

From THE DEPARTMENT OF CLINICAL NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

PERCEPTUAL INTEGRATION OF ODOR MIXTURES

Malin Schütze



**Karolinska
Institutet**

Stockholm 2014

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by AJ E-print AB

© Malin Schütze, 2014

ISBN 978-91-7549-471-5

ABSTRACT

Virtually all perceptions of environmental odors are based on an integration process of many volatile components, in many cases hundreds of components. By investigating the perceptual dimensions in this perceptual integration process and how it is affected by the neurophysiology of the olfactory system we can begin to understand the nature of complex odor perception. The aim of this thesis is to describe and model the perceptual integration process by investigating how two single components integrate into an odor percept.

Study I showed that the odor of an agonist, as hypothesized, was dominated by the odor of an antagonist.

Study II implicated that peripheral processing plays an important role in integrating odorants into the mixture perception, with higher intensities and more stable perception of quality when bypassing this level of interaction. Electrophysiological measurements converged with these perceptual effects.

Study III showed that the pleasantness of single odorants is dependent on *intensity* as described by a certain *family* of 2nd degree polynomials. The pleasantness of *mixtures* is dependent on the *quality* change and the *shift in intensity* that occurs when one odorant is added to another. The pleasantness of mixtures can be predicted along a *quality-weighted* average of the individual functions.

Study IV showed that mixture quality is not tied to any particular single component, which indicates that we perceive odor mixture more or less synthetically as a unitary percept. In addition, the study showed that the perceived quality and pleasantness of combined odorants is a simple function of the component qualities such that mixture quality is intermediate to its components' quality in perceptual space.

The combined results from these studies suggest that integration of odors into a mixture percept is dependent on the interaction at the peripheral level of the olfactory system, the receptor epithelia. In addition, the quality of an odor mixture tends to be intermediate to those of its components in a perceptual space and the odor mixture percept tends to be synthesized into a unitary homogeneous percept. Finally, a psychophysical model of mixture integration describing the interplay between fundamental dimensions of the odor percept: intensity, quality, and pleasantness is developed and tested.

LIST OF PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their roman numerals (Study I-IV):

- I. **Brodin*, M.**, Laska, M., & Olsson, M. J. (2009). Odor interaction between bourgeonal and its antagonist undecanal. *Chemical Senses*, 34, 625–630.
- II. **Schütze, M.**, Negoias, S., Olsson, M. J., & Hummel, T. (2014). Perceptual and processing differences between physical and dichorhnic odor mixtures. *Neuroscience*, 258, 84–89.
- III. **Schütze, M.**, Broman, D., Møller, P., & Olsson, M. J. (2014). The affective value of combined olfactory stimuli. *Manuscript submitted for publication*.
- IV. **Schütze, M.** & Olsson M. J. (2014). Synthesis and intermediacy in odor perception. *Unpublished manuscript*.

*) Author has changed last name from Brodin to Schütze

CONTENTS

1	Olfactory perception.....	1
1.1	Intensity.....	1
1.2	Quality.....	2
1.3	Pleasantness	3
2	The olfactory system.....	5
2.1	Olfactory epithelium	5
2.2	Olfactory bulb	6
2.3	Olfactory cortex.....	6
3	Odor mixtures.....	8
3.1	Perceptual integration of odor mixtures	8
3.1.1	Intensity of odor mixtures.....	9
3.1.2	Quality of odor mixtures	10
3.1.3	Pleasantness of odor mixtures	13
3.1.4	Physiological interactions of the olfactory system	15
4	Aim of the thesis.....	17
5	Empirical studies.....	18
5.1	Study I – Perceptual effects of receptor antagonism	18
5.1.1	Background and aim	18
5.1.2	Pilot experiment: <i>Assessing intensity of single components</i>	18
5.1.3	Main experiment: <i>Mixtures – Quality and pleasantness</i>	19
5.1.4	Conclusion.....	20
5.2	Study II – Central vs. peripheral processing of mixtures	20
5.2.1	Background and aim	20
5.2.2	Summary of methods	21
5.2.3	Main results	22
5.2.4	Conclusion.....	22
5.3	Study III – Predicting pleasantness of mixtures.....	22
5.3.1	Background and aim	22
5.3.2	Summary of methods	23
5.3.3	The model	23
5.3.4	General conclusions	24
5.4	Study IV – Conceptualizing odor integration	24
5.4.1	Background and aim	24
5.4.2	Experiment 1: <i>Intermediacy</i>	24
5.4.3	Experiment 2: <i>Physical mixtures</i>	25
5.4.4	Experiment 3: <i>Conceptual mixtures</i>	25
5.4.5	General conclusion.....	26
6	General discussion.....	27
6.1	Peripheral vs. central processing	27
6.2	Intermediacy	30
6.3	Synthesis of odor mixtures	32
6.4	Pleasantness of odor mixtures.....	33
6.5	Concluding remarks.....	35
7	Acknowledgements	36
8	References	37

1 OLFACTORY PERCEPTION

In pulses, synchronized with the breathing, we are constantly experiencing the world through our noses. The perceived quality and pleasantness of these objects, places and people, brings us joy, displeasure, and memories motivating us to take actions; to approach or avoid. To accomplish this we somehow need to represent the quality of the odor. This is a remarkable achievement in many ways; one being that most odors that we encounter in our daily life are comprised of hundreds of individually smelling components. For instance, the smell of your coffee in the morning is a mixture of hundreds of odorous substances. Together, these substances blend and are formed into *one* percept, coffee. This underlying integration process, that helps us combine all these components into a relevant representation of our odorous surroundings, is what this thesis will try to explain.

There are three main aspects of an odor percept: intensity, quality, and pleasantness. All these three aspects of odor perception will be necessary to take into account in order to understand the formation of odor mixture quality.

1.1 INTENSITY

The human sense of smell is often thought to be poor in comparison to other species for which the sense of smell is vital for their survival and for reproductive purposes. However, this notion might be somewhat mistaken. Studies have shown that the human nose can detect odorous substances in very small quantities, for some substances even in concentrations in the range of parts per billion (Devos et al., 1990).

There is some complexity to the intensity of odor substances that we need to consider. The intensity at a certain concentration level for one odorant is not necessarily comparable to another odorant's intensity at that concentration. Therefore, to have two odorants at equal intensities, i.e. iso-intense levels, the actual physical concentrations of those two odorants could vastly differ. Possible explanations for this effect are that odorant molecules differ in their tendency to penetrate the olfactory mucosa (Mozell & Jagodowiz, 1973) and that the level of airflow through the nostrils favors different types of molecular sorption (Mozell et al., 1991).

Perceived intensity of an odor increases with a concentration increase, which can psychophysically be described with a power function (Stevens' power law; Steven, 1957):

$$I = c S^n \quad \text{[Eq. 1]}$$

where I stands for perceived intensity, S stimulus concentration, and c and n are constants.

Another aspect that can influence the intensity function is trigeminal stimulation. Trigeminal nerve endings that end in the nasal cavities can be stimulated by chemical substances, and induce sensations like pain, coldness etc. Most odorants we encounter in

our daily lives are stimuli with mixed olfactory/trigeminal properties (Doty et al., 1978). It is quite rare that volatile chemicals only stimulate either the olfactory, or the trigeminal nerve. However, there are rare cases like vanillin and the rose-smelling, phenylethyl alcohol, which are generally used as pure olfactory stimuli (Chen & Halpern, 2008), and carbon dioxide, which is purely trigeminal (i.e., odorless). This trigeminal stimulation can interact with the olfactory sensation. According to Cain (1974), the trigeminal activation could account to up to 30% of the perceived intensity of certain odorants. However, Hummel and Livermore (2002) indicate that for a very strong chemosensory stimulus the trigeminal stimulation seems to suppress the perceived odor strength. The trigeminal stimulation increases with an increase in intensity and at lower concentration, little or low stimulation of the trigeminal nerve is seen.

1.2 QUALITY

Humans are surprisingly poor at identifying odors, which also holds true for regular household items. In fact, people are unable to name the odor of at least 50% of the household items they use daily (Cain, 1979, de Wijk et al. 1995, Lawless & Engen 1977). However, when it comes to smelling dangerous household products, even though children were poor at naming odors they correctly rated their edibility in 79% of the cases (de Wijk & Cain, 1994a). This could symbolize that the primary function of olfaction is to accurately avoid ingesting something inedible.

Our ability of naming odors improves when odor familiarity increases (Homewood & Stevenson 2001) and further improves with practice (Cain, 1979). When given semantic labels to choose from, rather than free recall, odor naming ability increases significantly (Cain, 1976; de Wijk & Cain 1994b). The perceived quality of an odor is also dependent on our other senses, like vision. When white wine was dyed red, students of oenology (i.e. the study of wines) assigned the wine typical red wine descriptors (Morrot et al., 2001).

The perception of odor quality is difficult to predict from its physical correlates. There have been many attempts on finding a molecular basis for odor quality, some with more success (e.g. Khan et al., 2007). However, connecting molecular structure of an odor to its perceived quality is rather erratic. In fact, structural similarity between odorants may in some cases give rise to similar qualities and in other cases they are perceived as very different (Sell, 2006). One aspect of the complexity of physiochemical structure is that of enantiomers (mirror-image molecules). Humans are able to discriminate between some pairs of enantiomers, like between (+) and (–) carvone; however others we cannot discriminate between (Laska & Teubner, 1999). For the two carvone isomers; one isomer smells like dill or caraway and the other like mint (Zawirska-Wojtasiak, 2006). Humans can also discriminate between some odorants with equal number of carbons, but differing functional groups (Laska et al., 2000), or between odorants with the same functional group that differ in carbon chain length by one carbon only (Laska & Freyer 1997).

In parallel to studies on taste quality, there have been several attempts to organize how we perceive odor quality by defining the dimensions of the odor space. Although different approaches have been used to do this, the results have been inconclusive. The pleasantness dimension is the one that different studies have best

agreed upon (Khan et al., 2007; Moskowitz, 1976; Moskowitz & Barbe, 1977;; Moskowitz & Gerbers, 1974; Yeshurun & Sobel, 2010). This suggests that one important function for the olfactory system is to guide approach/avoidance behavior. Odor pleasantness is in focus in Study III and IV of this thesis.

1.3 PLEASANTNESS

Humans are quite good at detecting and discriminating between smells, but are poor at naming them. However, research has shown, over and over again, that humans tend to readily apply labels in regard to odor pleasantness. In the current thesis, pleasantness is used as describing the bipolar range of the affective value of an odor, ranging from unpleasant, to pleasant. In olfaction, pleasantness has also been referred to as odor hedonicity (Stevenson & Mahmut, 2013) and odor valence (Lapid et al., 2008); however, pleasantness, valence, and hedonicity are often used interchangeably to describe the same aspect of the odor experience.

Pleasantness of an odor has shown to be the foremost dimension when estimating similarities of odorant descriptors (Khan et al., 2007; Moskowitz, 1976; Moskowitz & Barbe, 1977; Moskowitz & Gerbers, 1974). Pleasantness is often the first characteristic that participants spontaneously report in different olfactory tasks (Berglund et al., 1973; Schiffman et al., 1977; Schiffman, 1974). Stevenson and Mahmut (2013) suggest that, while odor labels might change, pleasantness or “liking” remains as the most basic function of the olfactory system. Interestingly, Khan et al. (2007), indicated that the pleasantness of an odor could be encoded in the physiochemical structure of the odorant.

Pleasantness of an odor/odorant is greatly *influenced by the intensity* of the odor (Doty, 1975; Henion, 1971; Moskowitz et al., 1976). However, the effect of intensity on odor pleasantness tends to differ depending on if the odorant starts out pleasant or unpleasant. An unpleasant odor seems to become more unpleasant with increasing intensity, while a pleasant odor initially becomes more pleasant as intensity increases. However, at high intensities, even the initially pleasant odors, become unpleasant.

The perceived pleasantness of an odor is *subject to learning*. Our familiarity with an odor alters the perceived pleasantness (Delplanque et al., 2008; Distel & Hudson, 2001; Distel et al., 1999; Jellinek & Köster, 1983), even by mere exposure (Cain & Johnson, 1978; Hudson, 1999). The pleasantness value of an odor can also be influenced by culture and can vary between individuals (Ayabe-Kanamura et al., 1998; Pangborn, 1975; Wysocki et al., 1991). There are studies suggesting that children, as small as newborns, have some basic preferences towards odors (Soussignan et al., 1997; Steiner, 1979). However, the pleasantness seems to be affected by learning since children are neither attracted nor repelled by odors as much as adults (Engen, 1974; Rozin, 1985; Stevenson et al., 2010).

The perceived pleasantness of an odor is *context dependent*. The pleasantness of an odor is clearly dependent on input from our eyes, as well as from semantic information that we receive (Case et al., 2006; de Araujo et al., 2005; Djordjevic et al., 2008; Herz, 2003, Herz & von Clef, 2001). Herz and von Clef (2001) presented the mixture of isovaleric and butyric acid to participants, either with the semantic label of *parmesan cheese* or with *vomit*. Even though the same odor was presented, the results show that

the rated perceived pleasantness was more negative when presented to “vomit” and more positive for “parmesan cheese”.

Pleasant and unpleasant odors are processed differently. Studies have shown that pleasant and unpleasant odors were evaluated at different speeds, with unpleasant odors having shorter response times (Bensafi et al., 2002). It has also been shown that pleasant and unpleasant odors show activation in different neural substrates as evidenced by electrophysiological recordings (Alaouilsmaili et al., 1997; Kobal et al., 1992; Masago et al., 2001; Pause & Krauel, 2000), as well as by other functional neuroimaging techniques (Anderson et al., 2003; Gottfried et al., 2002; Grabenhorst et al., 2007; Rolls et al., 2003; Royet et al., 2000; Zald & Pardo, 1997; Bensafi et al., 2012).

2 THE OLFACTORY SYSTEM

In comparison to other sensory modalities, there are two unique properties of the anatomy of olfaction. First, olfactory processing is mainly ipsilateral to the side of presentation. The human nose is comprised of two nasal chambers, divided by a septum, each leading up to an olfactory epithelium. From each olfactory epithelium olfactory receptor neurons project information ipsilaterally all the way to regions in the olfactory cortex, via its ipsilateral olfactory bulb. The projections are primarily ipsilateral (Lascano et al., 2010; Price, 1990; Shipley & Reyes, 1991), although there are possible contralateral connections via the anterior commissure, the corpus callosum, and the hippocampal commissure (Doty et al., 1997; Shipley & Ennis, 1996). However, these contralateral connections are so far shown to be marginal or non-existent in humans and that the human olfactory system seems to mainly project information ipsilaterally to the side of stimulation (Gottfried, 2006). This ipsilateral separation is, in comparison with audition and vision, quite unique.

Secondly, sensory information from the olfactory bulbs to central brain regions occurs without thalamic relay. This stands in contrast to other sensory modalities, for which all sensory input need to pass through the thalamus, before being delivered to its sensory-specific cortex.

Some studies have investigated the impact of side of stimulus presentation on brain activation, but the studies show varied results. Lascano et al. (2010) showed greater activation on left side of the brain when odorants were presented in the left nostril, as well as greater activation on right side when stimulated via the right nostril. However the study also shows that some activation was found contralateral to presentation. Savic and Gulyas (2000) concluded from their study using positron emission tomography that odors tended to be processed both ipsilaterally and contralaterally to the side of presentation. In addition, the right hemisphere showed a greater activation, irrespective of side of presentation. In a study by Stuck et al. (2006), left- and right- sided stimulation did not produce responses that were significantly different. Olofsson et al. (2006) showed no general effect of stimulated nostril side on olfactory event-related potential amplitudes. Although, pleasant odorants yielded larger N1/P2 amplitudes for left- compared to right-nostril stimulation.

The fact that the projections of sensory information within the olfactory system are remaining mainly on its ipsilateral side of presentation, is an aspect of the olfactory processing that is in focus in Study II of this thesis.

2.1 OLFACTORY EPITHELIUM

When we encounter an odor, the molecules of the odor travels up the nasal cavity to the olfactory epithelium, where the odorants lock onto olfactory receptors situated on the cilia of the olfactory receptor neurons. When an olfactory receptor is stimulated by an odorant it triggers a reaction which transforms into an electrical signal that travels up the cilia of the olfactory receptor neuron. There are about 1000 different receptor types in the

mammalian olfactory catalog (Buck & Axel, 1991), however genetic analysis has discovered that humans functionally express only about 350 of them (Glusman et al., 2001; Gaillard et al., 2004; Malnic, Godfrey, & Buck, 2004; Zozulya et al., 2001).

One olfactory receptor can be activated by an array of different odorants. The term odorant is considered a volatile chemical compound with low molecular weight. An odorant needs to have a low weight to be able to disperse through the air and be transported with an airstream leading up our nose.

An odorant can be recognized by multiple different olfactory receptors, which means that different odorants bind to and activate combinations of olfactory receptors (Araneda, 2000; Malnic et al., 1999). The olfactory receptors tend to bind to molecules that have similarities in the molecular functional group. Olfactory receptors have been thought to be broadly tuned, responding to many different odorants (Duchamp-Viret et al., 1999) but recent findings indicate that some receptor types are narrowly tuned to few odorants and others are more broadly tuned (Araneda et al., 2004; Hallem & Carlson, 2006; Sanz et al., 2005).

2.2 OLFACTORY BULB

The information from the olfactory receptor neurons are further transmitted to the ipsilateral olfactory bulb situated in the brain. All olfactory receptor neurons expressing the same type of receptor converge into the same region of the olfactory bulb, forming so called glomeruli, which are situated bilaterally in each bulb (Mombaerts et al., 1996; Ressler et al., 1994; Tsuboi et al., 1999; Vassar et al., 1994). Each glomerulus in the olfactory bulb represents one type of receptor neuron. This entails that odor identity can be represented in a spatiotemporal map of glomerular activation (Cleland et al., 2007; Leon & Johnson, 2003). Each olfactory bulb transmits information to the olfactory cortex ipsilaterally (Lascano et al., 2010; Price, 1990; Shipley and Reyes, 1991).

2.3 OLFACTORY CORTEX

Projections starting from the human olfactory bulb are, by means of mitral and tufted cells, connected with many regions in the brain. All brain regions that receive direct input from the mitral and tufted cell axons are defined as the primary olfactory cortex (Allison, 1954; Carmichael et al., 1994; de Olmos et al., 1978; Haberly, 2001; Price, 1973, 1987, 1990). There are many feedback projections from the regions in the primary olfactory cortex leading back to the olfactory bulbs (Carmichael et al., 1994), which could modulate olfactory information.

The piriform cortex is one of the major recipients of information projected from the olfactory bulb. It is the largest of the central olfactory areas and covers the area connecting the frontal and temporal lobes (Gottfried, 2006). There are multiple functions of the piriform cortex; it participates in basic olfactory processing like sniffing (Sobel et al., 1998), it is receptive to odor quality (Gottfried et al., 2006; Howard et al., 2009), it shows

activation to extremes in pleasantness (Gottfried et al 2002), and appears to be involved in olfactory learning and memory (Gottfried et al., 2004).

There are also projections from the olfactory bulb that terminate in the amygdala. In return, the amygdala sends back projections to the bulb and also to other subdivisions. The amygdala is commonly a part of emotional processing and is thought to be involved in the processing of odor pleasantness. However, the views of what part amygdala plays in processing of pleasantness are diverse. Some studies suggest that the amygdala is insensitive to pleasantness by showing similar activation for pleasant, neutral, and unpleasant odors (Gottfried et al., 2002), another that unpleasant odors have a higher activation than pleasant odors (Hudry, et al., 2003), whilst yet another show that the level of activation in the amygdala relates to the salience of the odor, by a combination of intensity and pleasantness (Winston et al., 2005). Perceived pleasantness is a central aspect of odor perception, and is in focus of this thesis.

3 ODOR MIXTURES

Most of the odors we perceive daily are composed of multiple odorous components, all contributing to the overall smell. Odors like coffee, banana, and vanilla are highly complex mixtures with more than hundred different components. The number of volatile chemicals for these odors clearly exceeds one's expectation, with 655 compounds for coffee, 350 for banana, and 190 for vanilla (Maarse, 1991). Even though these odors are in fact complex mixtures, it is important to accentuate that they are normally perceived as one, unitary odor. Additionally, people tend to not perceive odor mixtures as more complex than single odorants, which is demonstrated by the smell of a rose, which is comprised of about 260 components, but is not perceived as more complex than the rose-smelling phenyl ethyl alcohol (Keller & Vosshall, 2004).

3.1 PERCEPTUAL INTEGRATION OF ODOR MIXTURES

Even though the sense of smell plays an obvious role in people's lives, little is known about how the olfactory system works to create the percept of an odor. In fact, the process of integrating individual odor components into a unitary percept is still largely unknown.

What determines how an odor mixture will be perceived? With regard to odor quality, binary odor mixtures seem to follow a few general rules of thumb. Several studies have shown that an olfactory stimulus that gradually changes from odorant A to odorant B over mixtures of A and B yields a corresponding change in perception of quality A to B (Cain et al. 1995; Laing et al. 1994; Laska & Grimm 2003; Olsson 1994). In other words, it is feasible to describe the quality of a binary mixture in terms of its component qualities. It also has been demonstrated that the components' relative perceptual intensities before mixing determine the quality of the mixture. More specifically, an odorant with higher perceived intensity before mixing will usually dominate the mixture perception over a weaker odorant (Laing et al., 1984; Olsson, 1994, 1998). Sometimes, however rarely, perceptual asymmetries are found with mixtures in which components are perceptually isointense (Ferreira, 2011b), that is, a mixture of two equally strong odors, before mixing, may lead to a mixture percept for which one component quality dominates over the other.

In many experiments, there is of importance to control for intensity differences between the stimuli. Therefore a *substitution* procedure when mixing the odorants can be used. Here, odorants are mixed in relative proportion, so as the relative proportion of one odorant increases, the proportion of the other decreases. This typically leads to that the overall intensity is perceived to be isointense. However, an *additive* procedure can also be performed, in which odorants are actually added to each other such that we have more odorous material in the stimulus after mixing than before. This typically leads to that the *intensity* of mixtures is stronger than its components.

There are many factors, both physiological and cognitive, that could affect our perception of odor mixtures. Below, some important aspects will be presented. How, then, are the components of a natural odor combined into a more or less unitary percept?

3.1.1 Intensity of odor mixtures

The most studied aspect of odor mixture interaction is the intensity of odor mixtures and it is therefore not in focus of the work in the current thesis. However, here follows a brief summary of odor-intensity interaction. There are two main approaches to the investigation of odor intensity interactions, a psychophysical approach (e.g., Frijters & Oude Ophius, 1983; Schiet & Frijters, 1988) and a perceptual approach (e.g., Jones & Woskow, 1964; Patte & Laffort, 1979; Olsson, 1994, 1998). Within the psychophysical approach, researchers have attempted to explain the mixture by relating the intensity of the mixture to the intensities of the stimuli, the concentrations of the components, as well as the psychophysical functions for each substance. With the perceptual approach, explanations for the mixture interaction are sought by relating the perceived intensity of the odor mixture to the perceived intensity of the individual components.

The perceived intensity of a binary odor mixture I_{AB} is the result of adding the individual intensities of the two components, I_A and I_B . However, this rarely results in a clear additivity of the intensities of the components. By calculating the below ratio, σ , you get a normalized representation the level of this summation.

$$\sigma = \frac{I_{AB}}{I_A + I_B} \quad [\text{Eq. 2}]$$

However, when the intensity of the mixture I_{AB} would be a perfect summation of the intensities of the components, σ will be 1. This is called perfect or complete additivity and values that differ from 1, represent the deviation from this.

There are some important findings regarding mixture intensity. First, the perceived intensity of a mixture will rarely, if not never, surpass the sum of intensity of the components; i.e. σ is rarely larger than 1. Second, as mentioned above, most studies indicate that odor mixtures do not show perfect additivity, but instead, the intensity of responses is typically less than would be expected from simple additivity (Ferreira, 2012a). Moreover, individual components in the mixture percept can be more or less suppressed and the overall mixture quality can vary in how well individual components can be perceived (Jinks & Laing, 2001; Sinding et al., 2013).

Furthermore, an odor with a low perceived intensity will have little effect on the overall intensity, when mixed with an odor with strong perceived intensity. Olsson (Berglund & Olsson, 1993a, 1993b; Olsson, 1994, 1998) has argued that the relationship observed between the percept of a binary (two-component) mixture and of its components exhibit simple rules that are consistent across different pairs of odorants and at different levels of perceived intensity. From the perceived intensity of the individual components, we can predict the perceived intensity of the odor mixture. As mentioned above, one rule is that the intensity of the binary mixture never surpasses the sum of the intensity of the components. The perceived intensity of the mixture would neither be

weaker than the perceived intensity of the mixture's weaker component. It is also possible to predict both the perceived intensity and quality of the mixture more closely as a function of the perceived intensities of the individual components (Olsson, 1994, 1998). Along these lines, perceptual prediction models have been found to be more successful in describing consistent relationships between stimuli and mixture than psychophysical ones (i.e., models predicting intensities from concentrations and components' psychophysical functions; Berglund & Olsson, 1993c; Cain, 1995).

3.1.2 Quality of odor mixtures

When we investigate the perception of odor mixtures, it is of importance to look at the different views on just how the odorous components are processed and integrated into the mixture perception. One major question involves whether the components of odor mixtures form a heterogeneous or homogeneous percept. With a heterogeneous percept, the components of an odor mixture are perceived as separate odor entities, much like two voices sounding in unison. With a homogeneous percept, the components of the mixture are blended and synthesized into a uniform percept, like additive color mixtures. As mentioned above, when it comes to the complex mixtures that we encounter in our daily life, we tend to perceive these odor mixtures as unitary odor objects, hence homogeneous percepts. Another question centers on how we process our perceptions into heterogeneous or homogeneous percepts. These perceptual processes are often referred to as analytical (or dissociative, elemental), for heterogeneous percepts, whereas for homogeneous percepts, as synthetic (or associative, configural) (Berglund & Olsson, 1993a; Doty & Laing, 2003; Jinks and Laing, 2001; Linster and Cleland, 2004; Olsson, 1993). This question will be addressed in Study IV.

3.1.2.1 *The identification of components in a mixture*

A synthetic integration process of odors makes it possible for us to tag objects and events in our surrounding with unitary odor percepts. However, it also makes it harder for us to track known odor components in mixtures (Jinks & Laing, 1999). This can be illustrated by our lack of identifying components in mixtures. The ability to identify odor components is quite limited, with up to 3, or seldom 4 being the limit (Laing & Francis, 1989; Livermore & Laing, 1996, 1998a, 1998b). Hence, when presented with mixtures of four components the ability to detect the components seems to reach a plateau, which could be described as a ceiling of capacity to discriminate and identify the components of a mixture beyond chance. This limitation holds true for different types of mixtures; with familiar odor components (Laing & Francis, 1989), with no difference for odors that according to professional perfumers are considering "good"- or "poor blenders" (Livermore and Laing, 1998a), with complex odor essences (Livermore & Laing, 1998b). Also, even for experienced perfumers the number of correct identifications never surpassed four components (Livermore & Laing, 1996). When presented with a mixture with less than four components the participants are marginally able to discriminate and identify the components above chance and the process of analytically perceiving odor

mixtures might only work with a limited amount of components. Livermore and Laing (1998b) also showed that complex odors were processed as single entities, in a similar fashion as single substances were.

The extent to which we can identify components in odor mixtures does not necessarily mean that the mixture percept is heterogeneous. The judgments could instead be based on a perceptual *similarity* between the homogeneous mixture and its components.

3.1.2.2 Intermediacy

With the use of multidimensional analysis of odor similarity, many studies express that the quality of a mixture is intermediate to that of its components. In a study by Ekman and Engen (1962), they showed that mixtures of amyl acetate and n-heptanal was intermediate to their components in a two-dimensional similarity space. When investigating single odorants and their mixtures of 2 to 5 components, Moskowitz and Barbe (1977) showed that the estimates of similarity between odor stimuli demonstrated an overall tendency for mixtures to be intermediate to their components with all but one mixtures being in close proximity to the lines connecting the single components in the perceptual space. This means that the odor properties of the mixtures are well defined by the odor properties of the single components. Wise and Cain (2000) investigated the odor quality space by using discrimination errors and latencies of discrimination responses. The discrimination measures (d') were based on response latency and perfect additivity for the d' for A vs. B comparisons was found. In other words, the d' s for the A vs. AB and the B vs. AB comparisons added up to the d' for the A vs. B comparison. Dravnieks et al. (1981) found that mixtures of up to four components were well described by the arithmetic mean of the descriptor scores of the mixture components.

Another argument for intermediacy of quality for odor mixtures could be found in that for binary odor mixtures, the mixture quality can systematically be described in terms of its component qualities (e.g., Cain et al., 1995; Laing et al., 1984, 1994; Olsson 1994, 1998). Results from several studies (Atanasova et al., 2005; Olsson, 1994) would suggest that a balanced mixture, of equal intensities of odor A and B before mixing will yield reports of equal amounts of quality A and B.

However, other studies indicates that intermediacy is not always the case (Barkat et al., 2012; Le Berre et al., 2008b; 2010; Ferreira, 2011b; Sinding et al., 2013). Several authors indicate that a possible deviation from intermediacy concurs with a mixture being perceived as an object odor (e.g., the odor of pineapple), through a so-called “blending effect” (Barkat et al., 2012; Le Berre et al., 2008a, 2008b, 2010; Sinding et al., 2013). One description of this blending phenomenon states that “perceptual blending in odor mixtures can lead to the perception of a specific odor quality not present in any of the mixture’s components” (Le Berre et al., 2010: p. 156).

In some of these studies participants were using a typicality rating task, in which the subjects reported that they perceived a binary mixture as smelling like pineapple, while its components possessed other qualities (i.e., strawberry and caramel, respectively (Barkat et al., 2012; Le Berre et al., 2008a; 2010). Typicality measurements are suitable for providing evidence of blending in odor mixtures, and that the typicality

task would prevent participants from engaging in an analytical processing which in turn would disturb the perceptual blending (Le Berre et al., 2008a).

Sinding (2013), on the other hand, utilized a similarity sorting paradigm and compared the six-component “blending mixture” of Red Cordial (i.e., pomegranate-/raspberry cordial) with a mixture with no known blending effects. The multidimensional analysis discovered that the Red Cordial-mixture was statistically more discriminable from its components than the non-blending mixture, which was more intermediate to its components. However, it is unclear whether intensity differences between the components in the mixture could be affecting the discriminability.

The blending effect is sensitive to small changes in composition and therefore occurs at exact concentration ratios of the different mixture components (Le Berre et al., 2008b; 2010), and only for some, but not all, “blending mixtures” (Le Berre et al., 2010). Le Berre (2010) also showed that semantic and perceptual learning of odors could affect the perceptual mixture blending. However, the effect differed depending on odor mixture complexity and they propose that semantic learning with odor labels could have altered the perception of the more complex mixtures as being more typical to the odor label, while perceptual learning could have altered the perception of the less complex mixtures by enhancing the components qualities.

3.1.2.3 *The relative intensity of odor components*

Another way to investigate the odor quality of binary mixtures is through measures of the perceived intensity of the components as they are perceived in the mixture percept. By targeting multiple composition ratios of the mixture we can determine that the dominance of one odorant over the other in the perception of mixture quality is closely related to the relative intensities of the components in the mixture. This relationship can be illustrated by comparing τ , the relative intensity of components before mixing,

$$\tau_A = \frac{I_A}{I_A + I_B} \quad \text{and} \quad \tau_B = 1 - \tau_A \quad [\text{Eq. 3}]$$

with τ' , the relative intensity, I' , of components within the mixture,

$$\tau'_A = \frac{I'_A}{I'_A + I'_B} \quad \text{and} \quad \tau'_B = 1 - \tau'_A \quad [\text{Eq. 4}]$$

When comparing τ with τ' the mixture perception tends to follow a few rules of thumb. First, several studies have shown that an olfactory stimulus that is changed gradually from odorant A to odorant B over mixtures of A and B, will yield a gradual change in perception of the quality from A to B. Secondly, the components' relative intensities before mixing will highly affect the perceived quality of the mixture. That is, an odorant with a higher perceived intensity than the other odorant before mixing will most likely dominate the quality of the mixture (Cain et al., 1995; Laing et al., 1984, 1994; Olsson, 1994, 1998). The relationship between τ and τ' is represented by a sigmoidal

function (slightly S-shaped) as the stimulus is gradually shaped from A to B. In other words, the influence of the two components on mixture quality is symmetric or balanced when the function passes the point where τ and τ' both equal 0.5 (Atanasova et al., 2005; Laing et al., 1984, Olsson 1994, 1998). An example of how the analysis of the influences of component intensities before mixing affects the mixture quality measured in this way can be seen in Appendix: Study 1, Figure 1D and 1E.

3.1.2.4 *Plasticity of odor mixture quality*

Admittedly, the quality of mixtures is not under all circumstances a fix function of its components. Several learning processes can change mixture quality. For odors in general, learning in different forms has shown to alter odor perception. Conditioning of one of two enantiomers that were initially impossible to tell apart became discriminable from the other after repeated pairing with an electric shock (Li et al., 2008). Mere exposure or familiarization seems also able to affect judgments about mixture quality. In an experiment in which discrimination of an odor and the same odor with a minor adulterant was measured, familiarization of the adulterant enhanced the discrimination (Rabin & Cain, 1984). The familiarity to the odor boosts the distinguishing traits and sharpen the perceptual boundaries of the odor in both of these examples of olfactory learning affects the overall quality of the mixture.

Stevenson (2001) has shown that individual odors adopt each other's qualities after being presented in binary mixtures. This type of learning is, unlike classic conditioning, insensitive to extinction and does also not require consciousness of the relation of the conditioned and the unconditioned stimuli. This interesting type of learning may be specifically predominant among the chemical senses.

3.1.3 **Pleasantness of odor mixtures**

Everyday odors, like those of food, body odors, and other objects are composed of many different volatile compounds. Whereas we know more about how the interaction of components combine to form the perception of intensity (Ferreira, 2011a) and quality of odor mixtures (Ferreira, 2011b), pleasantness is instead quite poorly understood. Stimulus pleasantness (affective value, hedonics, and valence) is the primary dimension of olfactory perception (Khan et al., 2007, Moskowitz, 1976; Moskowitz & Barbe, 1977; Moskowitz & Gerbers, 1974). Thus, the pleasantness of odors is a major dimension underlying similarity estimates of odorant pairs. Whereas perceived quality is more stable across different suprathreshold concentrations, odor pleasantness, as noted above, is highly dependent on the perceived intensity (Doty, 1975; Henion, 1971). Unpleasant odors that are increased in intensity will smell more unpleasant whereas pleasant odors seem to have an optimal intensity for which a maximal pleasantness is perceived (Lawless, 1977).

3.1.3.1 *Predicting odor mixture pleasantness*

The attempts to explain the underlying principles of mixture pleasantness have so far had limited success. As a first formal attempt to explain the pleasantness of binary odor mixtures, Spence and Guilford (1933) reported that the pleasantness of the mixture tended to be intermediate to that of its components. That is, the mixtures was less pleasant than the most pleasant and more pleasant than the least pleasant odor. Moskowitz and Barbe (1977) also observed that the pleasantness ratings of mixtures in the majority of cases seemed to be intermediate to the components'. However, they had limited success when applying a regression analysis in order to predict the mixture's pleasantness from the pleasantness of its components.

These attempts did, however, not take into account the contribution of the components' intensities on the pleasantness of the mixture. Due to that the perceived pleasantness is highly dependent on intensity (Henion 1971; Doty 1975), it is of importance to take the intensities of the components into account when trying to predict mixture pleasantness. Therefore, in an attempt by Lawless (1977) to predict mixture pleasantness he tested the interaction of intensity and pleasantness of the components. He reported that the components in the mixture are normally perceived as weaker in the mixture than presented alone. In addition, the pleasantness of an odor is highly dependent on the intensity of that odor; a pleasant odor increases in form of an inverted U-shaped function, whereas an unpleasant odor normally gets worse with intensity. Lawless suggested a linear regression model for the pleasantness of the mixture, where he accounted for the pleasantness-/ intensity relationship of its constituents.

After a long silence on the topic of the pleasantness of odor mixtures, Lapid et al. (2008) proposed a new prediction model for the pleasantness of binary mixtures from the pleasantness and intensities of their separated constituents at different mixing ratios. To counteract the problem of mixture intensity as a confounding variable in prediction of the mixture's pleasantness, a substitution procedure for mixing was used, leading to that the intensity of mixtures and single substances will be roughly matched and therefore using the separate components' relative perceived intensities as weights. In most cases, the pleasantness of the mixture was intermediate to the pleasantness of the components and that it was also strongly influenced by the relative intensity of the constituents. However, the prediction model is only able to explain a mixture pleasantness that is intermediate to the pleasantness of the components.

The perceived intensity and the perceived quality of the mixture are important for predicting the pleasantness of the mixture. When combining odor components into a mixture, there will be a drastic shift in both perceived intensity and perceived quality in comparison to those of the individual components'. A model for predicting the pleasantness of odor mixtures by a quality- and intensity-weighted mixture model is presented in Study III.

3.1.4 Physiological interactions of the olfactory system

As mentioned, there are about 350 different types of receptors in the human olfactory epithelium (Crasto, 2001). Each receptor can be activated by more than one odorant and an odorant can activate more than one receptor, creating a combinatorial receptor code which distinguishes it from other odorants (Malnic et al., 1999).

All receptor cells carry only one type of receptor, but a receptor can be activated by multiple odorants. In such a system a single odorant is likely to activate a number of different receptor cells (Buck & Axel, 1991; Malnic et al., 1999). This suggests that different odorants would be identified, not by single receptors, but by their combinatorial activation of multiple receptors which creates a combinatorial receptor code for that particular odorant. A change in concentration could then imply that the receptor code for that odorant would change, through an activation of additional receptor types (Malnic et al., 1999). Although different odorant molecules could be registered by different receptor activation patterns, in some cases these receptor activations may overlap between the components and may therefore affect the overall mixture percept.

Odorant interaction has been observed at the periphery, for instance through competition, which is when one agonist competes for the same receptor site with another agonist or antagonist. Also noncompetitive interaction has been observed, e.g. allosteric interaction. That is, the main binding site is activated by an agonist, but an occupation at a second site modifies the binding or activation properties of the agonist at the main site (Rospars, 2013). Antagonism means that one ligand is blocking the active site of an olfactory receptor thus preventing interaction of another ligand, without inducing a response (Araneda et al., 2004; Duchamp-Viret et al., 2003; Jacquier et al., 2006; Oka et al., 2004; Rospars et al., 2008; Sanz et al., 2005). Antagonists will block the binding of an agonist at a receptor molecule, inhibiting the signal produced by a receptor-agonist coupling. Two important characteristics are *affinity* and *efficacy*. Affinity is the tendency to bind to a receptor and efficacy is how effective it activates the receptor. Antagonists display no efficacy to activate the receptors they bind. Antagonists do not maintain the ability to activate a receptor. Once bound, however, antagonists inhibit the function of agonists. The impact of specific receptor antagonism on odor mixture interaction will be tested in Study I of this thesis.

Whereas studies on fruit fly favor competitive interaction (Münch, 2013), studies on rat clearly indicate the occurrence of noncompetitive interactions. A study of several binary mixtures (Rospars et al., 2008) indicated that about half of the mixtures could be described by a syntopic interaction model, which is based on competitive interaction, and the other half suggested a noncompetitive interaction. The authors argued that this noncompetitive modulation added a new combinatorial dimension of olfactory coding that could contribute to the emergence of new perceptual qualities different from each component.

Odorant interaction can result in suppression and lateral inhibition of individual components perceptual qualities and this could have an important impact on the perception of odor mixtures. The odorants in a mixture can interact with each other by reducing the other odorants' capacity to activate receptors or by inhibiting the transmission through the olfactory system. These processes may also sharpen the odorant characteristics of single cells (Kurahashi et al., 1994). Competition for receptor sites between odorants has been discussed as a factor for inhibition. This competition could on

one hand, depend on the polarity of the odors, in that odors of similar chemical polarity could compete for the same types of receptors (Bell et al., 1987). On the other hand, there might be a time factor involved. Getchell et al. (1980) showed, in studies of the salamander that odorants could differ in the time it takes to register with a receptor. This could mean that a faster odorant could register with a receptor much quicker and therefore block the site for another component in a mixture, or it could reach the olfactory bulb quicker where it could inhibit transmissions from the contralateral bulb (Laing, 1987).

In addition to the interaction at the periphery (Chaput et al., 2012), more central processing may also be a part in forming the mixture percept (Boyle et al., 2009; Rouby & Holley, 1995; Zhou and Chen, 2009). Boyle et al. (2009) showed that by using a substitutional mixture procedure, the activation in the lateral part of the orbitofrontal cortex seems to respond to the impurity of a mixture in a graded fashion, and the anterior part seems to act more like an on-off detector for odor mixtures.

It has also been shown that the piriform cortex has a major role in mixture perception, in that there are neurons responding to mixtures but not to their components when presented separately (Kadohisa and Wilson, 2006; Wilson and Sullivan, 2011). The anterior part of the piriform cortex shows activation of mixture quality that differs from activations representing its components' qualities. The posterior part seems to show activations in regard to the similarity between the mixture and its parts (Gottfried et al., 2006; Kadohisa and Wilson, 2006; Wilson and Sullivan, 2011).

Psychophysically, the difference between peripheral and central processing can be investigated by utilizing the fact that the olfactory system seems to mainly project information ipsilaterally to the side of stimulation (Gottfried, 2006), and that the human nose is comprised of two nasal chambers, divided by a septum, each leading up to an olfactory epithelium. By comparing the effects of presenting a mixture of two substances in the same nostril (physical mixture) in comparison to presenting the same two odorants simultaneously into separate nostrils (dichorhnic mixture) the specific contribution of the interaction at the receptor sites can be estimated. A few such studies have been performed. Laing and Willcox (1987) showed that the suppression of the individual components' qualities that is typically observed as a result of mixing, were generally larger for physical than for dichorhnic mixtures. In line with these results, Cain (1975) reported that individual components were suppressed more in physical mixtures, and that the overall intensity of dichorhnic mixtures tended to be higher than that of physical mixtures. However, Cain concluded that the interaction of physical and dichorhnic mixtures was "similar". Rouby and Holley (1995) investigated temporal aspects of mixture interaction. They presented mixture components with some or no delay between components and found that compared to physical mixtures, dichorhnic mixtures showed, overall, a higher suppression of individual components' qualities. In conclusion, dichorhnic mixtures tend to be more intense than physical, although individual components have been shown to be both more and less suppressed in dichorhnic mixtures.

4 AIM OF THE THESIS

Virtually all perceptions of odors are based on an integration process of many volatile components, in many cases hundreds of components. By investigating how this perceptual integration process is affected by biological functions of the olfactory system, like the effect of receptor antagonism and route of odor mixture presentation, we can begin to understand the nature of odor perception. Why is it of interest to investigate odor integration? From a scientific point of view odor integration is of interest for understanding the very basis of olfactory perception; how the odor quality in a wider sense is formed. An interesting health application concerns the possibility to counteract, by modification, odor pollution in the environment. From a commercial point of view products are constantly developed aiming to improve the flavor of foods, the odor of objects, bodies, and the environment; this is a multibillion industry. The thesis intends to describe and model this perceptual integration process, by investigating how two single odorous components, binary mixtures, integrate into an odor percept. More specifically, the aims are:

1. To investigate the effects of peripheral and central processing of odor mixtures. More specifically, we investigate effect peripheral odor interaction has on our perception of odor mixtures; **a)** by mixing two odorous molecules with a known agonist-/antagonist relationship and **b)** by bypassing the peripheral odor interaction with dichorhnic mixtures (i.e., presenting the two odorants simultaneously, but into separate nostrils).
2. To assess how the odor mixture percept relates to those of its components in a perceptual space.
3. To test whether physical complexity affect the odor percept.
4. To describe and develop a model for predicting the pleasantness or valence of odor mixtures.

5 EMPIRICAL STUDIES

To enhance readability, statistical references and detailed description of the methods used, have been excluded from this brief summary of the empirical studies. The interested reader is referred to the full length articles that appear in the Appendix.

In all of the studies, the participants were healthy and normosmic (a normal sense of smell). Before participation, all participants gave informed consent and were paid for their participation at the end of the experiments.

5.1 STUDY I – PERCEPTUAL EFFECTS OF RECEPTOR ANTAGONISM

5.1.1 Background and aim

In a study by Spehr et al. (2003) a testicular receptor (hOR17-4), which mediates the human sperm chemotaxis, was identified. In addition they identified a strong agonist for this receptor, bourgeonal, and an antagonist, undecanal. In a following study, Spehr et al. (2004) revealed that the receptor type (hOR17-4) was also expressed in the human olfactory mucosa. Despite the likely combinatorial nature of olfactory coding of these substances, a strong inhibitory effect of undecanal on bourgeonal was also present at the perceptual level. More specifically, there was a significant decrease in perceived intensity of bourgeonal (agonist), after presentation of undecanal (antagonist). This inhibitory effect diminished as the concentration of the antagonist was lowered. The authors suggested that this observation reflected competitive receptor inhibition. Potential receptor antagonism between odors is of interest when trying to understand the formation of odor quality of a mixture. The finding of receptor antagonists for the human nose made it possible for us to, for the first time, investigate its effect on odor interaction.

5.1.2 Pilot experiment: *Assessing intensity of single components*

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

5.1.2.1 *Summary of Methods*

Ten participants, five men and five women participated in the study with ages ranging between the ages of 21 and 31 years (Mean [M] = 24.8, Standard deviation [SD] = 3.3). Three odorants were employed in the pilot experiment; n-butanol, undecanal and bourgeonal. The odorants were diluted in propylene glycol into 5 individual concentration levels, with each level estimated to be similar in perceived intensity. The stimuli were presented in polypropylene bottles with pop-up spouts.

The participants rated the intensity of a given stimulus in comparison to the standard stimulus that was set to a value of 100. The stimulus was presented one at the

time every 30 seconds and the participants were asked to sniff each stimulus for two seconds. For every stimulus, the participant estimated the perceived intensity in relation to the standard odor of lemon essential oil.

5.1.2.2 *Main results*

The group mean of rated intensities were calculated for each substance and concentration level. For each odorant the intensity scores were plotted against concentration and logarithmic functions were fitted to the data points. From these functions, one lower and one higher level of intensity were chosen. These two intensity levels of each odorant were used to predict intensity-matched concentrations for all three odorants and were later used as single components of the mixture series in the main experiment.

5.1.3 **Main experiment: Mixtures – Quality and pleasantness**

The experiment aimed to investigate the perception of quality and pleasantness of two types of binary mixtures. Of interest was to see whether the inhibitory effect of undecanal on bourgeonal previously seen in the study by Spehr et al. (2004), also would be present at simultaneous presentation (i.e., mixtures) of the agonist and antagonist. Hence, we tested whether bourgeonal paired with its antagonist undecanal would yield a mixture percept dominated by the odor quality of the antagonist (undecanal). As a control, we investigated the perceptual symmetry of bourgeonal paired with the control odorant n-butanol.

5.1.3.1 *Summary of methods*

Twenty-four individuals participated in the main experiment, 12 men and 12 women between the ages of 21 and 32 ($M = 24.4$, $SD = 2.4$), were divided into two groups balanced by gender. Each group was presented to one of the two types of mixtures.

In the experiment, we used a *substitution* procedure when mixing the odorants. Here, odorants are mixed in relative proportion, that is, when the relative proportion of one odorant increases, the proportion of the other decreases. This typically leads to that the overall intensity for all stimuli are perceived to be isointense.

For the two types of mixtures, bourgeonal/undecanal and bourgeonal/n-butanol, we employed one weaker and one stronger mixture series of 7 stimuli, each ranging from bourgeonal to an isointense undecanal or n-butanol over a 50/50 mixture. For the low and high series, bourgeonal concentrations were identical in both types of mixtures (i.e., bourgeonal/undecanal and bourgeonal/n-butanol). All stimuli were presented a total of five times.

For each stimulus, the participants were first asked to estimate the *overall intensity* of the odorant in relation to a lemon standard (which was set to 100) using the ratio scaling method of magnitude estimation (Baird et al., 1996). Secondly, the participants were asked to estimate the percentage of the components-specific intensity. Thirdly, the participants were asked to rate the perceived pleasantness of the stimuli with the help of a visual analogue scale running from -4 to 4, with zero being neutral.

5.1.3.2 Main results

For each individual and unique stimulus, the ratings of overall intensity, component-specific intensity, and pleasantness were averaged across five presentations. The main results of this experiment indicate that although there is no consistent dominance of the quality of undecanal in the mixtures with bourgeonal across both high and low mixture series. However, it does take a significantly stronger bourgeonal than undecanal to form a balanced, or symmetric, quality at higher concentrations. This supports the hypothesis that agonist/antagonist presentations can yield percepts dominated by the quality of the antagonist.

For the control mixtures of bourgeonal and n-butanol, level independency was indicated by the fact that odor quality, as a function of relative component intensities, was similar when we compared mixture series of high and low concentration.

5.1.4 Conclusion

For the control mixture, indeed odor quality tended to be dominated by the strongest component before mixing as would be suggested from previous studies (Cain et al., 1995; Laing et al., 1984, 1994; Olsson, 1994, 1998). In line with the hypothesis, the bourgeonal-undecanal mixture was dominated by the antagonist's quality, but only when mixed at higher concentrations, altogether suggesting the effects of a low-affinity receptor antagonism. This is the first example of effects of receptor antagonism on the perception of odor mixtures in humans.

5.2 STUDY II – CENTRAL VS. PERIPHERAL PROCESSING OF MIXTURES

5.2.1 Background and aim

Perceptual integration of sensory input from our two nostrils has received little attention in comparison to lateralized inputs for vision and hearing. Since the information received at the receptor level mainly is projected ipsilateral to the side of presentation; it allows us to investigate the difference between peripheral and central processing. By comparing the effects of presenting a mixture of two substances in the same nostril (physical mixture) in comparison to presenting the same two odorants simultaneously into separate nostrils (dichorhnic mixture) the specific contribution of interactions at the receptor site can be evaluated.

With the use of both behavioral and electro-physiological techniques, the current study investigated the effects of peripheral processing by comparing physical and dichorhnic mixtures. Since dichorhnic presentation of mixture components bypasses peripheral interaction, the resulting mixture percept depends on central processes. The aim was threefold: (1) to compare overall intensity for dichorhnic and physical mixtures; (2) to investigate whether such changes of mixture intensity would affect perceived mixture quality, and (3) to test whether the perception of physical and dichorhnic

mixtures was paralleled by according changes in early and late measures of olfactory event-related potentials.

5.2.2 Summary of methods

Twenty-four healthy volunteers, 12 men and 12 women, between the ages of 18 to 35 years participated in the study ($M = 23.9$, $SD = 3.6$). All participants were right-handed and non-smokers. Using the “Sniffin’ Sticks” threshold test (Hummel et al., 1997), all participants were screened for threshold differences between both nostrils separately. Those with differences over two threshold steps between the two nostrils were excluded (Gudziol et al., 2006).

We investigated whether a binary odor mixture of eugenol and l-carvone (smells of cloves and caraway) would be perceived differently if presented as a mixture in one nostril (physical mixture), vs. each of the two odorants presented in to separate nostrils (dichorhnic mixture). In the study there were 4 different types of stimuli, two physical mixtures (left and right side of presentation) and two dichorhnic mixtures (odor A to the right and odor B to the left and vice versa). We controlled for possible trigeminal (sensory irritation) effects of the odorants with a laterality test (Berg et al., 1998; Frasnelli et al., 2011; Hummel et al., 2003; Kobal et al., 1989; Roscher et al., 1996;). The results showed that at the concentrations used in the current study, the substances are believed to primarily stimulate the olfactory system. Trigeminal activation is important to rule out since the processing differences between physical and dichorhnic mixtures may differ between olfactory and trigeminal stimuli (Boyle et al., 2007).

Participants were asked to rate the composition of the stimuli on a visual analogue scale. Hence, they rated to which extent they perceived a single odorant, A or B, or a mixture. The participants also rated the overall intensity of the stimuli on a scale from not noticeable to extremely strong.

In addition, we investigated whether the different types of presentation resulted in differences in olfactory event-related potentials (OERPs). Event-related potentials are signals with high temporal resolution that are caused by activation of cortical neurons that generate electromagnetic fields, measurable with electrodes attached to the scalp.

The participants were seated comfortably in an air-conditioned room and electrodes were placed on their scalp. OERP were recorded at positions Fz, Cz, Pz, C3, and C4 of the international 10-20 System and eye movements were monitored for exclusion purposes. The odorous stimuli were presented with means of a Burghart olfactometer that generates a well-controlled stimulus with a very quick rise time of less than 20 ms, which makes the presentation optimal for recording OERPs. The olfactometer provides a flow of air to the test person, which is constant and non-smelling. The air is humidified up to 60% relative humidity and heated to body temperature, to not cause discomfort or sensory irritation, and to prevent the nasal mucosa to dry out. The olfactometer keeps a constant total airflow to each outlet, minimizing residues of odors in the nose.

5.2.3 Main results

Psychophysical ratings showed that the route of presentation altered the perceived intensity. When presented dichorhinely, mixtures were rated as more intense than a physical mixture presented monorhinely (Dichorhinic mixtures: $M=36.4$; $SD = 18.8$; Physical mixtures: $M=32.4$; $SD = 18.5$; $t(19) = -2.23$, $p = .038$). A tendency for shift in perceived quality was also observed. Both mixtures exhibited a nominal dominance of A (eugenol) over B (l-carvone); however, for physical mixtures this dominance was significant (odor A: $M= 57.1\%$ $SD = 13.9$; one-sample t-test against 50%; $t(19) = 2.26$, $p = .036$), but not for dichorhinic mixtures (odor A: $M= 53.1\%$, $SD = 9.9$; one-sample t-test against 50%, $t(19) = 1.38$, $p =$ not significant (*ns*). The dominance of odor A (i.e., 57.1% vs. 53.1%) tended to be larger for physical mixtures than for dichorhinic mixtures (paired t-test: $t(19) = 1.82$, $p = .084$).

For peak latencies, there was a significant main effect of mixture type on the P1 component and a tendency for the N1 component, such that latencies were shorter for dichorhinic than physical mixtures (P1: $F(1, 20) = 5.56$, $p = .029$; N1: $F(1, 20) = 3.41$, $p = .080$; P2: $F(1, 20) = 1.49$, $p = ns$). For peak amplitudes, we observed a main effect of mixture type on the N1 component, with higher amplitudes for dichorhinic than physical mixtures (P1: $F(1, 20) = .51$, $p = ns$; N1: $F(1, 20) = 11.45$, $p = .003$; P2: $F(1, 20) = .37$, $p = ns$).

5.2.4 Conclusion

The results indicated that dichorhinic mixtures are more intense than physical mixtures. Moreover, the perceived quality had a tendency to shift between mixture types. In parallel with these perceptual changes, the early “sensory” OERP components P1 and N1, but not the later “cognitive” component P2, exhibited shorter latencies and higher N1 amplitudes for dichorhinic mixtures.

There are two interpretations why dichorhinic mixtures yielded higher intensity than physical. First, it can be a general trait of the olfactory system that when distributing the stimulus across two epithelia rather than one, intensity is increased (cf. spatial summation). One previous study does contradict this interpretation (Cain, 1977; see General discussion and Appendix: Study II). Another possible explanation of higher dichorhinic intensity is that the inhibitory interaction at the receptor neurons, such as competition for sites, is bypassed during processing of dichorhinic mixture.

In sum, peripheral interaction between mixture components was analyzed by bypassing the first site of possible interaction, the receptor surface. This approach revealed changes in basic aspects of perception that were corroborated, for the first time, by electrophysiological measurements.

5.3 STUDY III – PREDICTING PLEASANTNESS OF MIXTURES

5.3.1 Background and aim

The perceived quality of odorant mixtures can typically be described in terms of its components' qualities. This means that if an odor A and B are mixed, its quality will exhibit some resemblance to its components in proportion to the relative perceived

intensity of the components. How about the *pleasantness* of odor mixtures? Will a similar averaging procedure be valid also in this case? The literature suggests this is not the rule.

Moreover, pleasantness of an odor is strongly dependent on the context which has been shown in both behavioral and imaging studies. These circumstances make the pleasantness of mixtures hard to predict, but an attempt is made here.

5.3.2 Summary of methods

Twelve participants (5 men, 7 women, 22-41 years) were tested three times on different days, each targeting a specific binary mixture type, AB, AC, and BC. The odorants used in the study were amyl acetate (Odor A; pleasant), n-butanol (Odor B; neutral), and pyridine (Odor C; unpleasant). For each mixture, stimuli were comprised of four different concentrations of each odorant, as well as all possible binary mixtures thereof including a blank, resulting in a total of 25 stimuli. The odors were presented with a computer controlled six-channel constant airflow olfactometer.

In the experiment, we used an *additive* procedure to mix the odorants. Here, odorants are actually added to each other such that we have more odorous material in the stimulus after mixing than before. This typically leads to that the *intensity* of mixtures is stronger than its components. Odor intensity in itself has an intricate relation to pleasantness. That is, the mixture differs both in quality and intensity from its components, both of which affects the mixture pleasantness.

After each presentation, the participants were asked to rate overall intensity, whether they smelled odor A, odor B, or a mixture of A and B. Last but not least they were asked to rate the pleasantness using a scale ranging from -10 to 10, where zero being neutral.

5.3.3 The model

To model the pleasantness of mixtures we did the following: First we analyzed the relation between odor intensity (*I*) and pleasantness (*H*) in *single* odorants. This relationship is well described by a family of 2nd degree polynomial functions indicating that pleasant odors have an optimal intensity for being maximally pleasant whereas unpleasant odors simply get worse with increased intensity. To model the mixture pleasantness, we assumed that the mixture pleasantness would follow the same family of functions as the single components, as the mixture intensity increases.

Second, we assumed that the mixture function should be a weighted average of the functions for the single components along which the predicted mixture pleasantness could be determined from the observed mixture intensity. These weights are defined by the relative dominance of one quality of the other as observed for each mixture (See Appendix; Study III for equations concerning the model). The accuracy of the model suggests that the pleasantness of mixtures of single odorants can be predicted, with highly significant correlations for all three types of mixtures, with explained variance (R^2) ranging from .85 for AB, .97 for AC, and .98 for mixtures of BC.

5.3.4 General conclusions

The aim of Study III was to develop a model for predicting the pleasantness of odor mixtures that also could predict the cases of odor mixture pleasantness when the perceived mixture pleasantness does not merely fall intermediate to the components' pleasantness.

There are five major conclusions to be made from the study; (1) the pleasantness of single odorants is dependent on *intensity* as described by a certain *family* of polynomials, (2) the family of curves suggests that no single odorant's pleasantness level is independent of intensity; nothing smells pleasant at high intensities, (3) basically two factors affect the pleasantness of *mixtures*: when two odorants are blended the *quality* changes, which is due to change in chemical composition and the outcome in pleasantness is also due to the *shift in intensity* that occurs when one odorant is added to another, (4) the pleasantness of mixtures can be predicted along a *quality-weighted* average of these functions, (5) all outcomes are possible ($H_{ab} > H_a, H_b$; $H_{ab} < H_a, H_b$; $H_a < H_{ab} < H_b$). A model relating intensity, quality and pleasantness of odor mixtures was generated and successfully tested.

5.4 STUDY IV – CONCEPTUALIZING ODOR INTEGRATION

5.4.1 Background and aim

We test two assumptions concerning the perceived quality of binary mixtures. The first assumption states that mixture quality is perceived as unitary (synthetically), and the second assumption that the perceived quality of mixtures is a simple function of the component qualities such that mixture quality is intermediate to its components' quality in perceptual space.

There were four aims of this study; **1)** to test whether odor mixture quality is intermediate to the qualities of its component (Experiment 1), **2)** to test whether and how participants could perceive the component-specific qualities in three binary mixtures that we changed gradually from the one to the other component (Experiment 2), **3)** to determine whether participants could track a certain perceived quality, changing in perceptual space, independent of whether the quality corresponded to a single substance or to a mixture (Experiment 3), and **4)** to assess whether odor pleasantness would vary together with odor quality as the concentration ratios of mixture components were changed. In all experiments of this study, a substitution procedure for odor mixtures was utilized; leading to that the overall intensity of the stimuli were kept at isointense levels.

5.4.2 Experiment 1: Intermediacy

We investigated whether odor mixture quality would be intermediate to the qualities of its component. Four odorants were employed in the study: amyl acetate, n-butanol, pyridine, and lemon essential oil (a standard stimulus). The study was comprised of a total of six stimuli: amyl acetate (A), n-butanol (B), pyridine (C), mixture of amyl acetate/n-butanol (X), mixture of n-butanol/pyridine (Y), and lemon essential oil (S). The

stimuli were presented using polypropylene plastic squeeze bottles with pop-up spouts. The concentrations of each odorants corresponded to an equal perceived intensity across all odorants, which had been extracted from psychophysical functions from a pilot study.

The participants rated the similarity between two stimuli presented on a scale between 0 and 100, with 0 described as “identical” and 100 as “maximal difference”. The ratings were subject to multidimensional scaling. The analysis confirms that odorants A, B, and C tend to lie on a straight line in the perceptual space, as intended, where odor B is intermediate to odor A and C. More importantly, the mixtures of A and B (“X”) and of B and C (“Y”) tend to be intermediate to their component qualities. That is, the qualities of the mixtures are not far from the lines connecting their components in perceptual space.

5.4.3 Experiment 2: *Physical mixtures*

In the second experiment, we studied the binary mixtures of the three single odorants used in the previous experiment. The aim was to see if component specific qualities in each mixture could be readily perceived, and if these qualities would be monotonically related to the components’ physical proportion of the mixture.

The odor substances used in the study were, again, amyl acetate (A), n-butanol (B), and pyridine (C). The stimuli were either mixtures of amyl acetate and n-butanol, mixtures of amyl acetate and pyridine, or mixtures of n-butanol and pyridine. In each of the mixture groups the odor mixtures were presented both at high and low concentration levels.

Participants rated the component-specific qualities approximately in proportion to the components’ relative concentration. This was true for both low and high concentration series. This indicates that the odor quality of these three mixture combinations can be expressed in terms of their component’s qualities. Therefore, when mixtures are maximally balanced, both qualities are perceived as equally intense.

5.4.4 Experiment 3: *Conceptual mixtures*

Based on the previous experiment, we know that an olfactory stimulus that is gradually changed from odorant A to odorant B over mixtures of A and B, will yield a gradual change in quality perception from A to B with a maximal probability of reporting both A and B around the middle of this physical continuum. We investigated if it would be possible to teach participants to recognize the mixture AB as being odor “X” and mixture BC as being odor “Y” and then to gradually change odor “X” to “Y” over a single odorant B, and to observe the same response patterns as in the former case. That is, that they would perceive an odor B as a mixture of “odor X” and “odor Y”.

The odors used were again amyl acetate (A), n-butanol (B), and pyridine (C). The results showed that participants were as likely to report both A and B in response to a balanced mixture of A and B, as they were to report both “X” and “Y” in response to stimulus B. Participants could clearly dissociate odor quality from a particular component. These results support the assumption of synthesis.

5.4.5 General conclusion

This study argues for a *synthetic* odor integration *process* that helps us to tag relevant objects with unitary and unique odor percepts, but that also makes it hard for us to track known odor components in mixtures (Jinks & Laing, 1999). We argue that the unitary quality of a mixture is strictly *intermediate* to the qualities of its components, not allowing for any synergistic effects on the odor quality, *per se*. A mixture of two odors can perceptually be described in terms of its components' qualities.

6 GENERAL DISCUSSION

6.1 PERIPHERAL VS. CENTRAL PROCESSING

In Study I and Study II, we focused on the effect that the peripheral processing has on perception (Aim 1). As mentioned in the introduction of this thesis, there are about 350 different types of functional receptors in the human olfactory epithelium (Crasto, 2001; Gaillard *et al.*, 2004; Glusman *et al.*, 2001; Malnic *et al.* 2004; Zozulya *et al.*, 2001). Each one of these receptors can be activated by more than one odorant and an odorant can activate more than one receptor, creating a combinatorial receptor code which distinguishes it from other odorants (Malnic *et al.*, 1999). When we mix two different odorants, the combinatorial receptor codes of each odorant may in some cases, dependent on similarities in molecular structure, overlap, and in other cases they may not.

In Study I, we wanted to test whether this overlap in receptor binding could affect the mixture perception. The odorants, bourgeonal and undecanal, had been identified by Spehr *et al.* (2003) to be an agonist and an antagonist for the same type of receptor (hOR17-4). In addition, they had shown that this relationship caused the perceived intensity of the agonist bourgeonal to be significantly reduced after a presentation of the antagonist undecanal (Spehr *et al.*, 2004). Potential receptor antagonism between odors is of interest when trying to understand the formation of odor quality of a mixture. There was no overall dominance by the quality of the antagonist undecanal over the mixture perception. However, at higher concentration levels, to reach a balanced/symmetric mixture quality a perceptually stronger agonist was demanded. This level dependency of the antagonistic effect can be discussed in terms of affinity and efficacy. The ability of the antagonist to bind to the receptor (affinity) and how well the antagonist blocks the receptor site could be dependent on concentration level; higher affinity and efficacy at higher concentrations.

Despite the complexity of overlapping combinatorial receptor activation, the perception of the mixture was affected by the antagonistic relationship. However, this perceptual effect could have been due to that the two odorants had additional activation of receptors in common. This study can only tell about the perceptual interaction of these two particular odorants, and further test of receptor activation is needed to clarify this.

The results in Study I, indicates that competition for receptor types may affect the perception of odor mixtures. As a complement, in Study II, we tested the effect of peripheral interaction on the perception of odor mixtures by bypassing the receptor level as a site for interaction. The results indicated that dichorhnic mixtures are perceived as more intense than physical mixtures, which is in line with results by Cain (1975). Hence, the measurements of the early “sensory” OERP components corroborated the perceptual measurement.

This higher perceived intensity for dichorhnic mixtures could be hypothetically explained by two different mechanisms. First, due to that the two odorous stimuli were distributed over two separate epithelia in the dichorhnic case, spatial summation may have increased the perceived intensity compared to the physical mixture. However, that spatial summation would yield higher perceived intensity in dichorhnic compared to

physical “mixtures” was not true when an odorant was added to itself (Cain, 1977). The second explanation would be more in line with the results from Study I: That higher dichorhnic intensity is due to that the inhibitory interaction at the receptor neurons assumed for physical mixtures is bypassed during processing of dichorhnic mixture. If the interaction at the receptor sites is bypassed, this would mean that there may also be effects on the perceived quality of the mixture percept. In fact, the perceived quality had a tendency to shift between mixture types, with the dichorhnic mixture perceived to be slightly more balanced in quality than the physical mixture. The reason for this result could be due to an asymmetry in proportions of shared receptor types between these odorants. However, there are studies indicating that interbulbar inhibition (for frogs: Levetau et al., 1993; for rats: Wilson, 1997), is a possible mechanism that could cause shift in odor quality between physical and dichorhnic mixtures. Laing and Willcox (1987) describes that the more one bulb is activated, the more it inhibits the contralateral bulb. However, this would have been illustrated by a shift in quality perception between physical and dichorhnic mixtures what would have been opposite to what the results really indicated.

Laing and Willcox (1987) studied physical and dichorhnic binary mixtures of (+)-limonene, alpha-pinene, and propionic acid. An earlier study had shown that the two former, the hydrocarbons, dominated over the acid (Bell et al., 1987). In the study by Laing and Willcox, participants rated the intensity of single components and of component qualities in the mixture. They indeed replicated the previous finding that the hydrocarbons in mixtures with the acid tended to dominate the odor quality of the mixture qualities. But what would have happened if we would have mixed odorants that interacted non-reciprocally in the periphery? Would then a dichorhnic mixture have smelled more balanced in quality than a physical one? In fact, a reanalysis of Laing and Willcox supports that notion. In Figure 1, we have reanalyzed the effect of mixture type, physical or dichorhnic in a τ / τ' – plot (See equations 3 and 4 in Chapter 3). Here, the dominance of the hydrocarbons over the acid is less noticeable for dichorhnic than physical mixtures, in both cases (Figure 1a and b), whereas the mixtures of the two hydrocarbons (Figure 1c) appear equally balanced between physical and dichorhnic mixtures (as for the odorant pair of Study II, eugenol and l-carvone).

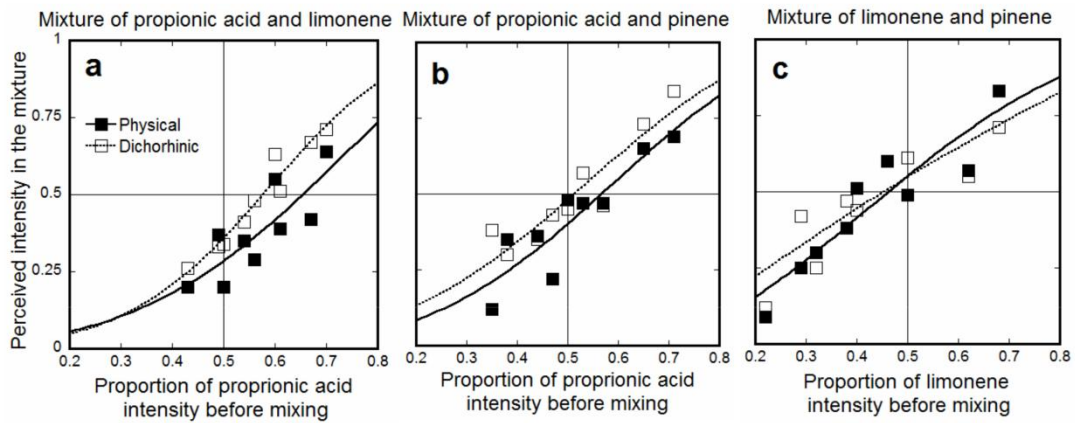


Figure 1. The panels display how relative intensity of components before mixing them relates to the relative intensity of those component qualities after mixing. Data are shown separately for physical and dichorhnic mixtures. In panel a, proportion of propionic acid (P) refers to intensity (I) of that odor in relation to the other component limonene (L), such that $I_P/(I_P+I_L)$. The ordinate denotes the relative perceived intensity of the quality of propionic acid in the mixture (I'_P) such that $I'_P/(I'_P+I'_L)$. The same relations are shown for the mixtures of propionic acid and Pinene (Panel b) and mixtures of Limonene and Pinene (Panel c).

Even if we cannot exclude the possibility of interbulbar inhibition, this inhibition does not seem to surpass the effects of the peripheral interaction. With the addition of the reanalysis of the data from Laing and Willcox (1987), we can further see that by bypassing the interaction of odorants at the epithelium, the mixture percept will be perceived as more balanced between component qualities, as well as more intense. This is in line with that the functionality of the connection between the bulbs in humans is shown to be marginal or possibly non-existent (Gottfried, 2006).

There are interactions at all stages of neuroanatomy, as well as feedback connections from the cortex, that are involved in mixture integration. However, the results of Study I and Study II indicate that the interaction at the receptor level plays a significant role in the perceptual integration of odor mixtures.

Future studies should consider another definition of physical mixtures in the comparison with dichorhnic mixtures (A to one nostril and B to another). Instead of presenting both A and B to one nostril, as a physical mixture, one could present half the concentrations of the previous physical mixture into both nostrils. By doing this not only the total concentration of stimuli, but also the stimulated area of the epithelia would be constant in the comparison between physical and dichorhnic mixtures. This would eliminate spatial summation as an explanation for potential differences between the modes of mixture presentation.

6.2 INTERMEDIACY

Several studies have found that odor mixture quality will for the most cases fall intermediate to the components' qualities in perceptual space (Ekman & Engen, 1962; Dravnieks et al., 1981; Moskowitz & Barbe, 1977; Wise & Cain, 2000). In Experiment 1 of Study IV, the results of a multidimensional analysis of similarity ratings of the single components A, B, and C, and their binary mixtures AB and BC, show that in a two-dimensional representation the component qualities are dispersed approximately on a straight line and, as hypothesized from the assumption of intermediacy, the mixture qualities tended to be intermediate to their respective components.

Several studies have shown that pleasantness of an odor is the foremost dimension when estimating similarities between odorant qualities (Khan et al., 2007; Moskowitz, 1976; Moskowitz & Barbe, 1977; Moskowitz & Gerbers, 1974). Along with these observations, similarity assessments in Study IV (See Appendix, Figure 2) indicated that the pleasantness dimension also appeared here. The odorants were citral (most pleasant), amyl acetate (pleasant), n-butanol (intermediate pleasantness; neutral), and pyridine (unpleasant). In line with previous studies, these pleasantness values map well onto one of the two dimensions of the multidimensional scaling solution.

As previously shown, the mixture quality can be described in terms of its component qualities (e.g., Atanasova et al., 2005; Cain et al., 1995; Laing et al., 1984; Olsson, 1994, 1998). The results here were congruent with these studies. We could show that for all the binary mixtures presented in Study I, Study III, and Study IV, the mixture qualities could be described in terms of the components' qualities. That is, when the stimulus is changed from odor A to odor B over different relative intensity or concentration proportions, the mixture quality also goes from one odor quality to the other. Although it may not be a strong argument for intermediacy, *per se*, it is a prerequisite for intermediacy to hold true.

It is not only the mixture quality that tends to be intermediate to that of its components; the perceived pleasantness of odor mixtures is typically intermediate to the components individual pleasantness. In Study I and Study IV (in which isointense mixture series was used), we can see that when mixing two odorants, the pleasantness of the mixtures will be intermediate to the single components' pleasantness values. In fact, as seen in Study IV, there is a highly significant correlation between pleasantness and quality, i.e., when quality changes from one odor to another the pleasantness follows (see Appendix; Study IV: Figure 7).

Blending mixtures have been used as an argument against intermediacy (Barkat et al., 2012; Le Berre et al., 2008, 2010). Their criterion for blending is that an imagined or actual target odor can be mimicked better by a mixture than by any of its components alone. For example, they showed that a three-component mixture of specific proportions of allyl-a-ionone (violet-like odor), ethyl isobutyrate (strawberry-like odor), and ethyl maltol (caramel-like odor) was judged more typical of a pineapple odor (target) than were any of the individual components alone. In our view the odor integration follows the same principles independently of whether the resultant mixture percept would match an odor or not. In other words, their observations are not in conflict with the assumption of intermediacy. In Figure 2 we depict why this is so.

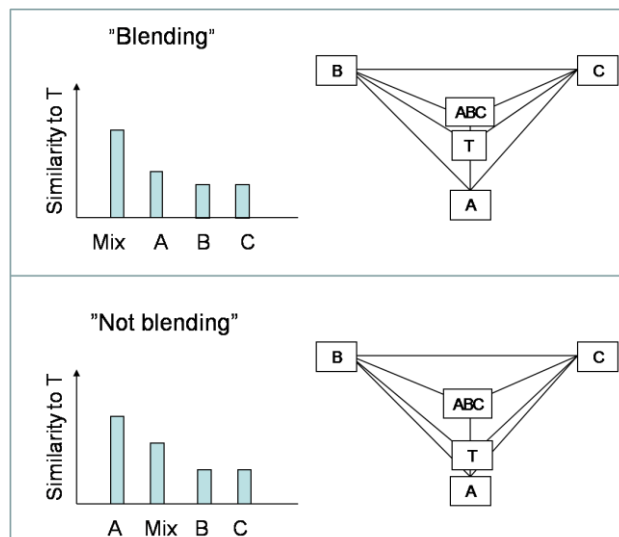


Figure 2. The figure depicts the difference between the mixture phenomena blending (upper) and not blending (lower) under the assumption of intermediacy. For each phenomenon, the left graph depicts the similarity judgments of three odorants (A-C) and their ternary mixture (ABC) to a target odor (T). The schematic to the right depicts how the odorants and their mixture would relate in a two-dimensional odor space according to the rule of intermediacy. That is, the mixture is thought to be intermediate to their component qualities. Only the position of the target varies between the two cases. In the blending case, the target odor closely matches the odor mixture. The target is better mimicked by the mixture than any of the single components. When blending is not the case, the mixture is not more similar to the target than any of the components. Although the cases differ in whether they meet or not meet the definition of blending, the underlying principle of odor integration remains the same.

The ecological validity of intermediacy can be illustrated in that it makes it possible for us to orient towards a target odor, not unlike that of sperm chemotaxis shown by Spehr et al. (2003). This orientation is directed by the means of similarity judgments between a preferred target odor, or to an odor of aversive properties that should be avoided, and the current odor. With a pronounced deviation of intermediacy, the useful information provided by a gradient in odor concentration, would then be obscured from the smeller.

In conclusion, there are both empirical and conceptual arguments for that quality of a mixture falls intermediate to the quality of the components. This does not exclude the possibility of top-down processes that could add to our perception of mixture intermediacy, for example the experience of blending. Indeed, such pop-out experiences and their basis in top-down processing should be further studied.

6.3 SYNTHESIS OF ODOR MIXTURES

As noted, odors that we encounter daily are mixtures of often hundreds of odorous molecules. Although of this physical complexity when it comes to the complex mixtures, we tend to perceive these odor mixtures as unitary odors, as homogeneous percepts.

When testing participants to recognize an odor as odor A and another as odor B, and asking them to rate how much of the individual odors they feel in a mixture, they are lead to judge the odor in a more analytical way. In this way we ask them to analyze the quality of the mixture and therefore they are trying to separate the qualities in the mixture, which could be assumed to lead to a more heterogeneous perception. However, the ability to analyze the ratios of component quality could also just represent a capability to estimate the ratios of similarities to the single components. Like in the case of orange, a mixture of red and yellow light, in itself a homogeneous perception, we are still able to estimate the similarities to its components, red and yellow, without perceiving these components in the mixture.

Livermore and Laing (1998b) argued that complex odors where processed as single entities, similar to single components. Our poor ability to identify components in a mixture shown in a number of studies (Laing & Francis, 1989; Livermore & Laing, 1996, 1998a, 1998b) can be the effect of a synthetic integration process. If complex mixtures, like in our natural environment, are processed in a similar way of single components, that instead of mixing substances, we are mixing percepts. In Experiment 3 of Study IV we investigated whether participants would track a certain perceived quality changing in a perceptual space independent of whether the quality corresponded to a single substance or to a mixture. We “mixed” two binary mixtures, by merging odor mixture X (AB) with mixture Y (BC), over the single component B, which both mixtures had in common. The results indicated that the quality X and Y of the mixtures evoked an unitary odor percept that could be tracked as the one mixture was gradually changed to be the other mixture via a single shared component positioned in between the two mixtures in the perceptual space (See Appendix; Study IV: Figure 2 and 4). These results support the assumption of synthesis.

When presented to the receptor epithelia, single odorants will activate a combination of receptors, as presented by Malnic et al. (2004). However, so will mixtures of odorants. This is in line with that that the olfactory system cannot easily tease apart whether it smells a single component or a mixture. Moreover, studies of the piriform cortex indicate that mixture-specific neurons are activated that either component separately does not activate. This is a possible neural substrate for the new quality that is the result of the synthetic processing of mixtures (Kadohisa and Wilson, 2006; Wilson and Sullivan, 2011). In conclusion, the current results indicate that a mixture is readily perceived as a unitary percept. This is consistent with earlier results (Livermore & Laing, 1998b), and concurs well with the perception of complex object odors in our natural environment. In addition, it aligns with what we know about receptor coding and how the qualities of mixtures are represented in the brain.

6.4 PLEASANTNESS OF ODOR MIXTURES

When keeping the overall perceived intensity constant for a mixture series, we can see that pleasantness strongly correlates with the shift in quality (see Appendix Study IV; Figure 7). Hence, a mixture of the more pleasant odorant amyl acetate (Odor A; smells of banana) with the unpleasant odorant pyridine (Odor C; smells like spoiled milk: Wysocki & Beauchamp, 1984) at isointense levels, we notice that as the quality changes from A (pleasant) to C (unpleasant), so does the pleasantness. Therefore, as we can see in both Study I and Study IV, when keeping the overall intensity constant we can see that the pleasantness tend to fall intermediate to the components individually perceived pleasantness.

However, it is not often in our natural environment that mixtures of odors are mixed by this sort of substitution technique, i.e., by changing the relative proportion of odorous compounds to keep the overall intensity more or less constant. Instead, naturally occurring odors are more commonly mixed additively and thus, creating a more intense overall mixture than the components in isolation, as we can see in Study III. The pleasantness of odors is highly dependent on odor intensity (Doty, 1975; Henion, 1971). Therefore, it is of great importance to take this increase in intensity into account when trying to predict the pleasantness of an odor mixture.

As shown in Study III, odor pleasantness is greatly affected by an increase in odor intensity. The psychophysical functions relating odor intensity to odor pleasantness can be fitted with 2nd degree polynomials (see Figure 3; Appendix Study III: Figure 2a). The weights of the 2nd degree polynomial functions of the single components in Study III are highly correlated ($R = .99$) and therefore assumed to belong to the same family. The fit with 2nd degree polynomial functions for the intensity-pleasantness relationship has previously been indicated by Lawless (1977). That is, an unpleasant odor in isolation will only get more unpleasant as the intensity increases. On the other hand, a pleasant odor will initially be more pleasant with an increase in intensity. However, at some point this increase in intensity will cause the odor to be less pleasant, and at very high intensities most odors are unpleasant. As can be seen in Figure 3, also pleasantness data extracted from Doty (1975) of 10 odorants can be well fitted (all $R > .95$) with 2nd degree polynomial functions altogether supporting the conclusion on the intensity-pleasantness relation.

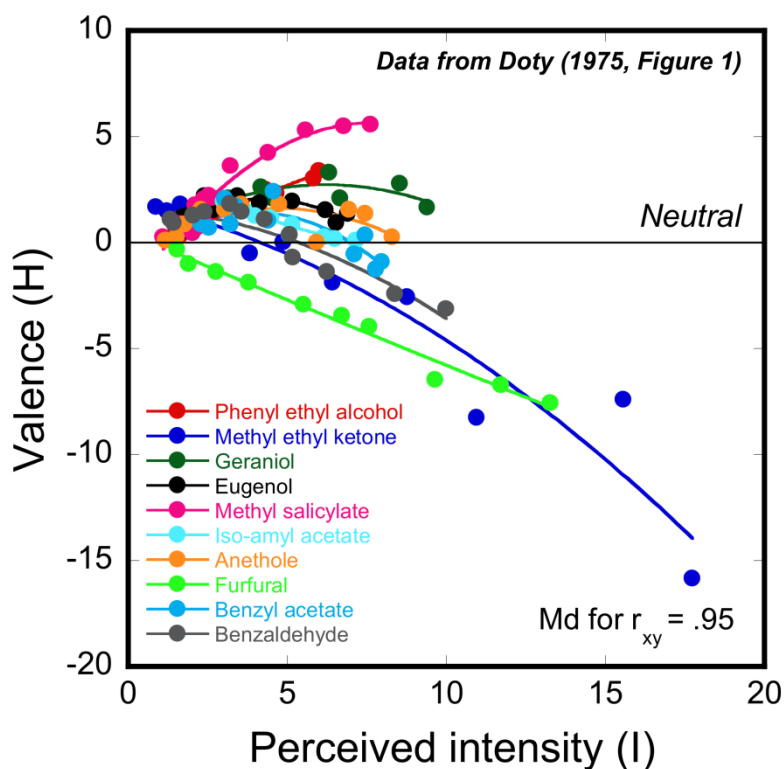


Figure 3. Pleasantness (valence) of 10 odorants plotted against their individual perceived intensity. The data are fitted with 2nd degree polynomials. Median (Md) of correlations (r_{xy}) is given. Data extracted from Doty (1975).

The pleasantness of odors, whether they are single components or mixtures, is clearly dependent on the context they are presented in. Visual and verbal labels have shown to clearly impact the perceived pleasantness of an odor (Case et al. 2006, de Araujo et al. 2005; Djordjevic et al., 2008; Herz 2003, Herz & von Clef 2001), as well as aspects of learning (e.g. Stevenson et al., 2010). In Study III of this thesis, we can see another effect of context on odor pleasantness. This contextual effect is caused by the other odor presented in the same experiment (cf., Poulton, 1989; Rankin & Marks, 2000). Depending of the pleasantness of the second odor presented in the pair, we can see that the perceptual ratings are shifted. When the pleasant smelling amyl acetate is presented together with the very unpleasant pyridine, amyl acetate is perceived as more pleasant than when presented to the more intermediate in pleasantness, n-butanol. These contextual effects on perceived pleasantness may seem to make it quite difficult to predict the pleasantness of an odor mixture. However, within a certain context of any two odorants, the model proposed is not sensitive to the context effect, simply because the model parameters are extracted in the context of the other odor.

In addition to the effect that intensity has on the perception, there is a second factor that affects the pleasantness of a mixture: the change in chemical composition when an odorants is added to another quite naturally calls for a change in *quality*.

As seen in both Study I and Study IV, the components' relative intensities before mixing will highly affect the perceived quality of the mixture. An odorant with a higher

perceived intensity than the other odorant before mixing will most likely dominate the quality of the mixture (Cain et al., 1995; Laing et al. 1984, 1994; Olsson 1994, 1998). For these reasons, it is of great importance when predicting pleasantness of odor mixtures, to take into account both the overall *mixture intensity* and the *relative intensity* of the components.

Aim 3 of this thesis was to develop a model for predicting the pleasantness of odor mixtures that could predict also the cases of odor mixture pleasantness when the perceived mixture pleasantness do not merely fall intermediate to the components' pleasantness (as all previous models are restricted to). The model accurately predicts the pleasantness of an odor mixture, with high correlations for all three types of mixtures. It also copes well with the context effects shown here.

Future studies should focus on testing the model under the influence other top-down effects such as those invoked by odor labels which have shown to have a striking effect on odor pleasantness. Moreover, the model should be generalized to other odorants. In particular, it would be interesting to test the model for integration of complex odors. That is, mixing odor percepts that are themselves complex mixtures.

6.5 CONCLUDING REMARKS

This thesis on perceptual integration of odor mixtures has aimed to develop a better understanding of the physiological basis of odor mixture interaction. Investigating the effect of peripheral interaction on the mixture perception has given us a better understanding of the importance of this first interaction level of the olfactory system. Hence, bypassing the level of receptor interaction will generally lead to a more balanced mixture percept, indicating that receptor interaction has a major influence on the perception. However, further research is needed to conclusively be able to say that an agonist-/antagonist relationship will have predictable effects on the odor mixture percept.

In addition, the thesis has provided support for some basic assumptions of odor mixture perception: *intermediacy* and *synthesis*. These principles are at the core of understanding the making of odor quality.

Three main aspects of the odor percept: intensity, quality, and pleasantness, need to be taken into account in order to understand the formation of odor quality. The current thesis has described the complex interplay of these fundamental dimensions by modeling how these aspects integrate to form complex object odors in our environment.

7 ACKNOWLEDGEMENTS

My doctoral studies started at the Department of Psychology at Uppsala University and continued at the Division for Psychology at Department of Clinical Neuroscience at Karolinska Institutet. A large part of my thesis work has been done in Dresden, Germany where I got the privilege to be included in the work at the Smell & Taste Clinic at the Department of Otorhinolaryngology at TU Dresden.

Foremost, I would like to express my sincere gratitude to my advisor Professor Mats J Olsson for his support and guidance during my doctoral studies. I really appreciate his patience, enthusiasm, and knowledge.

I would also like to thank my co-advisor Johan Lundström for his valuable advice in scientific writing and coping with the stress of a doctoral thesis.

I would also like to thank Professor Thomas Hummel, for his generous support and for making me feel welcomed as part of his lab group in Dresden.

My thanks also go to all my research colleagues and administrative staffs at the departments in Uppsala, Solna, and Dresden, as well as to other research colleagues within the field of Olfaction, for their support.

Special thanks go to my dear friend Jessika Karlsson, who has been a great motivational and emotional support during my doctoral studies and in life. “More intensity!!!”

I would also like to thank my parents, my sister Karolina, and my brother Johan, for their never ending support and love, to my brother-in-law Stefano, for his contagious passion for research, and to Elke and René, and Inge and Günther, for welcoming me into your family.

Last but not the least, I would like to thank my lovely daughter Elsa, for making me smile and my husband Alex, whom I never would have met if not for the experiments I carried out in Dresden, for his love.

Malin Schütze, Dresden 24th of January 2014

8 REFERENCES

- Alaoui-Ismaili, O., Robin, O., Rada, H., Dittmar, A., & Vernet-Maury, E. (1997). Basic emotions evoked by odorants: comparison between autonomic responses and self-evaluation. *Physiology & Behavior*, 62, 713–20.
- Allison, A. C. (1954). The secondary olfactory areas in the human brain. *Journal of anatomy*, 88(4), 481–488.
- Anderson, A. K., Christoff, K., Stappen, I., Panitz, D., Ghahremani, D. G., Glover, G., ... & Sobel, N. (2003). Dissociated neural representations of intensity and valence in human olfaction. *Nature neuroscience*, 6(2), 196-202.
- Araneda, R. C., Kini, A. D., & Firestein, S. (2000). The molecular receptive range of an odorant receptor. *Nature neuroscience*, 3(12), 1248-1255.
- Araneda, R. C., Peterlin, Z., Zhang, X., Chesler, A., & Firestein, S. (2004). A pharmacological profile of the aldehyde receptor repertoire in rat olfactory epithelium. *The Journal of physiology*, 555(3), 743-756.
- Atanasova, B., Thomas-Danguin, T., Chabanet, C., Langlois, D., Nicklaus, S., & Etiévant, P. (2005). Perceptual interactions in odour mixtures: odour quality in binary mixtures of woody and fruity wine odorants. *Chemical senses*, 30(3), 209-217.
- Ayabe-Kanamura, S., Schicker, I., Laska, M., Hudson, R., Distel, H., Kobayakawa, T., & Saito, S. (1998). Differences in perception of everyday odors: a Japanese-German cross-cultural study. *Chemical senses*, 23(1), 31-38.
- Baird, J. C., Berglund, B., & Olsson, M. J. (1996). Magnitude estimation of perceived odor intensity: Empirical and theoretical properties. *Journal of Experimental Psychology: Human Perception and Performance*, 22(1), 244-255.
- Barkat, S., Le Berre, E., Coureaud, G., Sicard, G., & Thomas-Danguin, T. (2012). Perceptual blending in odor mixtures depends on the nature of odorants and human olfactory expertise. *Chemical senses*, 37(2), 159-166.
- Bell, G. A., Laing, D. G., & Panhuber, H. (1987). Odour mixture suppression: evidence for a peripheral mechanism in human and rat. *Brain research*, 426(1), 8-18.
- Bensaïfi, M., Rouby, C., Farget, V., Vigouroux, M., & Holley, A. (2002). Asymmetry of pleasant vs. unpleasant odor processing during affective judgment in humans. *Neuroscience letters*, 328(3), 309-313.
- Bensaïfi, M., Iannilli, E., Poncelet, J., Seo, H. S., Gerber, J., Rouby, C., & Hummel, T. (2012). Dissociated representations of pleasant and unpleasant olfacto-trigeminal mixtures: an fMRI study. *PloS one*, 7(6), e38358.
- Berglund, B., Berglund, U., Engen, T., & Ekman, G. (1973). Multidimensional analysis of twenty-one odors. *Scandinavian journal of psychology*, 14(1), 131-137.
- Berglund, B., & Olsson, M. J. (1993a). Odor-Intensity Interaction in Binary Mixtures. *Journal of Experimental Psychology: Human Perception and Performance*, 19(2), 302-314.
- Berglund, B., & Olsson, M. J. (1993b). Odor-intensity interaction in binary and ternary mixtures. *Perception and Psychophysics*, 53(5), 475-482.
- Berglund, B., & Olsson, M. (1993c). Perceptual and Psychophysical Models for Odor Intensity Interaction. In A. Garriga-Trillo, P. R. Minon, C. Garcia-Gallego, P. Lubin, J. M. Merino, & A. Villarino (Eds.), *Fechner Day '93* (pp. 36-40). Palma de Mallorca, Spain: International Society for Psychophysics.

- Boyle, J. A., Frasnelli, J., Gerber, J., Heinke, M., & Hummel, T. (2007). Cross-modal integration of intranasal stimuli: a functional magnetic resonance imaging study. *Neuroscience*, 149(1), 223-231.
- Boyle, J. A., Djordjevic, J., Olsson, M. J., Lundström, J. N., & Jones-Gotman, M. (2009). The human brain distinguishes between single odorants and binary mixtures. *Cerebral Cortex*, 19(1), 66-71.
- Buck, L., & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*, 65(1), 175-187.
- Cain, W. S. (1974). Contribution of the trigeminal nerve to perceived odor magnitude. *Annals of the New York Academy of Sciences*, 237(1), 28-34.
- Cain, W. S. (1975). Odor intensity: mixtures and masking. *Chemical Senses and Flavor*, 1(3), 339-352.
- Cain, W. S. (1977). Bilateral integration in olfaction. *Nature*, 268, 51-53.
- Cain, W. S. (1979). To know with the nose: Keys to odor identification. *Science*, 203, 467-470.
- Cain, W. S., & Johnson Jr, F. (1978). Lability of odor pleasantness: influence of mere exposure. *Perception*, 7(4), 459-465.
- Cain, W. S., Schiet, F. T., Olsson, M. J., & de Wijk, R. A. (1995). Comparison of models of odor interaction. *Chemical senses*, 20(6), 625-637.
- Carmichael, S. T., Clugnet, M. C., & Price, J. L. (1994). Central olfactory connections in the macaque monkey. *Journal of Comparative Neurology*, 346(3), 403-434.
- Case, T. I., Repacholi, B. M., & Stevenson, R. J. (2006). My baby doesn't smell as bad as yours: The plasticity of disgust. *Evolution and Human Behavior*, 27(5), 357-365.
- Chaput, M. A., El Mountassir, F., Atanasova, B., Thomas-Danguin, T., Le Bon, A. M., Perrut, A., ... & Duchamp-Viret, P. (2012). Interactions of odorants with olfactory receptors and receptor neurons match the perceptual dynamics observed for woody and fruity odorant mixtures. *European Journal of Neuroscience*, 35(4), 584-597.
- Chen, V., & Halpern, B. P. (2008). Retronasal but not oral-cavity-only identification of "purely olfactory" odorants. *Chemical senses*, 33(2), 107-118.
- Cleland, T. A., Johnson, B. A., Leon, M., & Linster, C. (2007). Relational representation in the olfactory system. *Proceedings of the National Academy of Sciences*, 104(6), 1953-1958.
- Crasto, C., Singer, M. S., & Shepherd, G. M. (2001). The olfactory receptor family album. *Genome Biology*, 2(10), 1027:1-1027:4.
- de Araujo, I. E., Rolls, E. T., Velazco, M. I., Margot, C., & Cayeux, I. (2005). Cognitive modulation of olfactory processing. *Neuron*, 46(4), 671-679.
- Delplanque, S., Grandjean, D., Chrea, C., Aymard, L., Cayeux, I., Le Calve, B., ... & Sander, D. (2008). Emotional processing of odors: evidence for a nonlinear relation between pleasantness and familiarity evaluations. *Chemical Senses*, 33(5), 469-479.
- de Olmos, J., Hardy, H., & Heimer, L. (1978). The afferent connections of the main and the accessory olfactory bulb formations in the rat: An experimental HRP-study. *Journal of Comparative Neurology*, 181(2), 213-244.
- Devos, M., Patte, F., Rouault, J., Laffort, P., & Van Gemert, L. J. (1990). *Standardized human olfactory thresholds*. Oxford, USA: Oxford University Press.
- de Wijk, R. A., & Cain, W. S. (1994a). Odor Identification by Name and by Edibility: Life-Span Development and Safety. *Human Factors: The Journal of the Human Factors and Ergonomics Society*, 36(1), 182-187.

- de Wijk, R. A., & Cain, W. S. (1994b). Odor quality: discrimination versus free and cued identification. *Perception & psychophysics*, 56(1), 12-18.
- de Wijk, R. A., Schab, F. R., & Cain, W. S. (1995). Odor identification. In F. R. Schab & R. G. Crowder (Eds.), *Memory for odors* (pp. 21-37). Mahwah, NJ: Erlbaum.
- Distel, H., Ayabe-Kanamura, S., Martínez-Gómez, M., Schicker, I., Kobayakawa, T., Saito, S., & Hudson, R. (1999). Perception of everyday odors - correlation between intensity, familiarity and strength of hedonic judgement. *Chemical Senses*, 24(2), 191-199.
- Distel, H., & Hudson, R. (2001). Judgement of odor intensity is influenced by subjects' knowledge of the odor source. *Chemical Senses*, 26(3), 247-251.
- Djordjevic, J., Lundstrom, J. N., Clément, F., Boyle, J. A., Pouliot, S., & Jones-Gotman, M. (2008). A rose by any other name: would it smell as sweet?. *Journal of neurophysiology*, 99(1), 386-393.
- Doty, R. L. (1975). An examination of relationships between the pleasantness, intensity, and concentration of 10 odorous stimuli. *Perception & Psychophysics*, 17(5), 492-496.
- Doty, R. L., Bromley, S. M., Moberg, P. J., & Hummel, T. (1997). Laterality in Human Nasal Chemoreception. In S. Christman (Ed.), *Advances in Psychology: Cerebral Asymmetries in Sensory and Perceptual Processing*, (pp. 497-542). Amsterdam: North Holland Publishing Co.
- Doty, R. L., Bruggger, W. E., Jurs, P. C., Orndorff, M. A., Snyder, P. J., & Lowry, L. D. (1978). Intranasal trigeminal stimulation from odorous volatiles: psychometric responses from anosmic and normal humans. *Physiology & Behavior*, 20(2), 175-185.
- Doty, R. L. & Laing, D. G. (2003). Psychophysical measurement of human olfactory function including odorant mixture assessment. In R. L. Doty (Ed.), *Handbook of olfaction and gustation* (pp. 203-228). New York: Marcel Dekker.
- Duchamp-Viret, P., Duchamp, A., & Chaput, M. A. (2003). Single olfactory sensory neurons simultaneously integrate the components of an odour mixture. *European Journal of Neuroscience*, 18(10), 2690-2696.
- Duchamp-Viret, P., Chaput, M. A., & Duchamp, A. (1999). Odor response properties of rat olfactory receptor neurons. *Science*, 284(5423), 2171-2174.
- Dravnieks, A., Bock, F. C., & Jarke, F. H. (1981). Sensory structure of odor mixtures. In H. R. Moskowitz & C. B. Warren (Eds.), *Odor Quality and Chemical Structure* (pp. 79-92). Washington DC: ACS Symposium Series.
- Ekman, G., & Engen, T. (1962). *Multidimensional ratio scaling and multidimensional similarity in olfactory perception*. University of Stockholm, the Psychological Laboratory.
- Engen, T. (1974). The potential usefulness of sensation of odor and taste in keeping children away from harmful substances. *Annals of the New York Academy of Sciences*, 237(1), 224-228.
- Ferreira, V. (2012a). Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 1: intensity and detectability. A review. *Flavour and Fragrance Journal*, 27(2), 124-140.
- Ferreira, V. (2012b). Revisiting psychophysical work on the quantitative and qualitative odour properties of simple odour mixtures: a flavour chemistry view. Part 2: qualitative aspects. A review. *Flavour and Fragrance Journal*, 27(3), 201-215.
- Frasnelli, J., Hummel, T., Berg, J., Huang, G., & Doty, R. L. (2011). Intranasal localizability of odorants: influence of stimulus volume. *Chemical senses*, 36(4), 405-410.
- Frijters, J. E. R., & Oude Ophuis, P. A. M. (1983). The construction and prediction of psychophysical power functions for the sweetness of equiratio sugar mixtures. *Perception*, 12(6), 753-767.

- Gaillard, I., Rouquier, S., & Giorgi, D. (2004). Olfactory receptors. *Cellular and Molecular Life Sciences CMLS*, 61(4), 456-469.
- Getchell, T. V., Heck, G. L., DeSimone, J. A., & Price, S. (1980). The location of olfactory receptor sites. Inferences from latency measurements. *Biophysical journal*, 29(3), 397-411.
- Glusman, G., Yanai, I., Rubin, I., & Lancet, D. (2001). The complete human olfactory subgenome. *Genome research*, 11(5), 685-702.
- Gottfried, J. (2006). Smell: Central Nervous Processing. In T. Hummel & A. Welge-Lüssen (Eds.), *Advanced Otorhinolaryngology: Taste and Smell. An Update*. (pp. 44–69). Basel: Karger.
- Gottfried, J. A., Smith, A. P., Rugg, M. D., & Dolan, R. J. (2004). Remembrance of odors past: human olfactory cortex in cross-modal recognition memory. *Neuron*, 42(4), 687-695.
- Gottfried, J. A., Winston, J. S., & Dolan, R. J. (2006). Dissociable codes of odor quality and odorant structure in human piriform cortex. *Neuron*, 49(3), 467-479.
- Gottfried, J. A., Deichmann, R., Winston, J. S., & Dolan, R. J. (2002). Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. *The Journal of Neuroscience*, 22(24), 10819-10828.
- Grabenhorst, F., Rolls, E. T., Margot, C., da Silva, M. A., & Velazco, M. I. (2007). How pleasant and unpleasant stimuli combine in different brain regions: odor mixtures. *The Journal of Neuroscience*, 27(49), 13532-13540.
- Gudziol, V., Lötsch, J., Hähner, A., Zahnert, T., & Hummel, T. (2006). Clinical significance of results from olfactory testing. *The Laryngoscope*, 116(10), 1858-1863.
- Haberly, L. B. (2001). Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. *Chemical senses*, 26(5), 551-576.
- Hallam, E. A., & Carlson, J. R. (2006). Coding of odors by a receptor repertoire. *Cell*, 125(1), 143-160.
- Henion, K. E. (1971). Odor pleasantness and intensity: a single dimension?. *Journal of experimental psychology*, 90(2), 275-279.
- Herz, R. S. (2003). The effect of verbal context on olfactory perception. *Journal of Experimental Psychology: General*, 132(4), 595-606.
- Herz, R. S., & von Clef, J. (2001). The influence of verbal labeling on the perception of odors: Evidence for olfactory illusions?. *Perception*, 30(3), 381-392.
- Homewood, J., & Stevenson, R. J. (2001). Differences in naming accuracy of odors presented to the left and right nostrils. *Biological psychology*, 58(1), 65-73.
- Howard, J. D., Plailly, J., Grueschow, M., Haynes, J. D., & Gottfried, J. A. (2009). Odor quality coding and categorization in human posterior piriform cortex. *Nature neuroscience*, 12(7), 932-938.
- Hudry, J., Perrin, F., Rylvlin, P., Mauguière, F., & Royet, J. P. (2003). Olfactory short-term memory and related amygdala recordings in patients with temporal lobe epilepsy. *Brain*, 126(8), 1851-1863.
- Hudson, R. (1999). From molecule to mind: the role of experience in shaping olfactory function. *Journal of Comparative Physiology A*, 185(4), 297-304.
- Hummel, T., & Livermore, A. (2002). Intranasal chemosensory function of the trigeminal nerve and aspects of its relation to olfaction. *International archives of occupational and environmental health*, 75(5), 305-313.

- Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., & Kobal, G. (1997). 'Sniffin'Sticks': Olfactory Performance Assessed by the Combined Testing of Odor Identification, Odor Discrimination and Olfactory Threshold. *Chemical senses*, 22(1), 39-52.
- Jellinek, J. S., & Köster, E. P. (1983). Perceived fragrance complexity and its relationship to familiarity and pleasantness II. *Journal of the Society of Cosmetic Chemists*, 34, 83-97.
- Jinks, A., & Laing, D.G. (1999). A limit in the processing of components in odour mixtures. *Perception*, 28, 395-404.
- Jinks, A., & Laing, D. G. (2001). The analysis of odor mixtures by humans: evidence for a configurational process. *Physiology & behavior*, 72(1), 51-63.
- Jones, F. N., & Woskow, M. H. (1964). On the intensity of odor mixtures. *Annals of the New York Academy of Sciences*, 116(2), 484-494.
- Kadohisa, M., & Wilson, D. A. (2006). Separate encoding of identity and similarity of complex familiar odors in piriform cortex. *Proceedings of the National Academy of Sciences*, 103(41), 15206-15211.
- Keller, A., & Vosshall, L. B. (2004). Human Olfactory Psychophysics. *Current Biology*, 14(20), R875-R878.
- Khan, R. M., Luk, C. H., Flinker, A., Aggarwal, A., Lapid, H., Haddad, R., & Sobel, N. (2007). Predicting odor pleasantness from odorant structure: pleasantness as a reflection of the physical world. *The Journal of Neuroscience*, 27(37), 10015-10023.
- Kobal, G. (1985). Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. *Pain*, 22(2), 151-163.
- Kobal, G., Hummel, T., & Van Toller, S. (1992). Differences in human chemosensory evoked potentials to olfactory and somatosensory chemical stimuli presented to left and right nostrils. *Chemical Senses*, 17(3), 233-244.
- Kobal, G., Van Toller, S., & Hummel, T. (1989). Is there directional smelling?. *Experientia*, 45(2), 130-132.
- Kurahashi, T., Lowe, G., & Gold, G. H. (1994). Suppression of odorant responses by odorants in olfactory receptor cells. *Science*, 265(5168), 118-120.
- Laing, D. G. (1987). Coding of chemosensory stimulus mixtures. *Annals of the New York Academy of Sciences*, 510(1), 61-66.
- Laing, D. G., & Francis, G. W. (1989). The capacity of humans to identify odors in mixtures. *Physiology & Behavior*, 46(5), 809-814.
- Laing, D. G., Panhuber, H., Willcox, M. E., & Pittman, E. A. (1984). Quality and intensity of binary odor mixtures. *Physiology and Behavior*, 33, 309-319.
- Laing, D. G., & Willcox, M. E. (1987). An investigation of the mechanisms of odor suppression using physical and dichorhnic mixtures. *Behavioural brain research*, 26(2), 79-87.
- Laing, D. G., Eddy, A., & John Best, D. (1994). Perceptual characteristics of binary, trinary, and quaternary odor mixtures consisting of unpleasant constituents. *Physiology & behavior*, 56(1), 81-93.
- Lapid, H., Harel, D., & Sobel, N. (2008). Prediction models for the pleasantness of binary mixtures in olfaction. *Chemical senses*, 33(7), 599-609.
- Lascano, A. M., Hummel, T., Lacroix, J. S., Landis, B. N., & Michel, C. M. (2010). Spatio-temporal dynamics of olfactory processing in the human brain: an event-related source imaging study. *Neuroscience*, 167(3), 700-708.
- Laska, M. (2005). Olfactory discrimination ability for aliphatic C6 alcohols as a function of presence, position, and configuration of a double bond. *Chemical senses*, 30(9), 755-760.

- Laska, M., & Freyer, D. (1997). Olfactory discrimination ability for aliphatic esters in squirrel monkeys and humans. *Chemical senses*, 22(4), 457-465.
- Laska, M., & Grimm, N. (2003). SURE, why not? The SUBstitution-REciprocity method for measurement of odor quality discrimination thresholds: replication and extension to nonhuman primates. *Chemical Senses* 28, 105-111.
- Laska, M., & Teubner, P. (1999). Olfactory discrimination ability of human subjects for ten pairs of enantiomers. *Chemical senses*, 24(2), 161-170.
- Lawless, H. T. (1977). The pleasantness of mixtures in taste and olfaction. *Sensory Processes*, 1, 227-237.
- Lawless, H., & Engen, T. (1977). Associations to odors: interference, mnemonics, and verbal labeling. *Journal of Experimental Psychology: Human Learning and Memory*, 3(1), 52-59.
- Le Berre, E., Thomas-Danguin, T., Béno, N., Coureaud, G., Etiévant, P., & Prescott, J. (2008a). Perceptual processing strategy and exposure influence the perception of odor mixtures. *Chemical senses*, 33(2), 193-199.
- Le Berre, E., Beno, N., Ishii, A., Chabanet, C., Etievant, P., & Thomas-Danguin, T. (2008b). Just noticeable differences in component concentrations modify the odor quality of a blending mixture. *Chemical senses*, 33(4), 389-395.
- Le Berre, E., Jarmuzek, E., Béno, N., Etiévant, P., Prescott, J., & Thomas-Danguin, T. (2010). Learning influences the perception of odor mixtures. *Chemosensory Perception*, 3(3-4), 156-166.
- Leon, M., & Johnson, B. A. (2003). Olfactory coding in the mammalian olfactory bulb. *Brain research reviews*, 42(1), 23-32.
- Leveteau, J., Andriason, I., & MacLeod, P. (1993). Interbulbar reciprocal inhibition in frog olfaction. *Behavioural brain research*, 54(1), 103-106.
- Li, W., Howard, J. D., Parrish, T. B., & Gottfried, J. A. (2008). Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. *Science*, 319(5871), 1842-1845.
- Linstner, C., & Cleland, T. A. (2004). Configurational and elemental odor mixture perception can arise from local inhibition. *Journal of computational neuroscience*, 16(1), 39-47.
- Livermore, A., & Laing, D. G. (1996). Influence of training and experience on the perception of multicomponent odor mixtures. *Journal of Experimental Psychology: Human Perception and Performance*, 22(2), 267-277.
- Livermore, A., & Laing, D. G. (1998a). The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. *Physiology & behavior*, 65(2), 311-320.
- Livermore, A., & Laing, D. G. (1998b). The influence of chemical complexity on the perception of multicomponent odor mixtures. *Perception & Psychophysics*, 4, 650-661.
- Maarse, H. (Ed.). (1991). *Volatile compounds in foods and beverages*. New York: Marcel Dekker.
- Malnic, B., Godfrey, P. A., & Buck, L. B. (2004). The human olfactory receptor gene family. *Proceedings of the National Academy of Sciences of the United States of America*, 101(8), 2584-2589.
- Malnic, B., Hirono, J., Sato, T., & Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell*, 96(5), 713-723.
- Masago, R., Shimomura, Y., Iwanaga, K., & Katsuura, T. (2001). The effects of hedonic properties of odors and attentional modulation on the olfactory event-related potentials. *Journal of physiological anthropology and applied human science*, 20(1), 7-13.

- Mombaerts, P., Wang, F., Dulac, C., Chao, S. K., Nemes, A., Mendelsohn, M., ... & Axel, R. (1996). Visualizing an olfactory sensory map. *Cell*, 87(4), 675-686.
- Morrot, G., Brochet, F., & Dubourdieu, D. (2001). The color of odors. *Brain and language*, 79(2), 309-320.
- Mozell, M. M., & Jagodowicz, M. (1973). Chromatographic separation of odorants by the nose: retention times measured across in vivo olfactory mucosa. *Science*, 181(4106), 1247-1249.
- Mozell, M. M., Kent, P. F., & Murphy, S. J. (1991). The effect of flow rate upon the magnitude of the olfactory response differs for different odorants. *Chemical senses*, 16(6), 631-649.
- Moskowitz, H. R. (1976). Multidimensional scaling of odorants and mixtures. *Lebensmittel-Wissenschaft & Technologie*, 9, 232-238.
- Moskowitz, H. R., & Barbe, C. D. (1977). Profiling of odor components and their mixtures. *Sensory processes*, 1(3), 212-226.
- Moskowitz, H. R., & Gerbers, C. L. (1974). Dimensional salience of odors. *Annals of the New York Academy of Sciences*, 237(1), 1-16.
- Münch, D., Schmeichel, B., Silbering, A. F., & Galizia, C. G. (2013). Weaker ligands can dominate an odor blend due to syntopic interactions. *Chemical senses*, 38(4), 293-304.
- Oka, Y., Omura, M., Kataoka, H., & Touhara, K. (2003). Olfactory receptor antagonism between odorants. *The EMBO journal*, 23(1), 120-126.
- Olofsson, J. K., Broman, D. A., Gilbert, P. E., Dean, P., Nordin, S., & Murphy, C. (2006). Laterality of the olfactory event-related potential response. *Chemical senses*, 31(7), 699-704.
- Olsson, M. J. (1993). *The Perception of Odors in Interaction* (Doctoral dissertation, Stockholm University, Department of Psychology).
- Olsson, M. J. (1994). An Interaction Model for Odor Quality and Intensity. *Perception & Psychophysics*, 55, 363-372.
- Olsson, M. J. (1998). An Integrated Model of Intensity and Quality of Odor Mixtures. *Annals of the New York Academy of Sciences*, 855, 837-840.
- Pangborn, R. M. (1975). Cross-cultural aspects of flavor preferences. *Food technology*, 29, 34-36.
- Patte, F., & Laffort, P. (1979). An alternative model of olfactory quantitative interaction in binary mixtures. *Chemical Senses*, 4(4), 267-274.
- Pause, B. M., & Krauel, K. (2000). Chemosensory event-related potentials (CSERP) as a key to the psychology of odors. *International Journal of Psychophysiology*, 36(2), 105-122.
- Poulton, E. C. (1989). *Bias in quantifying judgements*. East Sussex: Lawrence Earlbaum Associates Ltd.
- Price, J. L. (1973). An autoradiographic study of complementary laminar patterns of termination of afferent fibers to the olfactory cortex. *Journal of Comparative Neurology*, 150(1), 87-108.
- Price, J. L. (1987). The central olfactory and accessory olfactory systems. In T. E. Finger & W. L. Silver (Eds.), *Neurobiology of Taste and Smell* (pp. 179-203). New York: Wiley.
- Price, J. L. (1990). Olfactory system. In G. Paxinos (Ed.), *The Human Nervous System* (pp. 979-998). San Diego: Academic Press.
- Rabin, M. D., & Cain, W. S. (1984). Odor recognition: familiarity, identifiability, and encoding consistency. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, 10(2), 316.

- Rankin, K. M., & Marks, L. E. (2000). Chemosensory context effects: Role of perceived similarity and neural commonality. *Chemical Senses*, 25(6), 747-759.
- Ressler, K. J., Sullivan, S. L., & Buck, L. B. (1994). Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell*, 79(7), 1245-1255.
- Rolls, E. T., Kringelbach, M. L., & De Araujo, I. E. (2003). Different representations of pleasant and unpleasant odours in the human brain. *European Journal of Neuroscience*, 18(3), 695-703.
- Roscher, S., Glaser, C., Hummel, T., & Kobal, G. (1996). An easy method for separating olfactory from trigeminal stimulation. *Chemical senses*, 21, 492-493.
- Rospars, J. P. (2013). Interactions of Odorants with Olfactory Receptors and Other Preprocessing Mechanisms: How Complex and Difficult to Predict?. *Chemical senses*, 38(4), 283-287.
- Rospars, J. P., Lansky, P., Chaput, M., & Duchamp-Viret, P. (2008). Competitive and noncompetitive odorant interactions in the early neural coding of odorant mixtures. *The Journal of Neuroscience*, 28(10), 2659-2666.
- Rouby, C., & Holley, A. (1995). Temporal competition between odorants: effect of different time intervals on the perception of monorhnic and dichorhnic binary mixtures. *Perception*, 24, 1083-1083.
- Royet, J. P., Zald, D., Versace, R., Costes, N., Lavenne, F., Koenig, O., & Gervais, R. (2000). Emotional responses to pleasant and unpleasant olfactory, visual, and auditory stimuli: a positron emission tomography study. *The Journal of Neuroscience*, 20(20), 7752-7759.
- Rozin, P., Fallon, A., & Augustoni-Ziskind, M. (1985). The child's conception of food: The development of contamination sensitivity to "disgusting" substances. *Developmental Psychology*, 21(6), 1075-1079.
- Sanz, G., Schlegel, C., Pernollet, J. C., & Briand, L. (2005). Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism. *Chemical senses*, 30(1), 69-80.
- Savic, I., & Gulyas, B. (2000). PET shows that odors are processed both ipsilaterally and contralaterally to the stimulated nostril. *Neuroreport*, 11(13), 2861-2866.
- Sell, C. S. (2006). On the unpredictability of odor. *Angewandte Chemie International Edition*, 45(38), 6254-6261.
- Schiet, F. T., & Frijters, J. E. (1988). An investigation of the equiratio-mixture model in olfactory psychophysics: A case study. *Perception & psychophysics*, 44(4), 304-308.
- Schiffman, S. S. (1974). Physicochemical correlates of olfactory quality. *Science*, 185, 112-117.
- Schiffman, S. S., Robinson, D. E., & Erickson, R. P. (1977). Multidimensional scaling of odorants: Examination of psychological and physicochemical dimensions. *Chemical senses*, 2(3), 375-390.
- Shipley, M. T., & Ennis, M. (1996). Functional organization of olfactory system. *Journal of neurobiology*, 30(1), 123-176.
- Shipley, M., & Reyes, P. (1991). Anatomy of the human olfactory bulb and central olfactory pathways. In D. G. Lang, R. L. Doty, & W. Breipohl (Eds.), *The human sense of smell* (pp. 29-60). Berlin Heidelberg: Springer.
- Sinding, C., Thomas-Danguin, T., Chambault, A., Béno, N., Dosne, T., Chabanet, C., ... & Coureaud, G. (2013). Rabbit Neonates and Human Adults Perceive a Blending 6-Component Odor Mixture in a Comparable Manner. *PloS one*, 8(1), e53534.

- Sobel, N., Prabhakaran, V., Desmond, J. E., Glover, G. H., Goode, R. L., Sullivan, E. V., & Gabrieli, J. D. E. (1998). Sniffing and smelling: separate subsystems in the human olfactory cortex. *Nature*, 392(6673), 282-286.
- Soussignan, R., Schaal, B., Marlier, L., & Jiang, T. (1997). Facial and autonomic responses to biological and artificial olfactory stimuli in human neonates: re-examining early hedonic discrimination of odors. *Physiology & Behavior*, 62(4), 745-758.
- Spehr, M., Gisselmann, G., Poplawski, A., Riffell, J. A., Wetzel, C. H., Zimmer, R. K., & Hatt, H. (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science*, 299(5615), 2054-2058.
- Spehr, M., Schwane, K., Heilmann, S., Gisselmann, G., Hummel, T., & Hatt, H. (2004). Dual capacity of a human olfactory receptor. *Current Biology*, 14(19), R832-R833.
- Spence, W., & Guilford, J. P. (1933). The affective value of combinations of odors. *The American Journal of Psychology*, 45(3), 495-501.
- Steiner, J. E. (1979). Human facial expressions in response to taste and smell stimulation. *Advances in child development and behavior*, 13, 257-295.
- Steven, S. S. (1957). On the psychophysical law. *Psychological Review*, 64, 153-181.
- Stevenson, R. J. (2001). Associative learning and odor quality perception: how sniffing an odor mixture can alter the smell of its parts. *Learning and Motivation*, 32(2), 154-177.
- Stevenson, R. J., & Mahmut, M. K. (2013). Detecting olfactory rivalry. *Consciousness and cognition*, 22(2), 504-516.
- Stevenson, R. J., Oaten, M. J., Case, T. I., Repacholi, B. M., & Wagland, P. (2010). Children's response to adult disgust elicitors: Development and acquisition. *Developmental psychology*, 46(1), 165-177.
- Stuck, B. A., Frey, S., Freiburg, C., Hörmann, K., Zahnert, T., & Hummel, T. (2006). Chemosensory event-related potentials in relation to side of stimulation, age, sex, and stimulus concentration. *Clinical Neurophysiology*, 117(6), 1367-1375.
- Tsuboi, A., Yoshihara, S. I., Yamazaki, N., Kasai, H., Asai-Tsuboi, H., Komatsu, M., ... & Sakano, H. (1999). Olfactory neurons expressing closely linked and homologous odorant receptor genes tend to project their axons to neighboring glomeruli on the olfactory bulb. *The Journal of neuroscience*, 19(19), 8409-8418.
- Vassar, R., Chao, S. K., Sitcheran, R., Vosshall, L. B., & Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. *Cell*, 79(6), 981-991.
- Wilson, D. A. (1997). Binaral interactions in the rat piriform cortex. *Journal of neurophysiology*, 78(1), 160-169.
- Wilson, D. A., & Sullivan, R. M. (2011). Cortical processing of odor objects. *Neuron*, 72(4), 506-519.
- Winston, J. S., Gottfried, J. A., Kilner, J. M., & Dolan, R. J. (2005). Integrated neural representations of odor intensity and affective valence in human amygdala. *The Journal of Neuroscience*, 25(39), 8903-8907.
- Wise, P. M., & Cain, W. S. (2000). Latency and accuracy of discriminations of odor quality between binary mixtures and their components. *Chemical Senses*, 25(3), 247-265.
- Wysocki, C. J., & Beauchamp, G. K. (1984). Ability to smell androstenone is genetically determined. *Proceedings of the National Academy of Sciences*, 81(15), 4899-4902.
- Wysocki, C. J., Pierce, J. D., & Gilbert, A. N. (1991). Geographic, cross-cultural, and individual variation in human olfaction. In T. V. Getchell (Ed.), *Smell and taste in health and disease* (pp. 287-314). New York: Raven.

- Yeshurun, Y., & Sobel, N. (2010). An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. *Annual review of psychology*, 61, 219-241.
- Zald, D. H., & Pardo, J. V. (1997). Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. *Proceedings of the National Academy of Sciences*, 94(8), 4119-4124.
- Zawirska-Wojtasiak, R. (2006). Chirality and the nature of food: authenticity of aroma. *Scientiarum Polonorum Technologia Alimentaria*, 5(1), 21-36.
- Zhou, W., & Chen, D. (2009). Binaral rivalry between the nostrils and in the cortex. *Current Biology*, 19(18), 1561-1565.
- Zozulya, S., Echeverri, F., & Nguyen, T. (2001). The human olfactory receptor repertoire. *Genome biology*, 2(6), 1-12.