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Effect of Interstimulus interval on Olfactory Event Related Potentials

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List of Abbreviations

ISI	**Inter stimulus Interval**
OERP	**Olfactory Event Related Potential**
ERP	**Event Related Potential**
CSERP	**Chemosensory event-related potential**
10s	**10 Seconds**
30s	**30 seconds**
ORN	**Olfactory Receptor Neurons**
MRI	**Magnetic Resonance Imaging**
TDI Score	**Threshold, Discrimination and Identification score**
tERP	**trigeminal Event Related Potential**
EEG	**electroencephalography**
PEA	**Phenyl Ethyl Alcohol**
H_2S	**Hydrogen sulfide**
CO_2	**Carbon dioxide**
P1	**Positive 1**
P2	**Positive 2**
N1	**Negative 1**
S/N	**Signal-to-Noise**
NMP	**negative mucosal potentials**

1. Introduction

1.1 Overview

The sense of smell is considerably different from other senses such as sight and hearing. Its importance is frequently underappreciated by many people. Often, smell is considered as a non-essential sense, since its value is not immediately evident compared to other senses. However, its importance can be found in its function as an alert system for potentially harmful environmental influences like spoiled food, fire or gas leaks (Welge-Lüssen and Hummel 2014).

Further, different studies have indicated that a loss of sense of smell has an effect on the quality of life for a human being and may lead to a variety of diseases such as depression (Hüttenbrink et al. 2013).

The sense of smell remains of little understanding to researchers. This is mainly due to the difficulties related to examining it, especially for those who have lost their sense of smell since changes are often not perceived directly, unlike for loss of vision –(closing eyes) – or hearing (covering ears).

In the nineteenth century, the groundwork research of the olfactory system was laid by Valentin (1810–1883) and Zwaardemaker. Their work explained the main aspects of the anatomy and physiology of the olfaction and developed specific methods for its examination (Philpott et al. 2008). Many studies about the olfaction have followed since then, yet the greatest developments in olfactory tests have been made over the last 30 years. During this period, different reliable psychophysical smell tests have been introduced, for example, the University of Pennsylvania's Smell Identification Test (Doty, Shaman, & Dann, 1984) or the Sniffin' Sticks tests (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997; G Kobal et al., 1996). In addition, electrophysiological measures of olfactory function have been introduced, such as olfactory event-related potentials (G. Kobal, 1981).

1.2 **Anatomical and physiological bases of the olfactory sense**

1.2.1 Macroscopic structure of the olfactory organ

The human nose is the organ responsible for olfaction due to the presence of special cells (Olfactory Epithelium) and the air passage to the larynx and lungs. The nose is divided longitudinally into two halves by a bony structure called the nasal septum which serves as the medial wall for both sides. The lateral wall has three long, narrow and curled bone shelves which are called inferior, medial and superior nasal turbinate (or concha). In the nasal cavity there is a group of four paired air-filled spaces that surround the nasal cavity (maxillary sinuses), above the eyes (frontal sinuses), between the eyes (ethmoidal sinuses), and behind the ethmoids (sphenoidal sinuses). The sinuses are named after the facial bones in which they are located (see Figure 1.1).

The nasal cavity is covered by olfactory and respiratory epithelial cells, the former being located at the top of the nasal cavity under the base of the skull. This part contains various olfactory receptors made up of membrane proteins and connected to olfactory neurons. The olfactory neurons run from the nasal cavity through the cribriform plate and end in the olfactory bulb (Figure 1.1).

The respiratory epithelial cells cover the nasal cavity, nasal sinuses and turbinate and continue to the back of the throat (Hornung et al., 1987; Hummel & Welge-Lüessen, 2006). This epithelial layer is very rich in blood vessel supply; therefore, a change in blood flow in the dense capillary network can lead to a change in the size of the nasal cavity. The epithelial layer is covered by a watery mucus layer designed to warm and humidify the air which passes through the nose to the respiratory tract. In addition, this layer serves as a filter by trapping air-borne particles larger than 2-3 micrometers.

Figure 1.1: Olfactory nerves are embedded in olfactory epithelium in the dorsal posterior recess of the nasal cavity. The olfactory nerves pass through the cribriform plate to the olfactory bulb of the brain.

(Fauci et al., Harrison's Principles of Internal Medicine, 17th Edition).

1.2.2 Microscopic structure of the olfactory mucosa

Olfactory epithelium: Most of the nasal cavity is covered by respiratory mucosa which is pseudo-stratified ciliated columnar epithelium with goblet cells (which produces the mucus). This is only present in the olfactory cleft which is the region located in the upper nasal passage covered by the olfactory epithelium, the area responsible for the sense of smell. The size of human olfactory epithelium is approximately 2x5cm^2, extending from the superior nasal turbinate to the top of the middle turbinate and made up of different cell types, such as olfactory receptor neurons (ORN), basal cells, Bowman's glandular cells, supporting cells and microvillar cells. **The olfactory receptor neurons (ORN)** are bipolar cells. The apical part contains immobile cilia which are coated by the olfactory mucus and play an important role in the transduction process (Lowe and Gold 1993). The other side of the ORN extends to the apical dendrites of the surface neuroepithelium and sends unmyelinated axons through the basal lamina and cribriform plate of ethmoid bone, terminating in the glomeruli of the olfactory bulb (Figure 1.2). The ORN have a relatively short life span in comparison to other neurons, ranging from one month to several months depending on the types of toxic and infectious agents that they are exposed to (Doty, 2003).

Figure 1.2: Shows the construction and cell contents of the olfactory epithelium

(http://droualb.faculty.mjc.edu/Course%20Materials/Physiology%20101/Chapter%20Notes/Fall%202007/chapter_10%20Fall%202007.htm**).**

The basal cells form the basis of the olfactory epithelium (Figure 1.2), which can be divided into two types of basal cells: horizontal basal cells are flat and produce cytokeratin, while globose basal cells are round in shape and produce several markers (Doty, 2003). The basal cells are adult stem cells and can be converted into neural progenitor cells from which immature and, eventually, mature neurons arise.

Due to their ability to regenerate, these nerve cells occupy a special position in the mature nervous system.

Bowman's glandular cells are located at the base of the epithelium at the Lamina propri. This gland is responsible for secreting the mucus layer which covers the surface of the olfactory epithelium. The mucus acts as a dissolvent for odorant chemical materials and brings them into contact with the olfactory receptor.

The supporting cells are located between the ORN and their purpose is to support the ORN. The majority of these cells are located in the upper third of the olfactory epithelium. **Microvilli cells** are located at their apical free end.

1.3 Mechanism of Smell

At least four different systems can be involved in the perception and transmission of olfactory signals: the (main) olfactory system, the trigeminal system, the accessory olfactory system and the terminal nerve. The olfactory system is the respective system for smelling volatile chemical substances. Its function for the perception of non-smelling and non-volatile substances in man is unclear. The trigeminal system is responsible for the perception of cold, pungent or burning sensations (Hummel, 2000). The accessory olfactory system is the respective system for the perception of non-smelling and non-volatile substances like pheromones, at least in nonhumans. The function of the terminal nerve is still unclear but its chemosensory stimulation seems to be related to reproductive behavior.

The cilia of the sensory receptor cells contain the seven domain transmembrane receptors, which interact with the incoming odorants. These odorants reach the nasal mucosa via the inhaled air and must be hydrophilic or lipophilic to dissolve in the mucus to reach the cilia (Doty 2003b). After stimulating the olfactory cells and eliciting a receptor potential within the cilia of the bipolar cell that trigger the action potentials, the afferent signal is transduced via the fila olfactoria to the olfactory bulb. The synaptic transmission in the olfactory bulb is the only synapse intervening between the impingement of a stimulus on the olfactory receptor and the arrival of the nerve impulse at the cerebral cortex. The major second-order neurons in the olfactory bulb are the mitral and tufted cells whose firing rates are under a considerable

modulation via inhibitory processes. From the olfactory bulb the axons of the mitral and tufted cells project as olfactory tract directly to the primary olfactory cortex without synapsing with the thalamus. The olfactory cortex is comprised of the anterior olfactory nucleus, the prepiriform cortex, the lateral entorhinal cortex, the olfactory tubercle and the cortical nucleus of the amygdala. Projections to the secondary olfactory cortex seem to be diffused. Evidence exists that the primary olfactory cortex projects to the hypothalamus, and that the medial thalamus, the nucleus basalis meynert, the hippocampus, the septal region, the substantia innominata, the mesencephalic reticular system and the orbitofrontal cortex are involved in the further processing of olfactory impulses. Due to the direct projection to the limbic system, the prominent emotional connotation of olfaction (hedonic component) is transmitted. Major neurotransmitters in the olfactory system include glutamate as an activating transmitter and gaba as an inhibiting transmitter in the olfactory bulb, olfactory tubercle, amygdala and septal region. Additionally, dopamine and a number of neuropeptides influence the transmission within the olfactory system.

In addition to the main olfactory system, an accessory olfactory system (AOS) exists in many species, which seems relevant for the processing of chemosensory substances related to social communication and sexual behavior.

The Vomeronasal system consists of the vomeronasal organ (VNO), containing the receptor sites and distinct afferences vi the vomeronasal nerve to the accessory olfactory bulb and further to hypothalamic areas that mediate specific behavioral and hormonal responses to specific chemosensory stimuli (Halpern 2003). The VNO is located in the base of the nasal septum. It is stimulated by pheromones, which are substances secreted by individuals of the same species and which induce a behavioral reaction. In mammals, pheromones are present in a variety of secretions from various origins like the skin, sperm and vaginal secretions (Monti-Bloch et al. 1994). Chemical senses play an important role in invertebrates and a great number of vertebrates. These senses seem to be essential for distal and proximate communication with other members of the same species, the detection of food and enemies and the identification of dangerous food. The chemical senses are phylogenetically old senses. Probably, with the development of an efficient visual or auditory system in humans the chemical senses lost their impact on distal communication, i.e., long distance perception of species members. However,

evidence exists that chemical senses still have strong influences in many species, including mammals and humans, on proximal social interaction with species members, mainly on reproductive behavior and territoriality.

1.4 Smell disorder

Olfactory dysfunction, in terms of a functional anosmia, occurs with an incidence of 1-5% in the population. It is one of the most common ailments presented at ear, nose and throat (ENT) clinics, with approximately 79,000 patients per year in Austria, Germany and Switzerland and around 200,000 patients per year in the USA (Welge-Lüssen and Hummel 2014).

The occurrence of olfactory dysfunction correlates with increasing age. Usually, patients remain unaware of the dysfunction, because it mostly develops gradually. The intensity of the dysfunction is further related to a decrease in the regeneration ability of olfactory receptors, in contrast to other senses like vision and hearing loss which are easily recognized by the patient or family members.

The triggers of smell disorders are classified as post-infectious, post-traumatic, post-operative, toxic, congenital and idiopathic.

1.5 Diagnosis of smell disorder

1.5.1 Case history

What is most important in the diagnosis of smell disorder is the detailed history which will lead us to its causes. For example, the patient must be asked how he noticed the smell disorder for the first time and whether it started suddenly or gradually over a long period of time. After this, the patient is asked to recall whether he recognized the loss of smell after drug intake (if yes, what this medicine was), after exposure to chemical materials or after an acute infection such as rhinitis or rhinosinusitis.

Other questions for the patient include whether or not he has experienced any smell loss before or if this is the first time, and if there are any other nasal symptoms such as discharge, rhinorrhea and nasal bleeding.

Other systemic diseases include hepatic disease, thyroid, renal and cardiopulmonary disease.

Family medical history is also important and the patient should be asked if a member of his family has ever experienced neurodegenerative diseases like Parkinson disease and Alzheimer disease.

All of these questions will be asked in a special questionnaire (data sheet) which can be given to the patient (Figure 1.3).

1.5.2 Clinical examination

An ENT examination focuses on the nose and the head. First, the shape of the nose is checked for an extremely clear nasal deviation and then anterior nasal rhinoscopy is conducted in order to check the anterior part of the nose through the nasal opening. After that, a nasal endoscopy is conducted in order to look at the nasal mucosa in terms of its texture and color (healthy pink or pathological red), and to see if there is any nasal discharge such as pus or serous. Further, if the nasal septum is extremely deviated and if the nasal sinuses have any discharge then they must be examined. Signs of acute or chronic sinusitis should be evaluated since polyps can conceal the nasal cavity.
Finally, the examiner will pass deeply through the nose with an endoscope to see whether the olfactory cleft is free or concealed due to a pathological cause.

Questionnaire:
History of smell/taste disorder

(self-adhesive label)

Phone (home):

Phone (other):

What kind of problem do you have? *You may check more than one box*	☐ a smell problem ☐ a taste problem concerning aromas (subtle taste perception) ☐ a taste problem concerning the perception of sweet, sour, bitter, or salty
When was the onset of your problem?	☐ less than 3 months ago ☐ 3 to 24 months ago ☐ more than 2 years ago ☐ it has been there as long as I can remember ☐ I don't know
How did the problem start?	☐ slowly ☐ suddenly ☐ I never could smell in all my life ☐ I don't know
How has the situation changed?	☐ there was an improvement ☐ the situation is unchanged ☐ it has become worse
What could have been the cause of your problem?	☐ accident ☐ cold / infection ☐ medication ☐ surgery ☐ breathing through the nose/nasal polyps/sinusitis ☐ dry mouth ☐ dentures ☐ others (please name)
Do you have chronic nasal problems?	☐ no ☐ yes—please indicate: running nose, nasal obstruction, sneezing, allergy, polyps, facial pain ....
Is your condition fluctuating or constant?	☐ fluctuating ☐ constant ☐ I don't know ☐ if fluctuations are dependent on certain circumstances, please describe:
How badly does the problem affect you?	☐ very badly ☐ badly ☐ medium badly ☐ mildly ☐ hardly at all ☐ not at all
How would you describe your nasal patency?	☐ very good ☐ good ☐ bad ☐ very bad ☐ I cannot breathe through the nose at all
The following questions concern **taste** dirorders only	
The taste disorder mainly concerns the perception of:	☐ sweet ☐ sour ☐ salty ☐ bitter ☐ spicy ☐ none of these
Do you suffer from any constant oral sensation?	buning mouth ☐ yes ☐ no bitter taste ☐ yes ☐ no salty taste ☐ yes ☐ no sour taste ☐ yes ☐ no dry mouth ☐ yes ☐ no foreign body sensation ☐ yes ☐ no

To be completed by the **physician**	
Weight loss due to problem?	☐ no ☐ yes .....kg/.....years
Medication?	☐ no ☐ yes – which?
Chronic diseases?	☐ no ☐ yes – which? ☐ diabetes ☐ high blood pressure ☐ neoplasia ☐ others: .....
Head surgery?	☐ no ☐ yes – which? ☐ sinuses ☐ nasal septum ☐ nasal ployps ☐ nasal turbinates ☐ palatal tonsils ☐ adenoids ☐ middle ear ☐ left ☐ right ☐ dental surgery: ..... ☐ others.....
Flu shots?	☐ no ☐ yes – when?
Smoker?	☐ no ☐ yes – extent?
Alcohol?	☐ no ☐ yes – ☐ occasionally ☐ regularly
Diagnostic imaging?	**CT** ☐ no ☐ yes **rad.-sinuses** ☐ no ☐ yes **MRI** ☐ no ☐ yes Findings: .....
Profession?	Specific exposure to gaseous, powdered, or other chemicals? ☐ no ☐ yes If **YES**, which? ..... duration (years)? ..... hours per day? .....
If idiopathic etiology is suspected:	Parkinson disease among relatives ☐ no ☐ yes Alzheimer disease among relatives ☐ no ☐ yes
Parosmia ☐ no ☐ yes ☐ left ☐ right	☐ daily ☐ not daily ☐ very strong ☐ mild ☐ weight loss due to parosmia ☐ no weight loss
Phantosmia ☐ no ☐ yes ☐ left ☐ right	☐ daily ☐ not daily ☐ very strong ☐ mild ☐ weight loss due to phantosmia ☐ no weight loss

Test results

"Sniffin' Sticks" T: D: I:

Taste Strips (x out of 32): .....

Taste sprays (4 Sprays): .....

Retronasal (x out of 20): .....

Suspected etiology:

☐ post traumatic ☐ post-infectious
☐ sinunasal ☐ idiopathic
☐ toxic ☐ congenital
☐ neurodegenerative ☐ others

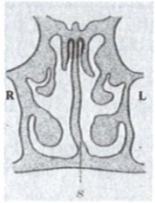
Nasal findings

Septal deviation ☐ left ☐ right ☐ none

Olfactory cleft visible ☐ left ☐ right

Polyps left: ☐ 0 ☐ I ☐ II ☐ III

right ☐ 0 ☐ I ☐ II ☐ III



Examiner (name / signature)

Figure 1.3: Family medical history questionnaire for patients with smell disorders. (Adapted from Hummel and Welge-Lüssen. Riech und Schmeckstörungen: Physiologie, Pathologie und Therapeutische Ansätze. Stuttgart: Thieme; 2005).

1.6 Tests to assess the sense of smell

Today, different methods exist to quantify olfactory capacities; some of those apply psychophysical measures, which are the most important and easiest methods, and electrophysiological tests, which come from a well-established method, in addition to imaging methods (functional magnetic resonance, MRI and volumetric assessment of the olfactory bulb).

1.6.1 Psychophysical assessment of olfaction

Several tests have been established for psychophysical assessment of olfaction. Most of them are based on the same ideas such as odor detection and recognition (threshold), difference threshold, discrimination, odor recognition and identification. The psychophysical assessment is the simpler and less expensive method. It also does not require a very experienced examiner in comparison to other assessment methods such as electrophysiological and imaging. However, these tests require the patient's cooperation with the examiner and they would be therefore very difficult to carry out on children or on patients with cognitive impairment.
Some examples involving the psychophysical test:

University of Pennsylvania smells identification Tests (UPSIT)

This test is commercially available for smell identification to test the function of an individual's olfactory system. The test only takes a few minutes and consists of 40 different odorants on "scratch and sniff" strips which are microencapsulated odorants embedded on paper. Upon scratching the odor is released and the patient has to detect the odor from multiple choice item lists. The test is scored out of a total of 40. The score is compared to scores in a normative database from 4000 normal individuals and its results show the level of absolute smell function (Doty et al., 1984). The score also indicates the patient's level in accordance to their age group and gender.

Sniffin' sticks TDI score

This psychophysical test provides more detailed information about the olfactory function. The test can be done by using many odorized felt-tip pens (see Figure 2.2). The whole test is divided into three parts: odor threshold (phenethyl alcohol testing by means of a single staircase method), odor discrimination (16 pairs of odorants, triple forced choice) and odor identification (16 common odorants, multiple forced choices from four verbal items per test odorant). After that the summation of results from these three parts provides the final result of the TDI score. For more detailed information see Sniffin' sticks in the material and methods chapter.

1.6.2 Electrophysiological test olfactory event related Potential (OERP)

Event related potential is an EEG-derived polyphasic signal; it is created through the activation of cortical neurons due to different sources of stimuli (visual, audio or olfactiory). The higher the amount of neurons involved and synchronized, the larger the amplitude obtained at the scalp surface.

Olfactory event related potential is important in clinical use for a number of reasons. OERP (1) directly correlates neural activation unlike the signals that are seen in functional MRI, for example; (2) has a high temporal resolution in a range of micro-seconds; (3) allows the investigation of the sequential processing of olfactory information; (4) can be obtained independently of the subject's cooperation (therefore, it can be recorded in children, older patients, and malingering patients).

As known, the EEG features noisy signals that contain activity from many cortical neurons. Therefore, the ERP needs to be isolated from this noisy background. The best solution for this problem is to calculate an average of the individual responses to stimuli (visual, auditory or olfactory); such that random activity would cancel itself out, while the non-random activity would remain (Hummel & Kobal, 2002).

However, calculating the average of many ERPs involves difficulties such as artifacts like blinking movement and muscular activity, which have to be excluded while performing the test in order to gain best results of ERPs signal.

Repeated stimulus at intervals (ISI) of 30-40s is necessary in order to avoid adaptation and habituation (Lötsch & Hummel, 2006). This means the examination

takes a long time to record the OERP from one subject and it is difficult for old people.

The aim of our study was to find out whether it is possible to obtain OERPs despite using a shorter ISI (in our case 10 sec.) as so far recommended. At the same time, we wanted to investigate the effects of a decreased ISI on the signal to noise ratio, amplitude and latency of OERPs. We hypothesised that OERPs conducted using a 10 s ISI might improve signal to noise ratio without impairing OERP signal.

2. Materials and Methods

2.1 Ethics

All the subjects and patients who agreed to participate in this study were fully informed about the course, procedure and the scientific background of the study, orally and in writing. Following this, they were asked to sign a written informed consent for the scientific use of the collected data. Participants were informed that their results and any information concerned with their participation in this study would be treated in accordance with the rules of medical confidentiality.

The study was conducted according to the Declaration of Helsinki of the World Medical Association on "Ethical Principles for Medical Research Involving Human Subjects" (World Medical Association General Assembly, 2004). The request to conduct the study was reviewed by the Ethics Committee of the Medical Faculty of the Technical University of Dresden and approved (EK 115042013) on the 16th of May, 2013. Data was collected from June 2013 to March 2014.

2.2 Participants

The total number of participants in this study was n=101 (50 women and 51 men) with an age range of 18-80 years, (mean age 44.3 years, SD 17.5 years).

Participants were divided into three groups (normosmic, hyposmic and functional anosmic) according to their Threshold, Discrimination and Identification score (TDI) using the “Sniffin’ Sticks” test battery (Burghart Instruments, Wedel, Germany). According to Kobal et al., a TDI score of ≤16.5 was considered as functional anosmic, a TDI score between 16.75 to 30.5 as hyposmic and a TDI score of >30.5 as normosmic (Kobal et al 2000).

The normosmic group consisted of 42 participants (22 women and 20 men) with a mean age of 32.1 years (SD 11.8 years, age range 19-69 years). The volunteers were paid a modest sum of 20 Euros for participation in this study. None of these participants had complaints about any smell or taste problems. In addition, they did not suffer from any chronic diseases (e.g. diabetes mellitus, depression etc.), which are known to possibly affect the sense of smell or cause acute disease of the nose or nasal sinus (e.g. chronic rhinosinusitis (CRS), influenza, upper respiratory tract

infection etcetera.). The hyposmic group consisted of 19 patients (11 women and 8 men). They were patients who had come to the TU Dresden Uniklinikum (Uni Clinic) because they suffered from olfactory problems. The mean age of the hyposmic patients was 49.1 years (SD 16.5 years, age range 18-80 years).

The functional anosmic group included 40 patients (17 women and 23 men). They were patients who came to the TU Dresden Uniklinikum because they complained about problems with their sense of smell. The mean age of the functional anosmic patients was 54.8 years (SD 25.2 years, age range 19-79 years) (see Figure 2.1).

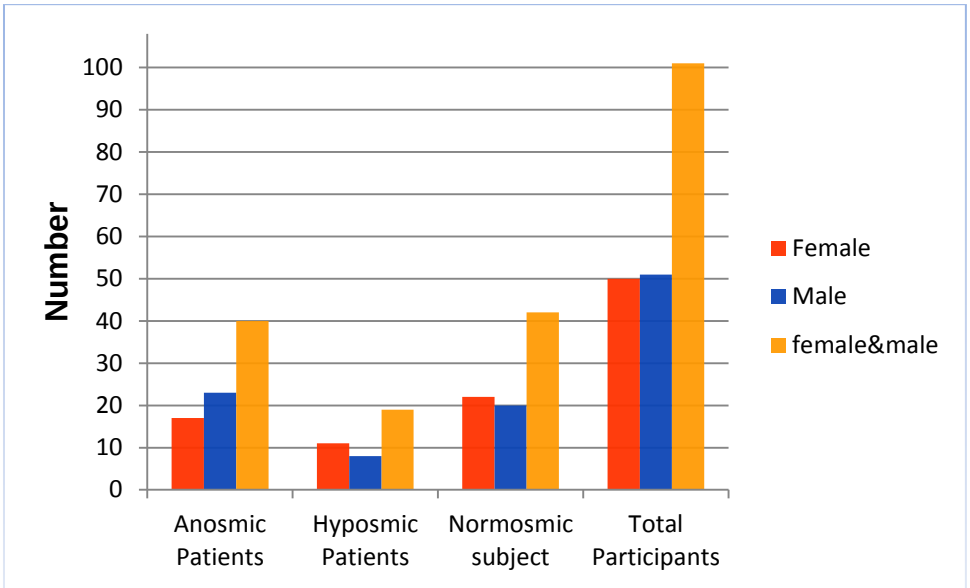


Figure 2.1: Shows the number of participants in each group and the differences in number between females and males in the study.

2.3 Psychophysical testing (“Sniffin’ Sticks”)

The psychophysical testing of the olfactory function was performed by means of the “Sniffin’ Sticks” test battery (“Sniffin’ Sticks” Burghart GmbH, Wedel, Germany). This test can be done by using odorized pens, such as commercially available felt-tip pens. The pen had a length of 14 cm, with an inner diameter of 1.3 cm. To release the odor, the cap of the pen was removed for three seconds and then the tip of the pen was placed approximately 1-2 cm in front of the nostrils (Hummel et al., 1997).

The “Sniffin’ Sticks” test was used to evaluate the olfactory function. It is divided into three sub-tests: 1. Odor threshold test (T), 2. Odor descrimination test (D) und 3. Odor identification test (I). These three tests make up the TDI score (the acronym TDI represents the first letter from each test). Acording to the results of the “Sniffin’ Sticks” test, it is possible to determine the smell ability of the subjects.

Figure 2.2: “Sniffin’ Sticks” test battery used in the psychophysical testing of the olfactory function (“Sniffin’ Sticks” Burghart GmbH, Wedel, Germany).

Odor threshold

The odor threshold test consisted of 16 pen triplets. Each triplet had one pen containing PEA diluted in propylene glycol (dilution ratio 1:2, starting at 4%). The stronger concentration was in pen no.1 and then the concentration was diluted for the

following pens. The last one, pen no.16, had the lowest concentration. The dilution process was in a fixed ratio of 1:2, which means pen no.2 had half the concentration of pen no.1, pen no.3 had half the concentration of pen no.2, and so on. The other two pens in each triplet were odorless, containing only propylene glycol.

During the examination, subjects were blindfolded with a sleeping mask to prevent visual identification of the odorized pens (Hummel et al., 1997; Kobal et al., 2000). One triplet of odor pens was presented to the subjects at a time and subjects were asked to choose the pen in each triplet which contained the odor. If subjects could not smell anything, they were told to guess which pen contained the odor. The test started with the lowest concentration (pen no.16). Two successive correct identifications or one incorrect identification of the pen containing the odor triggered a reversal of the staircase to the next higher or the next lower dilution step respectively. Seven reversals were conducted. The last four reversals were averaged in order to obtain the odor threshold (Doty, Smith, Mckeown, & Raj, 1994; Hummel et al., 1997). The testing procedure and the results were recorded on the data sheet displayed in Figure 2.2.

Riechtest - SDI

Sniffin' Sticks

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

Name:_____Vorname:_____

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

SNIFFIN' STICKS - SCHWELLE (beidseitige Testung)

Ergebnis : _____

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

SNIFFIN' STICKS - DISKRIMINIERUNG (beidseitige Testung)

	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)

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 : _____

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

Figure 2.3: The data sheet used during the TDI score examination.

Odor discrimination

The odor discrimination test also consisted of 16 pen triplets. Two pens from each triplet had the same odor and one pen had a different odor. All three pens in the same triplet were subjectively isointens. As in the olfactory threshold test, participants were blindfolded using a sleeping mask. One triplet of odor pens was presented to the participant at a time. The participant's task was to identify the pen with a different odor in each triplet. In this manner, all 16 triplets (starting from no.1 to no.16) were presented to the participant. The answers were recorded on the data sheet (Figure 2.2) and the correct answers were added up to calculate the odor discrimination score.

Odor identification

During the odor identification test, 16 pens containing different odors were presented to the participants. The odors used were familiar every-day life odors such as orange, cinnamon, garlic or coffee. Participants were asked to identify the odor from a list of four descriptors. The odor identification score is represented by the summation of the correctly identified odors.

2.4 Olfactometer (Olfactory Stimulator)

Chemosensory stimuli are produced by an air-dilution olfactometer (OM2S, Burghart, Wedel, Germany). With this device, these stimuli are delivered to the nasal mucosa without any mechanical or thermal irritation. This is achieved by a constant air flow rate (6.4 l/min), temperature (36-38°C) and humidity (80% relative humidity) during the examination.

The olfactometer has two airflows directed towards its outlet. Both of the airflows have the same flow rate, temperature and humidity. One of these systems contains an odorant at a defined concentration (Odorant "O" + Dilution "D") and the other contains odorless air (Control "C").

Stimulus concentrations can be adjusted by changing the ratio of the odorant and dilution, respectively, in the odorant air stream. The sum of both odor and dilution (O+D), should always be equal to the control airflow (C).

The olfactometer has a vacuum system to ensure fast switching between odorant and control airflow. This switching is faster than 20ms (Hummel & Kobal, 2002). During the interstimulus interval (ISI), the airflow delivered from olfactometer to the participants consists of the control airstream (odorless air), while the O+D airflow is sucked away by the vacuum. During odor presentation the vacuum is switched, leading to the delivery of the O+D airstream to the participant. In this case the C airflow is sucked away by the vacuum. For a detailed description of the airflow see Figure 2.3.

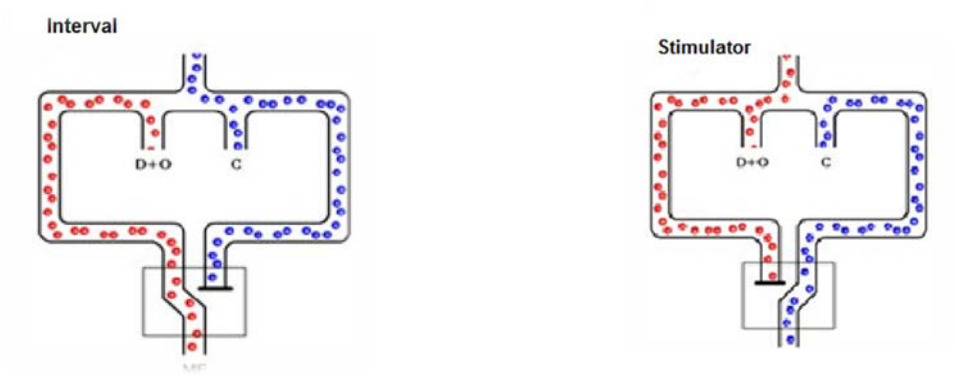


Figure 2.4: Switching principle of the olfactometer OM2S in interstimulus interval (left) and during stimulation (right), (ME = Main Exhaust (vacuum), C = Control / clean air, D = dilution / dilution, O = odor / fragrance); Burghart Medical Technology, Wedel, Germany.

The olfactometer has an air pump. This pump supplies the device with dry air, which passes through a heater. The heater adjusts the air temperature to 36-38°C. The air then passes through a water chamber leading to a humidified airstream of approximately 80% relative humidity. This is because a cold and dry airstream would affect the nasal mucosa and produce nasal congestion, mucus discharge and pain

(Lötsch, Weiss, Kobal, & Geisslinger, 1998; Mohammadian, Hummel, Loetsch, & Kobal, 1997), whereas the subjects can adapt to a warm and humidified airstream.

The olfactometer is connected to a computer from which the valves of the olfactometer are controlled. The program by which the olfactometer is controlled is called OM2s1.4.1 (Burghart GmbH, Wedel, Germany). This program determines the airflow of each air stream (as in the control airstream C) and changes the odor concentration of the stimulus by changing the percentage of air directed through the odorant and the dilution respectively.

Figure 2.5: Air-dilution olfactometer (OM2S, Burghart, Wedel, Germany).

2.5 Stimuli used for recording chemosensory event-related potentials

Two different stimuli were used in this study: phenyl ethyl alcohol (PEA) and carbon dioxide (CO_2):

- Phenyl ethyl alcohol (PEA) is a colorless liquid and a pleasant odor thought to selectively stimulate the olfactory nerve. It was used in a concentration of 50 v/v%.
- Carbon dioxide (CO_2) is a colorless gas which specifically stimulates the trigeminal nerve. It was used in a concentration of 50 v/v%.

2.6 EEG setup

The EEG was recorded from five positions Fz, Cz, Pz, C3, and C4. Electrode sites were used according to the 10/20 system (Klem et al. 1999). In addition to this, Fp2 for controlling vertical eye movements. All electrodes were referenced against A1 and A2 and ML and MR were used for grounding. All electrodes used in this study were gold-coated electrodes (Grass Instruments Division, Astro Med Inc. Warwick, RI, USA). After applying the electrode to its appropriate place on the scalp, they were connected to the EEG amplifier (EEG, Nihon Kohden, Tokyo, Japan).

EEG segments of 2048 ms were digitally recorded including a 500 ms pre-trigger period. Off-line (band-pass filter low-pass 15 Hz) averaging yielded ERP. Recorded EEG segments that involved eye blinks or other disturbances were excluded. Peak-to-peak amplitudes (p1n1 and n1p2) and peak latencies (p1, n1 and p2) of CSERP recordings were analyzed by Letswave5 (free Matlab toolbox). This software was developed by André Mouraux (Institute of Neuroscience, Université catholique de Louvain, Belgium).

2.7 Study design

Chemosensory event-related potentials (CSERP) are a well-known method for the clinical evaluation of olfactory function. Although this method is widely used, there is still room for improvement. Therefore, in this study we tried to increase the reliability of event-related potentials by trying to increase the signal-to-noise ratio by increasing the number of stimulus repetitions.

Two different ISI were used for PEA (10s and 30s) to obtain the OERP. In addition we used CO_2 to obtain trigeminal event-related potentials with a short ISI of 10s.

This study consisted of three sessions with a total recording time of 46 min.

1. PEA 10s ISI: This session took 20 minutes, 60 repetitions of PEA stimulation were used for each nostril (left and right).
2. PEA 30s ISI: This session had duration of six minutes, 16 repetitions from each nostril were recorded (left and right).

CO_2 10s ISI: The duration of this session was 10 minutes, 30 repetitions from each nostril were recorded (left and right).

2.8 **Examination process**

2.8.1 Preparation of participants

The procedure was started by psychophysically examining the participants' olfactory function. According to their TDI score, participants were assigned to one of the three groups in our study (functional anosmic, hyposmic, normosmic).

After evaluating the participants' olfactory function, the chemosensory event-related potential recordings procedure was explained to the participant in great detail. During the recording, the participants were asked to play a computer game to stabilize vigilance. The participants' task was to keep a white dot, which was controlled by a computer mouse, inside a randomly moving square. This results in relatively stable eye movements and therefore minimizes the primary source of EEG artifact. The computer game further stabilizes the attention and vigilance of the participants, which is very important in a long recording session with a duration of 46 minutes (Hummel et al. 2003).

After that the participants were instructed to remain relaxed and sit as still as possible in the olfactometer chair, which is equipped with arm, leg and head rests. The whole session should be conducted in a well-ventilated room with good air circulation to prevent odor contamination.

The EEG electrodes, Fz, Cz, Pz, C3, and C4, were arranged according to the 10/20 system as mentioned before (Klem et al. 1999). A tape measure was used to measure the distance between the nasion and inion to determine the five points for placing the electrodes. These points were then marked by using a special marker pen (Eyeliner Pencil).

The skin at the marked points was treated with a cotton swab and a special cleaner (Skin Pure, Nihon Kohden, Tokyo, Japan) for degreasing and improvement of conductance. Then a thin layer of highly conductive electrode paste (EC2 ™, Grass Instruments Division, Astro Med Inc., Warwick, RI , USA) was applied. In addition, the tips of the electrodes were covered with the highly conductive paste and pressed onto the pretreated skin.

Five additional electrodes were used as well as the previous mentioned electrodes: Fp2, placed over the right eyebrow for detecting eye movements and blinking artifacts, the reference electrodes A1 and A2 on the left and right ear lobes, and the grounding electrodes ML and MR at the left and right mastoid. These electrodes should be fixed at their positions after cleaning the skin by using the cotton swab and the special cleaner as described above (Skin Pure, Nihon Kohden, Tokyo, Japan).
All ten electrodes, attached to the scalp, ear lobes, mastoids and over the right eyebrow, were connected to the EEG amplifier (EEG, Nihon Kohden, Tokyo, Japan) according to their designated positions.

After these preparations the nose piece for odor delivery was adjusted. The nose piece was mounted on a user-adjustable telescopic arm and could therefore be optimally adapted to the size and sitting position of the participant.
Subsequently, the headphones were placed in the participant's ears. White noise with approximately 60 dB was presented to the participants in order to remove surrounding and, in particular, olfactometer noise, which can produce auditory-evoked potentials during the stimulation and thereby affect the measurements. Participants were asked to breathe through the mouth during the recording in order to minimize effects of respiratory air movements on the presentation of the odorous stimulus (G. Kobal, 1981). In addition, we asked the subject to avoid extensive eye blinking to avoid EEG artifacts.

A curtain was used to separate the participants from the investigator and to isolate the participant from any other visual stimulus which could affect his vigilance. In order to keep the vigilance of participants at the same level, we asked them to do a special task with a computer game (a free moving square was shown on a screen and the participants had to follow it with the mouse pointer). After finishing the preparations, the computer game and the program for EEG were started. Throughout the measurement the participants were monitored with a camera in order to observe their compliance as well as to notice any necessary adjustments regarding the nose piece.

2.8.2 Recording of chemosensory event-related potentials

This study consisted of three recording sessions. The total time of these three sessions was approximately 46 minutes.

The duration of the first session was 16 minutes. During this session, the odor stimulus was repeated 32 times. The stimulus concentration was fixed to PEA 50% and the stimulus duration was set to 200ms (G. Kobal, 1981). The ISI was randomized around 30s (28-32s). After eight stimulus repetitions the nose piece was shifted to the other nostril. The nose piece was shifted back to the first nostril after another eight stimuli until 16 stimuli from each nostril (left and right) had been recorded. The nostril site of first stimulation was alternated between the participants.

The second session took 20 minutes and 120 stimulus repetitions were presented. Again, the stimulus concentration was PEA 50% with stimulus duration of 200ms. In this session, the ISI was randomized around 10s (8-12s). The nose piece was switched between nostrils every 30 stimuli. At the end, 60 stimuli had been recorded from each nostril.

The duration of the third session was 10 minutes. Sixty stimulus repetitions were presented during this session. CO_2 was used at a concentration of 50%. Again the stimulus duration was fixed at 200ms and the ISI was set to 10s. In this session, 30 stimuli were presented to each nostril.

2.8.3 Evaluation the results of OERP

The recorded EEG data was saved on a computer with the file extension ".td". The file format had to be changed to ".txt" in order for it to be readable by the EEG analyzing program Letswave5. A small program, called evokconv2 (courtesy of Alexander Croy, Dresden), was used to convert the EEG data file from ".td" to ".txt".

Letswave5 is a free Matlab toolbox for analyzing EEG and other neurophysiological data. This software was developed by André Mouraux (Institute of Neuroscience, Université catholique de Louvain, Belgium). For analyzing the data, Matlab2013a (Natick, Massachusetts, USA) was used with the letswave5 toolbox. After importing the data file, a band-pass frequency filter (0.3 – 20 Hz) was applied. This was followed by a baseline correction. For the reference interval, the 500 prestimulus

intervals were used. After that the recordings were then analyzed separately for the left and right nostrils.

After this separation, EEG artifact rejection was processed manually. Recordings with amplitudes of greater ± 50μV and visible blinking artifacts were removed. A minimum of eight artifact-free EEG epochs were considered as the limit which allowed further interpretation of the elicited EEG responses (Covington et al. 1996; Hummel et al. 2000).

Finally, the remaining epochs were averaged in each participant in order to obtain the event-related potential.

The Event Related Potential Signal (ERP) consists of three components (P1, N1 P2) (see Figure 2.6). These components are represented by a negative-positive complex consisting of an initial positive peak (P1: latency > 250 ms) followed by a negative peak (N1: latency: 290 – 490 ms, amplitude < –2 _V) and a positive peak (P2: latency: 460 – 820 ms, amplitudes > +2 _V) (Rombaux et al. 2009). For the signal recording, we recorded the latency in msec. and amplitude in μv for each peak of the signal (P1, N1 and P2) from all electrodes (Cz, Fz, Pz, C3 and C4).

Signal-to-Noise ratio is usually recorded by calculating the signal which is equal to the peak of N1+P2, and the noise levels were calculated as the average of two selected maxima and minima of spontaneous EEG during the 500 ms prestimulus interval. Dividing the N1P2 amplitude by the average noise level yielded the signal-to-noise (S/N) ratio (Boesveldt and Haehner 2007).

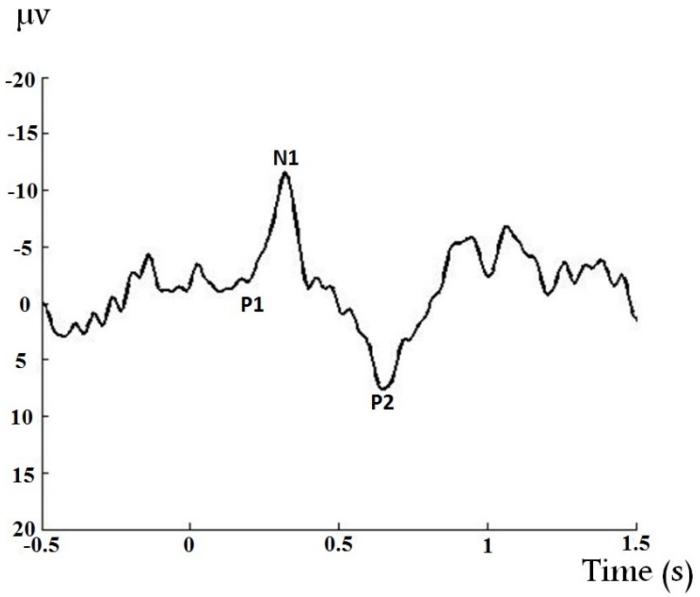


Figure 2.6: Event Related Potential (ERP) Components: first positive (P1), first negative (N1) and second positive (P2), the stimulus starts at 0 and lasts for 200 ms stimulus (0 - 0.2), the ISI was 10s and the stimulus was PEA.

3. Results

3.1 The causes of olfactory disorders

The causes of olfactory dysfunction in our functional anosmic and hyposmic group belonged to different diseases (Post traumatic, Idiopathic, Post viral, Sinusitis, Postoperative, among others).

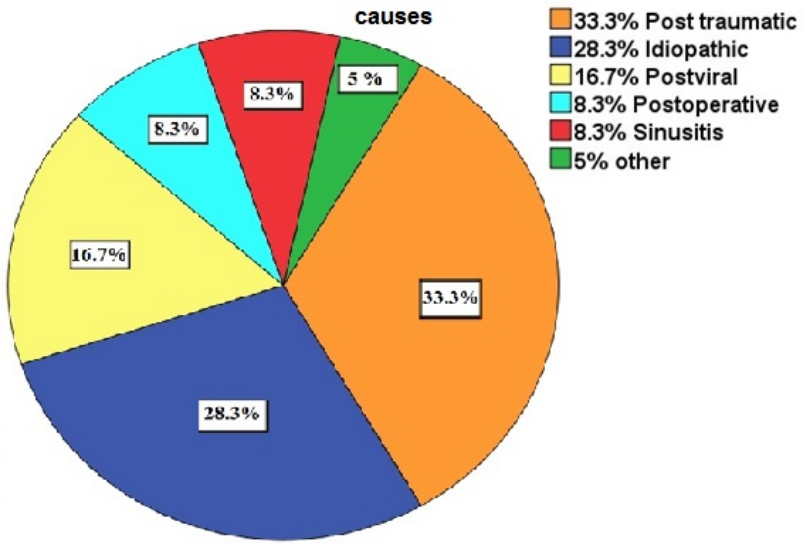


Figure 3.1: The percentage of different causes of olfactory loss.

3.2 Effects of Age on TDI score

There was a significant effect of age on the TDI score (the minimum age of the participants in our study was 18 years old). We found an inverse relationship between age and the TDI score with the younger subjects having higher TDI scores and the older subjects having smaller scores (r = - 0.32, P = 0.04).

Figure 3.2: Relation between age and the TDI score.

3.3 The presence of parosmia and phantosmia

Among the total number (101) of participants, we found a presence of parosmia and phantosmia.

-10 participants (10% of total participants) exhibited parosmia.
-14 participants (13.7% of total participants) exhibited phantosmia.
We found that most of the parosmic patients were in the hyposmic group and the larger numbers of participants with a phantosmia were in the functional anosmic group (Table 3.1).

	Functional anosmic	**Hyposmic**	**Normosmic**	**Total**
Participants	40 subjects	19 subjects	42 subjects	N=101 subjects
Parosmia	2 subjects	6 subjects	2 subjects	N= 10 subjects
Phantosmia	8 subjects	5 subjects	1 subjects	N= 14 subjects

Table 3.1: Percentage of participants with a phantosmia & parosmia in all groups

3.4 **ERP detection**

3.4.1 The detection of OERP in both ISI (30 & 10s) in normosmic group

We found that in the normosmic group there was no significant difference in OERP detection between both ISI (30 & 10s).

In this study, we had 42 normosmic participants. We detected the OERP with 30s ISI in 36 participants (85.7 %) and with 10s ISI the OERP in both nostrils was detected in 38 participants (90 %).

Additionally, the difference in recordings between the nostrils (left and right) in both ISI was not significant as shown (*Table 3.2*).

Normosmic	**Left side**	**Right side**	**Both sides**
PEA30s ISI	31	34	36
PEA10s ISI	37	33	38

Table 3.2: *the OERP detection in 42 normosmic* participants

The results also show that there were no significant differences in OERP detection between females and males in the normosmic group which consisted of 22 females and 18 males (*Table 3.3*).

Normosmic	**Female (*n*=22)**	**male(*n*=18)**
PEA30s ISI	20 (90.9%)	16 (88.88%)
PEA10s ISI	21 (95.4%)	17 (94.4%)

Table 3.3: *The OERP detection in females and males in the normosmic group*

3.4.2 The detection of OERP in both ISI (30 & 10s) in all participants

The OERP detection was higher in 10s ISI than 30s ISI (86% vs. 91% P = ns) in the normosmic group. OERP detection was better in 30s ISI than 10s ISI in functionally anosmic and hyposmic groups.

Participants		**Functional anosmic (*n*=40)**	**Hyposmic(*n*=20)**	**Normosmic (*n*=42)**
PEA30s ISI	Left side	7 (18%)	8 (40%)	31 (74%)
	Right side	11 (28%)	7 (35%)	34 (81%)
	Both side	14 (35%)	13 (65%)	36 (86%)
PEA10s ISI	Left side	4 (10%)	5 (25%)	37 (88%)
	Right side	7 (18%)	8 (40%)	33 (79%)
	Both side	9 (23%)	10 (50%)	38 (91%)

Table 3.4: *OERP detection in left and right side in both ISI (30 & 10s).*

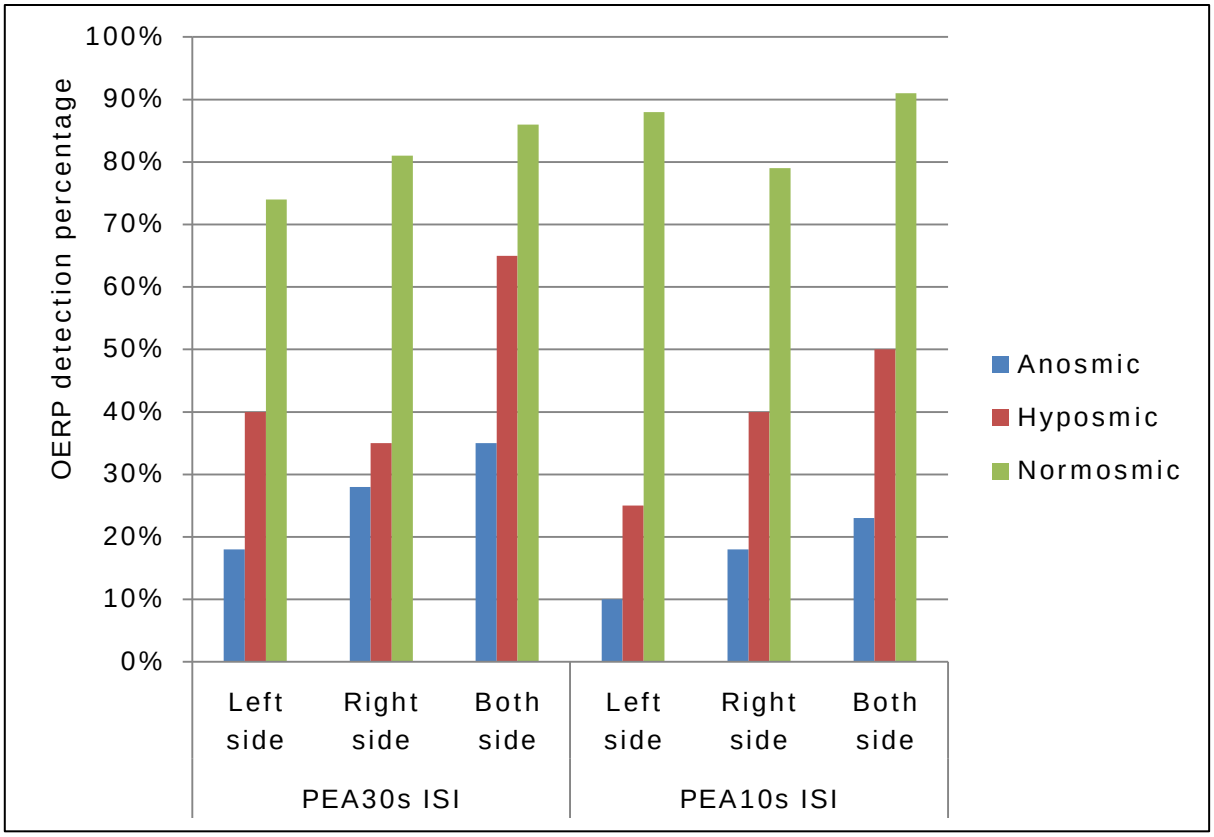


Figure 3.3: OERP detection in left and right side in both ISI (30 & 10s).

3.4.3 The detection of trigeminal ERP

The trigeminal ERP detection was higher in the normosmic group than in the functionally anosmic and hyposmic groups, as well as there being no significant difference between functionally anosmic and hyposmic groups.

Participants	**Functional anosmic (*n*=40)**	**Hyposmic (*n*=20)**	**Normosmic (*n*=42)**
Left side	19 (48%)	11 (55%)	31 (74%)
Right side	20 (50%)	10 (50%)	28 (67%)
Both side	28 (70%)	13 (65%)	34 (81%)

Table 3.5: *Trigeminal* ERP *in all* participants *groups.*

3.5 **The OERP latency in the normosmic group**

3.5.1 P1 Latency

The results from our study show that P1 latency was not significantly different (P=0.41) between the two ISI (30 &10s), just as the sites on the scalp (Cz,Fz,Pz) made no significant difference for P1 latency.

3.5.2 N1 Latency

The results show that there is a significant difference in N1 latency recorded from Cz and Fz (P=0.027), as well as between Fz and Pz (P=0.042).
However, there was no significant difference between the N1 latency recorded from Cz and Pz (P=0.626).
There was interaction between the electrodes' positions and ISI. This shows that in 30s ISI N1 latency recorded from the Fz electrode occurred later than Cz and Pz electodes, and in 10s ISI there was a difference in N1 latency recorded from Cz, Fz and Pz .

3.5.3 P2 Latency

The results show that there is no significant difference in P2 latency which was recorded from different positions (Cz,Fz and Pz) as well as no significant difference between different ISI (10 & 30s).

3.6 The OERP amplitude in normosmic group

3.6.1 P1 amplitude

The P1 amplitude showed that there was no significant difference in electrodes' positions (Cz, Pz and Fz) as well as between both ISI (10 and 30s) and the recorded nostril (left and right).

3.6.2 N1 amplitude

The results show a significant difference in amplitude (P=0.01) between the 10s and 30s ISI, but there was no significant difference in N1 amplitude which recorded from the different positions Cz, Fz and Pz (P=1.0), as well as there being no different results between each of the nostrils (left and right).

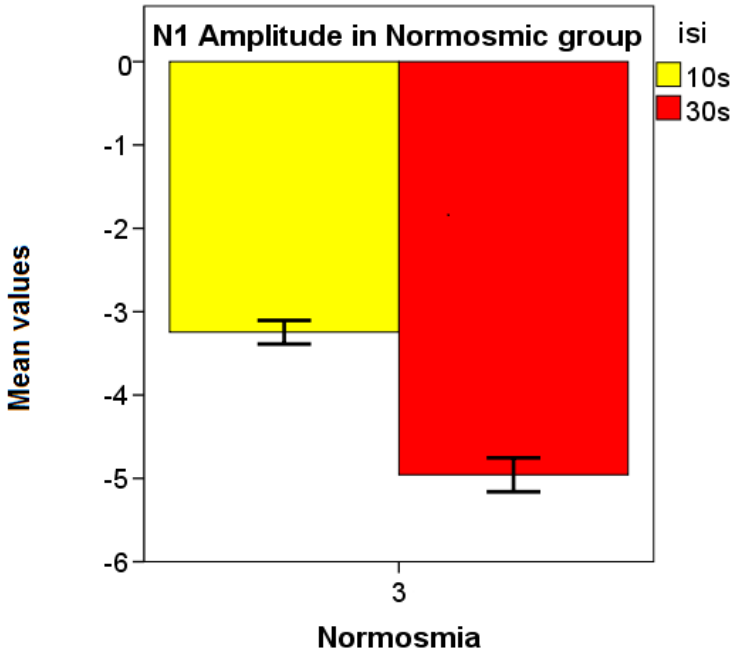


Figure 3.4: N1 amplitudes recoded by using 10s and 30s ISI in normosmic group.

3.6.3 P2 amplitude

The results show a significant difference in amplitude (P=0.001 and P=0.01) in the recording amplitude from different positions. The P2 amplitude was higher in Cz and Pz electrodes than in the Fz electrode, and there was no significant difference between Cz and Pz.

Additionally, there was a significant difference in amplitude (P=0.001) in the P2 amplitude between the 10s and 30s ISI. The P2 amplitude was higher in 30s ISI (median= 5.59 µV) in comparison to 10s ISI (median=2.99 µV).

However, we did not find any significant differences between P2 amplitudes recorded from the left and right sides.

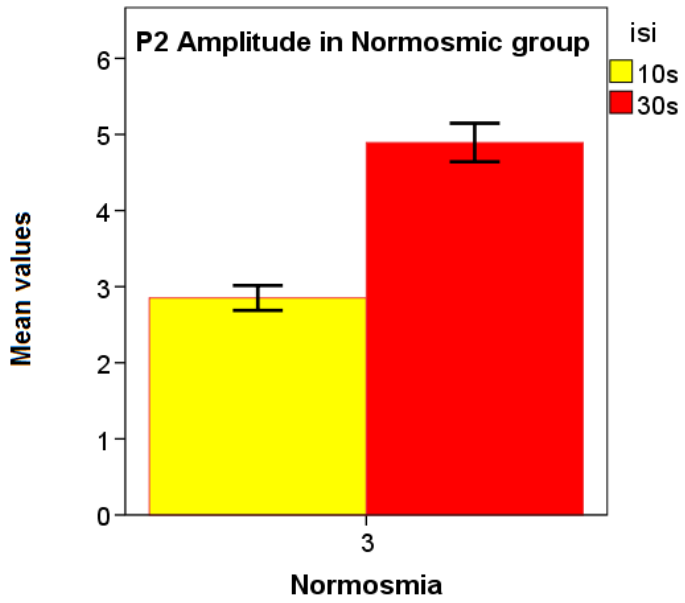


Figure 3.5: P2 amplitudes recoded by using 10s and 30s ISI in normosmic group.

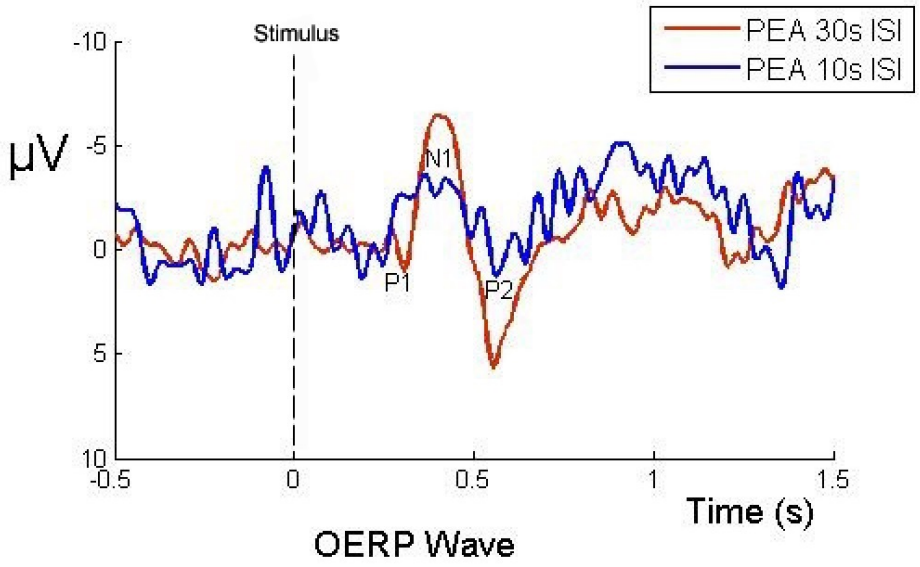


Figure 3.6: OERP wave recoded by using 10s and 30s ISI from Left nostril in normosmic group.

3.7 **The signal amplitude**

3.7.1 The signal amplitude (N1P2)

-To record the amplitude of ERP, we took the summation of the N1 and P2 amplitudes. In our results, we recognized that N1P2 amplitudes were larger (P=0.001) at Cz and Pz compared to the Fz recording site.

- Additionally, we found a significant difference between the N1P2 amplitude which was recorded from 10s ISI and 30s ISI. The results show that the amplitude was bigger in 30s ISI (median=10.423) than 10s ISI (median=6.284)
- No significant difference was found in the N1P2 amplitudes recorded after stimulating the left and right nostrils.

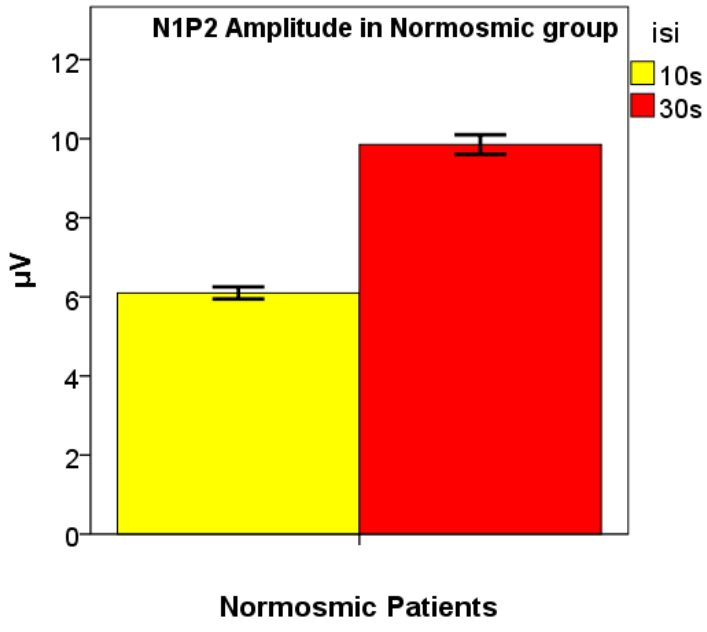


Figure 3.7: N1P2 amplitudes recoded by using 10s and 30s ISI in normosmic group.

3.7.2 The Signal to Noise Ratio

- We recorded the noise amplitude by taking the average of the two larger peaks before giving the stimulus (0s to -0.5s). The results show that noise peaks were significantly different in position between Fz-Pz (p=0.031), the noise peak was bigger in Fz electrodes than Pz.
However, there were no significant differences between Cz-Fz and between Cz-Pz.

- The noise peak also showed a significant difference (P=0.00) between 30s ISI and 10s ISI. The noise peak was larger in 30s ISI (median=6.237) than in 10s ISI (median=3.191)

- There was no significant difference in noise peak recorded from left and right nostrils.

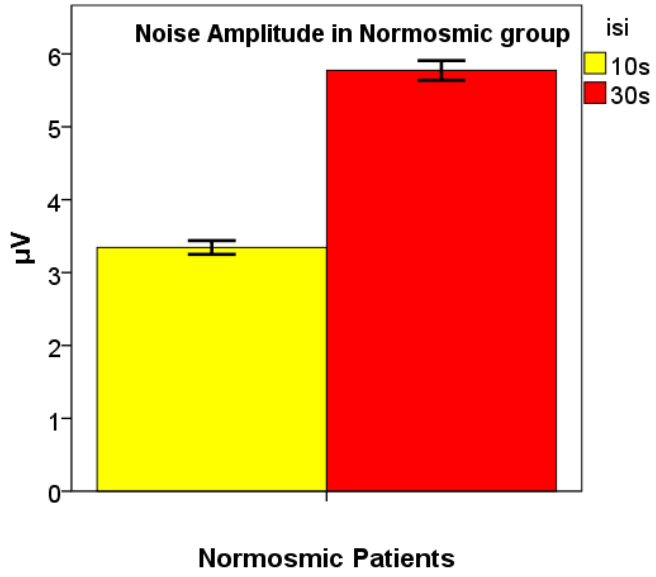


Figure 3.8: Noise amplitude recoded by using 10s and 30s ISI in normosmic group.

3.7.3 The Signal-Noise ratio

- The Signal-Noise ratio showed a difference between the positions of electrodes. There was a significant difference (P=0.001) between the Cz-Fz electrodes, as well as between Pz-Fz electrodes (P=0.010), and there was no significant difference between the Pz-Fz electrodes.
- According to the ISI, the results show that there was a significant difference in Signal-Noise ratio between 10s and 30s ISI (P=0.009). The Signal-Noise ratio was larger in 10s than 30s ISI which means that the 10s ISI improved the Signal-Noise ratio.

The results also show that there was no significant effect of the recording between stimulating nostrils site (left or right) on the Signal-Noise ratio.

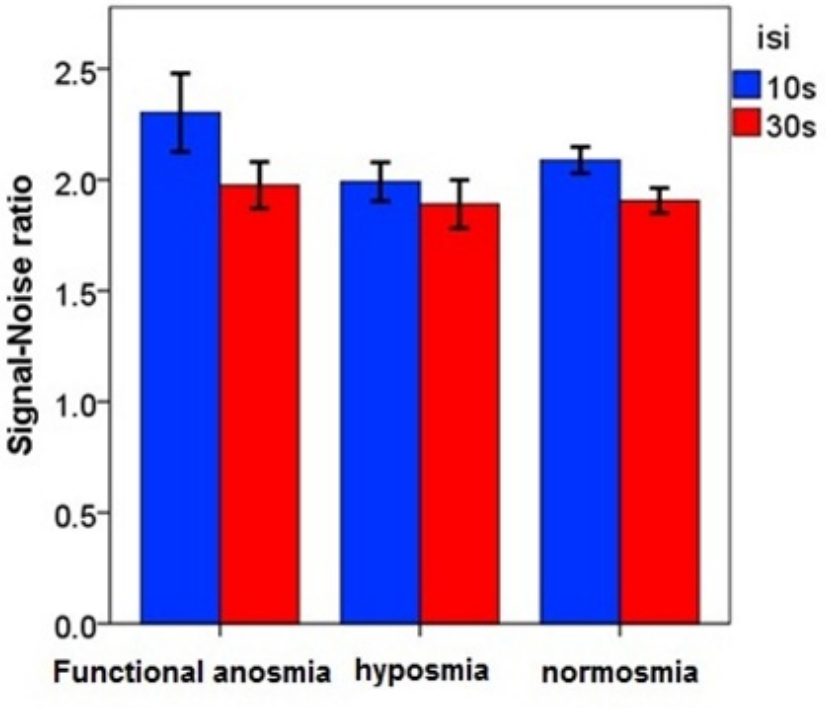


Figure 3.9: Relation in signal to noise ratio in all groups (Functional anosmia, hyposmia and normosmia) the 10s ISI show bigger signal-noise ratio than 30s ISI.

4. Discussion

The major findings of our study were, firstly, the applicability of using shorter ISI (10s) instead of longer ISI (30s) when recording OERP, which resulted in collecting meaningful data. Secondly, the Signal/Noise ratio, which recorded by using short 10s ISI, was significantly more improved than longer ISI of 30s. Thirdly, as expected from the literature, the CSERP's amplitude was smaller in short ISI (10s) in comparison to the longer ISI (30s). Fourthly, the latency was not different in both short and long ISI (10s and 30s). Lastly, there was an interaction between the olfactory and the trigeminal response to chemosensory stimuli (CO_2), which means functional anosmic and hyposmic patients were less sensitive to trigeminal responses than normosmic subjects.

As known, the CSERP is affected by many different factors such as stimulus quality, stimulus duration or ISI and we need to control and adjust these factors in order to obtain the CSERP. In this study, we wanted to examine the effect of ISI on CSERP by comparing two different ISI in order to see if this affected the CSERP's detection, amplitude and latency.

The ISI used in this study were 10s and 30s, Results show that the detection of OERP in the normosmic group was better for an ISI of 10s than 30s. Although the difference was not significant, we can still conclude that 10s ISI provide the benefit of increasing the stimulus repetition which in turn improves the signal/noise ratio (Boesveldt et. al, 2007). In addition, the use of an ISI of 10s decreases the overall time of OERP recording which makes the test easier and more reliable for patients during examination.

In previous studies (G. Kobal, 1981), Kobal used eucalyptus and linalool odors as stimuli with different ISI (12s, 22s, 32s, 42s and 52s). He found that there was a marked increase of OERP's amplitude between 12s to 42s ISI, and increasing the ISI more had little effect. For this reason, he concluded that the ideal ISI should be between 42 - 52s.

In other studies (Kassab et al. 2009; Schaub and Damm 2012) different ISI (30s, 20s, 10s) were used and compared in order to see if meaningful data could be obtained by using the shorter ISI, They also wanted to decrease the recording time of OERP.

They found that the OERP can be recorded by using the shorter ISI despite the difference in the amplitude which was significantly smaller than that recorded by the longer ISI. However, in these studies they only used a small group (n=10) of normosmic participants. For this reason we wanted to use a larger group of participants (n=102) in our study, who were normosmic, hyposmic and functional anosmic, in order to see if the short ISI is applicable in OERP detection and to try to decrease the Noise/Signal ratio in order to make the signal clearer.

In order to see the relation between the olfactory and trigeminal system, a study was done by Gudziol, Schubert, & Hummel (2001). They examined a normosmic group (n=96) and a functional anosmic group (n=72) for trigeminal threshold in response to formic acid. They observed that the functional anosmic group had a higher threshold than the normosmic group and there was also a difference depending on the cause of anosmia in the functional anosmic group. They found that the threshold was higher in patients with post traumatic anosmia compared to anosmics with sinonasal disease. They also concluded that those with olfactory loss would have a decreased sensitivity to trigeminal stimuli.

4.1 OERP detection by using 10s and 30s ISI in normosmic subjects

Our results show that the delectability of OERP was slightly higher in 10s than 30s ISI, although it did not reach the level of significance. Nevertheless, the data we obtained from 10s ISI is still valuable and can be used in the routine examination of OERP as concluded in the Kassab study (Kassab et al. 2009). The explanation for this may be due to the fact that with 10s ISI we have more stimulus repetitions than with 30s ISI. When recorded with approximately the same duration, with 10s ISI we recorded 120 stimulus repetitions during 20 minutes, while with 30s ISI we recorded 32 stimulus repetitions during 16 minutes. Following this, in order to calculate the average for OERP we had to reject the epoch which contained artifact signals from eye blinking and then to take the average of the remaining artifact-free signals. In this way, averaged epochs recorded from 10s ISI will have more repetitions than averaged epochs recorded from 30s ISI during approximately same amount of time.

It is also known from a previous study that the stimulus repetition will improve the signal (Hummel and Kobal 2002) (Boesveldt et al., 2007). These studies show that to record an OERP we must have a minimum of eight records for stimulus repetitions for it to produce valuable data (Covington et al. 1996; Hummel et al. 2000).

In yet another study such as Boesveldt et al., 2007, they used up to 160 stimulus repetitions with 25-35s ISI which meant that the recording time would be at least 100 minutes. This amount of time is impractical because it is a very long time for the patient to sit in the same position and keep their attention on the same level.

4.2 The effect of ISI on the OERP's amplitude

As expected, the results show that the amplitude was significantly smaller with 10s ISI than 30s ISI, and this is compatible with the previous studies which showed that the short ISI will decrease the amplitude of the CSERP (Hummel and Kobal 1999; Kassab et al. 2009; Schaub and Damm 2012).

The explanation for a decrease in amplitude remains unclear but it is thought to be due to an increased predictability of the stimulus occurrence after each repetition.

This results in a smaller amplitude which is therefore clearer in a shorter ISI than a longer one (Hummel and Kobal 1999).

The other cause of decreased amplitude in the short ISI may be due to adaptation which can occur at multiple levels in the olfactory system and can be both peripheral (receptor level) and more central (post-receptor). Olfactory adaptation can happen due to elevations in odor thresholds and in reduced responsiveness to suprathreshold stimulation (Dalton 2000), which could explain the decrease in the amplitude of ERP by using the short ISI (Morgan et al. 1997; Hummel and Kobal 1999).

On the other hand, the decrease in amplitude by using 10s ISI in this study may be due to increasing the number of averaged repetitions. With 30s ISI we recorded 16 repetitions for each side (left and right), unlike the 10s ISI in which we recorded 60 repetitions for each side. When we calculated the average, the amplitude of the signal was smaller due to a slight difference in the latency between the multiple repetitions (jitter) and the lack of signal in some of them. Despite the decrease of the signal's amplitude, it is still possible to detect it by using 10s ISI. The noise amplitude will be very small due to the jitter of the OERP and therefore the response amplitude will become smaller with averaging.

4.3 The Signal/Noise ratio

In this study we tried to improve the signal/noise ratio by increasing the number of averaged stimulus repetition trials. An additional aim was to avoid the long examination time, which can affect the participant's vigilance. For these reasons, we decreased the ISI to 10s (increased the repetition by using the same examination duration).

In a previous study (Boesveldt et. al, 2007), they tried to improve the signal/noise ratio for the ERP for olfactory and trigeminal (PEA, H_2s and CO_2) by increasing the number of averages and comparing them to the recorded ERP from lower repetitions. They observed that for the ERP from PEA and H_2s the signal/noise ratio significantly increased by increasing the number of repetitions up to 80 trials (averaging more than 80 trials did not show further improvement of S/N N1P2). On the other hand, with CO_2, the optimal signal/noise ratio was at an average of 60 trials and after that no significant difference was shown. $_{Ho2wever}$, t_he problem with this study is that they used a long ISI (25-35s) which means it took 40 minutes to record ERP for each side. This is a very long time and we therefore tried to decrease the ISI to 10s and increase the number of trials in our study.

Our results show that there was a significant difference in signal/noise ratio between the 30s and 10s ISI despite a decrease in the amplitude of signal and noise with 10s ISI, which was thought to be due to the effect of stimulus expectation, habituation and the effect of averaging more trials as mentioned previously.

We observed that the significant improvement in the signal/noise ratio when using 10s ISI and increasing the average number of trials gave us the chance of more repetitions in less amount of time in comparison to 30s ISI. This means that we have a higher possibility of rejecting the artifact amplitude which is caused by eye blinking without causing a big effect on the number of averaged repetitions. This will give us a clearer Yes/No olfactory response which is very important in medico legal cases in order to make a decision about whether the patient is able to smell or not, as well as having the benefit of reducing investigation time which makes it easier and more eligible for most people.

4.4 The interaction between the olfactory and trigeminal response

The results show that there was a relation between the response to olfactory and trigeminal stimulus. They show that the hyposmic and functionally anosmic patients have lower responses to trigeminal chemical stimulation (CO_2) than normosmic subjects, which means that the decrease of olfactory efficiency will decrease the efficiency of the trigeminal system in response to chemical stimulation. Many studies have been done to observe this relation and to understand its causes (Hummel & Livermore, 2002; Livermore, Hummel, & Kobal, 1992).

Our results were compatible with the previous studies (Hummel et al., 1996), which examined 32 participants, 16 with normal olfactory functions and 16 with decreased olfactory functions (hyposmic and functional anosmic patients), by using CO_2 as a stimulus to record the tERP and compare the amplitude and latency between the two groups. It showed that the amplitude in participants with normal olfactory functions was larger than the amplitude in patients with decreased olfactory functions and no significant difference was found in the tER's latency. However, it was not clear whether the interaction was at the peripheral (olfactory region and nasal mucosa) or central at the brain cortex. For this reason, other studies have been done in order to specifically locate where the interaction happened (Frasnelli and Hummel 2007). They examined two groups (a healthy control group and acquired anosmic subjects group). They recorded the response to trigeminal chemical stimulation from central electrophysiological responses, such as tERP, and peripherally such as negative mucosal potentials (NMP), which is an electrophysiological reflection of trigeminal activation on the level of the respiratory epithelium. They observed that in the acquired anosmic group the NMP was larger but the tERP was smaller than in the healthy control group. According to this result, they proposed a model of mixed sensory adaptation/compensation in the interaction between the olfactory and trigeminal systems. In this model, the primary trigeminal activation was increased on the peripheral mucosal level in subjects with olfactory loss, possibly due to adaptive processes. In healthy subjects, the olfactory system was involved in an amplification of trigeminal activation at a central nervous level. This amplification was not found in subjects with a loss of sense of smell.

5. Conclusion

Our study results show that there was no significant difference in OERP detection when using 30s or 10s ISI. However, the signal to noise ratio significantly improved with 10s ISI.

These results suggest, firstly, that we can use the 10s ISI in the electrophysiological test for recording OERP with a shorter test time, which will be more applicable and easier for patients, especially for those who are not able to stabilize their vigilance for a long time, or for children since it is difficult to keep them calm for long time.

Secondly, the test can be more applicable for routinely clinical examinations with a shorter time for and less cost.

Thirdly, it is easier for the examiner to make a decision about whether there is an OERP or not due to the increase of the signal to noise ratio.

It is recommended that more research should be carried out regarding using 10s ISI, especially for hyposmia and functional anosmia patients, because a very large amount of data is required in order to be able to study the effects of short ISI in OERP detection and signal to noise ratio.

6. Summary

The measurement of olfactory event-related potentials (OERP) is an established method to objectively assess olfactory function and it has been covered widely in research and studies, but there is still room for improvement to increase the detectability / improve the signal-to-noise ratio.

One hundred-two participants (51 female, 51 male) with a mean age of 44.3±17.5 years were included in the study. The participants were normosmic, hyposmic and functionally anosmic as ascertained by means of the "Sniffin' Sticks" test battery. OERPs in response to phenyl ethyl alcohol were measured separately for the left and right nostrils. The inter-stimulus interval (ISI) was set to either 30 or 10 seconds with 16 and 60 stimuli repetitions, respectively. OERPs were recorded from five electrodes (Cz, Fz, Pz, C3 and C4). In addition to the signal-to-noise ratio amplitudes and latencies were measured for OERP components N1 and P2.

When the ISI was set to 30 seconds, amplitudes of N1 and P2 were larger in comparison to amplitudes obtained with 10 seconds ISI (p=0.001). The signal-to-noise ratio was significantly different between the 10s and 30s ISI (p=0.009). In normosmic subjects it was more likely to obtain an OERP with the shorter ISI (both nostrils together 91 vs. 86%, n.s.).

Although it is not statistically significant, the detectability for OERP in normosmic adults was slightly higher with a 10s ISI compared to a 30s ISI, However the signal to noise ratio has significantly improved with 10s ISI. As a results of OERP recording with a shorter time, it will be easier for examiner to make a decision about whether there is an OERP or not; as well as; for patients who are not able to stabilize their vigilance for a long time, or for children since it is difficult to keep them calm for a long time.

Zusammenfassung

Die Messung der olfaktorischen ereignisbezogenen Potenzialen (OERP) ist eine etablierte Methode, um die olfaktorische Funktion objektiv einzuschätzen und ein bekannter Gegenstand in Forschung und Studien, jedoch gibt es noch Forschungsbedarf zur Verbesserung ihrer Nachweisbarkeit und des Signal-Rausch-Verhältnis.

In die hier vorliegende Studie wurden 102 Teilnehmer (51 weiblich, 51 männlich) mit einem mittleren Alter von 44.3±17.5 Jahren einbezogen. Die Teilnehmer waren normosmisch, hyposmisch und funktionell anosmisch, was mit Hilfe der "Sniffin Sticks Test Batterie" getestet wurde. OERPs wurden als Reaktion auf Phenyl-Ethyl-Alkohol getrennt für das linke und rechte Nasenloch gemessen. Das Inter-Impuls-Intervall (ISI) wurde für 30 oder 10 Sekunden mit 16 und 60 Reizimpulswiederholungen eingestellt. Die OERPs wurden von fünf Elektroden (Cz, Fz, Pz, C3 und C4) aufgezeichnet. Neben dem Signal-Rausch-Verhältnis wurden für die OERP Komponenten N1 und P2 auch Amplituden und Latenzen gemessen.

Wenn der ISI auf 30 Sekunden festgelegt wurde, waren die Amplituden von N1 und P2 größer im Vergleich zu den Amplituden die für einen ISI von 10 Sekunden festgestellt wurden ($p = 0,001$). Das Signal-Rausch-Verhältnis hat sich zwischen den ISI von 10 und 30 Sekunden signifikant unterschieden ($p = 0.009$). In normosmischen Teilnehmern war es wahrscheinlicher ein OERP mit dem kürzeren ISI zu erhalten (beide Nasenlöcher zusammen 91 vs. 86 %, n.s.).

Obwohl nicht statistisch signifikant, war die Nachweisbarkeit für OERP bei normosmischen Erwachsenen mit einem 10 Sekunden ISI im Vergleich zu einem 30 Sekunden ISI leicht höher. Jedoch hat sich das Signal-Rausch-Verhältnis bei einem 10 Sekunden ISI deutlich verbessert. Als ein Ergebnis der OERP Aufnahme mit einer kürzeren Zeit kann festgestellt werdem, dass es dem Prüfer hilft, zu entscheiden, ob es ein OERP gibt oder nicht. Außerdem erleichert es den Nachweis für Patienten, die nicht in der Lage sind, ihre Wachsamkeit für eine lange Zeit zu stabilisieren, sowie für Kinder, da es schwierig ist, dieser für eine lange Zeit zu beruhigen.

7. Literature

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8. List of Figures

9. List of Tables