Orientation in space using the sense of smell

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Abstract

Several studies reported that respiration interacts with olfactory perception. Therefore, in the pilot study of this experiment series human breathing was investigated during an olfactory experiment. Breathing parameters (respiratory minute volume, respiratory amplitude, and breathing rate) were quantified in response to odor stimulation and olfactory imagery. We provide evidence that respiration changed during smelling and during olfactory imagery in comparison to the baseline condition. In conclusion, olfactory perception and olfactory imagery both have an impact on the human respiratory profile, which is hypothesized to be based on a common underlying mechanism named sniffing. Our findings underline that for certain aspects of olfactory research it may be necessary to control and/or monitor respiration during olfactory stimulation.

The human ability to localize odors has been investigated in a limited number of studies, but the findings are contradictory. We hypothesized that this was mainly due to differential effects of olfactory and trigeminal stimulation. Only few substances excite selectively the olfactory system. One of them is hydrogen sulphide (H_2S). In contrast, most odorants stimulate both olfactory and trigeminal receptors of the nasal mucosa.

The main goal of this study was to test the human ability to localize substances, which excite the olfactory system selectively. For this purpose we performed localization experiment using low and high concentrations of the pure odorant H_2S, the olfactory-trigeminal substance isoamyl acetate (IAA), and the trigeminal substance carbon dioxide (CO_2).

In preparation for the localization study a detection experiment was carried out to ensure that subjects perceived the applied stimuli consciously. The aim of the detection study was to quantify the human sensitivity in response to stimulation with H_2S, IAA, and CO_2. We tested healthy subjects using an event-related experimental design. The olfactory stimulation was performed using an olfactometer.

The results showed that humans are able to detect H_2S in low concentration (2 ppm) with moderate sensitivity, and possess a high sensitivity in response to stimulation with 8ppm H_2S, 50% v/v CO_2, and 17.5% v/v IAA. The localization experiment revealed that

subjects can localize H_2S neither in low nor in high concentrations. In contrast to that, subjects possess an ability to localize both IAA and CO_2 stimuli. These results clearly demonstrate that humans are able to localize odorants which excite the trigeminal system, but they are not able to localize odors that stimulate the olfactory system exclusively, in spite of consciously perceiving the stimuli.

Zusammenfassung

Es gibt Hinweise darauf, dass sich olfaktorische Wahrnehmung und Atmung gegenseitig beeinflussen können. Deshalb wurde in einer Pilotstudie die Atmung gesunder Testpersonen während eines olfaktorischen Experiments untersucht. Es wurden Atemparameter (Atemminutenvolumen, Atemamplitude und Atemfrequenz) während einer Geruchsstimulation und während olfaktorischer Imagination im Vergleich zu einer Kontrollbedingung untersucht. Die Ergebnisse dieser Studie zeigten, dass die Atmung sich sowohl während des Riechvorganges, als auch während olfaktorischer Imagination im Vergleich zur Kontrollbedingung verändert. Zusammenfassend konnte festgestellt werden, dass sowohl das Riechen, als auch die olfaktorische Imagination zur Änderungen im Atemprofil von Menschen führen. Diese Änderungen können durch den motorischen Vorgang des Schnüffelns ("Sniffing") erklärt werden. Unsere Ergebnisse sprechen dafür, dass es bei olfaktorischen Experimenten erforderlich sein kann, die Atmung der Testpersonen zu überwachen und/oder zu standardisieren.

Die menschliche Fähigkeit, Duftstoffe zu lokalisieren, wurde bislang nur in wenigen Studien untersucht, deren Ergebnisse allerdings widersprüchlich sind. Wir stellten die Hypothese auf, dass dies auf unterschiedliche Effekte der Stimulation olfaktorischer und trigeminaler Rezeptoren zurückzuführen ist. In der Natur gibt es nur wenige Substanzen, die eine selektive olfaktorische Wirkung aufweisen, eine davon ist Schwefelwasserstoff (H_2S). Die meisten Geruchsstoffe stimulieren dagegen sowohl olfaktorische wie trigeminale Rezeptoren in der Nasenschleimhaut.

Das Hauptziel dieser Studie war es deshalb zu untersuchen, ob Menschen über die Fähigkeit verfügen, Substanzen, die selektiv das olfaktorische System aktivieren, zu lokalisieren. Hierfür wurde H_2S in zwei unterschiedlichen Konzentrationen untersucht. Darüber hinaus wurden zum Vergleich die olfaktorisch-trigeminale Substanz Isoamylacetat (IAA) und der rein trigeminale Wirkstoff Kohlenstoffdioxid (CO_2) getestet. Eine wichtige Voraussetzung für die Untersuchung der Lokalisation war es sicherzustellen, dass die Probanden die applizierten Stimuli bewusst wahrnehmen können. Daher wurde zuerst ein Detek-

tionsexperiment durchgeführt, mit dem Ziel, die Sensitivität der Probanden auf die ausgewählten Testsubstanzen zu untersuchen. Es wurden ausschließlich gesunde Probanden untersucht. Das Experiment basierte auf einem ereigniskorrelierten ("event-related") Studiendesign. Die Stimulation erfolgte mit Hilfe eines Olfaktometers.

Die Ergebnisse des Detektionsexperimentes zeigten, dass die Versuchspersonen den Duftstoff H_2S in niedriger Konzentration (2 ppm) mit moderater Sensitivität detektieren konnten, während sie H_2S in hoher Konzentration (8 ppm) mit einer hohen Sensitivität wahrnehmen konnten. Die Erhöhung der Duftstoffkonzentration und dadurch auch der Sensitivität hatte allerdings keine signifikante Auswirkung auf die Ergebnisse der Lokalisationstudie, da die Probanden weder 2 ppm noch 8 ppm H_2S erfolgreich lokalisieren konnten. Im Gegensatz dazu zeigte diese Studie, dass Menschen Duftstoffe, die bimodal (olfaktorisch und trigeminal) wirken (IAA), sowie auch trigeminale Substanzen (CO_2) sehr gut lokalisieren können. Zusammenfassend ergab diese Untersuchung, dass Menschen über die Fähigkeit verfügen, Duftstoffe, die das trigeminale System anregen, zu lokalisieren. Rein olfaktorisch wirkende Substanzen können hingegen von Menschen nicht lokalisiert werden.

1 Introduction

Behavioral studies reveal that mammals are able to orientate themselves in space by using their sense of smell. In other words they can routinely extract spatial information from odor perception. For example dogs and rats use this capability for scent tracking, and pigs use it for truffle hunting (Ackermann, 1995; Thesen et al., 1993). Thereby, it is unclear which specific background mechanisms facilitate the extraction of the spatial information. Furthermore, the question whether humans are able to directional smelling remains contradictory.

Directional smelling describes the ability to localize an odor source in space by perceiving the differences of the odor's concentration reaching both nostrils (concentration gradient), with a higher odor concentration on the nostril closer to the odor source (Kobal et al., 1989). The highest concentration gradient can be reached by presenting an odor to only one nostril and odorless air to the other nostril (monorhinal stimulation).

It is a well-known fact that mammalian sensory systems, like audition or vision, integrate information from bilateral receptive fields to generate spatial representations (Barlow et al., 1967; Knudsen and Konishi, 1979). The possibility that mammals extract spatial information from smell in a similar way, by comparing input across nostrils was supported by a study of von Békésy (von Békésy, 1964). He found that differences in odorant concentration as well as differences in time of stimulus arrival across the two nostrils, enable humans to spatially localize odors. During a later study by Porter and colleagues (Porter et al., 2005) the results of von Békésy were replicated, thereby confirming the hypothesis that humans similar to rats (Rajan et al., 2006) are able to extract spatial information from smell.

Contrary to these findings, several studies demonstrated that humans are not able to localize odors that selectively stimulate the olfactory system, but they can localize odors, which additionally excite the trigeminal system (Frasnelli et al., 2009; Kobal et al., 1989; Radil and Wysocki, 1998; Schneider and Schmidt, 1967; Wysocki et al., 2003). Authors of previous studies agree upon the localization of mixed olfactory-trigeminal stimuli, however,

it is still contradictory if humans are able to monorhinally localize pure odorants.

The breathing control is very important for the measurement of olfactory perception. There is some evidence that breathing interacts with olfactory perception and therefore can influence the results of localization experiments (Schneider and Schmidt, 1967; Porter et al., 2005). In general, when testing odor localization, stimuli can be presented in two different ways. The first possibility is to apply the stimuli within a constant air flow. In this case the subject does not have to sniff in order to convey the odor to the olfactory mucosa. During this kind of stimulation, called "passive stimulation", the subjects are required to use the breathing technique "velopharyngeal closure" to avoid respiratory airflow in the nose (Kobal, 1985). Passive stimulation is opposed to active stimulation, where the odor reaches the olfactory mucosa during the active sniffing process (quick and frequent inhalations through the nose).

When subjects were tested with pure odorants under passive stimulation, they were consistently found to be unable to identify the stimulated nostril (Frasnelli et al., 2008; Kobal et al., 1989; Radil and Wysocki, 1998; Schneider and Schmidt, 1967). However, a slightly different result was obtained by active stimulation. Whereas in one study, subjects were unable to localize the pure odorant phenyl ethyl alcohol, even after extensive training (Wysocki et al., 2003), other groups found that subjects were able to localize pure odorants (von Békésy, 1964; Porter et al., 2005).

Recently, Frasnelli and colleagues (Frasnelli et al., 2009) demonstrated that humans cannot localize pure odorants independent of the stimulus delivery method. They investigated the effect of active sniffing and passive stimulation on the localization prozess of both pure odorants and olfactory-trigeminal stimuli, finding no differences in the localization score between sniffing and passive stimulation. This suggests that breathing should not have an influence on the results of localization experiments.

Notwithstanding, previous research has shown that sniffing is not only a simple stimulus delivery method but also necessary for olfactory perception and important for generating neural activity in olfactory brain areas (Mainland and Sobel, 2006; Sobel et al., 1998b). Sniffing plays a major role in the formation of the olfactory perception (Bensafi et al., 2003; Sobel et al., 1999; Zelano et al., 2005). It has been suggested that sniffing facilitates odorant detection (Sobel et al., 2000a) as well as odor discrimination (Laing, 1986). Therefore, the investigation of respiration (indirect sniffing) is very important, and can be very helpful for a better explanation and understanding of olfactory perception.

1.1 Theoretical background

1.1.1 Biological importance of the sense of smell

The sense of smell appertains to the group of chemical senses and it is one of the oldest human senses. The biological importance of the sense of smell particulary appears in the signalization of a food source as well as in danger. An important function of the sense of smell is the food control (recognition of spoiled food) and the human communication as well. Odors are also necessary in the context of social relationships, reproduction, vegetative and hormonal regulation (Schmidt et al., 2005; Deetjen et al., 2006).

1.1.2 Anatomy of the olfactory organ

Nasal cavity

The nasal cavity is divided into a right and a left nostril by a bony and cartilaginous divider called nasal septum. The top of the nasal cavity is separated from the anterior cranial cavity by a bone called the cribriform plate, and is dorsally connected to the pharynx. Inside the nasal cavity three nasal conchae (lying upon each other) and three nasal passages are situated. They result in an enlargement of the surface area. The nose, the nasal conchae, and the nasal passages are lined by a tissue called mucosa (respiratory and olfactory epithelium).

Olfactory epithelium

The olfactory epithelium is located on the roof of the two nasal cavities of the human nose, between the eyes. The size of the human olfactory epithelium by human is about 5 cm^2 (Deetjen et al., 2006). The olfactory epithelium consists of three distinct types of cells:

- olfactory cells,
- supporting cells, and
- basal cells.

Humans possess about 30 million olfactory cells, which regenerate themselves from the supporting and basal cells. The olfactory cells are bipolar neurons with many tiny hair, called cilia, on the apical poles. Ciliae enable these cells the contact with the outside world. The olfactory axons accumulate to bundles called fila olfactoria. The fila olfactoria, which

are also termed olfactory nerve, pass through the lamina cribrosa and enter the olfactory bulb (Benninghoff and Drenckhahn, 2004; Schmidt et al., 2005).

1.1.3 Functionality of the sense of smell

Olfactory perception occurs in several steps: absorption and transportation of odor molecules to the mucosa, binding of odorants to specific receptor proteins, activation of the signal cascade, and generation of excitation in the olfactory neurons.

Olfactory receptors

Olfactory receptors (ORs) are members of the G-protein-coupled receptor family. In vertebrates, they are located in the cilia of the olfactory sensory neurons (Malnic et al., 2004). In the human genome exist approximately 1000 genes, which encode for ORs. However, not all of these potential ORs genes are expressed and are functional. According to an analysis of data derived from the human genome project, humans own approximately 400 functional genes coding for olfactory receptors whereas the remaining 600 candidates are pseudogenes (Young and Trask, 2002).

The olfactory receptor cells are bipolar neurones in the nasal epithelium. It is thought that each olfactory receptor neuron (ORN) expresses one type of receptor only. The ORNs are unique to the extent that they are capable of regeneration. They possess cilia which project into the mucus and, on the other end, axons that project to the olfactory bulb. 10-100 axons form up into bundles and terminate in the olfactory bulb, converging on synaptic glomeruli. ORNs expressing the same receptor protein synapse onto the same glomerulus in the olfactory bulb (Young and Trask, 2002).

The reason for the large number of different odor receptors is to provide the system for discriminating between as many different odors as possible. Even though, each odor receptor does not detect a single odor. Most odors activate more than one type of odor receptor. Since the number of combinations and permutations of olfactory receptors is almost limitless, the olfactory receptor system is capable of detecting and distinguishing between a practically infinite number of odorant molecules (Buck, 2004).

Signal transduction

Once an odorant has bound to the odor receptor, the receptor undergoes structural changes, binds and activates the olfactory G-protein inside the olfactory receptor neuron. The G-

protein in turn activates the lyase - adenylate cyclase - which converts ATP into cyclic AMP (cAMP). The cAMP opens cyclic nucleotide-gated ion channels which allow calcium (Ca^+) and sodium (Na^+) ions to enter the cell, depolarizing the olfactory receptor neuron and elicit an action potential which carries the information to the brain (Jones and Reed, 1989).

1.1.4 Central olfactory system

The central olfactory system is divided into primary-, secondary-, and tertiary olfactory cortex (Albrecht and Wiesmann, 2006; Weismann et al., 2001; Wiesmann et al., 2004).

Primary olfactory cortex

Olfactory information from the olfactory receptors is transferred to the olfactory bulb via the olfactory nerv. According to the neuroanatomical criteria, olfactory bulb constitutes the primary olfactory cortex (Albrecht and Wiesmann, 2006; Boyle et al., 2007). Contrary to these references, in the literature the olfactory bulb is often disregarded as a part of olfactory cortical area (Zatorre et al., 1992).

The olfactory bulb is located in a bony groove (Fossa olfactoria) formed by the osseous cribriform plate. This plate possesses perforations on both sides. Through these perforations the filae olfactoriae permeate the cranium and achieve the ipsilateral olfactory bulb. Within the olfactory bulb the axons of olfactory neurons synapse with the dendrites of mitral and tufted cells. Multiple synapses form a glomerulus. The axons of olfactory neurons which express the same receptor protein synapse onto the same glomerulus in the olfactory bulb.

Secondary olfactory cortex

The axons of the mitral cells of the olfactory bulb leave the olfactory bulb in the olfactory tract and reach the posterior part of the orbital surface of the forebrain. In the anterior perforate substance, in a region called olfactory trigone, the olfactory tract divides into three roots (striae). Whereas the medial olfactory stria guides to the septal region, the lateral olfactory stria leads to the medial surface of temporal lobe. The medial and lateral striae delineate the anterior perforated substance. The intermediate olfactory stria leads to the olfactory tubercle (Weismann et al., 2001).

All regions receiving direct projections from the lateral olfactory tract constitute the secondary olfactory cortex. It consists of anterior olfactory nucleus, olfactory tubercle, piriform cortex, parts of amygdala, periamygdaloid cortex, and a small part of the entorhinal cortex. All these regions, excepting the olfactory tubercle, send out feedback projections to the olfactory bulb. The projections of the olfactory bulb are mostly unilateral. Nevertheless, there are fibers which reach the contralateral bulb via the anterior commissure (Albrecht and Wiesmann, 2006; Weismann et al., 2001).

In humans the piriform cortex (PC) is the largest olfactory cortical area. It is situated along the lateral olfactory tract on the caudolateral part of the orbital cortex, near the junction of the frontal and temporal lobes, and continues onto the dorsomedial aspect of the temporal lobe (Weismann et al., 2001). The piriform cortex can be divided in two parts: the anterior (frontal) piriform cortex, which is responsible for the basal olfactory perception, and the posterior (temporal) piriform cortex, which is involved in the valence encoding of olfactory stimuli (Gottfried et al., 2002a; Gottfried, 2006).

Several fMRI studies demonstrated that the piriform cortex is activated during the smelling process (Cerf-Ducastel and Murphy, 2003; Gottfried et al., 2002a; Kareken et al., 2003; Poellinger et al., 2001; Savic et al., 2000; Savic and Gulyas, 2000; Sobel et al., 2000b; Zatorre et al., 1992). Nevertheless, a study of Sobel et al. (Sobel et al., 1998a) showed that the piriform cortex is also activated during sniffing in spite of odorant absence. These findings lead to the assumption that sniffing prepares the piriform cortex for an optimal odor perception. Furthermore, there is evidence that the piriform cortex is involved in the olfactory learning process and memory (Dade et al., 2002; Savic et al., 2000), olfactory imagination (Djordjevic et al., 2005), and the recognition of odors (Plailly et al., 2005).

The amygdala is also involved in the odor processing. It plays an important role during affective reactions, valence as well as intensity encoding of odorants (Zald and Pardo, 1997; Hudry et al., 2003; Winston et al., 2005). Additionally, the amygdala is involved in the associative learning processes (Gottfried et al., 2003; Gottfried and Dolan, 2004) and emotional odor memory (Herz et al., 2004).

Tertiary olfactory cortex

The information from secondary olfactory cortex is transmitted to the tertiary olfactory areas including: orbitofrontal cortex, parts of hippocampus and thalamus, and agranular insular cortex (Weismann et al., 2001).

The largest region of the tertiary olfactory cortex is the orbitofrontal cortex (OFC).

It forms the ventral surface of the frontal lobe. The caudal part of OFC is involved in the detection of odorants and passive smelling (Gottfried et al., 2002a; Zatorre et al., 1992). The rostral part of OFC participates in a higher olfactory processing like associative learning (Gottfried et al., 2002b; Gottfried and Dolan, 2004) and olfactory memory (Dade et al., 2001; Savic et al., 2000). The OFC receives not only olfactory, but also gustatory, visual, thalamic, and visceral projections. Therefore, it is supposed that OFC controls several complex functions like multimodal integration, reward, specific learning, and behavior (Gottfried, 2007, 2006). In addition to that, the OFC is involved in cognitive tasks like ratings of intensity (Zatorre et al., 2000), familiarity (Royet et al., 1999), and valence (Royet et al., 2001) as well as in discrimination tasks (Savic et al., 2000).

Another important region of tertiary olfactory cortex is the insula. An activation of the insula was found in several studies of the olfactory system (Savic, 2002b,a; Small et al., 2005; Wiesmann et al., 2006; Zald and Pardo, 2000). The insular cortex is responsible for the integration of olfactory and taste perception (Small et al., 2004; Small and Prescott, 2005).

Other areas involved in odor processing

There are brain areas, which are not directly a part of olfactory pathways, but still are integrated in the olfactory information processing, e.g. cingulate gyrus and cerebellum.

The cingulate gyrus is involved in the information processing of various kinds. More specifically, the anterior cingulate is frequently involved in tasks requiring attention to sensory features of the environment (Devinsky et al., 1995). Several olfactory studies reported activation in anterior and posterior parts of the cingulate gyrus (de Araujo et al., 2005; Levy et al., 1997; Small and Prescott, 2005; Yousem et al., 1997). Additionally, activations of the cingulate gyrus have been found in studies investigating sniffing in absence of odor (Koritnik et al., 2009). Interestingly, the cingulate gyrus has also been reported to be of critical importance in the processing of painful sensations (de Leeuw et al., 2005).

Referring to several olfactory studies, cerebellum is also involved in the olfactory processing (Cerf-Ducastel and Murphy, 2001; Mainland et al., 2005; Sobel et al., 1998b; Smejkal et al., 2003), but the functional significance of these findings remains unclear. Sobel et al. (Sobel et al., 1998b) compared the effects of smelling versus sniffing on cerebellar activation and hypothesized that the cerebellum maintains a feedback mechanism that regulates sniff volume in relation to odor concentration. Further studies are needed to elucidate the role of the cerebellum in olfaction.

1.1.5 Interactions between olfactory and trigeminal system

The olfactory and trigeminal systems have a close relationship (Brand, 2006; Frasnelli et al., 2007; Livermore and Hummel, 2004). Most odorants activate both the olfactory and trigeminal system (Cain, 1976; Doty et al., 1978). The first neurobiological study focused on olfactory and trigeminal responses has been published by Beidler and Tucker (Beidler and Tucker, 1956). The authors simultaneously recorded electrophysiological responses of olfactory and trigeminal fibers on rabbit nasal epithelium and found that the trigeminal nerve response was observed with most odors which stimulated olfactory receptors.

Electrophysiological studies suggested that trigeminal stimuli have an inhibitory effect on olfactory afferents to the brain (Kobal and Hummel, 1988). Inversely, single neuron responses to odorant stimulation in rats were enhanced when the trigeminal afferent activity was blocked by a local anesthetic (Inokuchi et al., 1993).

Several differences between olfactory and trigeminal perception were proven in numerous psychophysical studies. One of these differences concerns the unilateral localization of stimuli. Some groups have reported that selective olfactory stimulants presented to one nostril cannot be localized to that cavity; however, this is not the case with trigeminal stimulants (Cometto-Muñiz and Cain, 1998; Kleemann et al., 2009; Kobal et al., 1989; von Skramlik, 1925; Wysocki et al., 1992). Another difference was found in the anosmic population. Interestingly, it has been demonstrated that anosmics can detect the presence of a nasal stimulus via pungency (Doty et al., 1978; Cometto-Muñiz and Cain, 1990). However, anosmic subjects show reduced trigeminal sensitivity compared to healthy controls (Gudziol et al., 2001; Hummel et al., 1996). Some studies demonstrated that the age-related decrease of intranasal trigeminal sensitivity appears similarly to the decline of olfactory sensitivity (Laska, 2001; Stevens et al., 1982; Wysocki et al., 2003). An experiment of Cain and Murphy (Cain and Murphy, 1980) demonstrated an inhibitory influence of trigeminal stimulation on the olfactory perception. They showed that carbon dioxide (CO_2) suppressed the intensity of amyl butyrate, which stimulates both the olfactory and the trigeminal system (bimodal stimulus), if both these substances were applied as a mixture. On the other hand, the irritation induced by CO_2 was suppressed by amyl butyrate. Conversely, it has been shown in normosmic subjects that trigeminal stimuli are perceived as more intense, when being accompanied by olfactory stimulation (Livermore et al., 1992). Specifically, hydrogen sulphide (H_2S) as well as vanillin, both considered as pure olfactory stimulants (Doty et al., 1978; Kobal and Hummel, 1998), produced an increase of the perceived intensity of CO_2 stimuli, which selectively activated the intranasal trigeminal

system.

Studies using event-related potential (ERPs) or reflex responses to intranasal trigeminal stimuli (Hummel et al., 1996; Kendal-Reed et al., 1998) confirmed the psychophysical results presented above, i.e. a loss of olfactory sensitivity resulted in a decrease of the response to trigeminal stimuli (Lundström et al., 2005; Lundström and Hummel, 2006).

In conclusion, the interactions between olfactory and trigeminal systems have been shown previously. However, the question how these interactions coexist, and how one system influences the other, appears not clearly determined until today. This uncertainty could be due to the fact that only few studies specifically focused on these interactions. It can be argued that several studies focused on olfaction were inaccurately interpreted as they used odors which activate the trigeminal system simultaneously. Hence, electrophysiological and imaging studies using pure odors and pure trigeminal stimulants are certainly the most promising approaches for a better understanding of olfactory-trigeminal interactions.

1.2 Objectives

The aim of this project was to investigate whether humans possess an ability to orientate themselves in space using the sense of smell.

Studies of human olfaction are complicated by two facts: Firstly, olfactory perception requires respiration, and central control of respiration may be linked to the cortical system involved in the processing of olfactory information. Secondly, the nasal mucosa contains not only olfactory receptors, but among others, trigeminal receptors. It is known that most odorants stimulate both olfactory and trigeminal receptors. Therefore care must be taken to investigate whether any given effects of olfactory stimulation are indeed the result of olfaction instead of being caused by trigeminal perception.

Because some studies indicated an interaction between respiration and olfactory perception (Laing, 1986; Porter et al., 2005; Schneider and Schmidt, 1967; Sobel et al., 2000a) we initiated a first experiment to investigate the breathing parameters during an olfactory experiment (see chapter 2). The primary goal of this part of the study was to quantify the breathing parameters: respiratory minute volume, respiratory amplitude, and breathing rate, in response to odor stimulation and olfactory imagery. The second aim of this experiment was to evaluate a breathing sensor, which was developed for measurement of respiration during olfactory stimulation, and the measurement of respiraton inside an MR scanner. If necessary, this sensor could have been used in further localization experiments.

To investigate the differential effects of olfactory and trigeminal perception in olfactory localization studies, we first selected substances, which stimulate exclusively the olfactory system (hydrogen sulphide, H_2S), stimulate both the olfactory and the trigeminal system (isoamyl acetate, IAA), and stimulate the trigeminal system (carbon dioxide, CO_2) exclusively.

Then we performed a detection experiment to ensure that the substances used for the localization testing were detectable for the subjects. We used H_2S in low (2 ppm) and high (8 ppm) concentrations because this stimulant is known to be a pure odorant in these concentrations (Bensafi et al., 2008; Boesveldt et al., 2007; Frasnelli et al., 2006; Kobal et al., 1989; Stuck et al., 2007). For comparison the olfactory-trigeminal substance isoamyl acetate (IAA) (Doty et al., 1978; Porter et al., 2005), and the trigeminal substance carbon dioxide (CO_2) were tested (Bensafi et al., 2008; Boesveldt et al., 2007; Boyle et al., 2007; Iannilli et al., 2008; Livermore and Hummel, 2004; Stuck et al., 2006).

Finally, the localization experiment was carried out (see chapter 3) with the objective to test the human ability to localize odorants, which stimulate the olfactory system selectively,

and whether there are differences between localization of low and high concentrations of the olfactory substance H_2S. For comparison localization experiments were performed using IAA and CO_2.

The major hypothesis of this study was that humans can localize odorants, which excite both the olfactory and trigeminal systems, but are not able to localize pure olfactory substances, in spite of consciously perceiving the applied stimuli.

2 Investigation of breathing parameters during odor perception and olfactory imagery

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Investigation of Breathing Parameters during Odor Perception and Olfactory Imagery

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Abstract

Compared with visual and auditory imagery, little is known about olfactory imagery. There is evidence that respiration may be altered by both olfactory perception and olfactory imagery. In order to investigate this relationship, breathing parameters (respiratory minute volume, respiratory amplitude, and breathing rate) in human subjects during olfactory perception and olfactory imagery were investigated. Fifty-six subjects having normal olfactory function were tested. Nasal respiration was measured using a respiratory pressure sensor. Using an experimental block design, we alternately presented odors or asked the subjects to imagine a given smell. Four different pleasant odors were used: banana, rose, coffee, and lemon odor. We detected a significant increase in respiratory minute volume between olfactory perception and the baseline condition as well as between olfactory imagery and baseline condition. Additionally we found significant differences in the respiratory amplitude between imagery and baseline condition and between odor and imagery condition. Differences in the breathing rate between olfactory perception, olfactory imagery, and baseline were not statistically significant. We conclude from our results that olfactory perception and olfactory imagery both have effects on the human respiratory profile and that these effects are based on a common underlying mechanism.

Key words: olfaction, olfactory imagery, respiration, sniffing

Introduction

Perception is the process by which information is acquired, selected, and interpreted from the sensory systems. By contrast, imagery occurs when perceptual information is accessed from memory, giving rise to the experience of ''seeing with the mind's eye,'' ''hearing with the mind's ear,'' ''smelling with the mind's nose,'' and so on (Kosslyn et al. 2001; Stevenson and Case 2005).

Olfactory imagery is defined as the ability to experience a sensation of smell when an appropriate stimulus is absent. Compared with visual and auditory imagery, this process is relatively unknown. Some researchers suggest that in the visual, auditory, and motor systems a similar neural mechanism underlies perception and imagery. For instance, eye movements that were detected during visual imagery were similar to those of visual perception (Spivey and Geng 2001; Laeng and Teodorescu 2002; Mast and Kosslyn 2002). An analogue mechanism is suggested for olfaction. Bensafi et al. (2003, 2005) described that olfactory imagery is accompanied by olfactomotor activity, similar to that during odor perception. The primary sensory motor component for olfaction is sniffing, which is often compared with the movement of eyes to accommodate the vision as well as with the movement of ears to accommodate audition in most mammals (Johnson et al. 2003).

The sensation and perception of olfactory stimuli is widely dependent on sniffing, which is an active stage of stimulus transport. The sniff volume is inversely proportional to the concentration of an odorant (Laing 1983; Sobel et al. 2001). Sobel et al. (1998a) suggested that the cerebellum maintains a feedback mechanism that regulates the sniff volume in relation to odor concentration. In summary, previous research has shown that sniffing is not only a simple stimulus

delivery method but also necessary for olfactory perception and important for generating neural activity in olfactory brain areas (Sobel et al. 1998a; Mainland and Sobel 2006).

Bensafi et al. (2003, 2005) measured the airflow when subjects were trying to imagine various sights, sounds, or smells and showed that olfactory imagery, but not visual or auditory imagery, was accompanied by spontaneous sniffing. Moreover, the properties of the sniff during olfactory imagery resembled those of sniffing during olfactory perception. Analogous to real odor perception, when imagining a pleasant odor subjects took a larger sniff, and when imagining an unpleasant odor, they took a smaller sniff. Furthermore, blocking the nasal passage reduced the quality of olfactory imagery, and encouraging sniffing increased the quality of olfactory imagery. These results suggest that sniffing plays an important functional role not only in olfactory perception but also in olfactory imagery.

A functional imaging study of Djordjevic et al. (2005) reported that activation patterns during olfactory imagery are similar to those during olfactory perception. In this study, the authors were able to demonstrate that participants did imagine odors. They found increased activation in sensory regions specific for olfaction and in regions involved in mental imagery across different sensory modalities. They also demonstrated a positive relationship between activation of the secondary olfactory cortex and odor imagery performance. These findings demonstrate partially overlapping neural substrates for olfactory imagery and perception in agreement with findings in other modalities including vision, audition, touch, and motion.

Djordjevic et al. (2005) also measured respiration using a polygraph instrumentation system. This system recorded the respiratory movement (expansion and contraction) with 2 stretchable elastic belts attached around the chest and the abdomen of the subjects. However, due to the higher number of artifacts associated with the chest measurement belt, the results reported were based on the data collected with the abdominal belt. Two parameters, the mean amplitude and frequency, were extracted for each subject for all conditions. With their experimental approach, this group did not found any significant differences between the imagery, odorant, and baseline condition.

The goal of the current study was to quantitate the following breathing parameters: respiratory minute volume, respiratory amplitude, and breathing rate, in response to odor stimulation and olfactory imagery. Respiratory minute volume is the volume of air that is inhaled (inhaled minute volume) or exhaled (exhaled minute volume) by a human lung in 1 min. The respiratory amplitude is defined as the depth of inspiration. The respiration rate is the number of breaths taken within 1 min. We analyzed all important breathing parameters supposing that the analysis of a single breathing parameter (e.g., breathing rate) is insufficient to make a statement about the changes in the breathing or sniffing behavior. Secondary we investigated the breathing pattern by analyzing the shape of breathing profiles and searching for local maxima, which could be an indication for potential sniffing behavior. We also performed the fast Fourier transform (FFT) analysis of the breathing patterns in order to compare the frequency spectra between conditions for all individual subjects.

The hypothesis of the present study was that the breathing pattern in human subjects varies during olfactory perception as well as during olfactory imagery. More precisely it was hypothesized that the breathing parameters increase not only when the subjects smell an odor, but also if they imagine it. We supposed that the sniffing behavior, which attends odor perception and olfactory imagery, induces alterations in the breathing shapes and evidences a distinct characteristic, depending on the tested conditions.

Material and methods

Subjects

Fifty-six healthy volunteers (35 females) aged 21–42 years (mean age 28.9 ± 5.2 years) participated in this study. Their olfactory function was verified using the validated olfactory Sniffin' Sticks test (Kobal et al. 1996; Hummel et al. 1997). The protocol was approved by the local Ethics Review Committee, and the study was conducted in accordance with the Declaration of Helsinki/Edinburgh. All subjects gave their written, informed consent. The subjects were informed about the course of the experiment, but they were unaware of the real intention so as not to bias the results of this study.

Odor stimuli

Four odors (banana, rose, coffee, and lemon) were selected from the Sniffin' Sticks test battery (Burghart Instruments, Wedel, Germany), which consists of 16 odors based on pen-like odor-dispensing devices and is an established method to measure nasal chemosensory function (Kobal et al. 1996; Hummel et al. 1997). We employed 4 instead of only one odor in order to avoid adaptation of the olfactory system. Pleasant odors were used because they have been shown to induce a stronger breathing effect than unpleasant odors (Bensafi et al. 2002; Bensafi et al. 2007).

Experimental procedure

The experiment was based on a block design paradigm (Figure 1) with 3 kinds of conditions: odor perception (odor), olfactory imagery (imagery), and baseline. Both odor and imagery blocks were repeated 4 times. In every odorant block (duration 16 s), one of the 4 odors—banana, rose, coffee, and lemon in that order, was presented. In the imagery blocks (duration 16 s), the subjects were required to imagine the smell that was presented in the preceding odor block. The odor and imagery blocks were separated by a baseline condition (duration 32 s).

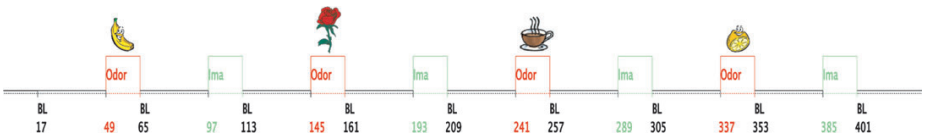

Figure 1 Experimental paradigm (timeline in s). Block design with 3 conditions: odor perception (Odor), olfactory imagery (Ima), and baseline (BL). In every odorant block, 1 of the 4 odors—first banana, then rose, coffee, and at last lemon, was presented. In the imagery blocks the subjects were required to imagine the smell that was presented in the preceding odor block.

The respiratory sensor (OL014, Burghart Instruments) used for this experiment is based on a pressure measuring principle. This sensor measures the pressure difference that arises between the nostril and the environment during breathing. The differential pressure sensor detects the deformation of a thin membrane under pressure using capacitive methods. The signal was recorded at 100 Hz using Lab View 7.0 software (National Instrument, Austin, TX).

During the whole experimental session, the nosepiece of the respiratory sensor was placed in the left nostril of the subjects. Because in other publications of our group, the left nostril was used for monorhinally olfactory stimulation (Weismann et al. 2001; Wiesmann et al. 2004; Wiesmann et al. 2006; Albrecht et al. 2008), we decided to do so to keep the testing conditions constant and reliable across our studies. In order to avoid the slipping out of the nosepiece, the subjects were requested to keep the sensor positioned with their hand. Additionally they were blindfolded and instructed to breathe through the nose. The presentation of odors in the odor condition was not communicated with the subject, whereas the imagery blocks were initiated with the command START and terminated with the command STOP. To detect the offset value of the respiratory sensor, the data recording started 10 s before subjects inserted the nosepiece of the sensor into the nose and ended 10 s after subjects removed the sensor out of the nose. Each experimental session consisted of 2 runs, which were interrupted by a 10-min break. The total experimental duration was half an hour.

Data analysis

Data were processed using Matlab 6.5. We calculated the offset value (mean value of the data recorded before the nosepiece of the sensor was placed in the subjects' nostril and after the sensor was removed from the nostril) and normalized the collected data for each subject about this value. In a second step, the data were smoothed using moving average filter. The window size for the moving average was set at 10.

The respiratory minute volume was determined by computing the integral of the breathing curve during the baseline, odor, and imagery condition. To find the respiratory amplitude, the global maxima of the breathing cycles were detected and subsequently averaged for each subject. The mean interval between 2 ensuing breathing cycles, represented by the global maxima, was calculated as the breathing rate, for all tested conditions.

To enable the comparison of breathing profiles within a condition, local maxima within each breathing cycle were identified. An increase in the number of local maxima is indicative of smell-induced sniffing behavior. The number of local maxima was averaged across all single breathing cycles for each condition. In this case, the global maxima were not considered.

Additionally, an FFT was carried out to analyze the spectra of frequency components for odor, imagery, and baseline condition. The FFT was performed for all breathing cycles within each condition for all subjects.

For the statistical analysis, SPSS for Windows (Statistical Package for the Social Science, Version 17.0, SPSS Inc, Chicago, IL) was used. Data (respiratory minute volume, respiratory amplitude, breathing rate, and the number of local maxima) were submitted to repeated-measures analyses of variance using the general linear model with the "within subject factor" condition (baseline/odor/imagery). We looked for main effects as well as for second-order interactions between these factors. Existing second-order interactions were corrected using Bonferroni correction. The alpha level for all tests was set at 0.05.

Results

Analysis of the recorded data showed that there were significant differences in the respiratory minute volume ($F_{2,222}$ = 23.89, $P < 0.001$) and respiratory amplitude ($F_{2,222}$ = 8.31, $P = 0.001$) across all conditions. The respiratory minute volume was significantly increased when the participants smelled an odor (mean: 4.77 ± 3.02 l/min, $P < 0.001$) or imagined an odor (mean: 4.74 ± 3.10 l/min) compared with the baseline condition (mean: 4.13 ± 2.38 l/min, $P < 0.001$). In other words, respiratory minute volume increased by 15.5% in the odor condition and by 14.8% in the imagery condition in comparison to baseline (Figure 2). We also found significant differences in the respiratory amplitude between the imagery (mean: 0.30 ± 0.19 l) and baseline condition (mean: 0.28 ± 0.16 l, $P = 0.002$) as well as between the imagery and odor condition (mean: 0.29 ± 0.18 l, $P = 0.03$). The amplitude rose by 6.2% in the imagery condition in comparison to baseline (Figure 3) and by 2.9% in comparison to the odor condition. An increase of 3.2% in the respiratory

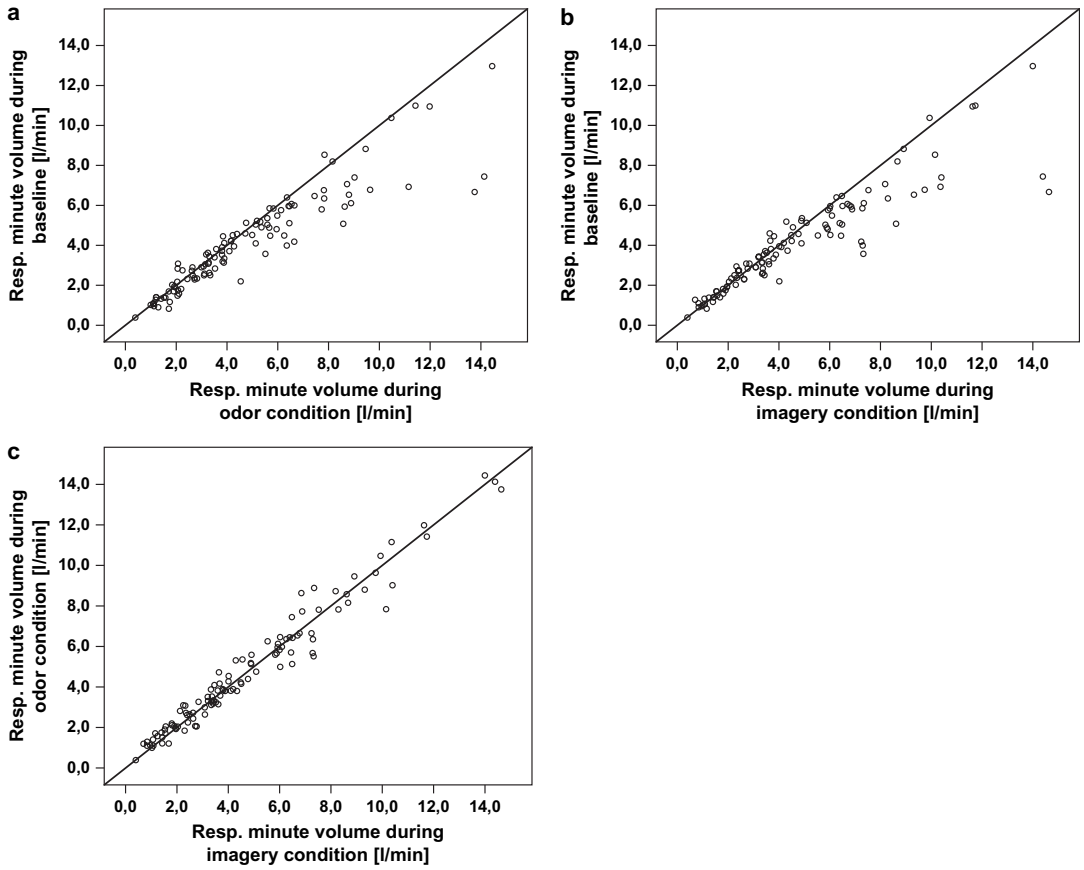

Figure 2 Paired comparison of respiratory minute volume (n = 56) between tested conditions. When there are differences across conditions, the points are mostly above or below the unit slope line. When the conditions are equal, the data points accumulate around the line. **(a)** The difference in respiratory minute volume between olfactory perception and baseline condition was statistically significant ($P < 0.005$). **(b)** The difference in respiratory minute volume between olfactory imagery and baseline condition was also statistically significant ($P < 0.005$). **(c)** The difference in respiratory minute volume between olfactory imagery and olfactory perception was not statistically significant.

amplitude between the odor condition and baseline was observed, although this increase was not statistically significant ($P = 0.13$). Differences in the breathing rate between the conditions were also not statistically significant ($F_{2,222} = 1.94$, $P = 0.15$, see Figure 4). An overview about the different breathing parameters is presented in Table 1.

Analysis of the breathing profiles showed that the number of local maxima in the breathing cycles within the odor and olfactory imagery condition contained significantly more local maxima than the breathing cycles in the baseline condition ($F_{2,222} = 22.41$, $P < 0.001$, see Figure 5 and Figure 6). The average number of local maxima in the baseline condition was 4.63, in the imagery condition 5.68, and 6.21 in the odor condition. Correspondingly, the frequency spectra demonstrated a different distribution of frequency components among conditions (see Figure 7, Figure 8, and Figure 9). The highest spectrum peak in all conditions corresponded to the breathing frequency (about 0.25 Hz). Interestingly, the second highest peak was found at a frequency of about 0.7 Hz, and its amplitude varied within the conditions. This peak had its lowest frequency power (amplitude) in the baseline condition (15.32) and its highest power (amplitude) in the imagery condition (25.55). In the odor condition, the peak frequency amplitude was 19.44. This indicates that the frequency of 0.7 Hz occurs more often in the imagery and odor condition in comparison to baseline.

The differences in respiratory minute volume, respiratory amplitude, and number of local maxima between conditions suggest that olfactory perception as well as olfactory imagery are accompanied by sniffing. According to the Fourier transformation analysis, the human sniffing frequency is in the range of 0.7 Hz.

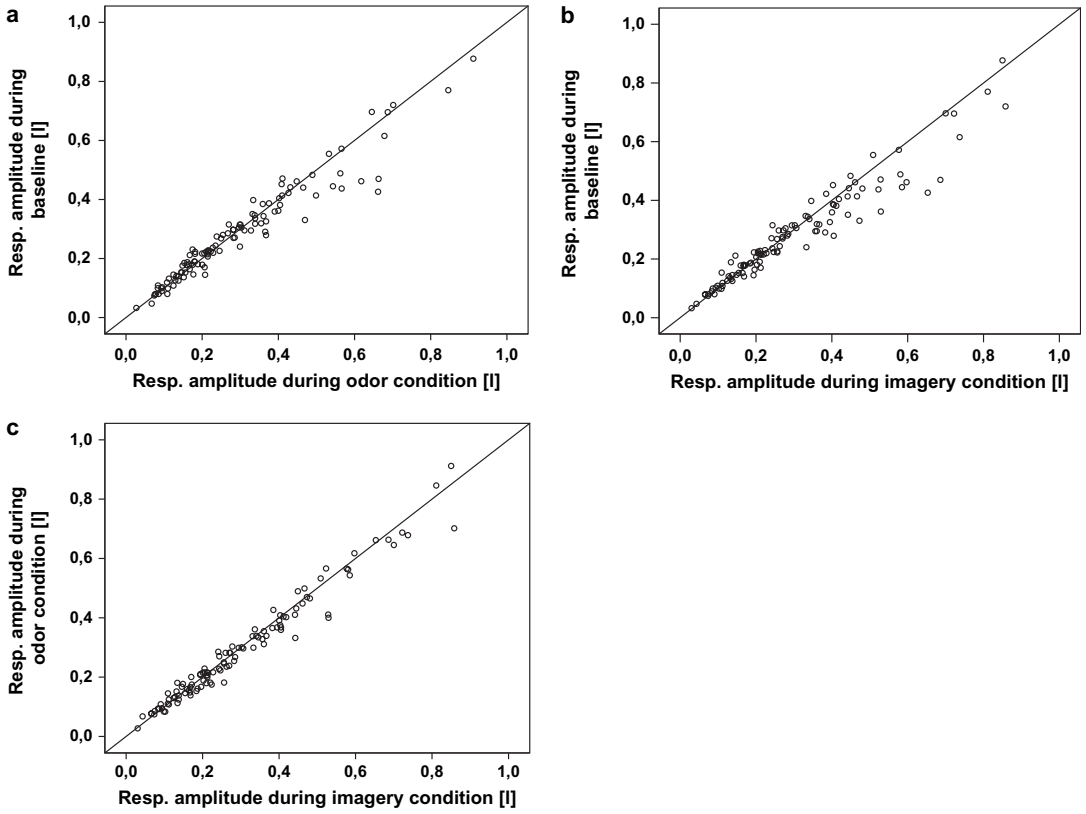

Figure 3 Paired comparison of respiratory amplitude (n = 56) between tested conditions. When there are differences across conditions, the points are mostly above or below the unit slope line. When the conditions are equal, the data points accumulate around the line. **(a)** The difference in respiratory amplitude between olfactory perception and baseline condition was not statistically significant. **(b)** The difference in respiratory minute volume between olfactory imagery and baseline condition was statistically significant ($P < 0.005$). **(c)** The difference in respiratory minute volume between olfactory imagery and olfactory perception was statistically significant ($P < 0.05$).

Discussion

The aim of the present study was to investigate the behavior of the breathing parameters respiratory minute volume, respiratory amplitude, and breathing rate in response to odor stimulation and odor imagery. Measurements were performed on 56 healthy subjects using a respiratory sensor. The results supported our hypothesis, demonstrating that the minute respiratory volume and the respiratory amplitude increase if humans smell or imagine an odor. Intuitively, an increase in the minute respiratory volume can be induced by an increase in the respiratory amplitude or an increase in the breathing rate. In this study however, the breathing rate showed no significant differences between the conditions, and the respiratory amplitude behaved differently from respiratory minute volume. Therefore, we suggest that the differences in the minute respiratory volume are rather caused by changes in the shapes of the breathing profiles. To quantify the changes in the shapes of the breathing profiles in all conditions, the local maxima within one breathing cycle were identified. This parameter was chosen because potential smell-induced sniffing behavior can be expressed as a local increase in the breathing profile. The significant differences in the number of local maxima between experimental conditions suggest that the shape of the breathing profile caused the changes in the minute respiratory volume. Also, the FFT confirms our assumption that both smell and olfactory imagery are accompanied by sniffing. The FFT showed that the second dominant frequency (0.7 Hz) is present in all conditions but has significantly higher amplitude in the odor and imagery conditions compared with baseline.

Our results are consistent with the findings of Bensafi et al. (2003, 2005). They showed that olfactory imagery is accompanied by olfactomotor activity similar to that during odor

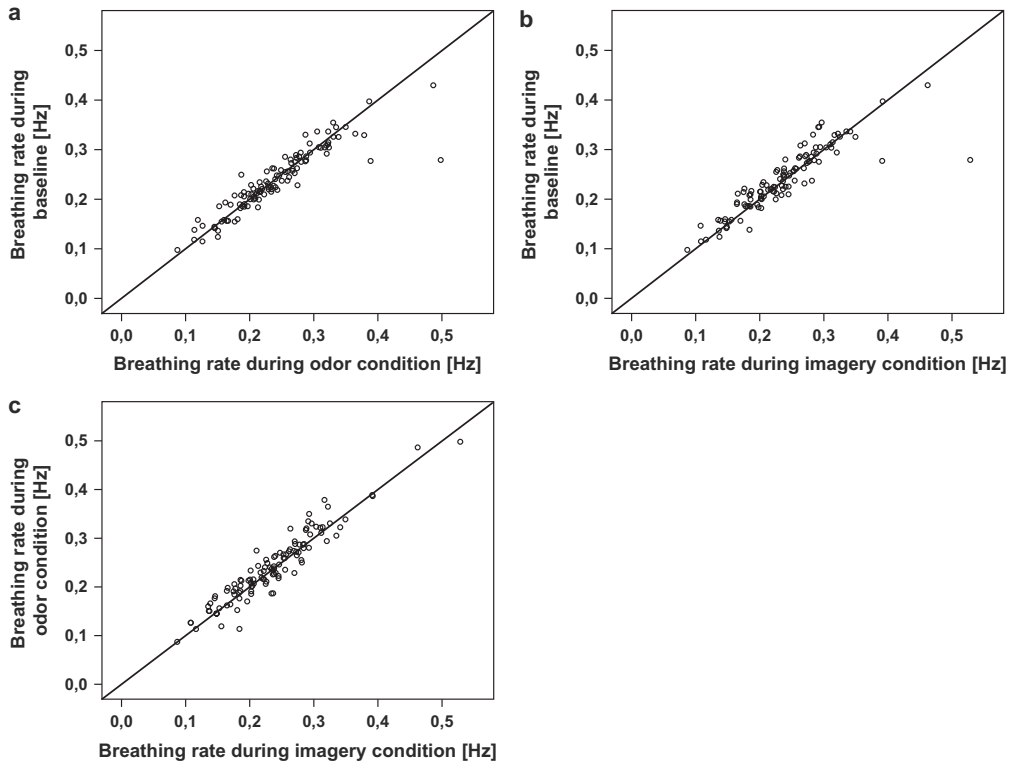

Figure 4 Paired comparison of breathing rate (n = 56) between tested conditions. When there are differences across conditions, the points are mostly above or below the unit slope line. When the conditions are equal, the data points accumulate around the line. **(a)** The difference in breathing rate between olfactory perception and baseline condition was not statistically significant. **(b)** The difference in breathing rate between olfactory imagery and baseline condition was not statistically significant. **(c)** The difference in the breathing rate between olfactory imagery and olfactory perception was also not statistically significant.

perception (Bensafi et al. 2003). The results of their experiments clearly pointed out that the sniff was spontaneously generated when participants were trying to imagine a smell but not when trying to imagine sight or sound, and their sniffs were more vigorous during imagery of pleasant versus unpleasant odors. It was also ascertained that the overall vividness of imagery was reduced during the sniff-blocked condition for olfactory but not for visual imagery. Additionally our findings contribute an important extension, namely the fact, that the measurement of the breathing rate is not enough to make a statement about the changes in the breathing or in the sniffing behavior. From the finding that breathing/sniffing rate was equal across conditions cannot be concluded that there are no differences in the breathing (or sniffing) across conditions. The results of this study confirm that it is necessary to analyze the breathing patterns (e.g., amplitude, minute volume, and shape) to evaluate breathing or sniffing behavior.

Our results confirm that sniffing is involved in both olfactory perception and olfactory imagery. Sniffing is a robust motor activity that is required for the transport of the olfactory stimuli and for olfactory perception. It is also very important for generating neural activity in olfactory brain areas (Sobel et al. 1998a, 1998b). Using functional magnetic imaging (fMRI) Sobel et al. (1998a) found that sniffing induces activation in the human piriform cortex whether an odorant is present or absent. The authors postulated that sniff-induced

Table 1 Breathing parameters (respiratory minute volume, respiratory amplitude, breathing rate) and the number of local maxima per respiratory cycle during tested conditions (baseline, odor perception, olfactory imagery). The mean values and standard deviations are presented.

	Baseline	Odor perception	Olfactory imagery
Respiratory minute volume (l/min)	4.13 ± 2.38	4.77 ± 3.02	4.74 ± 3.10
Respiratory amplitude (l)	0.28 ± 0.16	0.29 ± 0.18	0.30 ± 0.19
Breathing rate (Hz)	0.24 ± 0.06	0.24 ± 0.07	0.24 ± 0.07
Number of local maxima per respiratory cycle	4.63 ± 3.91	6.21 ± 5.54	5.68 ± 5.64

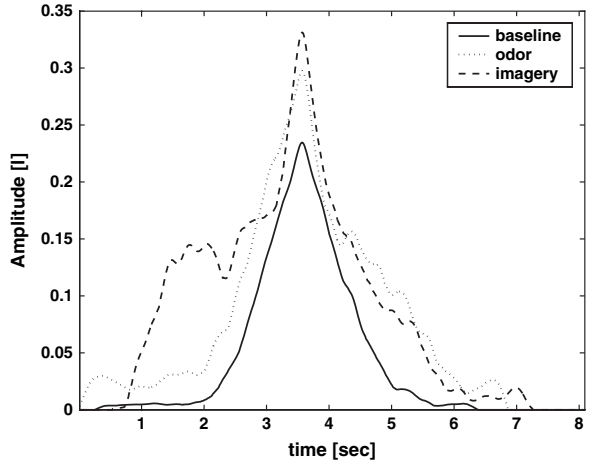

Figure 5 Representative examples of averaged breathing cycles for one subject.

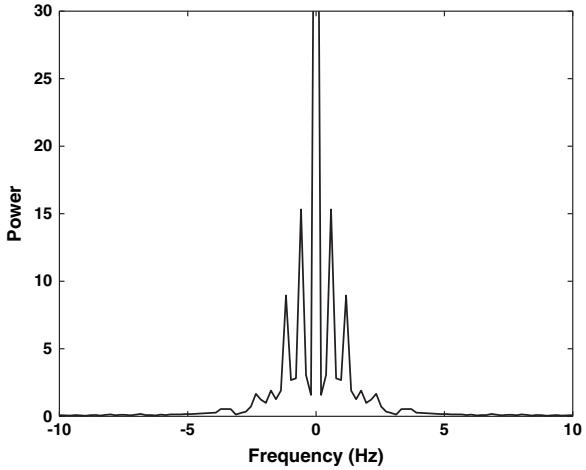

Figure 7 FFT of breathing curves in the baseline condition (n = 56).

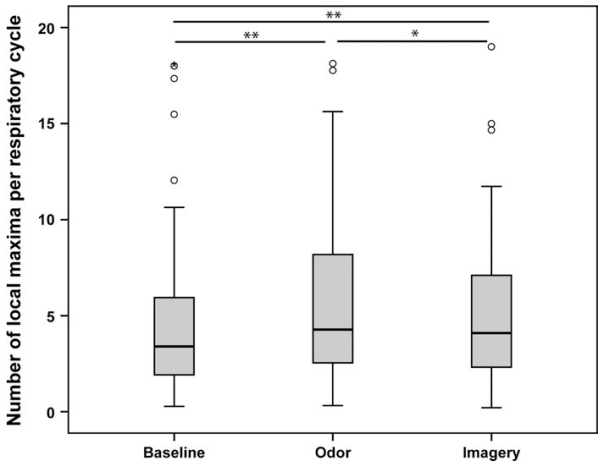

Figure 6 Box plot comparing the number of local maxima per respiratory cycle during olfactory perception, olfactory imagery, and baseline condition. The differences between the conditions were statistically significant (*significant $P < 0.05$; **significant $P < 0.005$).

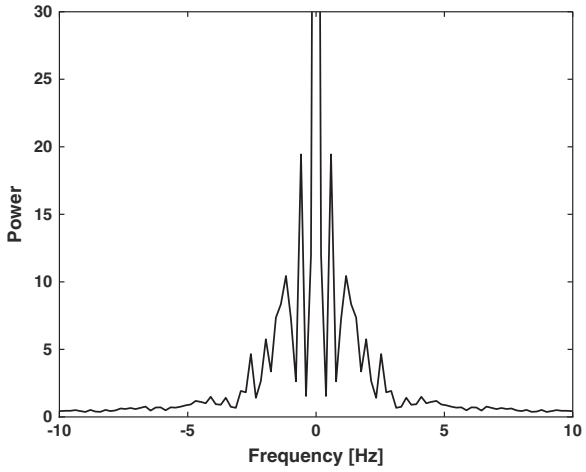

Figure 8 FFT of breathing curves in the odor condition (n = 56).

brain activation is in fact somatosensory stimulation that is caused by airflow through the nostril. Moreover, this group demonstrated that the cerebellum plays a role in human olfaction (Sobel et al. 1998b; Johnson et al. 2003). Given that the sniff volume correlates with the odorant concentration (Laing 1983; Sobel et al. 2001), they suggested that the cerebellum maintains a feedback mechanism that regulates sniff volume in relation to odor concentration.

Our findings confirm the hypothesis that sniffing, the motor component of olfaction, is very important for smelling and functionally involved in odor imagery. During the measurements, we observed that the sniff was spontaneously generated when the participants smelled an odor and when they were trying to imagine a smell. This indicates that both phenomena are based on a similar neural mechanism.

Referring to the mental imagery debate, which contains 2 main theories, the ''perceptual anticipation theory'' (Kosslyn et al. 1995, 2001; Kosslyn and Thompson 2003) and the ''propositional theory'' (Pylyshyn 1973; Pylyshyn 2003), our results support the first theory. The ''perceptual anticipation theory'' posits that the strong anticipation of perceiving an object or scene can actually lead to the creation of a descriptive representation in the early visual cortex resulting in a mental image. In contrast, the propositional theory postulates that mental images are not images at all but rather

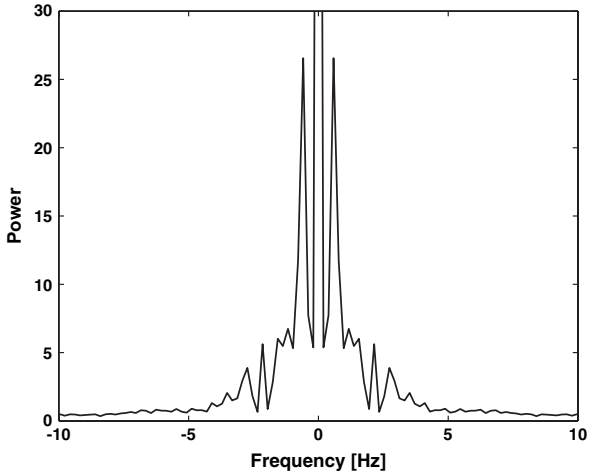

Figure 9 FFT of breathing curves in the olfactory imagery condition (n = 56).

rely on mental descriptions no different in kind from those that underlie language. Our findings, which show that sniffing accompanied not only smelling but also olfactory imagery, are consistent with the perceptual anticipation theory. We were able to show that olfactory imagery exists, and both processes are regulated by similar underling mechanism.

The respiratory sensor we used in the current study was shown to be a suitable device for the measurement of breathing parameters. An additional advantage over other sensors is that our sensor contains no magnetic elements and therefore can be use inside of MRI scanner.

The results of our study demonstrated that it is possible to detect differences in breathing between olfactory imagery, olfactory perception, and baseline. Similar to the study of Djordjevic et al. (2005), we detected no significant differences in the breathing rate. In contrast, we found that the largest differences between conditions were apparent in the minute respiratory volume. Further analysis permitted us to draw the conclusion that main changes in breathing were caused by changes in the breathing profiles. Furthermore, we conclude that the changes in the breathing profile result from sniffing that accompanied both olfactory perception and olfactory imagery.

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3 Trigeminal perception is necessary to localize odors

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Trigeminal perception is necessary to localize odors

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ABSTRACT

The human ability to localize odorants has been examined in a number of studies, but the findings are contradictory. In the present study we investigated the human sensitivity and ability to localize hydrogen sulphide (H_2S), which in low concentrations stimulates the olfactory system selectively, the olfactory-trigeminal substance isoamyl acetate (IAA), and the trigeminal substance carbon dioxide (CO_2). A general requirement for testing of localization was the conscious perception of the applied stimuli by the participants. Using Signal Detection Theory, we determined the human sensitivity in response to stimulation with these substances. Then the subjects' ability to localize the three different substances was tested. We found that humans can detect H_2S in low concentration (2 ppm) with moderate sensitivity, and possess a high sensitivity in response to stimulation with 8 ppm H_2S, 17.5% IAA, 50% v/v CO_2. In the localization experiment, subjects could localize neither the low nor the high concentration of H_2S. In contrast, subjects possessed the ability to localize IAA and CO_2 stimuli. These results clearly demonstrate that humans, in spite of the aware perception, are not able to localize substances which only activate the olfactory system independent of their concentration, but they possess an ability to localize odorants that additionally excite the trigeminal system.

1. Introduction

There is evidence that the nature of an odor influences the human ability to localize it [1–5]. The human nasal mucosa contains in addition to olfactory receptors, cells that respond to tactile, thermal, or nociceptive stimulation. What we experience as a "smell" is in most cases an integrative perception of different sensory modalities [6–8]. Although the activation of olfactory receptors is integral to the smell process, most odorants concurrently stimulate the olfactory and the trigeminal system [9]. Therefore the influence of the trigeminal, or irritant, component of smell must be considered when analyzing the olfactory localization processes.

The localization of olfactory stimuli has been investigated, but the resulting data have not been consistent. A number of studies demonstrated that humans are not able to localize odors that selectively stimulate the olfactory system, but they can localize odorants which additionally excite the trigeminal system [10–17].

In contrast, von Békésy [18] showed that humans are able to localize several chemosensory substances. He postulated that human sense of smell bears resemblance to directional hearing and assumed that a smell could be localized by differences in time or intensity of odors reaching the nostril. A study of Porter et al. [19] replicated the results of von Békésy, and confirmed the hypothesis that humans are able to localize olfactory stimuli.

The investigation of odor localization by humans is not quite trivial. Fundamental for this is an experimental design, which should approve olfactory stimulation exclusively. Furthermore, the selection of odors and their concentration is very important for a localization study. The concentration of tested odor may not be too high, because this can induce trigeminal irritation; nevertheless the experimenter has to assure that the subjects consciously perceive the presented stimuli. For this reason it is very important to verify, if the test substance is detectable for participants. Therefore, we first carried out the detection study and ensured that the stimuli are detectable for the subjects and based on this knowledge, we performed the localization study.

In the detection experiment we determined the human sensitivity based on the Signal Detection Theory (SDT) [20,21], which enabled us to separate the signal (the relevant input event – stimulus) from noise (background activity or irrelevant inputs –

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blank), in response to stimulation with the above mentioned substances. Based on these results the localization experiment was carried out.

In the localization study, we investigated the ability of humans to localize odorants, which stimulate selectively the olfactory system and whether there are differences between localization of low (2 ppm) and high (8 ppm) concentrations of the olfactory substance hydrogen sulphide (H_2S). We used H_2S because this stimulant is known to be a pure odorant in these concentrations [10,22–27]. As a comparison we used the olfactory-trigeminal substance isoamyl acetate (IAA) [9,19,28] and the trigeminal substance carbon dioxide (CO_2) [22,23,25,26,29,30]. We hypothesized that in spite of consciously perceiving the applied stimuli, subjects can localize only odorants which excite both the olfactory and trigeminal systems, but are not able to localize pure olfactory substances.

2. Materials and methods

2.1. Subjects

Twenty subjects (11 females) aged 22–43 years (mean age 28.3 ± 5.5 years) were tested with H_2S, 20 subjects (13 females) aged 21–43 years (mean age 28.7 ± 6.8 years) were tested with CO_2, and 23 subjects (16 females) aged 22–44 years (mean age 28.3 ± 5.9 years) were tested with IAA in both the detection and the localization experiment. There was a sub-group of 8 subjects tested with all three stimuli in both experiments. All subjects were healthy non-smokers.

Before the beginning of the experiments, the olfactory function of all subjects was verified using the validated Sniffin' Sticks test battery [31–34]. The protocol was approved by the local ethics committee and the study was conducted in accordance with the Declaration of Helsinki/Edinburgh. All subjects gave their written, informed consent. Subjects were free to end the testing session or their participation in the study at any time.

2.2. Odor stimulation

The odorant hydrogen sulphide (20 ppm H_2S, test gas mixed with nitrogen N_2; Linde Gas Therapeutics GmbH, Unterschleißheim, Germany), which has an unpleasant smell of rotten eggs, was chosen to selectively activate the olfactory system. Two concentrations of H_2S were used: a low concentration of 2 ppm (10% v/v), and a high concentration of 8 ppm (40% v/v). The olfactory-trigeminal odorant, isoamyl acetate in concentration of 17.5% (IAA, natural banana odor; Fluka Analytical, Sigma-Aldrich Chemistry GmbH, Buchs, Switzerland) was utilized for the bimodal stimulation (70% v/v of 25% IAA diluted in propylene glycol; Sigma-Aldrich Laborchemikalien GmbH, Steinheim, Germany). The trigeminal substance carbon dioxide in 50% v/v (99.9% CO_2, odorless medical gas; Linde Gas Therapeutics GmbH, Unterschleißheim, Germany) was used for the trigeminal stimulation. All odor stimuli were delivered in a constant air flow of 4 l/min to both nostrils of the subjects by using an olfactometer (OM6b, Burghart Instruments, Wedel, Germany) [35–37].

3. Detection experiment

3.1. Experimental procedure

The experiment was prepared based on the Signal Detection Theory (SDT) [20,21]. The paradigm consisted of 20 olfactory stimuli (H_2S/IAA/CO_2) and 10 blanks, which were applied randomly to both nostrils. The task of the subjects was to separate the signal, in this case the olfactory stimulus, from noise (blank stimulus). Both H_2S/IAA/CO_2 and blank stimuli were presented for 500 ms. The stimuli were prepared using the same proportion of gas (H_2S/CO_2 for odor stimuli and N_2/neutral air for blanks) and dilution (moistened) air. In case of IAA stimulation, which is available in a liquid form, the blanks consisted of dilution air only. Additionally, in order to assure the symmetrical design of the study and thereby rule out any asymmetrical tactile stimulation, the stimuli were presented simultaneously to both nostrils of subjects. For example, if H_2S/IAA/CO_2 stimulus was presented to the left nostril, an empty stimulus (blank) was presented to the right one. The interstimulus interval amounted to 30 ± 3 s.

During the experiment, subjects were laying in a supine position with their eyes closed [38]. White noise of approximately 80 dB (SPL) was delivered through earphones to minimize the perception of the olfactometer switching and other external auditory stimuli. Subjects were trained and requested during the experiments to breath using "velopharyngeal closure" [35,36]. This technique prevents respiratory air flow in the nasal cavities. The subjects were requested to pay attention to the applied stimuli. An auditory signal was presented 2 s after each stimulus. The subjects were instructed to respond to this signal and press the left mouse button if the stimulus contained a H_2S/IAA/CO_2 substance and the right mouse button if they perceived no stimulant. The response signal was recorded using a LabView 7.0 software (National Instruments, Austin, Texas, USA). The experimental session for H_2S contained two runs in pseudo-randomized order, to test the low (2 ppm) and the high (8 ppm) concentration. These runs were interrupted by a 20 to 30 min break to avoid olfactory adaptation. All other stimuli were tested in a single session. The average experimental duration of one run was approximately 17 min for each tested substance.

3.2. Data analysis

The response data were processed using Matlab 6.5 (Mathworks, Sherborn, MA, USA). To extract the individual responses, only the responses in a time window of 8 s starting from the auditory signal were considered. The data were analyzed based on the SDT [20,21]. For any event 4 outcomes were possible: hit (correct detection if signal is present), correct rejection (correct detection that the signal is absent), miss (failure to detect signal when this is present), and false alarm (incorrect detection of signal when this is absent). Based on these outcomes, the characteristic SDT parameters sensitivity and response criterion were calculated. Sensitivity (d') is the keenness of the sensory system, and is defined as the distance between the signal and noise peaks. The greater this distance the easier is the correct detection of the signal. $d'<0.5$ corresponds to a low sensitivity, if d' ranges between 0.5 and 2 the sensitivity will be referred to as moderate, $d'\geq 2$ corresponds to a high sensitivity. The response criterion (β) represents a subjective criterion level that produces "signal present" response when exceeded. $\beta<1$ corresponds to a low criterion i.e. the subjects tend to answer with "yes", $\beta=1$ means neutral criterion, $\beta>1$ corresponds to a high criterion i.e. the subjects tend to answer with "no".

4. Localization experiment

4.1. Experimental procedure

The experimental paradigm included 20 H_2S/IAA/CO_2 stimuli of 500 ms duration. The randomly distributed stimuli were presented to both nostrils of the subjects (10 to the left, 10 to the right nostril). In order to assure the symmetrical design of the study, when H_2S/IAA/CO_2 stimulus was presented to one nostril, a blank stimulus was presented to the other nostril. Blank stimuli were prepared using the same proportion of gas (N_2/neutral air) and dilution as the H_2S/CO_2 stimulant. In case of IAA stimulation, the blank stimuli consisted exclusively of dilution air. The interstimulus interval amounted to 40 ± 3 s. During the experiment, subjects were laying in a supine position with their eyes closed [38]. White noise of approximately 80 dB (SPL) was delivered through earphones to minimize the perception of the olfactometer switching

Table 1
Subject detection responses for H_2S ($n=20$), IAA ($n=23$) and CO_2 ($n=20$).

Substance	Hit	Miss	False alarm	Correct rejection
2 ppm H_2S	14.65 ± 2.91 (73.25%)	5.35 ± 2.91 (26.75%)	1.35 ± 1.27 (13.5%)	8.65 ± 1.27 (86.5%)
8 ppm H_2S	16.55 ± 1.82 (82.75%)	3.45 ± 1.82 (17.25)	0.9 ± 1.12 (9.0%)	9.1 ± 1.12 (91.0%)
17.5% IAA	16.96 ± 2.98 (84.78%)	3.04 ± 2.92 (15.22)	0.31 ± 0.7 (3.04%)	9.69 ± 0.7 (96.96%)
50% v/v CO_2	16.1 ± 4.63 (80.5%)	3.9 ± 4.63 (19.5%)	0.35 ± 0.67 (3.5%)	9.65 ± 0.67 (96.5%)

The mean, standard deviation, and percent total values (in parentheses) are presented.

and other external auditory stimuli. To avoid respiratory airflow in the nose, the subjects were trained to breath using "velopharyngeal closure" [36]. The subjects were requested to pay attention to the applied stimuli. They were instructed to press the left mouse button if the H_2S/IAA/CO_2 stimulus was applied to the left nostril, and the right mouse button if the H_2S/IAA/CO_2 stimulus was applied to the right nostril. The response was prompted by an audio signal, which was presented 2 s after each stimulus. The response signal was recorded using a LabView 7.0 software (National Instruments, Austin, Texas, USA). The experimental session for H_2S odor contained two runs in order to test the low (2 ppm) and high (8 ppm) concentration. The order of both runs was pseudo randomized. The runs were interrupted by a 20–30 min break to avoid the olfactory adaptation. The average experimental duration of one run was 14 min for each tested substance.

4.2. Data analysis

The response data were processed using Matlab 6.5 (Mathworks, Sherborn, MA, USA). Again, the responses in an 8 s time window starting with the auditory signal were used for the evaluation. The statistical analysis was carried out using SPSS software for Windows (Statistical Package for the Social Science, Version 15.0, SPSS Inc. Chicago, IL, USA). The statistical significance of the results was tested using one sample Student *t*-test. To compare the responses between nostrils, a paired sample Student *t*-test was used. The alpha level for all tests was set at $p=0.05$.

5. Results

5.1. Detection experiment

The results of the detection experiment for two concentrations of H_2S, IAA and CO_2 are presented in Table 1. Based on the response distribution SDT parameters sensitivity (d') and response criterion (β) were calculated. Values of these parameters are given in Table 2.

Participants were able to detect both: the low and the high concentrations of H_2S, the 17.5% of IAA, as well as the 50% v/v of CO_2. The sensitivity in response to 2 ppm H_2S was rather moderate ($d'=1.72$) whereas an increase of H_2S concentration to 8 ppm resulted in a higher sensitivity ($d'=2.29$). The response criterion on the other hand, showed that the behavior of the participants remained unchanged, independent of the concentration of H_2S ($\beta=1.51$ for 2 ppm H_2S and $\beta=1.56$ for 8 ppm H_2S). For both concentrations of H_2S, the subjects behaved with caution and tended to answer with "no", setting the decision criterion high ($\beta>1$). Subjects showed a high sensitivity in response to stimulation with 17.5% IAA ($d'=2.91$) and 50% v/v CO_2 ($d'=2.67$). Even more conservative subjects behavior was observed for the stimulation with 17.5% of IAA and 50% v/v of CO_2. For the trigeminally stimulating odors, subjects set strict decision criterion ($\beta=3.40$ for IAA and $\beta=3.56$ for CO_2), behaving conservatively and tending to answer with "no".

Table 2
Behavioral parameters sensitivity (d') and response criterion (β) according to SDT for H_2S ($n=20$), IAA ($n=23$) and CO_2 stimuli ($n=20$).

Substance	Sensitivity (d')	Response criterion (β)
2 ppm H_2S	1.72	1.51
8 ppm H_2S	2.29	1.56
17.5% IAA	2.91	3.40
50% v/v CO_2	2.67	3.56

Table 3
Results of the localization experiment for 2 ppm and 8 ppm H_2S ($n=20$), IAA ($n=23$) and CO_2 ($n=20$).

Substance	Correct assignment	Left	Right	Mistake
2 ppm H_2S	10.25 ± 3.02 (51.25%)	5.3 (51.71%)	4.95 (48.29%)	9.85 ± 3.02 (48.75%)
8 ppm H_2S	10.9 ± 2.88 (54.5%)	5.35 (49.08%)	5.55 (50.92%)	9.1 ± 2.88 (45.5%)
17.5% IAA	16.70 ± 4.10 (83.48%)	8.48 (50.78%)	8.22 (49.22%)	3.30 ± 4.10 (16.52%)
50% v/v CO_2	17.50 ± 3.24 (87.5%)	8.75 (50.0%)	8.75 (50.0%)	2.50 ± 3.24 (12.5%)

A total of 20 stimuli were applied to the subject for each odorant. The table shows the mean and standard deviation of correct localization. Additionally, the percent values of the correct localization are presented in parentheses.

5.2. Localization experiment

The results of the localization experiment are presented in Table 3 and Fig. 1. Subjects were unable to localize H_2S odor both in the low concentration of 2 ppm (mean of correct assignment = 10.25 ≙ 51.25%, $t(1,19)=0.37$, $p=$ n.s.) and in high concentration of 8 ppm (mean of correct assignment = 10.9 ≙ 54.5%, $t(1,19)=1.4$, $p=$ n.s.). By contrast, subjects could localize 17.5% IAA (mean of correct assignment = 16.70 ≙ 83.48%; $t(1,22)=7.84$; $p<0.001$) and 50% v/v CO_2 (mean of correct assignment = 17.5 ≙ 87.5%; $t(1,19)=10.36$; $p<0.001$). In all tested substances we found no significant difference in the localization rate between the nostrils.

6. Discussion

The aim of the present study was to test the human ability to localize odorants. In particular, we were interested in whether

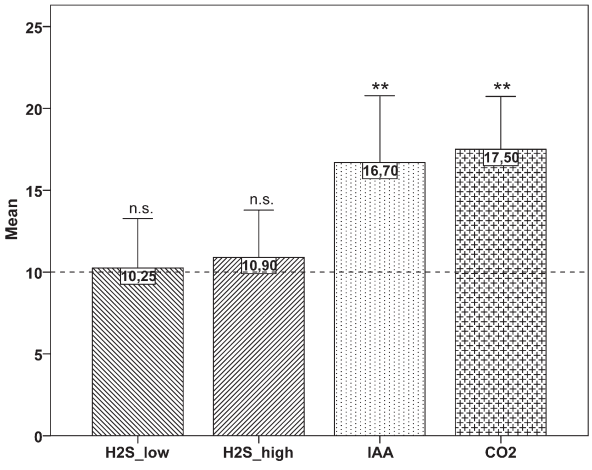

Fig. 1. Mean values of correct assignment in localization experiment for 2 ppm and 8 ppm H_2S ($n=20$), IAA ($n=23$), and CO_2 stimuli ($n=20$). Bars represent the standard deviation (** significant $p<0.001$; n.s. – not significant), the dotted line represents the chance score, in this case 10 (50% of trials).

humans possess an ability to localize odorants that exclusively activate the olfactory system. A general requirement for testing of localization was the participant's conscious perception of the applied stimuli. Therefore, we first performed the detection experiment, in order to prove the human sensitivity to detect the tested substances.

Our results demonstrated that subjects cannot localize such pure odorants. These results are consistent with the results of Kobal et al. [10], who found that subjects were not able to localize pure odors, in their case H_2S and vanillin. Stimulation with the trigeminal substance CO_2 or menthol on the other hand, yielded localization rates of more than 96%. Kobal et al. tested the H_2S odor in a relatively low concentration of 2.06 ppm. In the detection experiment we showed that the sensitivity to detect H_2S odor at this concentration (2 ppm) was only moderate. We show however, that even though the sensitivity to detect H_2S at a higher concentration of 8 ppm was quite high, subjects were still unable to localize H_2S at this concentration. To our knowledge this is the first localization study, in which H_2S in two concentrations was tested. In addition we were able to demonstrate that humans possess the ability to localize both the bimodal odor IAA and the trigeminal stimulus CO_2.

Radil and Wysocki [13] also demonstrated that it is not possible to determine which nostril is being stimulated, when the olfactory substances phenyl ethyl alcohol or vanillin are administrated into one nostril and an odorless, solvent blank into the contralateral nostril during the same inspiration cycle. Their study failed to obtain any evidence to support the notion that a pure olfactory stimulus could be localized when the odorant and blank stimulus were presented simultaneously.

Contrary to the aforementioned findings, von Békésy [18] demonstrated that humans are able to localize several chemosensory substances. He showed that subjects were able to determine the position of a small odorous ball placed 8 cm away from the nose with a precision of 7°–10° from the midline. In summary, von Békésy postulated that the human sense of smell bears resemblance to directional hearing and that smell could be localized by differences in time or intensity of the odors reaching the nostril. However, von Békésy [18] used only odorants that contained a trigeminal component (benzol, cloves, eucalyptus, lavender). Furthermore, only three subjects participated in the study and they had more than 1 year experience with localization experiments.

In a recent study Porter et al. [19] replicated the results of von Békésy and showed that humans, similar to rats [39], are able to localize olfactory stimuli. They tested the following odors: amyl acetate (banana), propionic acid (vinegar), phenyl ethyl alcohol (rose), and eugenol (cloves). Whereas the two first mentioned odors definitely include a strong trigeminal component, phenyl ethyl alcohol and eugenol can also induce trigeminal perceptions, when the applied odor is used in a high concentration [40–43]. Both groups (von Békésy, Porter), do not report the concentrations of the tested odors.

We decided to use H_2S (2 ppm and 8 ppm) because it is known to be a pure odorant [10,22–27]. Nevertheless it has also been shown that H_2S in high concentrations may have an irritant, even toxic properties [44–46]. According to these data, however, H_2S exerts no irritative effects in human experiments in concentrations up to 20 ppm. When higher concentrations of H_2S are used the exposition time has to be limited. In this study, we not only used comparably short stimulus durations, but also applied H_2S concentrations which have a moderate to high sensitivity but are still well below the trigeminal threshold.

There are several differences between the studies, which reported that olfactory stimulants can be localized [18,19], and our own experiment, which suggests that odorants can only be localized if they activate the trigeminal system. In contrast to other published studies we carried out the detection study and ensured that the stimuli are detectable for the subjects and based on this knowledge, we performed the localization study. Furthermore, in our study, and in the study of Kobal et al. [10], the odors were delivered in a constant air flow directly into the nostrils using an olfactometer [35–37] and the subjects used the breathing technique “velopharyngeal closure”, whereas other groups used a custom-designed nasal mask (Porter et al. [19]) or a custom-made tubing apparatus (von Békésy [18]) in combination with sniffing. Frasnelli et al. [11] however, demonstrated that humans cannot localize pure odorants independent of the stimulus delivery method. They investigated the effect of active sniffing and passive stimulation on the localization of both pure odorants and olfactory-trigeminal stimuli, finding no differences in the localization score between sniffing and passive stimulation. Therefore, it is not likely that the breathing technique influences the results of localization experiments.

Above all, we used a symmetrical study design, i.e. when an H_2S/IAA/CO_2 stimulus was presented to one nostril a blank stimulus was presented to the other one. Thereby, the blank stimuli were prepared using the same proportion of gas and dilution air as H_2S/IAA/CO_2 stimuli. By using this form of stimulus application, we excluded the influence of other sensations, such as touch or temperature, on the localization process.

Based on the results of the present study, we conclude that olfactory localization in humans is only possible when the odor also excites the trigeminal somatosensory system. Directional smelling mediated exclusively by the olfactory system appears to be absent in humans. Other studies have shown that animals, including mammals, are able to orientate themselves in space using their sense of smell [39,47,48], which would suggest that humans lost this property during the process of evolution. On the other hand, from the literature it is not fully clear whether animals localize based on purely olfactory cues, or if they also rely on trigeminal activation for this purpose. Nevertheless, based on neuroanatomic information it is possible that nostril-specific olfactory stimulation leads to site-specific activity in the human brain [19]. This should be investigated in further studies including functional magnetic resonance imaging.

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4 Further projects

The results of the presented studies clearly demonstrated that olfactory localization in humans is only possible, if the odor excites the trigeminal somatosensory system. Directional smelling mediated exclusively by the olfactory system appears to be absent in humans. In spite of clear findings this behavioral study does not explain the mechanisms, underlying the process of chemosensory localization in humans. Based on neuroanatomical information it could be possible that nostril-specific olfactory stimulation leads to site-specific activity in the human brain (Porter et al., 2005).

To investigate the neural substrates which underlie the behavioral mechanisms, we conducted a left versus right odorant localization study using functional magnetic resonance imaging (fMRI). We used two odors: 8 ppm H_2S (hydrogen sulphide), which is known to be a pure odorant in this concentration, and 17.5% isoamyl acetate (IAA) as an olfactory-trigeminal stimulus. We tested 22 healthy subjects with H_2S and 24 subjects with IAA. Functional images were acquired using a 3T MR scanner. The odorant stimulation was performed using an olfactometer. The experiment was carried out based on an event-related design, and the stimulus length was 500 ms. After every stimulus the participants were asked to discriminate between the H_2S/IAA stimuli perceived either from the left or from the right nostril.

We found activations of brain areas specific for olfactory stimulation (piriform cortex, orbitofrontal cortex, insula) for both odors. Using region of interest (ROI) analysis we found differences in the secondary olfactory cortex comparing left vs. right odorant stimulation in case of IAA odor, but not for H_2S odor. These results support our previous behavioral findings and confirm the hypothesis that nostril-specific differences in brain activation are functionally linked to the successful odor localization (original paper in preparation).

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