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# Synergy and Masking in Odor Mixtures: An Electrophysiological Study of Orthonasal vs. Retronasal Perception

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## Abstract

Perceptual interactions in a model of wine woody–fruity binary mixtures were previously reported in a psychophysical study performed through orthonasal stimulation only. However, recent studies suggested that the perception of food-like and nonfood-like odors may depend on the route of stimulation. The aim of the present study was two-fold: first to examine the neural correlates of perceptual interactions using electroencephalogram (EEG)-derived event-related potentials (ERPs) and second to test the influence of the stimulation route on quality perception. Therefore, we designed an experiment with 30 subjects to study perceptual interactions in woody–fruity mixtures and compared ortho- vs. retronasal stimulation sites on perceived odor quality and ERPs. The results revealed synergy or masking of the fruity component, depending on the woody component level. Synergy was supported by larger N1 amplitude of the ERP. Furthermore, mixtures including a medium level of the woody odor elicited a strong increase of P2 amplitude only retronasally. This study evidenced for the first time electrophysiological correlates of both perceptual synergy and masking on the early component of the ERPs and confirmed that retro- vs. orthonasal stimulation route induces different neural processes that are reflected in the late component of the ERP.

**Key words:** electroencephalography, event-related potential, masking, odor mixture, synergy

## Introduction

The variety of sensory perceptions observed when mixing odorants result from qualitative (odor quality) and quantitative (odor intensity) perceptual interactions between odorant perception (Laing et al. 1984). However, perceptual interactions between volatile compounds in combination remain difficult to predict in food or beverages, even when the complexity of the combination is reduced to binary mixtures in synthetic solutions (Berglund et al. 1973; Frijters 1987; Laffort 1989; Olsson 1994; Thomas-Danguin and Chastrette 2002).

A previous study on binary mixtures composed of woody and fruity odorants naturally present in wine (e.g., isoamyl acetate/whiskey lactone or ethyl butyrate/whiskey lactone), indicated that most subjects were unable to identify both components, even when equal intensity levels of the two components were mixed (Atanasova, Thomas-Danguin,

Chabanet, et al. 2005). Consequently, a sharp change in quality identification from the woody to the fruity odor was observed when the proportion of fruity compound increased in the mixture. These observations were in agreement with those of Laing and Willcox (1983), showing that most mixtures are perceived qualitatively as a single odor.

Olfactory binary mixture perception can lead to several perceptual qualitative interactions that include masking (whereby one quality is suppressed, totally or partially, by the other) and synergy (whereby one quality is enhanced by the other). In a recent investigation, we showed that according to the concentrations used, the perceptual interaction between fruity and woody components of wine may result in a masking of the fruity component by the woody component when presented at supra-threshold concentrations (Atanasova et al. 2004). In another study, a synergy

of the fruity odor by sub-threshold and peri-threshold levels of the woody component was observed (Atanasova, Thomas-Danguin, Langlois, et al. 2005). However, the above data were established only when stimuli were presented in the orthonasal pathway, and as suggested by Small et al. (2005), olfactory perception may strongly depend on the route of stimulation (orthonasal vs. retronasal).

Recent studies shed light on the importance of retronasal stimulation in the perception of foods and beverages; as suggested by Rozin (1982), "the same olfactory stimulation may be perceived and evaluated in two qualitatively different ways, depending on whether it is referred to the mouth or the external world." Heilmann and Hummel (2004) presented identical volumes of odorized air either orthonasally or retronasally and recorded both olfactory event-related potentials (ERPs) and psychophysical thresholds. The experiment showed that for the same odorant, thresholds, intensity, and physiological recordings differed according to the route of stimulation. One explanation of the differences between ortho- vs. retronasal perception of the same odorant could rely on the brain representation of food as shown by Small et al. (2005). Using functional imaging, these authors showed that a food odor (chocolate) activates areas that are activated to a lesser degree by nonfood odors (butanol or lavender); thus the central processing of an odorant may differ when the stimulant represents a food.

In a study on the binary mixture of CO<sub>2</sub> (trigeminal stimulus) and carvone (mixed trigeminal/olfactory stimulus), it was observed (Livermore et al. 1992) that the psychophysical suppression (masking) of CO<sub>2</sub> intensity ratings by carvone was paralleled by a reduction of olfactory ERPs amplitudes. These results suggest the possibility to relate the amplitude of ERP components to perceptual interactions in odorants mixtures (masking and synergy). Thus, the first aim of the study was to set out to examine the neural correlates of the masking and synergy effects described for binary mixtures of woody and fruity odorants using ERP recordings. To achieve this aim, we tested the hypothesis that a masking of the fruity odor should decrease ERP amplitudes and a synergy for the fruity odor should increase ERP amplitudes.

Livermore et al. (1992) also showed that in olfactory ERPs, the amplitudes of N1 and P2 seemingly reflect different aspects of stimulus processing, with the P2 component reflecting the endogenous processing of stimulus quality to a larger degree than N1. This was confirmed and extended by Pause et al. (1996). It allows expecting that the late positive component (P2) may reflect potential differences in odor processing between orthonasal and retronasal stimulation. The second aim of the study was thus to test the influence of the route of stimulus on quality perception of food-like (fruity) and less food-like (woody) components of mixtures. To achieve this aim, we hypothesized that the food-like component would be more meaningful through the retronasal path (vs. orthonasal), and this effect would be associated with a variation in P2 amplitude.

## Materials and methods

### Odorants

Isoamyl acetate (described as "fruity" or "banana"-like) represented the fruity odor and  $\beta$ -methyl- $\gamma$ -octalactone (generally named whiskey lactone, described as "woody" or "coconut") represented the woody odor. Both odorants were purchased from Sigma-Aldrich, Saint-Quentin Fallavier, France. Stimulations were isoamyl acetate ("F") at a medium concentration in air, whiskey lactone at a medium concentration ("W"), whiskey lactone at a low concentration ("w"), or their mixtures ("Fw" and "FW"). The concentrations were chosen to be as close as possible to those used in the work by Atanasova et al. (2004).

### Odorants delivery

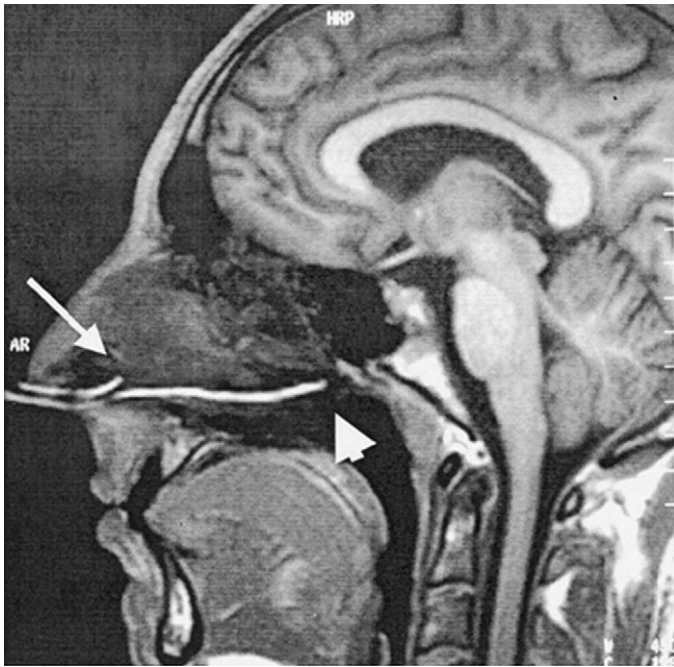
A computer-controlled olfactometer based on air dilution birhinal olfactometry (OM6b, Burghart, Wedel, Germany) was used to deliver odor stimuli. The air was humidified and temperature stabilized at 36 °C at the olfactometer's outlet. The total airflow from each outlet was kept constant at 6.0 l/min. Stimulus duration was 250 ms, the inter-stimulus interval (ISI) was ranged and randomized between 30 and 40 s. One of the birhinal channels was used for stimuli delivery and the other one was used for control (odorless air).

Each odorant was set in an olfactometer chamber without solvent. Pure medical dry air went through the liquid odorant to produce odorized air. This odorized air was diluted with humidified odorless air at the outlet of the olfactometer. The odorized air flow/total air flow ratio of each stimulus was 31% for fruity medium level odor (F), 41% for woody medium level odor (W), and 14% for woody low level odor (w). The mixtures (Fw and FW) were obtained keeping these ratios in the total air flow (6.0 l/min).

Two plastic tubes were placed into the nasal cavity under endoscopic control (Figure 1). These plastic tubes (3.3 mm outer diameter, 15 cm length) were attached to each other in order to separate their opening at a distance of 6.5 cm. Then, the tubings were inserted into the nasal cavity. Thus the orthonasal odor stimulus was delivered approximately 1 cm inside the nostril, whereas the retronasal stimulus was delivered approximately 7.5 cm in the epipharynx (Heilmann and Hummel 2004). Both tubes were attached to the nose by adhesive tape. These tubes were connected to the outlet of the olfactometer while the subject was sitting comfortably in a chair, the airflow was maintained constant through both tubes, and the subjects had no external cue as to where the stimuli were delivered.

### Stimulus chemical analyses

Gas chromatography analyses were carried out to determine the concentration of the odorants at the olfactometer outlet (Table 1). Teflon bags (49 × 49 cm, 20-l capacity, equipped with a Teflon connector; Interchim, Montluçon, France) was connected to the outlet of the olfactometer and filled with odorized



**Figure 1** Positioning of the tubes in the nasal cavity for stimulate ortho- or retronasally (Heilmann and Hummel 2004).

**Table 1** Concentrations of the odorants delivered by dynamic olfactometry

Chemical name	Odor label	Concentration (ppm v/v)
Isoamyl acetate	Fruity (F)	8.73 ± 1.45
Whiskey lactone	Woody strong (W)	3.23 ± 0.11
	Woody weak (w)	1.45 ± 0.07

air. Then, 500  $\mu$ l of the gas phase contained in the Teflon bag was injected into a GC system (HP6890 series, Hewlett Packard, Palo Alto, CA) equipped with a split-splitless injector, a DB-WAX column (Agilent technologies, Santa Clara, CA; 30 m, 0.32 mm inner diameter, 0.5  $\mu$ m thickness) and a flame ionization detector (FID). Injection was performed in splitless mode. The oven was programmed to increase temperature from 40 to 200  $^{\circ}$ C at a rate of 5  $^{\circ}$ C/min. Quantitative values were obtained by the calibration graph build by replicate analyses with dichloromethane solutions containing known amounts of the odorants.

### Subjects

Thirty subjects (18 females and 12 males) were recruited around the University of Dresden Medical School where experiments were performed. Regular smokers were excluded from the study. The subjects were asked not to eat, not to drink except water, and not to smoke 1 h before the experiment. They were also asked not to wear any perfume. Their age range was 19–27 years (average 24.3 years old). For 12 subjects, the tubes were inserted into the left nostril, and in the right nostril for 18 other subjects. Subjects were slightly

compensated for their participation, all signed an informed consent form. The study was conducted according to the declaration of Helsinki and approved by the Ethics Committee of the University of Dresden Medical School.

### Procedure

#### Screening

One week before the main experiment, subjects were submitted to the “Sniffin’ Sticks” test (Hummel et al. 1997) in order to examine their abilities for orthonasal olfaction. To examine their retronasal olfactory abilities, orally applied aromas had to be identified from lists of verbal descriptors (“Schmeckpulver” test, Heilmann et al. 2002). All subjects were found to be normosmic, both ortho- and retronasally. There was, however, no correlation between results from the 2 tests ( $r = 0.20$ ,  $P = 0.29$ ). During this session, subjects were familiarized with the processes of breathing with velopharyngeal closure and positioning of tubes inside the nose.

#### Main experiment

The main experiment was performed 1 week after the screening and lasted approximately 2 h. Two experimental sessions were run during the same day. In session 1, olfactory ERPs were obtained from the participants for F, Fw, and FW stimuli. These 3 stimuli were chosen to evaluate synergy and masking of the fruity odor by the woody one. In session 2, odor intensity ratings were obtained for the single odorants (F, w, and W) and quality ratings recorded for their mixtures (Fw and FW).

#### ERP recordings

The EEG was recorded at 5 positions (Cz, Fz, Pz, C3, and C4). EEG at Fp2 position was recorded to monitor eyeblinks. The left and right mastoids (Mz1 and Mz2) were used for grounding, and linked earlobes (A1 and A2) were used as the reference. Each stimulus (F, Fw and FW) was presented 16 times orthonasally and 16 times retronasally. Following presentation of each odor stimulus, subjects rated stimulus intensity for 2 descriptors (woody and fruity) on 2 on-screen visual analog scales (Atiken 1969): For the fruity odor, the left-hand end of the scale was defined as “extremely weak” (0%) and the right-hand end as “extremely strong” (100%). The same principle was applied for the woody odor.

During this session, subjects were sitting in an armchair and received white noise through headphones to mask switching clicks produced by the olfactometer. During the ISI, they performed a simple tracking task to stabilize vigilance and eyes moving (Hummel and Kobal 2001): Using a joystick, they had to keep a small square inside a larger one, which moved unpredictably on a monitor, at a distance of about 1.5 m, in front of the subjects.

#### Psychophysical measurements

In the second part of the sessions, odor intensity of each single odorant (F, w, and W) and odor quality of the mixtures

(Fw and FW) was evaluated. Odor intensity ratings were performed on a linear paper-and-pencil scale. Odor quality ratings were performed through a 4 alternative forced choice questionnaire ("fruity", "fruity and woody," "woody", and "no odor"). Each stimulus was presented 3 times ortho-nasally and 3 times retronasally in a randomized order.

### Statistical analysis

All statistical analyses were conducted using SAS vs. 8.2 (SAS Institute INC., Cary, NC). The repeatability of the subjects' individual responses was examined through a Control of Panelist Performances ("CAP analysis," Schlich 1997): Subjects who did not produce repeatable responses were excluded from the analyses. At the end, responses of 24 subjects were included in psychophysical data analyses. For ERP data, recordings contaminated by motor artifacts or eyeblinks were discarded. The first positive peak, which occurred typically at latencies of >250 ms from the stimulus onset, was named P1, and the following major negative peak and the late positive complex were, respectively, named N1 and P2 (Hummel and Kobal 2001). N1 and P2 amplitudes and latencies were then collected for further data analysis. Analysis of variance (ANOVA) was performed on the odor intensity ratings using the MIXED procedure of SAS. Multinomial analysis was performed on odor quality results using GENMOD procedure of SAS. ANOVA was performed on olfactory ERPs data using the MIXED procedure of SAS. The alpha level was 0.05. Paired *t*-tests on least-square means were used for post hoc comparisons. A first analysis revealed nonsignificant differences between left and right nostril stimulation. Consequently, data were pooled in further analyses.

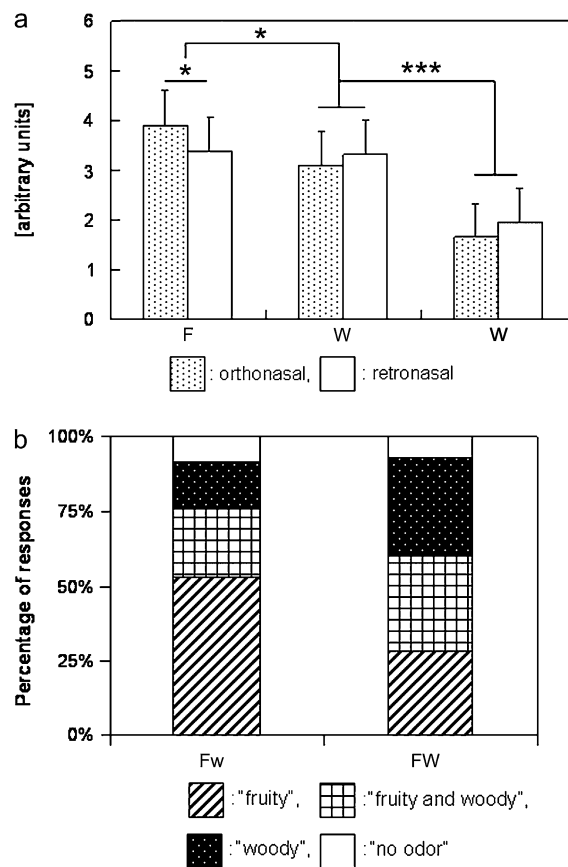
## Results

### Intensity of single odorants

Intensity ratings of each single odorant (F, w, and W) perceived either orthonasally or retronasally were recorded during the second session of the main experiment (Figure 2a). A two-way repeated-measures ANOVA with stimulation site (orthonasal and retronasal) and stimulus (F, w, and W) as within-subject variables was performed on intensity ratings and revealed a significant main effect of stimulus ( $F(2,403) = 51.7, P < 0.0001$ ) and a significant interaction between stimulus and stimulation site ( $F(2,403) = 3.07, P = 0.047$ ). Post hoc analysis showed that only the intensity of the fruity odorant (F) was lower retronasally than orthonasally ( $t(403) = 2.04, P = 0.042$ ).

### Quality of odor mixtures

Figure 2b showed quality ratings of odorant mixtures (Fw and FW), recorded during the second session of the main experiment. A multinomial analysis with stimulation site (orthonasal and retronasal) and stimulus (Fw and FW) as



**Figure 2** (a) Means of intensity ratings of single odors perceived either orthonasally (dotted bars) or retronasally (white bars). F: fruity single odorant; w: low level of woody odorant; W: medium level of woody odorant. Vertical bars indicate 95% confidence interval. \* $P \leq 0.05$ , \*\*\* $P \leq 0.001$ . (b) Means of quality ratings of odor mixtures. Fw: mixture of fruity odorant and low level of woody odorant; FW: mixture of fruity odorant and medium level of woody odorant.

factors was performed on quality ratings and revealed a significant effect of stimulus ( $\chi^2 = 9.7, P = 0.002$ ). Fw mixture was perceived as fruity only in more than 50% of the responses. FW was perceived as equally fruity and woody. It is noteworthy that FW mixture tended to be perceived as more woody retronasally ( $\chi^2 = 3.2, P = 0.08$ ).

### Fruity odor synergy and masking in mixtures

#### Psychophysical measurements

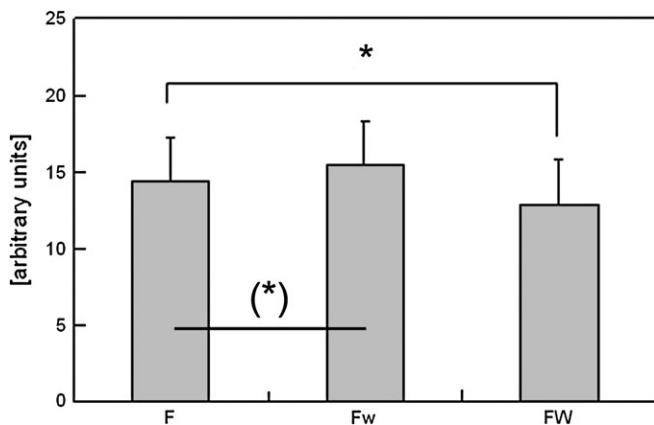
A two-way repeated-measures ANOVA with stimulation site (orthonasal and retronasal) and stimulus (F, Fw, and FW) as within-subject variables was performed on intensity ratings for fruity recorded during the first session of the main experiment. The results indicated a significant main effect of stimulus ( $F(2,2272) = 8.8, P = 0.0002$ ) and a significant main effect of stimulation site ( $F(1,2272) = 5.6, P = 0.018$ ). No significant interaction between variables was found. Post hoc analysis showed that fruity intensity of the Fw mixture tended to be higher than the fruity intensity of the F single

odorant ( $t(2272) = 1.82, P = 0.07$ ). This result suggested a perceptual synergy for the fruity odor in the Fw mixture (Figure 3). Intensity of the fruity odor in the FW mixture was significantly lower than the one in the F single odorant ( $t(2272) = 2.36, P = 0.02$ ). This result indicated a perceptual masking of the fruity odor in FW mixture (Figure 3).

### Neurophysiological measurements

In order to find neurophysiological correlates supporting the synergy and the masking observed at the psychophysical level, a three-way repeated-measures ANOVA with stimulation site (orthonasal and retronasal), stimulus (F, Fw, and FW), and recording position (Fz, Cz, Pz, C3, and C4) as within-subject variables was performed separately on N1 and P2 amplitude and latency. A significant main effect of stimulus was found for the amplitudes of N1 ( $F(2,687) = 13.1, P < 0.0001$ ) and P2 ( $F(2,687) = 20.5, P < 0.0001$ ) and a tendency was found for latency of N1 ( $F(2,687) = 2.8, P = 0.06$ ). A significant main effect of recording position appeared for the amplitudes of N1 ( $F(4,687) = 3.3, P = 0.01$ ) and P2 ( $F(4,687) = 17.8, P < 0.0001$ ). A significant main effect of stimulation site was found for N1 ( $F(1,687) = 19.9, P < 0.0001$ ) and P2 ( $F(1,687) = 20.9, P < 0.0001$ ) latencies. An interaction between stimulus and stimulation site was significant for P2 amplitude ( $F(2,687) = 7.7, P = 0.0005$ ) and latency ( $F(2,687) = 4.15, P = 0.016$ ).

Post hoc analysis revealed differences in ERP between F single odorant and Fw mixture which may support psychophysical measurements suggesting a perceptual synergy of the fruity odor in Fw mixture. There was a significant increase of N1 amplitude in Fw mixture as compared with F single odorant ( $t(687) = 3.66, P = 0.0003$ ; Figure 4a). N1 latency showed no difference between F and Fw stimulus presentation (Figure 4b). A decrease of P2 latency was observed for Fw mixture as compared with F single odorant but only orthonasally ( $t(687) = 2.61, P = 0.009$ ; Figure 5a). As



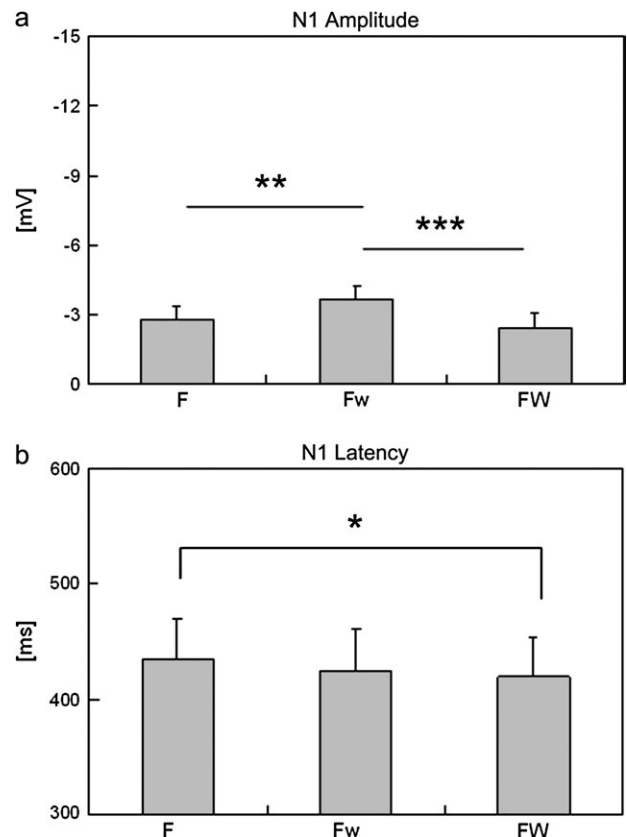
**Figure 3** Means of fruity intensity ratings. F: fruity single odorant; Fw: mixture of fruity odorant and low level of woody odorant; FW: mixture of fruity odorant and medium level of woody odorant. Vertical bars indicate 95% confidence interval. (\*) $P \leq 0.1$ , \* $P \leq 0.05$ .

regards masking, post hoc tests underlined differences in ERP between F single odorant and FW mixture which may support psychophysical observations. There was a significant decrease of N1 latency between F single odorant and FW mixture ( $t(687) = 2.32, P = 0.02$ ; Figure 4b) but no significant decrease or increase of N1 amplitude between these 2 stimuli (Figure 4a). We also observed an increase of P2 amplitude between F and FW stimuli but through the retronasal route only ( $t(687) = 6.51, P < 0.0001$ ; Figure 5a). P2 latency was smaller for FW than F stimulus but only orthonasally ( $t(687) = 2.27, P = 0.02$ ; Figure 5b).

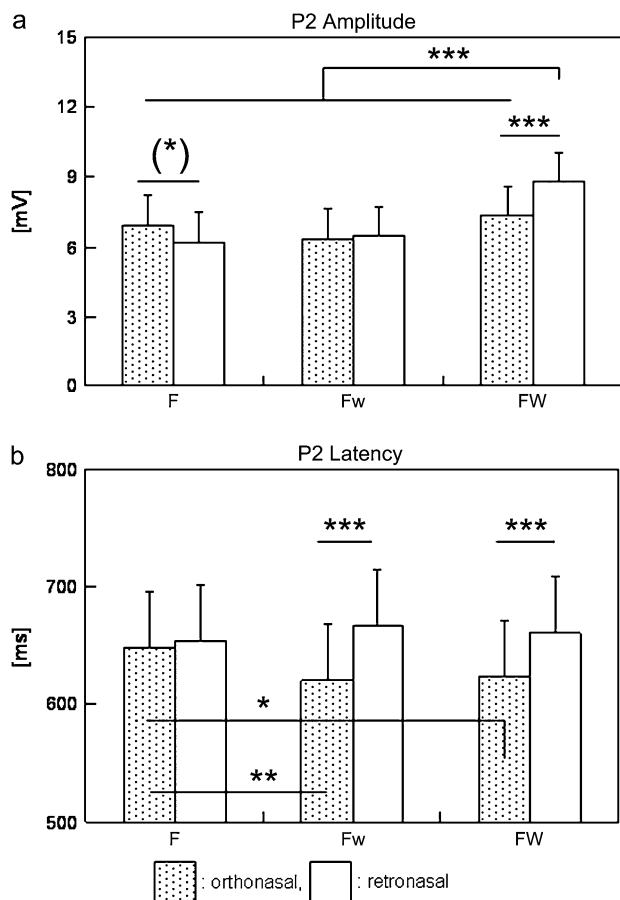
### Differences between ortho- vs. retronasal sites of stimulation

#### Psychophysical measurements

The above reported two-way repeated-measures ANOVA (stimulation site and stimulus) performed on intensity ratings for fruity indicated a significant main effect of stimulation site ( $F(1,2272) = 5.6, P = 0.018$ ). Post hoc tests revealed that the fruity intensity of the fruity odorant alone was higher orthonasally ( $t(2272) = 2.27, P = 0.02$ ). When the fruity



**Figure 4** Means of N1 components of olfactory ERPs. (a) N1 amplitudes. (b) N1 latencies. F: fruity single odorant; Fw: mixture of fruity odorant and low level of woody odorant; FW: mixture of fruity odorant and medium level of woody odorant. Vertical bars indicate 95% confidence interval. \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .



**Figure 5** Means of P2 components of olfactory ERPs. **(a)** P2 amplitudes. **(b)** P2 latencies. F: fruity single odorant; Fw: mixture of fruity odorant and low level of woody odorant; FW: mixture of fruity odorant and medium level of woody odorant. Vertical bars indicate 95% confidence interval. (\*) $P \leq 0.1$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

stimulus was mixed with the woody one, no significant difference in fruity intensity between stimulation routes could be observed (Figure 3).

#### Neurophysiological measurements

The above reported three-way repeated-measures ANOVA (stimulation site, stimulus, and recording position) performed on N1 and P2 amplitude and latency indicated a significant main effect of stimulation site for N1 ( $F(1,687) = 19.9$ ,  $P < 0.0001$ ) and P2 ( $F(1,687) = 20.9$ ,  $P \leq 0.0001$ ) latencies. An interaction between stimulus and stimulation site was significant for P2 amplitude ( $F(2,687) = 7.7$ ,  $P = 0.0005$ ) and latency ( $F(2,687) = 4.15$ ,  $P = 0.016$ ). Post hoc tests indicated that latencies were more prolonged through the retronasal than the orthonasal route (N1:  $t(687) = 4.46$ ,  $P < 0.0001$ ; P2:  $t(687) = 4.57$ ,  $P < 0.0001$ ). P2 amplitude was found to be higher retronasally for FW mixture ( $t(687) = 3.66$ ,  $P = 0.0003$ ; Figure 5a) and tended to be higher orthonasally for F single odorant ( $t(687) = 1.79$ ,  $P = 0.07$ ;

Figure 5a). No difference was observed for Fw mixture ( $t(687) = 0.17$ ,  $P = 0.9$ ). Thus, it seems that the more woody odor is added to fruity odor, the larger P2 amplitude for retronasal stimuli. As regards P2 latencies, no difference between stimulation sites was observed for F single odorant ( $t(687) = 0.4$ ,  $P = 0.7$ ), whereas for both mixtures (Fw and FW), P2 latencies were much longer with retronasal stimulus presentation (Fw:  $t(687) = 4.18$ ,  $P < 0.0001$ ; FW:  $t(687) = 3.34$ ,  $P = 0.0009$ ; Figure 5b).

## Discussion

### Synergy for fruity odor

The psychophysical data suggested a perceptual synergy for the fruity odor in the mixture with a low intensity of woody odor (Fw). This result was supported by electrophysiological data. Indeed, there was a significant increase of N1 amplitudes in response to the mixture with a low intensity of woody odor as compared with the fruity single odor. The psychophysical observations are in accordance with previous studies on the same fruity–woody mixture of isoamyl acetate and whiskey lactone. Atanasova, Thomas-Danguin, Langlois, et al. (2005) demonstrated that fruity–woody mixtures containing sub- or peri-threshold levels of whiskey lactone could be differentiated in a discrimination test. Moreover, these authors indicated that the difference between the mixtures and the fruity odorant alone was mainly due to perceived intensity rather than quality changes. In another study (Atanasova et al. 2004), the same authors reported that adding a peri-threshold concentration of whiskey lactone to a supra-threshold concentration of isoamyl acetate led to a perceptual synergy of the fruity odor. The present results replicated these observations with another panel of subjects in another laboratory and following another method. Consequently, the psychophysical results seem to rely on robust odor interactions rather than, for example, cultural conventions.

To the best of our knowledge, our results demonstrated for the first time neurophysiological correlates of such a synergy in odor mixture. Namely, there was an increase in N1 amplitude between the fruity single stimulus (F) and its binary mixture with a weak woody stimulus (Fw). Indeed, when synergy was observed at a psychophysical level, it was supported by a higher N1 amplitude, an ERP component that may be related to odor intensity (Pause 2002). Considering that the Fw mixture is perceived as mainly fruity and that psychophysical measurements of fruity intensity show a synergy effect, one may suggest that the increase of N1 amplitude for Fw mixture as compared with F alone reflected the perceptual synergy for fruity odor.

### Masking of fruity odor

The psychophysical results revealed a perceptual masking for the fruity odor in the mixture with a medium intensity of woody odor (FW). Indeed, psychophysical data showed

lower fruity odor intensity in this fruity–woody mixture. This masking effect was not reflected by ERP recordings. Indeed Livermore et al. (1992) reported that, in an olfactory/trigeminal mixture, the suppression of CO<sub>2</sub> by carvone was reflected in a suppression of ERP amplitudes. In our study, the decrease of N1 amplitude between the fruity single stimulus (F) and the mixture (FW) was not statistically significant. However, considering that the FW mixture contained medium intensities of both fruity and woody odors, the total number of molecules in FW stimulus is larger than in F single odor stimulus; if the peripheral events were simply additive, one could have expected an increase of N1 amplitude for FW mixture compared with F alone. On the contrary, a nonsignificant decrease for FW mixture as compared with F was observed. Moreover, considering that the FW mixture is perceived as equally fruity and woody and that psychophysical measurements of fruity intensity show a decrease of fruity odor quality in this mixture, it can be hypothesized that the absence of difference of N1 amplitude for FW mixture as compared with F alone reflects the perceptual masking of the fruity odor. In addition, a significant decrease of N1 latency was observed when adding a medium intensity of woody odor in the fruity one (F vs. FW). Our psychophysical observations are in accordance with previous orthonasal studies on these fruity–woody mixtures. Atanasova, Thomas-Danguin, Chabanet, et al. (2005) demonstrated a perceptual dominance of the woody odor in supra-threshold mixtures of whiskey lactone and isoamyl acetate which could account for the masking potential of the woody odor on the fruity one. Furthermore, in another study, these authors (Atanasova et al. 2004) evidenced that adding a supra-threshold concentration of whiskey lactone in a supra-threshold concentration of isoamyl acetate led to a perceptual masking of the fruity odor. Our results replicated these observations with a different panel in another country (Germany vs. France) underlying the robustness of these odor mixture interactions.

As a matter of fact, the neurophysiological observations related to N1 amplitude and latency partly supported the psychophysical observations of synergy and masking. If one considers that the early component of the ERP reflects exogenous influences, it might be that the synergy and masking effects are early ones and that they correspond to more peripheral physiological events.

#### **Differences between orthonasal and retronasal stimulation sites**

Our psychophysical data showed that the fruity odor alone (F) was perceived stronger when presented orthonasally than retronasally. However, in mixtures with a woody component (Fw and FW), this difference disappeared. Considering olfactory ERPs, this observation was supported by P2 amplitude and not reflected by N1 components. Indeed for the fruity odor alone, the P2 amplitude tended to be higher

for orthonasal stimulation. This difference disappeared in the mixture including a low level of woody odor (Fw), and it was the reverse in the mixture including a medium level of woody (FW): In the latter case, the amplitude of P2 became significantly larger when stimuli were presented retronasally. No difference of P2 latency between ortho- and retronasal stimulation sites was observed for the fruity component alone; however, adding woody odor at both low and medium level led to a relative prolongation of P2 latencies for retronasal stimuli.

P2 is known to be related to the attentional investment of the subject, but in addition, it is sensitive to the stimulus probability and task relevance (Pause 2002). Moreover, P2 amplitude is larger in response to infrequent stimuli, which has been interpreted in terms of a so-called context-updating model (Donchin and Coles 1988). They stated that P2 is thought to be elicited when an updating of a representation of the environment is called for. A large P2 component might be due to inherent emotional significance of odors (Pause et al. 1997). Furthermore, Lorig et al. (1996) found the P2 response to odors to be larger during exhalation than during inhalation. They attributed that effect to the evocation of surprise when odors are perceived during phases of exhalation. In our study, inhalation and exhalation phases varied between the odor stimulations, and these phases could be related to the sensation differences between orthonasal and retronasal stimulation sites. However, because subjects did breathe through the mouth (with the velopharyngeal closure) and had no external cue as to where the stimuli were delivered, odors could occur at any time in the breathing cycle, and this surprise cannot be related to breathing, but to retronasal presentation of the stimuli. Consequently, the increase of P2 amplitude to retronasal stimulation suggested that the retronasal perception of the woody odor was relatively more unusual (and thus more surprising) resulting in an increased response to the retronasal presentation of the medium level of woody odor in the fruity–woody mixture.

Olfactory ERP components latencies are an indicator of the speed of neuronal brain activity related to odor perception and also known to shorten in a concentration-related manner. Under specific experimental conditions, ERP latencies also exhibited a stronger relationship to changes in stimulus intensity compared with ERP amplitudes (Covington et al. 1999). In the present study, the fact that there was no difference of P2 latencies between ortho- and retronasal stimulation with the fruity single odor suggests that the neuronal activation speed was similar between stimuli presented at the different sites. Although this result does not seem to be in line with previous findings with 2-phenylethanol (PEA) and H<sub>2</sub>S presented ortho- and retronasally (Heilmann and Hummel 2004), P2 latencies increased in response to retronasal stimulation with increasing concentrations of woody odor added to the fruity odor. Thus, in line with the findings of Heilmann and Hummel (2004), it took longer time to process unusual odors, namely, when the retronasal stimulus



contained a woody odor component indicating a deeper processing of this unusual stimulus.

It has been shown that orthonasal and retronasal perception of odors is the result of the differential processing of this olfactory information (Small et al. 2005). This is confirmed by the present results. We especially demonstrated that a woody component in fruity–woody mixtures is not perceived in the same way when presented orthonasally and retronasally. These psychophysical observations are supported by electrophysiological findings where P2 amplitude and latency were modified in relation to ortho- and retronasal stimulus presentation. In the case of the presently used odors—only an example of odor mixture found in wine flavor (Aubry et al. 1999)—it seems that these differences between stimulation sites are related to the significance of the odor and to a food or nonfood dimension of the odor. Thus the fruity odor could be processed as a usual food odor when presented retronasally, whereas the woody odor, when presented through the same route, would cause surprise or at least be perceived as an unusual food odor. These considerations should be further examined in a study measuring pleasantness or familiarity of these stimuli when presented ortho- or retronasally. Moreover, it would be interesting to further investigate whether in mixtures including a food and a nonfood odor, synergy or masking are more likely to occur ortho- or retronasally, as suggested by our results.

## Conclusion

Our findings evidenced for the first time electrophysiological correlates of both synergy and masking on the early component of the ERPs. Moreover, the results confirmed that ortho- versus retronasal stimulation sites give rise to different neural processes as supported by differences in the late component of the ERP.

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