Leesversie proefschrift

Sensorische en Instrumentele Analyse van Voedselgeuren

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1 General introduction

According to popular belief, one of the world's best-kept secrets is the Coca-Cola recipe. In spite of the secrecy surrounding this recipe, detailed ingredient lists and preparation descriptions are available, allegedly derived from a retrieved notebook of J.S. Pemberton (the founder of the company) and statements by a Russian co-worker that defected the company (Pendergrast, 2000). Besides a number of non-aromatic ingredients like water, sugar, citric acid, caffeine, fluid-extracted alkaloids from cola leaves and carbon dioxide, the available recipes list a number of aromatic ingredients: Vanilla, lime juice, caramel and essential oils from orange, lemon, nutmeg, cinnamon, coriander, and neroli. Other sources also mention the addition of essential oils from lavender and cassia (Pendergrast, 2000). Each of these aromatic ingredients contributes tens up to hundreds of different volatile components to the drink, each of which is a potential odorant. Hence, a very complex mixture of odorants produces one of the worlds most known aromas, holistically perceived as cola.

To understand how an aroma percept is formed, it is likely to be important to know which chemical substances contribute to the aroma mixture, and how these individual substances are perceived (a decompositional approach). The present thesis deals with an analytical technique that combines the instrumental pre-treatment of food-born odorants with their subsequent chemical, analytical and sensory detection: gas chromatography olfactometry (GCO). This method unifies two scientific traditions in which very distinct methodological languages are spoken. The chemical analytical tradition excels in the nearly deterministic assessment of odorant quantities, only satisfied with instruments that show test-retest reliabilities approximating 100%. The perceptual psychological tradition, on the other hand, accepts probabilistic models of human behaviour as their daily practice, and is hardly surprised by the

fact that a human observer practically never generates identical responses to identical odorant concentrations.

In the eyes of a flavour chemist, GCO panellists are unreliable instruments. There is no better alternative available yet, so until that day flavour chemists will continue to use man as a method to study matter. However, psychological knowledge may help to improve the reliability of the human instrument. This is discussed in the first section of the thesis. In contrast, GCO experiments are interesting natural experiments in the eyes of a perceptual psychologist. Here, the perceptual system is subjected to a number of conditions similar to those known from scientific experiments on human perception. Much about perceptual mechanisms is known and, hence, much of the variability of GCO panellists' responses may be understood by the perceptual psychologist. However, the GCO practice has led to a number of assumptions regarding human perception that were not studied thus far. In that respect, for the perceptual psychologist, the experimental practices used in GCO provide an interesting approach to study man. This will be discussed in the second section of the thesis.

I - Man as a method to study matter

Gas chromatography

Chromatography is a method used to decompose complex mixtures of chemicals into their constituents. In essence, the method entails the forced transfer of chemical components along an adsorptive or dissolvent material, which usually is packed in a column or which constitutes the inner lining of a column. The affinity for the adsorbent differs over chemicals and their retention times on the column differ accordingly. Hence, chemicals that are forced through the column simultaneously will elude separately.

The principle of separating chemicals due to their differing affinities to an adsorbent dates back to the work of chemists like Schönbein and Goppelsröder (Beneke, 1999). In the 1860s they used filter paper to separate chemicals contained in liquids that were absorbed in the paper through the capillary effect. Later, it was the Russian botanist Mikhail Tswett who, driven by the motive to characterize and separate the chemicals that contributed to the colour of leaves, used various solvents to extract 'colour' from fresh and dried leaves (Tswett, 1906b; Tswett, 1906a). He then separated these pigments by having them adsorbed differentially to precipitated calcium carbonate, inulin or powdered sucrose. Subsequently, Tswett characterised these components chemically by measuring their spectral absorption patterns. In the words of Tswett, "the components of a pigment mixture separate on a column like the light rays of a spectrum – allowing for both a qualitative and a quantitative analysis". Hence, the separation process was coined the "chromatographic method" and the resulting quantitative absorption patterns of the mixture components a "chromatogram". This procedure – the extraction of the chemicals, their separation on an adsorbent material and their consecutive quantification and characterisation – is still at the core of modern-day chromatography. Tswett presented his work for the first time at a St. Petersburg convention in 1901 and presented it as the chromatographic method in 1906 (Tswett, 1906b; Tswett, 1906a). Tswett, who worked under unfavourable conditions with solvents like CS_2 (his favourite), petroleum ether, benzene, xylene, toluene, various alcohols, chloroform, acetone and acetaldehyde, died from a chronic throat inflammation in 1919 at the age of 47. Possibly due to the turbulent period that Russia went through in the early 20th century and its political and social isolation, Tswett's work went into oblivion until nearly three decades after its first presentation in 1901.

Although the technique of the adsorption of chemicals to solids by Tswett enabled the separation of chemicals dissolved in liquids in spatial terms, a revolutionary new conceptualisation of chromatography by Martin and Synge (Martin and Synge, 1941) allowed the

improved separation of a greater variety of dissolved chemicals in temporal-spatial terms. Their method of 'partitioning chromatography' employed a column containing counter-current liquids between which dissolved chemicals partitioned. Due to differences in partitioning behaviour, the chemicals separated spatially and could be captured at the end of the column, due to their separation in time. This fundamental improvement allowed for the thorough study of complex mixtures of chemicals. It won the authors a Nobel Prize in 1952. In the 1941 publication that introduced liquid-liquid chromatography, Martin and Synge discussed the possibilities of gas-liquid chromatography according to the same working principle of partitioning. Indeed, gas-liquid chromatography was realised in the early 1950s (James and Martin, 1952). Its principle - the separation of gaseous chemicals by forcing them through a capillary column lined with a liquid phase that retains the gaseous components at different rates - is still used to decompose mixtures of gaseous chemicals, including aromas. We will further refer to this technique as gas chromatography.

Gas chromatography olfactometry (GCO)

When odorous chemicals elude from a capillary column, their presence may be detected by instruments like flame ionisation detectors (FID) or by mass spectrometry (MS). Although this allows for a reliable quantification of the chemicals, the found quantities are poor estimates of the intensity of the odour sensations that these chemicals invoke. Due to large differences in detection thresholds between odorants, the capacity of chemicals to invoke odour sensations at a given concentration level varies strongly. Hence, relative quantities of the components in the mixture are poor indicators of their relative contributions to the mixture's aroma. A better estimate of each component's contribution to the aroma may be obtained by sensory evaluation of the separated constituents. Thus, by replacing the FID with a sufficiently large panel of subjects that sniff the effluents of the gas chromatograph in an effort to detect and characterize

the odour-active chemicals, a new method called gas chromatography olfactometry (GCO) was introduced (Fuller *et al.*, 1964; Dravnieks and O'Donnell, 1971).

Figure 1.1 shows a schematic overview of a GCO session. The physical detection and identification of volatile components by FID/MS is combined with the responses of panellists to perceived odours on one unifying time scale. Because human subjects are inconsistent responders – they do not always detect the same odorants under the same conditions – sessions need to be repeated between- or within-subjects to obtain acceptable reliabilities of detection scores. Therefore, responses over a number of identical GC sessions need to be aggregated before odour impacts may be assessed. The requirement of session repetition poses a threat to the reliability of odour impact assessment in lengthy GCO experiments. GC column characteristics may change due to repeated usage and variations in carrier gas pressure and in oven temperature may occur. As a consequence, retention times of odorants may vary, due to which timed responses to odorants by panellists may not co-occur in time. Because a number of GCO methods quantify the impact of odorants by employing the co-occurrence of responses, a low reliability of retention times will reduce odour impact measures. To prevent this, panellists'

Figure 1.1. Schematic representation of a GCO session. Volatile components (represented by coloured circles) are injected simultaneously at time T0 through an injector (Inj.) into a capillary which is gradually heated up in an oven. Being forced through the capillary, the volatile components separate spatially and are released separately in time (T1, T2, T3 etc.) from the capillary and simultaneously detected by FID (top graph) and a human subject at the sniffing port (SP). In this example, subjects pushed a button whenever an odour was perceived at the SP (bottom graph).
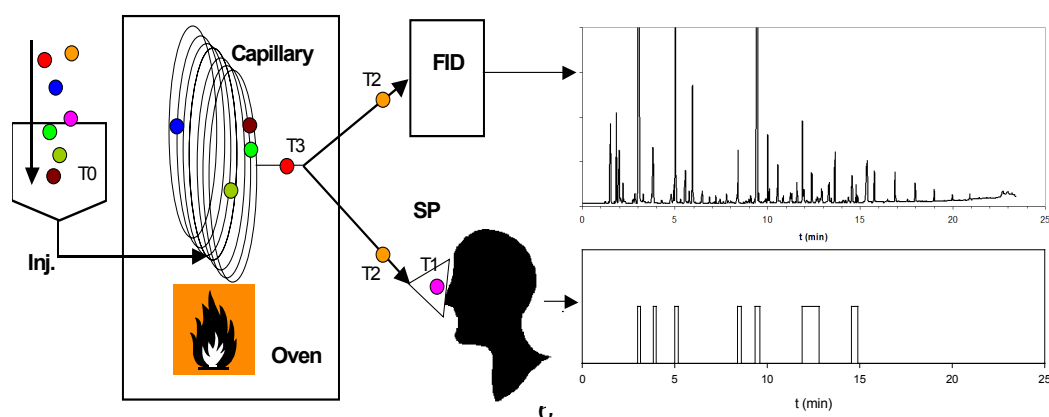
response times may be corrected according to the retention times of known components in the evaluated mixture. This may be done by linear interpolation of response times in relation to normalised elution times, which should raise the signal to noise ratio of combined panel responses. This issue will be discussed in chapter 2 of this thesis.

Assessing odour impact in GCO experiments

To quantify the sensory impact of the effluents, several sensory methods are in use. These methods fall into three categories:

1. *Flavour Dilution (FD)* methods use the number of times a sample needs to be diluted until it is detected by less than 50% of the panellists as a measure of odour impact. Examples of this approach are CHARM Analysis (Acree *et al.*, 1984) and Aroma Extract Dilution Analysis (Ullrich and Grosch, 1987).

2. *Detection Frequency (DF)* methods employ the number of coinciding panel detection responses to a stimulus as an indicator of its odour impact (Van Ruth and Roozen, 1994; Pollien *et al.*, 1997; Pollien *et al.*, 1997). The more panellists respond simultaneously to an odorant, the higher the estimated odour impact. This method is also referred to as Olfactory Global Analysis (Le Guen *et al.*, 2000; Grosch, 2001).

3. *Intensity rating* methods like the Osme method (Da Silva *et al.*, 1994; McDaniel *et al.*, 1990) use panellists' intensity ratings of undiluted GC effluents to assess their odour impact. The higher the intensity ratings the higher the odour impact.

The three methods generate highly comparable results when used to determine the main contributors to an aroma (Van Ruth and O'Connor, 2001; Le Guen *et al.*, 2000). However, a number of fundamental shortcomings have been noted regarding their use and the interpretation of their results.

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First of all, FD and DF methods falsely assume that the perceived odour impact relates linearly to odour threshold concentrations. In the case of FD methods, odour impact is conceptualised as the number of dilution steps needed for a panellist to reach detection threshold (the odour activity value or OAV). A common critique of this approach is that perceived intensity is not a linear function of the OAV (Frijters, 1978; Abbott *et al.*, 1993). Instead, odour intensity tends to approximate a power function of odorant concentration (Mitchell, 1971; Stevens, 1975) with threshold levels at a wide range of odorant concentration values. On their turn, detection frequency methods assume implicitly that detection thresholds differ between panellists. Panellists that respond to an odorant have thresholds below the presented concentration so that the number of responding panellists reflects the generally perceived intensities. Although detection probabilities may relate to odorant intensity/impact, the often Gaussian or even multimodal (more than one modus) sensitivity distributions in populations (Pollien *et al.*, 1997) allow, at best, ordinal models describing this relation.

Second, FD and intensity rating methods lack reliability estimates for odour detection. GCO sessions consist of vigilance tasks in which subjects are asked to detect and respond to unannounced signals. Often, the only indications for the presence of odours are the panellist reports, because very-low threshold concentrations rarely lead to FID responses. Therefore, the flavour chemist wonders whether he or she can reliably conclude that the panel perceived an odorant at a certain retention time or not. In this respect, it is generally assumed that FD and intensity rating measures are more informative than mere odour detections (DF), since the former consist of a variety of dilutions steps (FD) or responses on nearly continuous response scales (intensity ratings), whereas that latter (DF) provides dichotomous results. As a consequence, fewer subjects (and repetitions) are employed in dilution- and, especially, intensity studies compared to DF studies (Table 1.1). However, in spite of this general assumption, in the current

practice of FD and intensity rating procedures, the reliability of the answer to whether an odour was detected or not remains unsure.

First, odour impact assessment methodologies all share the problem that they need to define cut-off scores: How many panellists should respond simultaneously to ascertain that an odour was perceived? In FD methodology that number is 50% and in DF methodology it is the percentage of panellists that responded simultaneously in a blank session. However, from vigilance studies (Vickers *et al.*, 1977; Swets, 1977; Warm *et al.*, 1991) it is known that panellists adjust their decision criterion depending on the perceived stimulus probability: the number of responses will decrease when perceived stimulus probabilities decrease and the number will increase when perceived stimulus probabilities increase (Williges, 1969; Colquhoun and Baddeley, 1967). Hence, cut-off scores suffer from the occurrence of systematic errors.

In addition, cut-off scores are generally estimated on the basis of one average. This practice does not allow for the estimation of cut-off score reliability intervals. Therefore, cut-off scores are also subject to random errors of unknown proportions.

In the case of intensity ratings, higher-than-zero intensity ratings suggest odour detection. However, because false detections will also be accompanied by non-zero intensity ratings, the above question translates to: "At which intensity level can I reliably state that the panel perceived an odorant?" To resolve this, group intensity ratings may be compared between different odorants or against zero level to assess whether they differ significantly. Unfortunately, such tests have not been performed up to now and, therefore, any intensity rating above zero may be regarded as equally informative as a mere odour detection in answering the question of whether an odour was present or not.

Finally, compared to FD and DF methodology, intensity rating methodology adds extra task load to the basic detection process, viz. labelling the odour with an intensity label. In contrast

Table 1.1. Overview of recent GCO studies, showing the method used, the average number of subjects, and the average number of subjects x replicates per product.

Studies	Method [a]	# Subjects (SD)	# Subjects x reps (SD) [b]	Remarks
(Komthong *et al.*, 2006; Morales *et al.*, 2005; Avsar *et al.*, 2004; Selli *et al.*, 2004; López *et al.*, 2004; Mau *et al.*, 2003; Kim *et al.*, 2003; Zehentbauer and Reineccius, 2002; Fukami *et al.*, 2002)	FD	4.7 (2.8)	3.8 (1.3)	AEDA (6x) OAV (3x)
(Varlet et al., 2006; Arena et al., 2006; Jirovetz et al., 2005; Solina et al., 2005; Wu et al., 2005; Bult et al., 2004; Venkateshwarlu et al., 2004; Machiels et al., 2004; Van Ruth, 2004; Van Ruth et al., 2003; Bücking and Steinhart, 2002; Pennarun et al., 2002)	DF	8.9 (3.3)	10.9 (5.6)	Thresholds [c]: 3 of 10 (4x) 2 of 12 (1x) 5 of 10 (1x) unknown (6x)
(Kamadia et al., 2006; Varlet et al., 2006; Pérez-Silva et al., 2006; Gómez-Míguez et al., 2006; Gürbüz et al., 2006; Guillot et al., 2006; Campo et al., 2006; Solina et al., 2005; Warren et al., 2005; Avsar et al., 2004; Frank et al., 2004; Van Ruth, 2004; Garruti et al., 2003; Högnadóttir and Rouseff, 2003; Ferreira et al., 2003)	I	3.2 (1.3)	5.7 (3.7)	

[a] applied method for odour impact assessment: FD=Flavour Dilution, DF=Detection Frequency, I=Intensity rating; [b] number of subjects x number of replicates per product equals the total number of repetitions per product; [c] thresholds refer to the lowest number of coinciding panel scores that is assumed to signal a significant odour detection.

with FD and DF, extra task load may be detrimental for odour detection performance, which could make detection less reliable in intensity rating tasks.

Reliability estimates in DF methodology

Some users of the DF method have used arbitrary noise levels (Varlet *et al.*, 2006; Solina *et al.*, 2005; Le Guen *et al.*, 2000) without substantiating why the specific cut-off score would apply to their data. Others conducted stimulus-free sessions to determine the level at which coincidences in stimulus-present sessions should be interpreted as noise (Van Ruth and Roozen, 1994; Van Ruth *et al.*, 1996; Van Ruth *et al.*, 1995). In this approach, the highest response coincidence level encountered in stimulus-free sessions is considered the critical noise level for the stimulus-present sessions. As suggested above, the practice of using blank (no odour) sessions to estimate DF cut-off scores, may lead to decreased tendencies of panellists to respond at all. This suggests that the use of blank sessions in DF methodology will result in underestimated cut-off scores.

In 1997 Pollien et al. proposed an alternative to the use of blank sessions to assess the reliability of panel's DF scores. Their method employs detection frequency measures called nasal impact frequency (NIF), or a composite of detection frequency and the time during which it occurs (Surface NIF or SNIF). The method assesses reliability intervals of NIF or SNIF scores, which allows the assessment of the lowest NIF or SNIF that is significantly higher than 0. As such, the method estimates the minimal number of simultaneous responders that is required to conclude that subjects are indeed responding to an odorant. Although being a methodologically important advancement in GCO, it has some practical disadvantages. For instance, estimates of panel repeatability have to be made for each specific combination of odorants and panellists because each odorant gives rise to a separate reliability test and each panellist has different sensitivities to each odorant. Estimates of panel reliability may be made using jack knife

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techniques provided that panels are sufficiently large. To evade labour intensive reliability estimates, the authors proposed a heuristic based on empirical data, stating the minimum difference in NIF (30%) or SNIF (2000) that may be considered significant at a 5% confidence level. It is by this heuristic that some recent GCO-DF studies estimated the minimum detection frequency to be considered a significant odorant response (Table 1.1, DF remarks).

In the context of detection frequency methodology, it appears useful to have a reliability assessment technique that models panel responses in the (temporary) absence of stimuli and that does not rely on panel responses to each odorant. Such a model may serve as a reference to estimate the probability of the occurrence of simultaneous responses to any odorant. It can reduce panel work without making sacrifices to test reliability.

The first part of this thesis

In conclusion, in several ways the experimental validity of GCO-DF experiments may be improved. i) Before data analysis, panellists' responses may be corrected according to the retention times of known components in the evaluated mixture. ii) Ideally, the method used for estimation of the reliability of panel detections should not depend on the specific combination of odorants in an experiment; it should be based on the false alarm behaviour in the (temporal) absence of odorants. iii) In addition, false alarm behaviour of panellists should be modelled according to false alarm response distribution parameters rather than maximum response coincidences.

Studies that address these requirements are presented in chapter 2 (i) and chapters 3 and 4 (ii, iii).

II - Matter as a method to study man

Sensory research entails the study of sensory properties of food. Although the term 'sensory' allows some freedom of interpretation, the traditional term 'organoleptic analysis' narrows it down very clearly: analysis pertaining to the sensory properties of a particular food or chemical. As such, the food is in the focus of attention and the sensory panellist is considered an instrument to measure the sensory consequences of changing food recipes. Considering panellists as instruments often leads to the implicit assumption that panellists' reliabilities should be comparable to that of mechanical instruments. The major part of this thesis focuses on identifying fallacies in this implicit assumption.

In the psychological literature, a number of fields may be relevant in the context of sensory science. Possible biases in panellist behaviour may be found in studies of odorant mixture perception, studies of task context on stimulus evaluation, studies of the effects of memory and previous exposure on stimulus evaluation, and studies of sequential effects on rating behaviour. In this thesis, research questions were derived from knowledge obtained in these fields and these questions were tested empirically.

The perception of odorant mixtures and their components

The human capacity to identify components in mixtures of odorants is low (Laing and Francis, 1989). By presenting mixtures of 1-5 odorants from a collection of 7 distinctively smelling odorants, Laing and Francis found that subjects identified all three components in tertiary mixtures in only 14% of the cases. If no concurrent false identifications were allowed then this proportion even dropped below 0.07. For binary mixtures these proportions were 0.35 and 0.12, respectively. Furthermore, training or being an expert perfumer did not improve discriminative ability significantly (Livermore and Laing, 1996). In subsequent studies, Laing and co-workers ruled out a number of other possible explanations for this limited discriminative ability: olfactory adaptation (Laing and Glemarec, 1992), low qualitative distinctiveness of the odorants in the mixture (Livermore and Laing, 1998), odorant-perception-onset time (Laing and MacLeod, 1992) and focussing attention on certain components in the mixture (Laing and Glemarec, 1992).

Wilson and Stevenson (Wilson and Stevenson, 2003) presented a perceptual interpretation of earlier neuro-physiological findings, explaining the limited capacity of humans to identify odorants in mixtures. They suggest that in the processing of olfactory information odorant-specific activation patterns are not preserved beyond the level of peripheral encoding. Instead, mixtures of odorants are thought to generate holistic, mixture-specific neural activation patterns

that allow odour recognition and evaluation. In support of this hypothesis, Wilson (Wilson, 2000) showed that rats habituate to complex mixtures of odorants after few presentations although these mixtures were new to the rats on the first trial. Structural changes in the rat piriform cortex could be linked to the onset of this habituation process, indicating that rats had unique representations of the uniform mixture odour at the level of the first cortical projection after the olfactory bulb. Drugs acting on the same piriform cortex inhibited this cortical plasticity and forestalled habituation. In contrast, mitral cell activation patterns at the level of the olfactory bulb are not sensitive to previous exposure (Giraudet *et al.*, 2001).

Behavioural studies on humans further evaluated neural plasticity in the context of aroma learning: The mere exposure to an odorant in a context that renders meaning to the odour creates a conscious mental representation of the stimulating odour (Stevenson *et al.*, 2003; Stevenson and Boakes, 2003; Stevenson *et al.*, 2000), including verbal descriptions, elements of the context in which it was presented (accompanying tastes, odours, views or sounds), object category, and so on. These representations are continuously refined by experience. In this thesis, these mental stimulus representations will be referred to as stimulus concepts. Rather than the reductionistic, mechanistic thinking that odour perception is consciously constructed from physical elements, i.e. the odorants in a mixture of odorants, this thesis is adopts the view that aroma concepts are the basic conscious reference for odour recognition and evaluation. Although many people may know the smells of orange, lemon, coriander, caramel, nutmeg, cinnamon and vanilla, their first and probably only evaluation of the flavour of cola will be 'cola', although each of the mentioned smells contributes to it.

Effects of task context on stimulus evaluation

The psychological literature is full of studies in which tasks are completed under a variety of well-controlled task instructions, with the intention to study the dependency of task performance

on instruction. In a classic study by Festinger and Carlsmith (Festinger and Carlsmith, 1959), students had to perform very boring and meaningless tasks. After doing so for some time, they were asked to convince others to participate in the same experiment, an activity for which they were paid $1 or $20, depending on the experimental group they were assigned to. Although the students did not like the task, they had to invent arguments to present the task as being attractive. After task completion, the students rated how attractive they thought the task really was. It turned out that the students that were paid less to convince others rated the task as more attractive. The authors attributed this difference to humans' tendency to align beliefs and opinions with the justification at hand: if students are paid a lot, this justifies the boring nature of the task. If on the other hand monetary reward is low, the dissonance between experienced task fulfilment and reward is reduced by changing belief: "The task was actually not that bad at all, otherwise I had never accepted 1$ to convince others to do the same".

An impressive illustration of the effects of task instruction on food perception is provided by Frandsen et al. (Frandsen *et al.*, 2003). In their study, subtly differing milks had to be discriminated. In one condition, subjects were told the emotionally negative arousing story that some milks were produced by foreign competitors trying to take over the local market. This story was not told in the control condition. Results showed that milks with subtle flavour variations were discriminated better if they were accompanied with the negative emotionally arousing story. The authors aimed at maximising the subjects' use of implicit knowledge regarding the evaluated stimulus, part of which is expected to consist of emotional knowledge. Doing so optimises the use of knowledge that was learned during earlier experiences with similar stimuli. Many stimulus evaluation tasks may profit from the pre-activation of the proper stimulus knowledge. Likewise, this thesis will study the effects of presenting holistic stimulus information (product description rather than 'mixture of odorants') on the ability of humans to identify odorants from mixtures.

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Effects of memory and previous exposure on stimulus evaluation

If humans perceive, describe and recognise odorants by tapping from conceptual knowledge that was built from previous exposure, a logical consequence would be that current odour evaluations are influenced by the frequency and nature of previous exposures. There is ample evidence for such an influence in the literature. Previous exposure appears to increase odorants' perceived pleasantness (Distel *et al.*, 1999; Ayabe-Kanamura *et al.*, 1998; Distel *et al.*, 1997; Rabin and Cain, 1989) and improves the ability to discriminate these odorants from other odorants (Lesschaeve and Issanchou, 1996; Jehl *et al.*, 1995; Rabin and Cain, 1989; Rabin, 1988).

When odorants are perceived in the complete absence of cues indicating their provenance, humans show remarkably low recognition and naming abilities. Even for known and familiar aromas, performance may be as low as 50% correct identifications (Cain, 1979). However, if knowledge of odorants is available due to the proper activation of relevant stimulus concepts, the recognition, naming and discriminability of odorants improves drastically (Herz, 2003; Lesschaeve and Issanchou, 1996; Christie and Klein, 1995; Rabin and Cain, 1989; Rabin, 1988).

Sequential effects in odour perception

Besides the effect that exposure exerts over periods of days up to years, also short-term exposure effects occur. One such effect is that the presentation of odorants at intervals of seconds up to minutes may cause subsequent odours to appear less intense (Hulshoff Pol *et al.*, 1998; Cometto-Muñiz and Cain, 1995; Berglund and Engen, 1993; Evans and Starr, 1992; Stevens *et al.*, 1989; Berglund *et al.*, 1978; Berglund *et al.*, 1971; Cain, 1970). Such successive suppression of odour intensity is attributed to fatigue of receptors and sensory pathways due to previous stimulation by identical odours (self-adaptation) or different odours (cross-adaptation).

However, when adaptation is prevented by increasing inter-stimulus intervals or sniffing clean air, other sequential effects may still occur. When a non-uniform distribution of concentrations of a single stimulus quality is presented, this may induce subjects to expand their intensity rating range for frequent concentrations and to compress their rating range for infrequent stimulus intensities (Parducci, 1982; Riskey *et al.*, 1979). The resulting stimulus-response relations deviate from those that would have been obtained if equal numbers of stimuli were presented for each concentration level. In taste research, Kroeze found that, when taste qualities differ, the repeated presentation of one taste quality raises the observed intensity of a subsequently presented stimulus of a different quality (Kroeze, 1983).

Besides successive stimulus contrast effects, also response assimilation effects have been observed. This effect may be understood as the tendency to stick to the level of the previous response if the current stimulus has a similar concentration as the previous stimulus (Baird *et al.*, 1996). With auditory stimuli, a negative correlation of observed stimulus intensity with previous stimulus intensity and a positive correlation with the previous response was found (Ward, 1985; DeCarlo, 1994; Mori and Ward, 1990) just like for olfactory and taste stimuli (Gregson and Paddick, 1975; Ward, 1982; Jesteadt *et al.*, 1977). So, stimulus contrast and response assimilation processes generally occur in concert. At times, this may result in mutual compensation of both influences on observed taste intensity (Schifferstein and Frijters, 1992), sound intensity (DeCarlo and Cross, 1990; DeCarlo, 1992) and even for affective ratings (Schifferstein and Kuiper, 1997).

The second part of this thesis

Given the studies discussed above, many implicit assumptions regarding panel reliability in GCO studies do not seem to hold. Most panellists have never experienced single odorants from mixtures that constitute food aromas. Nonetheless, it is generally assumed that the qualitative

description of these singular odorants have predictive value for their contribution to the mixture aroma (Lorrain *et al.*, 2006; Pennarun *et al.*, 2002; Czerny *et al.*, 1999; Wagner and Grosch, 1998; Guth, 1997b; Guth, 1997a; Hofmann and Schieberle, 1995; Guth and Grosch, 1994; Blank *et al.*, 1992). In chapter 5 of this thesis, a close investigation of the validity of this assumption is performed using an apple aroma model.

The Frandsen study showed the possibility to improve discrimination performance by changing task instruction alone. Analogously, with respect to the task to identify single odorants in mixtures, the adopted stimulus-concept framework suggests that performance on the identification task should improve if it were presented as a task to identify the aspects of a known aroma, rather than to identify odorants within a mixture. In doing so, stimulus concepts of the complex aromas are activated in such a way that singular odorants may stand out as subtle variations in the holistic aroma percept. An empirical study that puts this hypothesis to a test is presented in chapter 6.

Besides many supra-threshold odorants, food aromas consist of a large number of sub-threshold, i.e. not perceivable, volatile components. One may wonder if changes to aroma mixtures as subtle as the addition of these sub- or peri-threshold volatile components could make a noticeable difference to the food aroma, provided that the aroma is well-known and the appropriate stimulus concept is activated. This research question was subjected to a test in chapter 7 of this thesis.

Finally, the detection of odorants at a sniffing port constitutes a task in which stimuli have to be evaluated sequentially. Whereas well-controlled studies generally employ fixed intervals in between consecutive stimuli, GCO experiments are notorious for variable stimulus intervals. Furthermore, the unique relation between odorant composition and odorant release time implies that the sequence of odorants that are released from a specific column is fixed. Also, food aromas consist of chemically related and perceptually similar odorants. Therefore, cross-adaptation

processes and sequential context effects may influence GCO results. The specific combination of variable intervals and chemically similar odorants poses a question that has not been studied so far: what are the effects of chemical and perceptual similarity in combination with variable stimulus intervals on the perceived intensities of odorants. This question resulted in a study that aims at estimating the unbiased "true" odorant score from intensity-interval functions for mutually dissimilar, similar and identical odorants. This allows the study of sequential effects in terms of diminution and enhancement rather than negative or positive autocorrelations. In addition, the focus on time dependencies may allow for the identification of different processes involved if these processes have different decay rates. This study will be presented in chapter 8.

Finally, the relevance of the presented results for the practice of GCO and for perceptual psychology will be discussed in chapter 9.

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2 Retention time indexing of coincident panel responses improves the sensitivity of gas chromatography olfactometry

Abstract

In gas chromatography olfactometry, panellists sniff the odorous effluents of a gas chromatograph and respond whenever they perceive an odorant. Their responses are used to derive measures of odour impact: the relative contribution of a volatile substance to a food aroma. Usually, multiple sessions are conducted, and panellists' responses need to be aggregated over sessions. However, due to small fluctuations in column gas flow, temperature program, and column properties, odorant retention times may vary over repeated sessions, which decreases the coincidence of panellist responses. In this study, response events are corrected for retention time variations by linear interpolation between odorant retention times. The effect of the method is illustrated by an increased statistical sensitivity of detection frequency scores when using queuing system theory testing. The interpolation method is especially advantageous in longitudinal studies with gas chromatograph columns that age relatively rapidly.

Introduction

What defines a food's aroma? This question has kept many flavour scientists and food chemists occupied during the past few decades. It is known that aromatic foods release mixtures of many different volatile chemicals, an unknown number of which contribute to the aroma. Gas chromatography (GC) has enabled the physical separation of these mixtures and the subsequent quantification of constituents. Linking the GC with mass spectrometry (MS) allowed the identification of constituents, often providing us with baffling long lists of chemicals (Maarse *et al.*, 1989). So, which of the chemicals from these lists contribute to an aroma? To answer this question, some decided to sniff the GC effluents to assess those chemicals that actually produce odours (Fuller *et al.*, 1964). The method of GC sniffing soon became standardised and optimised for assessor-friendliness (Dravnieks and O'Donnell, 1971). Since then, this technique has become the prevalent method for assessing the relevance of volatile chemicals for food aromas. It is commonly referred to as gas chromatography olfactometry (GCO).

The perceived intensities, or odour impacts in GCO terminology, of volatile chemicals in GCO sessions vary to a large extent, due to the large variability of chemicals' detection thresholds and concentration levels. A number of methods is in use for odour impact assessment (Grosch, 2001; Le Guen *et al.*, 2000; Van Ruth, 2001), utilising measures like intensity ratings, the number of dilution steps above odour threshold and the number of simultaneously responding assessors to each odorant. In spite of their very different approaches to quantify odour impact, these methods generate comparable odour impact distributions for identical mixtures of volatiles (Van Ruth and O'Connor, 2001; Le Guen *et al.*, 2000).

Because in GCO responses are aggregated over different GCO sessions, identical odorants have to be released at exactly the same retention times in different sessions. Therefore, the instrumental conditions at which each session is held have to be identical. This accuracy may be

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threatened by factors like differences in the column gas flow, the temperature program and the column properties. Although such variations may be as small as 6-10 seconds, if panellists' responses last shorter than 6-10 seconds they may fail to coincide. Figure 2.1 illustrates this for three GCO sessions with headspace samples of caramelised sugars (indices refer to identical components for each of the three samples). Although identical columns, temperature programs, carrier gas pressures and amounts of injected sample were employed, retention times in session B are shorter. After approximately 2 minutes, retention times of session B start to advance and retention times are advanced approximately 6 seconds around 4 minutes. Therefore, component 1 has identical retention times for al three sessions whereas component 7 is released approximately 6 s earlier in session B than in sessions A and C. This resulted in identical retention times of component 6 in sessions A and C and component 7 in session B. As a consequence, responses to component 7 in session B may fail to coincide with responses to component 7 in session A and C. In addition, responses to component 6 in sessions A and C may coincide with responses to component 7 in session B.

To compare results from GCO sessions with different instrumental settings, e.g. different temperature programs or different gas pressures, Acree and co-workers (Acree *et al.*, 1984) proposed the use of retention *indexes* rather than retention *times* to measure response events. Acree and co-workers analysed *n*-paraffin standards in separate sessions using the same instrumental conditions as used for the aroma samples. The retention times of these standards were used to construct a stable scale for the interpolation of response event times. This method yields retention indexes for response events that are stable across different instrumental conditions. It can be used whenever instrumental settings are changed during a series of GCO sessions.

Although the use of external standards (*n*-paraffins or any other set of components) may correct systematic changes in response times due to systematic changes in retention times, the method does not correct the effects of non-systematic retention time variations. Unintended variations of gas flow- and temperature or occasional column overloading with sample solvents

Figure 2.1. Three chromatograms obtained from three consecutive GCO evaluations (A,B,C) of caramelised sugar aromas.
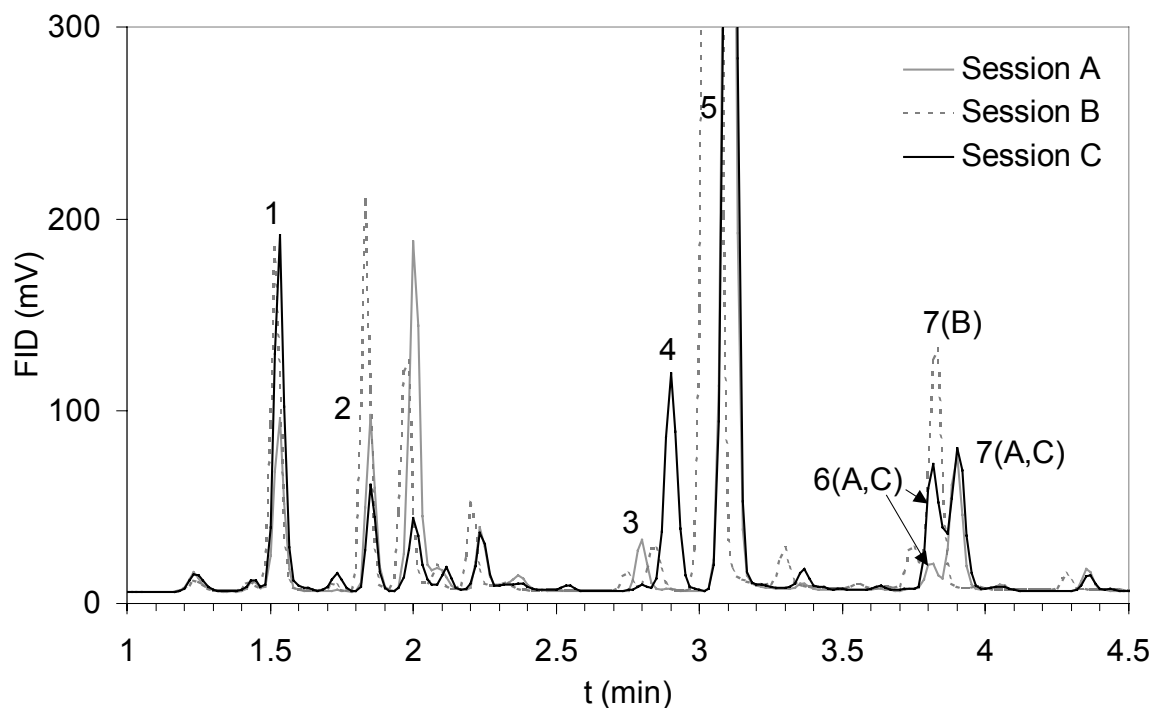
may occur during some GCO sessions. Furthermore, if an experiment involves many repetitions of aroma sample analyses under the same instrumental conditions, possible effects of column ageing on retention times cannot be accounted for. To solve this problem, we propose to use internal standards in all aroma samples. Since it is irrelevant which standards are used as long as they cover the retention-time range of the odour-active components, components already present in the sample may serve as well. This has two advantages. First, because the same components are both odorant and standard, peak retention indices are not interpolated but measured directly. Second, no extra standards have to be included in the sample. As a consequence, subjects cannot be influenced by any components apart from the aroma constituents.

In the present study, we separated a mixture of components derived from an apple aroma on the commonly used DB1 and Supelcowax 10 capillary columns. Different stationary phase polymers are known to suffer differentially from ageing - that is degradation of the polymer through oxidation or bleeding at high temperatures (Heyden *et al.*, 1996). Low-bleed DB1 columns tolerate temperatures up to 350°C whereas the Carbowax 20M polymer is guaranteed for usage up to 280°C. Because identical temperature programs with maximum GC temperatures of 272°C were employed for both columns, it is expected that the Supelcowax 10 column will suffer more from ageing than the DB1 column. Therefore, retention times are expected to vary more over repeated analyses on Supelcowax 10 than on the DB1 column. This would offer an opportunity to compare, between columns, the effects of retention time shifts on coincidence scores and the contributions of retention indices to the improvement of coincidence scores. Hence, response coincidences will be calculated for unadjusted and index-corrected response times. The resulting GC-olfactograms are compared for both 'time' conditions and for each of the two columns.

Materials and Methods

Assessors

Nineteen paid volunteers from the local community participated in the experiment, six were male and thirteen were female. Ages ranged from 20 to 53 years (average 27.6 years). Twelve were experienced assessors who had participated in olfactory attribute rating experiments, discrimination tasks, and GCO experiments over the course of two years. Seven new assessors were selected and familiarised with the GCO method during a 45-minute sniffing session with the same stimulus material as was used in the main study. All were non-smokers and had no history of olfactory dysfunction. Assessors were in good health and gave written informed consent.

Sample preparation

Nine odour-active compounds that are key contributors to a natural apple aroma (see chapters 4, 7 and 8) were dissolved in n-pentane at 4°C at concentrations listed in Table 2.1. The mixture was subsequently stored at 4°C. Immediately before the start of a sensory experiment, the solution was taken from storage and 0.075 μL was transferred to a glass tube containing Tenax (Tenax TA, 35/60 mesh; Alltech Nederland, Zwijndrecht, the Netherlands), a granulated absorbent material. If evaluated individually, these compounds would produce a wide range of odour intensities as can be inferred from the ratio of odorant masses to the corresponding odour thresholds (Table 2.1).

Instrumental analysis

The compounds were thermally desorbed from Tenax at 260°C for 300 s and cryofocused at -120°C by a cold trap/thermal desorption device (Carlo Erba TDAS 5000; Interscience, Breda, the Netherlands). During a period of 7 weeks, identically prepared samples of compounds were

alternately separated on either a DB1 capillary column (J&W Scientific, 60 m × 250 μm i.d, 0.25 μm film thickness, Tmax = 350°C) or a Supelcowax 10 capillary column (Supelco, 60 m × 250 μm i.d, 0.25 μm film thickness, Tmax = 280°C). These two columns were exchanged every four or five days in a Carlo Erba MEGA 5300 gas chromatograph (Interscience b.v., Breda, The Netherlands).

Table 2.1. Odorant quantities, their respective sniffing port masses, reported threshold concentrations in air and water of these odorants and their retention times.

Component name	In 10-ml n-pentane stock solution (mg)	Mass at sniffing outlet[f] (ng)	Reported detection threshold concentration in air (ng/L)	Retention time [rank] DB1[a] (min ± SD)	Retention time [rank] Supelco-wax[a] (min ± SD)	Descriptors used in the study [Dutch terms used]
diacetyl	0.25	0.74	5.0[b]	7.29 ± 0.05 [1]	13.63 ± 0.35 [2]	Butter-sweet [boter zoet]
propyl acetate	4.44	13.32	200-7000[c]	11.31 ± 0.04 [2]	13.46 ± 0.34 [1]	Fruity-acetone [fruitig aceton]
isobutyl acetate	8.68	26.04	1.7-17[c]	14.26 ± 0.06 [3]	15.28 ± 0.41 [3]	Sweet-lacquer [zoet lak]
hexanal	25.02	75.06	30-53[d]	15.37 ± 0.11 [4]	19.13 ± 0.63 [6]	Macaroon-hedge [bitterkoekjes heg]
butyl acetate	39.69	119.07	30-180[c]	16.61 ± 0.12 [5]	18.51 ± 0.58 [5]	Nail polish [nagellak]
trans-2-hexenal	21.15	63.45	340[c]	18.59 ± 0.33 [6]	25.34 ± 0.68 [8]	Bittersweet rum [bitterzoet rum]
ethyl 2-methyl butanoate (E2MB)	0.22	0.65	0.1-0.3[c]	19.03 ± 0.18 [7]	17.27 ± 0.53 [4]	Fruity sweet [fruitig zoet]
2-methyl-1-butyl acetate	35.04	105.12	90-200[c]	20.76 ± 0.05 [8]	21.26 ± 0.67 [7]	Sour hard-boiled candy glue [zuurtjes lijm]
hexyl acetate	43.50	130.50	2.3[c]	26.34 ± 0.36 [9]	27.04 ± 0.67 [9]	Pear apple [peer appel]

For both columns, the oven temperature was initially kept at 40°C (4 min), then increased to 75°C (3.0°C/min) and subsequently to 80°C (1.0°C/min). After a final increase to 272°C (15°C/min), oven temperature was kept at this temperature for another 5 min. This program allowed for an optimal separation of retention times.

Column effluents were split at the end of the capillary column. The ducts from splitter to sniffing outlets were kept at oven temperature to prevent condensation of volatiles. Of the total flow, 20% was directed to a Flame Ionisation Detector (FID) while the two sniffing outlets each received 40%. Column effluents were mixed with humidified nitrogen at the sniffing outlet.

Sensory evaluation

Each assessor evaluated column effluents in at least three DB1 sessions and at least three Supelcowax sessions. During an experimental session, one or two assessors were seated with their nose positioned at the sniffing outlet connected to the GC. In total, the nineteen assessors completed 76 sessions during 42 runs on DB1 and 91 sessions during 56 runs on Supelcowax. To ensure that the solvent peak would not be inhaled, sniffing started 6 minutes after the initiation of the GC procedure. Sniffing analyses finished after 36 minutes. Hence, all assessors completed sniffing sessions of 30 minutes. Assessors were instructed to inhale slowly through their nose at an even pace and to exhale at a higher pace. In this way, the dilution of sniffed odorants with the surrounding air was minimised while the net observation time was maximised. On observing an odour, assessors immediately stroke one key on the keyboard of a laptop (Acer Extensa 501T, Pentium II) placed in front of them. They had to strike that key again when the odour could not be perceived anymore. Immediately after this response, a set of odour descriptors was presented from which assessors had to select the one that best described the odorant that was responded to. If none of the

descriptors applied, the category "other" could be selected. These descriptors were

derived from the smell of the used odorants (see chapters 5 and 8) and assessors had

been trained on using these descriptors by sniffing aqueous solutions of the pure

components. Response times were registered electronically and synchronously with

FID registrations. Room temperature was kept at 21°C. The air inside the room was

ventilated and filtered.

Data treatment: retention time indexing of response times

For each column condition, one session was selected as the reference session. For this

session, the onset times of its components showed the least summed deviation from

the averaged onset times over all sessions. Stimulus onset times of all other sessions

Figure 2.2 Example of indexing sniffing response times. Onset odorant retention times in session A are delayed with respect to the reference session for peaks 6, 7 and 9 to the extent that corresponding panel responses fail to coincide (raw coincident responses graph). After indexing response times according to the algorithm described in the data treatment section, responses coincide for all released odorants (indexed coincident responses graph).
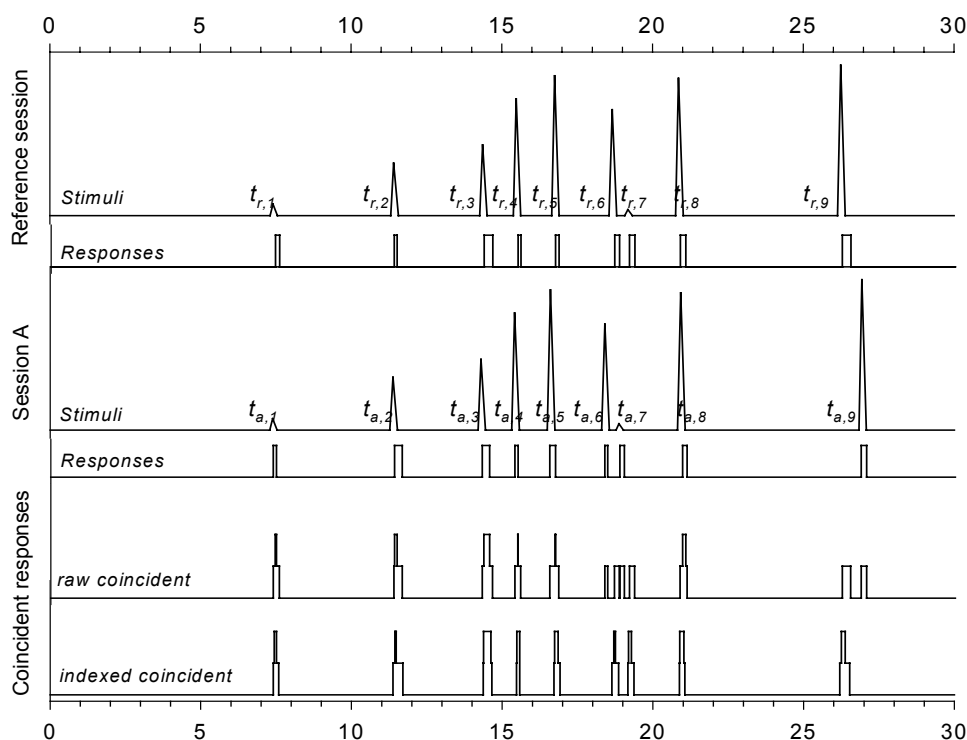
in each column condition were then indexed by assigning them the corresponding onset times in the reference session, i.e. $t_{a,i} = t_{r,i}$ with $t_{a,i}$ and $t_{r,i}$ being the onset times of component number i in session a and in the reference session, respectively (see Figure 2.2). Assessor response actions that took place in between two subsequent stimulus onsets were then indexed by linear interpolation. Formally, response onset times (t_{onset}) in between the onset times of stimuli i-1 and i in session a ($t_{a,i-1}$ and $t_{a,i}$) are assigned the indexed onset times

$$norm(t_{onset}) = t_{r,i-1} + \frac{(t_{onset} - t_{a,i-1})}{(t_{a,i} - t_{a,i-1})} \cdot (t_{r,i} - t_{r,i-1}) \tag{1}$$

with $t_{r,i-1}$ and $t_{r,i}$ being the stimulus onset times in the reference session corresponding with $t_{a,i-1}$ and $t_{a,i}$ in session a, respectively. For response events that took place before the first stimulus onset, $t_{a,i-1}$ and $t_{r,i-1}$ do not exist and were set to zero. For events that took place after the last stimulus onset, we assumed that $norm(t_{onset}) = t_{r,i} + t_{onset} - t_{a,i}$ with $t_{r,i}$ and $t_{a,i}$ being the onset times of the last stimulus in the reference session and session a, respectively.

Data treatment: significance of sniffing peaks

In the detection frequency (DF) method (Van Ruth and Roozen, 1994; Van Ruth *et al.*, 1995), the number of coincident assessor responses is not only used as a measure of odour intensity but also as an indicator for the probability that an odorous component was present at all. Higher detection frequencies reflect a higher probability that assessors responded to an actual odorant. In the context of the DF method, the highest number of coinciding responses during an odorant-free, i.e. blank session was used as the threshold value for determining whether a component was smelled or not (Van Ruth and Roozen, 1994). However, this approach has several major drawbacks (see chapter 3). First, assessors tend to adjust their willingness to respond according to

the stimulus incidence that they perceive. Therefore, responses during odorant-free sessions do not accurately represent responses that may be given during odorant-present sessions. Second, critical detection frequencies are based on incidental-, rather than global observations: One extreme case of many coinciding responses may determine the overall noise level. Third, the probability of finding a high number of coinciding detections in blank sessions increases as session length increases. Therefore, session length affects the critical coincidence levels for odorants, which is undesirable. Fourth, more confident responses to odorants are expected to last longer. A measure of coincidence level significance should, therefore, also depend on score lengths. To eliminate these drawbacks, an alternative method, based on queuing system theory, was proposed to test the significance of coincident sniffing responses (see chapters 3 and 4). In short, this method uses the probability distribution of the lengths and heights of separate sniffing peaks for the case that no odorants were presented to assess the probabilities of these measures observed in stimulus-present sniffing sessions. In the present study, an automated version of this method was used. Since sniffing peak significances will not only depend on the observed coincidence level but also on the observed duration of that level within the peak, sniffing peaks may be significant at different coincidence levels. Whereas one peak may be significant at a given coincidence level, other peaks may not.

Results and Discussion

Raw and indexed response event times were used to produce coincident response graphs (aromagrams). Together with the FID chromatograms of the respective reference sessions, aromagrams are shown for DB1 data in Figure 2.3 and for Supelcowax data in Figure 2.4.

Most sniffing peaks have an onset time that is delayed with respect to the onset time of the respective odorants. Short delays may be expected because assessors can

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only perceive an odorant once its concentration has exceeded the threshold concentration. Furthermore, an extra delay is expected due to the transfer time needed to transport odorants from the sniffing port to the olfactory cleft in the nose and, subsequently, through the mucosa layer to reach the olfactory receptor cells in the olfactory epithelium. In addition, assessors may take extra time to consider whether they will respond. Although this delay may raise doubt as to which odorant was responded to, the attributes selected after detection responses give additional information that may resolve this identification problem. Although descriptor analysis is not the central interest of this article, we will use it occasionally to clarify DF results.

DB1 results

QST parameters for the raw and the indexed sniffing data were assessed for the stimulus-free windows in all stimulus present sessions (see chapter 3). The estimates for λ and μ were 0.107 and 3.46, respectively, for raw response times and 0.104 and 3.54, respectively, for indexed response times. For both raw and indexed aromagrams, QST testing revealed significant peaks for all components except propyl acetate (2). Because this component was not released in the vicinity of other odorants on DB1, it can safely be concluded that propyl acetate was of sub-threshold concentration.

Even in the light of the expected delay of panel responses after odorant onset, responses following E2MB (7) and hexyl acetate (9) were considerably more delayed than responses to other odorants. Because *trans*-2-hexenal partly blended with E2MB on release, assessors may have responded to the latter only once its odour quality could be distinguished from the odour quality of earlier released *trans*-2-hexenal. This would explain the delayed responses to E2MB. Responses to hexyl acetate are not only delayed to a large extent – the sniffing peak started approximately 1 minute after initiation of the component's release at 25:40 minutes in the standard FID session –

but sniffing response times for this component also varied to a large extent. As a consequence, the corresponding sniffing peak has a reduced height and a long duration from approximately 27:00 to 30:00 minutes. The large delay of these responses could either be due to an additional condensation of the odorant in the duct that leads from the oven to the sniffing outlet or to lingering of the odorant at the outlet. Inspection of the qualitative descriptors learns that responses were most often associated with the descriptor of hexyl acetate (pear apple: 11 times) and second most often with none of the given descriptors (7 times). The other responses (30 in total) were associated with the eight remaining descriptors. This result may partly reflect the masking effect of odorants produced by the apparatus at high oven temperatures (approximately 173°C at the moment of release of hexyl acetate). In an earlier study, employing a regular panel of 16 assessors and using the same instrumental configuration (see chapter 4) assessors failed to detect hexyl acetate under similar analytical conditions. Hence, the exceptionally large panel size in the present study allows us to conclude that not only masking effects, but also lingering/condensation at the outlet may have contributed to this result. Because retention time indexing relies on the observable variation of component retention times, response variations due to condensation and lingering in the SP duct cannot be corrected.

Added value of retention time indexing for DB1 results

Although retention time standard deviations occasionally reached approximately 20 s on DB1 (Table 2.1), the aromagrams from DB1 sessions suggest that the indexing of raw data did not substantially improve sniffing peak separation: Between raw- and indexed aromagrams, individual sniffing peak heights were approximately equal. However, the coincidence levels from which sniffing peaks were significant at $\alpha = 0.01$ were lower for peaks 3, 4 and 6 in the indexed aromagram than in the raw aromagram. Furthermore, a slight improvement may be noted with respect to the

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separation of responses to ethyl 2-methyl-butanoate (E2MB, component 7 in the FID chromatogram) and *trans*-2-hexenal (6). In the raw aromagrams, responses to E2MB appear to have blended with responses to *trans*-2-hexenal. In the indexed aromagram, however, responses to these two components are separated better, which can be concluded from the additional peak in the tail of the major sniffing peak for *trans*-2-hexenal (6) in the indexed aromagram between 20:10 and 20:29 minutes.

Figure 2.3. Gas chromatogram of the nine apple-model compounds in n-pentane separated on a DB1 capillary column (bottom). Indices correspond with compound indices in column 5 of Table 2.1. The corresponding coincident responses to these components are given for the raw aromagram (middle) and the indexed aromagram (bottom). Significant coincident scores are coloured light-grey (confidence level = 95%) and dark-grey (confidence level = 99%) above the lowest significant coincidence level of each sniffing peak.
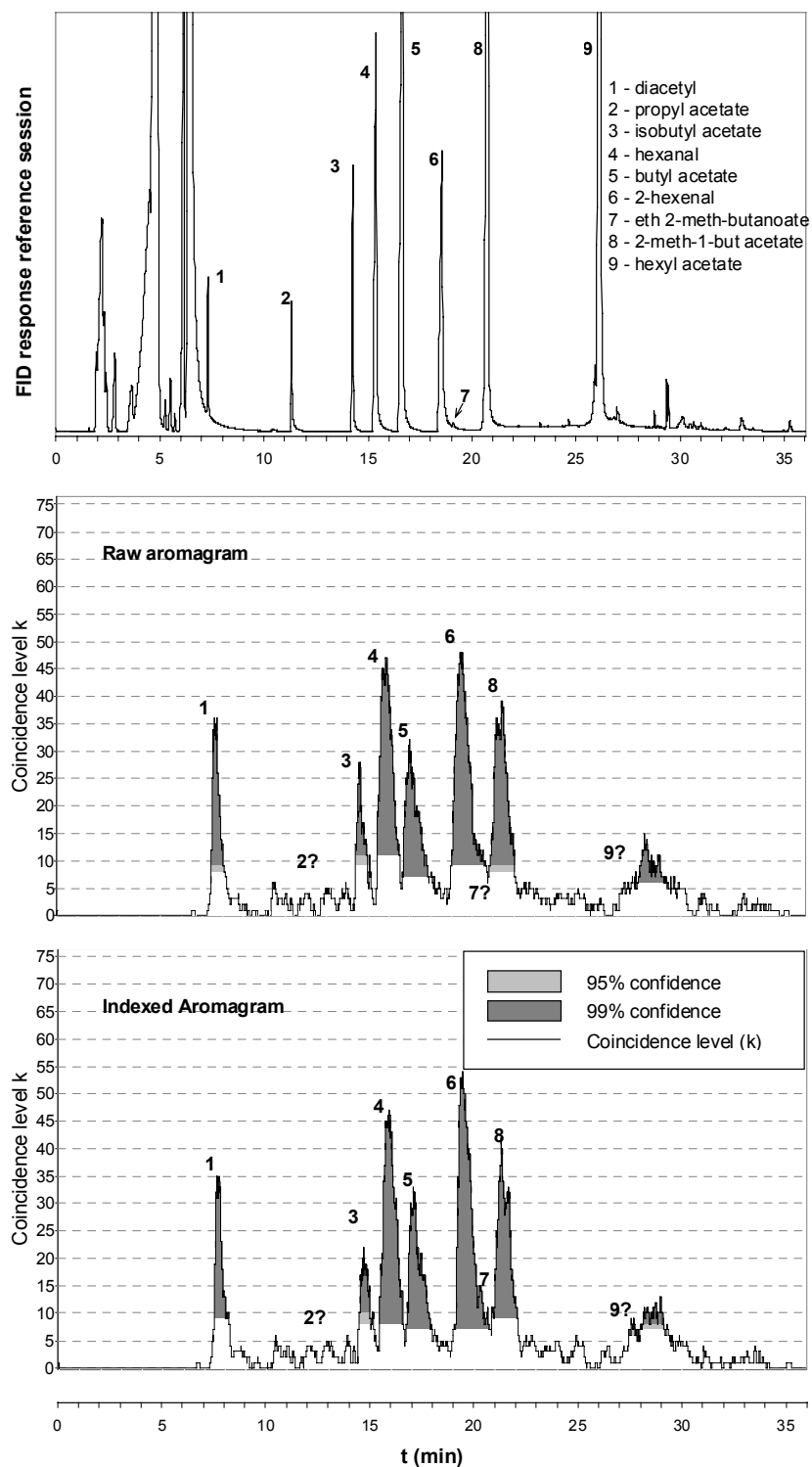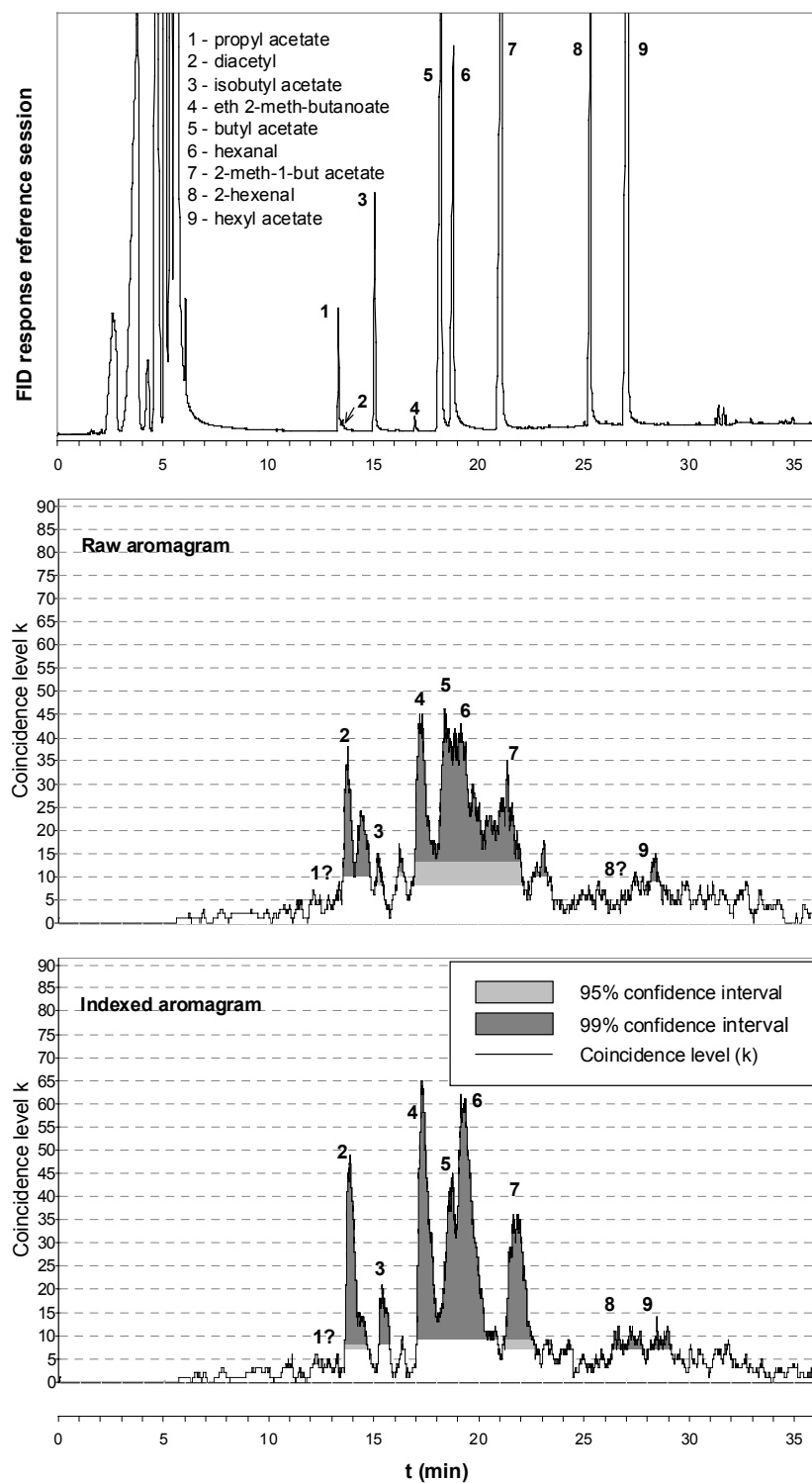
Supelcowax 10 results

QST parameters for the raw and the indexed sniffing data were assessed for the stimulus-free windows in all stimulus present sessions. The estimates for λ and μ were 0.154 and 3.55, respectively, for raw response times and 0.131 and 4.09, respectively, for indexed response times. The DB1 session results suggested that propyl acetate (1 in Figure 2.4) was presented at sub-threshold concentration. From this we infer that the first significant sniffing peak (in the raw- and the indexed aromagram) must have been caused by the release of diacetyl (2). This is confirmed by the associated descriptor selections: In the time interval from 13:34 to 15:01 minutes assessors made 77 selections of which 48 were the descriptor relating to diacetyl (butter sweet).

Although sniffing peaks related to *trans*-2-hexenal (8) and hexyl acetate (9) are both delayed considerably on Supelcowax as well, inspection of selected descriptors supported the suggested relation between components and peaks: from 26:07 to 27:57 minutes (the first significant peak) 11 responses were associated with *trans*-2-hexenal (bittersweet rum) and another 11 with hexanal (macaroon hedge), which odour is perceived as similar to *trans*-2-hexenal. For the other 25 responses one of the other descriptors was chosen, of which only 3 were associated with hexyl acetate (pear apple). In the consecutive time window from 27:57 to 31:02 minutes, the descriptors associated with hexyl acetate and E2MB (fruity sweet) were selected 17 and 11 times respectively. Both describe fruity odours. In contrast, the other 29 responses were characterised by 7 other odour descriptors, of which only the one for propyl acetate (fruity acetone) had a fruity character. These results support the conclusion that assessors have been responding to trans-2-hexenal during the first window and to hexyl acetate in the second window. The massive response delays are in line with what was observed for the late-released components on DB1. In addition, 3 sniffing

peaks just fell outside the 95% reliability interval during the final 12 minutes of in the indexed aromagram (at 30:00, 31:13 and 31:15). This also suggests that the apparatus may have produced some odours at high oven temperatures. We conclude that the same masking or condensation/lingering processes that were discussed for DB1 were responsible for these results and that retention time indexing cannot improve the resolution of the aromagram for these components.

Figure 2.4. Gas chromatogram of the nine apple-model compounds in n-pentane separated on a Supelcowax-10 capillary column (top). Indices correspond with compound indices in column 6 of Table 2.1. Layout of the raw aromagram (middle) and the indexed aromagram (bottom) is as described for Figure 2.3.

Added value of retention time indexing for Supelcowax results

In contrast to the results for DB1 sessions, a considerable improvement is observed when sniffing response times from Supelcowax sessions are indexed (Figure 2.4). This outcome was expected, given that the standard deviations of retention times were considerably larger than for DB1 (Table 2.1). First, nearly all significant peaks in the raw aromagram have increased in height after indexing and are separated better. This is most striking for the four consecutive sniffing peaks relating to E2MB (4), butyl acetate (5), hexanal (6) and 2-methyl-1-butyl acetate (7). Butyl acetate (5) and hexanal (6), which blend in the raw aromagram, are largely separated in the indexed aromagram. In addition, responses to *trans*-2-hexenal (8) and hexyl acetate (9) merged into one significant peak in the raw aromagram whereas two distinct and significant peaks occurred in the indexed aromagram. Furthermore, the minimum levels at which sniffing peaks differed significantly from noise were lower for all peaks.

General Discussion

The procedure of retention time indexing resulted in improved aromagrams for GCO experiments employing both DB1 and Supelcowax 10 columns. Although improvements for the DB1 experiment were modest, the results showed better separation of sniffing peaks and significance was reached at lower coincidence levels for some sniffing peaks. The Supelcowax 10 column produced far more retention time variation than the DB1 column. Therefore, retention time indexing has contributed considerably to the resolution of aromagrams in the Supelcowax 10 experiment. Throughout the aromagram, sniffing peaks were separated better and sniffing peaks could be matched better to respective odorants. In addition, significance was reached at lower coincidence levels in the indexed aromagram than in the raw aromagram, most markedly for the 99% reliability intervals.

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We conclude that in GCO DF experiments response time indexing is a useful technique to improve sniffing peak separation. In cases were component retention times are rather stable over repeated experiments, indexing will not be as necessary as in cases where retention times are unstable. The latter case is more likely to occur for less stable polymer column linings.

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3 Modeling coinciding panel detections by Queuing System Theory

Abstract

In continuous vigilance tasks, the number of coincident panel responses to stimuli provides an index of stimulus detectability. To determine whether this number is due to chance, panel noise levels have been approximated by the maximum coincidence level obtained in stimulus-free conditions. This study proposes an alternative method by which to assess noise levels, derived from Queuing System Theory (QST). Instead of critical coincidence levels, QST modeling estimates the duration of coinciding responses in the absence of stimuli. The proposed method has the advantage over previous approaches that it yields more reliable noise estimates and allows for statistical testing. We propose that QST may be used as an alternative to Signal Detection Theory for analyzing data from continuous vigilance tasks.

Introduction to QST

A central question in the domain of the psychology of perception is how to distinguish stimulus-induced responses from responses to noise. In the present paper, we use a quantitative approach to estimate noise levels for a panel of subjects responding to a sequence of events in a vigilance task. Traditionally, the theoretical framework that has been used in experiments in which signals have to be distinguished from noise has been Signal Detection Theory (SDT). Since this approach is difficult to apply in vigilance tasks in which stimulus incidences are low, we propose a different framework based on Queuing System Theory (QST), a framework originally developed to model the dynamics of electronic networks. First, we will introduce the vigilance task paradigm and discuss the disadvantages of using SDT in this context. Then, we will introduce QST and demonstrate how it can be used to model the response behavior of a panel of observers under the assumption that no stimuli are presented. Finally, in a simulation study we derive a heuristic that yields the variances of panel response lengths at each level of coinciding responses. With these variances, critical values of response lengths may be calculated to enable statistical testing of empirically encountered response coincidences.

Vigilance tasks and SDT

The vast majority of human psychological experiments involving stimulus presentations have been conducted using highly structured, time controlled presentation trials. Vigilance tasks form an exception to that rule. In its elementary form, a vigilance task consists of a sequence of randomly timed presentations of identical stimuli. The subject is asked to give a simple response, generally by pressing

a button, whenever he or she believes that the critical event - a stimulus presentation - has occurred. Two presentation modes can be distinguished: Either stimuli may be presented during distinct observation intervals or stimuli may be presented at any point in time without observation intervals being defined. The former, which is the most common mode, can be described as a 'discrete-events' task whereas the latter is known as a 'continuous' or 'free response' task {Egan, 1961 806 /id} {Egan, 1961 809 /id}. Although the vigilance paradigm covers a small portion of the literature on perception, it represents a wide range of daily life situations ranging from motorists checking their car's instrument panel to lifeguards watching over bathers' safety. Vigilance tasks require sustained attention from the observer: The critical event requiring a response might occur at any moment. The incidence of critical events, however, is often very low. It is not by the hour that a bather's life is in danger, nor does a car's oil pressure drop drastically once every day. Task difficulty is high when critical event intensities are weak or when events have to be discriminated from background noise. A bather's call for help might easily go unnoticed in the cacophony of sounds at the beach.

SDT offers a framework for the quantification of the ability to detect weak stimuli or to discriminate stimuli from noise {Swets, 1961 616 /id} {Green, 1966 811 /id}. Since the early 1960s, SDT has been applied in vigilance experiments, in both the auditory {Egan, 1961 809 /id} and the visual {Mackworth, 1963 810 /id} domain. Its usefulness is still recognized to date {Craig, 1987 829 /id} {See, 1997 817 /id}. SDT assumes that each observation is projected on an arbitrary sensory continuum. The location on the continuum is determined by the projected stimulus (signal) intensity and normally distributed noise. Assuming that noise (N) and stimulus + noise (SN) magnitudes have equal variances, the detectability index d' of a stimulus is defined as

the difference between the means of the probability density distributions of N and SN, expressed in standard deviation units. Under the assumption that N and SN magnitudes are Gaussian distributed for a given stimulus condition, the detectability d' can be calculated from observed proportions of true detections ('hits' in SDT) and false detections ('false alarms' in SDT) {Green, 1966 811 /id}. The probability that an observer detects a stimulus correctly increases with increasing d'. In addition, this probability depends on an arbitrary, observer-dependent critical value on the sensory continuum (x_c) which is the lower bound sensation magnitude for the observer to decide that a stimulus has been presented. A higher x_c will result in a lower probability of "yes" responses (both hits and false alarms) and a higher probability of "no" responses (both true rejections and misses). This observer-dependent decision criterion is expressed by the likelihood ratio $\beta = P(x_c|SN)/P(x_c|N)$, which equals the probability of a hit divided by the probability of a false alarm. Since the average of the SN distribution is at least equal but usually higher than the average of the N distribution, high decision criteria ($\beta > 1$) generally reflect high x_c (exceeding the average of the SN- and the N distributions).

However useful SDT may be in a wide range of perception experiments, free-response vigilance tasks are a special case in which the use of SDT has some serious disadvantages. During a vigil, a stimulus may occur occasionally and at any point in time. The low presentation rate in combination with the lack of temporal structure introduces difficulties with respect to the qualification of responses in terms of SDT. On the one hand, SDT requires sufficiently high rates of both hits and false alarms to ensure a reliable estimation of d'. On the other hand, the lack of structured presentation intervals produces difficulty in deciding whether a delayed affirmative response event is still to be regarded as a hit or as a false alarm {Watson, 1976 808

/id}. Furthermore, in classical signal detection experiments signals generally are presented at constant duration and last less than 1000 ms, which is not comparable to many practical situations in which stimulus duration is highly variable, influencing the probability of stimulus detection.

In the present study, we will present an alternative, QST-based conceptual approach to estimate noise levels in continuous vigilance tasks. Only false alarms serve as input for the model. Hence, no hits are needed and no structured presentation intervals need to be defined. In QST-modeling, response length relates directly to detection probability, which respects the variability of stimulus duration under natural conditions and thus improves its ecological validity in comparison with SDT.

QST applied to vigilance tasks

Queuing systems are described as systems of flow - that is, systems "in which some commodity flows, moves, or is transferred through one or more finite-capacity channels in order to go from one point to another" {Kleinrock, 1975 776 /id}. Examples are the flow of automobile traffic through a road network, the transmission of telephone messages through a telecommunication network, or the flow of data through a time-sharing computer system. In terms of vigilance tasks, the flow system is reflected by panelists' processing of responses to a specific sequence of stimuli. The number of members in the panel defines the channel capacity and the commodity is formed by the panelists' responses to the stimuli presented. The state of the flow system is the number of panelists responding simultaneously, which is expressed as the number of coinciding responses: the coincidence level. Transitions between coincidence levels follow individual response onsets and offsets. An example of how the timed responses of four panelists to detected odorants are combined to form coincidence levels is given in Figure 3.1.

Under the assumption that all responses are false alarms (i.e., no stimuli are presented), QST can formalize response behavior by modeling the probabilities of coincidence levels. This allows for the definition of panel noise levels of coinciding responses. From false alarm responses, two measures are used: the *length* of responses and the *latency* between consecutive responses (see Figure 3.1). Response length and

Individual responses

Panel cumulatives (aromagram)
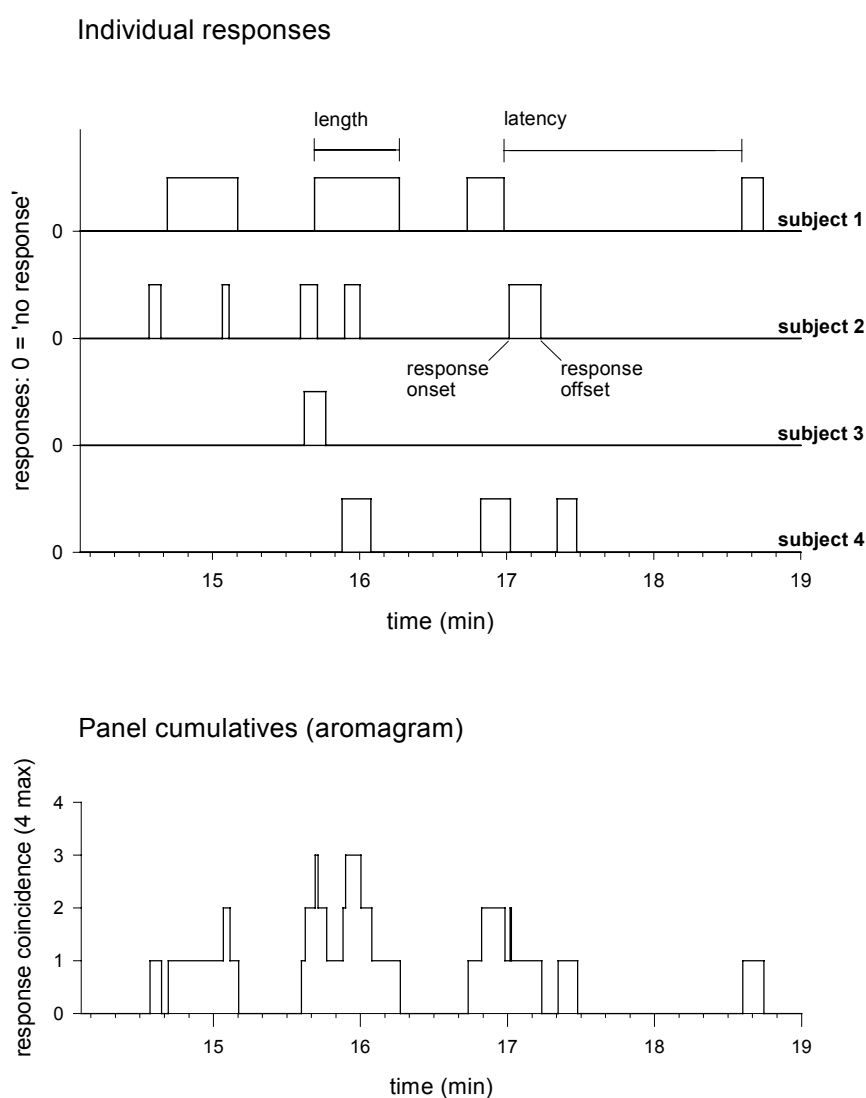
Figure 3.1. Determining coincidence levels from individual responses. The upper panel shows responses generated by four subjects to identical odorant stimulus sequences. The lower panel shows the aggregate coincidence levels as cumulatives on the time axis (aromagram).
response latency together determine response incidence. If the average latency increases, response incidence decreases. On the other hand, an increase of response

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lengths reduces response incidence. However, since response length is generally short compared to response latency, changes in response length will have a smaller impact on response incidence than changes in response latency.

To model coincidence level probability in QST terms, two requirements should be met with respect to the model. The QST model should allow for coincidence levels that range from zero up to and including the panel size. In addition, each response action should instantly produce a transition in coincidence level. This implies a channel capacity that equals the number of panelists, so that waiting times are zero. QST modeling makes two assumptions with respect to the occurrence of responses in SF sessions. First, a transition in coincidence level is assumed to take place one step at a time. Consequently, the model assumes that two panelists never initiate or end a response at exactly the same moment. Second, transition probability as a function of time is defined by the current coincidence level only. It does not depend on the past coincidence levels nor on the length of the time interval at the actual coincidence level. In other words, the queuing system is memory-less.

A simple response system that fits these requirements is the response onset-only system. An imaginary person presses a button repeatedly although no stimuli are presented. Every button press signifies a response onset. Following the memory-less property of the system, the probability density function of the length of the time period between two consecutive onsets can be described by the exponential decay function {Kleinrock, 1975 776 /id}

$$a(t) = \lambda e^{-\lambda t} \quad t \geq 0 \tag{1}$$

where t is the time since the start of the observation, $a(t)$ is the probability density at time t, and the *onset rate* λ is the expected number of button hits per unit of time. The exponential decay function satisfies the assumption that the probability of a

response onset as a function of time t depends neither on the moment that registration started nor on the number of previous responses. If the response onset times are exponentially distributed, the probability of the number of responses being k at time t is Poisson distributed:

$$P_k(t) = \frac{(\lambda t)^k}{k!} e^{-\lambda t} \qquad (k \geq 0, t \geq 0)$$

(2)

To account for response offsets, a measure of response length is introduced, reflecting the interval during which a subject perceives an ongoing stimulus. In SF conditions requiring little stimulus processing, the exponential function (Equation 1) accurately describes response length distributions {Luce, 1986 788 /id}. The probability density function of the length of a single response can be defined using the offset rate μ, which equals the expected mean number of response intervals per unit time. The expected mean length of a single response then equals the reciprocal of μ:

$$E(\overline{length}) = \frac{1}{\mu}$$

(3)

The rate at which a panel initiates responses depends on the panel size, the individual onset rates, and the number of panelists already responding. For instance, the probability that any panelist initiates a response, given that all panelists are currently responding, is zero. This rate will increase with the number of non-responding panelists available. Given a panel of size M and assuming that individual onset rates are identical and additive, the collective onset rate of a panel in which k panelists are currently responding (λ_k) can be calculated from the individual rates for any given coincidence level k:

$$\lambda_k = (M - k)\lambda \quad \text{for} \quad 0 \leq k \leq M$$

(4)

Under the same assumptions, the collective offset rate μ_k can be calculated by:

$$\mu_k = k\mu \quad \text{for} \quad 0 \le k \le M \tag{5}$$

From Equations 1, 4 and 5, Kleinrock {Kleinrock, 1975 776 /id} arrives at a model describing the probability that k out of M subjects are responding simultaneously at an arbitrary moment in time given that no stimuli are presented:

$$P_k = \frac{\left(\frac{\lambda}{\mu}\right)^k \binom{M}{k}}{(1+\frac{\lambda}{\mu})^M} \quad (0 \le k \le M \quad \text{and} \quad \sum_{k=0}^{M} P_k = 1) \tag{6}$$

Given λ and μ, the model can be used to calculate the expected proportion of the total session length at which k subjects will be responding simultaneously, under the condition that no stimuli are present. The expected cumulative length of time (T_k) at coincidence level k can then be calculated by multiplying P_k by the length of the experimental session (l_{session}):

$$T_k = P_k \cdot l_{\text{session}} \tag{7}$$

Following $\sum_{k=0}^{M} P_k = 1$, T_k are also linearly dependent because $\sum_{k=0}^{M} T_k = l_{\text{session}}$.

Parameters λ and μ can be assessed empirically. For each subject, the offset rate μ is estimated by the reciprocal of the average length of responses and can be calculated by:

$$\hat{\mu} = \left(\overline{length}\right)^{-1} = \frac{n_{\text{responses}}}{\sum length} \tag{8}$$

where length is the response length (see Figure 3.1) and $n_{\text{responses}}$ the number of responses involved. Individual onset rate is estimated by the reciprocal of the average latency and can be calculated by:

$$\hat{\lambda} = \left(\overline{latency}\right)^{-1} = \frac{n_{\text{responses}}}{l_{\text{session}} - \sum length} \tag{9}$$

where latency is the length of the interval between successive responses (see Figure 3.1). In the present study, we will use the results of each individual panelist to estimate individual onset- and offset rates. In addition, we will estimate overall panel onset- and offset rates. The latter requires concatenating responses from several individuals as if these were generated by one person during a session of length $M \cdot l_{session}$.

By substituting the empirical estimates $\hat{\lambda}$ and $\hat{\mu}$ (Equations 8 and 9) in Equation 6, estimates of P_k ($\widehat{P_k}$) can be obtained. However, it is virtually impossible to derive an exact probability distribution of $\widehat{P_k}$ analytically from the exponential probability distributions of $\hat{\lambda}$ and $\hat{\mu}$. Instead, we simulated SF sessions under varying conditions of session length, λ and μ. The resulting distributions of $\widehat{T_k}$ were used to model $Var(\widehat{T_k})$ as a function of session length, λ and μ. Results of these simulations will be discussed in the next section.

Deriving a heuristic to calculate T_k variance

Introduction

Variances of $\widehat{T_k}$ are needed to estimate critical values for $\widehat{T_k}$, which, in their turn enable the statistical testing of observed panel response coincidences. Because $Var(\widehat{T_k})$ cannot be derived analytically from $\widehat{T_k}$, $\hat{\lambda}$ and $\hat{\mu}$, a heuristic for the calculate of $Var(\widehat{T_k})$ should be derived. This section presents the simulation of vigilance task responses, assuming response distributions that can be described by the QST model. By systematically varying onset rates, offset rates, panel sizes and session lengths,

results can be used to derive the model relating $\widehat{T_k}$ to Var($\widehat{T_k}$). In this section we discuss the routines and derive the heuristic. Subsequently, we propose a *post hoc* test for durations of coincidence levels within individual sniffing peaks.

Method

Simulation of sniffing data

Sniffing experiments were simulated using uniformly distributed random deviates u ($0 \leq u < 1$) generated by the Ran3 routine {Press, 1989 812 /id}. To obtain exponentially distributed latencies, uniform deviates u_1 were transformed by

$$latency = -\ln(1-u_1)/\lambda \tag{10}$$

where λ is the onset rate. Similarly, exponentially distributed response lengths were calculated from uniform deviates u_2 by

$$length = -\ln(1-u_2)/\mu \tag{11}$$

where μ is the offset rate. In a simulated session, initial simulated latencies all start at $t = 0$, which systematically lowers P_k values during the first minutes of a session. Consequently, we simulated 120-minute sessions of which only responses given during the final 60 minutes were used for calculation of P_k. All algorithms were written as Pascal routines embedded in a Delphi 4 environment {Borland, 1998 866 /id}.

Results from multiple simulated sessions may be used to calculate P_k and Var(P_k). Because the QST model and the simulations both assume exponential decay functions for onset- and offset times, simulated P_k are best predicted by model estimates of P_k, using the same λ and μ in Equations 6 and 7. Therefore, we first cross-validated simulated P_k with P_k predicted by the QST model in simulation 1. In this simulation,

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P_k were obtained by simulating SF sessions using the values of λ and μ specified in Table 3.1. These onset- and offset rates represent a range of realistic values (see chapter 3). The simulated panel size was 20. One thousand sessions were simulated for each of the twenty-five possible combinations of λ and μ. Subsequently, the resulting averaged P_k were cross-validated with modeled P_k (Equation 6).

Estimation of P_k variances supplementing model estimates of P_k

As implied by the central limit theorem, raising response incidences in the time window used to estimate λ and μ results in narrower probability distributions for $\hat{\lambda}$ and $\hat{\mu}$ and, consequently, in narrower probability distributions for $\widehat{P_k}$. Therefore, the expected number of responses per time unit should be considered for the calculation of Var($\widehat{P_k}$). If the number of responses per time unit increases (when λ increases and/or μ decreases) Var($\widehat{P_k}$) will decrease. To estimate the number of responses per time unit, we assess the number of completed responses that fit one time unit. The time needed to complete a response is defined as the average time before responding ($1/\lambda$) plus the average response length ($1/\mu$). The number of completed responses per time unit is the reciprocal thereof:

$$n_{\text{expected}} = \frac{1}{\lambda^{-1} + \mu^{-1}} = \frac{(\lambda \cdot \mu)}{(\lambda + \mu)} \tag{12}$$

Furthermore, because P_k are proportion scores, their distributions will have a positive skew at sufficiently small P_k, approximating a Gamma distribution (see the Appendix). Being approximately Gamma-distributed, P_k relate linearly to Var(P_k) {Winer, 1991 870 /id}. Later in this simulation section we will determine up to which P_k values this linear relation is valid. As for now, we assume the model to be linear. The coefficient λ/μ, expressed as the parameter ρ, is a measure for the relative

length of a response with respect to its incidence. Relatively long responses result in larger variances. Therefore, ρ is expected to relate positively to $\text{Var}(\widehat{P_k})$. The coincidence level k relates inversely to $\text{Var}(\widehat{P_k})$ because individual responses do not coincide for the full length of the responses but rather overlap partially, causing the length of coincidences to decrease with increasing k. Summarizing, in its simplest linear form, a heuristic relating $\text{Var}(\widehat{P_k})$ to $\widehat{P_k}$ would read:

$$Var(\widehat{P_k}) = c \cdot \frac{\rho \cdot \widehat{P_k}}{k \cdot n_{\text{expected}}} \qquad (k > 0) \qquad (13)$$

where $\text{Var}(\widehat{P_k})$ is the variance of the proportional length of responses at coincidence k, c is an arbitrary constant and $\rho = \lambda / \mu$. $\text{Var}(\widehat{T_k})$ can be obtained by multiplying $\text{Var}(\widehat{P_k})$ with the session length (see also Equation 7). This concise model does not define variances at $k = 0$. Yet, this will not interfere with testing empirical T_k in detection experiments, because coincidence levels of 0 are irrelevant in that context.

To test whether the assumptions underlying the heuristic are plausible and to determine the size of the constant c, four extra series of experiments were simulated in which the parameters n_{expected}, l_{session} and ρ were systematically varied. The designs of these four series are shown in Table 3.1. In Simulation 2, n_{expected} values were manipulated by varying onset and offset rates, maintaining a constant ρ. In Simulation 3, ρ was manipulated by using 25 factorial combinations of 5 different λ and 5 different μ. As a control for the influence of panel size, we ran the same series of 25 experiments again for a different number of subjects (Simulation 4). Length of experimental sessions was manipulated in a series of four experiments with varying session lengths (simulation 5). Each of these simulations was conducted 100 times.

Table 3.1. Simulated experiments. Specified are: simulation experiment numbers as referred to in the text; onset rates (λ) and offset rates (μ); the ratio of λ and μ (ρ); length of the data collection window ($l_{session}$); the number of replications of each simulation experiment that were used to calculate averages of $\widehat{T}_k$ and Var($\widehat{T}_k$); the number of panelists that were simulated (*M*). All simulated experimental windows from which data were used were preceded by 60-minute windows from which data were discarded.

Simulation number	λ (min^{-1})	μ (min^{-1})	ρ	$l_{session}$ (min)	number of replications
1	0.01, 0.05, 0.10, 0.15, 0.25	1, 2, 3, 6, 10	Variable	60	1000
2	0.01[a], 0.04[b], 0.16[c], 0.64[d]	0.5[a], 2[b], 8[c], 32[d]	0.02	60	100
3	0.01, 0.05, 0.10, 0.15, 0.25	1, 2, 3, 6, 10	Variable	60	100
4	0.01, 0.05, 0.10, 0.15, 0.25	1, 2, 3, 6, 10	Variable	60	100
5	0.01	0.5	0.02	60, 240, 960, 3840	100

[a,b,c,d]The parameters λ and μ were chosen in a way that ρ always equalled 0.02. Hence, four simulations (d) were performed by combining λ and μ that shared identical index letters.

$\widehat{P}_k$ and Var($\widehat{P}_k$) were calculated for each *M*, λ, μ, $l_{session}$ condition. Var(P_k)$_{simulation}$

was then evaluated against Var($\widehat{P}_k$) to estimate the constant *c* in Equation 13.

Testing observed T_k *and* post hoc *testing of separate coinciding panel responses*

If panelists observe stimuli, their responses will increase in number and in length, which will raise the probability of responses to coincide. Consequently, the cumulated time at coincidence levels $k > 1$ will increase accordingly. Because $\widehat{T}_k$ and Var($\widehat{T}_k$) are estimated from responses given when no stimuli were presented, these values can be used to test whether the probability of observed T_k is above chance level. Given some $\hat{\lambda}$ and $\hat{\mu}$, $\widehat{T}_k$ close to zero have skewed distributions because zero constitutes a

lower bound to observable T_k values. Therefore, it should be noted that simply

assuming T_k to be Gaussian distributed in order to calculate critical values of T_k might

seriously threaten test reliability (see the Appendix). In stead, we assume Gamma-

distributed T_k with variance $\mathrm{Var}(\widehat{T_k})$ and average $\widehat{T_k}$.

Observed T_k can be tested one-sided because relevant T_k are expected to increase

when stimuli are detected. However, because $\sum_{k=0}^{M} T_k = l_{session}$, an increased response

incidence can reduce T_k at low coincidence levels (i.e. $k = 0,1$) to the extent that these

would be significant if two-sided tests were used. Because we are interested mainly in

increases of response coincidences, and because the decrease of T_k at low k can be

regarded as a side effect of the increases we were interested in, we restrict ourselves

to the use of upper critical levels for T_k.

If T_k were independent and if an aggregate test of T_k was to be performed at some

significance level α, it would be advisable to adjust the significance levels for single

tests at each level of k to $1-(1-\alpha)^{1/k}$. This would result in strict significance levels per

level of k in order to preserve the desired overall level of significance. However, as

discussed earlier, T_k are linearly dependent. Furthermore, T_k can only be larger than

zero if $T_{k-1} > 0$. Hence, adjustment of the α levels is undesirable.

To identify which sniffing peaks contribute to significant T_k values, *post hoc* tests

should be performed at coincidence levels that produce significant T_k. Under the null

hypothesis that all responses are false alarms, it is assumed that coinciding responses

may occur at any point in time at a constant probability. Each partial T_k associated

with a single coinciding peak ($\mathrm{T}_{k,i}$; i being the peak index) can then be tested against

a critical value that is based on an observation window of the length $l_{session}/N_k$, with

N_k being the number of peaks that contain coinciding responses at level k. For a given

significance level α, the critical response length summarized over all peaks in a

session T_k' is the T_k value at which the corresponding cumulative probability

distribution equals 1-α. Accordingly, T_k' can be represented as a linear function of the

estimated standard deviation of T_k by $\widehat{T_k}$ +a·SD($\widehat{T_k}$) [a>0]. Per single-peak

observation window, the expected $\widehat{T_{k,i}}$ equals $\widehat{T_k}$ /N_k and, given the linear dependency

of Var($\widehat{T_k}$) on $\widehat{T_k}$, Var($\widehat{T_{k,i}}$) equals Var($\widehat{T_k}$)/N_k. From this, we calculate single-peak

critical values of $T_{k,i}$' by

$$ T_{k,i}' \quad = \quad \frac{\widehat{T_k}}{N_k} + a \cdot \sqrt{\frac{Var(\widehat{T_k})}{N_k}} \quad = \quad \frac{\widehat{T_k}}{N_k} + \frac{a \cdot SD(\widehat{T_k})}{\sqrt{N_k}} \tag{14} $$

with k being the coincidence level, i the peak index, and N_k the number of peaks

containing coincidences at level k. The constant a is derived from the critical value T_k'

and depends on the chosen α.

Results

Simulated $\widehat{T_k}$ were obtained per session and averaged over replications. Figure 3.2

shows that the corresponding $\widehat{P_k}$ obtained in simulation 1 perfectly fitted probabilities

predicted by the QST model. For the applied range of λ and μ values, these

measures proved to be identical up to the third decimal. Therefore, averages of the

simulated $\widehat{P_k}$ are considered unbiased estimators of model probabilities.

Consequently, we will use variances of the simulated $\widehat{P_k}$ to optimize the heuristic for

variance calculation.

In addition, simulated response lengths and latencies of simulation 1 were analyzed to see whether these corresponded with the μ and λ used in the simulation algorithm. For all used values of μ and λ, the average lengths and the average latencies indeed corresponded with their respective μ and λ according to the model specified in Equations 8 and 9. For example, for simulations with $\mu = 6$ and $\lambda = 0.15$ the resulting average response length was 0.167 min and the average latency was 6.67 min, resulting in $\hat{\mu} = 5.99$ and $\hat{\lambda} = 0.15$ respectively (Figure 3.3). Distributions of simulated response length and response latency also fitted their respective exponential probability distributions for all λ-μ combinations. This is illustrated in Figure 3.2 for $\lambda = 0.15$, $\mu = 6$ with $n_{simulations} = 1000$.

If the estimated variance $\mathrm{Var}(\widehat{P_k})_{model}$ equals the simulated variance $\mathrm{Var}(P_k)_{simulation}$, their quotient will equal 1. The observed ratio of $\mathrm{Var}(\widehat{P_k})_{model}$ by $\mathrm{Var}(P_k)_{simulation}$ produces an approximately horizontal line for P_k values ranging from 0.001 up to 0.1 (Figure 3.4). For sniffing sessions of 60 minutes, this P_k range represents T_k of 3.6 to 360 seconds, which largely covers empirically encountered T_k

Figure 3.2. Proportional densities of response length (left panel) and response latencies (right panel) as calculated by Equation 6 with stimulus onset rate λ = 0.15 (min⁻¹) and stimulus offset rate μ = 6 (min⁻¹) for 1000 simulations of one subject. Modeled probabilities are plotted in the same graph, coinciding perfectly with calculated probabilities. Average t (M_t) equal the reciprocal of the model parameters.
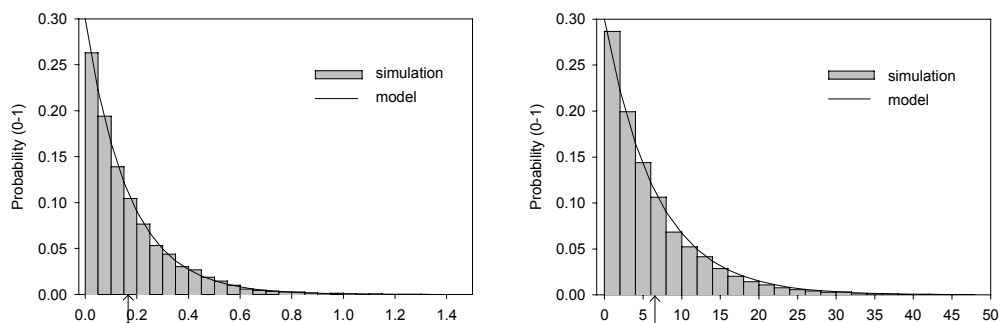
at k levels well above zero. Therefore, we optimized the value of c in the heuristic in

Equation 13 by fitting Var($\widehat{P_k}$)$_{model}$/Var(P_k)$_{simulation}$ ratios to a geometrical average

variance ratio of 1 for $0.001 < P_k < 0.1$ by the least sum of squares method using

logarithms of variance ratios. The use of the geometric mean gives the same weights

to identical proportional differences between ratios, either above or below the center

score. This analysis resulted in an optimal fit for c equal to 2.14. After solving for

$n_{expected}$ (Equation 12) and $\rho = \lambda / \mu$, Equation 13 then results in

$$Var(\widehat{P_k}) = \frac{2.14(\lambda + \mu)}{k \cdot \mu^2} \cdot \widehat{P_k} \qquad (k > 0) \qquad (15)$$

At P_k values above 0.1, ratios of variances rapidly increase, indicating that the

heuristic tends to overestimate variances for these P_k values. In 60-min SF sessions

these P_k values would represent T_k of 6 minutes and more. Such T_k values are only

found at $k=0$ or $k=1$, due to the generally frequent occasions that 0 or 1 panelist is

responding. Because we assess detection at values of k well above zero, P_k values

above 0.1 will not be relevant for our decisions on noise levels. At P_k below 0.001 the

variances appear to be slightly overestimated by the heuristic. This causes slightly

more conservative testing at these P_k values. Variance ratios at low P_k show larger

variability than ratios at high P_k (Figure 3.4). This is due to the small number of

observations involved in the calculation of simulated variances. Similarly, simulations

involving 10 subjects (Figure 3.4, simulation 4) produce a larger variability of

variance ratios than those involving 20 subjects when the same parameter settings are

employed (Figure 3.4, simulation 3). Again, this is due to fewer observations in

simulations of 10 subjects. However, panel size per se does not lead to different

variance estimates. This can be concluded from the fact that variances for panel sizes

10 and 20 produce distributions of variance ratios that center on the same optimal

variance ratio of 1 (Figure 3.4). No systematic effect of session length on variance estimates was observed (Figure 3.4: simulation 5) but increased session lengths do lead to more reliable estimates of T_k. If variance relates linearly to T_k. then critical values of T_k , being a linear function of SD(T_k), relate linearly to the square root of T_k,. Hence, increasing the session length would reduce the proportional difference between the model T_k and its upper critical value.

Because the model of Equation 15 uses the coincidence level k as a predictor of Var($\widehat{P_k}$), we evaluated possible effects of k on variance estimates. For separate categories of k, geometrical averages of Var($\widehat{P_k}$) were calculated from simulations 2 through 5. Variances were only calculated for P_k below 0.1. In analogy with Figure 3.4, ratios of Var($\widehat{P_k}$)$_{model}$ by Var(P_k)$_{simulation}$ are presented in Figure 3.5. Variance ratios are all close to one, which suggests an absence of an effect of k on model estimates. No systematic trend of variance ratios over values of k is observed.

Discussion

The primary objective of this study was to develop a method that uses the false alarms generated by a panel in a vigilance task to yield noise estimates at *a priori* significance levels, and to include response length as a factor, because stimuli are generally perceived during intervals of time, the length of which is partly related to the probability that a stimulus was actually presented. The QST-based approach meets these objectives and is applicable to conventionally registered timed responses. QST modeling allows for the calculation of critical values ($T_{k,i}$') for cumulated response lengths at each coincidence level k (T_k). Upper critical values of T_k' can be condensed *a posteriori* into peak-related critical values $T_{k,i}$', allowing the assessment of the minimal coincidence level at which observed $T_{k,i}$ are significant.

Figure 3.3. Response probabilities (modeled) and proportions of the total experimental session length (simulations) as functions of coincidence level *k*, and specified for different onset- (λ) and offset (μ) rates. Panel size is 20 subjects. Data points represent averages over 1000 simulated experiments.
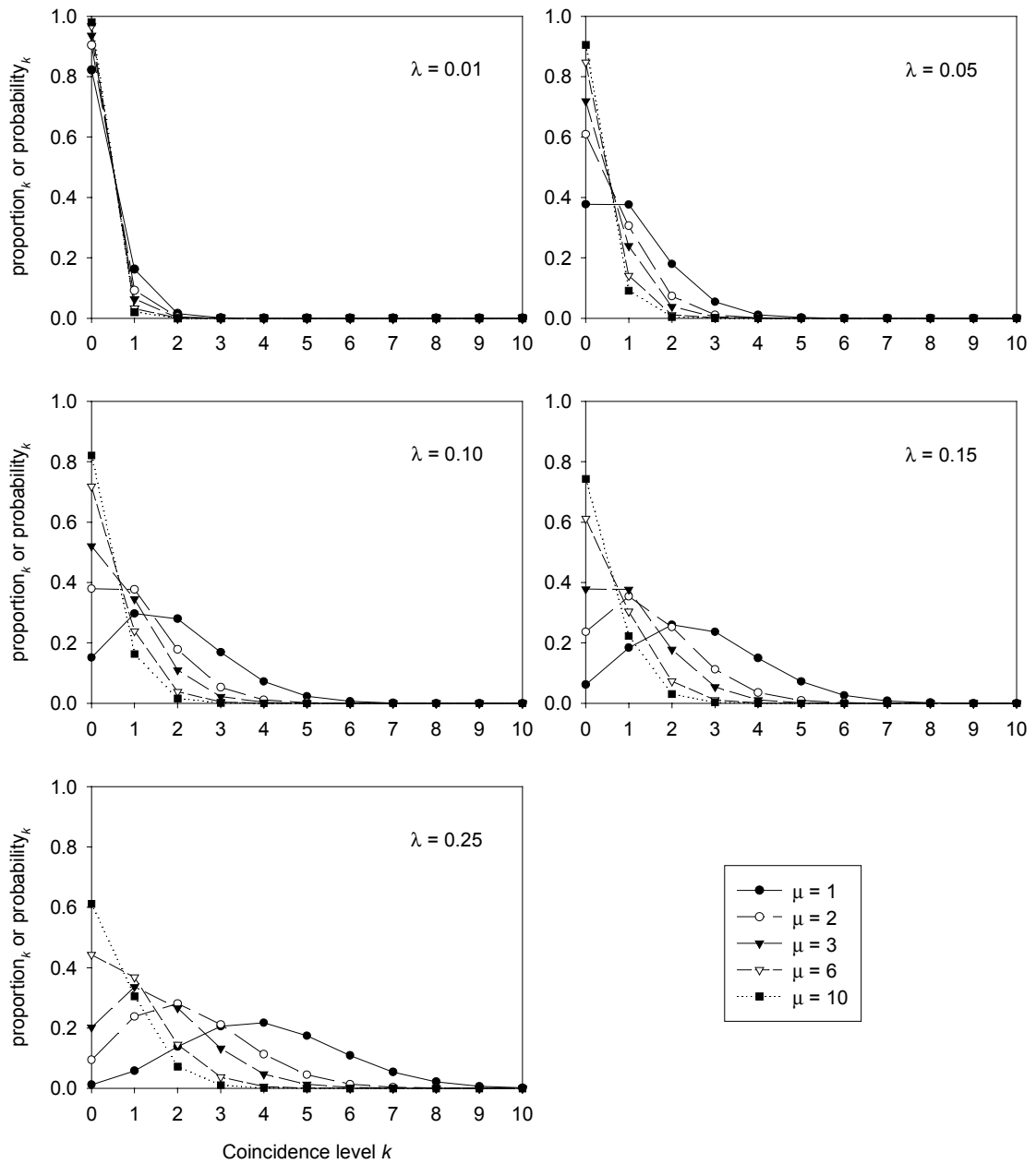
Figure 3.4. Ratios of modeled P_k variance by simulated P_k variance as a function of P_k. Variance estimates are calculated by the heuristic discussed in the text (equation 15).
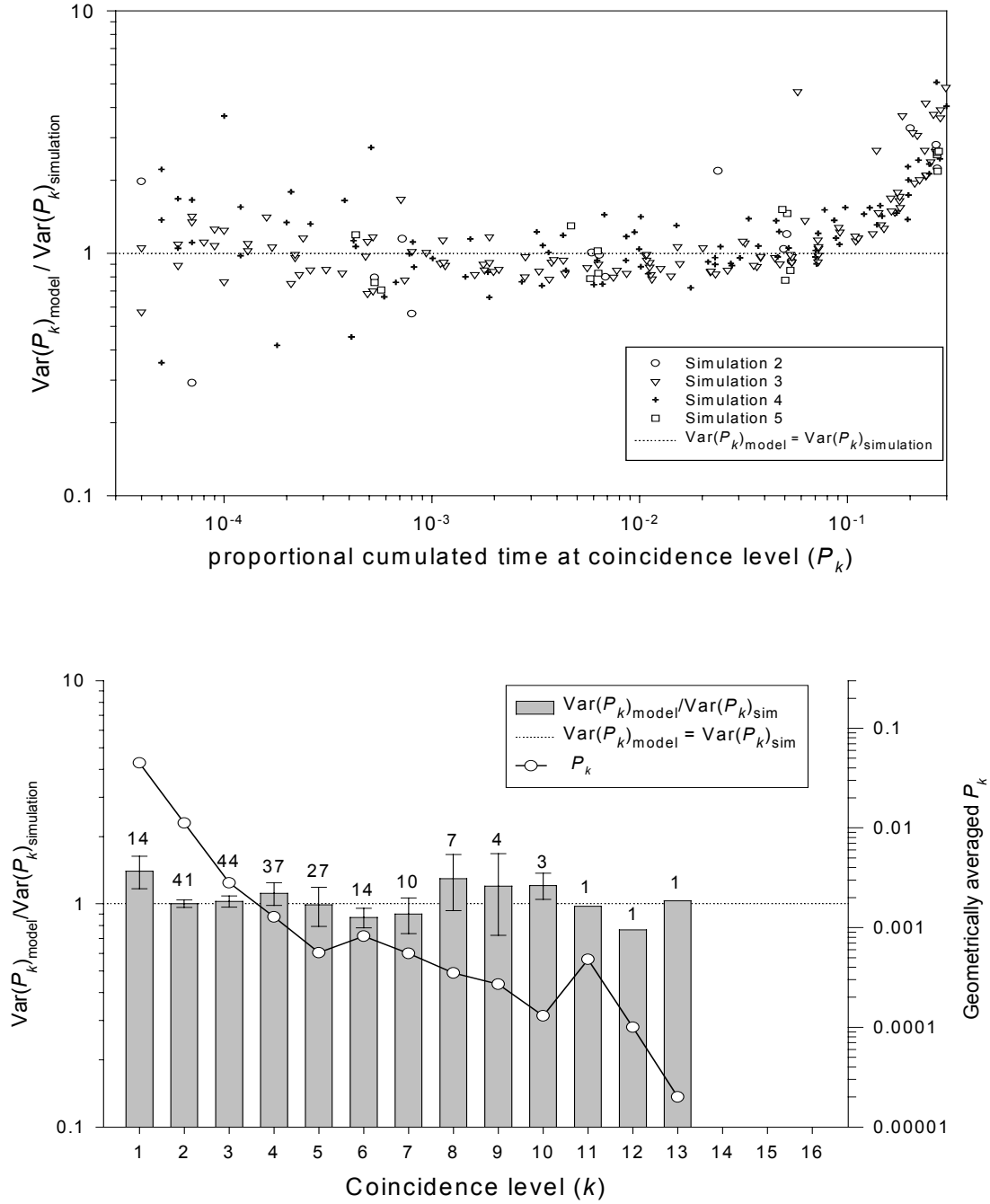
Figure 3.5. Vertical bars: Ratios ($\pm$SEM) of modeled P_k variance (calculated with Equation 15) by simulated P_k variance (using the same λ and μ), and plotted as a function of the coincidence level (k). Circles: Corresponding geometrically averaged P_k at corresponding coincidence levels. Data for which $P_k > 0.1$ are excluded from the analyses. The numbers of observations contributing to the average ratios are presented above respective bars.

Many vigilance tasks involve the visual or acoustic detection of brief signals. In these tasks, responses merely affirm that stimuli were perceived. These vigilance tasks are in line with the SDT paradigm that assumes short discrete and uniform observation intervals and a single 'yes' or 'no' response per interval. In contrast, tasks that involve a continuous evaluation of continuous stimuli are rare in the literature. They occur, however, in many situations in daily life: Do I hear the telephone ringing or not? Do I see a drowning person over there or not? Do I smell leaking gas or not? This thesis discusses the method of gas chromatography olfactometry, a technique in which panelists respond to odorants that are also presented at poor-defined moments in continuous observation periods. To be used in such tasks, SDT would need to account for the information that is enclosed in the length of responses. Therefore, the length of a single 'hit' trial should be variable. Although SDT has been used in continuous vigilance tasks by making *post hoc* assumptions of the length of observation intervals {Watson, 1976 808 /id}, the extra need for observation intervals to be variable requires fundamental modifications of SDT. For instance, it needs to be resolved how to interpret multiple consecutive 'hits' in response to a single lengthy stimulus. Also, the length of true rejection trials cannot be defined in a straightforward manner due to the non-uniform length of stimuli. In the present study, we introduced QST as a framework to model noise response behavior. QST merely utilizes false alarms, no hits, and it includes response length as a contributor to stimulus detection probability. Since QST does not require structured trials and uses only false alarms, it is easier to apply in vigilance tasks than SDT. Furthermore, the variability of trial length and the use of response length as a factor add to the ecological validity of QST in vigilance task applications. Therefore, in continuous vigilance tasks with variable response length QST is a good alternative to the

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fundamentally less appropriate SDT. For these reasons, QST is very well suited to

analyze the reliability of panel responses odorants in GCO experiments. An

application of QST in GCO is shown in the next chapter.

4 Testing detection frequencies in gas chromatography olfactometry by applying Queuing System Theory

Abstract

The QST method (see chapter 3) was applied in an olfactory detection experiment using sixteen panelists in stimulus-present (SP) and stimulus-free (SF) conditions. In contrast with traditional detection frequency (DF) techniques, QST also employs the length of coinciding panel responses as an index of the probability that an odorant was present. Due to this, the QST method resulted occasionally in significant sniffing responses at lower coincidence levels than traditional DF thresholds, whereas other sniffing peaks were only significant at coincidence levels higher than DF thresholds. In addition, it is shown that the use of SF sessions to assess sniffing peak reliabilities causes more liberal testing conditions than the use of SP sessions. It is recommended to calculate sniffing peak reliabilities on basis of QST parameters derived from SP sessions.

Introduction

This chapter demonstrates the use of QST in an olfactory vigilance experiment in which odorants have to be detected at a sniffing port. Panelist's responses that are generated in the absence of stimuli are used to estimate parameters of the QST model in two distinct stimulus contexts: stimulus-free (SF) sessions and stimulus-free windows of stimulus-present (SP) sessions. Subsequently, observed panel responses from SP tasks can be tested using the against the null hypothesis that no stimuli were present.

In the following empirical section, we apply QST modeling to the results from a continuous vigilance task, in which a panel had to detect odorants at a sniffing port connected to a GC outlet. We use the classical DF approach to assess noise levels and compare the results with best estimates of $\widehat{T}_k$ calculated by QST modeling. Response coincidence probabilities are estimated for SF sessions and for SF windows in SP sessions, using model parameters λ and μ that are estimated for the two conditions separately.

First, we will present a study of noise estimates in an olfactory vigilance task - that is gas chromatography olfactometry (GCO).

Gas chromatography olfactometry

Flavor chemists face the challenge of discriminating odorous components from the many odorless components in mixtures of volatiles constituting food aromas. Generally, gas chromatography is used to physically separate mixture constituents. It entails the pressurized transfer of mixture constituents through a capillary column under controlled temperature conditions. The time that each component needs to pass through the column depends on its specific physical-chemical interaction with the column lining. On release from the column, each component is quantified by a detector and identified by mass spectrometry. The mass release function typically shows a steep, approximately linear incline to a maximum, followed by more gradual and tailing decay to zero. We define the stimulus onset time as the point in time where the mass function starts to incline and the offset time as the point in time where the mass function returns to zero. Typically, between 5 and 45 seconds elapse between stimulus onset and offset.

However, the capacity of volatiles to invoke odor sensations at a given concentration level varies strongly, due to large differences in detection thresholds between odorants. Hence, relative quantities of the components in the mixture are poor indicators of their relative contributions to the mixture's aroma. A better estimate of each component's contribution to the aroma may be obtained by sensory evaluation of the separated constituents. Therefore, a method combining gas chromatography with sensory evaluation was developed, enabling the assessment of the olfactory impact of mixture constituents. With this method called GCO, subjects sniff the effluents of the gas chromatograph in an effort to detect and characterize the odor-active chemicals {Dravnieks, 1971 566 /id}.

To quantify the sensory impact of the effluents, several sensory methods have been proposed. Grosch {Grosch, 2001 805 /id} distinguishes three categories of GCO techniques: (i) Dilution methods use the number of times a sample needs to be diluted until it can no longer be detected as a measure of odor impact. Examples of this approach are Charm Analysis {Acree, 1984 352 /id} and Aroma Extract Dilution Analysis {Ullrich, 1987 639 /id}. (ii) Detection Frequency (DF) methods employ the number of coinciding panel detection responses to a stimulus as an indicator of its odor impact {Bult, 2001 508 /id}{Van Ruth, 1994 658 /id}{Pollien, 1997 630 /id}{Ott, 1997 797 /id}. This method is also referred to as Olfactory Global Analysis {Le Guen, 2000 796 /id}{Grosch, 2001 805 /id}. It reflects the vigilance task described above. Finally, (iii) intensity rating methods like the Osme method {Da Silva, 1994 842 /id}{McDaniel, 1990 844 /id} use panelists' intensity ratings of undiluted GC effluents to assess their odor impact. The three methods generate highly comparable results when used to determine the main contributors to an aroma {Le Guen, 2000 796 /id}. However, none of these methods allows statistical testing of

positive stimulus detections. In the present study, we attempt to improve the DF method by proposing a method that tests whether the observed DFs fit a distribution of DFs that is expected to occur in the absence of stimuli.

To the present, DF methods have been employed to obtain measures of odor impact without assessing the reliability of positive identifications. Users of the DF method used arbitrary noise levels {Le Guen, 2000 796 /id} or they have conducted SF sessions to determine the level at which coincidences in SP sessions should be interpreted as noise {Van Ruth, 1994 658 /id}{Van Ruth, 1996 631 /id}{Van Ruth, 1995 660 /id}. In the latter approach, the highest response coincidence level encountered in SF sessions was considered the critical noise level for the SP sessions. However, this approach suffers from fundamental shortcomings. First of all, the critical noise level estimates are affected by session length. If the probability that 12 responses coincide at least once during a one-hour session with a panel of 15 subjects equals 10^{-5}, then the aggregate probability of finding 12 coinciding responses at least once during one hundred consecutive hours equals $99 \cdot 10^{-5}$, assuming that subsequent responses of panelists are independent. Second, the use of SF sessions to estimate noise levels presupposes that the tendency to generate false alarms is independent of stimulus frequency. However, this assumption is contradicted by experimental findings from vigilance studies {Vickers, 1977 836 /id}{Swets, 1977 835 /id}{Warm, 1991 834 /id}. Subjects appear to adjust their decision criterion depending on the perceived stimulus probability: response frequencies decrease when perceived stimulus probabilities decrease and response frequencies increase when perceived stimulus probabilities increase {Williges, 1969 841 /id}{Colquhoun, 1967 840 /id}.

Method

Subjects

Sixteen paid volunteers from the local community, four males and twelve females served as subjects. Their ages ranged from 20 to 53 years (average 29 years). Twelve were experienced subjects who had participated in olfactory attribute rating experiments, discrimination tasks, and GCO experiments over the course of two years. Four new subjects were selected and familiarized with the GCO method during a 45-min training session. The selection criteria for all subjects comprised the ability to generate and use refined odor attributes and inter-subject coherency in the use of graphic rating scales. The subjects were naïve as to the objectives of the experiment. All were non-smokers and had no history of olfactory dysfunction. The subjects were in good health and gave written informed consent.

Stimuli

A model mixture was used, consisting of 9 chemical components that are commonly found in natural apple aromas. The concentrations of seven components (Table 4.1, numbers 2;3;4;5;6;8 and 9) matched the relative concentrations of these components in the saturated headspace of a bottle containing fresh apple juice {Bult, 2001 508 /id}. The other two odorants, diacetyl and ethyl 2-methylbutanoate are also found in apples {Maarse, 1989 219 /id}. They were added because of their distinct smells, and because they would increase the span of stimulus onset times. Their concentrations would produce intensities in the same range as the other components (see Table 4.1). The components were dissolved in 10.0 ml of n-pentane (4°C) at concentrations shown in Table 4.1, and stored at 4°C after preparation. Immediately before the start of a sensory experiment, 0.075 μL of this stock solution was

transferred to a glass tube containing Tenax (Tenax TA, 35/60 mesh; Alltech

Nederland, Zwijndrecht, the Netherlands), a granulated absorbent material.

Stimulus production

The injected components were thermally desorbed from Tenax at 260°C for 300 s

and cryofocused at -120°C by a cold trap/thermal desorption device (Carlo Erba

TDAS 5000; Interscience, Breda, the Netherlands). Components were separated on a

Table 4.1. Stimulus onset times, reported threshold concentrations in air and water of the odorants used, their mean sniffing port masses and respective standard deviations (SD) in the present study.

Peak index	Component name	Onset time [a]	Reported detection threshold concentration		In 10-ml stock solution	Mass at sniffing outlet [l]	
		(min)	Water (ppb vol/vol)	Air (ng/L)	(mg)	(ng)	(
1	Diacetyl	7.21	1.4[b]	5.0[b]	0.25	0.74	0
2	propyl acetate	11.25	-	200-7000[c]	4.44	13.32	0
3	isobutyl acetate	14.17	10[d]	1.7-17[c]	8.68	26.04	1
4	Hexanal	15.20	4.5[e]	30-53[f]	25.02	75.06	1
5	butyl acetate	16.40	66[g]	30-180[c]	39.69	119.07	0
6	trans-2-hexenal	18.30	17[e,g]	340[c]	21.15	63.45	0
7	ethyl 2-methyl butanoate	19.01	0.006-0.008[h,I]	0.1-0.3[j]	0.22	0.65	2
8	2-methyl-1-butyl acetate	20.48	5[g]	90-200[c]	35.04	105.12	0
9	hexyl acetate	25.68	2[g]	2.3[c]	43.50	130.50	5

[a]normalized to stimulus onset times of a selected reference session (see text); [b]{Hall, 1983 509 /id}3); [c](V Nettenbreijer, 1977), thresholds were mostly calculated from water concentrations in water-headspace sy employing either empirical or literature partition coefficients ; [d](Ong & Acree, 1998); [e](Buttery & Ling, 1 Schieberle, & Grosch, 1998); [g](Flath, Black, Guadagni, McFadden, & Schultz, 1967);[h](Takeoka, Buttery Dao, Edwards, & Berrios, 1998); [i](Takeoka, Buttery, Turnbaugh, & Teranishi, 1991); [j](Kollmannsberger 1992); [l]equals 40% of the total amount that was injected on column. [m]actual concentrations of inhaled co depend on the sniff vigor and, therefore, ratios are merely relative measures of odor impact.

DB1 column (J&W Scientific, 60 m × 0.25 mm i.d.; film thickness = 0.25 μm). Oven temperature was initially kept at 40°C (4 min), then increased to 75°C (3.0°C/min) and subsequently to 80°C (1.0°C/min). After a final increase to 272°C (15°C/min), oven temperature was kept at this temperature for another 5 min. This program allowed for an optimal separation of stimulus onset times. Column effluents were split. The ducts from splitter to sniffing outlets were kept at oven temperature to prevent condensation of volatiles. Of the total flow, 20% was directed to a Flame Ionization Detector (FID) while the two sniffing outlets each received 40%. Column effluents were mixed with humidified nitrogen at the sniffing outlet. On presentation, odorants are expected to have variable intensities, as may be inferred from the ratio of odorant masses at the sniffing outlet to their corresponding odor thresholds (Table 4.1).

Procedure

The experiment was part of a larger GCO study that was originally undertaken to investigate the effects of stimulus onset time and stimulus order on sensory evaluations. Originally, two ordering conditions were used, each comprising a different stimulus order and different stimulus onset times. Per subject, four sessions were randomly assigned to one ordering category and four other sessions to the other ordering category. In the present study, data were used from one SP session only, containing the same stimulus order for each subject. For each subject, one SF session was included to provide a reference for response behavior due to noise. This SF session could occur on any experimental day with the exception of the first day, at which only SP sessions were run. On average, the SF session occurred on day 4.0. One SP session was chosen at random from the four sessions with the same stimulus orders. In the group of sixteen subjects, this session occurred six times before a SF

session and ten times after a SF session, and occurred on average on day 4.2 in the sequence.

During a session, one or two subjects were seated with their nose positioned at the sniffing outlet connected to the GC. A blind prevented visual contact between subjects, who were not allowed to interact with each other in any way. To ensure that the solvent peak would not be inhaled, sniffing started 6 minutes after the initiation of the GC procedure. Sniffing analyses finished after 36 minutes. Hence, all subjects completed sniffing sessions of 30 minutes. The subjects were instructed to inhale slowly through their nose at an even pace and to exhale at a higher pace. In this way the dilution of sniffed odorants with the surrounding air was minimized while the net observation time was maximized. To respond as quickly as possible to stimulus events, the subjects chose one easy accessible key on the first day, usually the spacebar on the keyboard placed in front of them. Throughout the experiment, the subjects were instructed to strike this key at stimulus onset and again at stimulus offset. This procedure was chosen for the following reasons. First, it compels subjects to attach equal significance to the decision that a stimulus has started and to the decision that a stimulus has terminated. Second, this procedure is physically less fatiguing than when one key has to be pressed continuously until the stimulus stops. Finally, this method is less prone to registration errors, because it is easier to strike a key shortly than to keep it pressed continuously. In between the two keystrokes, visual feedback on the computer screen indicated that a response was being recorded. No cues as to the occurrence of a stimulus were given. After each second keystroke, the subjects had to rate odor quality by selecting descriptors from a list. These ratings are not analyzed in the present study. The registration of responses was electronically

synchronized with FID registrations. Room temperature was kept at 21°C. The air inside the room was ventilated and filtered.

Data analysis

Model parameters λ and μ were estimated according to Equations 3.8 and 3.9 for both SF and SP sessions. For SF sessions, these estimates could be calculated using all response events that were generated during the session. The SP data, however, were first trimmed by omitting all response intervals that coincided with stimulus presentations, as indicated by FID readings. Because subjects may persist responding after the stimulus itself has disappeared, we prolonged SP intervals by 20 seconds. Parameters of the QST model were then estimated on the basis of the responses given during the SF windows of the SP sessions. To illustrate the impact that stimuli have on parameter estimates, QST parameters were also calculated for SP sessions without trimming SP windows.

Although the sequential order of component peaks did not differ between sessions, the stimulus onset times of identical FID peaks varied slightly. This is due to small variations between sessions in oven temperature and column gas pressure. In the present study, the average absolute deviation from the mean stimulus onset time was 8.5 sec. Because subjects' response times for identical components will vary accordingly between sessions, responses may fail to coincide in time. To correct for effects of stimulus onset time variation on resulting response coincidences, we normalized the stimulus onset times and response times with respect to the session that showed the least cumulated deviation from mean stimulus onset times. This procedure was described in chapter 2 of this thesis.

Upper critical values of T_k (T_k') were calculated for all coincidence levels k at significance levels 0.05 and 0.01.

Results and Discussion

The tendency to initiate false alarms varied considerably over subjects, as can be concluded from the individual $\hat{\lambda}$ and $\hat{\mu}$ in Table 4.2. In SF sessions, five subjects did not respond at all, whereas the two most eager subjects responded 9 and 14 times, respectively. These two subjects were experienced panelists. In addition, closer inspection of the FID readings of the respective sessions showed no evidence of irregularities that might have caused these outcomes. Therefore, all data were used for the analyses.

Although subjects clearly varied in the number of false alarms, they showed fairly consistent intra-individual response behavior over experimental sessions. In general, high responders in the SF sessions were also high responders in the SF windows of the SP sessions, and vice versa, low responders in the SF sessions generally showed low response behavior in the SF windows of the SP sessions. Accordingly, the subjects' numbers of responses correlated significantly between the two experimental conditions (*Pearson r* = 0.52, p = 0.040). Because responses in the SF sessions and in the trimmed sections of SP sessions consisted of false alarms only, we attribute individual differences in onset rates to different decision criteria.

Colquhoun and Baddeley (1964) showed that after previous sessions with high stimulus probabilities, the initial decision criterion β in the current session was lower than after sessions with low stimulus probabilities. Furthermore, β gradually increased during the current session. The authors argued that this was caused by unfulfilled expectations of stimulus probability. The increasing response conservatism that follows from the gradual increase of β is seen as an adaptive response to the

decreasing anticipated stimulus probability in order to optimize response behavior. In the present study, the stimulus density dropped from 9 per SP session to 0 per SF session. Because SF sessions were preceded by 1 to 7 SP sessions and because the stimulus probability had been identical for all SP sessions, it is likely that subjects had strong *a priori* expectations of stimulus probability when they embarked on the SF session. In contrast to the study by Colquhoun and Baddely (1964), where changes of stimulus probability could readily be attributed to experimental conditions, subjects in the present study probably attributed the unexpected and complete absence of stimuli to their own inability to smell: Many subjects reported to be frustrated with their own smelling inability on completion of the SF session. Instead of increasing their β in response to externally attributed changes in stimulus frequency, subjects would have to decrease their β if they wanted their response frequencies to remain at the level of previous sessions in order to compensate for their alleged smelling inability. This would result in higher onset rates λ in SF sessions than in trimmed SP sessions.

Table 4.2. Estimates of onset rate ($\hat{\lambda}$) and offset rate ($\hat{\mu}$) for individual subjects and the panel, based on trimmed stimulus-present sessions (false alarms), stimulus-free sessions (false alarms) and non-trimmed stimulus-present sessions (all responses).

Subject	Trimmed stimulus-present (SP) Observation window = 17.74 min			Stimulus-free (SF) Observation window = 30.00 min			Stimulus-present SP) Observation win min	
	$\hat{\lambda}$ (min^{-1})	$\hat{\mu}$ (min^{-1})	Nr. resp	$\hat{\lambda}$ (min^{-1})	$\hat{\mu}$ (min^{-1})	Nr. resp	$\hat{\lambda}$ (min^{-1})	(min
1	0.115	6.08	2	0.067	13.57	2	0.206	6.
2	0.000		0	0.306	15.15	9	0.101	12
3	0.000		0	0.102	4.38	3	0.275	9
4	0.305	3.66	5	0.252	3.22	7	0.456	3
5	0.000		0	0.000		0	0.219	2
6	0.056	45.45	1	0.000		0	0.184	1
7	0.229	15.00	4	0.481	15.92	14	0.420	8
8	0.277	1.12	5	0.231	1.27	8	0.459	1
9	0.000		0	0.000		0	0.219	4
10	0.172	7.55	2	0.000		0	0.287	7
11	0.057	2.44	1	0.102	5.75	2	0.257	2
12	0.000		0	0.033	11.02	1	0.129	2
13	0.114	16.67	2	0.168	26.02	5	0.226	18
14	0.127	1.02	2	0.000		0	0.278	0
15	0.115	7.38	2	0.067	8.18	2	0.247	8
16	0.113	19.67	2	0.067	30.74	2	0.249	7
Panel [a]	**0.106**	**3.03**	**29**	**0.120**	**4.90**	**56**	**0.283**	**2.**

[a]Panel parameters were estimated by concatenating the results of the 16 panelists, as if one person performed was 16 times as long.

Whereas in stimulus-free vigilance conditions the response onset rate is primarily associated with the decision criterion β, the offset rate, being a measure of response length, does not have a direct counterpart in terms of SDT. A possible factor influencing the offset rate in the case of false alarms may be response confidence. Once an observer has initiated a response, he or she will continuously evaluate whether the stimulus is still present or even whether it has ever been present at all. Having lowered their decision criteria in response to an unexpected absence of stimuli

in SF sessions, subjects' response confidence may also drop. As a result, they would terminate responses (being false alarms) earlier in SF sessions than in trimmed SP sessions, resulting in higher offset rates in SF sessions than in trimmed SP sessions. In summary, both λ and μ are expected to be smaller in trimmed SP sessions than in SF sessions, implying that the average latency and the average response length are expected to be larger in trimmed SP sessions than in SF sessions. Furthermore, the presence of stimuli in the non-trimmed SP session is expected to increase the average response length (lower μ) and to decrease the average latency between responses (higher λ) in comparison with the trimmed session.

We tested individual values of λ and μ for these hypothesized effects of stimulus condition (trimmed SP, SF, and non-trimmed SP). To obtain metrically comparable measures suitable for analysis of variance, reciprocals of all observed individual λ and μ were calculated, yielding measures that reflect response latency and response length, respectively. Some subjects failed to generate false alarms in SF or trimmed SP sessions and, hence, did not produce estimates of response length (Table 4.2). As a consequence, effects of stimulus condition were analyzed by repeated-measures analysis of variance on complete data sets of the remaining 8 subjects. Where necessary, test results were corrected for deviations from sphericity by multiplying the numerator and denominator degrees of freedom with Greenhouse-Geisser's ε.

Significant condition effects were observed for both response latency {$F(2,14) = 6.04, p = 0.015$} and response length {$F(2,14) = 4.40, p = 0.037$}. As expected, panel values of λ and μ were lower for trimmed SP sessions than for SF sessions ($\lambda = 0.106$ vs. 0.120 and $\mu = 3.03$ vs. 4.90, respectively). However, contrasts comparing the reciprocals of λ and μ between trimmed SP sessions and SF sessions were not

significant for latency {$F(1,7) = 0.225$, $p = 0.650$} nor for length {$F(1,7) = 4.39$, $p = 0.074$}. Tests of separate contrasts showed that the significant overall test results could be attributed to the difference between the effects of trimmed SP and non-trimmed SP sessions on latency (corresponding λ are 0.106 and 0.283, respectively) {$F(1,7) = 10.12$, $p = 0.015$} and the difference between the effects of SF and non-trimmed SP sessions on response length (corresponding μ are 4.90 and 2.95, respectively) {$F(1,7) = 6.53$, $p = 0.038$}. Obviously, these effects are caused by responses to stimuli in the non-trimmed data.

It should be realized that the results of the repeated-measures analysis of variance do not provide the optimal conditions for demonstrating a differential influence of stimulus condition on QST noise models. Obviously, excluding 8 out of 16 subjects from the analysis has reduced the power of the test considerably. Also, the exclusion of non-responding panelists from the repeated-measures analysis causes a systematical underestimation of the average panel latency. A subject that generates zero responses during a SF period contributes as much to the estimate of panel QST parameters as a subject that responds at least once. Therefore, statistical tests of effects of stimulus presence on empirically obtained QST-model parameters are poor indicators of the effects on the sensitivity of QST tests of DFs.

DFs of the 16 panelists in the SP session are shown in Figure 4.1A, along with the stimulus onset times of the 9 stimuli. The corresponding DFs in the SF session are shown in Figure 4.1B. Simulations of sessions based on $\hat{\lambda}$ and $\hat{\mu}$ from trimmed SP sessions and SF sessions are shown in Figures 4.1C and 4.1D, respectively. On visual inspection, these simulation results are very similar to the empirical results from the SF session in Figure 4.1B, regardless of the stimulus condition in which the parameters were estimated. The SF session yields a maximum DF of 3. Hence,

according to the rule-of-thumb used in the DF method, DFs above 3 in the SP session are considered stimulus detections. Thus, 6 stimuli are detected, i.e. stimuli 1, 3, 4, 5, 7, and 8.

In terms of QST, the observed T_k and the estimates $\widehat{T_k}$ for trimmed SP and SF sessions are shown in Table 4.3. Observed T_k exceed QST estimates $\widehat{T_k}$ at all coincidence levels ($k > 0$) and for parameter estimates from both SF and trimmed-SP sessions. Upper critical values $\widehat{T_k}$ in Table 4.3 are estimated by equations 3.14 and the variance heuristic (equation 3.15) to test the significance of observed T_k and single sniffing peaks.

Table 4.3. Modeled cumulative time at response coincidence level k (T_k), the respective critical values of $\widehat{T}_k$ at significance levels 0.05 and 0.01, and observed times T_k at specified coincidence levels. Modeled $\widehat{T}_k$ and respective upper critical values are shown for model parameters estimated from stimulus-free and trimmed stimulus-present sessions.

coincidence level (k)	T_k (min) observed in non-trimmed stim-pres session[a]	Stimulus-present (trimmed); $\hat{\lambda} = 0.106$ (min^{-1}), $\hat{\mu} = 3.03$ (min^{-1})			Stimulus-free; $\hat{\lambda} = 0.120$ (min^{-1}), $\hat{\mu} = 4.9$ (min^{-1})		
		model $\hat{T}_k$ (min:s)[a]	Upper critical $\hat{T}_k$ at α=0.05 (min:s)[a]	Upper critical $\hat{T}_k$ at α=0.01 (min:s)[a]	model $\hat{T}_k$ (min:s)[a]	Upper critical $\hat{T}_k$ at α=0.05 (min:s)[a]	Upper critical $\hat{T}_k$ at α=0.01 (min:s)[a]
0	*15:08.2*	17:18.3	-	-	20:22.2	-	-
1	*09:27.9*	09:41.2	14:26.3	16:55.5	07:58.9	11:19.4	13:01.4
2	*02:00.9*	02:32.5	04:18.4	05:18.1	01:28.0	02:31.0	03:06.9
3	*01:01.4*	00:24.9	01:02.2	01:28.8	00:10.1	00:28.9 *	00:43.7 **
4	*00:36.4*	00:02.8	00:14.4 *	00:23.9 **	00:00.8	00:05.7 *	00:10.4 **
5	*00:32.6*	00:00.2	00:03.4 *	00:06.4 **	00:00.0	00:01.2 *	00:02.4 **
6	*00:26.7*	00:00.0	00:00.8 *	00:01.6 **	00:00.0	00:00.2 *	00:00.5 **
7	*00:12.6*	00:00.0	00:00.2 *	00:00.4 **	00:00.0	00:00.0 *	00:00.1 **
8	*00:18.2*	00:00.0	00:00.0 *	00:00.1 **	00:00.0	00:00.0 *	00:00.0 **
9	*00:14.3*	00:00.0	00:00.0 *	00:00.0 **	00:00.0	00:00.0 *	00:00.0 **
10	*00:01.0*	00:00.0	00:00.0 *	00:00.0 **	00:00.0	00:00.0 *	00:00.0 **
11	-	00:00.0	00:00.0	00:00.0	00:00.0	00:00.0	00:00.0
12	-	00:00.0	00:00.0	00:00.0	00:00.0	00:00.0	00:00.0

[a] $\wedge T_k = \widehat{T}_k$; * $p < 0.05$; ** $p < 0.01$

Figure 4.1. Response coincidences of 16 subjects plotted against experiment time. A) Empirical results of the stimulus-present condition: stimulus onset times are indicated by dotted lines. Index numbers correspond with peak numbers in Table 1. Individual responses were normalized before calculation of response coincidences. Therefore, the times used on the abscissa reflect stimulus events in the reference session (see text). B) Empirical results of the stimulus-free condition. C) Simulated results: example of a coincidence plot generated using $\hat{\lambda}$ and $\hat{\mu}$ values calculated from the stimulus-free windows in stimulus present sessions. D) Simulated results, example of a coincidence plot generated with $\hat{\lambda}$ and $\hat{\mu}$ values calculated from de results in stimulus-free sessions.
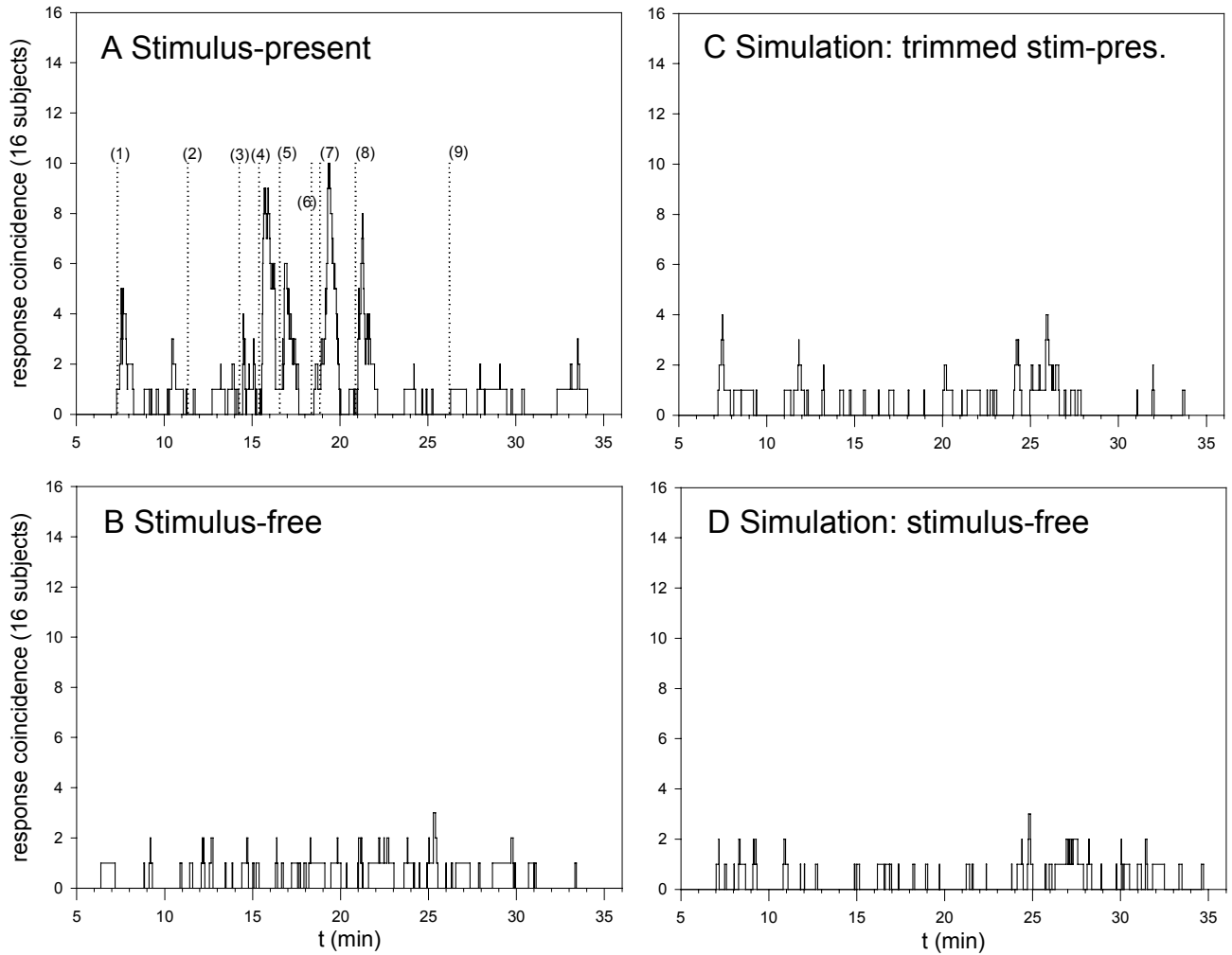
Statistical tests of sniffing task data by QST modeling

The observed panel onset- and offset rates (Table 4.2) produce Var($\widehat{P_k}$) values of

$0.73 \cdot \widehat{P_k} \cdot k^{-1}$ and $0.45 \cdot \widehat{P_k} \cdot k^{-1}$ for trimmed SP and SF sessions, respectively (Equation

15). The decreased variance estimates for SF sessions must be caused by the increased

offset rates because the increased onset rates could only have increased variance

estimates (see Equation 15). In general, changes in offset rates will affect the

calculation of variance by Equation 15 more than proportionally identical changes in

onset rates. As a consequence of the decreased variance estimates, critical values for

T_k based on SF sessions are smaller than critical values based on trimmed SP sessions

(Table 4.3). Smaller critical values of T_k promote the occurrence of significant effects.

Hence, parameter estimates from SF sessions lead to more liberal testing than

estimates from trimmed SP sessions. Due to this, $k = 3$ was the lowest coincidence

level at which the observed T was significantly above noise ($\alpha=0.05$ and $\alpha=0.01$) for

parameter estimates from SF sessions whereas trimmed SP sessions produced

significant T from coincidence level 4 and up (Table 4.3). Hence, QST noise models

indicate that subjects have been responding to at least one stimulus at coincidence

level 4 if trimmed SP sessions produced model parameters and at level 3 if SF

sessions produced model parameters.

Subsequent *post hoc* testing of partial T_k for separate sniffing peaks showed that

the significant results at $k = 3$ (SF sessions, $\alpha=0.05$ and $\alpha=0.01$) can be attributed to

stimuli 5, 7 and 8 (Table 4.4). When the noise model based on trimmed SP sessions

was used, stimuli 5, 7 and 8 were detected at $k = 4$ ($\alpha=0.05$) or at k values 5, 5 and 4

($\alpha=0.01$), respectively (Table 4.4). Stimuli 1 and 4 were detected at $k = 4$ and 5

respectively, regardless session context or significance level.

Summarizing, 5 out of 9 stimuli, i.e. 1, 4, 5, 7, and 8, were detected unambiguously because they coincided in time with significant sniffing peaks (Figure 4.1A). Four out of sixteen subjects responded simultaneously when stimulus 3 (isobutyl acetate) was presented. Nonetheless, the short $T_{k,i}$ of this sniffing peak did not result in a significant detection. This illustrates the elementary difference between the QST method and traditional DF methods. The DF methods test whether the observed coincidence level is high enough, whereas the QST method tests whether the *duration* of coinciding responses lasts long enough at a certain coincidence level. Thereby, it gives secondary importance to the coincidence level.

Of the other three stimuli, two remained undetected and the status of the third remains ambiguous. The failure to detect stimulus 2 (propyl acetate) can be attributed to its low concentration, as is suggested by the ratio of mass and detection threshold given in Table 4.1. Stimulus 9 (hexyl acetate) was not detected by the panel, although its mass/threshold ratio suggests a supra threshold concentration when compared to other components. The failure of a GCO panel to detect hexyl acetate was observed earlier (Bult et al., 2001). It was attributed to the masking effect of extraneous stimuli that may be generated by the apparatus at high oven temperatures, i.e. at relatively high stimulus onset times. These extraneous stimuli can be characterized as burnt smells that may either be caused by column bleeding at high oven temperatures or by the synthesis of volatiles and airborne particles in the casing of the GC oven. Hexyl acetate was released at an oven temperature of approximately 173°C, a likely oven temperature for extraneous stimuli to be observed. Stimulus 6 (*trans*-2-hexenal) seems to elicit only 2 responses. Since this component is released immediately before stimulus 7, it remains unclear whether some of the responses to stimulus 7 were in fact delayed responses to *trans*-2-hexenal. An analysis of the qualitative odor

descriptions might elucidate this. However, this would go beyond the objectives of the present study.

In the present study, five out of nine stimuli were significantly detected using QST modeling. Using the DF method, stimulus 4 would have been classified as detected as well, since this stimulus corresponds with a sniffing peak of 4 coinciding responses (Figure 4.1B), which exceeds the maximum DF of 3 in the SF session. This does not imply that the QST method lacks sensitivity, but rather that the traditional DF approach underestimates the probability that short peaks occur accidentally at low coincidences.

Table 4.4. Results of the *post-hoc* analyses of single sniffing peaks. Significant peaks are indicated by the indices of the coinciding stimuli (see Figure 4.1 and Table 4.1). Shown are (i) the lowest coincidence level k at which the total response time $T_{k,i}$ within that peak was significantly above chance level, (ii) the observed $T_{k,i}$, (iii) the critical value $T_{k,i}'$ that constitutes the minimal value of significant $T_{k,i}$ and the onset times and offset times of the sniffing peaks at corresponding incidence levels. Results are presented for tests based on QST parameters obtained from stimulus-present and stimulus-free sessions and for tests at $\alpha = 0.05$ and $\alpha = 0.01$.

Significant sniffing peaks at $\alpha = 0.05$

Index of coinciding stimulus	Stimulus-present					Stimulus-free				
	Lowest significant k	$T_{k,i}$ observed (s)	$T_{k,i}'$ (s)	T_{onset} (min:s)	T_{offset} (min:s)	Lowest significant k	$T_{k,i}$ observed (s)	$T_{k,i}'$ (s)	T_{onset} (min:s)	T_{offset} (min:s)
1a	-	-	-	-	-	4	3.3	1.7	7:33.4	7:37.3
1b	4	9.0	4.2	7:39.4	7:50.5	4	9.0	1.7	7:39.4	7:50.5
4	5	12.2	1.2	15:36.4	16:19.0	5	12.2	0.4	15:36.4	16:19.0
5	4	16.6	4.2	16:49.4	17:14.1	3	16.6	7.0	16:49.3	17:28.6
7	4	11.8	4.2	19:10.7	19:52.4	3	11.8	7.0	18:56.3	19:54.1
8	4	13.8	4.2	21:03.0	21:24.6	3	13.8	7.0	21:01.4	21:45.0

Significant sniffing peaks at $\alpha = 0.01$

Index of coinciding stimulus	Stimulus-present					Stimulus-free				
	Lowest significant k	$T_{k,i}$ observed (s)	$T_{k,i}'$ (s)	T_{onset} (min:s)	T_{offset} (min:s)	Lowest significant k	$T_{k,i}$ observed (s)	$T_{k,i}'$ (s)	T_{onset} (min:s)	T_{offset} (min:s)
1a	-	-	-	-	-	4	3.3	3.3	7:33.4	7:37.3
1b	4	9.0	7.3	7:39.4	7:50.5	4	9.0	3.3	7:39.4	7:50.5
4	5	12.2	2.4	15:36.4	16:19.0	5	12.2	0.9	15:36.4	16:19.0
5	5	4.4	2.4	16:49.4	17:05.7	3	16.6	11.6	16:49.3	17:28.6
7	5	11.0	2.4	19:10.7	19:47.9	3	11.8	11.6	18:56.3	19:54.1
8	4	9.6	7.3	21:03.0	21:24.6	3	13.8	11.6	21:01.4	21:45.0

General discussion

QST method

In the present olfactory vigilance experiment we showed that QST-modeling was

successful in modeling detection processes. We think that a noise model based on data

from SF sessions is not a good baseline for SP conditions. Critical values of $\widehat{T}_k$ based

on parameters obtained in a SF session were lower than those obtained in a trimmed

SP session. Furthermore, *post hoc* analysis showed that the lowest significant

coincidence levels of 3 out of 5 sniffing peaks were lower when SF sessions instead

of trimmed SP sessions were used to estimate the QST noise model (Table 4.4).

However, in the present study these differences would not have led to a different set

of significantly detected stimuli. Nevertheless, we propose that noise level estimations

for GCO-DF experiments should be based on trimmed SP sessions rather than on SF

sessions.

During SF sessions, the generation of unintended (extraneous) stimuli may cause

artificially high coincidence scores. Traditional DF noise estimates based on

maximum coincidence levels may be affected easily by such artifacts. In the present

vigilance study, coincidence noise levels were calculated from empirical estimates of

μ and λ at *a priori* significance levels. Although estimates of μ and λ from SP

sessions may also be affected by panelist's responses to extraneous stimuli, noise

estimates will be affected to a much lesser extent since these are based on global

parameters and not on incidental coincidence scores. As a consequence of the QST

model assumption that the occurrences of false alarms of separate observers are not

related temporally, actual coincidences are not processed but only latency and length measures are used. The approximately homogenous distribution of responses over the 30-minute sessions shows no evidence of extraneous stimuli in SF or SP sessions (Figure 4.1). Hence, however small the effect would have been, the QST noise model was not biased by extraneous stimuli in the present study.

Factors affecting decision criteria

For various modalities, it has been observed that the length of the time period that someone has already been performing a vigilance task correlates negatively with the frequency of both hits and false alarms: increased watch lengths cause lower response frequencies (Swets, 1977). This effect, which is referred to as the 'vigilance decrement', is generally attributed to an upward shift in the decision criterion β, an adaptive response of the subject to a stimulus probability that is lower than anticipated. No such tendency was observed in our SP- or SF sessions (Figures 4.1A and 4.1B). In stead, a rather stable response frequency was observed in both SF windows of SP sessions and in SF sessions. This absence of vigilance decrement may be explained by the fact that subjects were largely experienced in low-stimulus-incidence GCO tasks and, hence, were not inclined to adjust their expectations regarding stimulus probability.

The general observation that subjects tend to adjust their response criterion β to the observed stimulus probability suggests that observed discrepancies between expected- and observed stimulus probability are usually attributed externally – that is, to changes in the actual stimulus probabilities. In the present study, the subjects might have attributed discrepancies internally – that is, to weak sensory abilities, if strong expectations of stimulus frequency preexisted. This hypothesis is supported by two other vigilance studies. Williges (1969) made subjects believe that stimulus

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frequencies were five times higher or lower than the actual stimulus frequency. Subjects adapted their β to the believed stimulus frequency. Despite the experienced discrepancy between expected and observed stimulus frequency, subjects maintained stable β throughout the session. In a visual vigilance study, Sullivan and co-workers (Sullivan, Warm, Schefft, Dember, O'Dell, & Peterson, 1998) reported a drastic increase of false alarm percentage for brain injured patients in the final phase of the task. In contrast, a control group showed the usual vigilance decrement throughout the task. The authors suggested that the increased false alarm rate of the brain injured was due to an internal attribution of the discrepancy between perceived- and expected stimulus probability. Hence, the strategy of β adjustment may indeed depend on whether stimulus probability discrepancy is attributed internally or externally.

Olfactory vigilance

The methodology discussed here relates to continuous vigilance tasks, implying a continuous observation interval. In visual and auditory tasks this is readily realized by asking a subject to watch a screen constantly or to listen to an audio-speaker. Although odors can be presented at any time on a continuous time scale, they cannot be observed continuously. The repetitive breathing pattern of an observer imposes observation intervals on continuous presentations. Inevitably, olfactory presentations are discrete and self paced by the observer. However, we made an effort to minimize detrimental influences of breathing patterns by giving breathing instructions. The aim of these instructions was to reduce the relative length of the exhalation phase and to increase the relative length of the inhalation phase to achieve, at best, nearly continuous observations.

A second point that needs to be considered with respect to the use of olfaction in vigilance tasks is that its adaptive function may be fundamentally different from the adaptive functions of vision and audition. Whereas vision and audition permanently guide us in our interactions with our environment, olfaction will often only ask for attention in cases in which immediate action is desirable. The latter involves mental states that tend to be associated with increased vigilance. In animal studies, it was shown that behavioral responses indicating increased vigilance were invoked by unannounced unconditioned presentations of odors signaling either a threat or the presence of food (Dielenberg, Carrive, & McGregor, 2001; Jones & Roper, 1997; Terlouw, Boissy, & Blinet, 1998). In two visual detection studies with human subjects, the presentation of odorants raised vigilance as was concluded from the increased hits/false alarms ratios (Sullivan et al., 1998; Warm et al., 1991). Results from the studies by Warm *et al.* and Sullivan *et al.* suggest that overall vigilance during olfactory tasks may be higher than in auditory or visual tasks. This may explain why no vigilance decrement was observed in this study. Although this failure to find a decrement in vigilance does not affect the data analysis, it may cause differences in typical results of vigilance tasks.

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5 Sensory Evaluation of Character Impact Components in an Apple Model Mixture

Abstract

Food aromas generally are complex mixtures of volatiles. In the present study, we investigated the joint effects of hexyl acetate, *trans*-2-hexenal and 1-hexanol on the multi-attribute perception of an apple aroma. The first two substances were identified earlier as positive contributors to the apple aroma (high character impact), whereas the third component was identified as an irrelevant or negative contributor (low character impact). Aroma quality was quantified using a set of eight graphic rating scales. All three components had significant effects on the aroma profiles. These effects consist mainly of an effect of each component on the attribute that described its individual character and an effect of all three components on ratings on the main character attribute 'apple'. As expected, the high impact components increased 'apple' ratings, whereas the low character impact component decreased 'apple' ratings. Furthermore, intensity ratings on the attribute that corresponded with the odour of the low impact component were suppressed by the presence of high impact components. These results indicate that the contributions of odorants to the mixture's aroma are not linear combinations of separate odour intensities, because sensory interactions were observed. In addition, humans detect components in complex mixtures more accurately than studies on identification performance have suggested. We conclude that for an adequate assessment of the effects of multiple mixture components on changes in aroma perception, it is sufficient to employ multiple response scales measuring intensities of attributes that are distinctive with respect to the expected

qualitative changes. Results of this approach should be subjected to multivariate

methods of statistical analysis.

Introduction

Food aromas generally comprise extensive mixtures of volatile constituents. A large number of these constituents produce odours if presented alone at similar concentration levels. Others, however, might not produce noticeable odours at all. An important objective in aroma research is to minimise the number of components in a modelled aroma by selecting those volatile components that contribute most to the original aroma. In general, this selection is made on the basis of two sensory properties: the relative perceived intensity of that component presented in isolation and the extent to which its character resembles the quality of the particular food aroma. Both higher perceived intensity and higher typicality of a component's odour quality result in a higher *character impact* of the component on the mixture's aroma (Buttery and Ling, 1998).

Character impact components (CICs) are usually identified by sensory analysis of mixture constituents after decomposing the mixture by gas chromatography (Dravnieks and O'Donnell, 1971). The constituents are evaluated with regard to their unmixed odour qualities and intensities. In doing so, one disregards that the contribution of an odorant to the mixture's aroma depends not only on its sensory characteristics when presented in isolation, but also on sensory interactions that occur when the odorant is perceived in the presence of other components.

Perceptual mixture interactions

In olfaction, partial mutual masking of mixture components is the most commonly observed interaction, even in mixtures consisting of as few as 2 components (Cain, 1975; Laing and Willcox, 1987; Lawless, 1987; Laing *et al.*, 1994). If one knows a component's psychophysical function, which relates component concentration to its

perceived intensity, the intensity of a 'mixture' of that component with itself can be predicted from the sum of the two respective concentrations. However, the intensities of binary mixtures of two different supra-threshold components are often lower than expected on basis of their respective psychophysical functions (Moskowitz and Barbe, 1977; Berglund and Olsson, 1993a; Berglund and Olsson, 1993b). On the other hand, indications of synergetic effects were observed when mixing sub-threshold components (Guadagni *et al.*, 1963; Laska and Hudson, 1991).

The mutual perceptual suppression of odorant intensities in multi-component mixtures is often observed (Moskowitz and Barbe, 1977; Moskowitz, 1979). Cain (Cain, 1975) hypothesised that the masking power of supra-threshold odorants is positively related to either the chemical or the perceptual complexity of the masker. In the taste modality, the masking power of two substances in concert was indeed observed to be larger than the masking power of each of the substances alone (Stevens and Traverzo, 1997). In the case of olfaction, Laing and co-workers demonstrated that humans perform increasingly worse with increasing numbers of masking components when they are asked to identify odorous constituents in mixtures (Laing and Francis, 1989; Laing and Glemarec, 1992; Livermore and Laing, 1996; Jinks and Laing, 1999). Even the seemingly easy task of identifying the qualities of odorous constituents in binary mixtures yields probabilities of correct detections far below perfection (Olsson, 1994). A similar relationship between mixture complexity and masking power was observed when the mixture components themselves were multicomponent mixtures, each mixture representing a familiar object odour (Livermore and Laing, 1998).

Although humans experience great difficulties in recognising the contribution of single components to the aroma of complex mixtures, they are able to discriminate

between complex mixtures of odorants that are identical except for one component (Laska and Hudson, 1992). This can be explained by assuming that some or all of the odorants in a complex mixture blend perceptually into an intrinsically new aroma (Livermore and Laing, 1998). The omission of components from a complex mixture may then be detectable as a change in aroma quality, but not as an omission as such. Food aromas as well as many other object-related aromas generally consist of complex mixtures of odorants that, nonetheless, are perceived as homogeneous aroma blends. It is therefore, rather speculative to assume that omitting components from a mixture would only affect the perceived intensity of their respective characters in the aroma of the mixture.

In recent years, scholars at the 'Deutsche Forschungsanstalt für Lebensmittelchemie' have recognised the relevance of studying the contribution of CICs *in* the mixture. In a number of studies they evaluated the impact of components on mixture aroma by assessing the effect of omitting these from the mixture (Blank *et al.*, 1992; Guth and Grosch, 1994; Schieberle and Hofmann, 1997; Reiners and Grosch, 1998). In a study on the aroma of french fries (Wagner and Grosch, 1998), the authors determined the components with high ratios of mixture concentration versus detection threshold concentration, called odour activity values (OAVs). Omitting these supra-threshold components from a model mixture often resulted in a significant discrimination of the aromas of the reduced versus the complete mixture. When reduced and complete mixtures were significantly perceived as different, panellists characterised the aroma qualities of the mixtures by rating intensities of attributes describing the odours of mixture components. In this study, omitting the component with the second highest OAV from the mixture was not detected in the discrimination task. After subsequent omission of additional components, however, a

number of panellists gave higher ratings on the attribute describing the component with the second highest OAV. This post-hoc evaluation suggests that this component was perceived only after it had been released from suppression due to a number of masking components. Indications for 'release from suppression' effects were also found in similar studies on an Arabica coffee model (Czerny *et al.*, 1999) and a white wine model, as reviewed by Grosch (Grosch, 2000).

Evaluating aroma differences

The effect of component concentration on aroma quality can be quantified by measures of discriminability between mixtures, by similarity ratings, or by ratings on attributes describing the aromas. However, these methods vary with respect to their sensitivity to differences in aroma quality and their ability to characterise aroma quality.

If one only wishes to test hypotheses with respect to perceptual discriminability of stimuli, discrimination tasks may suffice. Trials in discrimination tasks yield binary-scaled results: a subject either does, or does not distinguish correctly between differing stimuli. Proportions of correct stimulus discriminations can be calculated from repeated stimulus comparisons and are tested against the expected chance proportion of a correct discrimination. A psychophysical application of this method conceives the probability of correctly detecting a difference between stimuli as a measure of the sensory difference between stimuli (Thurstone, 1927; Frijters, 1980). It expresses sensory difference as the perceptual distance between stimuli on an arbitrary sensory continuum. Such measures of perceptual distance have been used to detect mixture interactions (Lawless and Schlegel, 1984).

Similarity ratings are used to measure the degree of sensory similarity between stimuli using discrete or continuous rating scales. Hence, the rated dissimilarity of stimuli can be conceived of as a measure of the distance between stimulus representations on an arbitrary sensory continuum. This sensory continuum may either represent a quality continuum or an intensity continuum, depending on the nature of the difference between stimuli.

Although discrimination tasks and similarity ratings are sensitive in detecting differences in both intensity and quality, they do not allow for semantic interpretations of results in terms of odour quality characterisations. Therefore, to study the qualitative nature of sensory interactions, methods that directly address odour quality are needed. Attribute ratings reflect perceived intensities of odour characteristics indicated by odour quality descriptors. This makes attribute ratings an adequate tool to study and describe mixture interactions in both qualitative and quantitative terms. However, when the descriptor set is not distinctive with respect to the characteristic on which the stimuli differ (Callegari *et al.*, 1997), attribute ratings may have less discriminative power than similarity ratings. This may explain why Lawless and Schlegel (Lawless and Schlegel, 1984) found a taste-odour interaction in mixtures with variable sucrose and citral concentrations when using sensory distances calculated from discrimination task results, whereas no interaction was observed when attribute ratings were used. Intensity ratings on the attributes 'sweetness' and 'lemon odour' were merely statistically additive for the used stimulus set. In a meta-analysis, Callegari *et al.* showed that 25 to 30 distinctive descriptors are needed to cover the perceptual space for olfaction alone. Therefore, in the cross-modal study of Lawless and Schlegel, a set of two descriptors may have lacked the discriminative power needed to measure interactions. Dravnieks and colleagues (Dravnieks *et al.*,

1978) showed that over different panels, similarity ratings were at least as consistent as measures derived from attribute ratings. Summarising, discrimination tasks and similarity ratings may be more sensitive or reliable methods to detect mixture interactions than attribute ratings, especially if selected descriptors are not distinctive. Nevertheless, the latter are to be preferred if the qualitative nature of these interactions should be assessed, provided that these are distinctive with respect to the characteristics on which stimuli differ.

Where no perceptual blending occurs in a mixture of odorants, we expect that the effect of changing a constituent's concentration in that mixture is best reflected by ratings on the component's corresponding descriptor. Panellists can generate these descriptors on presentation of the unmixed odorants, in which case these odorants can be used as standards to train panellists on the use of descriptors. This helps to align panellists' conceptual representations of attributes and, hence, may improve consistency of panel responses (O'Mahony, 1991; Lesschaeve and Sulmont, 1996). A descriptor set so designed may include as many descriptors as there are components in the mixture, which will often be less than the number of 25 – 30 recommended by Callegari *et al.* (Callegari *et al.*, 1997). Although a small selection of attributes covers merely a part of the olfactory universe, we argue that it is still a sensitive tool for describing the interactions in the mixture under investigation if component-derived descriptors are used.

Statistical interaction versus sensory interaction

When systematically manipulating the presence of a number of components in a mixture according to a factorial mixing design, one faces the task of deriving sensory mixture interactions from factorial plots of intensity measures. Note that this is not identical to identifying statistical interactions in the factorial plot. In general,

statistical methods assume linear models relating dependent variables to independent variables. Psychophysical studies, however, have shown that the relationship between stimulus concentration and its perceived intensity rarely approaches linearity, but generally yields negatively accelerating curves fitting power functions with exponents ranging from 0.1 to 1.0 (Stevens, 1961; Cain, 1969; Baird *et al.*, 1996). It can be shown that, due to the non-linearity of psychophysical functions, factorial mixing plots of two-component mixture intensities will generally show converging lines, even when the 'constituents' are the same substance (De Graaf and Frijters, 1988). This may lead to a statistically significant interaction, while no sensory interaction is present. Only in those rare cases where the factorial mixing plot shows a set of diverging lines, a statistical interaction effect supports a sensory interaction: a case of extremely strong synergism (De Graaf and Frijters, 1988; Schifferstein and Frijters, 1990; Schifferstein, 1995). In addition, sensory interactions are evident when a component suppresses an aspect of the mixture's aroma to which the component does not itself contribute. This will appear as a significant, negative statistical main effect and/or interaction effect of the suppressing component on intensity ratings. In the latter case, the suppressive effect does not necessarily coincide with a significant statistic interaction although it does concern a sensory interaction. In this paper we will first report the outcomes of statistical tests and, subsequently, discuss these outcomes in terms of their implications with respect to sensory interactions.

The present study

In the present study, we investigated whether and how sensory interactions affect the perception of CICs in a complex mixture of odorants that observers recognise as a natural food aroma. To study the contribution of different CICs, we omit CICs systematically from the mixture. If a component's odour does not blend into an aroma

at all, its impact can be measured using intensity ratings on its respective quality descriptor. Suppression of this component's intensity by other components can be measured accordingly. If, on the other extreme, all components contribute to one aroma blend, the main character descriptor of the aroma can be used to measure the impact of constituent components. The omission of a CIC should then reduce intensity ratings on the main character descriptor. In order to be able to describe aromas with different degrees of blending, we used a detailed aroma-profiling task involving both single component descriptors and a main character descriptor. If components contribute to the main aroma character and also remain individually distinguishable, effects on both the main character descriptor and on specific quality descriptors will be observed.

Generally, food aromas are elicited by odorous components of varying odour intensities. Intensity is likely to be an important factor influencing the impact of an odorant on the mixture's aroma. In the present study, however, we wish to study processes involving odour *quality* only. To eliminate the effect of odour intensity, we matched the intensities of the three unmixed components under investigation before evaluating their effects in a multi-component model solution. These three components were selected according to their expected character impact: two components rated high on the target quality and one component rated low on this target quality.

Pilot Study

Materials and Method

Subjects

Eighteen paid volunteers, five men and thirteen women, served as subjects. They were recruited from the local Wageningen community and were selected on the basis of their ability to generate and use refined odour attributes. In addition, they showed high inter-subject coherency in the use of graphic rating scales. This implied that subjects generated inter-subject-correlating profiles when they rated various aroma intensities. All subjects were experienced olfactory panellists (Bult *et al.*, 2001) but they were naïve with respect to the objectives of the experiment. Their ages ranged from 19 to 51 (average 29 years). All were non-smokers and none had any history of olfactory dysfunction. Subjects were in good health during the experiments. All gave written informed consent.

Stimuli

The aroma model that is used in this study was derived from a headspace sample of fresh apple juice earlier at this laboratory. Although the model consists of a limited number of components, it was recognised and described as apple by 13 out of 23 subjects upon presentation of the olfactory stimulus and without any extra information being given (Bult *et al.*, 2001). As identification performance for many common odours is approximately 50% (Cain, 1979), this model was deemed appropriate for the present study. Although we expect that more authentic apple aromas can be made, the aroma model in the present study may validly be assumed to represent a recognisable food aroma.

An apple reference stimulus was prepared from ten components. Its composition was largely identical to that of the original apple mixture (Table 1). However, ethanol was excluded from the original mixture because it was of sub-threshold concentration, even after substantially increasing its concentration. Pre-testing revealed that the ethanol component did not induce any consistent olfactory sensations. Furthermore, the propyl propanoate concentration in the mixture was raised by a factor 5 to enable a more accurate stimulus preparation, thus improving stimulus reliability. Since this component still had a low intensity in the given concentration, we assume that this alteration did not have a significant impact on the character of the mixture aroma.

In addition to the mixture, ten one-component stimuli were prepared from each of the ten components in the apple model mixture. To obtain equi-intense stimuli, the concentration of each singular solution as well as the concentrations in the complete apple mixture were raised, so that all intensities matched the sensory intensity of an 80 µL/L solution of *trans*-2-hexenal. This was done in a preliminary study employing 4 faculty members. The resulting composition of the apple reference stimulus is given in the sixth column of Table 1. The concentrations of the singular solutions are given in the fourth column of Table 1. Mixtures and single component dilutions were prepared using distilled water. All stimuli were prepared at least 2 hours and not earlier than 26 hours before presentation. Stimulus solutions were stored in the dark at 4 °C and were presented at ambient temperature (21 ±1°C).

Table 5.1. Substances used for the stimuli with their nominal purities and concentrations

Component	Nominal purity (%)	Attributes generated by the sensory panel (translated)	Concentration in water in pilot study (μL/L) mixture	Base (base) or additional component	Concentration in water for apple reference aroma in pilot study (μL/L)	
1-butanol[c]	~	sour dairy	20	base	15	
2-methyl-1-butyl	>94	sour hardboiled	12	base	7.5	1
butyl acetate[c]	>99	nail polish	40	base	12.5	
hexanal[c]	>98	macaroon[2] hedge	25	base	12.5	
isobutyl acetate[a]	>99	sweet lacquer	20	base	0.75	1
propyl acetate[b]	>99	fruity acetone	12	base	2.5	
propyl propanoate[c]	>99	spicy eggnog	20	base	2.5	
1-hexanol[c]	>98	nuts musty	20	add	10	
hexyl acetate[a]	>99	pear apple	15	add	7.5	1
trans-2-hexenal[b]	>99	bitter sweet gum	80	add	40	

Obtained from: [a]Aldrich, [b]Janssen Chimica and [c]Merck.

[1]Sour hardboiled candy is a popular sweet in the Netherlands where it is referred to as 'zuurtjes'.

[2]Macaroon is a cookie that has bitter almonds as its major flavour.

[3]1-Hexanol was present in both 20μL/L and 300μL/L concentrations (see text).

Procedure

Stimuli were presented in 200 mL glass jars, closed by a low-odour plastic screw cap, which could be opened by one simple twist. Each jar contained 10 mL of solution. To prevent volatile components from migrating from the screw caps to the headspace, these two phases were separated with aluminium foil. The subjects had to open each stimulus jar by unscrewing the cap while keeping the jar just underneath their noses. Responses were to be given after taking a few short sniffs.

In the first session, the subjects generated odour attributes individually for all 10 unmixed stimuli. In the second part of this session, these attributes were discussed in a

plenary meeting of all subjects. Consensus on the use of attributes was reached after plenary consultation in the second session.

At the start of the third session, the model mixture with the apple aroma was presented as the reference stimulus for 'apple' aroma. Subsequently, the 10 singular component solutions were presented. Of each solution, subjects rated the intensity of its 'apple' character on 150 mm scales printed on paper, labelled 'no apple' at the left end and 'very much apple' at the right end. The two components that scored highest and the one that scored lowest on 'apple' were selected as respectively 2 CICs and 1 non-character impact component (non-CIC). Note that the definition of character impact used here is based on quality rather than perceived intensity, since all stimuli were approximately equally intense.

Statistical analysis

Ratings for all the ten stimuli were collected within subjects. To compensate for idiosyncratic scale usage, attribute ratings were normalised to obtain equal means and standard deviations for each subject. For convenience, the complete data set for the group of subjects was transformed linearly in order to obtain a group score range from 0 to 100. This resulted in an average 'apple' value of 39.1 (SD = 22.4) for every subject, over ten evaluated samples. Throughout the paper we used SPSS, version 7.5.2 (1997) for data analyses and 0.05 as the level of significance.

Results

Averaged normalised 'apple' scores (± SEM) are plotted for each component in Figure 1. Analysis of variance revealed a significant effect of Component on 'apple' responses [$F(9,179) = 10.9, p < 0.01$]. Subsequent post hoc testing, using Duncan's multiple range statistic, showed that the ten 'apple' scores could be grouped in four

Figure 5.1. Normalised (0-100) apple ratings for 10 equi-intense components, expressed as mean (± SEM) transforms of 150mm scale ratings. Components are grouped in homogeneous groups according to the results of Duncan's multiple range test on 'apple' ratings. Identical letters indicate homogeneous groups.
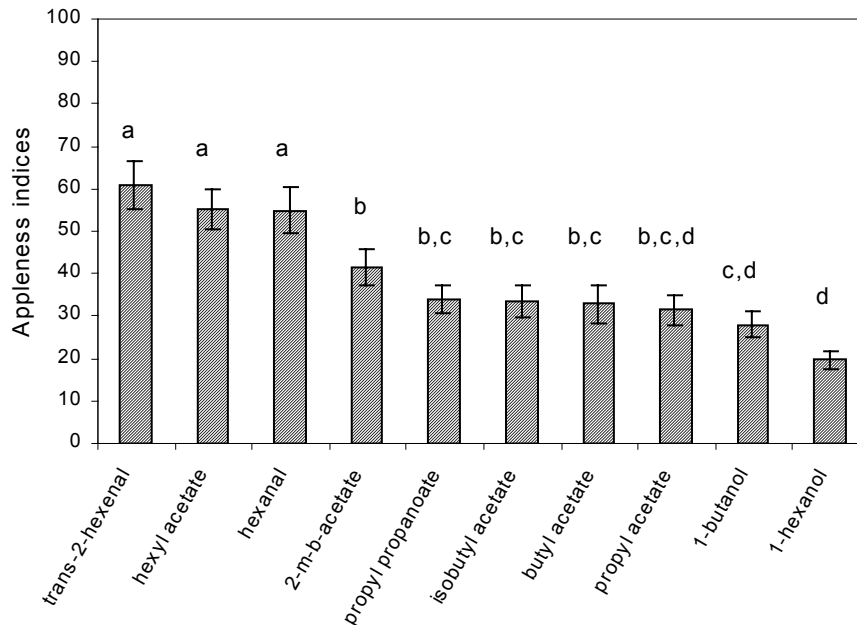
clusters of not significantly different means (Figure 1). From the cluster of

components rated highest on 'apple', hexyl acetate and *trans*-2-hexenal were selected

as CICs, whereas 1-hexanol was selected from the lowest ranking cluster as a non-

CIC.

Main Experiment

Materials and Method

Subjects

Eighteen paid volunteers, five men and thirteen women, served as subjects for the

main experiment. This group was identical to the group described in the pilot study,

except for one female subject who was replaced by another female subject. The new

subject met the criteria for admission to the panel as described for the pilot study.

Ages ranged from 19 to 51 years and the average age was 30 years.

Stimuli

Ten different stimulus mixtures were prepared from the ten selected components

by systematically adding combinations of the two CICs and the non-CIC to a base

solution of 7 components in distilled water. Concentrations of the base solution

components and the additives are given in the last column of Table 1. The three

additives – *trans*-2-hexenal, hexyl acetate and 1-hexanol – were either added in

singular, binary or ternary combinations, thus resulting in 2 (presence of hexyl acetate

= HYL) × 2 (presence of 1-hexanol = HOL) × 2 (presence of *trans*-2-hexenal = HAL)

= 8 mixtures (Table 2). To match the unmixed intensities of hexyl acetate and *trans*-2-

hexenal, the 1-hexanol component concentration had been increased to 300 μL/L

relative to its original 20 μL/L concentration in the apple mixture. In addition, two

mixtures containing the original – lower – concentrations of 1-hexanol were included

in the stimulus set. One mixture consisted of the base mixture with only the low

concentration 1-hexanol added (nr 9). The other also contained the two CICs hexyl

acetate and *trans*-2-hexenal (nr. 10, the original apple aroma).

Procedure

Table 5.2. Composition of stimulus mixtures derived from an apple model mixture.

	Mixture composition (x= present)								
constituents	1	2	3	4	5	6	7	8	9
base mixture	x	x	x	x	x	x	x	x	x
trans-2-hexenal (80 ppm)		x			x	x		x	
hexyl acetate (15 ppm)			x		x		x	x	
1-hexanol (300 ppm)				x		x	x	x	
1-hexanol (20 ppm)									x

Mixture #1 constitutes the fully 'stripped' apple aroma while mixture #10 represents de original apple aroma.

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Subjects performed a descriptive analysis of the aromas of the 10 different stimuli. The attributes that were generated for the 10 unmixed substances were reduced to 7 by letting each subject select three attributes that they considered the least appropriate descriptors for the full apple mixture. The attributes that were selected most frequently were discarded. In addition, an 'apple' attribute was included. Consequently, the eight attributes used in de descriptive analysis were 'sour hardboiled candy - glue'; 'macaroon - hedge'; 'sweet - lacquer'; 'fruity - acetone'; 'nuts - musty'; 'pear - apple'; 'bittersweet - rum' and 'apple'. The attribute names are translations of the Dutch terms used. Reference stimuli for the eight attributes (the apple mixture plus the respective components at concentrations identical to the pilot study) were presented prior to every experimental session in order to refresh odour-attribute associations. The use of the attributes 'apple' and 'pear-apple' may seem confounding because of their similarity. Subjects, however, perceived the respective qualities differently (see also Figures 2 and 3) and they considered these attributes the most appropriate for these aromas.

Stimulus preparation and presentation proceeded as described in the pilot study. One session lasted 40 to 50 minutes. Laboratory conditions conformed to the ISO 8589 standard (International Organization for Standardization, 1987). During a session, subjects were seated in separate booths. The uniform stimulus jars were coded with randomly generated three-digit codes and they were presented in random order, each individual receiving a separate order. Subjects were instructed to rate attribute intensities on eight linear 150 mm graphic rating scales that were presented on a laptop computer screen (Compaq Contura 80386 DX 25 MHz with monochrome display), using the left button of a two-button computer mouse. Between two stimuli, they waited for at least one minute, which was computer-paced. After completing 2

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training series in the first session, subjects completed 9 experimental series of 10 stimuli each in three separate sessions. Consequently, 9 evaluations of every distinct stimulus were recorded for every subject.

Statistical analysis

Data from the training sessions were discarded. Since no significant systematic changes in responses over sessions were observed, ratings were averaged over the nine repeated experimental sessions. Thus, data analyses were performed on averaged intensity scores on 8 attribute variables for 10 different stimuli per subject.

Perceived aroma quality is reflected in the aroma profile. This does not imply that specific alterations of single component concentrations are reflected exclusively in attribute ratings of their respective accompanying attribute. Therefore, we initially tested for differences between complete profiles due to stimulus composition by doubly multivariate repeated measures ANOVA (Stevens, 1996). These analyses permit simultaneous multivariate analyses of results on a set of dependent variables according to a repeated-measures design. The approach of initially performing a multivariate analysis also guards against spurious effects due to the increased overall significance level that results from performing successive univariate tests. Since the experiment had a fractional factorial design (10 categories from a 12 category full factorial design), the analysis was split into two consecutive multivariate analyses. First, CIC and non-CIC effects were tested in a 2×2×2, HOL (not present vs. high concentration) × HYL × HAL, design. Subsequently, the influence of all three 1-hexanol concentrations (not present – low concentration – high concentration) and the presence or the absence of both *trans*-2-hexenal and hexyl acetate was tested in a 3 (HOL) × 2 (HYLHAL) design.

Any effects in the multivariate space indicate that aroma changes are perceived due to the addition or the omission of the CICs and the non-CIC. However, they do not give insight into the qualitative nature of the differences perceived. Because results on single attribute ratings may elucidate this in part, we proceed with an analysis of single attribute data by univariate repeated measures ANOVA, as a post-hoc test after significant multivariate effects are found. Multivariate F-values were calculated according to Pillai's trace criterion.

Results

The multivariate effects of CICs and the non-CIC on the eight attribute ratings in the HOL $\times$ HYL $\times$ HAL analysis were found significant for HOL [$F(8,10) = 4.80$, $p=0.012$] and HAL [$F(8,10) = 13.12$, $p<0.001$], whereas HYL [$F(8,10) = 2.48$, $p=0.090$] failed to reach significance. A significant multivariate HOL $\times$ HAL interaction was also observed [$F(8,10) = 4.73$, $p=0.013$]. No three-way interaction was found. The HOL3 $\times$ HYLHAL analysis of all three 1-hexanol levels yielded significant multivariate effects for HOL3 [$F(16,56) = 2.07$, $p=0.023$] and HYLHAL [$F(8,10) = 3.97$, $p=0.023$]. The HOL3 $\times$ HYLHAL interaction was also significant [$F(16,56) = 2.16$, $p=0.017$].

Univariate repeated measures ANOVAs were performed for the main- and the two-way interaction effects that were significant in the multivariate analyses. Effects were found on four out of eight dependent variables. All effects of component presence on aroma that were found in the multivariate analysis had counterparts in one- or several of these univariate effects. These univariate effects, therefore, appear to explain the multivariate effects. Hence, further discussion of results will be restricted to the four dependent variables that showed significant effects. Table 3 shows the ANOVA

results for the HOL ´ HYL ´ HAL design grouped for each separate dependent

variable and specified for separate sources of variance. Apple ratings, that reflect

character impact of the three components in the mixture, show significant main

effects of HOL, HAL and HYL. No interactions were found with respect to 'apple'

ratings. In Figure 2A, the effects on aroma quality are illustrated. As may be expected

on the basis of the nature of character impact components, addition of the CICs (HYL,

HAL) to the base mixture increased 'apple' ratings. The addition of the non-CIC

(HOL) decreased 'apple' ratings.

Table 5.3. Repeated measures ANOVA of HOL × HYL × HAL.

Attribute	Source	$d.f._{effect}$, $d.f._{error}$	F	p
Apple	HOL	1, 17	18.67	<0.001**
Apple	HYL	1, 17	7.16	0.016*
Apple	HAL	1, 17	16.92	0.001**
Apple	HOL × HAL	1, 17	0.03	0.865
Nuts – musty	HOL	1, 17	34.21	<0.001**
Nuts – musty	HYL	1, 17	15.81	0.001**
Nuts – musty	HAL	1, 17	21.26	<0.001**
Nuts – musty	HOL × HAL	1, 17	28.62	<0.001**
Pear – apple	HOL	1, 17	9.38	0.007**
Pear – apple	HYL	1, 17	10.61	0.005**
Pear – apple	HAL	1, 17	1.05	0.319
Pear – apple	HOL × HAL	1, 17	3.07	0.098
Bittersweet – rum	HOL	1, 17	0.04	0.836
Bittersweet – rum	HYL	1, 17	2.52	0.131
Bittersweet – rum	HAL	1, 17	4.59	0.047*
Bittersweet – rum	HOL × HAL	1, 17	1.94	0.182

Only the univariate results of the four attributes with significant effects are shown (* $p<0.05$; ** $p< 0.01$)

Most pronounced were the effects on the 'nuts - musty' ratings, which were significantly affected by all three components. The 'nuts - musty' attribute describes the character of the 1-hexanol component, which is reflected by significant higher 'nuts-musty' ratings at high HOL levels (Table 3, Figure 2B). Furthermore, a

significant HOL × HAL interaction is found for 'nuts-musty' ratings. This interaction appears to be responsible for the multivariate HOL × HAL interaction found, since it is the only univariate interaction effect. It can be attributed to a masking influence of HAL on the 'nuts-musty' character introduced by HOL. The presence of *trans*-2-hexenal does not affect 'nuts-musty' ratings when 1-hexanol is not present. If 1-hexanol is present, however, the addition of *trans*-2-hexenal to the mixture suppresses the 'nuts-musty' character drastically (Figure 2B). Likewise, HYL, the other CIC, appears to exhibit a masking effect on the 'nuts-musty' character. Although the HOL × HYL interaction was not statistically significant, HYL had a significant main effect (Table 3) on 'nuts-musty' ratings comprising a decrease in 'nuts-musty' ratings due to HYL (Figure 2B).

HYL had a significant effect on its character descriptor 'pear - apple'. As may be expected, this effect comprised an increase of 'pear-apple' ratings after adding hexyl acetate to the mixture. Furthermore, 'pear - apple' ratings decreased significantly when 1-hexanol was added to the mixture (Figure 2C).

Ratings on 'bittersweet – rum' also increased significantly when its characteristic component, *trans*-2-hexenal was added to the mixture (Table 3, Figure 2D). No other effects were found for this descriptor.

Table 4 shows the ANOVA results for the HOL3 × HYLHAL 3 × 2 design, grouped for each separate dependent variable and specified for separate sources of variance. The results are similar to those presented in Table 3. High HOL levels suppress 'apple' and 'pear-apple' ratings and increase 'nuts-musty' ratings (Figure 2E, Figure 2G and Figure 2F, respectively). Ratings on 'apple' and 'pear-apple'

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increase when CICs (HYLHAL) are added (Figure 2E and Figure 2G respectively). The contribution of HOL3 to 'nuts-musty' is suppressed by HYLHAL (Figure 2F).

Applying orthogonal simple contrasts on HOL3 levels (none, low, high), comparing the levels 'none' to 'low', respectively 'none' to 'high', revealed significant effects for 'high' vs. 'none' on ratings for 'apple' [$F(1,17) = 14.48$, p=0.001], 'pear - apple' [$F(1,17) = 4.84$, p=0.042] and 'nuts - musty' [$F(1,17) = 36.16$, p<0.001]. No significant effects of 'low' vs. 'none' were observed. Likewise, the HOL3 × HYLHAL interaction could be attributed to the interaction between 'none' vs. 'high' (HOL) and HYLHAL. Therefore, all main and interaction effects of HOL3 were due to the influence of the highest 1-hexanol concentration level.

Table 5.4. Repeated measures ANOVA of HOL3 × HYLHAL for the same attributes as in Table 3

Attribute	Source	$d.f._{effect}$, $d.f._{error}$	F	P
Apple	HOL3	2, 34	12.13	<0.001**
Apple	HYLHAL	1, 17	19.22	<0.001**
Apple	HOL3 × HYLHAL	1, 17	0.70	0.499
Nuts – musty	HOL3	2, 34	32.34	<0.001**
Nuts – musty	HYLHAL	1, 17	23.86	<0.001**
Nuts – musty	HOL3 × HYLHAL	1, 17	17.17	<0.001**
Pear – apple	HOL3	2, 34	6.05	0.006**
Pear – apple	HYLHAL	1, 17	8.54	0.010**
Pear – apple	HOL3 × HYLHAL	1, 17	1.52	0.234
Bittersweet – rum	HOL3	1, 17	0.10	0.909
Bittersweet – rum	HYLHAL	1, 17	4.12	0.058
Bittersweet – rum	HOL3 × HYLHAL	1, 17	1.60	0.217

** $p < 0.01$

Discussion

If only 'apple' ratings are taken into account, the CIC's and non-CIC investigated here show statistical additivity. Adding HYL or HAL to the apple base mixture increases apple ratings. Whether HYL and HAL produce sensory hypo-additivity or hyper-additivity cannot be concluded on the basis of the present data, since this

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requires more information on the form of the psychophysical functions of these two substances (De Graaf and Frijters, 1988; Schifferstein, 1995). The presence of 1-hexanol suppresses 'apple' ratings for all mixtures, reflecting a marked sensory interaction. Correspondingly, 1-hexanol suppresses mixture ratings on the 'pear-apple' attribute.

When the *multivariate* HOL × HAL interaction is investigated further by studying single attribute effects, this interaction can be attributed to *trans*-2-hexenal suppressing the 'nuts-musty' intensity at high 1-hexanol levels. In other words, the CIC *trans*-2-hexenal suppresses the contribution of 1-hexanol to the ratings on its corresponding attribute. This adds to the status of *trans*-2-hexenal as 'character impact component' since it suppresses part of the effect of a non-character impact component on the mixture's aroma. Summarised, the observed interactions show that sensory interactions among mixture components are present and that these interactions pertain to ratings on a number of attributes.

In experimental investigations of mixture aroma quality, a single attribute describing the main character of the aroma cannot sufficiently reflect contributions of all components to the aroma. Had in this study, for instance, only 'apple' ratings been used, then the important *trans*-2-hexenal × 1-hexanol interaction would have gone unnoticed. Therefore, we argue that the use of multiple attribute ratings should be preferred to one-dimensional measures in food aroma studies. However, a limited set of eight attributes is rather small according to recommended sizes of 25 to 30 (Callegari *et al.*, 1997). Therefore, some concern is justified with respect to the validity of the operationalisation of aroma quality in the present study. When using the common technique of odour profiling as an operationalisation of aroma quality, one assumes a linear additive model for contributions of each attribute to the overall

aroma. Therefore, an observed mutual suppression of components cannot result from the chosen operationalisation technique. Hence, we attribute the sensitivity for mixture interactions of our characterisation method to the use of descriptors generated on basis of the constituent odours. This enabled the training of attribute usage and allowed for direct measurement of mutual suppression of odorants in the mixture. Although the used operationalisation used here may not allow full representation of perceived aromas, it proved sufficient for the assessment of mixture interactions.

To eliminate the effect of odour intensity, the intensities of the two CICs and the non-CIC were matched before their contribution to a multi-component model solution was investigated. This involved raising the concentration of the non-CIC component. Although this may have altered the quality of the aroma, it was necessary to do so in order to be able to attribute effects exclusively to the influence of odour quality. Interestingly, the highest rating on the apple attribute was given for the original apple model (Figure 2E) in which the non-CIC was present in low concentration.

The two CICs and the non-CIC showed main effects on their corresponding odour attributes. From this, it can be concluded that these components did indeed influence the perceived aromas. More specifically, when the three studied components were added to the mixture, ratings for the three respective character descriptors increased significantly. This suggests that panellists were able to recognise the unique contribution of each of the three manipulated components to the mixture's aroma. This is surprising given that Laing showed that humans have great difficulty recognising as few as 3 or 4 components in mixtures containing up to 5 or 6 odorants (Laing and Francis, 1989; Laing and Glemarec, 1992; Livermore and Laing, 1996). This discrepancy in results is unlikely to be due to differing similarities between the used odorants in each study. Laing et al. employed dissimilar odorants, which should

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have maximised the number of correctly identified components, whereas at least one odorant in the present study was, both structurally and perceptually similar to a CIC (i.e. hexanal and *trans*-2-hexenal). Also, differing mixture complexities cannot have caused this outcome since the mixture in the present study is more complex, creating a more difficult task.

An explanation for the seemingly enhanced performance of subjects in the present experiment can be found in the methodology employed. In the present study, the subjects were aided by being provided with specific descriptors that directed them in rating specific feature intensities. Subjects were not requested to focus on physical components, as was the case in the study by Laing et al. Furthermore, Laing et al. gave their subjects dichotomous decision options: an odorant is present in the mixture or it is not. Under this regime a subject has a complex task: he or she has to assess the intensity of component-specific contributions to the mixture *and* has to decide on the relevance of the perceived intensities to the question whether components are present or not. In contrast, the present study employed continuous attribute scales that enabled subjects to express the intensity of sensations. No absolute decisions on presence had to be made.

Figure 5.1. Mean intensity ratings (± SE) for the attributes 'apple', 'nuts - musty', 'pear - apple' and 'bittersweet - rum'. Panels A-D show the ratings for the base mixture with 1-hexanol – HOL (not present; present in high concentration), *trans*-2-hexenal – HAL (not present; present) and hexyl acetate - HYL (not present; present). Panels E-H show the ratings for the base mixture with 1-hexanol (not present, low concentration and high concentration) and a combination of *trans*-2-hexenal and hexyl acetate (not present, present).

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6 Effects of task instruction on the capacity of humans to identify odours in mixtures

Abstract

Studies in which people had to identify odorants in mixtures of odorants suggest that humans have no access to the neural codes relating single odorants in complex mixtures to respective singular receptor activations. Nevertheless, one may get familiarised with the unitary mixture percept without being exposed to single odorants. This may explain why the 'analytic' task of identifying single components in a mixture has proven to be so difficult. However, identification tasks become 'synthetic' by formulating them as 'identify aroma notes in a unitary aroma'. Thus, performance is expected to improve. We tested this expectation with apple and orange aromas. Subjects with the synthetic task to identify odour notes in an apple/orange aroma performed better than subjects with the analytic task to identify odorants in a mixture. The latter task produced less correct identifications for the apple aroma and more false identifications for the orange aroma.

Introduction

Humans possess impressive discriminative abilities in their visual, tactile and auditory senses. They can discern large numbers of objects or colours in an eye blink, they can easily select their favourite candy from a paper bag by recognising its texture, they can single out the constituting voices during a cocktail party and listen selectively to the various instruments in a band. In this context, it is surprising that the human capacity to identify smells of different sources (the components of a mixture of odorants) is rather low. In their classic study, Laing and Francis (Laing & Francis, 1989) composed mixtures of 1-5 odorants from a collection of 7 distinctively smelling odorants. They found that the proportion of correct identifications of all three components in tertiary mixtures was as low as 0.14. If no false identifications were allowed then this proportion even dropped below 0.07. For binary mixtures these proportions were 0.35 and 0.12, respectively.

Because training allows musicians to improve their analytical hearing abilities, it may be hypothesised that olfactory discriminative ability may also improve through training. However, training or being a trained expert perfumer did not increase discriminative ability significantly (Livermore & Laing, 1996). In subsequent studies, Laing and co-workers ruled out a number of other possible explanations for this limited discriminative ability: olfactory adaptation (Laing & Glemarec, 1992), low qualitative distinctiveness of the odorants in the mixture (Livermore & Laing, 1998a), odorant perception onset time (Laing & MacLeod, 1992) and the focus of attention to certain components in the mixture (Laing et al., 1992).

Most natural aromas are perceived as unitary smells although they emanate from complex mixtures of odorants. So, regardless of the chemical complexity of the stimulus, its neural encoding culminates in an activation pattern that is associated with

a unitary smell and, depending on how and where it was experienced, labelled as such (Stevenson & Boakes, 2003). Because humans perform equally poor in discerning single odorants from mixtures as in discerning complex odorant mixtures with unitary smells from their respective mixtures (Livermore & Laing, 1998b), it seems plausible that the most elementary neural code that is consciously accessible in aroma perception is already unitary. This notion proposed by Wilson & Stevenson (Wilson & Stevenson, 2003; Wilson, 2000) was supported by literature that shows that neurons in the piriform cortex of rats developed enhanced selectivity in their responses to different aromas after repeated exposure. In contrast, mitral cells in the olfactory bulb, which are involved in analytical, stimulus-feature recognition at peripheral and consciously inaccessible process stages, did not show this plasticity in developing odorant-specific responses. Hence, after repeated exposure, new aromas become encoded as unitary, non-dissectible objects at the primary olfactory cortex. The authors argue that the mere exposure to an odorant creates a mental representation of the stimulating odour, representing verbal descriptions, elements of the context in which it was presented (accompanying tastes, odours, views or sounds), object category, and so on. These learned and continuously refined representations, which we will further refer to as *stimulus concepts*, then become the reference for future odour recognition and interpretation. This conceptualisation has been worked out in full detail by Stevenson and Boakes in their mnemonic theory of olfaction (Stevenson et al., 2003).

Since single odorant information is not readily available in the unitary stimulus concepts of chemically complex aromas, one might presume the analytical identification of separate odorants in mixtures to be hindered. Nonetheless, differences between aromas due to small changes in constituting odorants may still be

perceived, because each percept can be compared in detail with the most similar stimulus concept available. Many studies show that the availability of a stimulus concept, as reflected in stimulus familiarity, enhances stimulus discrimination, recognition and even increases perceived intensity (Rabin & Cain, 1984; Rabin, 1988; Jehl, Royet, & Holley, 1995; Christie & Klein, 1995; Lesschaeve & Issanchou, 1996). Even stimulus modifications as minor as adding peri-threshold odorants to a supra-threshold mixture of odorants may result in a detected aroma change, provided that subjects have activated the appropriate stimulus concept (Bult, Schifferstein, Roozen, Voragen, & Kroeze, 2001). This qualifies olfactory perception as a top-down process, in which it is necessary to activate the stimulus concept for the complex mixture before stimulus details may be discriminated.

Using qualitative descriptors of single odorants instead of odorant names to rate the quality of mixtures of these odorants, Jinks and Laing (Jinks & Laing, 2001) found no improvement in the human ability to identify odorants in mixtures. As a consequence, Jinks and Laing proposed the 'configurational hypothesis of olfaction', stating that complex mixtures preclude that features of single odorants stand out for recognition and that, therefore, mixtures can only be perceived synthetically. However, because their task did not focus on the details of a unitary percept but rather requires bottom-up processing, we think that their task did not fully exploit the advantages of the use of stimulus concepts.

Activating an appropriate stimulus concept for the complex mixture prior to an identification task will facilitate the detection of discrepancies from the concept, and, therefore, it will facilitate the identification of odour notes in that aroma (synthetic / top-down task). The present study tests this hypothesis by presenting single odorants and mixtures thereof to two groups of subjects. Both groups sniffed the single

odorants first. One group is told that these odours represent odour notes that may be perceived in apple and orange aromas (synthetic condition). The other group is told that the odours are produced by odorants, which may be also present in mixtures (analytic condition). The results will be presented in terms of the classical odour-mixture identification paradigm and in terms of signal detection theory.

Materials and Method

Subjects

Subjects consisted of students from an introductory sensory science course, an audience interested in the Food Science program at Wageningen University and colleagues from that university's Food Chemistry department. In the 'synthetic' panel 24 male and 33 female subjects participated (ages ranging from 19 to 56 years), whereas in the 'analytical' panel 19 male and 26 female subjects participated (ages ranging from 19 to 63 years). Subjects did not report any olfactory dysfunctions and were in good health during the experiments. Apart from the given task instructions, subjects were naïve with respect to the objectives of the experiment.

Stimuli

Two model aromas were prepared by diluting odorous components in distilled water. The used mixture compositions were derived from literature reports on apple (Bult et al., 2001) and orange (Ahmed, Dennison, Dougherty, & Shaw, 1978) aromas. From the reported components, the five with the highest perceived singular intensities were selected for each aroma, provided that the two aromas did not share these components. The apple aroma consisted of 40ppm *trans*-2-hexenal; 7.5 ppm 2-methyl butylacetate; 0.12 ppm ethyl 2-methylbutylacetate; 7.5 ppm hexyl acetate and 12.5 ppm butyl acetate (concentrations are vol/vol). The orange aroma consisted of 190

ppm *d*-limonene; 0.06 ppm octanal; 0.4 ppm ethyl butanoate; 0.78 ppm citral and 3 ppm acetaldehyde. The latter component is not always mentioned in the literature because of difficulties in identifying it by gas chromatography olfactometry, but is considered an important contributor to orange flavour (Shaw, Moshonas, & Buslig, 1995).

Limonene cannot be dissolved in water at the concentration used. We chose not to use an emulsifier but to prepare a micro-emulsion using a high-speed ultra-turrax mixer. The limonene was pipetted into the solution of the other four components while the mixer was running at 6000 rpm. Mixing then continued for another 60 s. The resulting emulsion is relatively stable during one hour. Presentation took place within that period of time. Thermodynamically, flavour release from an emulsion is intrinsically different from the diffusion of odorants from a water solution. Nevertheless, the headspace concentration of limonene is proportional to the concentration of limonene in the emulsion (De Roos, 2000).

For each of the two mixtures, 4 out of 5 single odorants were also diluted in water at the same concentration as was used in the mixtures. Acetaldehyde and 2-methylbutyl acetate were not used as single odorants from the orange aroma and the apple aroma, respectively. Limonene was prepared analogously to the method described above.

Ten ml of each stimulus was poured in 200 ml glass jars, closed by a low-odour plastic screw cap. To prevent volatile components from migrating from the screw caps to the headspace, these two phases were separated using a sheet of aluminium foil. Before presentation to the panel, the headspace concentrations in the jars were allowed to equilibrate during 10 minutes.

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Procedure

Stimuli were presented in a classroom setting. Stimulus jars were placed on trays. The eight single-component dilutions were placed randomly on the right half of the tray and the two mixtures were placed on the left half of the tray. Single odorant jars were numbered with random, three-digit codes. The apple and orange mixtures were indexed with roman numbers I and II respectively. At every occasion when sessions were held, subjects were separated in two groups and gathered in separate classrooms. Subjects were given one of the following two instructions:

Synthetic instruction:

'Food aromas may be described as a collection of odour notes. For instance, wine aromas may be described by woody, floral and fruity notes. Odour notes may be used to give refined descriptions of any food aroma. You will participate today in an experiment in which you will describe an apple aroma and an orange aroma. These two aromas are contained in jars I and II respectively. You may start by sniffing the first food aroma and then continue by sniffing the 8 jars that contain odour notes that could apply to any or both aromas. Proceed by indicating which of these notes describe part of the food aroma. If desired, you may go back to the food aroma to compare the notes. Repeat these steps for food aroma number II. Each odour note may be present in one food aroma, both food aromas or in none of the food aromas.'

Analytical instruction:

'Food odours consist of chemical components. Each has its own odour and all of these odours contribute to the aroma of the mixture. Humans are able to distinguish the contributing odorants from the mixture, after sniffing these odorants separately. We ask you to do the same in this experimental session. You may start by sniffing the first mixture aroma and then continue by sniffing the 8 jars that may contain odorants

from any or both aromas. Proceed by indicating which of these are present in the mixture. If desired, you may go back to the mixture to compare the odours. Repeat these steps for mixture number II. Each component may occur in one, in both or in none of the mixtures.'

Subjects opened a jar by twisting off the cap while keeping the jar just underneath their noses. Only short sniffs were allowed. Besides indicating which components were perceived in the two mixtures, subjects rated aroma intensity, liking and familiarity for the two mixtures on 7-point category scales, anchored with 'not perceivable' and 'extremely strong' for the intensity scale, 'extremely unappetising' and 'extremely tasty' for the liking scale and 'not at all familiar' and 'extremely familiar' for the familiarity scale.

Data analysis

Proportions of correctly identified components ((0-4)/4) and falsely identified components ((0-4)/4) were calculated for each subject and each mixture. Student's T-Tests for instruction effects were subsequently performed on the logit transforms of these proportions. Proportions were also used to calculate corresponding Z-scores. From these, discriminability indices *d'* of components/aroma notes in each mixture aroma can be calculated as Z(Correct Identifications) – Z(False Identifications). For this calculation, proportions of correct or false identifications equal to 0 or 1 are first converted to $1/(2N)$ and $1-1/(2N)$, respectively, as suggested by Macmillan and Creelman (MacMillan & Creelman, 1991). ANOVA is subsequently performed on these d' values for effects of Mixture (apple or orange) and Instruction (synthetic or analytic). All tests were performed against significance levels $\alpha = 0.05$.

Results

In terms of the number of correct- and false identifications, both the apple mixture and the orange mixture showed significant effects due to the Instruction condition. Logit-transformed proportions of correct identifications differed significantly for apple, [$t(100) = 2.30$, $p = 0.023$] but not for orange [$t(100) = -0.66$, $p = 0.51$], whereas logit-transformed proportions of false identifications did not differ for apple [$t(100) = -1.30$, $p = 0.19$] but did for orange [$t(100) = -1.98$, $p = 0.050$]. For apple, more correct identifications were given in the synthetic condition whereas for orange, less false identifications were given (Figure 1).

Effects of Instruction and Mixture on *d'* values were tested in a repeated-measures ANOVA, with Instruction as a between-subject factor and Mixture as a within-subject factor. A significant Instruction effect was found [$F(1,100) = 5.56$, $p = 0.020$]. No significant Mixture or Mixture x Instruction interaction effects on d' were found. Inspection of d' (Figure 1) learns that the identification of odorants in the aroma mixtures improved under the synthetic instruction for both fruit aromas.

Intensity ratings [5.05, 5.13, 4.11, 4.42], hedonic ratings [4.23, 3.89, 4.53, 4.96] and familiarity ratings [4.51, 4.49, 4.86, 4.93] for synthetic apple, analytic apple, synthetic orange and analytical orange, respectively, were compared over Instruction conditions (between-subject) and Mixture conditions (within-subject) in a repeated measures ANOVA. Main effects of Mixture were found on intensity ratings [$F(1,98)$ = 52.3, $p < 0.001$], hedonic ratings [$F(1,98) = 33.4, p < 0.001$] and familiarity ratings [$F(1,98) = 6.12, p = 0.015$]. The apple aroma was generally perceived as more intense, less familiar and less pleasant than the orange aroma. Interestingly, for the apple aroma the hedonic ratings were higher for the panel that received synthetic instructions whereas for the orange aroma these were lower than those for the analytical panel, resulting in a significant Instruction x Mixture interaction [$F(1,98)$ = 6.63, $p = 0.012$].

Discussion

Figure 1. Number of correct identifications (Correct, M +SEM), false identifications (False Alarms, M +SEM) and discriminabilities (d', M +SEM) of components from the apple and the orange mixtures, shown for panels receiving synthetic and analytical instructions.
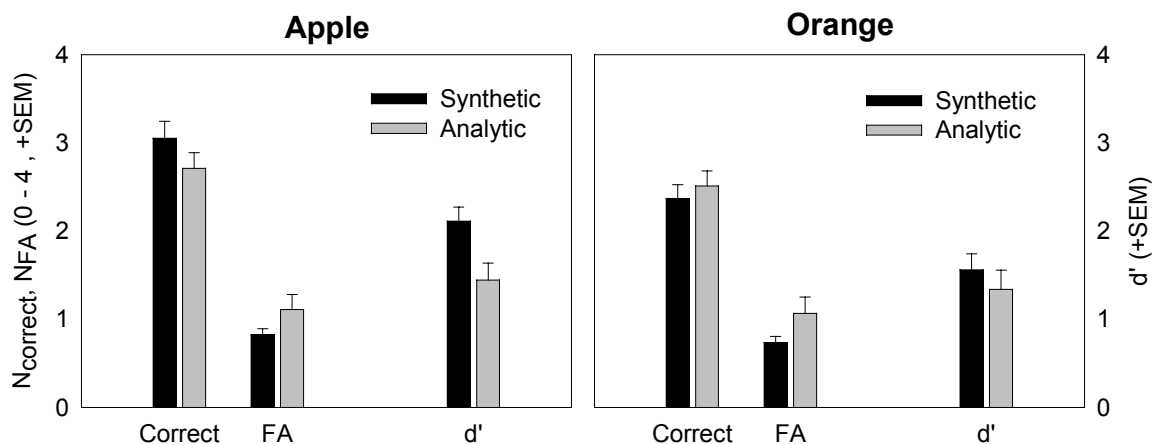
The primary objective of the present study was to test whether performance on component identification tasks would improve by providing subjects with a task instruction that would facilitate top-down stimulus processing. A short task instruction was given with the objective to either activate stimulus concepts for the complex mixtures or not. No extra training for the two used instruction conditions was given. Therefore, subjects may be assumed to differ only in the activation level of stimulus concepts, not in the elaborateness of stimulus concepts per se. Indeed, subjects' performance improved when attention was directed to the holistic qualitative nature of the mixture aroma, by asking to identify odour notes in the aromas (the synthetic instruction) rather than asking to identify components from a mixture. Usually, the use of verbal odour descriptors promotes intentional odour learning and recognition (Møller, Wulff, & Köster, 2004). However, this could not explain the better performance of subjects in the synthetic condition of the present study, because the components to be recognised were not labelled.

The experimental conditions appear comparable to some of the conditions in previous studies regarding the used number of odorants contained in the mixtures (5) and the number of odorants (8) from which constituents could be chosen (Laing et al., 1989; Laing et al., 1992; Livermore et al., 1996; Livermore et al., 1998b; Livermore et al., 1998a; Jinks & Laing, 1999; Jinks et al., 2001). Nonetheless, proportions of correctly identified components were considerably higher in the present study. Whereas mixtures consisting of 4 or 5 odorants generally produce correct identification proportions below 0.5, the mixtures in the present study produced proportions of correct identifications above 0.6, regardless of the instruction condition. This difference may be explained by the fact that the mixtures used reflected existing aromas, in contrast to many previous studies. Even though subjects

in the analytic panel were not provided with further information on the aromas, the rather high familiarity scores for both panels suggest that task performance in the analytic panel may also have benefited from familiarity with the aromas and their components.

Although d' scores suggest that apple and orange mixture percepts were decomposed better by the synthetic panel than by the analytic panel, it should be noted that most of the improved discriminability for orange components depended on lower proportions of false identifications. The finding that identification of odorants that are *not* part of the mixture profits more from the availability of a stimulus concept than the identification of odorants that are part of the mixture, is in line with the suggestion by Köster that odour memory is intrinsically tuned to the detection of change (Köster, 2005). In other words, odour perception allows for better perception of what is not there, instead of what is there, provided that a representation of the prototypical aroma, viz. the stimulus concept, exists.

In the present study, we compared analytical performance with stimulus-concept driven synthetic performance. By presenting the natural provenance of an aroma, we aimed at activating subjects' complex of stimulus-related knowledge in the synthetic condition. In a similar vein, Frandsen and co-workers (Frandsen, Dijksterhuis, Brockhof, Nielsen, & Martens, 2003) provided an emotionally arousing label for the stimuli by suggesting that some milks might be from foreign competitors on the Danish market. This resulted in an improved discriminative performance in comparison with the analytical condition, although the milks were not labelled individually. The authors suggested that by providing the emotional setting, subjects were stimulated to tap from their implicit affective knowledge regarding milks in general. In comparison to the present study where we activated implicit knowledge by

presenting an overall, synthetic construct, Frandsen et al. may have been able to activate even more specific, affective implicit knowledge. In line with the thinking in the present study, this suggests that any instigation to tap stimulus knowledge in stimulus evaluation may increase performance.

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7 The influence of olfactory concept on the probability of detecting sub- and peri-threshold components in a mixture of odorants

Abstract

The headspace of apple juice was analysed to obtain an ecologically relevant stimulus-model mixture of apple volatiles. Two sets of volatiles were composed; a set of eight supra-threshold volatiles (MIX) and a set of three sub-threshold volatiles. These sets were used to test the hypothesis that sub-threshold components can change the quality of a familiar smelling mixture of odorants, when added to this mixture. In order to test this hypothesis, three successive dilutions of the sub-threshold volatiles were prepared in such a way that the strongest was at threshold concentration and the two lower concentrations were below threshold. The detection probabilities of the sub-threshold components in a blank stimulus were compared to the detectabilities in MIX. The sub- and peri-threshold volatiles were not detected better in MIX than in a blank. On the contrary, sub- and peri-threshold volatiles were better detected alone than when added to MIX. However, when the group of subjects was split into two subgroups, employing either a rough or a detailed concept definition of the target stimulus respectively, the subjects with highly refined concepts were better able to detect the presence of sub-threshold volatiles in MIX than those with poorly refined stimulus concepts. The effect of stimulus concept definition occurred independently of the proportions of correct detections of sub-threshold volatiles in a blank.

Introduction

In food science it is a common practice to formulate complex food aromas from their odorous constituents as identified in isolation. A widely used method in this field is gas chromatography olfactometry (GCO). This method, developed during the 1960s, is still being applied according to the design that first was described by Dravnieks and O'Donnell (1971). In typical GCO experiments human subjects detect odorous mixture constituents by sniffing components that elute sequentially from the gas chromatograph at a sniffing port (SP). These detected constituents are assumed to be the relevant contributors to the aroma of the mixture. A general finding, however, is that the thus recomposed aroma, albeit similar, usually is not identical to the original aroma. So far, a satisfactory reconstruction of a complex food aroma by means of GCO/SP identification of constituents has not yet been accomplished. The finding that the perceived smell of GCO-reconstructed mixtures differs from the original smell, while the concentrations of the odorous constituents are identical in both mixtures is referred to here as the 'reconstruction discrepancy'.

One explanation for reconstruction discrepancy could be that components not detected by GCO/SP do play a role in the perception of the original mixture. However, since these components have sub-threshold intensities at the SP they are not selected for construction of the mixture. The contribution of sub-threshold components to the overall percept can be understood using concept formation theory (Miller and Johnson-Laird, 1976; O'Mahony, 1991). According to this theory, the extent to which a subject can discriminate between stimuli depends on the refinement of the subject's conceptual representation of that particular stimulus. This is supported

by the fact that the ability to discriminate between instances of a set of qualitatively different odours is positively related to the subject's familiarity with these stimuli (Rabin and Cain, 1984; Rabin, 1988; Jehl et al., 1995). Therefore, we hypothesise that manipulating a familiar smelling mixture of odorants (MIX) by adding sub-threshold components will result in a mixture (MIX+) that is easier discriminable from MIX than that the sub-threshold components (BLANK+) will be discriminable from a blank stimulus (BLANK). One should note that if this hypothesis holds, the most familiar smelling mixture would be MIX+, since this is the mixture that optimally approximates the composition of the original food aroma.

Most of the available studies on olfactory mixture perception can be characterised by two features: a *limited number* of mixture constituents eliciting *unfamiliar odour qualities* (e.g. Olsson, 1994; Schiet and Frijters, 1988; Berglund and Olsson, 1993; Laing and Glemarec, 1992). In recent years however, the importance of studying complex, familiar smelling and ecologically relevant mixtures was recognised. Livermore and Laing (1998) studied subjects' capacity to identify mixture constituents when these constituents themselves were familiar smelling, complex mixtures of odorants. Others studied the effect of changing the concentrations of odorants in complex mixtures, modelled after food aromas (Blank et al., 1992; Guth and Grosch, 1994; Schieberle and Hofmann, 1997; Guth, 1997; Czerny et al., 1999).

Likewise, in the present study we compose a mixture that reflects the complexity of a natural food aroma. In contrast to previous studies, however, this mixture is used to investigate the influence of adding *sub-threshold* components. Few cases of sub-threshold components affecting olfactory mixture perception have been reported (Guadagni et al., 1963; Laska et al., 1990; Laska and Hudson, 1991; Patterson et al., 1993). These studies reported additive or even synergetic effects in conditions where

all mixture constituents were at peri- or sub-threshold concentration. In the present study, however, the sub-threshold components are added to a supra-threshold mixture.

A sample taken from the headspace of an apple juice dilution is used to select the constituting components for a model mixture. The components in the headspace sample are identified sensorically by GCO/SP. In parallel, the components are instrumentally identified by Mass Spectrometry (MS) analysis. Components, identified in both analyses, will be used to reconstruct the original food aroma as a mixture of odorants (MIX). In addition, several components with concentration levels similar to those of the selected odorants but not detected sensorically at the SP will be selected (Experiment 1). The latter components (BLANK+) will constitute the sub-threshold mixture. Using the components selected in Experiment 1, we investigate the effect of adding sub-threshold components on the perceived odour quality of MIX. To test the hypothesis that the degree of odour-concept refinement influences component detectability, we also investigate whether identifying the target stimulus as an apple aroma affects the probability with which MIX is discriminated from MIX+ (Experiment 2).

Experiment 1

A number of quantification methods relating the amount of an odorous component to its intensity in the mixture have been proposed in literature. Several methods are based on subjects' direct intensity judgements. Dilution methods derive a measure of intensity from the number of dilution steps a component is above its threshold level. The detection frequency method relates the number of panellists' coincident responses to the amount of an eluting component at the SP. Aroma extract dilution analysis (AEDA), introduced by Ullrich and Grosch (1987), is an example of the dilution method while Pollien et al. (1997) and Van Ruth and Roozen (1994) used the detection frequency method. A hybrid method is CHARM analysis proposed by Acree et al. (1984). Essentially a dilution method, CHARM analysis also encompasses the use of the number of coincident responses to infer a component's odour intensity. Although these methods are not psychophysically quantifying odour intensities, reliable relationships between the stimulus concentrations and the number of coincident respondents have been reported (Van Ruth et al., 1996; Van Ruth et al., 1996; Pollien et al., 1999). In this experiment we employ the detection frequency method presented by Van Ruth and Roozen (1994).

Materials and Method

Subjects

Sixteen paid volunteers, 10 female and 6 male, ranging in age from 18 to 43 years, participated in the experiment. They were recruited from the local Wageningen community. All were non-smokers and none had any history of olfactory dysfunction.

Participants were selected according to their performance on an odour-recognition and attribute-generation test, designed especially for this purpose. Subjects were naive with respect to both the nature of the stimuli and the objectives of the experiment. During the experimental sessions none of the subjects suffered from colds, allergic reactions or other adverse conditions of the respiratory tract.

Preparation of stimulus material (GCO)

Commercial quality 'Jonagold' apples, taken from a batch picked in France in October 1997 and subsequently stored for six months under controlled atmosphere conditions, were processed sequentially during a three-week period. Fresh from storage, the apples were peeled and their cores were removed. Subsequently, they were homogenised using a food processor (AEG) that yielded filtered apple juice. Three parts of this juice were diluted with 2 parts of distilled water. The complete process took no more than 3 minutes. Immediately after preparation, 15 mL of the diluted sample was poured into the container of a 'purge and trap' device (Van Ruth et al., 1995) and heated to 30 °C. The solution was then purged for 10 minutes with purified nitrogen gas (30 ml min^{-1}) while being stirred constantly at a rate of 250 rpm. Volatile components thus extracted from the dilution were trapped on granulated organic adsorbent material (Tenax TA, 35/60 mesh, Alltech Nederland b.v., Zwijndrecht, The Netherlands).

Instrumental analysis

Volatiles were thermally desorbed from Tenax at 260 °C for 300 s and trapped at –120 °C by a cold trap/thermal desorption device (Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). Subsequently, the volatiles were analysed on GC (Carlo Erba MEGA 5300, Interscience b.v., Breda, The Netherlands) equipped with a

Supelcowax 10 capillary column with a 0.25 mm inner diameter and a length of 60 m. The oven temperature was initially 40 °C for 4 minutes, after which it was increased to 92 °C at a rate of 2 °C min^{-1}, followed by an increase to 272 °C at a rate of 6 °C min^{-1}. The total running time was 65 min. Column effluents were split: the total flow was divided over the Flame Ionisation Detector (FID) and two sniffing ports (SP) in a 1:2:2 ratio, respectively. Desorbed volatile components were identified using combined GC/FID and SP. The chemical identities of the components were determined additionally by mass spectrometry (MS) analysis with VG MM 7070 F (Fisons Instruments Weesp, The Netherlands) on duplicate samples.

Calibration curves for a number of identified components were determined on this GC-system by transferring series of 10 linearly incrementing amounts of every pure component dissolved in hexane to Tenax. Desorption and subsequent analysis of the components was executed using the same GC-system settings as used for the apple aroma samples. The pure components used are listed in Table 7.1.

Table 7.1. **Components used for the model mixture, their derived partition coefficients, aqueous dilutions based on the presumption that SP-detected masses were present in 1 ml of air and the final aqueous dilutions which were chosin such a way that headspace detectabilities maximally mirrored the SP-detectabilities.**

Component	Nominal purity (%)	Mean mass (ng) at GC/SP	Partition coefficient[d] (30°C, mixture)	Derived aqueous dilutions (mg/L)	Model mixture (µL/L)
propyl acetate[b]	> 99%	48	1.3×10^{-2}	3.9	0.5
propyl propanoate[c]	> 99%	4.7	2.1×10^{-2}	0.23	0.1
butyl acetate[c]	> 99%	341	1.8×10^{-2}	19	2.5
hexanal[c]	> 98%	286	1.3×10^{-2}	22	2.5
2-methyl-1-butyl acetate[a]	> 94%	335	2.9×10^{-2}	12	1.5
trans-2-hexenal[b]	> 99%	231	3.3×10^{-3}	70	8.0
hexyl acetate[a]	> 99%	426	3.5×10^{-2}	12	1.5
isobutyl acetate[a]	> 99%	23	2.3×10^{-2}	0.99	0.15
ethanol[c]	> 99%	5.9	3.0×10^{-4}	22	2.0
1-butanol[c]	> 99%	16	5.4×10^{-4}	30	3.0
1-hexanol[c]	> 98%	21	9.5×10^{-4}	22	2.0

Calibration curves for all components on headspace-GC/FID had precision indices ranging from $R^2 = 0.995$ to $R^2 = 1.000$.

Obtained from: [a]Alldrich, [b]Janssen Chimica and [c]Merck.

[d]Determined for components when in mixture, partition coefficients are averages over three determinations at aqueous concentrations of respectively 25 ppm, 5 ppm and 1 ppm.

Sensory Analysis

Subjects participated in two identical GCO sessions. The first session was regarded as a training session and, therefore, the results were discarded. During a session, two subjects were simultaneously involved in analysing the effluents at a SP. A screen prevented the subjects from seeing each other. Subjects were not allowed to interact in

any way. Room temperature was kept at 21 °C by air conditioning. Effluents from the SP were humidified prior to presentation.

The subjects were positioned behind the SP. Keystroke responses were recorded on a laptop computer that was placed in front of them. These recordings were synchronised in time to the FID registrations. The subjects were instructed to strike a key (any key) whenever they noticed an odour at the SP. The moment they stopped perceiving the odour they had to strike a key again. The computer provided visual feedback on whether a key-strike was being registered. After they indicated that no odour was being perceived anymore, subjects gave a precise description of the odour smelled. Total session time was 60 minutes.

Data Analysis

Subjects' responses during SP-sessions were recorded in 1 ms intervals. Before aggregation they were corrected for GC-retention time differences. Since the SP-sessions were repeated with identically prepared samples, the FID-profiles should be similar. Retention times, however, are subject to variation due to fluctuations in GC-performance. Therefore, all chromatograms, along with the accompanying sensory time events, were matched according to the retention times of nine selected reference components in one of the FID-chromatograms. Time scores of events that occurred between two reference peaks were interpolated linearly.

The number of subjects responding simultaneously at a specific time interval (1 ms) was calculated and plotted as a coincident response chromatogram (Van Ruth and Roozen, 1994). If this number was below 4 it was considered to be noise. The noise level was determined according to the method of Bult et al. (in preparation). With this method, noise levels are estimated using the response time distributions derived from

'stimulus present' data. Thus, in order to be selected in the model mixture, the volatiles had to be identified by at least 4 of the 16 subjects. In addition, the descriptions of the lower scores had to be similar (for instance, "sweet fruity" and "strawberry" would be considered similar descriptors). Components not detected by the subjects, but still detected by FID were selected for the set of sub-threshold components. Concentration levels of these non-perceived components were in the same range as the levels of the sensorically detected components.

Results

A typical FID chromatogram of the Jonagold aroma mixture is shown in Figure 7.1, along with the time-corrected, cumulated subject responses in the corresponding response chromatogram. Ten sniffing peaks scored above noise level. However, not all of these sniffing peaks coincided unambiguously with FID peaks. The first sniffing peak, located at 12 minutes, could not be related to any specific component. The accompanying descriptions (see Table 7.2) did not give any further indication for identification either.
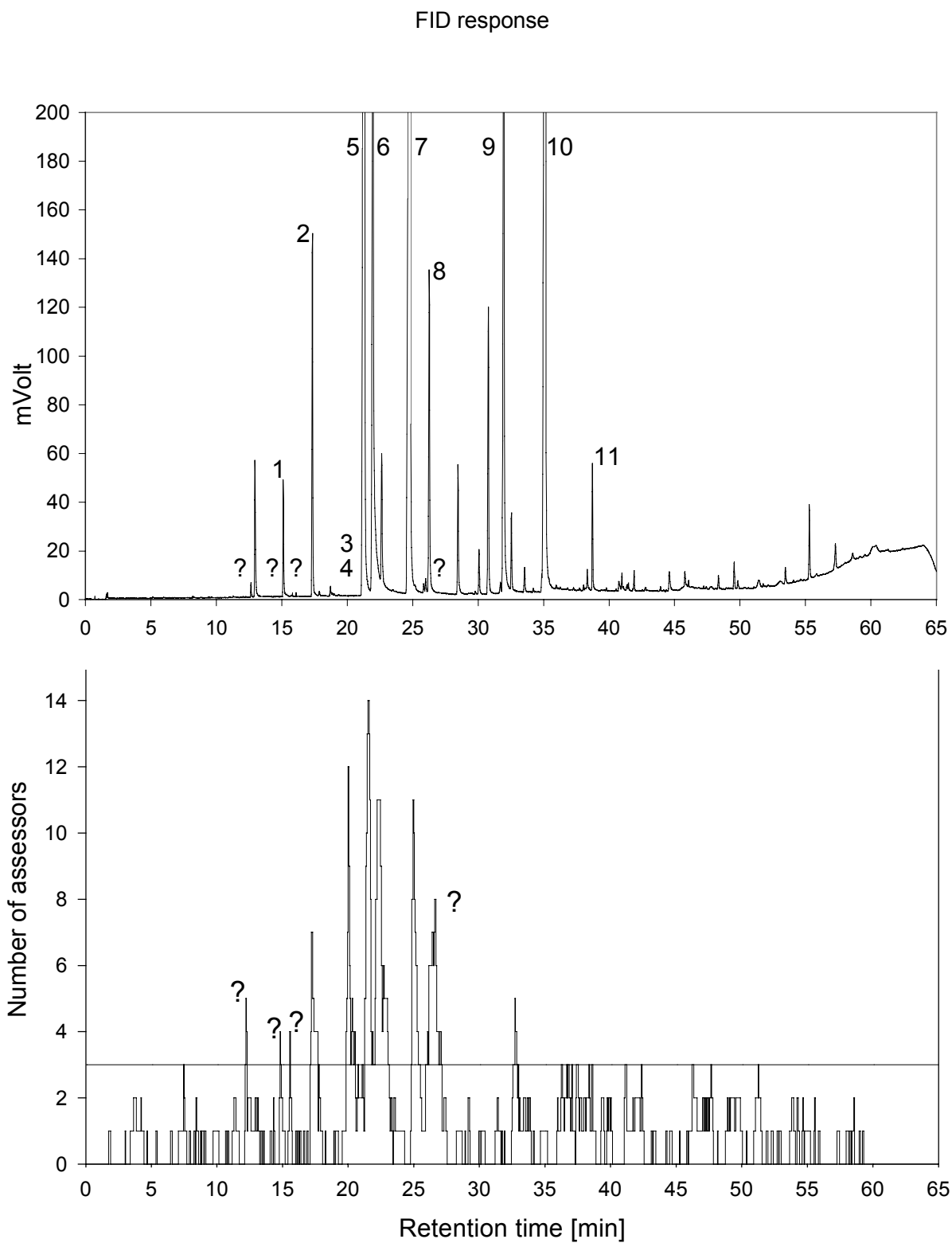
Figure 7.1. **Combined FID-chromatogram (top) and olfactogram (bottom) of volatile components obtained from purge-and-trap apple-juice samples. The olfactogram is composed of the results from 10**

Clear matches with respect to both response timing and consistency of odour descriptions were found for propyl acetate (2); butyl acetate (5); hexanal (6); 2-methyl-1-butyl acetate (7); and *trans*-2-hexenal (9). The numbers between parentheses refer to the indexes in Table 7.2 and Figure 7.1. The fifth SP peak, with an onset time closely to 20 minutes, was immediately preceded by FID readings of propyl propanoate (3) and isobutyl acetate (4). Therefore, and because of the typical descriptors that accompanied these two components, they were both selected, assuming that the ensemble had been responsible for the predominantly sweet and fruity descriptions (see Table 7.2). Hexanol (10) was clearly present in the FID-chromatogram but was not detected in the sniffing sessions. Therefore, hexanol was selected for the list of sub-threshold components. Ethanol (1) and 1-butanol (8) seem to coincide with sniffing peaks. However, the detection threshold of ethanol, being ±100 ppm (Flath et al., 1967), is considerably higher than the concentration found in this experiment while the accompanying odour descriptions for 1-butanol are far from consistent with typical descriptions reported for this component, e.g. 'alcohol like', 'chemical' and 'paint like' (Dravnieks, 1985). Possibly, ethanol and 1-butanol eluted close to low-threshold components, not detected by FID and MS. Therefore, ethanol and 1-butanol were included in the list non-detected components. Also hexyl acetate (11) was included in this list since it was not detected at SP although it was clearly present in de FID chromatogram. The two sniffing peaks that nearly coincided with the elution of ethanol and the sniffing peak that coincided with the elution of butanol were ascribed to the presence of components not identified by FID/MS. This selection procedure resulted in an initial set of 7 components being the supra-threshold mixture and a set of 4 components constituting the sub-threshold mixture.

With the exception of ethanol, calibration curves for the identified components allowed for reliable estimates of their masses at SP (Table 7.2). Due to its hydrophilic nature, however, ethanol has a lower affinity for Tenax (Novák et al., 1981). Even the use of a syringe to transfer an ethanol solution to the central region of the Tenax tube did not result in a precision index higher than $R^2 = 0.837$.

Table 7.2. Mean component masses at SP, R^2-quality indices of the calibration curves for the GC/SP system, number of analyses on which these were based and the corresponding odour descriptions.

Component	peak in Figure	R^2 of the GC-SP/FID calibration curve	mean mass at GC/SP[d] (ng)	Number of analyses	Descriptors generated during SP-sessions
SP-DETECTED COMPONENTS					
Unknown	?				'sweet' 'pungent sweet' 'sweet warm mu… wood' 'sulphide' 'apple sour'
Unknown	?				'flowery spicy' 'sweet plums' 'strawberry 'light sweet blossom'
Unknown	?				'sweet dough warm vanilla' 'butter' 'butt 'unpleasant'
propyl acetate	2	0.997	48	15	'fresh lemon like' 'sour candy' (2×) 'swe 'chemical alcohol sweet' 'petrol' 'sweet strav candy'
propyl propanoate[a]	3	0.996	4.7	13	'sweets' (4×) 'sweet' (2×) 'sweet sour' ' strawberry' 'strawberry lollipop' 'chewing gu 'sweet fruity' 'flowery'
isobutyl acetate[a]	4	0.994	23	15	see propyl propanoate
butyl acetate	5	0.995	341	14	'glue' (5×) 'paint' 'glue paint' (3×) 'pain acetone' 'lacquer' (2×) 'sour'
hexanal	6	0.999	286	14	'grass plant' 'grass' (5×) 'plant hedge' 'g grass hedge' (2×) 'apple' 'green plant'
2-methyl-1-butyl acetate	7	0.994	335	15	'sweet green' 'glue' (5×) 'sweet apple-lik 'sweet sour' 'sour candy' 'lacquer paint'
Unknown	?				'plant' (2×) 'sour apple' (2×) 'grass' 'gre 'apple' 'banana'
trans-2-hexenal	9	0.995	231	15	'heavy sweet' 'spicy plant' 'spicy musty' like pricking marzipan' 'pine needles' 'rum'
NON-SP-DETECTED COMPONENTS					
ethanol[b]	1	0.837	5.9	14	-
1-butanol[b]	8	0.997	16	14	-
hexyl acetate	10	0.997	426	14	-
1-hexanol	11	0.997	21	14	-

[a] propyl propanoate and isobutyl acetate eluted simultaneously

[b] descriptors that coincided with ethanol and butanol are attributed to co-eluting non-detected low-threshold comp

[c] coefficients of variation ranged between 0.19 and 0.65

[d] FID masses are 50% of the masses that are given here for each single sniffing port (see text)

Experiment 2

Materials and Method

Subjects

The panel of 16 subjects that participated in Experiment 1 was extended with 7 subjects to form a panel of twenty-three paid volunteers, 17 female and 6 male (age range 18-51 years). Selection and specific requirements with respect to health and habits equalled those of Experiment 1. Subjects were naive with respect to both the nature of the stimuli and the objectives of the experiment.

Determination of partition coefficients

Partition coefficients of the eleven components dissolved in distilled water were determined for equilibrated static headspaces at 30 °C. Because unexpected matrix interactions might alter partition coefficients, components were dissolved as one mixture. The headspaces were sampled from vials of 12.25 ml, containing 3.0 ml of solution. They were loaded by an automated sampling unit (Fisons HS800) and subsequently injected on a HRGC 5300 Mega Series gas chromatograph (Carlo Erba Interscience b.v., Breda, The Netherlands) equipped with a DB-wax column (30 m × 0.542 mm). The oven temperature initially was 40 °C for 10 minutes and was then raised to 220 °C at 15 °C min^{-1} where it remained for another 3 min. Component detection was done by FID. The concentrations that were used to determine the partition coefficients were 1, 5 and 25 ppm (Vol/Vol) with duplicate samples for every concentration.

Calibration curves on this GC-system were determined by manually injecting a series of 10 linearly incrementing amounts of every pure component in hexane on the DB-wax column. The temperature program and system settings were similar to those used for the partition coefficient determinations.

Formulating the mixtures from the identified constituents.

Volatile concentration levels at the SP cannot be derived directly from the amount of eluting volatiles. Concentration levels depend on the dilution of volatiles in the air immediately after their release at SP. This dilution depends heavily on the respiratory capacity of the subject and the exact positioning of the nose. Therefore, an attempt was made to adjust the concentrations of the volatiles in a way that all components previously perceived at SP were also perceivable in the static headspace of the model mixture. This implied diluting the model mixture using distilled water. In doing so, we kept the concentration-ratios in the mixture identical to the mass-ratios in the extraction.

Initially, all derived aqueous dilutions were calculated as if the SP-masses from Experiment 1 were present in 1 mL of air, whereas the final diluted aqueous model was obtained after a subsequent 10-fold dilution in water. At this dilution rate, presumed sub-threshold components were not discriminable whereas supra-threshold components were, with the exception of hexyl acetate (10). This component, not perceived by any of the subjects at SP, was clearly perceivable when presented in static headspace. For this reason hexyl acetate was transferred to the set of supra-threshold components (MIX). The concentrations of the aqueous solutions of the components in the mixture are listed in Table 7.1.

Stimuli

For the construction of the model mixture eleven components were used (Table 7.1). Solutions were made in distilled water. Four stimuli were prepared: BLANK, consisting of distilled water only; BLANK+, that is, the sub-threshold components dissolved in water; MIX, the supra-threshold components dissolved in water and MIX+, all eleven sub- and supra-threshold components dissolved in water. These four stimuli were prepared at three concentration levels: High (the initial solution, as in the last column of Figure 7.1); Medium (1:4 dilution of initial solution) and Low (1:16 dilution of initial solution). In these dilutions the relative concentrations of the various components remained constant.

Pre-testing assured that the combination of the components in the BLANK+ mixture was at detection threshold level for the highest concentration whereas the two lower concentrations were at sub-threshold levels. The MIX mixture was of supra-threshold concentration at all three concentration levels.

Design

A 2 (Complexity) x 3 (Concentration) x 2 (Concept) full factorial design was used. The three variables were defined in the following way.

The detectability of three sub-threshold components was studied under two different Complexity conditions that varied within subjects: a simple condition in which the three sub-threshold components had to be discriminated from distilled water (BLANK+ versus BLANK) and a complex condition in which the sub-threshold components were contained in a complex mixture of 8 supra-threshold components. This complex mixture had to be discriminated from the supra-threshold mixture alone (MIX+ versus MIX). The Concentration factor also varied within

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subjects: the comparison tasks were carried out at three concentration levels. The Concept factor was a between subjects variable. Subjects were assigned to one of two groups, depending on whether they used a poorly refined or highly refined definition of the target stimulus (an apple aroma).

Sensory Analysis (Discrimination test)

Since it was not possible to predetermine any sensory attributes on which MIX and MIX+ could be distinguished, the duo-trio method was applied to measure perceived qualitative differences (Ennis, 1990; Ennis, 1993). A typical duo-trio discrimination trial comprises the presentation of three stimuli: one designated as a standard and, subsequently, two stimuli designated as comparison stimuli. One of the comparison stimuli is identical to the standard. The subject has to decide which of the two comparison stimuli differs from the standard.

The discrimination threshold concentration for BLANK+ was defined as the concentration at which the group proportion of correct responses in discriminating BLANK+ from BLANK equalled 0.75. Using the duo-trio method, a 0.75 probability of correct scores corresponds to a 0.5 probability of correct detections. The highest concentration of the BLANK+ mixture was chosen according to this criterion from pre-test results.

Stimuli were presented in 200 mL glass jars, closed by a low-odour plastic screw lid that could be opened by one simple twist. Every jar contained 10 mL of solution. To minimise the possible migration of odorous components from the lids to the headspace, the lids were separated from the jar by a sheet of aluminium foil. The stimuli in the discrimination test were prepared at least 2 hours before presentation. Presentation was at ambient temperature. Every sample was used only once.

In each session, a subject was presented with all stimuli from the full factorial design: Concentration (3) X Complexity (2). Four possible duo-trio presentation sequences were used in a randomised order within each of the 6 design cells. For each concentration level all eight duo-trio trials were carried out in succession. The subject could either receive the four MIX to MIX+ comparisons first or the four BLANK+ to BLANK comparisons. The ordering of oncentration levels and the order of complexity within concentration levels was randomised over subjects and sessions.

For each concentration level a sequence of 8 sets (24 jars) was presented on a tray. Subjects had to twist off the caps from the jars and remove the aluminium foil while holding the jar close to their nose. They were instructed to sniff the headspace in small sniffs after opening the jar. Subjects could proceed in a free-paced manner within each trial. Between trials, however, the subjects had to rest for 60 seconds. A complete session for all three 'Concentration' levels (24 trials) lasted no more than one hour.

The subjects were trained for two hours on the experimental procedure. Results from these sessions were discarded. Subsequently, four experimental sessions were completed. This resulted in 16 responses per cell per subject (4 sessions, 4 duo-trio replications each). So every subject completed 96 trials (6 cells in the design, 16 replications each). The time interval between two sessions was approximately 2 weeks for every subject.

Before the first training session subjects were instructed to verbalise individually the odour qualities of the samples. Although the subjects were not informed on the nature of the stimuli, they were encouraged to form some kind of mental representation of the odour quality throughout the experiment. Following the last session, subjects were, again, presented with a high-concentration apple odour

stimulus (MIX). They were then asked to verbally describe the odour impression they had. According to their responses subjects were assigned to one of two categories: The 'highly refined' category was used for respondents referring to a clear conceptual representation of the apple aroma, the 'poorly refined' category was used for non-specific or blurred qualifications. Instances of the 'highly refined' category are 'apple' or more refined instantiations of this category like 'ripened apple'. 'Poorly refined' are qualifications like 'sweet', 'fruity', 'pungent' and the like.

Data-analysis

The subjects in this experiment had to discriminate between nearly identical aromas. Therefore, these aromas used can be considered a complex background against which one is asked to detect a weak stimulus (i.e. the sub-threshold components). Signal detection theory (SDT) provides a psychophysical framework to interpret results from such an experiment (Swets, 1961). SDT is built upon the Thurstonian point of view that stimulus magnitudes are projected on a psychological continuum by means of representation processes that introduce variability into that projection. The probability distribution of the resulting psychological representation is hypothesised to be Gaussian (Thurstone, 1927). The probability that a subject will discriminate between two or more stimuli relates to the probability density functions of the stimulus representations. As a result, SDT provides an index for sensory difference. This index, known as δ, is expressed in the number of standard deviations of the Gaussian distribution. It can be calculated for a specific discrimination task, depending on the sensory comparisons that the subject is assumed to make. In a duo-trio discrimination task, the probability of a correct response depends on δ according to the model (David and Trivedi, 1962; Ennis, 1993):

$$P(correct) = F(\delta) = 1 - \Phi(\delta / \sqrt{2}) - \Phi(\delta / \sqrt{6}) + 2 \cdot \Phi(\delta / \sqrt{2}) \cdot \Phi(\delta / \sqrt{6}) \qquad (1)$$

where Φ denotes the cumulated standard normal distribution.

We calculated d' scores, empirical estimates of the perceived sensory difference, from proportions of correct responses per cell and per subject. Unfortunately, this conversion compresses all proportions below 0.5 to the exact score of 0.5 since lower proportions are not allowed in this deterministic model. However, if one wishes to analyse empirical responses in a repeated-measures design, a proportion of correct responses below 0.5 can very well occur due to random variation. Therefore, the proportions of correct responses were also transformed using the logit transformation (McCullagh and Nelder, 1983), which does not compress individual scores below 0.5. Like d' conversion, this conversion yields metrically comparable data, suitable for analyses of variance. The logit conversion, however, lacks the psychophysical relevance of the d' conversion. We considered both sets of transformed data in parallel.

Transformed scores were subjected to repeated-measures analysis of variance ANOVA using SPSS 7.5 software (SPSS, 1997) with Concentration and Complexity as within-subjects variables. To study the modulating influence of Concept refinement, the transformed proportions of correct MIX vs. MIX+ discriminations, irrespective of mixture concentration, were plotted as a function of the corresponding BLANK vs. BLANK+ discriminations for 'highly refined' and 'poorly refined' concept groups separately. Because all BLANK+ vs. BLANK correct discrimination proportions higher than 0.75 are above threshold by definition, we discarded these observations from the analysis together with the corresponding MIX vs. MIX+ responses. The remaining BLANK+ vs. BLANK correct discrimination proportions

were allocated to three categories (low, medium and high discriminability for BLANK+ vs. BLANK) so that the numbers of proportion-correct scores in each category were approximately equal. We tested whether the BLANK+ vs. BLANK discriminability category and the Concept factor had an effect on transformed proportions of correct MIX vs. MIX+ discriminations by means of ANOVA. Independent variables were BLANK+ vs. BLANK difference (3 categories, within subjects) and Concept (2 categories, between subjects).

Throughout this paper $p < .05$ was used as the level of significance.

Results

Proportions of correct discriminations in comparing MIX with MIX+ or BLANK with BLANK+ did not vary significantly between experimental sessions for the three Concentration levels. Therefore, data were aggregated over the four sessions. Mean proportions of correct discriminations between stimuli with or without sub-threshold components are plotted as a function of concentration in Figure 7.2. The mean proportion of correct discriminations between the highest concentration of the BLANK+ vs. BLANK (i.e. 0.78) is not significantly higher than the expected proportion for threshold concentrations (i.e. 0.75) [1-tailed $Z = 1.50$, $p = 0.07$] so, all concentrations are on (or below) detection threshold level.

Mean proportions of correct responses for the BLANK+ vs. BLANK comparisons are higher than mean proportions of correct responses for the MIX vs. MIX+ comparisons. Furthermore, the mean correct response proportions increase with concentration for both the BLANK+ vs. BLANK comparison and the MIX vs. MIX+ comparison (Figure 7.2). A repeated measures ANOVA on the logit-transformed

proportions confirmed these outcomes by significant effects for Concentration, $[F(2,44) = 11.6, p < 0.001]$; Complexity, $[F(1,22) = 116.2, p < 0.001]$; and their interaction, $[F(2,44) = 4.2, p < 0.05]$ which can be ascribed to the weaker effect that Concentration has on discriminability in the MIX+ vs. MIX comparisons compared to the BLANK+ vs. BLANK comparisons. An identical test for d'-converted data revealed similar results: effect of Concentration, $[F(2,44) = 12.7, p < 0.001]$; Complexity, $[F(1,22) = 106.6, p < 0.001]$; and their interaction, $[F(2,44) = 3.3, p < 0.05]$.

Figure 7.3 shows the proportions of correct MIX vs. MIX+ detections plotted as a function of correct response proportions on 'BLANK+ vs. BLANK comparisons for the two 'Concept' groups. ANOVA on logit-transformed proportions did not reveal an effect of 'BLANK+ vs. BLANK proportion correct' on 'MIX vs. MIX+ proportion correct', $[F(2,44) = 0.8. p > 0.1]$. In other words: The responses on 'BLANK+ vs. BLANK' comparisons do not predict the responses on the paired 'MIX vs. MIX+' comparisons. However, the level of concept refinement does. Subjects having highly refined stimulus concepts (13 out of 23) scored higher proportions correct on the 'MIX vs. MIX+' comparisons than the subjects having poorly refined stimulus concepts (10 out of 23) $[F(1,44) = 4.6, p < 0.05]$. Concept refinement was not found to interact with BLANK+ vs. BLANK discriminabilities, $[F(2,44) = 1.5, p > 0.1]$.

Discussion

The first objective of the present study was to compose a mixture of odorants that reflects a natural food aroma with respect to its complexity and its perceived familiarity. Although four SP-detected peaks could not be identified by FID, we

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obtained a mixture generating descriptions related to apple aroma in more than half of the subjects. Since neither visual-, nor verbal clues were given, identification depended exclusively on the mixture's aroma. This suggests that the mixture's aroma reflected the natural apple aroma rather well. Because we failed to identify several SP-detected components, the model mixture may be expected to differ in aroma quality from the original apple aroma. Nonetheless, we consider it acceptable to use the present mixture aroma in studying possible causes for reconstruction discrepancy, since merely the total absence of a distinct apple quality would interfere with the objective to relate stimulus concept refinement to the discriminability of minor aroma changes.
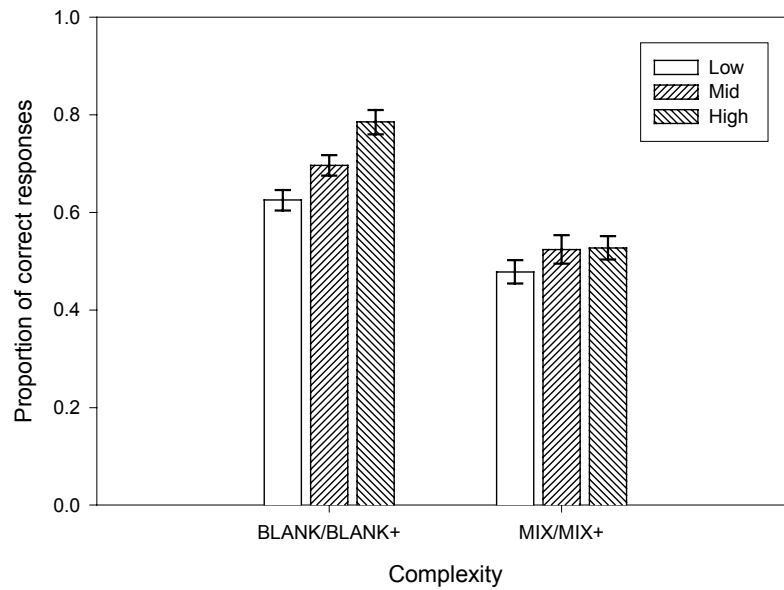
Figure 7.2. **Proportions of correct discriminations (means ± SEM) between mixtures with either a sub- or peri-threshold set of components present or not. These proportions are shown for three different concentrations (i.e. Low, Mid and High) and grouped for the two paired Complexity manipulations.**
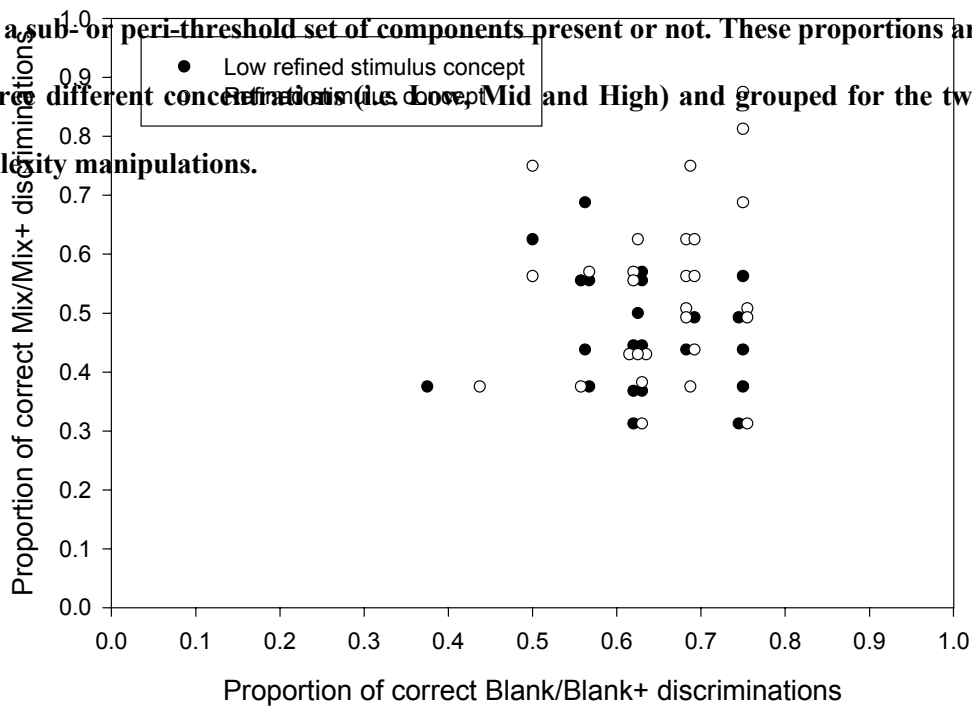

Figure 7.3. **Proportions of correct MIX/MIX+ discriminations as a function of the proportion of correct BLANK/BLANK+ discriminations and marked for the two levels of refinement of the stimulus concept. The clustered dots represent identical proportions. The chance level for both axes is 0.5.**

The probability of detecting peri- or sub-threshold components did not increase in the presence of a mixture of odorants representing an apple aroma. On the contrary, the apple aroma (MIX) decreased detectability of peri- and sub-threshold components. Although this contradicts our hypothesis that sub-threshold components could cause the reconstruction discrepancy, this outcome is not surprising if we relate it to studies that focus on sensory suppressive effects in mixtures. In both the olfactory and the gustatory domain the effect of suppression of certain odour- and taste qualities by other agents is well documented (Berglund and Olsson, 1993; Olsson, 1994; Schifferstein and Kleykers, 1996; Schiet and Frijters, 1988; Cain et al., 1995). Moreover, Stevens and Traverzo (1997) observed that a multi-component mixture of tastants was more effective in masking a dissimilar tasting component than each of the mixtures' components was alone. In fact, their data suggest complete masking additivity of the two masking agents that were used. When applied to the present study, we would expect the complex apple base mixture to act as a powerful masker for the, already nearly discriminable, sub- or peri-threshold components. The observed suppression perfectly fits this line of expectation.

Regardless of the masking effect of MIX, the availability of a well-defined stimulus concept was found to improve the ability to discriminate between similar stimuli. This effect, however, was too small to counteract the suppressive influence of the apple aroma mixture. Note that the 'concept facilitation effect' and the 'mixture suppression effect' are unlikely to be located at the same level of stimulus processing. Mixture suppression has been attributed to various levels of interaction, ranging from peri-receptor levels (Ennis, 1996) to central processing levels (Rouby and Holley, 1995; Algom and Cain, 1991). Most likely, mixture suppression is due to concurrent peripheral and central interactions (Laing and Willcox, 1987; Cain, 1975). This

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suppression takes place during bottom-up information processing, i.e. the integration of information from physical stimuli resulting in a cognitive representation that allows an adequate response or further cognitive processing. In contrast, the concept facilitation effect depends on the influence of memorised sensory representations on the processing of the incoming stimulus information. This is an example of top-down processing. Top-down processing is known to facilitate stimulus recognition in a number of sensory domains. For example, the positive relation between odorants' familiarity and discriminating ability (Rabin and Cain, 1984; Rabin, 1988; Jehl et al., 1995) may be attributed to top-down processing. Therefore, the top-down processing involved in concept facilitation is more central to and intrinsically different from the bottom-up processing involved in mixture suppression.

In this study concept formation theory was proposed as the theoretical framework from which reconstruction discrepancy can be explained. We hypothesised that sub- and peri-threshold components can change aroma quality under the condition that the subject has a well-refined stimulus concept. In part, this hypothesis was supported by the results: subjects who employed a refined stimulus concept showed improved discrimination ability. Since food aromas are familiar to many subjects, we did not choose to manipulate the formation of stimulus concepts in a randomised experimental design. Instead, a quasi-experiment was designed: subjects were assigned *post-hoc* to either of both 'Concept' groups according to their existing concept refinement. Since the sequence of effects was not controlled for, the design permits an alternative conclusion on the direction of the causal relation: *good discriminators develop well-refined stimulus concepts*. Nevertheless, we argue that the hypothesised causal direction – *well-refined concepts make good discriminators* – is the most plausible one. To compare a set of stimuli on a sensory property, a subject

has to employ (meta-) knowledge of stimulus properties and, therefore, discriminating processes can not occur without the involvement of cognition. Since sensory discriminating ability starts out from cognitive processing, sheer discriminators do not exist.

In the present case, a general conclusion should be that peri- and sub-threshold components were not detected better in an apple-like mixture than in isolation. However, stimulus concept refinement did affect discriminability between aromas. For the case of the presented apple model, peri- and sub-threshold components could account for reconstruction discrepancy, albeit with stimulus concept as a confounding factor. The results of this study indicate that semantics are an important factor in odour mixture research because the ecological relevance of the aroma affected its sensory evaluation.

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8 Sequential effects in olfaction: an integrated approach

Abstract

Different conceptualizations of sequential effects are discussed and integrated into a unifying research approach. In an experiment, 4 odorants producing pairs with identical, similar or dissimilar odor qualities were presented sequentially at 'high' or 'low' concentrations using inter-stimulus intervals (ISI) of 20, 60, or 180s. Identical preceding stimuli decreased subsequent graphic intensity ratings and induced stimulus contrast. Dissimilar preceding stimuli raised successive judgments without causing stimulus contrast. Enhancing- and diminutive effects decayed for increasing ISI, with the highest decay rate for enhancing effects. The results support a model comprising two complementary mechanisms causing stimulus effects: chemosensory adaptation and the adaptive property of the receptive system to emphasize qualitative contrast. Time-series analysis of response residuals evidenced effects by previous responses and by non-stimulus-related cognitive processes.

Introduction

When stimuli are presented sequentially, previous stimuli and previous responses may influence the magnitude of the current intensity judgment. Traditionally, two distinct conceptualizations of stimulus context, which we will refer to as global context and local context, are used in studies of stimulus context effects. Global stimulus context effects are observed as sustained modifications of stimulus-response functions that remain relatively stable over successive trials within one session. Relevant sources of global stimulus context are the proportional distribution of preceding stimulus intensities, the stimulus spacing and the span of the range of stimulus intensities. These factors were found to influence intensity judgments within or across various modalities: vision (Mellers & Birnbaum, 1982), audition (Marks, 1992), taste (Schifferstein, 1995), olfaction (Hulshoff Pol et al., 1998) and taste vs. olfaction (Rankin, 1993; Rankin & Marks, 2000; Nordin, 1994). In contrast, local stimulus context effects, generally referred to as sequential effects, are induced by immediately preceding stimuli or responses. As a consequence, sequential effects have a transient character. Sequential effects are usually observed as correlations of judgments with directly preceding judgment- or stimulus magnitudes. They have been observed for audition (McKenna, 1984; DeCarlo & Cross, 1990), vision (Festinger et al., 1970; Wagenaar, 1968), taste (Schifferstein, 1996), olfaction (Baird et al., 1996), numerical stimuli (Wagner & Baird, 1981), haptic stimuli (DeCarlo, 1994), and mixed-modality stimuli (Ward, 1985; Baird et al., 1980). The main focus of the present study is on these sequential effects. First, a taxonomy integrating different sequential effects observed when using different methods is proposed. A class of

influential data analysis methods, called dynamic regression methods is reviewed followed by a review of sequential effect studies in olfaction. A unifying approach to study the anticipated sequential effects in an olfactory setting is proposed. This approach uses aspects from dynamic regression methods and allows the identification of mechanisms of causality. Finally, this approach is worked out in an olfactory experiment.

A taxonomy of sequential effects

Sequential effects on intensity judgments manifest themselves in various ways. Which class of sequential effects is observed depends usually on the chosen method of investigation. Consequently, there is no coherent taxonomy of sequential effects. In addition, effect descriptors are sometimes loosely defined and used inappropriately. Therefore, we will first discuss and categorize four elementary sequential effects together with their associated experimental method. *Assimilation* is the systematic tendency of the current judgment to shift towards the relative magnitude of the previous event (stimulus or judgment). Hence, assimilation is observed as a positive correlation of judgments with previous event magnitudes. Instead of calculating correlations, conditional means may also be used. In that case, assimilation follows from high-average judgments after previous high-magnitude events and low-average judgments after previous low-magnitude events. *Contrast* is the opposite of assimilation, as judgments deviate away from the relative magnitudes of the previous event. Therefore, contrast is observed as a negative correlation of judgment magnitudes with previous event magnitudes. In terms of conditional means this implies low-average judgments after previous high-magnitude events and high-average judgments after previous low-magnitude events. We define *successive enhancement* as a systematic increase of judgments due to previous events, regardless

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of the magnitudes of these events. Furthermore, we define *successive diminution* as a systematic decrease of judgments due to previous events, again regardless of the magnitudes of the previous events. If, for instance, 0.1 ppm, 1 ppm and 10 ppm dilutions of an orange aroma all cause an increase of the intensity judgments of a successively presented apple aroma, then the orange aroma enhances intensity judgments of the apple aroma. Because enhancement and diminution effects are unconditional with respect to the magnitude of previous events, they may occur whilst correlations of judgments with previous events remain zero.

Schifferstein and Oudejans (Schifferstein & Oudejans, 1996) performed an illustrative study that may give a good understanding of the nature of these elementary effects and of the pitfalls involved when studying them. They studied effects of preceding taste solutions on the saltiness rating of a sodium chloride solution as a function of the judged qualitative dissimilarity and the judged saltiness intensity similarity. Preceding stimuli included water, aqueous solutions of quinine, citric acid, sucrose, sodium chloride, and aqueous solutions of sucrose mixed with sodium chloride. Successive enhancement of saltiness ratings was observed for all preceding stimuli that contained no sodium chloride, with the exception of citric acid. Although the enhancement is observed independent of the intensity of the preceding stimulus, this effect is generally being referred to as successive *contrast* (Kroeze, 1983; Schifferstein & Oudejans, 1996; Schifferstein & Frijters, 1992) or sequential *contrast* (Lawless, 1991; Lawless et al., 1991). Here, the term contrast relates primarily to the source of the effect, which is the qualitative contrast between stimuli, rather than the effect itself, which is enhancement. Nonetheless, contrast effects as defined in the present study were also observed by Schifferstein and Oudejans. They reported a decrease of the observed successive enhancement effect that culminated

into successive diminution when they increased the sodium chloride content in preceding stimuli. Hence, increasing the sodium chloride content of stimuli caused decreasing saltiness ratings of subsequent sodium chloride solutions, which implies a negative correlation between previous sodium chloride concentration and the saltiness rating of the current stimulus.

Regression models

Notwithstanding the contributions of sequential effects, a substantial part of the variation on current intensity judgments (R_t) is introduced by the physical intensity of the stimulus that is responded to (S_t). Correspondingly, previous responses at trial *t-k* (R_{t-k}) correlate highly with previous stimulus concentrations (S_{t-k}). As a result, the correlation between subsequent responses (R_t and R_{t-k}) is substantially confounded by the correlation between R_{t-k} and S_{t-k}. To distinguish between the exclusive contributions of S_{t-k} and R_{t-k} on R_t, Jesteadt and co-workers (Jesteadt et al., 1977) assumed a linear multiple regression model specifying each contribution separately:

$$\log R_t = \gamma \log S_t + \sum_{j=1}^{N} \alpha_j \log S_{t-j} + \sum_{k=1}^{M} \beta_k \log R_{t-k} + \delta + \varepsilon_t \qquad (j,k \in \mathbb{N}) \qquad (16)$$

Here, the intercept δ is a constant related to the values on the response scale and ε is an error term that is assumed to be Gaussian-distributed. The regression coefficients γ, α_j and β_k are the weights of the variables that may contribute to the current response: S_t, S_{t-j} and R_{t-k} respectively. If no sequential effects occur, the coefficients α_j and β_k will equal zero, thus reducing the model to the log-log transform of the psychophysical power function with exponent γ. Employing auditory stimuli, Jesteadt and co-workers found a negative coefficient α_1 and a positive coefficient β_1, suggesting that intensity judgments assimilate toward previous responses and contrast with previous stimuli. Responses and stimuli at lags larger than 1 did not contribute

significantly to the multiple regression. Others reported stimulus contrast and response assimilation effects for 1 up to 6 lags (Ward, 1979; Ward, 1982; Ward, 1985; Mori & Ward, 1990; Schifferstein & Frijters, 1992).

Response assimilation is generally found when using models of judgmental sequential effects that include previous responses. In the various theoretical frameworks that were proposed to explain this effect, response assimilation is generally attributed to 'educated guessing' (Ward, 1979; Mori & Ward, 1990; Wagner & Baird, 1981; Green et al., 1977; Petzold, 1981). Responses are guessed within limits set by assumptions of randomness or stimulus probability based on recent stimuli. Generally, these theories predict that, if intensities of previous and present stimuli converge, the observer will increasingly rely on his previous response in the production of the present response. This is reflected in the commonly observed increase of correlation between consecutive responses if the observed intensity difference of the consecutive stimuli decreases e.g. (Jesteadt et al., 1977). This dependency is generally referred to as the *second-order dependency.* When plotting the response autocorrelation as a function of intensity difference (from negative, through zero, to positive), the second-order dependency effect typically produces an inverted V-shaped figure.

DeCarlo and Cross (DeCarlo & Cross, 1990) noted that the stimulus contrast effect at lag 1, often observed when using Equation 1, contradicts the typical stimulus assimilation effect that is found when regression models are used that do not include R_{t-k} as a regressor ($\beta_k = 0$ for $k > 0$). They resolved this discrepancy by establishing that the error term in the model comprising only the S_{t-k} regressor is autocorrelated:

$$\log R_t = \gamma \log S_t + \alpha_1 \log S_{t-1} + \delta + \rho e_{t-1} + u_t \tag{17}$$

In this equation, $\log R_t = \delta + \gamma \log S_t$ is the log-log converted psychophysical function and S_{t-1} is the physical intensity of the previous stimulus. The error term is decomposed into a first-order autocorrelive part ρe_{t-1} and a random variable u_t with zero mean and not correlated with any u_{t-k} ($k \in \mathbb{N}^+$) or e_{t-k}. The parameters γ, α and ρ are weights of the model factors. DeCarlo and Cross showed that if a positively autocorrelated error term was not included in Equation 2, a negative S_{t-1} coefficient would have been obtained, suggesting a stimulus contrast effect. This was supported by a review of a number of auditory and visual studies that presented coefficients for Equation 1. The authors evaluated the effect of omitting the R_{t-k} term from the model by recalculating the S_{t-1} coefficients that would have resulted if Equation 2 had been used. This resulted in a shift from negative to slightly but consistently positive S_{t-1} coefficients, which implies a small but consistent assimilation of R_t to S_{t-1} rather than a stimulus contrast. The authors concluded that Equation 2 is a parsimonious model, providing a better account of the data than Equation 1 or Equation 2 without the autocorrelated error term. DeCarlo and Cross further suggested that the first-order autocorrelated error term may reflect non-monitored processes such as attention, memory, motivation or strategy that affect response behavior over more than 1 time period. If so, the contribution of the cognitive variable to residual autocorrelation would depend on task instruction. To test this hypothesis, subjects were instructed either to refer to one fixed stimulus-response pair (free magnitude estimation) or to use the immediately preceding stimulus-response pair (ratio magnitude estimation) as a reference for current auditory intensity judgments. As predicted, ratio magnitude estimation led to the highest contribution of the autocorrelated error term to R_t variance.

Studies of sequential effects in olfaction

Studies on sequential effects in olfaction are scarce. Two studies have investigated sequential effects for odor intensity. Baird *et al.* (Baird et al., 1996) reported response assimilation of magnitude estimation judgments as indicated by positive correlations between R_{t-1} and R_t. In line with the commonly reported second-order dependencies, response assimilation was highest if subsequent stimuli were equal. Gregson and Paddick (Gregson & Paddick, 1975) studied sequential effects using a linear regression model relating R_t to S_{t-k} ($k \in \mathbb{N}^+$) not accounting for an autocorrelated error term. They observed contrasting effects of S_{t-1} on R_t for separate series of eugenol or acetophenone at various concentrations. According to the authors, these contrast effects were probably caused by olfactory adaptation.

In addition to these studies on odor intensity, studies have been performed in which the odor qualities varied in the sequence. Lawless (Lawless, 1991) studied the effect of the odor quality of preceding stimuli on the intensity judgments on two character descriptors of dihydromyrcenol (DHM). This odorant elicits an ambiguous odor comprising both a citrus-like and a woody sensation. After initial presentation of odorants with a predominantly citrus-like character, the woody aspect of DHM was rated higher than after initial presentation of woody odorants. In contrast, citrus intensity judgments of DHM were higher after initial presentation of woody odorants than after initial presentation of citrus-like odorants. These results may either be caused by successive enhancement after presenting a stimulus with a different character or successive diminution after a stimulus with a similar character. Because stimulus concentrations did not vary in this study, no conclusions can be drawn with respect to contrast and assimilation effects.

Gregson (Gregson, 1983) presented binary odorant mixtures and recorded magnitude estimates of the character descriptors of each odorant in the mixture (indexed i and j). Judgments of each odorant's intensity at time t in the sequence ($R_{i,t}$ and $R_{j,t}$) were modeled as functions of previous judgments and previous and present stimulus components ($S_{i,t-k}$ and $S_{j,t-k}$, $k \in \mathbb{N}^+$). Besides a mutual mixture suppression of the intensity of the character descriptor of one odorant by the other odorant (effects of $S_{i,t}$ on $R_{j,t}$, and $S_{j,t}$ on $R_{i,t}$ respectively), successive diminution was observed as an effect of each substance on ratings of it's character descriptor for the following stimulus ($S_{i,t-k}$ on $R_{i,t}$ and $S_{j,t-k}$ on $R_{j,t}$, respectively). In summary, these studies show that sequential dependencies also occur for the sense of smell, and that the size and the nature of these effects depend on both the quality and the intensity of the used stimuli. In short, successive diminution is observed when previous stimuli are of the same odor quality (Gregson, 1983) and contrast is found under similar circumstances if a correlative method is used (Gregson & Paddick, 1975). Like for taste (Schifferstein & Oudejans, 1996), either dissimilar odor qualities induce enhancement or identical qualities induce diminution (Lawless, 1991; Lawless et al., 1991). Assimilation appears to be a response-induced cognitive effect, whose occurrence depends on the similarity of successive stimulus qualities (Baird et al., 1996).

From the picture that emerges from the discussed studies we distilled the following working hypotheses with respect to olfactory sequential effects. The correlative effects of contrast and assimilation are directly or indirectly related to stimulus intensity variation. Contrast depends on the intensity of the previous stimulus and has been associated with olfactory adaptation. Assimilation depends on the previous response magnitude if intensities of two consecutive stimuli are similar and, hence, is likely to be related to response generation processes. The level effects of successive

diminution and enhancement are related to variation in stimulus quality. Enhancement

is caused by dissimilar preceding stimuli and diminution is caused by identical

preceding stimuli. In line with DeCarlo and Cross (DeCarlo & Cross, 1990), we

suggest that autocorrelation of residuals is caused by cognitive factors like attention,

memory, motivation or strategy. In contrast with the suggested sources of correlative

and level sequential effects, these factors are not directly related to specific stimulus

evaluations. An overview of the hypothesized effects and their associated methods is

presented in Table 8.1.

The present study

In the present study, we intend to gain a better understanding of the sources of sequential effects in olfaction by testing the above hypotheses. To accomplish this, we present odorants sequentially, using a factorial design in which the stimulus intensity and the similarity between subsequent stimuli are varied systematically. This design allows the calculation of correlation effects (contrast and assimilation). However, to calculate level effects (enhancement and diminution), it is essential to compare observed averages against the intensity that judged stimuli would have had in the absence of preceding stimuli. To obtain such unbiased estimates of judged intensities,

Table 8.1. Taxonomy of sequential effects that may be observed. Specified are the experimental methods that are sensitive to the corresponding sequential effect, the task variables that are possible sources of the effects and the hypothesized processes involved.

Sequential effect	Experimental method	Hypothesized source	Possible proces involved
Successive enhancement	conditional R_t averages for previous qualitative similarity categories	qualitative stimulus contrast	cognitive (stimu concept compai
Successive diminution	conditional R_t averages for previous qualitative similarity categories	qualitative stimulus similarity	periphery (neur adaptation) cognitive (habit
assimilation	conditional R_t averages for categories of previous event magnitudes correlating R_t with previous event magnitudes	previous judgment magnitude (identical stimulus quality)	cognitive (judgi processes)
contrast	conditional R_t averages for categories of previous event magnitudes correlating R_t with previous event magnitudes	previous stimulus magnitude (identical stimulus quality)	periphery (neur adaptation)
residual autocorrelation	auto-correlating residuals from the best fitting model of R_t averages	mental state	Cognitive (glob factors like atte memory, motiv strategy)

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we follow the approach used by DeCarlo (DeCarlo, 1992) to study the decay of sequential effects in time. In an auditory task, he varied inter-stimulus intervals and observed that S_{t-1} assimilation decreased in time, becoming nearly absent at the longest inter-stimulus intervals. Accordingly, we vary inter-stimulus intervals to obtain decay functions of level effects for all degrees of similarity between a specific stimulus and preceding stimuli of various qualities. By estimating the level of convergence of these decay functions, the unbiased intensities of each specific stimulus may be estimated. This design will give insight into the processes underlying sequential effects because different decay times may indicate the involvement of different processes. Table 8.1 shows some processes possibly involved in the discussed sequential effects. Two distinct levels of information processing are the peripheral and the central level. At the central level, conscious processing may be required to some extent. Because conscious information processing is more readily impaired by a limited processing capacity of the system than peripheral processing, sequential effects caused by central processes may be less robust against the effects of new stimulus presentations than sequential effects caused by peripheral processes. The design of this experiment allows for the examination of the robustness of sequential effects for interceding stimuli. In spite of its great explanatory potential, a multi-factor experiment has, to our knowledge not yet been used in studies of sequential effects.

Preliminary experiment: selection of odorants of three similarity levels

In the present study we wish to manipulate the qualitative similarity of subsequent stimuli. This section describes the selection of two pairs of stimuli that are perceived as similar within-pairs but dissimilar between pairs. The selection took place on the basis of chemical and perceptual dissimilarity.

Materials and Method

Subjects

Table 8.2. Substances used to make odorous stimuli, their nominal purity, their corresponding quality descriptors and their aqueous concentrations in the preliminary and main experiment.

Component	Nominal purity	Attributes in the main experiment (translated from Dutch)	Concentration in prelim. experiment ($\mu L/L$)	Concentrations in training session ($\mu L/L$)	Concentrations (Low-High) in main experiment ($\mu L/L$)	
1-hexanol[c]	> 99.5%	nuts[g]	130	50	25	100
2-methyl-1-butyl	> 94%	sour	7.5	7.5	7.5[m]	
Hexanal[a]	> 98%	hedge[g]	2.5	3.0	1.5	6
Isobutyl acetate[a]	> 99%	lacquer[g]	10	10	10[m]	
Octanal[c]	> 98%	fatty[h]	1.0	1.0	1.0[m]	
R-(+)-limonene[b]	> 99%	orange[i]	40	45	22.5	90
2-octanone[c]	> 99%	blue[j]	2.0	4.0	4.0[m]	
citral[d,f]	> 98%	lemon[k]	1.0	1.25	0.625	2.5

Substance are obtained from: [a]Alldrich, [b]Sigma, [c]Merck and [d]Acros.

[e]Sour candy is a popular hardboiled sweet in the Netherlands where it is referred to as 'zuurtjes'.

[f]Racemic mixture of neral and geranial.

[g](Bult et al., 2001), [h](Rychlik et al., 1998), [i](Livermore & Laing, 1996), [j]Odor is very similar to the odors o similar methyl-ketones 2-heptanone and 2-nonanone which are key aroma components in blue cheese (Lub et al., 1997), [k](Kuenzel & Bahri, 1990), [m]Concentrations of the four components that are used as distracters

Employees and students from the Food Chemistry department participated voluntarily in a panel of 10 subjects, 5 men and 5 women. Their average age was 24.1 (SD = 3.0). At the day of the experiment, subjects were not allowed to eat spicy food or to use any fragrance.

Stimuli

Eight substances were selected so that the odors varied from very similar to very dissimilar. Approximately equally intense stimulus concentrations were obtained after preliminary consultation of 10 co-workers at the faculty using an intensity-ranking task. Initial concentrations were adjusted in an iterative way according to the average ranks of the eight substances after each of two sessions. The resulting stimuli had moderate intensities that were well above threshold. All but one substance were dissolved in distilled water according to the concentrations mentioned in Table 8.2. At room temperature, the given solution concentration of R-(+)-limonene could not be achieved in water. Therefore, this substance was prepared as an oil-in-water emulsion using a high-speed blender (Ultra Turrax T25, Janke & Kunkel IKA labortechnik) at 20,500 min^{-1} during 60 s. Assuming that headspace concentrations of dissolved components depend on molecular diffusion at the interface between the water phase and the headspace, emulsions will produce headspace concentrations proportional to the concentration dissolved in water, and not to the amount present in oily volume elements in the emulsion. Nevertheless, stimulus intensity is still related to the total amount of oil fraction in the emulsion due to eddy diffusion (De Roos, 2000). Hence, the chosen stimulus preparation of R-(+)-limonene allowed manipulation of stimulus intensities above the maximum solubility in water. To prevent aggregation of oily volume elements in the emulsion, R-(+)-limonene stimuli were prepared within one hour prior to presentation. The emulsion could not be discriminated visually from

other stimuli, as was confirmed by interviewing subjects after the experiment. Except for R-(+)-limonene, solutions were prepared and stored in the dark at 4 ℃ for 16 hours and poured into presentation jars 2 hours before each session.

Procedure

Subjects were seated in individually ventilated booths. Stimuli were presented in 200-mL glass jars that were closed by low-odor plastic screw caps. Each jar contained approximately 10 ml of solution. To prevent exchange of volatile components between the screw caps and the headspace, the jars were sealed with aluminium foil before being capped. Stimuli of eight different qualities were presented in pairs, each pair comprising two different qualities. All subjects received all 28 possible stimulus pairs. The order of stimuli in a pair and the order of pairs in the experiment were randomized per subject.

The subjects opened stimulus jars by unscrewing the caps while keeping the jars just underneath their noses. Immediately after opening the jar, they took one or a few shallow sniffs and closed the jar. They were not allowed to evaluate a stimulus twice. Subjects could proceed in a free-paced manner to the second stimulus of each pair. After sniffing the second stimulus, subjects judged the qualitative difference of the pair using a 150-mm graphic rating scale presented on a laptop computer screen. The left end of the scale was labeled 'very similar' and the right end was labeled 'very dissimilar'. Any intensity differences, if perceived, were to be disregarded. Forty-five seconds after filling in their response, the computer screen signaled that subjects could proceed with the next pair.

Stimulus intensities had been matched before the start of the experiment. Because new panelists participated in this study, we double-checked the equi-intensity

assumption. Therefore, after finishing the dissimilarity-rating task, the subjects ranked the 8 stimuli according to their perceived intensities.

Statistical analyses

Perceived dissimilarities were assumed to be independent of the order of stimulus presentation. Hence, each combination of two stimuli was presented in one arbitrary, randomly selected order, after which the inverse order of this pair was assigned the same dissimilarity score. Dissimilarities between identical stimuli were assumed zero. For each subject, dissimilarity scores were tabulated in an 8×8 square-symmetrical distance matrix. The dissimilarity matrices were used to estimate disparities between the eight odors in a group multidimensional Euclidean space using the Alscal multidimensional scaling algorithm in SPSS. Distance scores were treated as ordinal measures. The fit of the multidimensional model is expressed by Young's S-stress criterion, which is 0 for perfect fit and 1 for the worst possible fit. Results of the stimulus intensity-ranking test were subjected to the Kruskal-Wallis test of rank equality. Throughout this study a significance level of 0.05 is used.

Results

Multidimensional distance models were estimated from individual dissimilarity scores of the stimulus pairs. Subsequent S-stress scores for 1-, 2-, 3-, 4- and 5-dimensional models were 0.49, 0.29, 0.20, 0.13 and 0.09 respectively. Although stress-improvement is still considerable when the dimensionality of the model is raised from 3 to 4, we used the three-dimensional model (Figure 8.1) because the number of subjects used (10) does not allow for a reliable estimation of associations at higher dimensions (Kruskal & Wish, 1978). The disparities for the odorants in three-dimensional space were used to calculate Euclidean distances between stimulus

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projections on three dimensions (Table 8.3). Large distances represent dissimilar odorants whereas small distances represent similar odorants. Pairs of odorants that showed the smallest distances were selected as similar odorant pairs (distance ranks 1 and 2). This resulted in the selection of the similar pairs citral – R-(+)-limonene and 1-hexanol – hexanal. Odorants from different pairs showed inter-odorant distance ranks 12, 18, 16 and 22 (out of 28 ranked distances).

Individual scores on the stimulus intensity-ranking task did not yield averaged ranks that differed significantly [Kruskal-Wallis statistic $H(7) = 9.016$, $p = 0.251$]. Therefore, we considered the used concentrations (Table 8.2, column five) approximately equally intense.

Table 8.3. Euclidean distances between odorants as derived from the best fit 3-dimensional MDS solution in the preliminary experiment. Small distances represent similar odorants, whereas large distances represent dissimilar odorants. Indices are rankings of the respective distances from smallest (1) to largest (28).

	hexanol	methyl but	octanone	Citral	hexanal	Iso-but acet	limonene	octa
Hexanol	0	2.16	1.72	**2.61[12]**	**0.96[2]**	2.99	**2.80[16]**	2.38
Methyl but		0	1.98	2.99	2.86	1.19	2.87	2.17
Octanone			0	2.95	2.67	2.68	3.24	3.50
Citral				0	**2.83[18]**	2.72	**0.62[1]**	3.13
Hexanal					0	3.60	**2.97[22]**	2.26
Iso-but acet						0	2.46	2.56
Limonene							0	2.81
Octanal								0

Figure 8.1. Three-dimensional MDS solution for the eight stimuli used in the preliminary experiment. Small disparities represent similar odorants, large disparities represent dissimilar odorants. Euclidean distances within and between the two clusters of similar components are given in Table 8.3.
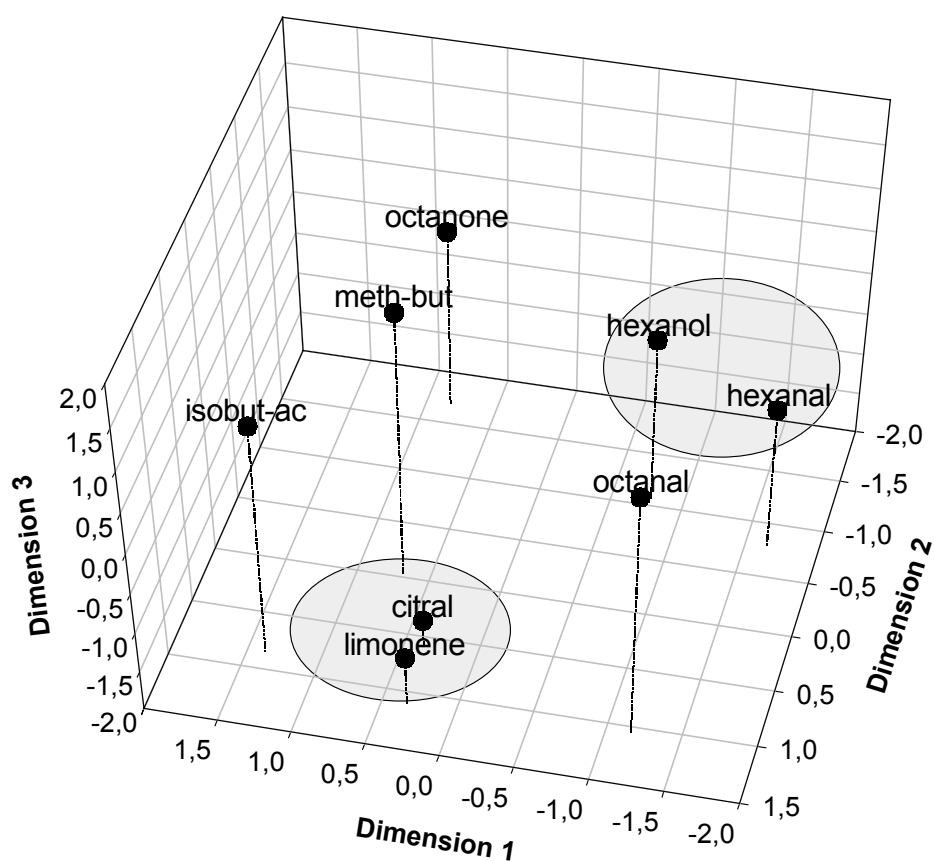
Main Experiment

Materials and Method

Subjects

Fifteen paid subjects, six men and nine women, average age 27.4 years (SD = 9.5), participated in the experiment. All subjects, recruited from the local Wageningen community, were selected on basis of their ability to generate refined odor attributes and their ability to use intensity scales consistently. All were non-smokers and none suffered from olfactory disorders. Subjects gave informed consent. They were naïve with respect to the objectives of the experiment. All subjects except one had experience in olfactory experiments.

Stimuli

The four equally intense substances that were selected in the preliminary experiment were used to form two sets of two substances that are qualitatively similar within, but dissimilar between sets. Four other substances of the same intensity were added as distracting stimuli. The concentrations of the experimental stimuli were multiplied and divided by two, to obtain high and low concentrations. Resulting stimulus concentrations of the eight experimental stimuli and the four distracters are given in Table 8.2. Stimuli were prepared as described in the method section of the preliminary experiment.

Stimulus sequencing

Stimulus sequences varied on three factors: stimulus quality (*Quality*, 4 levels: citral, R-(+)-limonene, 1-hexanol and hexanal), stimulus intensity (*Intensity*, 2 levels:

High and Low) and *ISI* (3 levels: 20 s, 60 s and 180 s). A stimulus sequence can be conceived of as a series of consecutive stimulus pairs:

$$\text{Sequence:} \quad S_1 \quad S_2 \quad S_3 \quad ... \quad S_k \quad S_{k+1} \quad ... \quad S_{n-1} \quad S_n \quad S_1$$
$$\Downarrow \quad \Downarrow \qquad \qquad \Downarrow \qquad \qquad \Downarrow \quad \Downarrow$$
$$\text{Pairs:} \qquad S_1S_2 \quad S_2S_3 \qquad \quad S_kS_{k+1} \qquad \quad S_{n-1}S_n \quad S_nS_1$$

where S_i is the ith stimulus in the sequence. Within each full sequence, the stimulus pairs comprised all possible combinations of *Quality*, *Intensity* and *ISI*. Four different odor qualities at two intensities per quality resulted in 8 different stimuli. Allowing for identical stimulus pairs, these combined to $8^2 = 64$ different sequences of two stimuli. Combining all unique pairs with the three ISIs resulted in 192 unique S_k-ISI_k-S_{k+1} combinations. For each subject, a computer algorithm generated a unique random stimulus sequence by randomly generating ISI-stimulus combinations and selecting only those that met the requirements of a full factorial design of pairs at lag 1. This sequence was split up into six consecutive sections that were presented at separate sessions during a three-week period. If consecutive sections are presented on different occasions, sequential effects are not transferred over the intersections. To prevent these discontinuities, sections were preceded by repetitions of the last four ISI-stimulus combinations of the preceding section. At the first session, the last four ISI-stimulus combinations of the last section preceded the section presented. As a result, each person's stimulus sequence could be conceived as a circular sequence with each of the 192 different ISI-stimulus combinations being preceded by at least four stimuli. We did not use responses to the four 'warm-up' stimuli in our analyses. This procedure also excludes responses generated while adopting a response strategy (Schifferstein & Kuiper, 1997), inclusion of which could harm stationarity of response data.

Although only responses to four different stimulus qualities were used for data analysis, the subjects were to anticipate any of 8 different stimulus qualities at any time. This was done to increase task difficulty. Four extra qualities of distracter stimuli were added to the sequences at each session. To ensure that these distracter stimuli did not influence performance on the main task, they were appended before (3 distracters) and after (1 distracter) the main sequence. Each session lasted approximately one hour. An example of a full stimulus sequence using two odor qualities, 2 intensities and 2 ISIs is shown in Table 8.4.

Procedure

Laboratory conditions and stimulus preparations were similar to those in the preliminary experiment. In an approximately one-hour training session, subjects were familiarized with the odorants and the experimental procedure. They were instructed to sniff the eight equi-intense odorants (Table 8.2, column 5) and to memorize the accompanying odor descriptors (Table 8.2, column 2). After memorizing odor-descriptor associations, they had to match descriptors to the eight odors until performance was perfect. Finally, a 30-minute version of the sequence experiment was presented to familiarize subjects with the routine.

The six experimental sessions had identical procedures. First, subjects did the

Table 8.4. Example of a randomized stimulus sequence. Distracting stimuli ($X_1 – X_4$) consist of equi-intense 2-methyl-1-butyl acetate, isobutyl acetate, octanal and 2-octanone. Warm-up stimuli are repetitions of the last four stimuli in the previous session, or the last four stimuli in the final session if the current session is the first session (see the outlined sections). The experimental sequence (bold typed letters) comprises a full factorial combination of all possible binary stimulus combinations with all possible *ISI*s (32 stimuli in this example and 192 stimuli in the experiment). The stimuli 'a' and 'A' are low and high concentrations respectively of one substance whereas 'b' an 'B' are low and high concentrations respectively of another substance. Numbers below the stimulus indices are the *ISI*s (s) between consecutive stimuli.

Session #	Distracting stimuli			Warm-up stimuli				Experimental sequence										Distracting stimuli
1	X_1	X_2	X_3	b	b	a	a	**B**	**B**	**b**	**A**	**a**	**a**	**B**				X_4
	60	20	20	60	20	60	20	**20**	**60**	**60**	**60**	**20**	**60**	**60**				20
2	X_1	X_2	X_3	A	a	a	B	**a**	**b**	**b**	**a**	**A**	**B**	**A**	**A**	**b**	**B**	X_4
	20	60	20	60	20	60	60	**60**	**20**	**60**	**20**	**20**	**20**	**20**	**60**	**20**	**60**	60
3	X_1	X_2	X_3	A	A	b	B	**b**	**B**	**a**	**A**	**b**	**A**	**A**	**B**	**B**	**A**	X_4
	20	60	60	20	60	20	60	**20**	**20**	**20**	**60**	**60**	**20**	**20**	**60**	**20**	**60**	60
4	X_1	X_2	X_3	A	B	B	A	**a**	**b**	**b**	**a**	**a**						X_4
	20	20	60	20	60	20	60	**60**	**60**	**20**	**60**	**20**						20

[1]The intervals preceding distracting stimuli were randomly chosen from the set of intervals used in the target sequence

matching test that was also used in the training session. After perfect performance was reached, they proceeded with the main experiment. Subjects were guided by a time-controlled computer routine that presented instructions on a 12-inch laptop color screen at 800×600-pixel definition (Acer Extensa 501T, Pentium II). Stimulus jars in the sequence were indexed with increasing numbers that corresponded with the numbers of the consecutive response fields on the computer screen. Each stimulus evaluation trial started with a grey screen showing the number of the upcoming stimulus. The subject had to keep the jar with that number underneath his/her nose. The jar was opened as soon as the background color of the computer screen turned to green. When the subject started to sniff a jar, (s)he struck a key registering the time the actual sniffing commenced. As soon as the subject was ready to indicate the stimulus quality and intensity, (s)he struck a key again after which a screen appeared with eight horizontal 150-mm graphic scales, each labeled on the left hand side with one of the eight odor descriptors (Table 8.2). The left end was labeled 'no perceivable odor' whereas the right end was labeled 'extremely strong odor'. From top to bottom, the descriptors were arranged in alphabetic order. By a single mouse click on the appropriate scale, subjects selected an odor descriptor and rated the odor intensity. Finally, subjects confirmed their choice by clicking a button in the response screen. Then the next trial's initial gray screen was shown. The signal that called for stimulus evaluation was time-locked with the call for the former stimulus evaluation, regardless of the in-between actions of the subject. This procedure was repeated until the last stimulus was evaluated. As an additional check on whether the proper ISIs were respected in the experiment, all response actions on the computer were timed and recorded automatically.

Data treatment and statistical analyses

Normalization of response data. To compensate for idiosyncratic scale usage, each subject's responses were normalized according to:

$$R_{t,subj} = \left(\frac{R'_{t,subj} - \overline{R}'_{subj}}{SD(R'_{subj})} \right) \cdot SD(R'_{all}) + \overline{R}'_{all} \tag{18}$$

Here, the normalized intensity score for an individual subject at time t [$R_{t,subj}$] is calculated as a function of the subject's response at time t [$R'_{t,subj}$], the average response value of that subject [$\overline{R}'_{subj}$], the standard deviation of the intensity scores of that subject [$SD(R'_{subj})$], the standard deviation of all subjects responses [$SD(R'_{all})$] and the average response intensity of all subjects [$\overline{R}'_{all}$].

Calculation of residuals. In compliance with the sequential regression model of Equation 2, we related judgments R_t to S_t and S_{t-1} and not to R_{t-1}. To prevent confusion with the physical stimulus concentrations S_t and S_{t-1}, we will specify the physical intensity levels by I_t and I_{t-1} ('low' or 'high'). In this study we studied effects on intensity judgments by the level of qualitative similarity with the previous stimulus (*QSim*: identical, similar and dissimilar), I_{t-1}, I_t, and *ISI* (20 s, 60 s and 180 s). The average panel response to a stimulus in a specific experimental condition is obtained by calculating the average of normalized responses of panelists for that condition. Hence, such panel averages can be interpreted as estimates of normalized responses for individual subjects. When averaged panel responses are used as estimators of individual responses, observed normalized responses by individual subjects may deviate from the mean panel response due to a limited number of factors. First, individual subjects may show different sensitivities to an odorant. This would cause differences in the shape of subjects' psychophysical functions and raise the inter-

subject variability of responses. Second, repeated evaluations by one observer of identical stimuli in identical contexts generally result in variable judgments due to irregularities in the transfer of the stimulus to the receptor and the transfer of information through the perceptional system. Due to their *ad hoc* nature, these two factors, i.e. inter-individual sensitivity variation and intra-individual variation of the responsiveness of the perceptual system, will only cause uncorrelated residuals. A third factor is the influence of cognitive variables that induce response bias, independent of the stimulus being evaluated (DeCarlo & Cross, 1990). For instance, a subject may be inclined to overestimate intensities during some consecutive trials as a result of an increased task motivation. This would result in systematic changes of response residuals over multiple stimulus evaluations (DeCarlo & Cross, 1990), inducing a positive autocorrelation of residuals. Finally, if the proposed regression model does not account for effects of R_{t-1} on R_t , and second-order dependencies of R_t on R_{t-1} occur, then these dependencies will contribute to the residual variance. Correlations of R_t with R_{t-1} then would cause autocorrelating residuals, but only for specific combinations of subsequent stimuli. In the present study, residual autocorrelation effects due to cognitive factors and response autocorrelation were studied using residuals of the full factorial *QSim* (3), I_{t-1} (2), I_t, (2), *ISI* (3) and *Odorant* (4) model of averages.

Conditional autocorrelation effects. Pairs of successive stimuli were categorized according to the similarity of their intensities. Intensity similarities (*ISim*) of I_t and I_{t-1} were either 'identical' (i.e. high$_{t-1}$ vs. high$_t$ or low$_{t-1}$ vs. low$_t$) or 'different' (i.e. high$_{t-1}$ vs. low$_t$ or low$_{t-1}$ vs. high$_t$). In two consecutive analyses, the dependencies of the Pearson product moment correlations (*r*) between successive residuals [residual(*t*) and residual(*t*-1)] on the joint effects of *ISim* and *QSim* and the joint effects of *QSim* and

ISI were calculated. Differences between residual correlations were then tested in a (2×3) *ISim* × *QSim* and a (3×3) *QSim* × *ISI* repeated-measures analysis of variance. Prior to the repeated-measures analysis, correlations were corrected for deviations from normality by *r'*- transformation of individual *r* (Fischer, 1921) by:

$$r' = 0.5 \cdot \ln\left(\frac{1+r}{1-r}\right) \tag{19}$$

Since 15 subjects produced data and because the number of within-subject categories was - depending on the test design – either six or nine, we used univariate tests for within-subjects effects (Stevens, 1996). Univariate test results were corrected for non-sphericity using Greenhouse-Geisser's epsilon. Analyses were performed with the SPSS v10 statistical software package. For all tests, a significance level of 0.05 was used.

Results

Threats to reliability of time series analyses and external validity

Learning effects may have occurred over the six consecutive sessions. If so, proportions of correct attribute selections, intensity judgments and, possibly, residuals may show trends. A prerequisite for the calculation of autocorrelations, referred to as weak stationarity, is that averages and standard deviations are constant for any subsection of a data series (Chatfield, 1996). Thus, the occurrence of trends in residuals over time would imply a violation of the stationarity of this data series and harm the reliability of autocorrelation calculations. An inspection of the proportions of correct attribute selections indicated no learning effects: over sessions, the proportions of correct attribute selections remained constant at approximately 0.7. A repeated measures ANOVA of logit-transformed proportions of correct scores did not

show significant differences between sessions [$F(5,70) = 0.694$, $p = 0.577$]. Average judgments were 42.1, 44.5, 46.2, 41.2, 41.0, 44.3 (for sessions 1 – 6 respectively) for low concentrated stimuli and 70.8, 72.6, 73.2, 68.3, 71.3, 72.3 (for sessions 1 – 6 respectively) for high concentrated stimuli. These scores did not show any trends over sessions.

In the preliminary study, we made up sets of substances constituting odorant pairs with different similarity qualifications. Since the panel in the preliminary study differed from the panel in the main study, we examined whether the panel in the main study agreed with the similarity categories. To test this, we assumed that similar stimuli would be confused with each other more frequently than dissimilar stimuli. This implied that the correct descriptor would be selected most frequently, and that similar stimulus descriptors would be selected more frequently than dissimilar descriptors. In Figure 8.2, proportions of responses on the *QSim* categories 'correct', 'similar' and 'dissimilar' are shown for the four substances. Proportions for each distracter category are also included. For all stimulus qualities, correct responses were most common, false identifications by using a 'similar' category were less common. Despite the fact that responses were cumulated over two 'dissimilar' categories, these categories received the lowest proportion of false responses. A (3 × 4) repeated-measures analysis of variance on logit-transformed proportions of correct responses to the three *QSim* categories for the four different odorants citral, R-(+)-limonene, 1-hexanol and hexanal (Odorant) resulted in a significant main effect of *QSim* [$F(2,28) = 64.31$, $p < 0.001$]. Significant effects were found for the contrasts comparing 'correct' with 'similar' proportions and 'similar' with 'dissimilar' proportions [$F(1,14) = 23.93$, $p < 0.001$; $F(1,14) = 47,29$, $p < 0.001$ respectively]. This implies that the probability of descriptor selection decreased with increasing dissimilarity

between descriptor and stimulus. A significant effect of Odorant [$F(3,42)=5.44$, $p = 0.007$] was also found. This was mainly due to the different frequencies with which distracters were selected per odorant (Figure 8.2). The relatively high proportions of distracter selections after presentation of 1-hexanol (Figure 8.2) also added to the external validity of the results. These high proportions corresponded with the small disparities between the two distracting substances octanone ('blue cheese') and octanal ('fatty') and the target substance 1-hexanol (Table 8.3 and Figure 8.1), as they were found in the preliminary study. No significant Odorant × *QSim* interaction was found [$F(6,84) = 1.36$, $p = 0.271$].

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insert Figure 2 approximately here

Effects of current stimulus intensity, former stimulus intensity, ISI and inter-stimulus similarity on intensity judgments

Normalized intensity judgments were averaged over substances. The influences of *ISI* levels (20 s, 60 s and 180 s), *QSim* levels ('identical', 'similar' and 'dissimilar'), I_t levels ('high' and 'low') and I_{t-1} levels ('high' and 'low') on normalized intensity judgments were tested within-subjects by repeated measures ANOVA. Significant main effects were found for I_{t-1} [$F(1,14) = 12.50$, $p = 0.003$], I_t [$F(1,14) = 415.71$, $p < 0.001$] and *QSim* [$F(2,28) = 40.55$, $p < 0.001$]. A significant interaction was found for *ISI* × I_t [$F(2,28) = 4.32$, $p = 0.025$].

To illustrate these effects, we presented averaged responses as functions of sub-sets of the independent variables in Figures 8.3, 8.4 and 8.5. Inspection of the mean intensity judgments in Figure 8.3 learned that the main effect of *QSim* was due to consistently lower judgments of stimuli preceded by identical stimuli. Furthermore, at

20 s ISI, stimuli preceded by dissimilar stimuli were judged more intense than stimuli preceded by similar stimuli. To determine whether *QSim* effects on intensity judgments reflected successive diminution or successive enhancement effects, we assumed that sequential effects reduce to zero at sufficiently large ISI. In other words: At infinitely large ISI, responses to a specific stimulus would all level out at a unique judgment value, which is free from enhancement- or diminution effects due to *QSim*. Hence, this asymptotic level reflects a judgment that is free from sequential effects. To estimate the asymptotic level from the data, normalized responses were modeled by exponential growth and decay functions of ISI:

$$R_{ISI} = R_0 + a_{QSim} \cdot b_{QSim}^{ISI} + e_{ISI} \qquad a, b \in \mathbb{R}, \quad 0 \le b \le 1, \quad ISI \ge 0 \tag{20}$$

where R_{ISI} is the response intensity at time *ISI*, R_0 is the asymptote of the model, representing the judgment in the absence of sequential effects at infinitely large *ISI*, and e_{ISI} is an error term. The index *QSim* indicates the qualitative similarity between the currently judged stimulus and the previous stimulus. In Figure 8.3, the least-sum-of-squares model fit is superimposed on the averaged intensities across *ISI* × *QSim* categories. It shows that intensity judgments decrease when preceded by qualitatively identical stimuli and increase when preceded by qualitatively dissimilar stimuli. The successive diminution effect of identical preceding stimuli can be observed from 20-s up to 180-s *ISI* while the successive enhancement effect of dissimilar preceding stimuli is only visible at 20-s *ISI*. When stimuli are preceded by similar stimuli, the respective intensity judgments do not differ from the asymptotic level at any *ISI*. Note that the estimated asymptotic judgment ($R_0 = 58.60$) is higher than the average judgment ($\bar{R} = 56.16$). This is caused by the massive successive diminution effect due to identical preceding stimuli.

The main effects of I_{t-1} and I_t are illustrated in Figure 8.4. The effect of I_t reflects the obvious stimulus-response relation: Intensity judgments are lower for low stimulus intensities than for high stimulus intensities. The effect of I_{t-1} is caused by higher judgments after low concentrated previous stimuli and lower judgments after high previous stimulus intensities (Figures 8.4 and 8.5). This indicates that the magnitude of the current judgment is negatively related to the intensity of the previous stimulus. The interpretation of the $ISI \times I_t$ interaction follows from Figure 8.4. The differences between intensity judgments for low and for high concentrated stimuli were relatively large at 20 s ISI whereas these differences were relatively small at 180 s ISI.

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insert Figures 3, 4 and 5 approximately here

The I_{t-1} stimulus-contrast effect (Figure 8.5) appears to decrease for increasing stimulus dissimilarity. However, the $I_{t-1} \times QSim$ interaction was not significant $[F(2,28) = 1.76, p = 0.198]$. Furthermore, the expected decay of enhancement- and diminution effects over ISI (Figure 8.3) expressed by the $ISI \times QSim$ test was not significant $[F(4,56) = 2.10, p = 0.107]$. Also the related interactions $ISI \times QSim \times I_{t-1}$ $[F(4,56) = 1.98, p = 0.130]$ and $ISI \times QSim \times I_t$ $[F(4,56) = 2.57, p = 0.064]$ failed to reach significance. Although the overall ANOVA did not show significant effects, the ANOVA of the hypothesized linear $(I_{t-1} \times QSim)$ contrast testing the decrease of stimulus contrast at increasing stimulus dissimilarity and the linear $(ISI \times QSim)$ contrast that tests convergence of intensity judgments of the three $QSim$ categories at increasing ISI were significant, $[F(1,14) = 5.15, p = 0.040]$ and $[F(1,14) = 12.71, p =$

0.003], respectively. No additional second- and higher order interactions on intensity judgments were found.

At lag 1, identical previous stimuli induced profound successive diminution effects that sustained over *ISI*s up to 180 s. Even after this large *ISI*, the average intensity judgment did not reach the asymptotic level (Figure 8.3). Therefore, we examined the effects of identical preceding stimuli spanning more than one sequential step. We averaged intensity judgments for stimuli that were preceded by identical stimuli (regardless their intensity) at lag 1, 2 and 3, under the restriction that intermediate stimuli had different qualities. Averages were calculated for each time interval between two identical stimuli. Due to the increasing number of possible *ISI* combinations at increasing lags, the number of time interval categories increases, leading to fewer judgments per average. The number of judgments per average was further limited by the restraint not to accept identical intermediate stimuli, since these would reinforce any existing effects of the preceding stimulus of interest. At lag 4, the average number of judgments that was used to calculate intensity averages had dropped from 240 judgments at lag 1 to 22.4 judgments per time interval. Therefore, data were not analyzed for lags higher than 3 (39.6 judgments per average). The three averages with the largest standard errors at lag 3 were calculated from respectively 10, 23 and 9 judgments. Results were plotted in Figure 8.6. A reference line for zero-enhancement is added, defined by the predicted asymptotic judgment level that was assessed by Equation 5. Independent of the number of intermediate stimuli, intensity judgments were well below the asymptotic level up to time intervals of approximately 180 s. Trends of intensity judgments over time intervals do not appear to be affected by the number of interceding stimuli.

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insert Figure 6 approximately here

Auto-correlation of residuals

Sequential dependencies of residuals were studied by calculating autocorrelation functions of lag (ACF) and partial autocorrelation functions of lag (PACF). The latter calculates the net autocorrelation at lags higher than one by separately accounting for compound autocorrelations at lower lags. ACF and PACF were calculated per subject and the significance of deviations of correlations from zero were tested by one-sample *T*-tests, on *r'* transforms using subjects as source of variance. Resulting ACF and PACF are plotted in Figure 8.7. Although autocorrelations are rather small, i.e. in between –0.1 and 0.1, they are significant at 6 out of 16 lags. Furthermore, autocorrelations are positive at all lags, with the exception of lag 1.

As was expected on basis of the external cognitive variable hypothesis, autocorrelations of residuals were positive at lags higher than 1. This is also observed for partial autocorrelations, implying that positive autocorrelations are not caused by progressing autocorrelations at lower lags. However, at lag 1 autocorrelations were significantly negative, a result that is not in line with the external cognitive variable hypothesis. To test whether this negative autocorrelation of residuals at lag 1 was induced by task related variables, we calculated autocorrelations of residuals at lag 1 for all possible combinations of present with previous stimuli separately. Thus, secondary dependencies of residual autocorrelations were tested in a *QSim* × *ISim* × *ISI* (3×2×3) repeated-measures analysis of variance on *r'*-transforms of individual autocorrelations. This analysis showed significant main effects of *QSim* [$F(2,28) = 3.87$, $p = 0.035$] and *ISim* [$F(1,14) = 79.90$, $p < 0.001$] and a significant *QSim* × *ISim*

interaction [$F(2,28) = 5.17$, $p = 0.017$]. Inspection of Figure 8.8 reveals that the *ISim* effect is caused by a pronounced positive autocorrelation of residuals when two subsequent stimuli are of the same intensity category vs. a negative autocorrelation of residuals when two subsequent stimuli are of dissimilar intensity categories. On average, autocorrelation magnitudes decreased at increasing dissimilarities of subsequent stimulus qualities (Figures 8.8 and 8.9). Figure 8.8 suggests that the effect of *QSim* is mainly due to decreasing positive autocorrelations for equal intensity stimulus pairs when the stimuli decrease in qualitative similarity. When the intensities in the consecutive stimuli are different, the qualitative similarity does not seem to have an effect on autocorrelation. This explains the significant *QSim* × *ISim* interaction. No significant effect of *ISI* [$F(2,28) = 1.07$, $p = 0.345$] is observed. Figure 8.9 shows no trace of the usual trend that sequential effects decrease over increasing *ISI*. No further significant interactions were observed.

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insert Figures 8 and 9 approximately here

Discussion

In the introduction we proposed a taxonomy of sequential effects that distinguishes between level effects on judgment magnitudes (i.e. enhancement and diminution) and correlative effects (i.e. assimilation and contrast). For reasons of clarity, results will be discussed accordingly, followed by a discussion of the possible sources of the observed effects. We conclude with discussing residual autocorrelation effects.

Data analyses were performed under the assumption that the panels employed in the preliminary and the main experiments agreed in their perceptions of odor similarity and that no learning effects took place between experimental sessions. On the basis of the proportions of responses in the 'correct', 'similar' and 'dissimilar' categories we conclude that the panels indeed agreed in their perceptions of odorant similarities. Since trends over sessions were observed neither for the proportions of correct responses nor for the average intensity judgments, we concluded that it is not likely that learning effects have affected response behavior.

The equi-intense concentrations of the used substances, made up in the preliminary experiment (Table 8.2), were multiplied or divided by two, to obtain a high and a low concentration of each substance, respectively. Although low concentrations resulted in low intensity judgments and high concentrations resulted in high intensity judgments, the intensity differences between low and high concentrations varied between substances: 1-hexanol = 14.2; hexanal = 24.7; citral = 36.7 and limonene = 37.1. This is probably due to differences between psychophysical functions of different odorants. Unfortunately, the similar pair consisting of limonene and citral showed large intensity differences between the low and the high concentrations whereas the other similar pair (hexanal and 1-hexanol) showed small intensity differences. Subjects may have exploited this as an aid in the descriptor selection. For instance, observed intensities of limonene and citral differ more from single-intensity distracters than observed intensities of hexanal and 1-hexanol. Therefore, exploitation of intensity differences would make distracter selection less likely if limonene or citral is presented. However, Figure 8.2 showed no clear evidence of such facilitation

of stimulus identification. Hence, distracter selection appeared to depend primarily on qualitative similarities.

Sequential level effects on judged intensity

A massive successive diminution effect was observed due to preceding stimuli with an identical chemical composition as the current stimulus. This successive diminution decreased for increasing *ISI* but failed to reach asymptotic level before 180s. A sequential enhancement effect was also observed, caused by preceding stimuli of a dissimilar quality. Full recovery from this enhancement was reached after approximately 60s, which is considerably faster than the observed recovery from diminution. Therefore, we expect that principally different mechanisms caused these two sequential effects. Averaged intensity judgments appeared not to be affected by preceding stimuli with a similar odor quality. This result may be explained in two ways. Either enhancement and diminution both took place in a compensatory fashion or neither took place. Because recovery times differed for enhancement and diminution effects, regardless the initial magnitude of the effect, mutual compensation of the two level effects was not possible at all ISI levels simultaneously. We therefore assumed that preceding stimuli of a similar odor quality induced neither enhancement nor diminution effects.

In the literature, successive enhancement effects are not uniformly defined. In a taste experiment, Schifferstein and Oudejans (Schifferstein & Oudejans, 1996) used the mean intensity judgment of a stimulus preceded by repetitions of itself as a reference for the calculation of the successive enhancement effect that occurred when this stimulus was preceded by repetitions of a different stimulus. Others have used the grand mean response to a specific stimulus as the reference level. Both methods may cause an underestimation of the reference judgment if diminution effects occur that

are as pronounced as in the present study. Such underestimation of the reference judgment would cause spurious successive enhancement effects. With this in mind, we predicted responses to stimuli at infinite *ISI* to calculate level effects. Nonetheless, the presently found successive enhancement effect that was induced by only one preceding dissimilar stimulus at an 20s ISI, was robust for this conservative calculation method. At lag 1, intensity judgments for identical, similar and dissimilar preceding stimuli converge at increasing *ISI*. This indicates that the predicted intensity judgment at infinite *ISI* is a reliable reference. It is therefore improbable that the successive diminution and enhancement effects are artifacts due to the data treatment that was used.

Besides the temporal decay of the magnitude of both successive enhancement and successive diminution, a temporal decay of response dynamics for I_t was also observed. Responses to high, respectively low concentrated stimuli converged for increasing *ISI* (Figure 8.4), resulting in a significant $ISI \times I_t$ interaction. Since this effect is observed independently of other sources of two-way interactions with *ISI*, e.g. *QSim* or I_{t-1}, we presume that this effect should be attributed to a changing response strategy over *ISI*. Schifferstein and Kuiper (Schifferstein & Kuiper, 1997) observed that when presenting sequences of taste stimuli at an ISI of 50 or 60 seconds, subjects would show incoherent response behavior over the initial 3 up to 12 trials. During these initial trials, the adoption stage, subjects are thought to adopt a stimulus-response strategy based on the experienced sensations and the available response scale. We suggest that in experiments that comprise variable ISIs, the adoption of a response strategy is a continuous process characterized by a shift towards more liberal scale usage after short ISI and increasing response conservatism after longer ISI.

Correlative sequential effects

Besides the discussed successive diminution and enhancement effects, also contrastive correlations between intensity judgments and preceding stimuli were observed. In general, intensity judgments were significantly lower after high-intensity preceding stimuli than after low-intensity preceding stimuli. This effect was observed for identical preceding stimuli and to a lesser extent also for similar preceding stimuli (Figure 8.5). In the case of *dissimilar* preceding stimuli, stimulus contrast is absent. This suggests that stimulus intensity alone does not induce contrast. We conclude that, at least to some extent, sequential contrast is a byproduct of successive diminution effects. As we saw earlier, diminution occurs after preceding stimuli with identical qualities. Contrast is observed when the magnitude of successive diminution depends on the intensity of the previous stimulus.

In the present study, level effects clearly decayed over increasing ISI. In literature, a decay of correlative sequential effects over increasing ISI has also been observed. DeCarlo studied sequential effects on intensity judgments of 1000 kHz tones at various sound levels (DeCarlo, 1992). Effects on coefficients and residuals of Equation 2 were compared within-subjects for two experimental sessions employing short (2 s-6 s) and long (15 s-20 s) inter-trial intervals (ITI). DeCarlo found S_{t-1} assimilation effects on R_t that were larger at short ITI than at long ITI, the S_{t-1} assimilation being nearly absent in the case of long ITI. The study by DeCarlo differs in two important aspects from the present study. Instead of chemosensory stimuli, DeCarlo presented auditory stimuli. Furthermore, stimuli only differed in intensity whereas in the present study stimulus quality was also manipulated. Nonetheless, DeCarlo's central contention that the influence of the previous stimulus intensity should decrease with an increase in ITI also finds support in the present study,

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provided that DeCarlo reported assimilation whereas we found contrast. Although the statistical test of this effect (the $ISI \times I_{t-1}$ interaction) failed to reach significance, the predicted trend is apparent in Figure 8.4: Independent of the current stimulus intensity, I_{t-1} contrast is highest for 20 s ISI (steepest downward slopes) and almost absent for 180 s (nearly horizontal slopes).

An adaptation hypothesis of sequential effects in chemosensation

Schifferstein and Frijters (Schifferstein & Frijters, 1992) reviewed visual and auditory experiments that used Equation 1 to calculate effects of S_{t-1} on intensity judgments. They calculated sequential effects for these experiments according to Equation 2 by using the correction suggested by DeCarlo and Cross (DeCarlo & Cross, 1990) and observed that the signs of nearly all S_{t-1} coefficients changed from negative into positive. Nevertheless, after correction their own taste data still produced negative S_{t-1} coefficients. Schifferstein and Frijters suggested that this robust stimulus contrast is typical for the sense of taste, implying that sequential effects in the sense of taste are at least partly mediated by processes unique for that modality.

In the olfactory domain, some authors suggested that sensory adaptation might be such a modality-specific process causing contrast effects (Gregson & Paddick, 1975; Lawless et al., 1991). We think that this study presents compelling evidence for the hypothesis that chemosensory adaptation causes successive diminution and contrast. To understand why adaptation may be an important factor causing sequential effects in the chemical senses, it should be noted that chemosensory information transfer is based on a physicochemical receptor interaction, whereas in other senses information transfer is based on a physical receptor interaction. Due to the longevity of the stimulus-receptor interaction and due to lingering of stimulus molecules in the mucus embedding the receptor area, tastants and odorants produce sustained receptor

responses. In contrast, physical stimuli in the other senses are readily coded into transient, neural responses. This may explain why in the chemical senses, as opposed to the other senses, the exhaustion, i.e. adaptation, of receptors and/or their afferent pathways is easily induced.

Olfactory adaptation is observed whenever the perception of a stimulus is impaired due to sensory fatigue invoked by previous presentations. It causes decreased perceived intensities of supra-threshold stimuli. Hence, larger decreases are observed for more intense or lengthy adapting stimuli (Ekman et al., 1967; Berglund et al., 1978; Cain, 1970; Cain & Polak, 1992; Todrank et al., 1991). Adaptation to an odorant that is identical to the evaluated odorant is referred to as self-adaptation, whereas adaptation to a chemically different odorant is referred to as cross-adaptation. Self-adaptation generally impairs stimulus perception more than cross-adaptation (Berglund et al., 1978; Berglund & Engen, 1993; Cain, 1970; Todrank et al., 1991). Furthermore, cross-adaptation effects appear to be related to the perceived similarity of the two odorants involved: increasing similarity between the adapting and the evaluated odorant induces more adaptation (Todrank et al., 1991; Cain & Polak, 1992). Both behavioral and psycho-physiological investigations showed that, even after a few sniffs, the olfactory system approaches full recovery from adaptation not earlier than 60 s and up to 360 s after stimulus presentation (Stevens et al., 1989; Morgan et al., 1997; Ekman et al., 1967; Cain, 1970; Cometto-Muñiz & Cain, 1995).

With adaptation sustaining over periods from 1 to 6 minutes and judged stimulus intensities decreasing with increasing concentrations of the adapting stimulus, adaptation may induce sequential effects in judgments of sequentially presented olfactory stimuli. These sequential effects are expected to be diminutive,

because adaptation lowers intensity judgments, and contrastive, because adaptation effects are larger after higher concentrated adapting stimuli.

In our study, regardless of the number of stimuli in between two identical stimuli, the successive diminution induced by the identical preceding stimulus disappears after approximately 3 minutes (Figure 8.6). This is a realistic recovery period for adaptation effects. Furthermore, as may be expected for adaptation effects, the observed successive diminution coincided with a stimulus contrast effect. This contrast effect decreased with increasing stimulus dissimilarity. Finally, the observed successive diminution effects were robust for interceding stimulus presentations (Figure 8.6). Because peripheral neural fatigue is not likely to be overruled by new stimulus presentations, in contrast with effects with a cognitive origin, this result provides extra support for a peripheral hypothesis of diminution effects.

A complementary mechanism for successive enhancement

Earlier, we suggested that two complementary mechanisms were responsible for the successive diminution and enhancement effects, respectively. Diminution effects could be attributed to a peripheral adaptation process. We suggest that the observed successive enhancement effects can be explained by an ecologically adaptive mechanism emphasizing new and contrasting information. For mammals, a primary function of olfaction is to warn the organism for potential danger (Jones & Roper, 1997), for instance by (Sullivan et al., 1998; Terlouw et al., 1998)increasing vigilance on perception of new odors (Warm et al., 1991; Warm et al., 1989). Perceptual mechanisms that emphasize contrasting or new information play an important role in this. For instance, to facilitate the perception of objects, visual contrast enhancement is achieved by the lateral inhibition of ganglion- and cortical cells. We suggest that

the presently observed successive enhancement is an adaptive response to the qualitative contrast (dissimilarity) between an odorant and its precursor.

Because no enhancement or diminution was observed at any ISI after stimuli with a 'similar' odor quality, we concluded that after preceding stimuli of 'similar' odor quality none of the two discussed complementary mechanisms was involved in the response production. However, the I_{t-1} contrast effect observed for 'similar' preceding stimuli (Figure 8.5) still indicates a possible involvement of adaptation processes. The mechanism causing successive enhancement only produces successive enhancement, which may have compensated diminution effects by adaptation but not its contrast effects (see the average intensities for high and low I_{t-1} of dissimilar preceding stimuli in Figure 8.5). Unfortunately, the present results do not allow a more critical testing of these rivaling explanations.

Alternative sources of the observed sequential effects

An alternative mechanism that could cause successive diminution is stimulus habituation. Repetitious presentations of a stimulus with inter-stimulus intervals that are sufficiently long to prevent adaptation may still habituate the observer to that stimulus (Kroeze, 1983). As a consequence, responses to a habituated stimulus may be lower than responses to the same, but not habituated stimulus. Although adaptation and habituation may both reduce judged intensities, the former acts at peripheral levels of processing whereas the latter acts at central levels of information processing. Therefore, adaptation effects can be introduced by merely one preceding stimulus, whereas habituation requires repetitious exposure to the same stimulus. In an experiment where stimulus solutions were applied directly to the observer's tongue, Kroeze observed pronounced habituation to sweet taste (Kroeze, 1983). However, in a similar study employing identical *ISI*s and identical stimuli but an active sip-and-spit

procedure, no habituation effects were observed (Schifferstein & Frijters, 1992). Apparently, human observers habituate more readily to passively administered stimuli than to actively administered stimuli. In the present study, we prevented repetitious exposure to one stimulus by allowing no more than two identical stimuli to be presented in succession. Furthermore, stimuli were presented actively by the subjects. For these reasons, it is unlikely that habituation has occurred.

Differential context effects are shifts in the intensity judgment of one kind of stimulus relative to another stimulus due to differences in the (averages of) intensity distributions of both stimuli (Rankin, 1993; Rankin & Marks, 2000). Accordingly, Rankin and Marks (2000) observed subjects judging a 70 dB tone of low frequency as being louder than a 70 dB tone at high frequency, provided that the low frequency tones in the experiment were predominantly weaker than the high frequency tones. One may be tempted to attribute successive enhancement after presentation of dissimilar stimuli to differential context effects (Rankin & Marks, 2000). However, this would be incorrect because successive enhancement does not pertain to a specific stimulus quality but to the mere fact that qualities differ. Since dissimilar, similar and identical stimuli all consist of equal portions of the used stimulus qualities, any differential contrast effect would occur within all three similarity categories and could, therefore, not explain enhancement effects.

Cardello *et al.* (Cardello et al., 2002) presented short sequences of orange flavor solutions at varying intensities. The authors showed that subjects' expectations of stimulus intensities assimilated towards previous stimulus intensities. Subsequently, it was shown that current intensity judgments contrasted with the prevailing intensities of as few as three preceding stimuli. The authors attributed this contrast effect to the mediating influence of stimulus expectations. This suggests that global context effects

mediated by stimulus expectations can be introduced by small subsets of stimuli. With cognitive factors like expectations being transient along trial sequences rather than sustained, correlative effects may very well be induced. One may wonder if, for instance, expectations could have contributed to the contrast effects that were observed in this study.

Autocorrelation of residuals of intensity judgments at lag 1

In line with DeCarlo and Cross (DeCarlo & Cross, 1990), we assumed that response assimilation effects are artifacts that can be attributed to autocorrelation of residuals. DeCarlo and Cross suggested that autocorrelated residuals reflect non-monitored processes such as attention, memory, motivation or strategy. Support for this was found in the present study by the exclusively positive autocorrelations at lag 2 and higher. However, the observed dependency of residual autocorrelations at lag 1 on the intensity difference between the present and the preceding stimuli implies that autocorrelation of residuals was in fact influenced by stimulus characteristics. These stimulus-specific second-order dependencies of residuals on previous stimulus characteristics may be explained by response assimilation or contrast effects. To illustrate this, we discuss an olfactory study by Baird *et al.* (Baird et al., 1996). These authors reported response assimilation and second-order dependencies evidenced by an inverted V plot of correlations between R_{t-1} and R_t with maximum correlations for identical stimulus pairs. If Equation 2 had been used to model their data, then the response variance that was originally explained by a positive autocorrelation of responses in Equation 1 would contribute to the variance of residuals, which would consequently be positively autocorrelated. Therefore, the observed second-order dependency of residual autocorrelation on stimulus intensity similarity may reflect

sequential response effects. These occur independently of residual autocorrelations due to stimulus-independent cognitive variables.

Besides the secondary dependency of residual autocorrelations on stimulus intensity category, a dependency on qualitative stimulus similarity is also observed. Residuals showed highest autocorrelations if respective stimuli had the qualitatively highest similarity (= identical) and residuals were lowest if respective stimuli had the qualitatively lowest similarity (= dissimilar). As with the effects of intensity similarity, this result fitted the hypothesis that subjects relied increasingly on previous responses when consecutive stimulus qualities were perceived as more similar.

Implications for aroma research

The present study was inspired by a research paradigm commonly used in the realm of food aroma research and practiced at our laboratory. Food aromas generally emanate from complex mixtures of volatile chemicals, not all of which contribute to the aroma (Grosch, 2001). Gas chromatography olfactometry (GCO) is used to identify the volatiles that elicit odor sensations (Abbott et al., 1993; Blank et al., 1992; Flath et al., 1967; Gasser & Grosch, 1988; McDaniel et al., 1990; Ong & Acree, 1998; Schieberle & Grosch, 1985; Van Ruth & Roozen, 1994; Bult et al., 2001). With gas chromatography, a mixture of volatiles is forced through a capillary column using an inert gas as vehicle. The retention time of each chemical component on the column depends on its unique physicochemical interaction with the column lining, thus causing components to elute separately in time. Retention time and chemical identity can be monitored with detectors placed at the outlet of the column. Since human olfactory sensitivity varies greatly over odorants (Van Gemert & Nettenbreijer, 1977), analytical measures of concentrations of volatiles are poor indicators of their odor impact. Therefore, human observers are used to evaluate the odors of column

effluents, which is the essence of GCO (Dravnieks & O'Donnell, 1971). In GCO, human observers are generally conceived of as detectors that, ideally, generate unbiased responses to the presented volatiles. However, as the present study indicates, GCO results may be affected by sequential effects. An extra complicating factor is that these effects are very complex to be understood due to the involvement of different stimulus qualities, stimulus intensities and ISI. GCO encompasses the presentation of relatively few, generally no more than 10 to 20 odorants. In studies that involve a sequential presentation of stimuli, the number of initial presentations during which subjects develop a response strategy amounts up to 12 (Schifferstein, 1996; Schifferstein & Kuiper, 1997). A straightforward estimation of the nature of sequential effects in GCO can therefore not be given. However, intensity reduction due to preceding stimuli is likely to occur whenever retention times of gas chromatograph effluents differ only seconds in time whilst components are structurally and perceptually very similar. As a matter of fact, structurally related, and therefore often also perceptually related components tend to have similar retention times. This makes sequential effects a probable thread to the validity of GCO evaluations.

General conclusion

In the present study we attempted to gain a better understanding of the sources of sequential effects in olfaction. To accomplish this, odorants were presented sequentially whilst three factors were varied systematically: (i) the qualitative similarity of subsequent stimulus qualities, (ii) the stimulus concentration and (iii) the length of the ISI between subsequent stimuli. To our knowledge, sequential effects on judgments of stimuli from different categories of odor similarity have not been studied so far. The used multi-factor approach including ISI has proven to be a

powerful technique to disentangle various kinds of sequential effects and allowed the

attribution of these effects to physiological and psychological mechanisms to some

extent.

Figure legends

Figure 8.2. Average proportions of descriptors (±SEM) that were selected on presentation of the four odorants in the main experiment. The four substances are indicated on the abscissa. Plain bars represent 'correct', 'similar' and 'dissimilar' descriptor categories. Striped bars represent distracter categories.

Figure 8.3. Average intensities (±SEM) of stimuli that were preceded by identical, similar or dissimilar stimuli as a function of *ISI*. Intensities, indicated by symbols, are averaged over both I_{t-1} categories. Lines are best-fit exponential models as given in Equation 4.

Figure 8.4. Average intensity judgments (±SEM) as a function of the intensity category of the preceding stimulus (I_{t-1}), the inter-stimulus interval (*ISI*) and the intensity category of the judged stimulus (I_t).

Figure 8.5 Average intensity judgments (±SEM) of stimuli that are preceded by identical, similar and dissimilar stimuli at both low- and high stimulus intensities.

Figure 8.6. Average intensity judgments (±SEM) of stimuli that are preceded by identical stimuli, without any interceding stimuli (lag=1), with one non-identical interceding stimulus (lag=2) or with two non-identical interceding stimuli (lag=3). Average judgments are plotted as a function of the total time that elapsed after presentation of the previous stimulus with an identical odor quality.

Figure 8.7. Autocorrelation function (ACF) and partial autocorrelation function (PACF) of residuals (* = significant at $\alpha = 0.05$).

Figure 8.8. Secondary dependencies of residual autocorrelations at lag 1 on qualitative similarity (QSim) and similarity of intensity categories (ISim) of successive stimuli.

Figure 8.9. Secondary dependencies of residual autocorrelations at lag 1 on qualitative similarity and inter-stimulus interval between successive stimuli.

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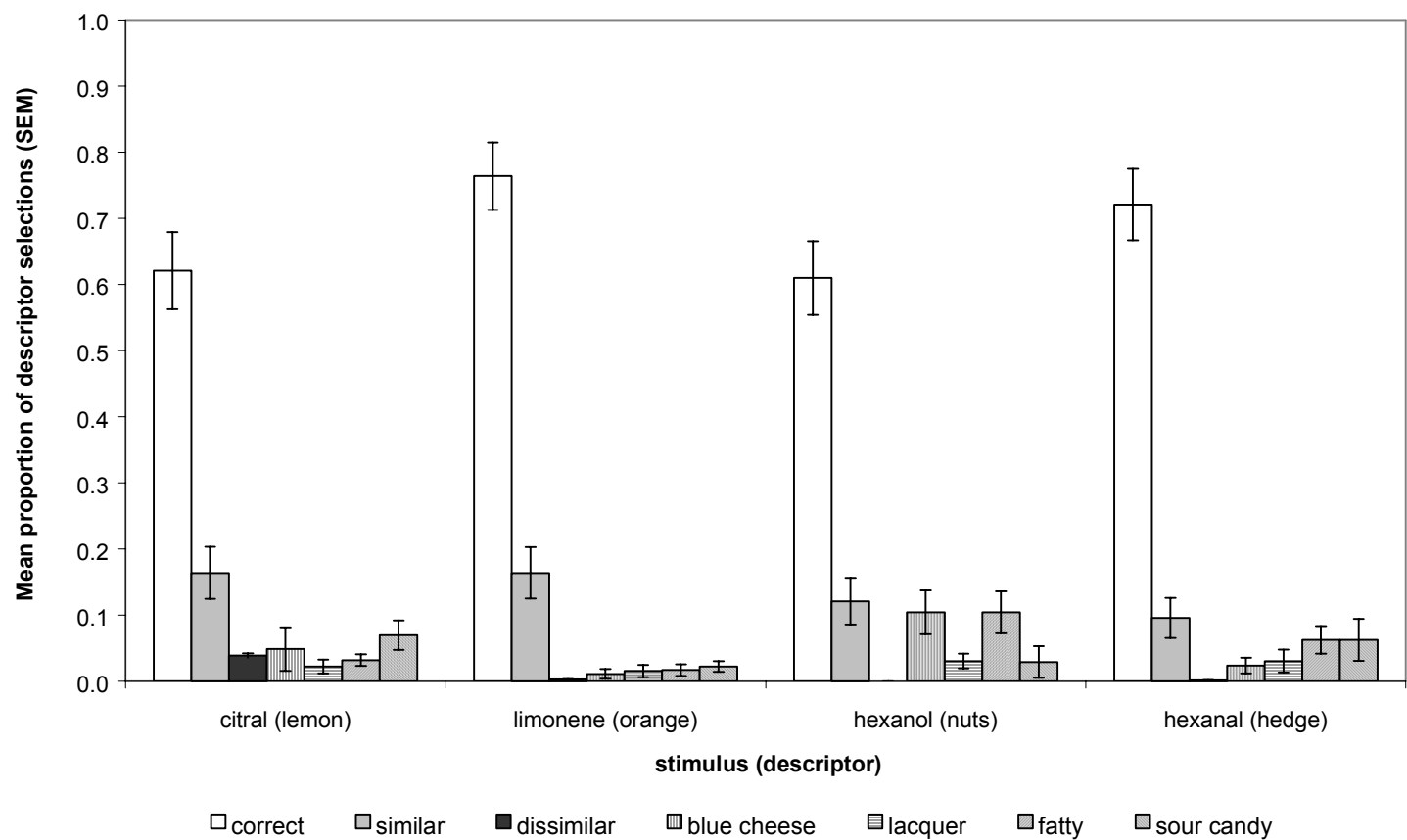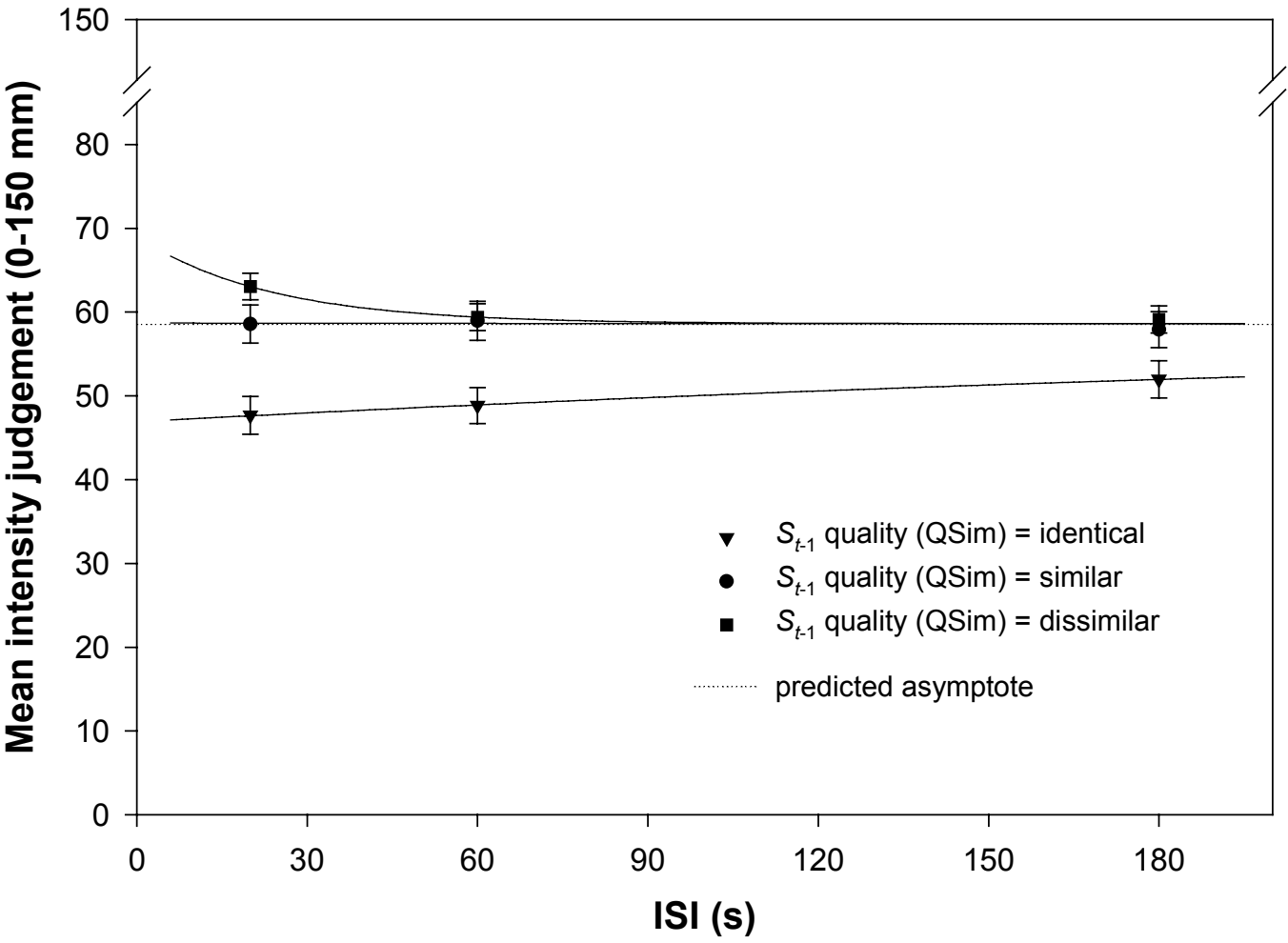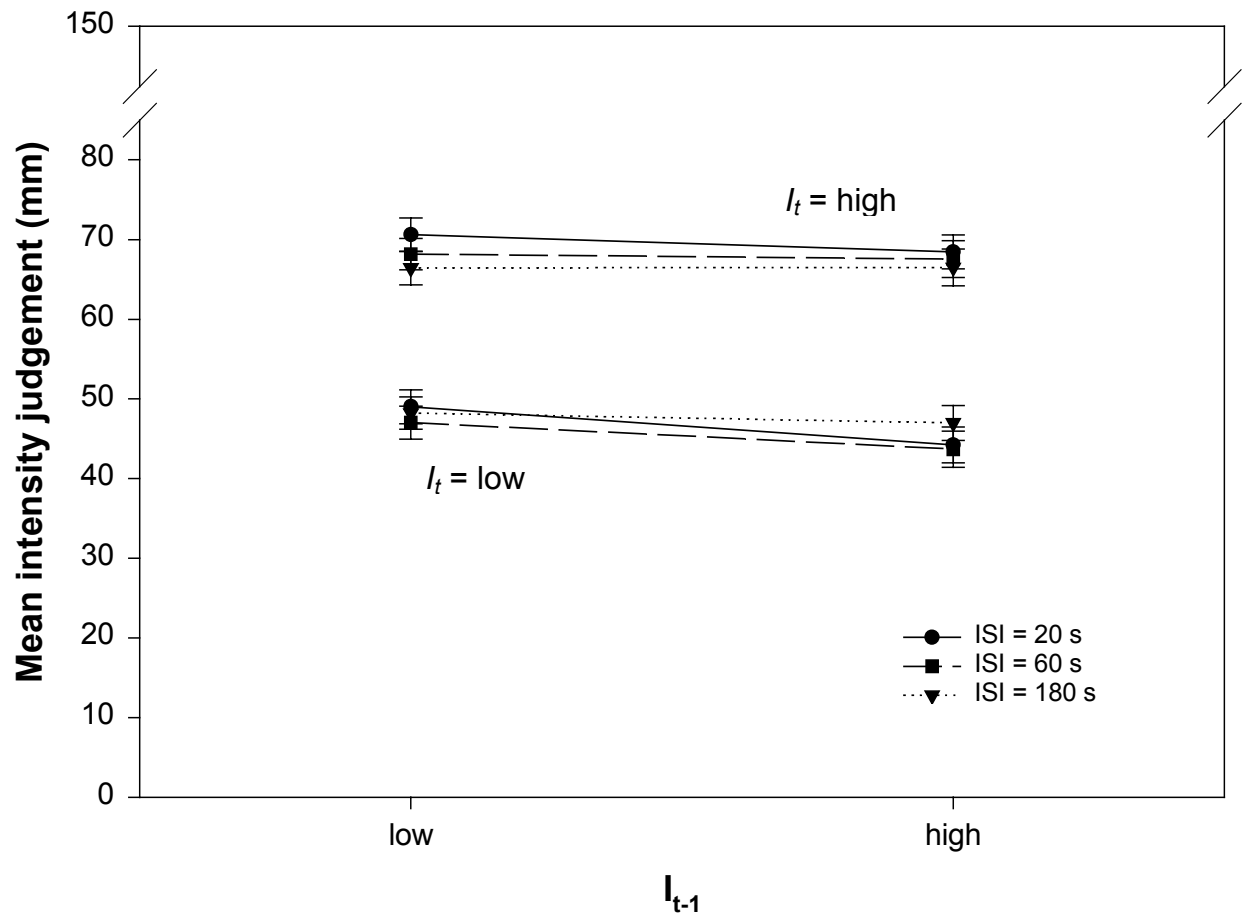
Figure 1

Figure 2

Figure 3

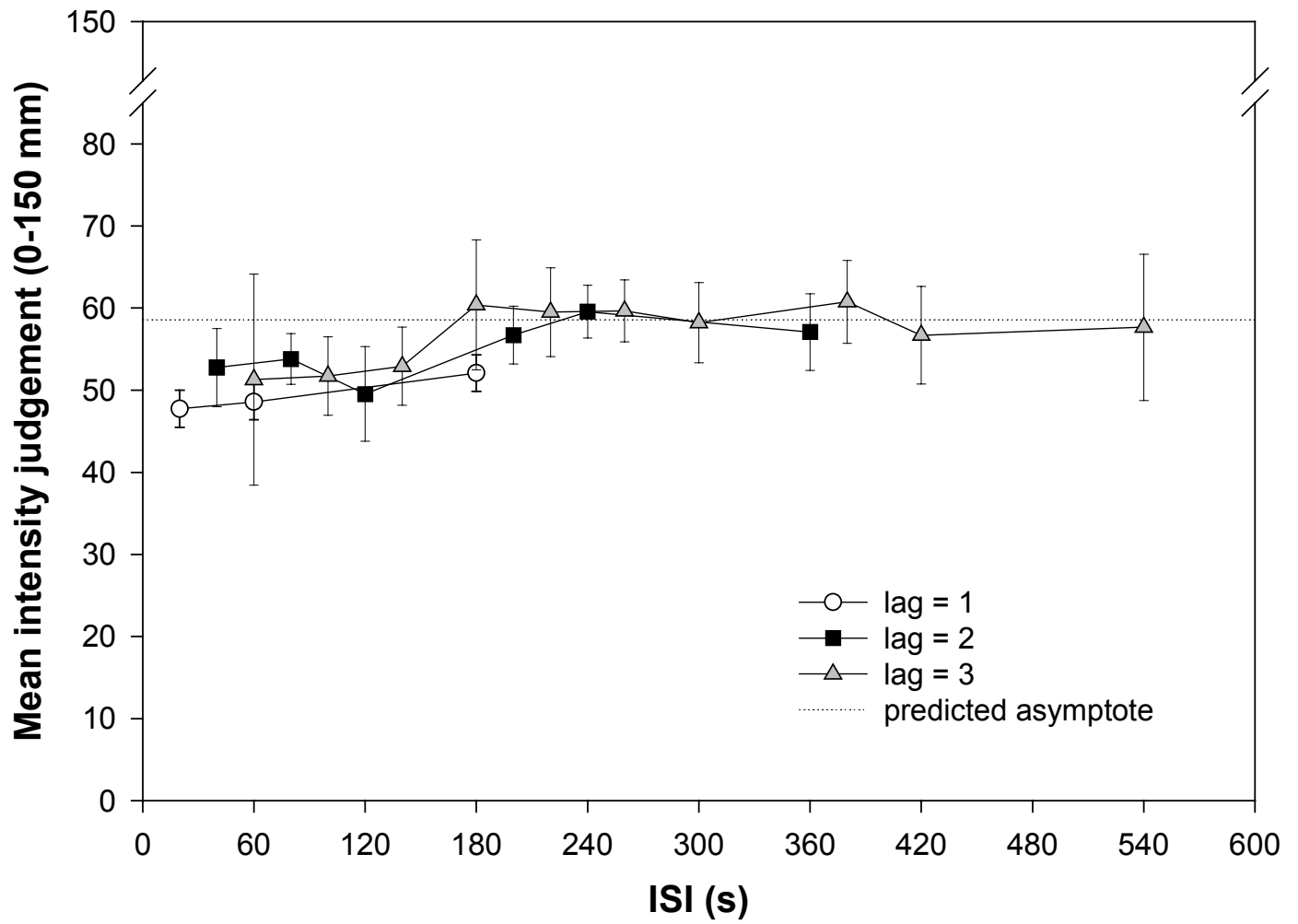**Figure 4**
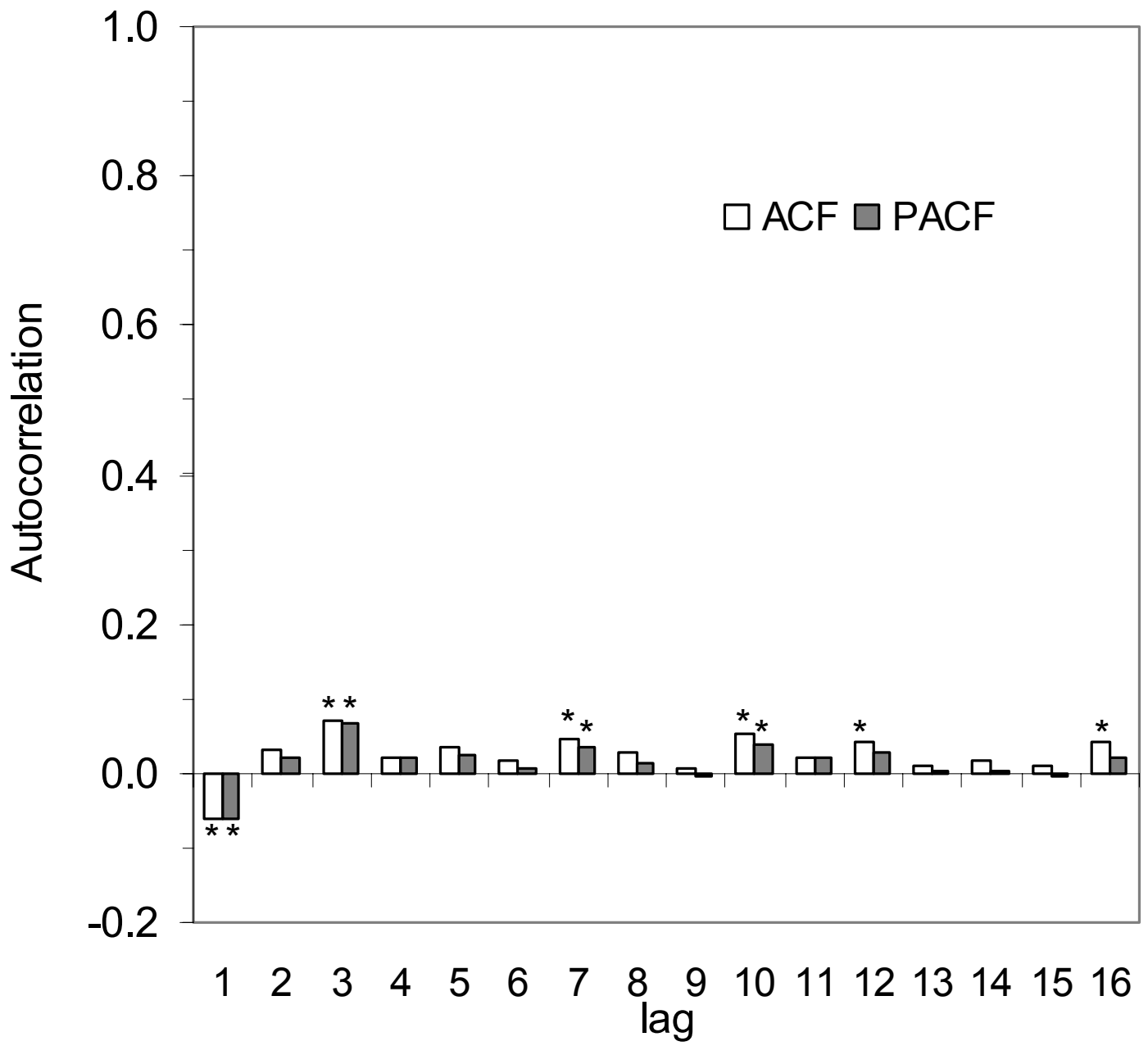
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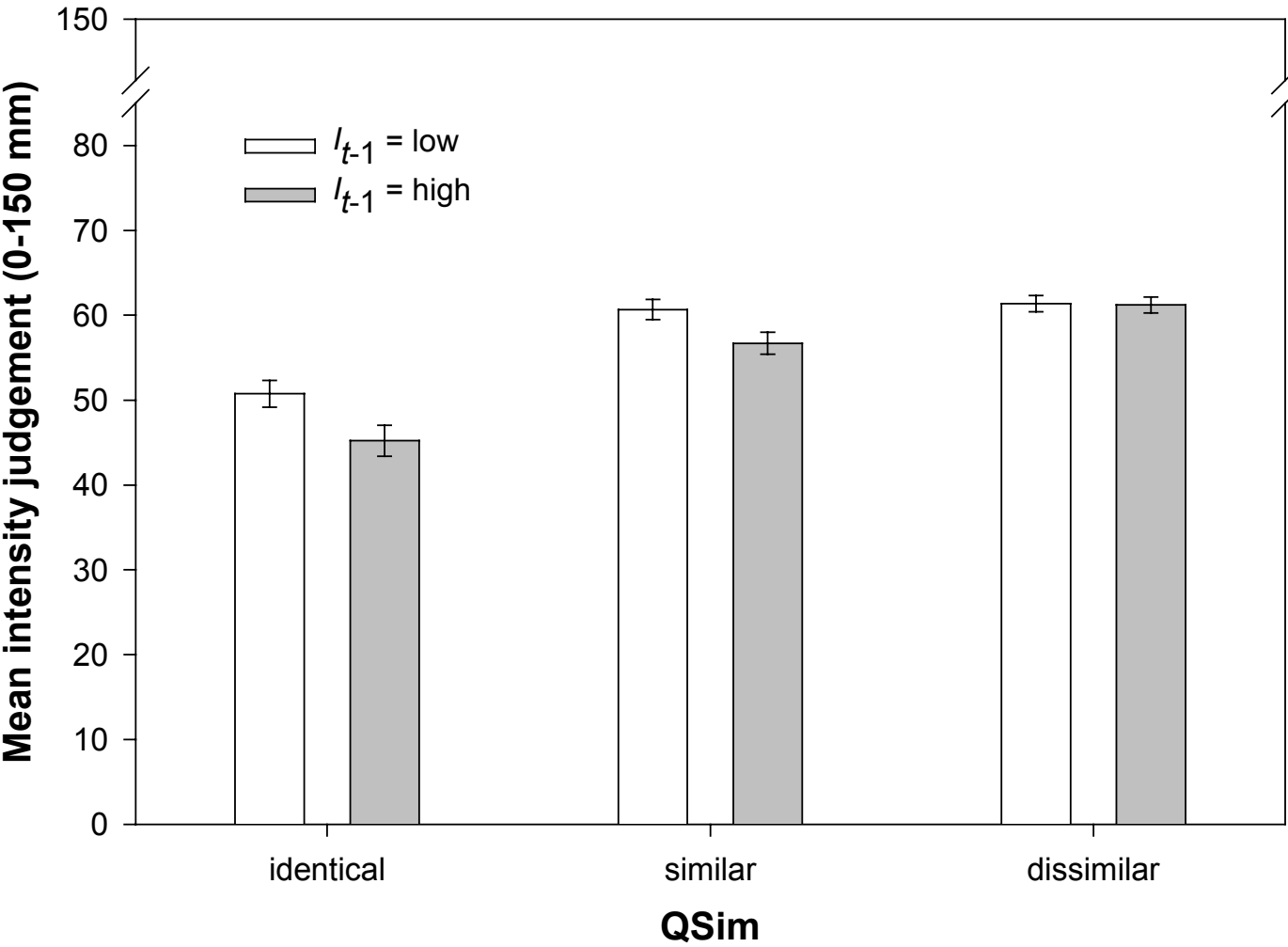
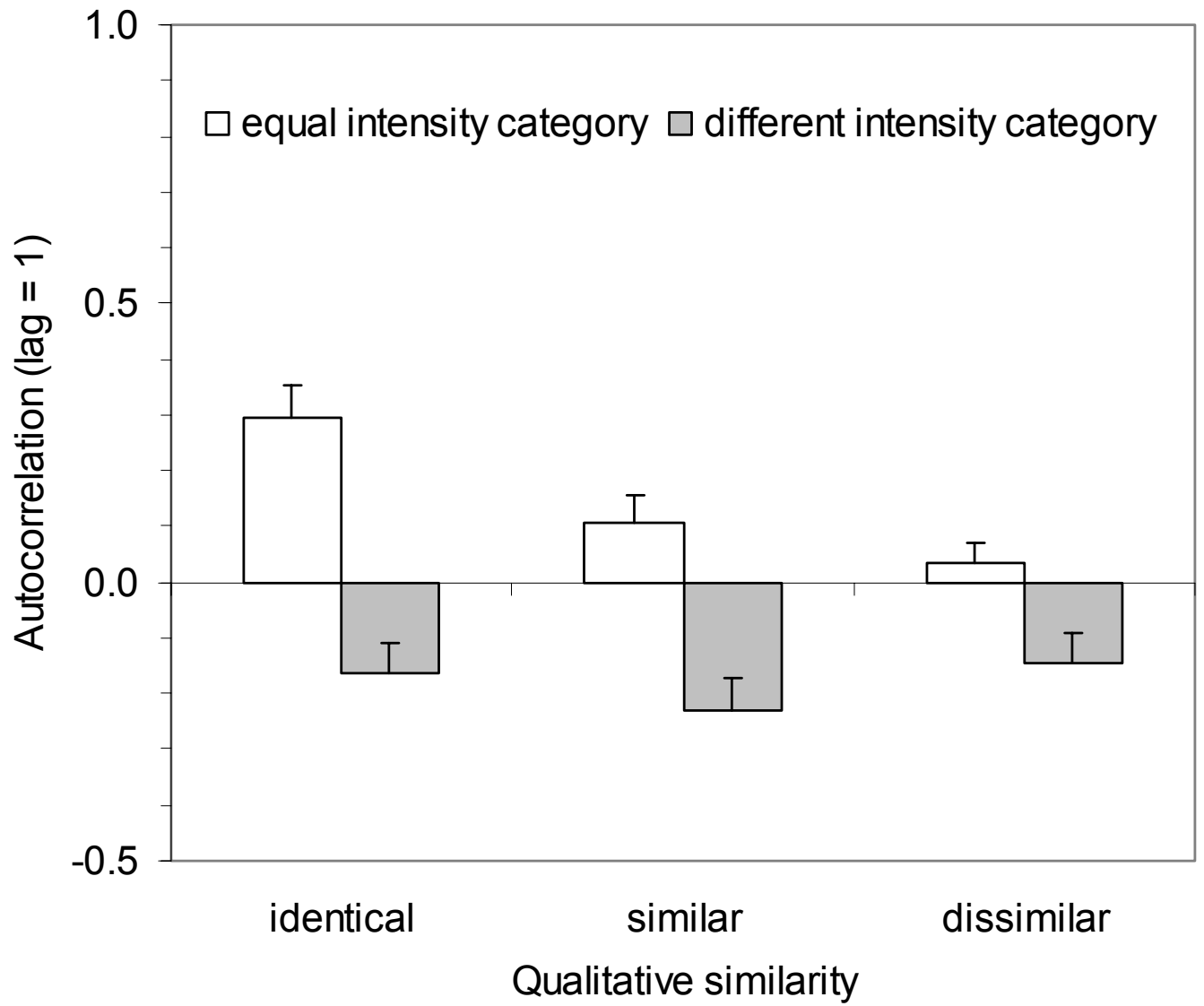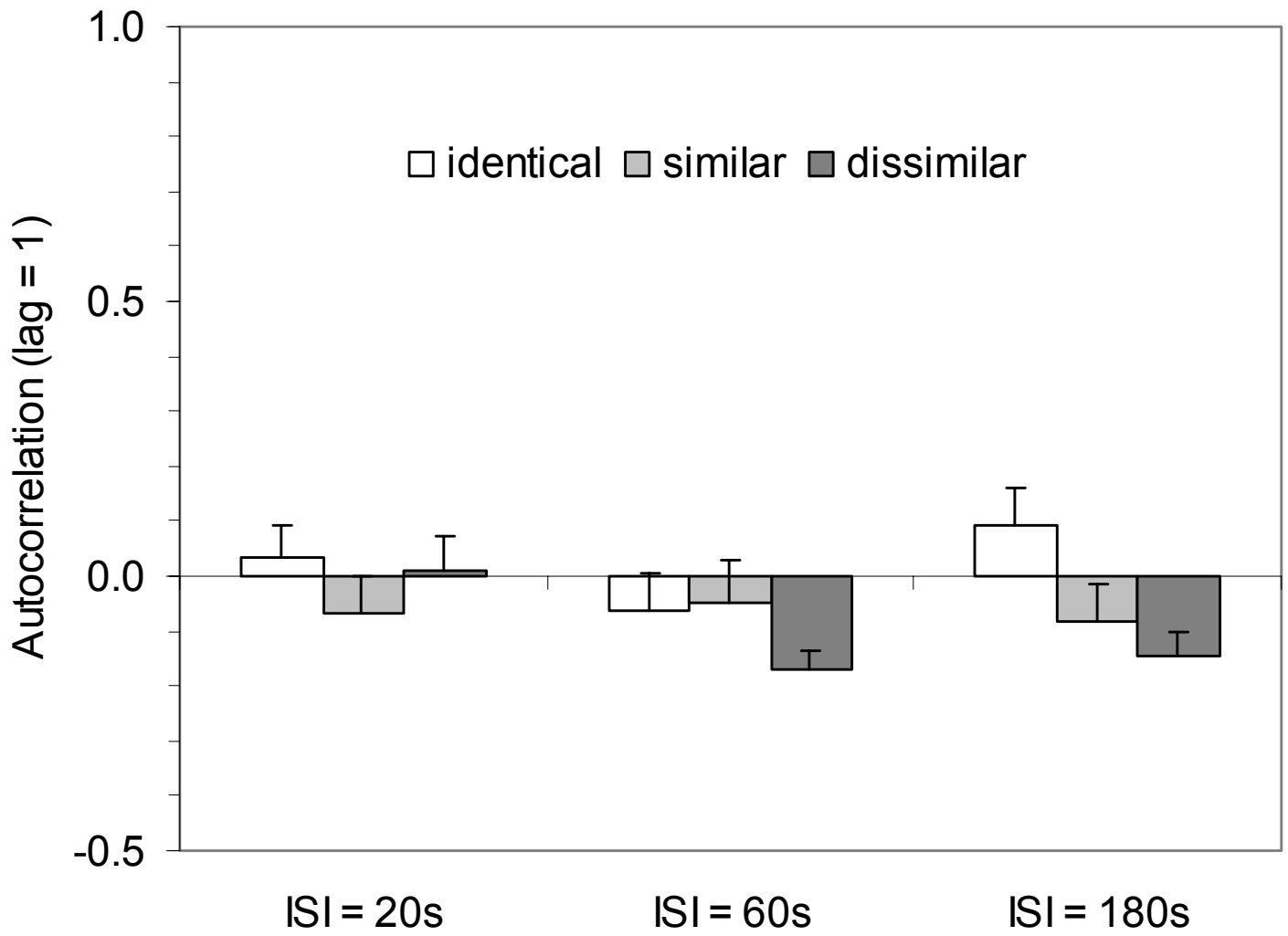**Figure 6**

Figure 7

Figure 8

Figure 9

9 General discussion

GCO is a method that employs panels of human detectors as an instrument to identify the odorants in mixtures of odorous and non-odorous volatile chemical components. As such, this method is based on paradigms from natural sciences for at least two reasons. First, it follows the well-accepted view that complex matter may be understood better by understanding its elements. This view suggests that decomposing complex mixtures of odorants emanating from foods and subsequently characterising these odorants by human detectors may provide a better understanding of the food aroma. Second, the instrument used to quantify properties of matter should yield valid results. Using human detectors to describe the olfactory properties of an aroma is likely to be a valid approach. What other instrument could better describe how humans perceive an aroma?

To detect and identify odorants, the human instrument proved both a blessing and a curse. So far, no instruments have been constructed that can mimic the versatility and the, at times, extreme sensitivity of humans in the detection of odorants. On the other hand, both the psychological literature and this thesis have illustrated that the human response depends on a multitude of factors besides the presence of the odorant alone. Therefore, the factors that affect the validity of the human responses shoud be taken into account when human observers are used as measuring instruments.

By approaching GCO from a combined psychological-technological perspective this thesis work had two main objectives: (i) to use knowledge of human perception to improve GCO methodology (the first part of this thesis) and (ii) to identify experimental GCO conditions in which humans perform like biased instruments and

to study the psychological mechanisms that govern such bias (the second part of this thesis).

The first part of this thesis

Two methodological improvements were suggested for the practice of GCO. The first suggestion entailed the correction of panel responses for variation in components' retention times, due to variation in the GC instrument. This correction improved the odorant detection power of the panel (chapter 2). Although this method was not born from any psychological considerations, it increased the sensitivity of the panel as an instrument. This was required before embarking on the second enterprise: the development of a psychologically valid method to assess the reliability of panel detections of odorants (chapters 3 and 4).

A GCO experiment is an exceptional task setting in psychological research. Subjects have to respond to unannounced stimuli that occur after irregular time intervals. Although this task may reflect many real life situations, e.g. noticing the faint ringing of your cell phone inside your pocket or hearing somebody calling for help, it is not a common situation in psychological laboratories. Here, detection experiments generally require subjects to respond to events that occur in well-defined time frames. Subjects are typically asked whether an event took place or not, rather than when it took place. Chapter 3 of this thesis presented a model of detection behaviour under unstructured tasks conditions. It models panel behaviour from responses that were generated during time windows in which stimuli were absent. With this model, it can be tested whether panel responses differ significantly from those expected under stimulus-free conditions. The method proved very useful for GCO applications and offers an ecologically valid alternative for signal detection

theory, the prevalent framework in the psychology of detection. Hence, the method used to model GCO sessions may be applied in other contexts relevant to psychology as well.

The second part of this thesis

When panellists rate stimulus intensity, characterise stimulus quality or even indicate the mere presence of stimuli, their responses depend on a multitude of factors besides the stimuli alone. Several relevant factors were identified earlier: stimulus frequency (Schifferstein and Frijters, 1992; Riskey *et al.*, 1979; Colquhoun and Baddeley, 1964); the stimulus context as provided by task instruction (Frandsen *et al.*, 2003; Herz and Von Clef, 2001; Herz, 2003) and the intensity and quality of the previous stimulus in combination with the time that has elapsed since its presentation (Ekman *et al.*, 1967; Cain, 1970; Bujas *et al.*, 1991). In this thesis, the impact of such factors on odorant perception was demonstrated in chapter 4 (stimulus frequency), chapter 6 (stimulus context as provided by task instruction), chapter 7 (stimulus meta-knowledge), and chapter 8 (the intensity and quality of the previous stimulus and the time that has elapsed since its presentation). All these factors pose potential threats to the reliability of GCO results.

Holistic stimulus perception in olfaction

For the modality of olfaction, there is compelling evidence that the neural encoding of stimulus information is holistic as from only few synapses beyond the olfactory bulb (Wilson and Stevenson, 2003; Wilson, 2000). Most of the detailed, odorant-related information seems to be lost before stimulus information reaches cortical stages of processing. Hence, complex, object-related aromas are thought to be processed in terms of the (food) source rather than in terms of constituting odorant

components (Stevenson *et al.*, 2003; Stevenson and Boakes, 2003; Stevenson, 2001).

Two studies in the present thesis dealt directly with predictions that can be derived

from this conceptualisation. In chapter 7, sub- and peri-threshold odorants were added

to a mixture of components that produced an apple aroma. Panellists found it easier to

discriminate these components in the aroma when they had recognised the aroma

(apple) than when they did not. In chapter 6, subjects proved to be better identifiers of

components in aroma mixtures when they were instructed to recognise aroma notes in

aromas of orange and apple than when they were instructed to recognise components

in mixtures of components.

The notion of holistic stimulus processing is anything but new and has been subject

of debates ever since the first experimental psychologists populated university

laboratories. In fact, it was a central point of dispute between Wilhelm Wundt, the

founder of the first psychological laboratory in 1879, and Oswald Külpe, one of his

most successful students (Leahey, 1987). Wundt was convinced that all abstract and

complex thought was constructed from simple elements that related directly to

sensory impressions. Although some complex thought could be more dominant than

its constituting parts, it still depended on sensory elements for its existence, Wundt

maintained. Hence, psychological research should deal with the study of those

elements of mental processes, whereas the direct study of higher mental processes

should be avoided for being unreliable. Külpe, on the other hand, embarked on

studying higher mental processes and concluded from these that 'imageless' thinking

exists. In other words, he concluded that complex mental states were possible without

a need for accessible or even existing representations of elementary stimulus input. In

the following century, this discussion reappeared in debates between extreme

atomists, who adhered to the idea that all mental processing could be reduced to

sensory elements, and new mentalists who believed that meaning, which was extracted from sensory input, formed the elements of memory, to Gestalt psychologists who claimed that every reduction of a holistic percept was artificial (Leahey, 1987). Recent studies in olfaction, including those in the present thesis, are supportive for the new mentalist view and even the Gestalt view with respect to olfactory information processing.

Suggestions to improve the validity of GCO experiments

Knowing that response bias occurs does not prevent it from occurring. However, a number of suggestions for improvement of GCO may be formulated on basis of the findings in this thesis. Odorants that are processed sequentially influence consecutive odorant evaluations (chapter 8). These effects were shown to be persistent over periods of 30 seconds up to 180 seconds, depending on the mechanism involved. Olfactory self-adaptation can only occur during the sniffing of one peak in GCO, because each odorant is released at one retention time. Self adaptation may affect intensity ratings but it cannot be prevented by changing GC settings. However, cross-adaptation effects for chemically related odorants at short retention time intervals may occur. To reduce the probability of not detecting odorants due to cross-adaptation, intervals between odorants should preferably not be shorter than 3 minutes (see chapter 8). In additon, to prevent sequential effects like successive contrast, any odorant sequence with intervals below 1 minute should be prevented. If modifications of gas chromatograph temperature programs do not enable such long intervals, it should be considered whether GCO sessions could be split into sections that are presented at different occasions.

Furthermore, sessions with very few noticeable odorants should be avoided. As was shown in chapter 4, the tendency to initiate a response in olfactory detection tasks

increases when the apparent odorant frequency decreases to low levels. In such cases, raising the concentration of presented volatiles might help.

In addition, it is premature to assume that 'odour impact' components may be recognized on basis of their qualitative similarity to the mixture aroma or on basis of their relatively high impact score. This thesis showed that the impact of components on the mixture aroma could neither be based on intensity (chapter 7) nor on quality (chapter 5). Regardless of how well trained a subject may be in recognising one odorant, it would not necessarily mean that the subject would predict better the impact of that odorant's absence in the complex mixture. A better alternative for the practice of impact component assessment is the technique introduced by (Blank et al., 1992; Guth and Grosch, 1994; Schieberle and Hofmann, 1997). They assessed odorant impacts by measuring the sensory implications of omitting these from the complex mixtures.

Future research

In recent years, the issue of response reliability and method validity has been addressed increasingly in GCO studies. Different methods of odorant impact assessment have been compared critically (Le Guen *et al.*, 2000; Van Ruth, 2004; Van Ruth and O'Connor, 2001; Van Ruth, 2001) and efforts were made to assess the reliability of odour impact scores (Pollien et al., 1997). In the absence of instrumental methods that may replace human subjects in their sniffing task, continued efforts to improve panel response reliability are expected. The psychological model that was developed (chapter 3) may contribute to that development.

This thesis provided further support for the involvement of central processes in odorant perception (chapters 6, 7 and 8). Much of this involvement could be attributed

to the integrative and holistic nature of aroma perception. If the contributions of separate ingredients are lost in the process of aroma perception and replaced by one holistic percept, one may wonder to what extent impressions from different sensory modalities merge into holistic percepts.

Since the late 1980s, multi-modal integration processes have been studied for odour-taste combinations. It was shown that smells may increase taste intensities or prolong taste perceptions and vice versa (Dalton *et al.*, 2000; Davidson *et al.*, 1999; Stevenson and Boakes, 1998; Frank *et al.*, 1989; Frank and Byram, 1988). This effect seemed to be found mainly for odour-taste combinations that co-occur in real life (Frank and Byram, 1988), for instance strawberry odour with sweet taste. In addition, the retronasal pathway for delivery of odorants to the olfactory epithelium, i.e. the natural pathway during eating, favours the occurrence of multi-modal integration in comparison with orthonasal delivery. For instance, if strawberry odour and sucrose taste are delivered orally in an aqueous solution, swallowing leads to more sweet-taste enhancement than the use of a sip-and-spit technique (Frank *et al.*, 1989). This effect is attributed to retronasally perceived strawberry odours due to the act of swallowing. In addition, when comparing sipping an odour/taste solution with sniffing the odour while tasting the taste solution, the relatively more retronasally presented odorants (sipping) contribute more to perceived taste than the orthonasally sniffed odorants (Rankin and Marks, 2000). These results support a holistic view of stimulus perception that goes beyond the boundaries of sensory modalities.

Multi-modal interaction effects are also found for combinations of touch (texture) and taste. A consistent finding is that by increasing the viscosity of solutions the perceived sweetness decreases (Mackay and Valassi, 1956; Mackay, 1958; Moskowitz and Arabie, 1970; Arabie and Moskowitz, 1971; Pangborn and

Szczesniak, 1974; Christensen, 1977; Christensen, 1980). One specific thickener, i.e. acid-modified starch, introduced synergistic texture effects on sweetness perception, which could be attributed to receptor interactions (Kanemaru *et al.*, 2002). However, for the general case of taste suppression by texture, it has not been proven whether texture-taste interactions occur due to receptor interactions, e.g. the lower availability of the sweetener due to the thickener, or whether it depends on central processes.

For the similar suppression of smell intensity by increased viscosity (Pangborn and Szczesniak, 1974) there is convincing evidence that cross-modal effects of texture on odour perception originate centrally: Perceived odour intensity also depends on texture when the odorant concentration in the nose remains unchanged. (Hollowood *et al.*, 2002; Cook *et al.*, 2005; Weel *et al.*, 2002; Bult *et al.*, 2006).

Extra support for the role of holistic perception in multi-modal integration processes comes from neuro-physiological studies. The cross-modal integration of taste and retronasally presented odours rely on shared cortical projections of olfactory activations in areas that are normally activated by oral stimulation (Small *et al.*, 2005). Other studies, employing electro-physiological measurements (Rolls and Baylis, 1994) demonstrated that besides the neurons responding to inputs from only one specific stimulus modality, neurons responding to specific combinations of stimulus modalities, e.g. odor-taste or odor-texture combinations, also exist in the orbito-frontal cortex and other areas (De Araujo *et al.*, 2003; Rolls and Baylis, 1994). Small et al. (Small *et al.*, 2004) demonstrated super-additive activations in specific brain areas for familiar taste-odour combinations (e.g., sweet-vanilla) but not for unfamiliar ones (e.g., salty-vanilla). It may be concluded that these studies provide new neuro-physiological support for the central theorem of Gestalt psychology: perceived events melt into holistic percepts that are inseparable after integration.

In a recent study, Bult *et al.* further illustrated the relevance of studying ecologically valid combinations of stimuli from various modalities (Bult *et al.*, 2006). The authors showed that the separate presentation of cream odours led to perceived increases of thickness, creaminess and flavour intensity of orally presented milks, but only when odorants were presented retronasally. Furthermore, these intensities did not increase when odours were presented during the filling of the mouth with the milk, they did increase when odours were presented during oral processing of the milk and they increased most when odours were presented during the swallowing of the milk. This 'presentation path' x 'odorant timing' interaction suggests that texture, flavour and even complex properties like creaminess were all influenced by a stimulus from one modality, under the condition that the stimulus is presented under natural presentation (path and timing) conditions. These research developments suggest that perception studies will continue to explore the mechanisms that govern multi-modal integration processes under ecologically valid conditions.

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Samenvatting behorende tot het proefschrift getiteld "Sensorische en instrumentele analyse van voedselgeuren"

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Levensmiddelenaromas worden in de regel als eenduidige percepten waargenomen. Zo ruiken gebakken aardappelen naar gebakken aardappelen en ruikt gebrande koffie naar gebrande koffie. Desalniettemin worden vrijwel alle levensmiddelenaromas veroorzaakt door meerdere vluchtige verbindingen die in wisselende samenstellingen aan zeer uiteenlopende aromas bijdragen. Van de honderden vluchtige verbindingen die vrijkomen uit gebakken aardappelen en gebrande koffie produceert een groot deel geen geur, vele tientallen produceren een geur en dragen bij aan een van de genoemde aroma's, vele andere aan beide aroma's. Desalniettemin worden voedselaromas niet als een verzameling afzonderlijke geurende verbindingen waargenomen.

Centraal in dit proefschrift staat een methode die aangewend wordt om de geurende verbindingen van een mengselaroma te scheiden, te identificeren en sensorisch te karakteriseren: gas chromatografie olfactometrie (GCO). In GCO worden vluchtige verbindingen die geïsoleerd zijn uit de gasfase die een levensmiddel omgeeft onder druk door een capillair gevoerd. Doordat de verblijftijd in de capillair van de verbindingen onderling verschilt kunnen proefpersonen deze bij het vrijkomen afzonderlijk waarnemen zodat per verbinding afzonderlijke sensorische informatie ingewonnen kan worden.

Panels van proefpersonen (sniffing panels) worden in levensmiddelentechnologisch onderzoek ingezet als instrument om de geurbijdrage van samenstellende vluchtige verbindingen vast te stellen. In de regel tracht men in technologische studies de instrumentele foutenmarges vast te stellen en te minimaliseren. De paradoxale situatie doet zich nu voor dat die praktijk zelden geldt voor sniffing panels. Er is geen valide en bruikbare methodiek voor het vaststellen van de betrouwbaarheid van panelresultaten en onderzoek naar systematische afwijkingen van panelresponsen is er niet. Bovendien zijn een aantal aannamen die gelden binnen GCO onderzoek psychologisch niet valide. Zo wordt algemeen aangenomen dat sterk en prototypisch geurende verbindingen een belangrijk aandeel in het mengselaroma zullen hebben. Waarneemstudies hebben echter aangetoond dat gemengde geuren elkaar wederzijds kunnen onderdrukken of anderszins beïnvloeden. Aannames voor de rol van aparte verbindingen in het mengselaroma op basis van GCO evaluaties zijn derhalve voorbarig.

In dit proefschrift worden methodes voor het vaststellen en het verbeteren van de betrouwbaarheid van GCO resultaten geïntroduceerd en in de praktijk getoetst (hoofdstukken 2,3 en 4). Bovendien zijn een aantal psychologisch twijfelachtige aannames van GCO geïdentificeerd en gebruikt als uitgangspunt voor empirische studies. Dit heeft achtereenvolgens zijn beslag gekregen in de studie van de invloed van stimulus context op de geschatte betrouwbaarheid van GCO resultaten (hoofdstuk 4), de invloed op mengselaroma's van GCO-geïdentificeerde 'character-impact' verbindingen (hoofdstuk 5), de studie van de invloed van drempelige geurverbindingen op bovendrempelige mengselaroma's (hoofdstuk 6), de studie van

de wederzijdse beïnvloeding van sequentieel aangeboden geurstimuli op de waargenomen geurintensiteit (hoofdstuk 7) en de studie van de invloed van taakinstructie op de herkenbaarheid van geurverbindingen in een levensmiddelengeur (hoofdstuk 8). Algemene conclusies zijn dat willekeurige panel responsen gebruikt moet worden om de betrouwbaarheid van GCO geur detectie vast te stellen en dat systematische responsvariaties gekend moeten zijn om voorbarige conclusies in GCO te voorkomen.

Abstract pertaining to the thesis, entitled "Sensory and Instrumental Analysis of Food Aromas"

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Food aromas are generally perceived as unitary aromas, i.e. fried potatoes smell like fried potatoes and roasted coffee smells like roasted coffee. Nonetheless, nearly all food aromas are produced by a multitude of volatile components that contribute to an extensive collection of aromas in various compositions. Of the hundreds of volatiles released from fried potatoes and roasted coffee a major selection does not produce an odour, many others do produce an odour that contributes to one of both aromas, many others contribute to both. In spite of this, food aromas are not being perceived as collections of discernable odours.

Central theme of this thesis is a method used to decompose, identify and characterise the odorous components in mixtures of odorants: gas chromatography olfactometry (GCO). GCO entails the pressurised transfer of volatiles through a capillary, after capturing these from the headspace surrounding a food. Since the capillary delays volatiles differentially, panellists may sniff these volatiles sequentially on their release from the capillary. This allows the separate sensory evaluation of components in the aroma mixture.

A well-established practice in technological studies is that measurement reliability of instruments is estimated and minimised. Although sniffing panels are generally

employed as instruments that assess the odour impact of chemical components in food aromas, reliability assessment is generally not applied to their application. Paradoxically, there are no practically available valid methods that may assess the reliability of panel responses. In addition, studies of systematic bias of panel responses in GCO studies are not available, although some common assumptions in GCO studies are not valid psychologically. For instance, intense odorants that are qualitatively similar to the aroma quality are generally identified as character impact components. However, perception studies showed that odorants may affect (viz. suppress) each others odour contribution when mixed. Therefore, assumptions regarding the contribution of singular odorants to mixture aromas on basis of GCO is premature.

This thesis introduces methodology to estimate GCO response reliability and reports the empirical testing of this methodology (chapters 2,3 and 4). In addition, several GCO assumptions considered psychologically disputable are evaluated in empirical studies. These studies entail the effect of stimulus context on the estimated reliability of GCO results (chapter 4), the evaluation of the contribution of GCO-identified 'character impact components' to an apple model aroma (chapter 5), the effects of peri-threshold components on a supra-threshold food aroma (chapter 6), the effects of sequentially presented odorants on their mutual odour intensities as a function of qualitative odour similarity (chapter 7) and the effects of task instruction on the identification of odorants in food aromas (chapter 8). The general conclusions of this thesis are that panel responses in the temporary absence of odorants must be used to estimate the reliability of panel odour detections and that mechanisms that systematically affect response variation should be known and considered in GCO.