

Electro-Olfactograms in Humans in Response to Ortho- and Retronasal Chemosensory Stimulation

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Abstract

Aim Ortho- and retronasal olfaction represent two aspects of a shared sensory system yet evoke different sensations. The differences between ortho- and retronasal olfaction have triggered a number of studies during the past years, which pointed towards a decreased sensitivity to odors presented through the retronasal olfactory pathway. Especially intensity was reported to be lower after retronasal olfactory stimulation. The aim of this study was to investigate how this compares to activation at the level of the olfactory epithelium in humans.

Methods Trigeminal (CO₂) and olfactory (H₂S, phenylethyl alcohol) stimuli were presented ortho- and retronasally. Electro-olfactograms (EOG) in response to chemosensory stimulation were recorded in 10 participants (6 women, 4 men, mean age 23.4 years).

Results Typical EOGs were demonstrable after either orthonasal or retronasal stimulation across the stimulus qualities. Overall, EOG amplitudes to retronasal stimulation were smaller when compared to those to orthonasal stimulation, but

a significant difference was obtained only in phenylethyl alcohol ($p = 0.048$).

Conclusions The present data indicate that the perceptual differences between ortho- and retronasal olfaction may start at the level of the olfactory epithelium. The data support the idea that the intensity of physically identical stimuli is lower after retronasal stimulation compared to orthonasal stimulus presentation for both olfactory and trigeminal stimuli.

Implications The current electrophysiological results are in line with reported differences in psychophysical properties of retro- and orthonasal stimulations meaning that different sensations are elicited through these two channels although the same molecule is presented.

Keywords Olfaction · Food · Olfactory receptors · Eating · Smell · Nose · Pharynx

Introduction

Retronasal olfaction represents a peculiar aspect of the olfactory system (Hummel et al. 2006). Contrary to the orthonasal pathway, it is evaluated within the interior of the body rather than from the external world (Rozin 1982). This olfactory pathway provides important information for the well-being of the individual by detecting potentially harmful substances that enter the mouth. In addition, it is the basis for the pleasures of eating and drinking. Retronasal application of odors seems to evoke different sensations compared to orthonasal olfactory stimulation, as shown in psychophysics and neuroimaging studies (Small et al. 2005). Similarly, certain foods in daily life are perceived differently depending on the mode of presentation (for review, see Halpern 2004).

Previous research highlighted lower olfactory performance of the retronasal system, but it can be improved by special

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breathing techniques or mouth movements optimizing odor transport towards the nasal cavity (Burdach et al. 1984; Burdach and Doty 1987). Attention to the interaction between the chemosensory modalities (e.g., taste, smell, and trigeminal system) may be responsible for differences between ortho- and retronasal olfaction where individuals find the separation of chemosensory components more difficult when perceiving stimuli in the mouth. Here, a major influence on olfactory perception is contributed to taste (e.g., Dalton et al. 2000). Furthermore, nasal patency seems to influence retronasal olfaction (Raudenbush and Meyer 2001) as well as trigeminally mediated sensations (Frasnelli et al. 2004).

Release of odor molecules from the oral to the nasal cavity occurs during mastication and after swallowing. A significant part of the transport of odorous molecules from the mouth to the nose is controlled by the soft palate. Pulses of odorized air are pumped from the oral cavity into the pharynx during mastication, depending on the consistency of the food, or during swallowing (Buettner et al. 2001). Therefore, food that requires more force to breakdown exerts more, stronger pulses of odorized air than softer foods of similar composition. Additionally, differences in relation to the perception of ortho- and retronasal stimuli are—at least to a certain degree—due to differences in airflow patterns. In fact, subtle changes in nasal anatomy may produce large differences in the perception of odors. For example, it has been shown that modifications of the volume of the anterior portion of the nasal cavity may lead to differences in odor thresholds or odor identification (Damm et al. 2002). Similarly, minute changes of intranasal anatomy remote from the olfactory cleft may produce significant changes of olfactory function (Damm et al. 2003; Landis et al. 2003).

Rats (Gautam and Verhagen 2012a) and mice (Rebello et al. 2015) have been found to detect or discriminate retronasal odors. Rats were found to have associations between odors and tastes (Gautam and Verhagen 2010), as well as between odors and illness (e.g., conditioned odor aversion; Chapuis et al. 2007, 2009) based on their learning experiences. Gautam and Verhagen (2012b) showed that retronasally presented odors induce not only smaller response amplitude but also longer response onset latency when compared to orthonasally presented odors in rat models. Furthermore, using electro-olfactogram (EOG) recordings in rats Scott et al. (2007) demonstrated that polar odorants activated the olfactory epithelium when they were orthonasally presented but not effective when they were retronasally presented. Non-polar or hydrophobic odorants were found to be more effective in activating the olfactory epithelium than hydrophilic odorants when they were presented through a retronasal route (Scott et al. 2007).

Building on previous findings that compared orthonasal and retronasal olfactory perceptions, this study aimed to compare human EOG recordings at the level of the olfactory epithelium between orthonasally and retronasally presented

olfactory and trigeminal stimuli. The EOG represents the sum of electrical potentials that response to olfactory stimulation at the olfactory epithelium (Getchell and Getchell 1991; Knecht and Hummel 2004) and provides neuronal information from the peripheral olfactory level (Lapid and Hummel 2013), thereby allowing us to compare ortho- and retronasal olfactory responses at the peripheral level.

Material and Methods

EOG recordings were attempted in 10 healthy subjects (6 women, 4 men, mean age 23.4 years; age 21–29 years). The protocol was approved by the Ethics Committee of the TU Dresden (Dresden, Germany) and this study was conducted in accordance with the Declaration of Helsinki for studies on human subjects.

All subjects had previous experience in the experimental conditions of EOG recording and more specifically, with a required breathing technique (velopharyngeal closure, Kobal 1981) which avoids respiratory flow inside the nasal cavity. For chemical stimulation, an olfactometer was employed delivering the odorants to the left nostril without altering mechanical or thermal conditions at the stimulated mucosa (Kobal 1981; Kobal and Hummel 1988). This monomodal chemical stimulation is achieved by mixing pulses of the stimulants in a constantly flowing air stream with controlled temperature (36.5 °C) and humidity (80 % RH).

For retronasal stimulation odorants were applied into the pharynx via tubing placed below the lower turbinate; a second tube placed in the vestibulum of the nasal cavity was used for orthonasal administration of stimuli. The following approach was used: two plastic tubes of 3.3 mm outer diameter and 15 cm length (suction catheter, Ch. 10; B. Braun Melsungen AG, Melsungen, Germany) were attached to each other so that the opening of the tubes was 6 cm apart. The tubes were placed inside the nose under endoscopic control, with the “retronasal” tube resting below the lower turbinate. The tubes were fixed to the bridge of the nose by adhesive tape so that the tube ending in the epipharynx was placed below the tube that ended in the nasal vestibulum (for details and visualization, see Heilmann and Hummel 2004).

Total flow rate was 6 L/min. Two thirds of the maximum stimulus concentration were reached at the olfactometer's outlet within 20 ms (Kobal 1981). For olfactory stimulation, phenylethyl alcohol (40 % v/v, PEA) and hydrogen sulfide (8 ppm, H₂S) were chosen which are regarded to specifically activate the olfactory system (Doty et al. 1978; Thiele and Kobal 1984; Hummel et al. 1991). In addition, carbon dioxide (CO₂; 60 % v/v; Air Liquide Forschung und Entwicklung GmbH, Frankfurt, Germany) was used at concentrations which produces a clear trigeminally mediated sensations (e.g., stinging, burning, or tickling). During the experiment

Table 1 Recordings from the olfactory epithelium in relation to the three stimulus qualities and the two stimulation sites: response amplitudes (in mV)

Odorants	Stimulation site	Number of subjects	Mean amplitude	Standard error of mean
Phenethyl alcohol (PEA)	Orthonasal	6	0.24	0.09
	Retronasal	4	0.02	0.01
Hydrogen sulfide (H ₂ S)	Orthonasal	9	0.26	0.13
	Retronasal	8	0.17	0.06
Carbon dioxide (CO ₂)	Orthonasal	6	0.37	0.19
	Retronasal	7	0.19	0.11

subjects were seated comfortably in an air-conditioned room. White noise of approximately 50 dB SPL was used to mask switching clicks of the stimulator.

EOG was recorded by means of a tubular electrode filled with Ringer-agar (1 %) which contained a silver-chlorided silver wire (impedance ≤ 10 k Ω at 1 kHz, Ottoson 1956). The outer diameter of the Teflon tubing was 0.8 mm. For reference, an electrode normally used for EEG recordings (sintered Ag/AgCl electrode) was placed on the contralateral bridge of the nose. Subjects were instructed to carefully insert the electrode approximately 7 cm deep into the nasal cavity until they felt that contact had been established between electrode and mucosa. The location of the electrode was endoscopically verified (Richard Wolf, Knittlingen, Germany; outer diameter 1.9 mm); final adjustments of the tip of the electrode were performed by the experimenter under endoscopic control. The recording electrode was placed above the middle turbinate under endoscopic control such that the distance of the recording site from the opening of the orthonasal and retronasal tubes was approximately the same. The recording site was marked in a sketch of the human nasal cavity (Lang 1989; Lapid et al. 2009).

After positioning of the electrode, it was stabilized by means of adjustable clips on a frame similar to lensless glasses (Knecht and Hummel 2004). After a response had been established after stimulation with PEA, H₂S, or CO₂, the three stimuli were randomly applied at an interval of approximately 60 s; each stimulus was used 2–16 times. Mucosal potentials were amplified, filtered (band pass 0.01–15 Hz; SIR, Röttenbach, Germany), and digitized (sampling frequency

125 Hz, segments of 8192 ms). Recordings started 200 ms prior to stimulus onset. After averaging the responses per site and for each stimulus over the different repetitions, maximum base-to-peak EOG amplitudes were analyzed for each subject using BOMPE software (Kobal, Erlangen, Germany).

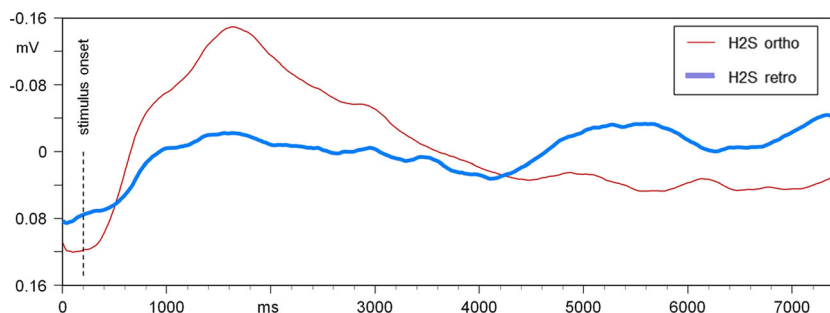
Using SPSS 23.0 (SPSS Inc. Chicago, IL, USA), data were statistically analyzed by means of Fisher's exact test (because EOGs were obtained in inhomogeneous groups of people and because the results did not fulfil the criteria for parametric statistics) to investigate differences between EOG amplitudes in response to orthonasal and retronasal stimulations for the different stimulus qualities.

Results

Responses from the epithelium could be obtained in all subjects; however, not all subjects exhibited responses to all classes (i.e., ortho- and retronasal stimulations, different stimulus qualities) probably due to the patchy distribution of the olfactory epithelium.

When comparing response amplitudes to ortho- and retronasal stimuli, those to orthonasal stimuli were on average larger than responses to retronasal stimuli (Table 1 and Fig. 1). These differences became significant for PEA (Fisher's exact test: $p = 0.048$), but not for H₂S and CO₂. When analyzing only those subjects in whom responses could be obtained ortho- and retronasally, responses to orthonasal compared to retronasal stimulation were larger in 4 of 4 subjects for PEA, in 4 of 7 for H₂S, and in 3 of 4 for CO₂.

Fig. 1 Grand means of electro-olfactograms averaged across subjects obtained after ortho- and retronasal (*thin* and *thick* lines, respectively) stimulation with hydrogen sulfide. Stimulus duration was 1000 ms



Discussion

The current results suggest that the amplitudes of peripheral responses to olfactory stimuli are larger after orthonasal stimulation compared to retronasal stimulation for the specific odor set tested, especially for PEA. Considering that increasing EOG amplitudes are correlated with increasing intensities (Lapid et al. 2009), this indicates that orthonasal stimuli are perceived with a higher intensity than retronasal ones.

Typically, odors applied ortho- or retronasally reach similar concentrations in the olfactory cleft (Small et al. 2005). Accordingly, ortho- and retronasal stimuli should elicit the same olfactory responses. However, the perceptual difference between the two routes of odor administration may be, among other factors, due to either differences in absorption patterns of odors to the olfactory epithelium depending on the site of odor application (Scott et al. 2014) or to the steepness of stimulus onset with retronasal stimuli being slightly less steep compared to orthonasal stimuli (Small et al. 2005), which seems to reflect differences in orthonasal and retronasal anatomy. Accordingly, retronasal stimuli might produce lower EOG amplitudes.

The current results are to some extent in line with previous findings. Heilmann and Hummel (2004) compared thresholds to ortho- and retronasal stimuli. A food-related (“chocolate”) and a non-food-related odor (“lavender”) were investigated. For both the food and non-food odor, orthonasal thresholds were significantly lower than retronasal thresholds, i.e., subjects required higher retronasal odor concentrations to detect an odor (Heilmann and Hummel 2004). On a suprathreshold level, this is also supported by a study from Pierce and Halpern who reported a diminished ability of odor identification through the retronasal pathway using the oral presentation of the vapor phase of solid odorous substances (Pierce and Halpern 1996).

The idea of higher thresholds (i.e., less sensitive) to retronasally presented stimuli also seems to be compatible with the thought that retronasal perception of foods and liquids typically encounters odors at higher concentrations than orthonasal perception of odors. Compared to the smell of a given food (e.g., cheese), odor release from that food is higher intraorally due to salivation, warming, and mastication (Burdach and Doty 1987). Because of these conditions, retronasal perception of odors, compared with orthonasal olfaction, may be adjusted to a higher range of odor concentrations.

Notably, the significant difference of EOG amplitudes between ortho- and retronasal stimuli was observed in PEA, but not in H₂S and CO₂, even though overall orthonasal stimulation showed larger amplitudes than retronasal stimulation. In other words, the difference between ortho- and retronasal olfactory responses at the level of the peripheral system may be dependent on odorant type, which are to some extent in line with the findings of rodent studies. In rats, the differences

between ortho- and retronasal responses at the peripheral level with respect to response amplitude and/or latency were found to be dependent on odorant type including polarity, hydrophobicity, sorption, and volatility properties (Scott et al. 2007, 2014; Gautam and Verhagen 2012b). Thus, to generalize the present findings in humans, further research should be conducted in a variety of olfactory stimuli.

In conclusion, response amplitudes to retronasal stimulation were typically smaller compared to responses obtained after orthonasal stimulation. These data indicated that differences between ortho- and retronasal olfaction may start as early as on the mucosal level. These electrophysiological experiments also support previous reports that the intensity of physically identical stimuli is lower after retronasal stimulation.

Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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