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Influence of simultaneous gustatory stimuli on orthonasal and retronasal olfaction

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ABSTRACT

Orthonasal and retronasal olfaction processes differ. The aim of this study was to examine whether congruent and incongruent simultaneous gustatory stimuli influence orthonasal and retronasal odorant perception, using olfactory event-related potentials as a measure. Thirty-two young, healthy subjects (16 men, 16 women) took part in two test sessions. Olfactory event-related potentials were recorded in response to a food-like odor, vanillin, and to an odor usually not associated with foods, the rose-like phenylethylalcohol. Each session consisted of four randomized blocks of 15 stimuli each which were applied either orthonasally or retronasally. Simultaneously, sweet or sour gustatory stimuli were applied. In response to retronasal vanillin, stimuli latencies P2 of the event-related potentials were significantly shorter in the congruent "sweet condition" than the incongruent "sour condition". In contrast, with orthonasal stimulation, shorter P2 latencies were seen for both odorants in the incongruent condition. Intensity of both odorants was perceived as less pronounced after retronasal stimulation than after orthonasal stimulation. In conclusion, application of a sweet taste significantly enhanced the processing of a congruent olfactory stimulus when presented through the retronasal route. Incongruent simultaneous gustatory stimulation applied during orthonasal olfaction seemed to induce conflict priming, also resulting in faster processing.

The way we experience our environment is largely based on the integration of multiple sensory inputs. Flavor perception is known to mainly consist of the interaction of retronasal olfaction and gustatory stimuli. Using functional magnetic resonance imaging (fMRI) techniques with a high spatial resolution, a network of regions consisting of orbitofrontal cortex, frontal operculum, ventral insula, amygdala, and anterior cingulated cortex has been shown to be specifically activated through retronasal stimuli [7,25,24].

In line with these results, electrophysiological studies in primates identified the caudal orbitofrontal cortex and agranular part of the insula as regions integrating multimodal inputs, such as olfactory and gustatory stimuli [4,21]. Using event-related potentials (ERP), effects of simultaneous olfactory and gustatory stimulation were studied in humans. Early processing of orthonasal chemosensory stimuli is modulated by concomitant gustatory stimulation [30]. While orthonasal stimuli are strongly associated with sniffing and the "outside" world, retronasal stimuli are related to food intake [22]. Therefore, it might be assumed that gustatory stimuli have differing effects on retronasal and orthonasal stimulation.

The aim of the present study was to examine whether orthonasal and retronasal stimuli, perceived as related or not related to food, are differently influenced by the simultaneous application of a related or unrelated taste stimulus.

The study was conducted according to the 'Declaration of Helsinki on Biomedical Research Involving Human Subjects', after having been approved by the Ethics Committee of the University of Basel, Switzerland. All participating volunteers provided written informed consent. Thirty-two healthy, right-handed subjects agreed to participate in the experimental sessions 1 and 2 (16 women, 16 men; mean age 27 years; range 21–34 years).

During the clinical examination prior to testing, no subject reported any history of major neurological, psychiatric, pulmonary, or otorhinolaryngological disturbances. Nasal endoscopy ruled out any mechanical obstruction of the anterior olfactory cleft and/or other major nasal pathologies. All participants were normosmic tested by means of the validated "Sniffin' Sticks" test kit, which included orthonasal testing of *n*-butanol odor threshold, odor discrimination, and odor identification [13,15]. Normogeusia was ascertained by a screening test based on administration of aqueous solutions presented at suprathreshold concentrations, as proposed by the Working Group on Taste and Smell of the German ENT Society (http://www.uni-duesseldorf.de/awmf/ll/017-052.htm).

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Two odors were used for olfactory stimulation, i.e., phenylethylalcohol (PEA, 40% v/v) and vanillin (40% v/v). Both odors are known to produce little or no activation of the intranasal trigeminal system when presented orthonasally at suprathreshold concentrations [8]. Moreover, both odorants can be identified correctly in 88% (vanillin) and 87% (PEA) of cases if presented retronasally using odorant delivery containers [6], while neither can be correctly identified after oral-cavity-only (OCO) presentation [6], nor can they be discriminated during OCO presentation [26]. This confirms the selective olfactory processing of the chosen stimuli.

Odors were presented using a computer-controlled air dilution olfactometer (OM2 s; Burghart, Wedel, Germany) that delivers rectangular odor pulses embedded in a continuous flow of odorless, humidified, and temperature-controlled air (relative humidity 80%, total flow 8 L/min, 36 °C) without perceptible tactile co-stimulation [14]. The aforementioned olfactory volatiles were delivered to the olfactory cleft either through an orthonasal or retronasal route as described previously [9]. This method provides a well-controlled and reproducible monorhinal stimulus environment. The model is commonly used for stimulus presentation in the laboratory setting although it may not fully reproduce the complex fluid dynamics, odorant distribution, and spatial-temporal patterns of normal orthonasal and retronasal olfaction during natural breathing.

Two soft polyvinyl tubes with an outer diameter of 3.3 mm and 15 cm length were joined, with their opening 6 cm apart. The tubes were inserted in the nose under visual control and placed with their openings for orthonasal stimulation just beyond the nasal valve and for retronasal stimulation close to the epipharynx (approximately 1.5 cm and 7.5 cm from the naris, respectively). As previously shown by Heilmann and Hummel [9], this setting allows well-controlled stimulus delivery irrespective of the route of administration. Specifically, odor concentrations in the olfactory cleft after either orthonasal or retronasal application exhibit a similar rectangular shape [9]. However, an online analytical technique (proton-transfer reaction mass spectrometry) revealed that the rectangular shape of odor pulses associated with the olfactometer differs from odorant signals achieved intranasally, suggesting a certain odorant loss after intranasal administration [1].

Gustatory stimulation was performed using sweet or sour stimuli. In both sessions, taste dispensers consisting of plastic bags (7.5 cm × 3 cm × 0.7 cm) were filled with glucose (approximately 7 g, "sweet") or filleted lemon (approximately 14 g, "sour") and then heat-sealed. Perforation of the lateral surfaces of the taste dispensers (16 holes per sour bag and 40 holes per sweet bag, needle of 1.7 mm diameter) provided sweet and sour sensations that were stable over a period of 10 min and were similar in intensity, as tested in preliminary experiments in a group of trained observers.

During the experiment, subjects were asked to suck on the taste dispensers while rating the intensity of the olfactory stimulus. At these moments subjects breathed normally, thereafter returned to the velopharyngeal closure breathing technique.

All subjects took part in both sessions. In each session, one odorant (either PEA or vanillin) was used, and the site of stimulation (orthonasal/retronasal) was paired with the gustatory stimulus (sweet/sour) in each session, resulting in four possible combinations ([orthonasal/sweet], [orthonasal/sour], [retronasal/sweet], [retronasal/sour]). Testing conditions were randomized and counterbalanced across subjects. In both sessions, each subject received the stimuli in the same sequence; sequences were different interindividually. Data acquisition per session lasted about 60 min.

Intensity ratings were collected after each olfactory stimulus. Using a visual analogue scale, subjects had to rate the intensity of olfactory stimuli on a computer screen by adjusting a marker according to stimulus intensity (right-sided end of scale: extremely strong intensity = 100 estimation units (EU); left-sided end: no stimulus detectable = 0 EU). In addition, after each of the four blocks, subjects had to specify (1) duration of gustatory perception (i.e., whether it lasted for the entire period of the functional run), and (2) mean gustatory intensity on a scale ranging from 0 (no taste) to 10 (extremely strong taste).

Olfactory ERP were recorded in response to orthonasal and retronasal stimulation; short olfactory stimuli were presented in the presence of continuous gustatory stimulation. Stimulus duration was 200 ms, and the average interstimulus interval was 40 s (range 36–44 s). Recording positions were Fz, Cz, Pz, C3, and C4 of the international 10/20 system (eight-channel amplifier; SIR, Röttenbach, Germany). Eye movements were monitored via the Fp2 lead, and linked earlobes (A1 plus A2) served as the non-lateralized reference for pseudo-monopolar ERP recordings. The sampling frequency was 250 Hz, and a pre-trigger period of 500 ms was chosen with a total time of 2048 ms per record (bandpass 0.02–30 Hz). Additionally, recordings were filtered offline (lowpass 15 Hz). Recordings confounded by muscular movements or eye blinking artifacts were discarded; remaining ERP segments were averaged and analyzed for amplitudes and latencies of the major peaks N1 and P2 by an experienced observer (TH).

To avoid respiratory airflow in the nasal cavity during ERP recording, subjects were instructed to apply a special breathing technique (velopharyngeal closure [14]). Subjects were comfortably seated and received white noise through headphones (60 dB) in order to mask switching clicks of the olfactometer.

Results were analyzed using analyses of variance for repeated measures. Post-hoc tests were performed using Bonferroni corrections. The alpha level was set at 0.05. Statistical analyses were performed using SPSS 12.0 (SPSS, Chicago, IL, USA).

In the Results section and in all figures, means and standard errors of the mean (SEM) are shown.

Due to technical problems, data were available for 29 subjects for intensity ratings of PEA, while data from all 32 subjects were evaluated for intensity ratings of vanillin. Intensity of orthonasal stimuli achieved higher ratings than did that of retronasal stimuli for both olfactory conditions (PEA: $F = 19.7$; $p < 0.001$; vanillin: $F = 32.3$; $p < 0.001$; see Table 1, Fig. 1). Odor stimuli were rated as more intense in the presence of the sweet rather than the sour stimulus (PEA: $F = 5.62$, $p = 0.025$; vanillin: $F = 5.77$, $p = 0.023$).

Sour taste perception was perceived significantly more intense than sweet taste perception ($F = 21.6$, $p < 0.001$) in the presence of PEA ($n = 32$). The same was true for vanillin ($F = 9.79$, $p = 0.004$; Fig. 2).

Table 1
Intensity ratings (means, standard error means = SEM) of odor (top, maximum = 100, minimum = 0) and taste sensations (bottom, maximum = 10, minimum = 0) separately for the two odors (phenyl ethyl alcohol = PEA [$n = 29$], vanillin [$n = 32$]) presented ortho- or retronasally, in the presence of sweet or sour taste stimuli.

Odor intensity		PEA	($n = 29$)	Vanillin	($n = 32$)
Olfactory stimulation site	Presence of sweet or sour taste	Mean	SEM	Mean	SEM
Retronasal	Sweet	34.1	3.92	23.8	2.74
	Sour	26.6	3.80	22.3	2.75
Orthonasal	Sweet	42.7	4.19	37.8	2.55
	Sour	39.0	3.73	33.7	2.66
Taste intensity		PEA	($n = 29$)	Vanillin	($n = 32$)
Olfactory stimulation site	Presence of sweet or sour taste	Mean	SEM	Mean	SEM
Retronasal	Sweet	6.0	0.31	6.0	0.28
	Sour	7.2	0.28	7.0	0.30
Orthonasal	Sweet	6.3	0.30	6.1	0.30
	Sour	7.2	0.31	6.7	0.30

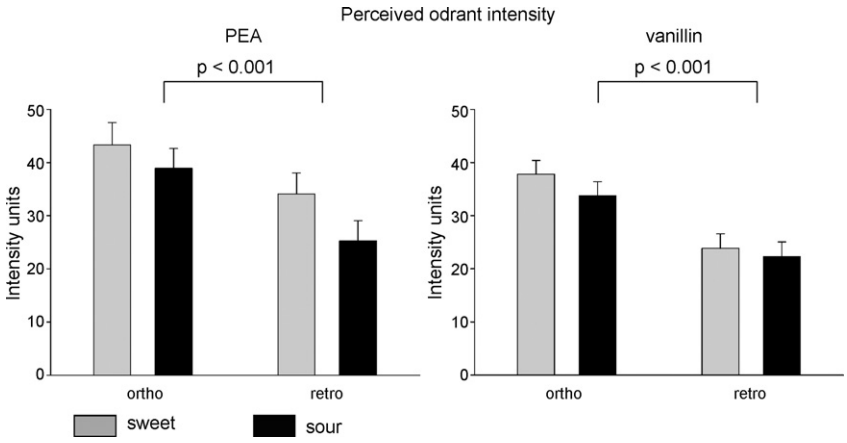

Fig. 1. Intensity of olfactory stimuli (vanillin and PEA) after orthonasal or retronasal stimulation in the presence of the gustatory stimulus.

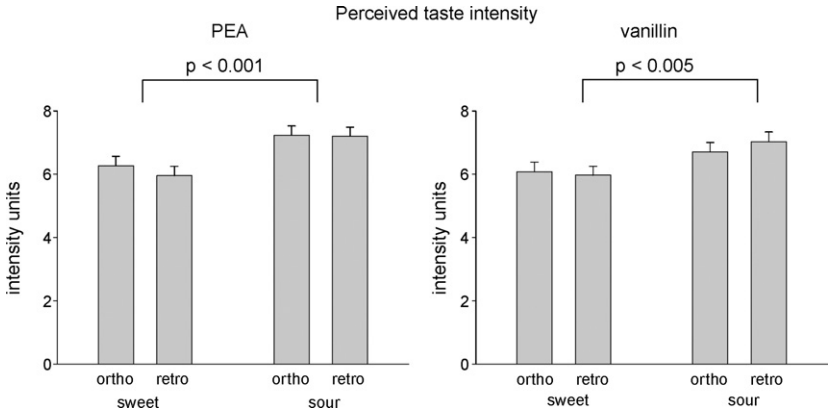

Fig. 2. Perceived intensity of taste stimuli (sweet/sour) in relation to olfactory stimulation.

Due to artifacts, data from only 26 subjects were available for evaluation. Latencies P2 (Cz) exhibited differences in relation to the stimulation site ($F = 20.7$; $p < 0.001$). Moreover, gustatory stimulation had a significant effect on the recordings made after orthonasal or retronasal stimulation ($F = 4.48$; $p = 0.044$). With orthonasal stimulation, independent of the olfactory stimulus, latencies of P2 (Cz) were significantly prolonged in the sweet-taste condition compared to the sour-taste condition. Retronasal olfactory stimulation, however, produced shortened latencies of P2 (Cz) in the presence of the sweet-taste stimulus, in contrast to the presence of the sour-taste stimulus (Fig. 3).

There were no statistically significant differences for other latencies and amplitudes of the ERP.

The results of our study show that (1) gustatory stimulation modulated PEA and vanillin perception in a different way, depending on the site of stimulation, and (2) orthonasal and retronasal stimulus intensities differed. As indicated by ERP peak latencies, vanillin was modulated more markedly by simultaneous gustatory stimulation than was PEA in the retronasal condition. However, this finding was reversed with orthonasal stimulation.

Numerous studies indicate that orthonasal and retronasal olfactory stimuli are processed differently [12,23]. Comparing the

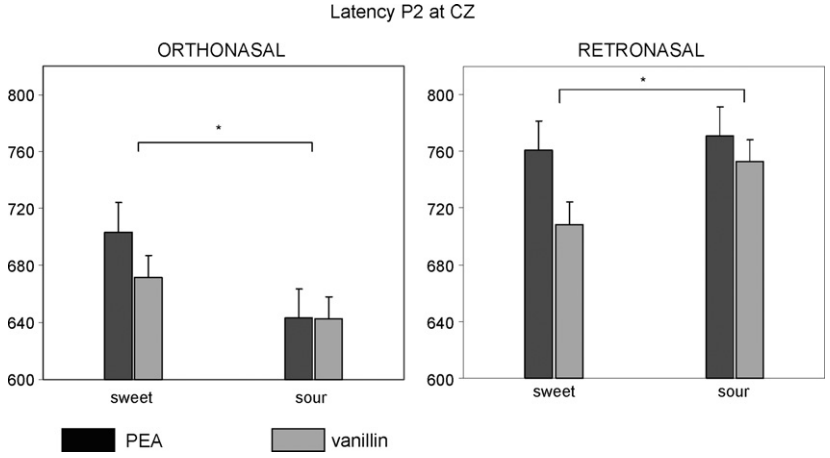

Fig. 3. Latency of P2 at position Cz for the two odorants, PEA and vanillin, after orthonasal and retronasal stimulation during simultaneous gustatory stimulation.

different studies, however, it has to be kept in mind that in many of these, retronasal olfactory stimuli were applied orally [5,10,20,3], thus potentially eliciting trigeminal and/or gustatory stimulation in the oral cavity which might interfere with retronasal olfactory perception. This source of errors can be overcome by using the intranasal route [9]. However, retronasal stimulation using an olfactometer with intranasal device is different from retronasal stimulation during normal breathing and food consumption. Normal food consumption is affected by oropharyngeal processes during swallowing and mastication [3] and is subject to variable topographical adsorption of odorants in both the intraoral pharyngeal mucosa and olfactory epithelium [2]. Nonetheless, we chose the intranasal route in our study to spatially separate retronasal olfactory and gustatory stimulation.

It is known that gustatory stimuli can influence olfactory processing. This was previously shown by using sweet and sour gustatory stimuli in combination with olfactory stimuli [30]. Typically, congruent olfactory-gustatory stimuli are processed more rapidly than incongruent stimuli. Our current results support this notion, suggesting that congruency and familiarity play an important role in stimulus processing. Hummel and Heilmann [11]reported that chocolate, a food-related odor, and lavender, an odor not related to food, are processed differently depending whether they are applied orthonasally or retronasally. Chocolate produced larger P2 amplitudes when presented orthonasally, in contrast to lavender that produced larger P2 amplitudes when presented retronasally. As interpreted by the authors, food-related odors presented at an "unusual" site appear to lead to an enhanced P2 component, due to the relative unfamiliarity of the context [11]. In our study, latency of P2 in response to vanillin, the food-linked odor, was influenced much more markedly during retronasal stimulation in the presence of the congruent ("sweet") gustatory stimulus. It seems that simultaneous sweet gustatory stimulation enhanced processing significantly while the non-food odor PEA was not significantly influenced by the concomitant gustatory stimulus during retronasal stimulation.

For orthonasal stimulation, our results suggest that in the presence of the sweet gustatory stimulus, vanillin was processed more rapidly than PEA. Interestingly, both vanillin and PEA were processed more rapidly even in the presence of the incongruent gustatory stimulus, i.e., the sour taste. These results seem to be in contrast to previous observations showing that latencies P1 and N1 were enhanced during congruent gustatory and olfactory stimulation [30]. However, it should be considered that in our former study [30] as well as in the study by Hummel and Heilmann [11], "early" ERP components (peaks P1 and N1) were mainly influenced, possibly pointing towards a priming effect. Usually, response latencies and error rates increase in incongruent conditions as seen in the so-called "Stroop task", an experimental paradigm presenting conflicting information [27], for example meaning and color of a written word. In a slightly modified paradigm presenting a task-irrelevant standard or deviant auditory stimulus first followed by a single congruent or incongruent visual word stimulus, Mager et al. showed that ERP revealed a more phasic early negativity after presentation of the deviant auditory stimulus [17]. The authors concluded that the deviant pre-stimulus might enable improved processing of the subsequent visual stimulus with incongruent stimulus features. Moreover, they hypothesized that this phenomenon may be due to "conflict priming". Accordingly, it has been reported that incongruent stimuli achieve more attention, a fact known to decrease latencies of N1 and P2 indicating faster sensory processing [16,19]. Even though the paradigm used in this study was different, the decreased latency of P2 in the incongruent orthonasal stimulation might be due to conflict priming indicating that incongruence increases arousal and therefore accelerates processing.

Notably, stimulus processing in the orthonasal and retronasal conditions differs. Incongruence in orthonasal stimulation might be considered as conflict priming, i.e., the situation in which the individual has to decide whether food can be ingested or not. Subsequently, retronasal olfaction starts to take place during swallowing. Incongruence is then evaluated differently leading to a prolonged latency.

As an additional finding, olfactory stimuli were rated less intense when presented retronasally, which is in line with the literature [29,9] and also confirms the validity of the current experiment. In addition, latencies of olfactory ERP typically exhibit smaller amplitudes and longer latencies [18,28] when presented retronasally. This observation was also confirmed by the present results.

In conclusion, the present study supports the idea that orthonasal and retronasal olfactory stimuli are processed differently. Moreover, congruent and incongruent concomitant gustatory stimuli appear to have a different impact on orthonasal and retronasal olfaction. Stimulus congruency in retronasal stimulation decreases P2 latencies. We hypothesize that in orthonasal stimulation, incongruent stimuli seem to induce conflict priming and, thus, also induce a decrease in P2 latency.

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