatte odøren ble testet. Hver deltaker fikk basert på dette en totalskår som mål for lukteevne (totalskår for TDI). MR-opptak ble gjort av hver deltaker for å kunne måle størrelsen av luktekolben samt dybden av sulcus olfactorius ved hjelp av "voxel based morphometry". SPSS ble brukt til å se på sammenhenger mellom OB-volumer, OS-dybder og lukteevnen.

Resultater: Resultatene viste en signifikant forskjell mellom de kvinnelige og mannlige deltakerne i studien når det kommer til cerebral prosessering av luktesansen. I den kvinnelige deltagergruppen fant vi signifikant korrelasjon mellom totaldybden til OS, dybden til høyresidig OS og lukteevnen. I den

mannlige deltagergruppen ble ikke dette funnet. Resultatene viste at det var en signifikant forskjell (p = 0,025) mellom unge og eldre kvinner med tanke på lukteevnen. Yngre kvinner hadde bedre luktesans enn eldre kvinner. Unge kvinner hadde også en signifikant bedre luktesansskår enn yngre menn (p = 0,025). Videre analyse av den kvinnelige deltagergruppen viste at det var en signifikant korrelasjon mellom lukteevnen og gjennomsnittlig totalt volum av OB og gjennomsnittlig total dybde av OS. Lignende signifikant korrelasjon ble funnet mellom gjennomsnittlig volum av OB og gjennomsnittlig dybde av OS på høyre side av hjernen og lukteevnen. Det ble ikke funnet en slik korrelasjon for gjennomsnittlig volum av OB og gjennomsnittlig dybde av OS på venstre side av hjernen og luktesansen. Det var også signifikant korrelasjon mellom intensitetsskår (som også er et mål for luktefunksjonen) og alder i den kvinnelige deltagergruppen. Blant den mannlige deltagergruppen ble det kun funnet en signifikant korrelasjon mellom gjennomsnittsvolumet av OB på venstre side av hjernen og lukteevnen.

Konklusjon: Disse resultatene indikerer (i) en høyresidig dominans av hjernen for prosessering av lukt hos kvinner, og (ii) signifikant korrelasjon mellom luktefunksjonen, volumet av OB og dybden av OS hos kvinner. Det er likevel behov for et større utvalg av deltagere og flere studier for å trekke en endelig konklusjon rundt dette. Disse resultatene kan være viktige for forståelsen av sykdommer som affiserer hjernen knyttet til luktforstyrrelser.

ABSTRACT

Introduction: Smell (olfactory) disorders are very common in the general population. It has been reported that 50% of the population over 65 years has smell disorders [1]. Furthermore, sense of smell is very important for intake of nutritional and healthy food. Studies have shown that 50% of patients with disturbed sense of smell express food complaints and have preference of high sugar intake [2]. High sugar intake may lead to dental caries and other systemic disorders. Cerebral changes in the olfactory cortex have been suggested as reason for olfactory disorders [3]. However, little is known about correlations between olfactory bulb (OB) volume, olfactory sulcus (OS) and smell function.

Aim: The aim of this thesis, therefore, was to investigate correlations between olfactory function, olfactory bulb volume and olfactory sulcus depth in healthy subjects.

Material and methods: The following data was collected from 31 healthy subjects: Participant's normal mental health was ascertained using a questionnaire. Their subjective perception of odor intensity was collected, and their olfactory function was assessed by using sniffin' sticks. Magnetic resonance images were obtained and voxel based morphometry was performed to assess each participant's' OB volume and OS depth. SPSS was used to look at correlations between OB volumes, OS depth and smell function.

Results: The results showed significant differences between male and female participants, when it comes to olfactory processing of sense of smell. In the female group, significant correlations were found between the total depth of the OS, the depth of the right OS and the sense of smell. However, in the male group no such significant correlations were found. The results showed that there was a significant difference ($p = 0,025$) between younger and older females, concerning the smell scores. Younger females had better sense of smell than older females. Also, young females exhibited significantly better sense of smell than young males ($p = 0,026$).

Further analysis in the female group showed that there was a significant correlation between sense of smell and mean total OB volume and mean total OS depth. Similar significant correlation was also found between mean OB volume and OS depth on the right side of the brain and the sense of smell. No such correlation was found between mean OB volume and OS depth on the left side of the brain and the sense of smell. There was also significant correlation between intensity ratings and age in the female group.
In the male group, we could only find a significant correlation between the mean volume of the left OB and the TDI scores.

Conclusion: These results indicate (i) right hemispheric dominance in the brain for olfactory processing in females, and (ii) significant correlations between the sense of smell, OB volume and OS depth in females. However, bigger sample size and more studies are needed to draw a conclusion. These findings illustrating the mapping of the olfactory cortex can be useful in understanding cerebral pathology in patients with olfactory disorders.

1. BACKGROUND

Our interest in this field of science started with the great work of our supervisor Dr. Preet Bano Singh and her colleagues at Munntørrhetklinikken (the dry mouth clinic) at the University of Oslo. A respectable amount of patients seek treatment and help due to problems regarding their sense of smell and/or taste. The scientific research in this field is yet not that vast and most clinicians are not aware of these problems, how to treat them or even where to refer these patients. These senses are connected to the oral cavity, where we as dentists are experts and should therefore have more knowledge in helping this group of patients. Rusthen et al., concluded that patients with primary Sjögren syndrome reported higher occurrence of dysgeusia (distortion of the sense of taste), burning sensation of the tongue (BST) and halitosis. These patients also reported a lower oral health-related quality of life because of these symptoms [4].

The initial plan for our project was to travel to the smell and taste clinic at the Technische Universität Dresden (TUD) to do a study on subjects with burning mouth syndrome (BMS). Unfortunately, the group of subjects were not fully ready for the project at the time of our arrival, and therefore we had to change our thesis. Together with prof. Thomas Hummel, we discussed different project topics, concluding with a study on the correlation of the volume of the olfactory bulb, the olfactory sulcus depth and the olfactory function in healthy subjects. We started collecting data for our new thesis cooperating with the department of psychology at TU. We were introduced to the research community that worked with the smell and taste clinic, and learned about the different tools we would use to analyze and collect the data for our project. We were also able to participate in the ongoing studies at the lab. During our stay we saw the importance of the cooperation between the different departments at the university of research, diagnostics and treatment of this patient group. This includes the departments of dentistry, psychology, otorhinolaryngology and neurology as the most relevant ones.

2. INTRODUCTION

2.1 Project background:

Even though the sense of smell (olfaction) may be thought of as one of the less important senses in humans, it plays an important role for survival and the quality of life [5]. It is due to our sense of smell that we can detect spoiled food and dangerous gases, but also enjoy odors that are pleasant to us, not to mention the important role the sense of smell has in the sense of taste [6].
Moreover, the sense of smell is important in detecting emotions [7]. Another study from the faculty of dentistry at the University of Oslo showed that dentists could smell if patients were anxious and that this could result in poor dental performance [7]. The study showed that when exposed to masked anxiety body odors, the test subjects' dental performance was significantly worse than when they were exposed to masked rest body odors and masker alone, indicating that their performance was modulated by exposure to the emotional tone of the odor. [7]

2.2 Anatomy of the olfactory system

The sense of smell is known as *olfaction* in the scientific context. The Olfactory system consists of a peripheral and a central structure. The peripheral olfactory system consists of the nostrils, nasal cavity, olfactory epithelium (with the olfactory receptor cells) and ethmoid bone (fig. 1).

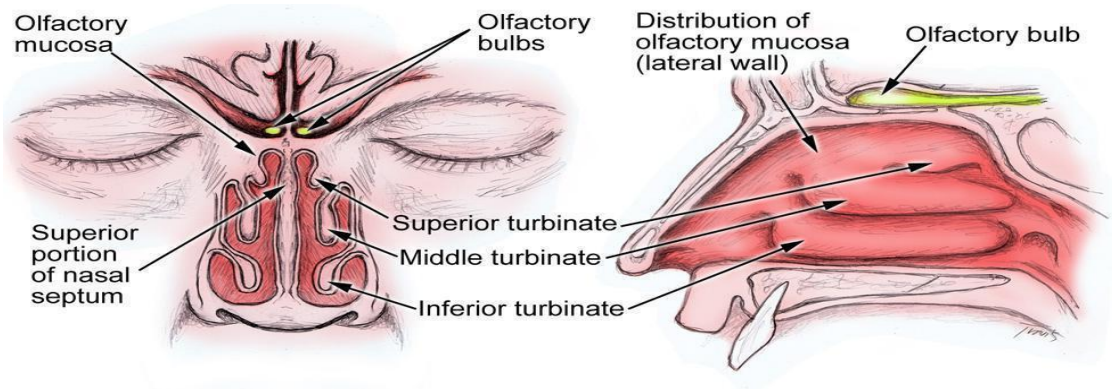


Figure 1: *Anatomy of the human peripheral olfactory system. (https://emedicine.medscape.com/article/835585-overview) 30.04.19.*

The central structure of the olfactory system consists of the olfactory bulbs, primary olfactory cortex and the secondary olfactory cortex (fig. 2). Primary olfactory cortex consists of the piriform cortex, amygdala and entorhinal cortex receiving direct input from the olfactory bulbs. The secondary olfactory cortex, receiving signals from the primary olfactory cortex consists of hippocampus, thalamus, insula and orbitofrontal cortex [8].

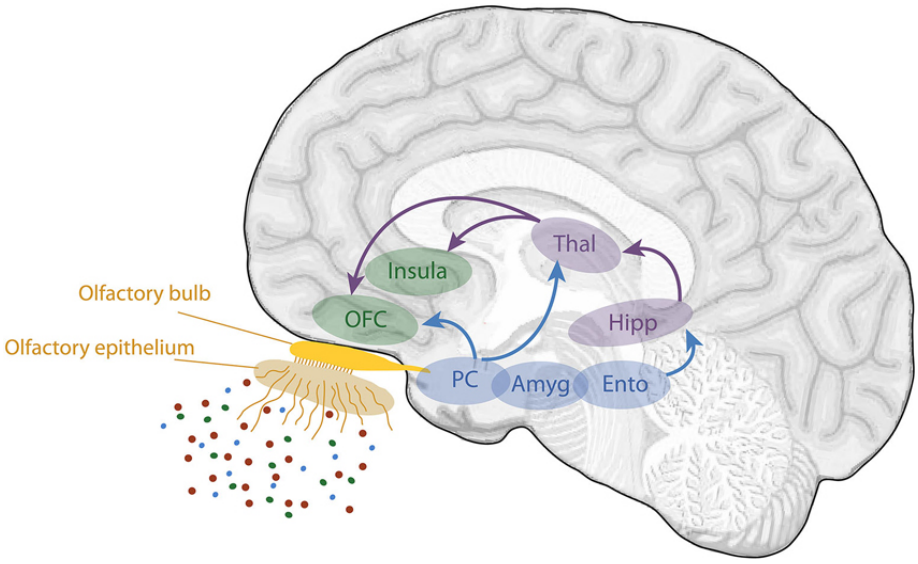


Figure 2: *Schematic view of the human olfactory system. The primary and secondary olfactory cortices are represented in blue and green, respectively. Amyg, amygdala; Ento, entorhinal cortex; Hipp, hippocampus; OFC, orbitofrontal cortex; PC, piriform cortex; Thal, thalamus*
(Royet et al., 2014).

Chemicals, which can be detected by the olfactory receptors, can enter the nasal cavity either by the nostrils or through nasopharynx. The olfactory receptors are located in the uppermost part of the nasal cavity, along the septum and on the superior concha. Because of their location and the position of the middle and inferior conchae, approximately 10% of the inspired air is directed toward the olfactory receptors. The remaining area of the nasal cavity is covered with respiratory epithelium to modify the air that enters the nostrils. Inspired air is brought to the temperature and humidity of the lungs. The hairs of the nostrils and the broad surface of the respiratory epithelium help protect the olfactory epithelium (OE) by removing particles that enter the nasal cavity with the inspired air [9].

Olfactory epithelium consists of multiple cell types. It is designed for detection of and protection from volatile stimuli. The apical layer, which is the outermost part towards the nasal cavity, consists of the supporting cells. They help protecting the epithelium by detoxifying potentially dangerous chemicals. In the intermediate layer, proximal to the apical layer, the core of the mature receptor cells is found. They have prolonged organelles (olfactory cilia) that extend out into the nasal cavity. Both the supporting and the mature receptor cells are anchored to the basal membrane proximately. The basal cells are located towards the basal membrane, which continuously develop to mature olfactory receptor neurons. Proximal to the basal membrane is the lamina propria, which consists of fibrous- and glandular tissue, blood vessels, unmyelinated axonal fibers and immune cells. Here the Bowman's capsule produce watery mucus is secreted into the nasal cavity through the secretory ducts (fig. 3) [10].

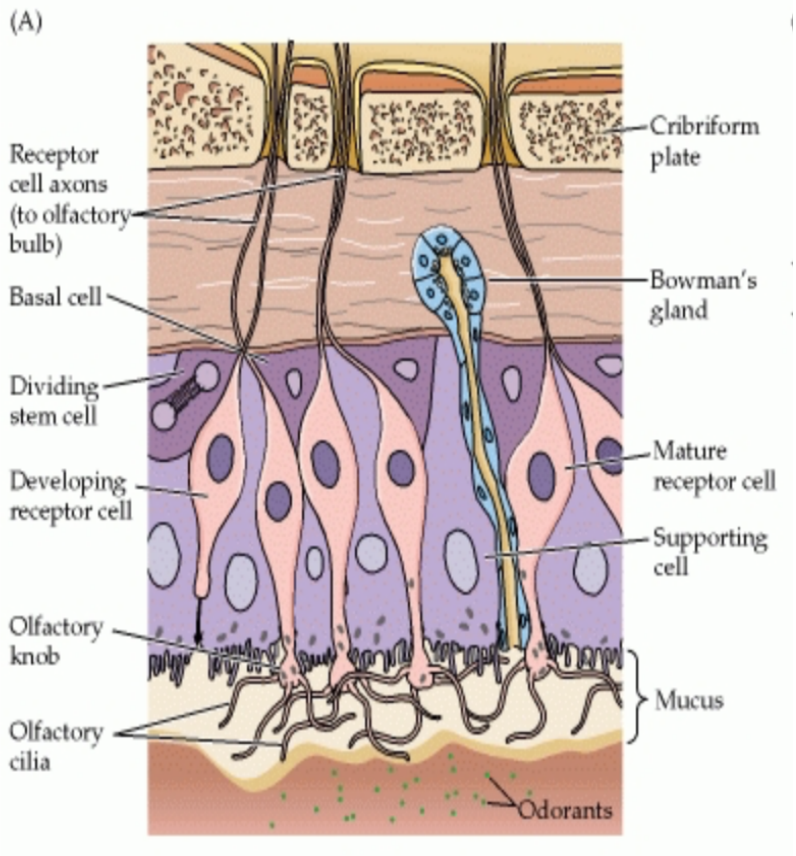


Figure 3: *Diagram of the olfactory epithelium showing the major cell types: olfactory receptor neurons and their cilia, sustentacular cells (that detoxify potentially dangerous chemicals), and basal cells. Bowman's glands produce mucus. Nerve bundles of unmyelinated neurons and blood vessels run in the basal part of the mucosa (called the lamina propria). Olfactory receptor neurons are generated continuously from basal cells.*
(https://www.ncbi.nlm.nih.gov/books/NBK10896/ fig. 15.5 30.04.19)

2.3 Physiology of the olfactory system

The perception of smell occurs when odorants enter the nasal cavity through the nostrils or nasopharynx. Molecules that enter the nasal cavity through air are dissolved in the nasal mucus produced by the olfactory glands. Cilia, which are prolonged organelles of the olfactory receptor neurons, are then able to bind to the odorants [11], and this results in an intracellular cascade that leads to an action potential. The receptor cells are bipolar neurons, which send their unmyelinated axons directly to the OB.

2.4 Significance of olfaction in dentistry

Smell (olfactory) disorders are very common in the general population. It has been reported that 50% of the population over 65 years has smell disorders [1]. Furthermore, sense of smell is very important for intake of nutritional and healthy food. Studies have shown that 50% of patients with disturbed sense of smell express food complaints and have preference of high sugar intake [2]. Furthermore, one of the most important factors in developing dental caries is diet consisting of a lot of carbohydrates, especially food with great amounts of sugar [12]. High sugar intake may also lead to other systemic disorders. Cerebral changes in the olfactory cortex have been suggested as reason for olfactory disorders [3]. However, little is known about correlations between olfactory bulb (OB) volume, olfactory sulcus (OS) and smell function.

Sufferers of smell disorders have been shown to bear significant psychosocial consequences [13]. A study for a support and information organization for sufferers of olfactory dysfunction, the Fifth Sense United Kingdom, showed that olfactory disorders have a negative impact on the quality of life for patients suffering from chemosensory dysfunctions. This study showed high rates of depression (43%) and anxiety (45%), and that the same group had impairment of eating experience (92%), felt isolated (57%) and had relationship difficulties (54%) [13]. There has been shown a significant relationship between depression and oral diseases [14], regarding higher amount of decayed,

missing or filled teeth (DMFT-score). It should therefore be important for us as dental professionals to investigate further about humans with olfactory disorders thus we know that these disorders can affect their oral health.

Smell disorders have been classified as such: **(1)** Anosmia, which describes the lack of ability to smell, and specific anosmia; the inability to smell a specific odor. **(2)** Hyposmia, which refers to a reduced ability to smell. **(3)** Parosmia is described as the "wrong" perception of odors. For example, being presented with an odor of roses and perceiving it as another odor. **(4)** Phantosmia describes the perception of odors in the absence of a relevant odor source. In addition **(5)** normosmia which refers to the normal ability to smell [15].

The four main causes of smell disorders are trauma, viral infections, sinusitis or polyposis nasi. Smell disorders associated with aging or neurological illnesses such as Parkinson's disease and Alzheimer's disease are also common causes. (Fig 1)

	Skull-brain trauma	Infection of the upper respiratory tract	Rhinitis/Sinusitis
Probable cause	Lesion of olfactory fibres in the region of the lamina cribrosa; Contusion of important olfactory areas of the brain	Viral damage to the olfactory epithelium	Mechanical shifting, oedema/functional impairment as a result of inflammation processes in the mucous membrane/olfactory bulb (?)
Occasional smell sensations	Seldom	Frequently	Frequently
Epithelium	Degeneration	Metaplasia, abnormal development of the ORN	Usually normal
Rate of occurrence of smell disorders	About 5%	About 1%	About 2/3 of all patients with chronic sinusitis
Typical age	20–50 years	Older than 50 years	30–60 years
Onset of smell disorders	Fast	Fast	Slow
Loss of smell	Severe	Moderate	Moderate
Rate of occurrence of parosmia	Frequent	Very frequent	Rather seldom
Probability of improvement of the smell disorder	Less often, improvement mostly in hyposmia patients	Often, improvement usually over several years	Very often, improvement due to OP or treatment with corticosteroids, however often only short-term

Figure 1: *Table displaying post-trauma, post-viral/infection and sinunasal smell disorders. (Hummel T, Landis BN, Hüttenbrink KB. Smell and taste disorders. GMS Curr Top Otorhinolaryngology Head Neck Surg. 2012;10:Doc04. doi:10.3205/cto000077)*

It has been shown that patients with isolated anosmia have significantly shorter OS depth than healthy persons [3]. Isolated anosmia meaning anosmia without evidence of other defects. Regarding the OB-volume, it has been shown a significant correlation between the volume and the olfactory function in children [16]. However, little is known about how the olfactory cortices are wired together to facilitate normal perception of sense of smell. Before one can start looking for pathology in the designated olfactory cerebral areas, it is necessary to map the wiring in olfactory cortices in healthy subjects.

3. AIM

The purpose of this study was to investigate if there is any correlation between the volume of the olfactory bulb (OB), depth of the olfactory sulcus (OS) and olfactory function.

4. MATERIALS AND METHODS

4.1 Participants and study design

Initially, thirty-nine participants (24 females, 15 males, aged between 18 and 36 years, mean age 24,2 years ± 4,1 SD) were invited to take part in a pre-test. The purpose of the pre-test was to make sure that the participants were healthy, and had normal olfactory function. Each participant was asked to answer a Patient Health Questionnaire (PHQ) to reveal potential mental health disorders concerning depression, anxiety, somatoform disorders and to reveal the participants diet and alcohol usage. None of them showed any abnormalities. Olfactory function was tested by using the «sniffin' sticks». Three participants were excluded because of hyposmia (reduced ability to detect odors). Further three participants were also excluded due to health impairments that were associated with olfactory function (e.g. diabetes mellitus, renal insufficiency) and acute or chronic sinusitis. Out of the remaining 33 participants, 31 showed up for further examinations (19 women, 12 men, aged between 20-38 years, mean age 26,2 years ± 3,79 SD).

The study followed the declaration of Helsinki on biomedical research involving human subjects (World Medical Association, 1997), and was approved by the University of Dresden Medical Faculty Ethics Review Board. All the participants provided written informed consent for the study.

4.2 Odor intensity testing

It has been shown that reduced odor intensity perception is associated with reduced olfactory receptor function [17].

Odors for the odor intensity testing were chosen randomly from the smell and taste clinic. The following odors were used: civet (musky scent), creamy butter (rancidificated), onion, syringa (flieder), milk, eucalyptus, orange oil, coffee,

cinnamon and lavender. In order to evaluate the precision of perception of bodily feelings, the heartbeat perception task (HBPT) was performed by every participant. The HBPT is a well-established test to depict the ability of interoceptive awareness [18, 19]. During this test, the subjects were asked to count their heartbeats by concentrating on their body (not by taking their pulse). The numbers were then reported and compared to the actual reading of the electrocardiogram (EKG) machine [20].

With the help of an olfactometer (device built to present odor stimuli in a standardized computer-controlled manner [21]), the participants were exposed to the ten different odors, three times each, in a random order. Stimuli were embedded in a constantly flowing air stream of controlled temperature (36.5° C) and humidity (80% relative humidity) which was directed into the nasal cavity by means of a 4-mm diameter polyurethane tubing (image 1). After each odor presentation the participants were asked to rate the intensity of the odors from 1 (not intense) to 5 (very intense).

Image 1: Olfactometer set up with test subject performing odor intensity test.
Photo: Sajad Maghsoudi©

4.3. Olfactory testing

Olfactory function was tested using the validated ”sniffin’ sticks” (Burghart, Wedel, Germany) method [22, 23], in which odor threshold (T), odor discrimination (D) and odor identification (I) was tested (Image 2). Each of the sub tests have their own set of pens. During the odor threshold and discrimination test, the subjects were blindfolded, to eliminate any visual cues. The whole experiment followed a forced-choice procedure, so that the participants always had to give an answer. The participants were presented with the odor only once. Overall olfactory function is expressed as the sum of the scores (TDI) from the three individual tests (T), (D); (I), and the maximum sum is 48 (Maximum 16 points for each of the subtests). A low score equals reduced olfactory function, and a high score equals normal or good olfactory function.

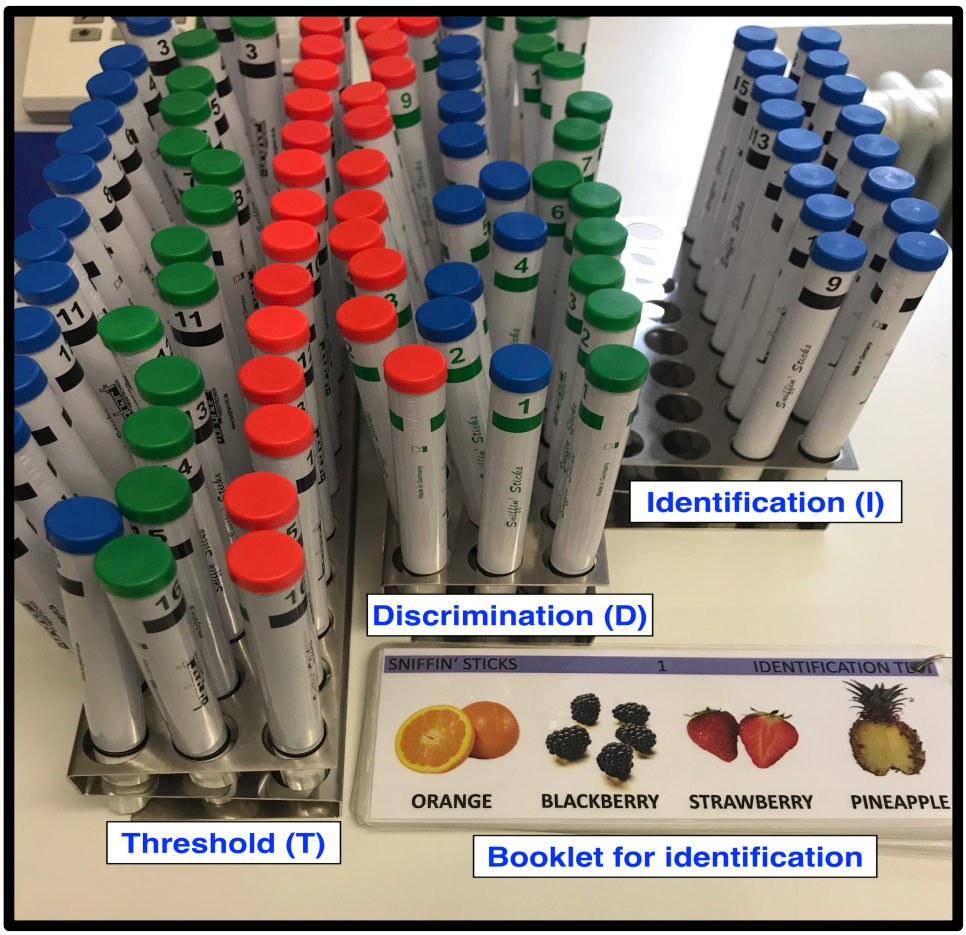


Image 2: Sniffin’ Sticks setup

Photo: Sajad Maghsoudi©

This data was collected by the lab workers at the Smell & Taste Clinic, Department of Otorhinolaryngology at the Carl Gustav Carus Universitätsklinikum, Technical University of Dresden. The same procedure for the test was done with every subject, to minimize the source of error. Each participant was therefore tested with similar testing kits and a lab worker presenting the sniffin' sticks was always using nitrile disposable gloves. The use of gloves eliminated any odors that could occur from the hands of the person executing the test, and minimized number of different odors presented to the participant. The odors were then presented one by one. The tester removed the cap from one pen, and held it approximately 2 cm below the participant's nostrils in a wave-like motion for a couple of seconds (image 3).

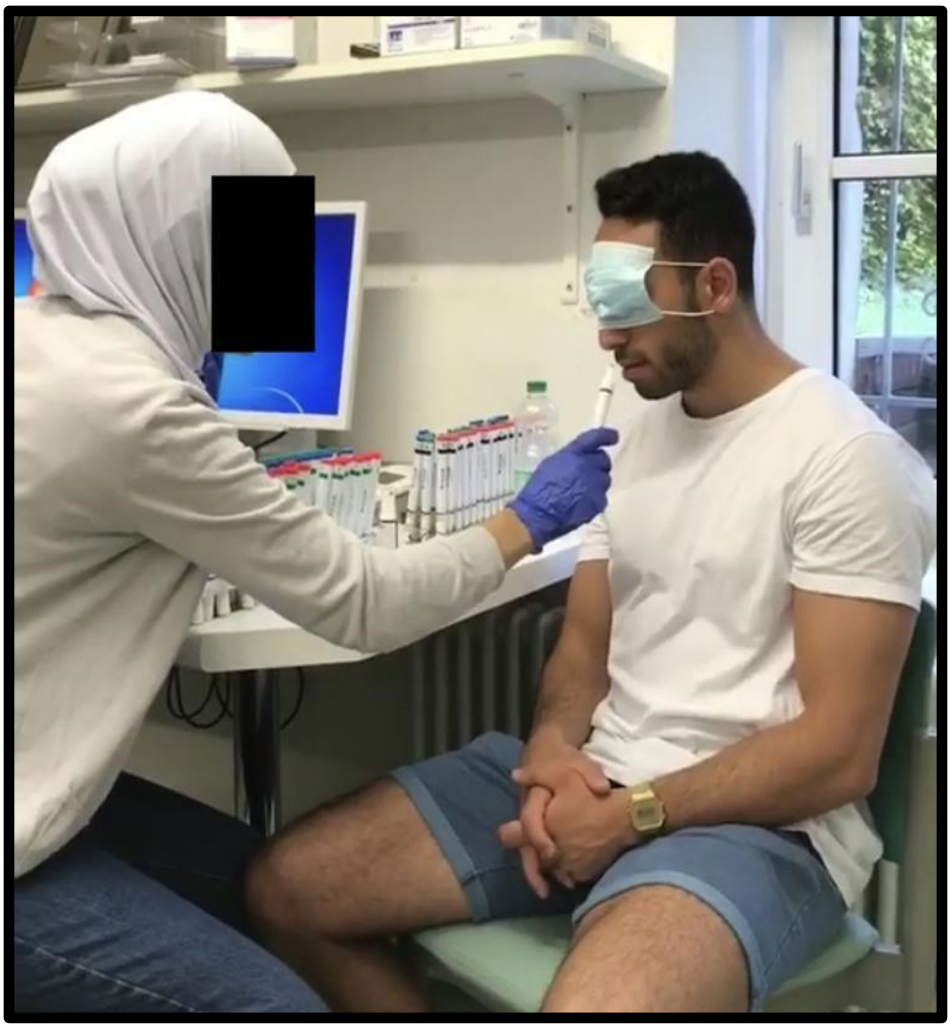


Image 3: Olfactory function testing using Sniffin' Sticks.
Photo: Eugén Treimo©

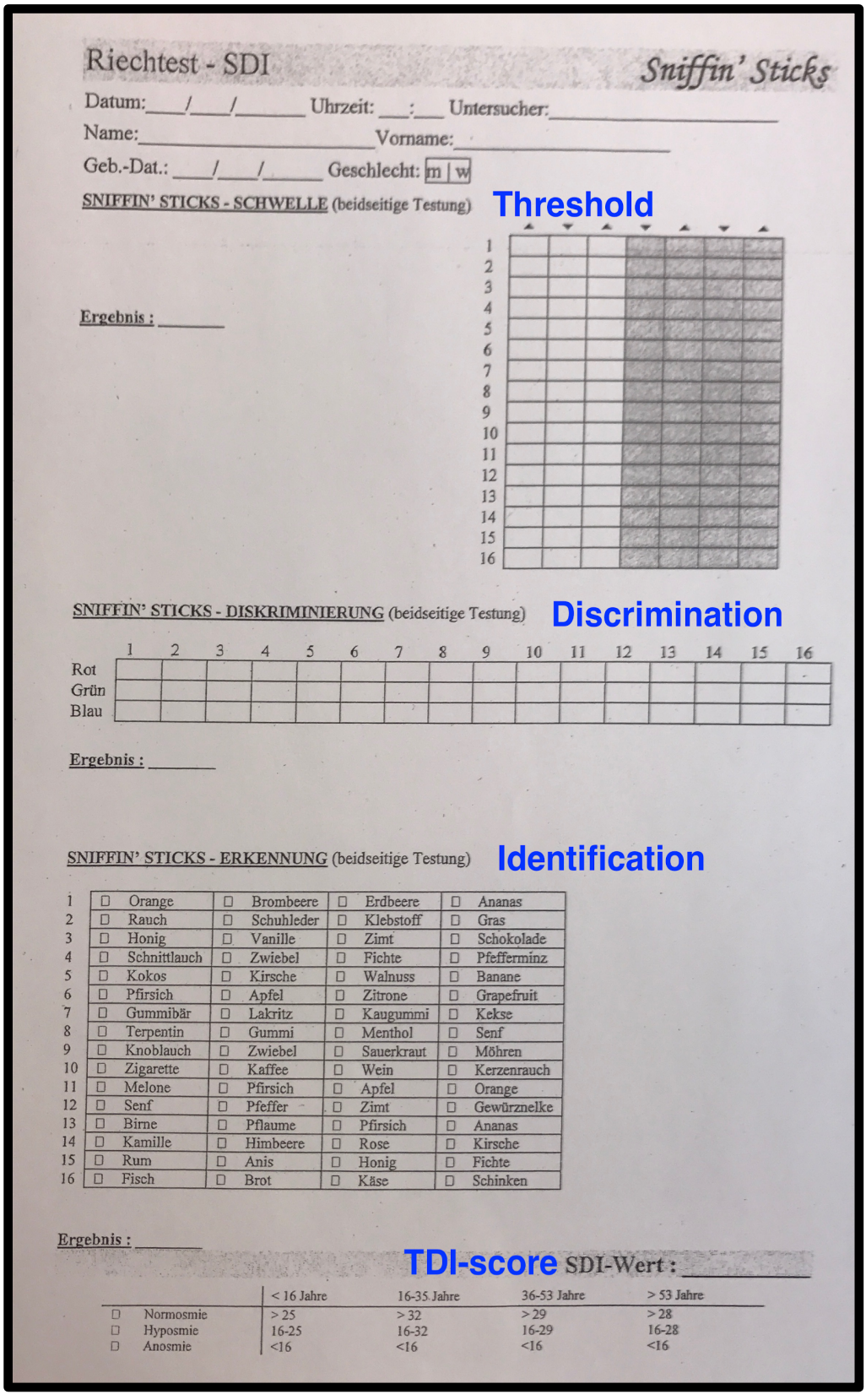
TDI:

Odor threshold was tested by presenting the subject with three different sniffin' stick pens, one of which containing odorant with a certain concentration, and two without any odor. While blindfolded, the subject must try to identify the pen that they believe contains the odorant. This test is repeated with 3 x 16 pens that vary in odor concentration to establish the subject's threshold for olfaction.

Odor discrimination was tested by presenting a triplet of sniffin' stick pens to the subject, two of which contained the same odor and one that had a different odor. The concentration of the odorants was the same in all of the pens. The subject, while blindfolded, had to pick the pen that they believed had an odor that differed from the other two. Odor discrimination was established after presenting the subject with 3 x 16 pens.

Odor identification was tested by presenting one "sniffin' stick" pen at a time to the subject. The participant was not blindfolded for this test. A booklet containing suggestions to what the odorant could be was given to the participant. The booklet had 16 pages, and each page was meant for a particular pen. The participant had to look at page 1, when pen number 1 was presented to the participant. There were four different suggestions (pictures and names) of what the odor could be. The participant had to choose the suggestion that they felt was the correct one. 16 different pens were presented to the subject, to establish odor identification.

Participants' responses were registered in standard form used at the Smell and Taste Clinic, Department of Otorhinolaryngology, Technische Universität, Dresden (image 4).



Riechtest - SDI

Sniffin' Sticks

Datum:___/___/_____ Uhrzeit: ___:___ Untersucher:_____

Name:_____ Vorname:_____

Geb.-Dat.: ___/___/_____ Geschlecht: m | w

SNIFFIN' STICKS - SCHWELLE (beidseitige Testung) Threshold

Ergebnis : _____

SNIFFIN' STICKS - DISKRIMINIERUNG (beidseitige Testung) Discrimination

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rot																
Grün																
Blau																

Ergebnis : _____

SNIFFIN' STICKS - ERKENNUNG (beidseitige Testung) Identification

1	☐ Orange	☐ Brombeere	☐ Erdbeere	☐ Ananas
2	☐ Rauch	☐ Schuhleder	☐ Klebstoff	☐ Gras
3	☐ Honig	☐ Vanille	☐ Zimt	☐ Schokolade
4	☐ Schnittlauch	☐ Zwiebel	☐ Fichte	☐ Pfefferminz
5	☐ Kokos	☐ Kirsche	☐ Walnuss	☐ Banane
6	☐ Pfirsich	☐ Apfel	☐ Zitrone	☐ Grapefruit
7	☐ Gummibär	☐ Lakritz	☐ Kaugummi	☐ Kekse
8	☐ Terpentin	☐ Gummi	☐ Menthol	☐ Senf
9	☐ Knoblauch	☐ Zwiebel	☐ Sauerkraut	☐ Möhren
10	☐ Zigarette	☐ Kaffee	☐ Wein	☐ Kerzenrauch
11	☐ Melone	☐ Pfirsich	☐ Apfel	☐ Orange
12	☐ Senf	☐ Pfeffer	☐ Zimt	☐ Gewürznelke
13	☐ Birne	☐ Pflaume	☐ Pfirsich	☐ Ananas
14	☐ Kamille	☐ Himbeere	☐ Rose	☐ Kirsche
15	☐ Rum	☐ Anis	☐ Honig	☐ Fichte
16	☐ Fisch	☐ Brot	☐ Käse	☐ Schinken

Ergebnis : _____

TDI-score SDI-Wert : _____

	< 16 Jahre	16-35 Jahre	36-53 Jahre	> 53 Jahre
☐ Normosmie	> 25	> 32	> 29	> 28
☐ Hyposmie	16-25	16-32	16-29	16-28
☐ Anosmie	<16	<16	<16	<16

Image 4: TDI-score registration form

Photo: Sajad Maghsoudi©

4.4 Magnetic resonance imaging

A 3T magnetic resonance imaging system (Trio, Siemens Medical, Erlangen, Germany) was used to perform all the examinations. A standardized protocol for OB analysis was used. The protocol included capturing: 1) a series of 3-mm-thick functional MRIs with no interslice gap, covering the whole brain, to rule out any organic brain disorders (Specific settings: TR=3000 ms, TE=40ms, FA=90deg, voxel size= 3 x 3 x 3mm); 2) 1,2-mm-thick T2 weighted spin-echo images without interslice gap in the coronal plane, covering the anterior and middle segments of the base of the skull. (specific settings: repetition time (TR)=6390ms, Echo time (TE)= 95ms, voxel size = 0,6 x 0,5 x 1,2 mm = 0,36 mm3).

4.5 Voxel based morphometry

Voxel based morphometry was used to measure the volume of the olfactory bulb and depth of the olfactory sulcus. Voxel based morphometry (VBM) is a technique using MRI that allows investigation of focal differences in brain anatomy. The volume of the olfactory bulb and the depth of the olfactory sulcus was detected by drawing regions of interest (ROIs) on the images of the MRI scans of the brain, and calculating the volume enclosed (image 5). [24]

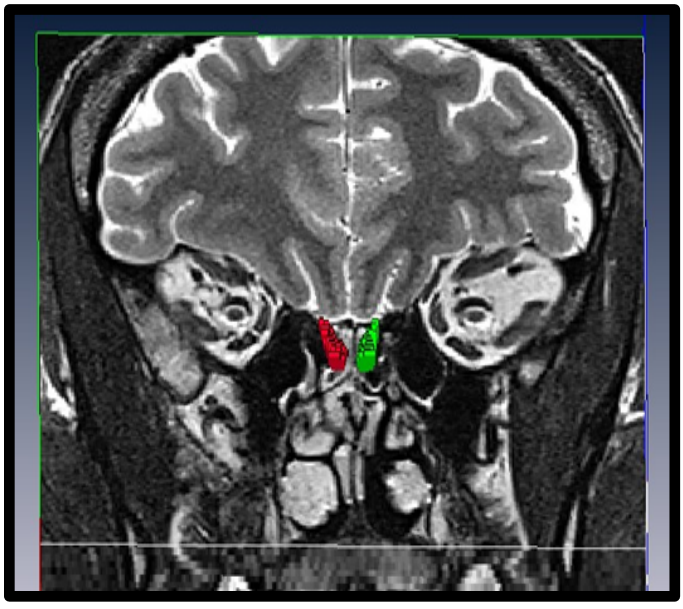


Image 5: 3D-presentation of counted voxels of the olfactory bulb in one of the test subjects

Photo: Sajad Maghsoudi©

Measurement of the OB volume

The measurements of the OB volumes were done by manual segmentation, using the AMIRA 3D visualization and modeling system version 5.4.1(Build 006-5e11b Visage Imaging, Carlsbad, USA). Two observers measured the bulbs of each patient, blinded by each other and the results from the olfactory function tests. Each observer looked through a sequence of approximately 30 slices in the coronal plane, for each patient. In every slice, areas thought to be a part of the OB were manually marked, approximately six to ten slices per participant. The sum of all voxels in every slice revealed the OB volumes in mm^3 for each side of the OB (image 6).

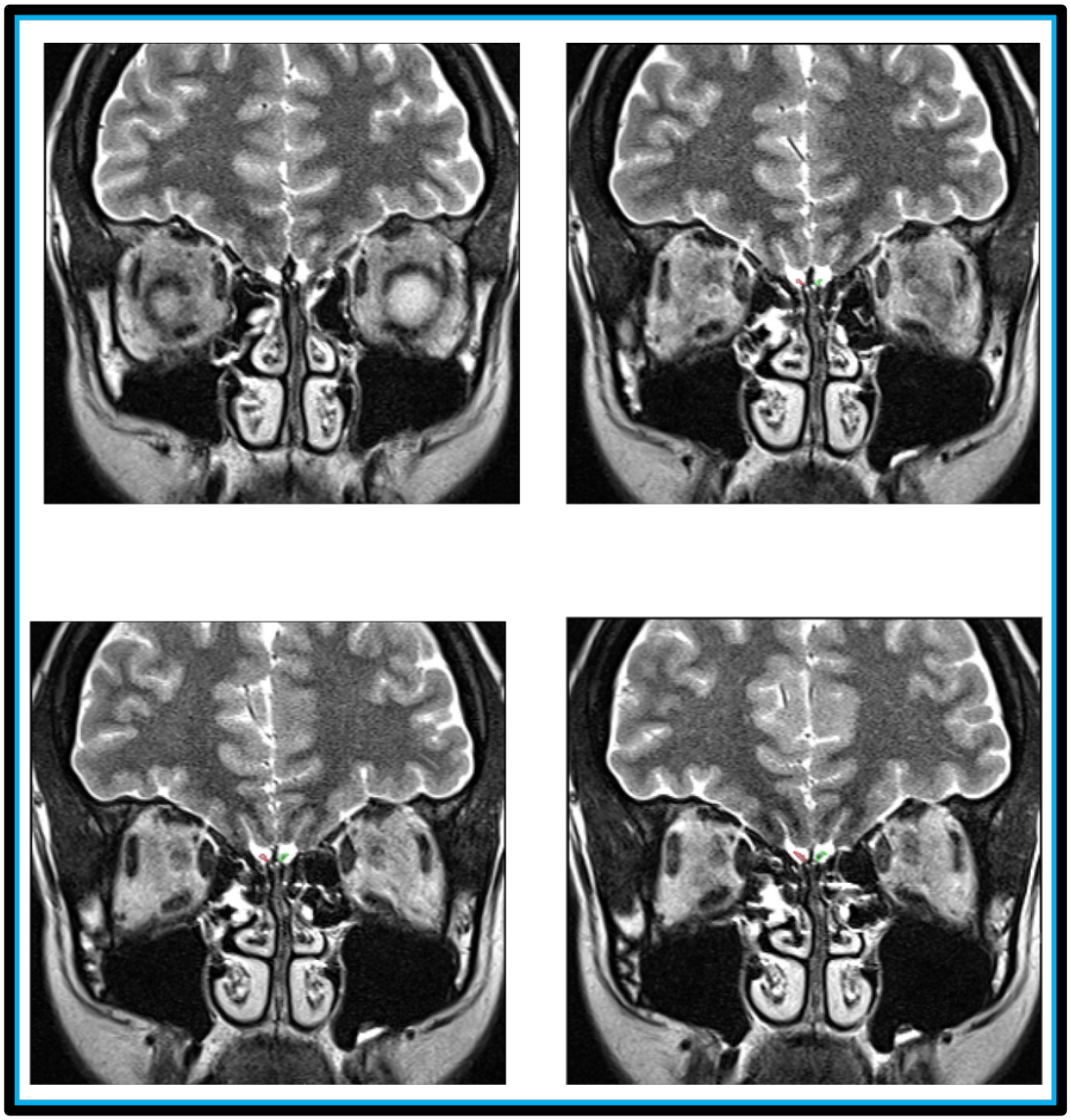


Image 6: The four pictures above show the counting of voxels through four different MRI-layers from one test subject. Photo: Sajad Maghsoudi©

Measurement of the OS depth

The depth of the olfactory sulcus was measured by manual measurement using the AMIRA 3D software. These measurements were done by two observers, blinded by each other and the functional olfactory tests. The observers looked through approximately 30 slices per patient, and picked the one where the eyeballs just no longer could be seen, when scrolling from anterior to posterior direction. This is where we have the plane of the posterior tangent through the eyeballs (PPTE), which cuts through the anterior mid segment of the OB [3]. From the chosen slice, a digital measurer in the software was used to find the depth of the OS in mm, from the deepest point of the sulcus until its opening into the OB.

4.6 Statistical analysis

All statistics were performed using IBM SPSS software version 24.0.0.0 (SPSS Inc, Chicago, Illinois, United States of America). Interclass analysis were performed with significance level at 0.01. Correlations and partial correlations were performed between OB volumes, OS depths, olfactory function and intensity scores, gender and age. These tests were computed according to Pearson, with the significance level ranging from 0.01 to 0.05.

5. RESULTS

5.1 Voxel based morphometry results

5.1.1 Inter-rater reliability in OB and OS measurements

The inter-rater reliability between the volumes measured by the two observers was significant (Left OB: r_{31} = 0,98 p = 0,01; Right OB: r_{31} = 0,97 p = 0,01). Meaning that the calculated OB-volume done individually by the two observers were significant similar to each other. The individual measurements of the OB volume in the participants had a rather big variance (Left OB: min= 0,00 mm^3 , max= 78,66 mm^3; Right OB: min= 0,00 mm^3, max= 59,22 mm^3). This shows the big variance in OB-volume between the different participants.

5.1.2 OB volume measurements

The mean measurements for the OB-volumes showed 41,85 mm^3, SD 18,2 on the left side, and 36,23 mm^3, SD 16,4 on the right side. These mean scores were calculated as the mean score of each individual participant of the two observers, based on the significance of the inter-rater reliability test. The correlation between the right and left volumes was also significant, r_{31} = 0,91 p = 0, 01 (Table 2).

5.1.3 OS depth measurements

The measurements of the depth of the OS between the observers also significantly correlated (Left OS: r_{31} = 0,89 p = 0,01; Right OS: r_{31} = 0,90 p = 0,01). The difference in depth measurements between the participants ranged from 3,50 mm to 14,80 on the left side and 4,26 mm to 15,72 on the right side. The mean measurements for the depth of the OS were on the left 8,26 mm SD 2,32, and on the right 9,28 mm SD 2,04. The correlation between left and right sulcus depth were calculated to be r_{31} = 0,835 p = 0,01 (Table 1).

	Mean	N	SD	SEM
Mean L_OB	41,84	31	18,10	3,26
Mean R_OB	36,22	31	16,40	2,94
Mean L_OS	8,26	31	2,30	0,41
Mean R_OS	9,27	31	2,00	0,36

Table 1 - Describing the mean Olfactory bulb volume(mm^3) and olfactory sulcus length(mm) in all subjects. OB: Olfactory bulb, OS: Olfactory sulcus, SD: Standard deviation, SEM: Standard error of the mean.

	N	Correlation
Mean L_OB & Mean R_OB	31	0,912*
Mean L_OS & Mean R_OS	31	0,835*

Table 2: Showing the correlation between the Left and right Olfactory Bulb volume and Olfactory sulcus depth.

**Significance p = 0.01 (2-tailed)*

5.2 Olfactory function (TDI) scores

Lowest threshold was 7,5 and the highest 15,25, out of a maximum 16. Discrimination gave the minimum score of 9, and the maximum of 15, out of 16 total. Identification scores ranged from 11 to 15, out of a total of 16. The total TDI scores out of a maximum of 48, ranged from 31,5 - 40, which is in the range of healthy young participants.

Looking at the scores in all the groups regarding the TDI-test, female participants generally scored a little bit higher than the male participants (Table 3), except from the identification part of the TDI-test.

Female (N=19)	Mean	SD	SEM
Threshold	11,06	2,01	0,46
Discrimination	12,42	1,64	0,37
Identification	13,11	1,32	0,30
TDI	36,59	2,42	0,55
Male (N=12)			
Threshold	10,83	1,92	0,55
Discrimination	11,75	1,13	0,32
Identification	13,25	1,05	0,30
TDI	35,83	1,23	0,35

Table 3 - TDI: the sum of the three subtests.

No correlation was found based on age and TDI-scores in this study. The participants' age ranged from 20 to 38 years with a mean of 26 years and SD 3,7.

Further analysis was done by splitting the male and the female group by age. Data showed that there was a significant difference ($p = 0,025$) between younger and older females, concerning the TDI scores. This meaning younger females had significantly better scores than older females. (Figure 1). There was also a significant difference ($p = 0,026$) between the young females and young males in TDI scores.

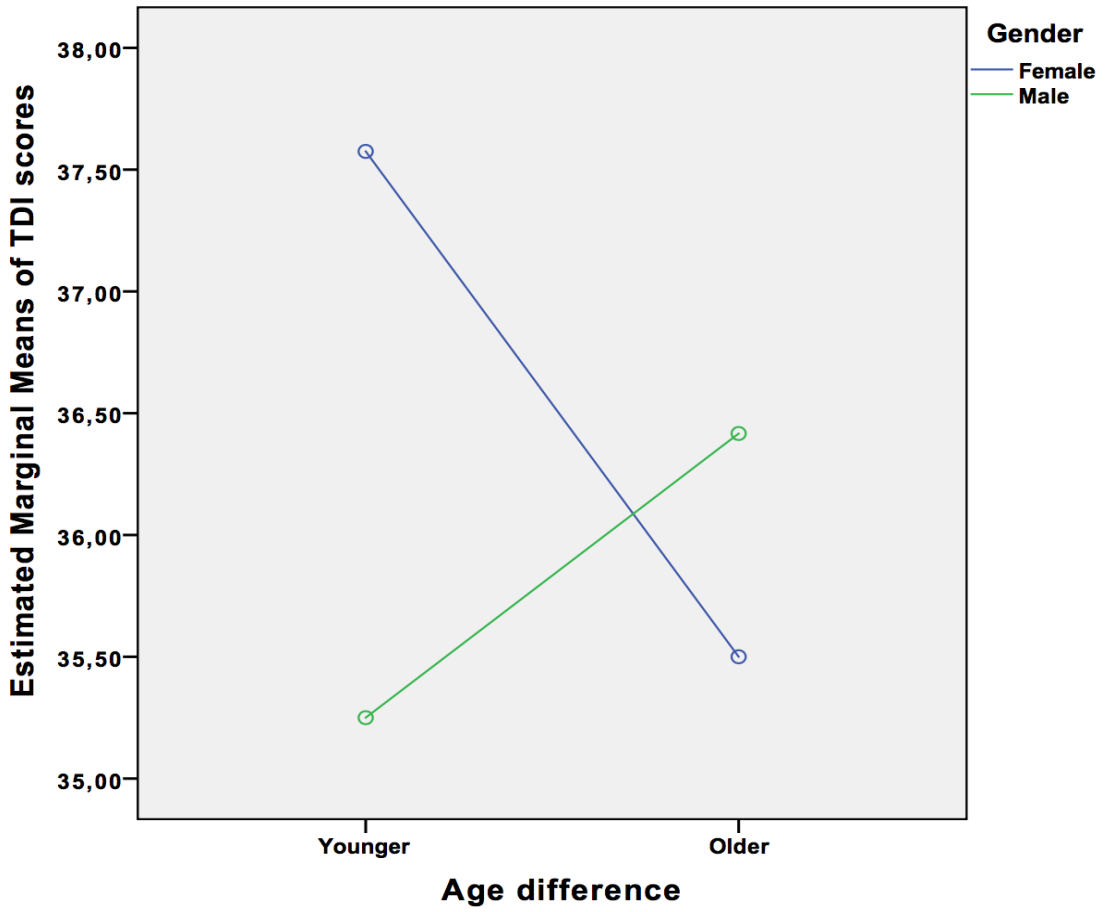


Figure 1: Comparing age difference to TDI-scores divided by genders. Males represented in blue color, and females in green.

Although there were no significant correlation between the total OB volumes and the functional olfactory scores, there were some correlations between OS depths and odor intensity ratings. These significant correlations were only found in the female group in this study.

Female (N=19)	Intensity	Significance
L_OS depth	- 0,43	0,063 (n.s.)
R_OS depth	- 0,49	0,030*
T_OS depth	- 0,49	0,032*
Age	0,58	0,008**

Table 4: Correlations between OS depth, age and intensity ratings in the female group.

n.s.: Not significant.

** Significant at 0,05 (2-tailed)*

*** Significant at 0,01 (2-tailed)*

Looking at the intensity ratings for the female participants, there was a significant correlation between the total depth of the OS, the depth of the right OS and intensity ratings (R_OS and Intensity: r_{19} = -0,49 p = 0,05; T_OS and intensity: r_{19}=0,49 p = 0,05). There was also a correlation between the age of the female participants and the intensity ratings (r_{19} = 0,58 p= 0,01). Further analysis was done by splitting the female, and male group by age, but no significant differences were found.

	Intensity	SD	SEM
Female (N=19)	3,7	0,5	0,1
Male (N=12)	3,5	0,4	0,1

Table 5: Mean intensity ratings in the female, and male group.

Due to the high variation in OB volumes in comparison to the low variation in the TDI scores, we did an extra analysis with the exclusion of the participants with extreme values. The exclusion criteria was to remove any participant with a mean total OB volume below 50 mm^3. The number of participants was reduced from 31 to 25 (14 females and 11 males).

The calculations of the female participants showed that there was a significant correlation between intensity ratings and mean total OB volume, mean total OS depth, mean OB volume and OS depth on the right side. There was also significant correlation between intensity ratings and age in the female group.

Females(N=14)	Intensity	Significance
Age	0,57	0,03*
Mean_R_OB volume	- 0,58	0,029*
Mean_T_OB volume	- 0,59	0,026*
Mean_R_OS depth	- 0,623	0,017*
Mean_T_OS depth	- 0,619	0,018*

Table 6: correlation between OB volume(mm^3), OS length(mm), age and intensity ratings in the female group.

** significant at 0,05(2-tailed)*

In the male group, we could only find a significant correlation between the mean volume of the left OB and the TDI scores.

Males(N=11)	Total TDI score	Significance
Mean_L_OB volume	0,62	0,039*

Table 7: The correlation between the left Olfactory Bulb volume and TDI scores in males.

** significant at 0,05(2-tailed)*

6. DISCUSSION

The aim of this study was to investigate whether there is a correlation between olfactory function, OB volume, and OS depth. In the female group, we found that there was a correlation between 1) right olfactory sulcus depth and olfactory function (intensity ratings), 2) total olfactory sulcus depth and olfactory function (intensity ratings) and 3) age and olfactory function (intensity ratings). We could not find any sustainable data in the male group. After the exclusion of every participant with a total OB volume under 50 mm^3 for a second analysis, we found a correlation between the volume of the OB on the left side and functional olfactory scores in the male group.

We were not able to find any correlation between the OB volumetric measurements and olfactory function (TDI-scores). Compared to earlier studies our sample size in this study was relatively small. In comparison to earlier studies with 87 [16], and 127 [25] participants, we only had 31. The small sample size gives us an increased variability within the group. On the other hand, the variability in the olfactory function data was very low, due to the fact that only normosmic participants were allowed to be a part of the study. The difference in variation between the OB volume measurements and the olfactory function scores makes it hard to find a correlation. It is worth mentioning that significant correlation between OB volume and olfactory function has been confirmed in earlier studies with a bigger sample size [16, 25].

Besides the participants with an unusual small OB, we also had a participant with no OB that could be detected on the MRIs. The OB volume can vary; the increase or decrease of the OB volume is dependent on the sensory input. With decreased sensory input, the OB volume will also decrease [26, 27]. Reversing this, may cause an increase in OB volume [28]. This goes to show that the OB volume in the population can vary. Since our sample size is small, these variations can easier come to light, and are not representative for a whole population.

Other studies have also reported participants with a functioning sense of smell that have either small or no OBs [29, 30]. This indicates that chemosensory perception may be based on other mechanisms, e.g. the trigeminal system. It may also be hypothesized that humans with small OBs are more prone to olfactory loss in the long run.

Our results show that women have superior olfactory abilities, and that they are more sensitive to odor than men. Other studies on sex differences in the sense of smell have also shown the same thing [31, 32, 33]. We also found that the younger part of the female group had better smelling abilities than the older part of the female group and the younger part of the male group. Other studies have shown that age and sex play a role in olfaction, suggesting that women have better olfactory abilities and that the sense of smell is affected by age [34, 35].

Results from the female group show that there is a correlation between the right OS depth, right OB volume and intensity ratings. This suggests that participants with smaller right OB and OS, have higher intensity ratings than participants with bigger OB and OS. Due to the fact that earlier studies with much bigger sample size, have shown the opposite [36, 37], we believe that these correlations are significant by coincidence.

We have also found a strong correlation between age and intensity ratings in the female group, with no significant difference within the group when split by age. The highest age difference in the female group is 8 years (youngest being 21 years old, and the oldest 29), thus there is no big variety in age in this group. Studies have shown that the healthy subjects' ability to rate their own ability to smell is correct most of the time, but also often wrong. Shu et al. [38] did a study with one thousand and five participants where they recorded self-rated olfactory function, and measured olfactory function with «sniffin' sticks». They found that self-ratings of olfactory function were unreliable at all ages. Landis et al. [39] investigated the accuracy of self-reported olfactory function in eighty-three healthy participants. The participants were asked to rate their own olfactory function, and olfactory function was assessed with the «sniffin' sticks». Their results indicate that self-ratings of olfactory function in healthy subjects are

unreliable. Due to the fact that the sample size of our study is not representative of a whole population, the possibility of unreliable self-ratings is higher, and therefore a misleading significant correlation like this, can occur.

7. CONCLUSION

These results indicate right hemispheric dominance in the brain for olfactory processing in females. Significant correlation was found between olfactory function, OB volume and OS depth in the female group. However, bigger sample size and more studies are needed to draw a conclusion. These findings can be useful in understanding cerebral pathology in patients with olfactory disorders.

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