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Odor Interaction between Bourgeonal and Its Antagonist Undecanal

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Abstract

The perceived quality of a binary mixture will, as a rule of thumb, be dominated by the quality of the stronger unmixed component. On the other hand, there are mechanisms that, in theory, suggest that this will not always be true; one example being receptor antagonism. Undecanal has been indicated as an antagonist for bourgeonal-sensitive receptors in the human olfactory epithelium. Therefore, we investigated mixtures of isointense concentrations of bourgeonal and undecanal and, as a control, mixtures of isointense concentrations of bourgeonal and *n*-butanol. Both mixture types were investigated at 2 levels of concentration. The particular aim was to see if the bourgeonal–undecanal mixtures would exhibit asymmetric odor quality favoring the perception of the antagonist and the control mixture would not. For the control mixture, indeed odor quality tended to be dominated by the strongest component before mixing as would be suggested from previous studies. In line with the hypothesis, the bourgeonal–undecanal mixture was dominated by the antagonist's quality, but only when mixed at higher concentrations, altogether suggesting the effects of a low-affinity receptor antagonism. This is, to our knowledge, the first demonstration of how antagonistic interaction at the level of the receptor can affect the perception of odor mixtures in humans.

Key words: antagonist, odorant mixture, olfaction, perception, pleasantness, quality

Introduction

What determines how an odor mixture will be perceived? With regard to odor quality, binary odor mixtures seem to follow a few general rules of thumb. Several studies have shown that an olfactory stimulus that gradually changes from odorant A to odorant B over mixtures of A and B yields a corresponding change in perception of quality A to B (Laing et al. 1994; Olsson 1994; Cain et al. 1995; Laska and Grimm 2003). In other words, it is feasible to describe the quality of a binary mixture in terms of its component qualities. It also has been demonstrated that the components' relative perceptual intensities before mixing determine the quality of the mixture. More specifically, an odorant with higher perceived intensity before mixing will usually dominate the mixture perception over a weaker odorant (Laing et al. 1984; Olsson 1994, 1998). Sometimes, however rarely, perceptual asymmetries are found with mixtures in which components are perceptually isointense (Atanasova et al. 2005), that is, a mixture of 2 equally strong odors, before mixing, may lead to a mixture percept for which one component quality dominates over the other.

There are several potential mechanisms for odor interaction at the level of the receptor through which the affinity or efficacy of an odorant could be affected by the presence of another odorant. One way to accomplish this is through "antagonism", that is, one ligand is blocking the active site of an olfactory receptor and thus prevents interaction of another ligand, without inducing a cellular response (Duchamp-Viret et al. 2003; Araneda et al. 2004; Oka et al. 2004; Sanz et al. 2005; Jacquier et al. 2006; Rospars et al. 2008).

Spehr et al. (2003) identified a testicular odorant receptor (hOR17-4) mediating human sperm chemotaxis with bourgeonal as a strong agonist and undecanal acting as an antagonist. Spehr et al. (2004) found that this receptor is also expressed in the human olfactory mucosa and, moreover, that undecanal despite the likely combinatorial nature of olfactory coding also in this case has a strong inhibitory effect on bourgeonal also at the perceptual level. Spehr et al. reported that the perceived intensity of bourgeonal significantly decreased after presentation of undecanal and that the effect faded as the concentration of the antagonist was lowered. It was suggested that this effect was caused by competitive receptor inhibition.

The present study investigated the principles of odor integration of 2 types of binary mixtures: One was a mixture of bourgeonal and *n*-butanol. For this mixture, we expected symmetry in line with what seems to be a general principle for other binary odorants. For the other mixture type, bourgeonal and its antagonist undecanal, we hypothesized that the odor quality of a mixture of bourgeonal and undecanal will be asymmetrical such that the undecanal odor would dominate the mixture percept when isointense components have been mixed. The logic is that, after mixing, the antagonist undecanal will reduce the intensity of the bourgeonal quality more than the agonist bourgeonal will reduce the intensity of the undecanal quality. To the best of our knowledge, the literature provides no examples of perceptual asymmetry for an odor mixture that have been tied to known interaction at the level of the receptor.

Pilot experiment: psychophysics of single odorants

This experiment aimed to determine the psychophysical functions for bourgeonal, its antagonist undecanal, and the control odorant *n*-butanol in order to select isointense concentrations of each odorant as was necessary for the following experiment on mixture perception.

Method

Participants

Ten individuals participated in the pilot study, 5 women and 5 men, ranging between the ages of 21 and 31 years (mean [M] = 24.8, standard deviation [SD] = 3.3).

Stimuli

Three odorants were employed in the pilot experiment; *n*-butanol, undecanal, and bourgeonal. The odorants were diluted in propylene glycol (Merck; purity ≥99%) into 5 concentrations. The concentrations employed for *n*-butanol (Sigma-Aldrich; purity ≥99%) were 3.00%, 1.00%, 0.333%, 0.111%, and 0.037%, for undecanal (Sigma-Aldrich; purity ≥99%) 5.84%, 1.783%, 0.594%, 0.198%, and 0.066%, and for bourgeonal (Biomol International; purity ≥99%) 16.1%, 5.37%, 1.79%, 0.596%, and 0.199%. A 1% dilution of lemon essential oil in propylene glycol (Lozano) was employed as a standard with a predefined intensity value of 100. An additional bottle with only propylene glycol was used as a ‘‘blank.’’ All stimuli were presented in 250-mL polypropylene squeeze bottles with pop-up spouts.

Procedure

The participants rated the intensity of a given stimulus in comparison to the standard stimulus that was set to a value of 100. The stimulus was presented one at the time every 30 s, and the participants were asked to sniff each stimulus for 2 s. For every stimulus, the participant estimated the perceived intensity in relation to the standard.

Every concentration of each of the odorants *n*-butanol, bourgeonal, and undecanal as well as the blank was presented 5 times. All 16 stimuli were randomly assigned into sets. All were presented before the next set was initiated. The lemon standard was presented 10 times during the session at even intervals.

Results

The group means of rated intensities were calculated for each substance and concentration level. The psychophysical functions of bourgeonal, undecanal, and *n*-butanol are shown in Figure 1A,C. One lower and one higher isointense level of concentrations were chosen for each substance. These concentration levels matched the perceived intensities 100 and 137, respectively, on all 3 functions.

Main experiment: the perceived quality of mixtures

This experiment investigated the perception of quality and valence of 2 types of binary mixtures. Of particular interest was to see whether the antagonistic effect of undecanal on bourgeonal sensitivity following successive presentations of stimuli shown by Spehr et al. (2004) would also be present following ‘‘simultaneous’’ presentation (i.e., mixtures) of the agonist and antagonist. More specifically, we tested whether bourgeonal paired with its antagonist undecanal would yield a mixture percept dominated by the odor quality of the antagonist. As a control, we also investigated the perceptual symmetry of bourgeonal paired with the control odorant *n*-butanol that is a common reference substance in olfactory psychophysics.

For the 2 mixture types, we also studied the odor integration at 2 different levels of concentration. Earlier research on odor intensity of mixtures has indicated that odor integration is ‘‘level independent’’ (Berglund and Olsson 1993a, 1993b; Olsson 1994). That is, the additivity of weaker mixtures is comparable with that of stronger mixtures. If this principle is universal, mixing odorants at a weaker concentration for any particular ratio R_a/R_b should therefore yield the same quality as for stronger odorants with the same intensity ratio. Olsson (1994) tentatively concluded that this principle was indeed valid for the quality of 36 mixtures of pyridine and *n*-butanol.

Method

Participants

Two groups, each of 6 men and 6 women, participated in the experiment. The 24 participants were between 21 and 32 years of age (M = 24.4, SD = 2.4).

Stimuli

For the mixtures of bourgeonal and undecanal, we employed one weaker and one stronger mixture series of 7 stimuli, each

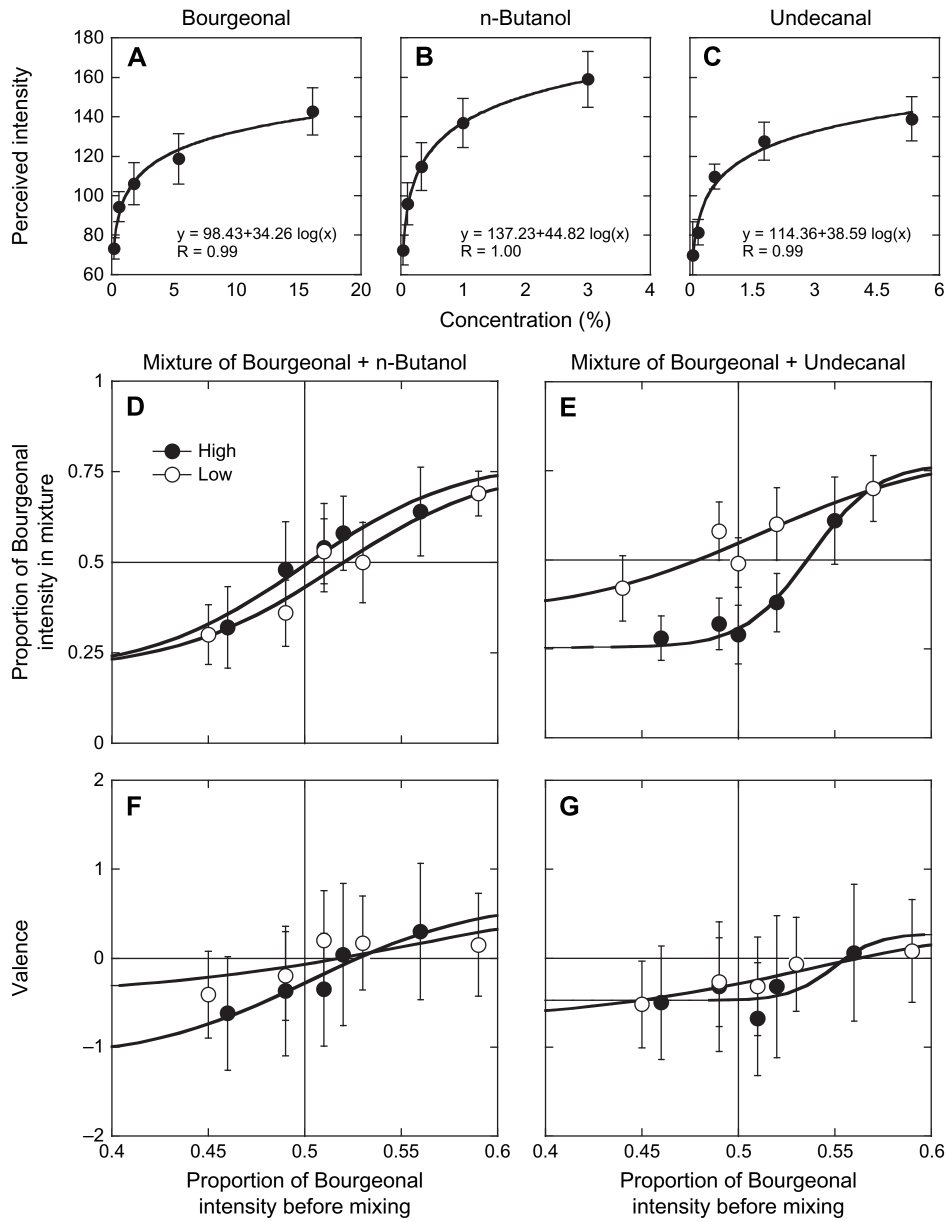

Figure 1 **(A–G)**. Psychophysical functions for group means (±standard error) of perceived intensity for bourgeonal **(A)**, *n*-butanol **(B)**, and undecanal **(C)** are shown. Odor intensity was rated in relation to a 1% lemon standard with the modulus of 100. Logarithmic functions were fitted to the data points. From these functions, we assessed the isointense concentrations used as pure endpoints of the mixture series in the main experiment. **(D)** The group means (±95% CI) of individual proportions of perceived intensity of bourgeonal in the mixture [$R'_{\text{bourgeonal}}/(R'_{\text{bourgeonal}}/R'_{n\text{-butanol}})$] are plotted against the group means of proportions of perceived intensity of bourgeonal before mixing [$R_{\text{bourgeonal}}/(R_{\text{bourgeonal}} + R_{n\text{-butanol}})$] for both the low and the high concentration mixture series. The logistic functions that are fitted also include the proportions of pure substances. These data points are not shown in the graphs for better resolution. **(E)** Same as in (**D**) but for the mixtures of bourgeonal and undecanal. **(F** and **G)** The group mean valence (±95% CI) of mixtures is plotted against the proportion of perceived intensity of bourgeonal. Fit of functions as in **(D)** and **(E)**.

ranging from bourgeonal to an isointense undecanal over a 50/50 mixture (see Table 1). This mixing by substitution has previously been shown to produce roughly isointense series of mixtures (Olsson and Cain 2000; Boyle et al. 2009). The particular concentrations for the unmixed substances for the high and low series were chosen from the functions in Figure 1A–C. The intensity of the concentrations was, as noted, set to 137 for the high series and 100 for the low series. The 6 concentrations of bourgeonal (Biomol International; purity ≥99%) employed with the high series were 13.3%, 10.7%, 8.00%, 6.67%, 5.33%, and 2.66% and of undecanal (Sigma-Aldrich; purity ≥99%) 3.88%, 3.10%, 2.33%, 1.94%, 1.55%, and 0.776%. For the low series, bourgeonal concentrations were 1.11%, 0.888%, 0.666%, 0.555%, 0.444%, and 0.222% and undecanal concentrations were 0.417%, 0.333%, 0.250%, 0.208%, 0.167%, and 0.083%. In

Table 1 Composition of the stimulus mixtures for hypothetical odorants A and B

	1	2	3	4	5	6	7
A (%)	100	80	60	50	40	20	0
B (%)	0	20	40	50	60	80	100

100% refers to concentrations of unmixed substances A and B. Stimulus 2, for example, consists of 80% of odorant A and 20% of odorant B in terms of liquid volume.

addition, a bottle containing 1% lemon essential oil (Lozano) in propylene glycol was used as a standard stimulus at a set value of 100, and a bottle containing only the diluent (see below) was used as an odorless blank. This amounts to 15 distinct test stimuli and a lemon standard. The stimuli were presented in 250-mL polypropylene squeeze bottles with pop-up spouts. Odorants were diluted in propylene glycol (Merck; purity ≥99%). In total, each bottle contained 6 mL of solution.

For mixtures of bourgeonal and *n*-butanol, we prepared 2 series of 7 stimuli, as above, each ranging from unmixed bourgeonal to unmixed *n*-butanol. For the low and high series, bourgeonal concentrations were identical to those in the mixtures with undecanal (above). The 6 *n*-butanol (Sigma-Aldrich; purity ≥99%) concentrations employed with the higher series were 0.972%, 0.778%, 0.583%, 0.486%, 0.389%, and 0.194% and with the low series 0.139%, 0.111%, 0.083%, 0.069%, 0.056%, and 0.028%.

Design and procedure

The 24 participants were divided into 2 groups, one group being tested with the bourgeonal/undecanal mixtures and the other group with the bourgeonal/*n*-butanol mixtures. Each of the 15 test stimuli was presented, in a random order, before any of them was presented again. As a consequence, a complete set of the test stimuli was presented before a new set started. Altogether, 5 sets were presented. Before the onset of each set, the lemon standard was presented. Then, participants were asked to identify an example of odors of A and B to check that they did not mix-up the labels of the 2 qualities. The order of the presentation of odor A and odor B was counterbalanced for each set.

When a stimulus bottle was presented, the participants were first asked to estimate the overall intensity of the odorant in relation to the lemon standard (which was set to 100) using the ratio scaling method of magnitude estimation (Baird et al. 1996). In all trials, including those with unmixed substances, participants were asked to estimate the percentage of the overall intensity that corresponded to components-specific odors. They were instructed that the percentages did not have to sum up to 100%. For example, if participants were presented with stimulus 2 in Table 1, they were first asked to rate the overall intensity in relation to the standard stimulus lemon (100), say 120. Next, they were asked to rate how much of the overall intensity was attributed to A and B, respectively. If they felt that neither A or B was especially prominent in the mixture, they could give percentages such as 40% and 30%, respectively. In addition, the participants were asked to rate the perceived valence of the stimuli with the help of a visual analog scale running from –4 to 4, with zero being neutral. The stimuli were presented 25–30 s apart depending on the time it took for the participants to answer all 3 questions. In each trial, they were allowed to smell the stimulus once (ca. 2 s) and to squeeze the bottle twice.

Results and discussion

For each individual and unique stimulus, the ratings of overall intensity (R), quality-specific intensity (R'), and pleasantness were averaged across the 5 presentations. This was done for the group presented with bourgeonal/*n*-butanol mixtures as well as for the group presented with the bourgeonal/undecanal mixtures.

We first set out to assess the symmetry of the components' contribution to mixture quality for the 2 mixture types. Here, we define perceptual symmetry in the odor mixtures as the case when a mixture of 2 isointense odorants A and B form a mixture percept in which both component qualities according to some measure are equally prominent (Olsson 1994; Cain et al. 1995; Atanasova et al. 2005). Therefore, we related the ratio of the perceived intensity of component-specific qualities in the mixture (R'), defined as $R'_a/(R'_a + R'_b)$ (where a and b represents any 2 odors), to the ratio of the perceived intensities of components before mixing (R), defined as $R_a/(R_a + R_b)$. (It should be noted that the perceived intensities of the components, R, were not directly assessed within this experiment but instead predicted from the psychophysical functions in Experiment 1; Figure 1A–C). These ratios are represented on the ordinate and abscissa, respectively, in the graphs of Figure 1D,E. From the functions, we can read how much stronger one component must be than the other component in order to generate a qualitatively symmetric or balanced mixture (i.e., the value on the x axis when the y axis is 0.5). Similarly, we can read how asymmetric the mixture quality is when we mix isointense components (i.e., the value of y when $x = 0.5$). To the extent that the functions pass through the coordinates 0.5 and 0.5 perceptual symmetry prevails.

Consider the quality of the control mixture of bourgeonal and *n*-butanol in Figure 1D. The results, functions and confidence intervals (CIs), indicate that there is a symmetrical relationship between the perceived intensity of unmixed components and the perceived intensity of component qualities in the mixture. Also, the functions for the weak and strong mixture series do not seem to differ significantly. This observation is in line with the assumption of level independency. Now, consider the same analysis for the mixtures of bourgeonal and undecanal in Figure 1E. Whereas the function for the weak mixture series does not seem to deviate significantly from the assumption of perceptual symmetry, according to the CIs, the function for the strong mixture series does. This latter observation indicates that to form a perceptually balanced mixture of the 2 odorants, we need to add a bourgeonal stimulus that is stronger than the undecanal stimulus into the mixture. Thus, the results for the stronger mixture series support the idea that undecanal would have an antagonistic effect on bourgeonal-sensitive receptors in the human olfactory epithelium.

Turning to the valence of odor mixtures (Figure 1F,G), we can see that valence follows quality quite well. The functions in Figure 1G indicate that valence, in line with quality (Figure 1E), shifts at different points on the x axis for high and low mixture series. The size of the CIs for the valence ratings, however, indicates that this difference is not statistically reliable in the case of valence.

The main results of this experiment indicate that although there is no consistent dominance of the quality of undecanal in the mixtures with bourgeonal across both high and low mixture series, it does take a significantly stronger bourgeonal than undecanal to form a balanced, or symmetric, quality at higher concentrations. This supports the hypothesis, as will be discussed below, that agonist/antagonist presentations can yield percepts dominated by that of the antagonist.

General discussion

In line with previous research (Laing et al. 1994; Olsson 1994; Cain et al. 1995; Laska and Grimm 2003), the results for the control mixtures of bourgeonal and *n*-butanol demonstrated that the best predictor of binary mixture quality is the relation between perceived intensities of components before mixing them. As components are mixed in different proportions, the qualities of mixtures exhibit a relatively sharp transition from being dominated by one component's quality to the other approximately at the point where components before mixing are isointense. This was also true for the perceived pleasantness of the odorants. For the control mixtures of bourgeonal and *n*-butanol, level independency prevailed: Odor quality as a function of relative component intensities was similar when we compared mixture series of high and low concentration.

The mixtures of bourgeonal and undecanal, on the other hand, were to some extent perceived differently. Whereas no asymmetry in the perception of mixtures of isointense components could be seen for the low concentration mixture series, undecanal quality dominated the mixtures of isointense odorants for the high series. Under the assumption that undecanal is an antagonist for bourgeonal-sensitive receptors, this outcome is plausible if undecanal in its role as an antagonist has a relatively low affinity and is more effective at higher concentrations (note that the high series has ca. 10 times higher liquid concentration). It should be noted that Spehr et al. (2004) observed a significant inhibition of bourgeonal sensitivity following preexposure to 100% and 10% of the antagonist undecanal but not significantly so following 1%. In the current study, we found antagonism for the stronger mixture series, comprising undecanal components between 0.776% and 3.88%. Thus, significant change of the perception of bourgeonal is observed following both simultaneous and successive presentations of undecanal in the same concentration range. In conclusion, the current study demonstrates, for the first time, how antagonistic interaction at the level of the receptor can affect the perception of odor mixtures in humans.

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