

# DIPLOMARBEIT

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### Multisensory Integration of Natural Volatile Stimuli

Verfasserin Laura Hackl

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#### ABSTRACT

Evolutionary pressure forces organisms to thoroughly observe their environment in order to detect potentially dangerous and beneficial stimuli, with the final aim of maximizing the chances of survival. Therefore, accurate perception of sensory stimuli presented in different modalities and their affective evaluation are important functions, which allow for the preparation of an individual for appropriate actions. These actions are proposed to be organized by at least two different motivational systems that enable approach or avoidance behaviors. In general, people tend to approach positively evaluated stimuli and to avoid negatively evaluated ones. A way to investigate approach-avoidance motor tendencies in humans is offered by experimental paradigms measuring the reaction time of arm movements used to evaluate the emotional valence of affective stimuli, such as the approach-avoidance-task. Participants are asked to perform arm movements that are either congruent or incongruent with their action tendencies. As an example, affect congruent arm movements require participants to evaluate a stimulus as negative by pushing a response lever away (arm extension) and to evaluate a stimulus as positive by pulling the lever towards them (arm flexion). On the contrary, affect incongruent actions require participants to follow opposite instructions (e.g., pull a lever to a negative stimulus or push a lever to a positive stimulus). By calculating the difference in motor reaction time between affect-congruent and affect-incongruent movements to positive and negative stimuli, it has been revealed that participants are faster to produce affectcongruent than affect-incongruent arm. This paradigm has been applied to multiple

studies, which have mostly examined the effect of motor-mediated approach and avoidance behavior in the visual domain. However, given that stimuli in all modalities can constitute relevant cues to navigate the environment, they can have the ability to trigger similar evaluative motor responses. In particular, odors, which are well known for their intimate interconnection with emotions and their influence on actions, constitute an interesting stimulus to be tested with this paradigm.

In order to investigate the effect of stimuli presented in modalities other than the visual one, the experimental participants were administered with a modified version of the AAT, which included visual social stimuli (i.e., human faces expressing positive and negative emotions) and olfactory stimuli, positive or negative common odors, or social chemosignals. The aim of the present project was to explore how odors of a social and non-social nature can influence our evaluative motor behavior when reacting to emotional positive and negative visual stimuli with a social meaning.

Three experiments were conducted by administering three separate samples of participants with the AAT in varied visual and visuo-olfactory contexts. Participants were required to respond by pushing or pulling a joystick lever to angry, happy and neutral human faces while no odor (Experiment 1), positive and negative non-social common odors (Experiment 2) and social odors (Experiment 3), were presented.

Experiment 1 was aimed to replicate earlier findings on the unimodal visual task. Five female and five male participants were tested, showing approach towards positive visual stimuli and avoidance towards negative visual stimuli, as previously proposed. Additionally, the inclusion of neutral faces revealed a slight tendency to produce an avoidance reaction.

Experiment 2 was aimed at ascertaining the behavioral response to the same social visual stimuli while presenting common odors with a differing valence – a neutral, a pleasant or an unpleasant common odor. Ten female and ten male participants responded to the visual and olfactory stimuli in the AAT task. Results revealed that the valence of common odors did not influence the approach avoidance motivational response in the AAT.

The result of Experiment 2 – namely, that positive and negative common odors have no influence on affective motor behavior – raised the hypothesis that a social chemosignal might instead influence the motor response to social visual stimuli in the AAT. Therefore, Experiment 3 was conducted by presenting a masked gender-matched body odor and a common odor while recording the motor approach and avoidance reactions to the same social visual stimuli used in the previous experiments. Given that body odors require a time consuming and very detailed collection procedure, only two female and two male participants were included in the sample. Results, although not significant possibly due to the limited sample size, indicate that the motor reaction time to positive and negative social visual stimuli is not sensitive to the influence of the common odor but shows an interesting pattern for the masked social chemosignal. Participants tended to avoid the angry faces and to approach the happy faces when they were exposed to the body odor. As in Experiment 1, a neutral face was avoided.

Taken altogether, the results of these experiments demonstrate that the valence of common odors, with no relation to the social affective visual stimuli, does not influence our motivational tendencies towards them. However, pairing the visual presentation with a social chemosignal, even when not perceived on a conscious level,

seems to produce a difference in the approach avoidance motor behavior. In order to fully disentangle this issue, future studies are needed.

#### ZUSAMMENFASSUNG

Durch evolutionären Druck sind Organismen gezwungen, ihre Umgebung genau zu beobachten um potentiell gefährliche oder günstige Stimuli zu detektieren, mit dem Ziel, das eigene Überleben zu sichern. Deshalb sind eine genaue Auffassung von sensorischen Stimuli in unterschiedlichen Modalitäten, sowie eine emotionale Evaluierung dieser wichtige Funktionen, die es einem Individuum erlauben, entsprechend zu reagieren. Mindestens zwei verschiedene Motivationssysteme sollen für diese Reaktionen, die Annäherungs- oder Abweisungsverhalten hervorrufen, verantwortlich sein. Menschen nähern sich positiv evaluierten Stimuli eher an und vermeiden negative Stimuli. Eine Möglichkeit, um Annäherungsbzw. Abwendungstendenzen gegenüber emotionalen Stimuli auf motorischer Ebene im Menschen zu untersuchen, bieten experimentelle Modelle, die Reaktionszeiten in Form von Armbewegungen messen, ein Beispiel ist der "Approach-Avoidance-Task' (AAT).

Hierbei werden Studienteilnehmer gebeten, Armbewegungen durchzuführen, welche entweder mit ihren Aktionstendenzen übereinstimmen, oder nicht. Zum Beispiel, in einer übereinstimmenden Armbewegung drückt der Studienteilnehmer den Joystick von sich weg (Arm-Streckung), wenn er einem negativen Stimulus ausgesetzt ist bzw. er zieht den Joystick zu sich, wenn er einem positiven Stimulus ausgesetzt ist (Arm-Beugung). In einer nicht-übereinstimmenden Armbegeweung werden die Teilnehmer gebeten, gegensätzliche Armbewegungen durchzuführen (zum Beispiel, den Joystick an sich ziehen bei einem negativen Stimulus beziehungsweise wegdrücken bei einem positiven Stimulus).

Beim Berechnen der Differenz der Reaktionszeiten zwischen übereinstimmenden und nicht-übereinstimmenden Bewegungen gegenüber positiven und negativen Stimuli konnte gezeigt werden, dass Teilnehmer schneller übereinstimmende Arm-Bewegungen ausführten, als nicht-übereinstimmende. Dieses Modell wurde in verschiedenen Studien angewandt, die meist die motorischen Annäherungs- beziehungsweise Abwendungstendenzen im visuellen Kontext allein untersuchten.

Nachdem Stimuli in allen Modalitäten relevante Informationen zur Umgebung geben können, haben sie die Möglichkeit ähnliche Antworten des motorischen Systems hervorzurufen. Besonders Geruchstoffe, die bekannt sind für die enge Verbindung mit Emotionen und deren Einfluss auf das Handeln, sind interessante Stimuli, die mit diesem Modell getestet werden können.

Um den Effekt von Stimuli abseits von der rein visuellen Modalität zu erforschen, führten die Studienteilnehmer eine modifizierte Version des AAT durch, wobei soziale visuelle (menschliche Gesichter, die positive und negative Emotionen zeigen) und olfaktorische (positive und negative gewöhnliche Gerüche oder soziale chemische Signale) Stimuli verwendet wurden. Das Ziel dieses Projektes war es, herauszufinden, wie Gerüche einer sozialen und nicht-sozialen Natur in Verbindung mit emotional positiven und negativen visuellen Stimuli mit sozialem Kontext das evaluative Motorsystem beeinflussen.

Dies führte zur Anwendung des AAT in drei verschiedenen Experimenten mit multimodalem Kontext. Die Teilnehmer zogen den Joystick an sich, oder drückten ihn weg als Antwort auf wütende, glückliche oder neutrale Gesichtsausdrücke, während

entweder kein Geruch (Experiment 1), positive oder negative gewöhnliche Gerüche (Experiment 2) oder soziale Gerüche (Experiment 3) präsentiert wurden.

Experiment 1 wurde durchgeführt, um frühere Ergebnisse (unimodal visuell) zu reproduzieren. Fünf weibliche und fünf männliche Teilnehmer wurden getestet. Es konnte, wie erwartet, eine Annäherung gegenüber positiven visuellen Stimuli und eine Abneigung gegenüber negativen Stimuli gezeigt werden. Zusätzlich konnte gezeigt werden, dass neutrale Gesichtsausdrücke eine leichte Abneigungstendenz hervorriefen.

Experiment 2 wurde durchgeführt, um das Verhalten gegenüber den selben sozialen visuellen Stimuli zu erfassen, während gewöhnliche Gerüche mit unterschiedlicher Wertigkeit – neutral, positiv oder negativ – präsentiert wurden. Zehn weibliche und zehn männliche Teilnhemer wurden mit den visuellen und olfaktorischen Stimuli getestet. Die Ergebnisse zeigen, dass die Wertigkeit eines Geruches keinen Einfluss auf das motorische Annäherungs- und Abweisungsverhalten im AAT hat.

Die Ergebnisse von Experiment 2 – positive und negative gewöhnliche Gerüche haben keinen Einfluss auf das motorische Wertungsverhalten – führten zur Hypothese, dass ein soziales chemisches Signal das Verhalten im AAT beeinflussen könnte. Deshalb wurden in Experiment 3 maskierte, auf das Geschlecht abgestimmte, Körpergerüche und ein gewöhnlicher Geruch präsentiert, während das motorische Annäherungs- und Abweisungsverhalten gemessen wurde. Es wurden die selben visuellen Stimuli wie in den Experimenten 1 und 2 verwendet. Nachdem die Sammlung von Körpergerüchen eine zeitaufwändige und sehr detaillierte Prozedur erfordert, wurden nur zwei weibliche und zwei männliche Teilnehmer getestet. Die Resultate sind nicht signifikant – was unter Umständen auf die geringe Anzahl an Studienteilnehmern

zurückgeführt werden kann – und zeigen, dass sich die Reaktionszeit zu den positiven und negativen sozialen visuellen Stimuli nicht verändert, wenn der gewöhnliche Geruch präsentiert wird. Es konnte jedoch ein abweichendes Verhaltensmuster gezeigt werden, wenn der maskierte Körpergeruch präsentiert wurde. Die Teilnehmer mieden die wütenden Gesichtsausdrücke eher und näherten sich den glücklichen Gesichtsausdrücken an. Neutrale Gesichtsausdrücke wurden – wie bereits im unimodalen Experiment 1 gezeigt – eher gemieden.

Zusammenfassend zeigen die Resultate der drei Experimente, dass die Wertigkeit von gewöhnlichen Gerüchen ohne Beziehung zu den emotionalen, visuellen Stimuli die Motivationstendenzen nicht beeinflusst. Wird der visuelle Stimulus jedoch mit einem sozialen, chemischen Signal gepaart, auch wenn dies nicht bewusst wahrgenommen wird, wird ein Unterschied im Annäherungs-/ Abwendungsverhalten festgestellt. Um dieses Verhalten jedoch vollständig zu evaluieren, müssten weitere Studien durchgeführt werden.

## **SECTION I**

### THEORETICAL BACKGROUND

#### **RATIONALE OF THE PRESENT THESIS**

The aim of the present project was to explore how odors of a social and non-social nature can influence our evaluative motor behavior when reacting to emotional positive and negative visual stimuli with a social meaning.

The thesis is organized as follows. The first part of this thesis provides a general overview on the sense of smell in humans. The main anatomical structures involved in the olfactory system (1.1.), the physiological processes mediating odor processing (1.2.) and the functional aspects pertaining human olfaction (1.3.) are briefly outlined.

The second part focuses on the performed experimental work. The Approach-Avoidance Task (AAT; Rinck & Becker, 2007) was employed to evaluate the approach and avoidance tendencies of young adults to human facial stimuli expressing positive and negative emotions in both a unimodal and a multimodal context in three experiments. In Chapter 3, the approach and avoidance behavior to emotional visual stimuli (angry, happy and neutral human faces) were examined. In Chapter 4, the response to the same visual stimuli, while presenting olfactory stimuli differing in valence: neutral, pleasant and unpleasant 'common' odors were examined. The use of common odors might reveal how our evaluative behavior is influenced by the valence of everyday odors without a social component. Subsequently, the effect of human social chemosignals (i.e., axillary body odor) on the approach avoidance response as measured with this paradigm was tested. This is justified by the fact that previous literature shows that body odors are stimuli conveying a social message, which is implemented differently in neural terms as compared to common odor processing

(Lundström et al., 2008). Therefore, in Chapter 5, the effects of a same-sex supra-donor body odor, originating from unknown individuals, and a common odor neutral in valence on the approach avoidance response measured via AAT were compared. In Chapter 6 the general discussion is presented where the main findings from the above-mentioned experiments are compared and then discussed in light of the current literature on the topic. Directions for future research questions are then suggested.

## 1. OLFACTORY SYSTEM: ANATOMY, PHYSIOLOGY, FUNCTION

Humans are commonly unaware of the influence the sense of smell have on their own behavior. This may depend on the fact that the sense of smell is considered a 'hidden' sense, meaning that odors are seldom the focus of our attention (Smeets et al., 2009). However, we use the sense of smell on several different occasions: it influences our consumption of food, it highlights environmental hazards and it is involved in social communication. To better understand how odor processing occurs and what organs of the human body are involved, a brief description of the anatomy, physiology and functional ability of the human main olfactory system will be provided.

#### **1.1 A**NATOMY

When thinking of olfaction, the first thing that comes to one's mind is most likely the nose. However, the nose and its underlying structures only provide the peripheral structures, while the brain serves as a central unit to decipher the signals of the received stimuli. The human nose comprises the nasal cavity, which is approximately 12-14 cm long. It extends from the nostrils to the nasopharynx and it is separated longitudinally by the cartilaginous nasal septum. Four angular turbinates (inferior, middle, superior and supreme) form each lateral nasal wall, which affects processes like airflow, filtration and mucus flow in the smelling process (Pinto, 2011; Figure 1).

The brain is responsible for the central processing of olfactory signals, more precisely the primary (piriform cortex, olfactory nucleus, tubercle, amygdala, entorhinal cortex) and the secondary (hippocampus, hypothalamus, thalamus, orbitofrontal cortex, cerebellum) olfactory regions (Pinto, 2011).

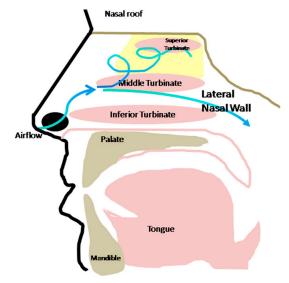


Figure 1: Structure of the Nasal Cavity (Pinto, 2011)

#### **1.2 PHYSIOLOGY**

The human nasal cavities host two specialized neural systems: the *main olfactory system* (cranial nerve I, CN I) and the *trigeminal somatosensory system* (CN V). CN I innervates special sensory axons that mediate the odor sensation and the processing of flavors. CN V innervates somatic sensory and somatic motor axons that mediate the sensation of touch (such as e.g. burning or irritation) and the movement of muscles, respectively (Bear et al., 2007).

Most animals have developed two olfactory systems. The main olfactory system serves to identify the flavor of foods, to evaluate potential toxins and to mark territory.

Therefore, this open system has to process a lot of external information that has been gathered from the environment without any a priori selection (Firestein, 2001). The second, accessory olfactory system is also known as vomeronasal system. The vomeronasal organ is a tubular structure in the nasal septum and serves – among other functions – to provide information about reproduction relevant topics (Firestein, 2001). In humans, however, the vomeronasal organ regresses after an initial development in utero (Bhatnagar & Smith, 2001). As the human vomeronasal epithelium is lacking neurons and vomeronasal nerve bundles it seems not to serve as a sensory organ in adulthood (Trotier et al., 2000).

In the present thesis the focus will be on the human main olfactory system, which comprises at the central level the olfactory epithelium, the olfactory bulb and central olfactory regions. The main sensory receptors for human olfaction are located in the *olfactory epithelium* of the nasal cavity. The surface of the human olfactory epithelium is about 10 cm<sup>2</sup> and it consists of several different cell types. The four most important cell types are olfactory receptor cells, supporting cells, duct cells of Bowman's glands and basal cells (Lapid & Hummel, 2013) and will be described in more detail (Figure 2). *Olfactory receptor cells* are neurons whose cilia reach into the mucus layer and whose axons penetrate into the CNS. More than 900 different odorant receptor genes have been found in the human genome; about 60 % of them are pseudogenes whose function is yet to be discovered. Therefore, humans possess less than 350 intact OR genes that are distributed among 21 chromosomes (Malnic et al., 2004), and code for about 50 million receptor cells in each human (Elsaesser & Paysan, 2007). Considering this large variety of non-monogamous olfactory receptor cells, it is not surprising that humans are

able smell an infinite number of various odor molecules (Buck & Axel, 1991) as the unique structure of each receptor gene enables it to bind different odorants. *Supporting cells* produce mucus, a water base with different dissolved mucopolysaccharides, different kinds of proteins (eg antibodies, enzymes, odorant binding proteins) and salts. The components of the mucus fulfill important purposes. Antibodies, for example, can defend the organism against some viruses and bacteria that could otherwise enter the brain directly via olfactory cells. Odorant binding proteins may also play a role as an odorant enhancer (Briand et al., 2002). The *Bowman's glands* excrete the multicomponent mucus. *Basal cells contain* progenitor cells that serve as the precursors for new receptor cells. Within a cycle of 4 to 8 weeks, olfactory receptors can regenerate, which is unique in the human nervous system (Bear et al., 2007).

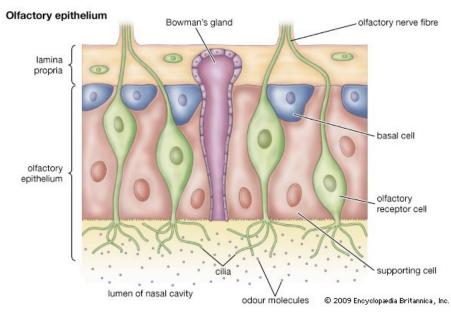


Figure 2: Cellular Structures of the Olfactory Epithelium (Encyclopedia Britannica, 2009)

Having reviewed the cellular structures in the main olfactory system leaves the question of how we are able to smell an odorous substance. Not every substance has an odor, but only volatile molecules can serve as odorants. The smelling process starts with a

sniff that drags molecules into the nasal cavity where they encounter the nasal epithelium. The odorants then dissolve in the mucus layer and bind to the cilia of the olfactory receptor and therefore activate a complex transduction process via G-protein coupled receptors (GPCR), in the case of olfaction Golf (Lapid & Hummel, 2013). Golf stimulation activates adenylyl cyclase, which eventually forms the second messenger cyclic AMP (cAMP). cAMP binds to specific ion channels and leads to an influx of Na<sup>+</sup> and  $Ca^{2+}$ . This triggers the opening of  $Ca^{2+}$  activated chloride channels and therefore a current flow and leads to a membrane depolarization (Figure 3). If the potential exceeds the threshold for action potentials in the cell body, the sensation will propagate into the CNS. On the opposite side of the olfactory receptor are thin, unmyelated axons which collectively form the CN I. Unlike other cranial nerves which form a single nerve bundle, these axons form clusters that penetrate a thin sheet of bone and then progress into the olfactory bulb where they form synaptic structures and converge into mitral cells (Bear et al., 2007). Eventually, the signals get transduced to primary and secondary olfactory regions in the brain (Pinto, 2011). Which cerebral regions get activated is highly dependent on the kind of odor that is encountered. At this point it is important, to draw a line between common odors and body odors. While the former activates areas within the olfactory cortices, the latter has a differing activation pathway and can activate areas that are known for processing emotional stimuli and regulating alertness (Lundström et al., 2008).

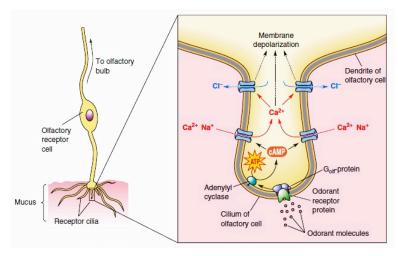


Figure 3: Transduction Mechanisms in Olfactory Receptor Cell (Bear et al., 2007)

#### **1.3 FUNCTION**

Olfaction is one of the oldest senses in evolutionary terms (Sarafoleanu et al., 2009) and the fact that its precursors can be found even in smallest organisms indicates its phylogenetic high significance. Unlike other mammals, humans appear to be less confident in their olfactory abilities. Both the comparably small size of our olfactory epithelium (Bear et al., 2007) and the small number of odorant receptor genes (Zhang & Firestein, 2002) could be an explanation for our relatively weak smelling abilities when compared to other animals. A lot of research has been conducted in the fields of vision and audition, while the chemical senses have not received that much attention for a long time, possibly because they were considered as being less important. It is only recently that the high significance of the chemical senses attracts researchers' and public interest. As a matter of fact, olfaction plays an important role for the survival of human beings as well as in everyday life. Olfaction is a protective sense as it allows detecting potential threatening dangers from a distance or the detection of food. When compared to the sense of vision, olfaction has the advantage that odorants can be detected in the dark, around obstacles and that it can be conducted unconsciously. It further gives an opportunity to place remote signals as it stays in the environment for extended periods (Sergeant, 2010). Also, its role in social communication should not be underestimated, as a variety of studies has proved (Chen & Haviland-Jones, 2000; Pause, 2012).

Social communication was established early during evolution. In order to survive, species had to communicate with the signals present in the environment. A signal can be e.g. an odor, but adding a social component to an odor can increase the information that a signal contains. When discussing odors in a social aspect, we usually mean body odors. As indicated earlier, body odors have to be distinguished from common odors in terms of odor perception and neural processing. The topic of *pheromones* has been investigated thoroughly in recent years. In animals, pheromones are chemicals that the body releases to signal reproductive behavior. In other words, they are defined as substances that are "secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction" (Karlson & Luscher, 1959). In humans, no receptor for pheromones has been found yet (Wysocki & Preti, 2004) and the signaling process remains unclear (Trotier, 2011). Nevertheless, our social life is highly dependent on our olfactory abilities as olfaction serves as a mode of communication, kin recognition (Cernoch & Porter, 1985; Weisfeld et al., 2003) and mating (Wedekind et al., 1995). Studies revealed that social communication through odors in humans can have an influence in menstrual cycle synchronization (McClintok, 1971) and female ovulation (Stern & McClintock, 1998) in humans. Considering this, it is not surprising that olfaction in consequence has an impact on mood (Jacob & McClintock, 2000) and emotion (Adolph & Pause, 2012; Zald & Pardo, 1997).

## **SECTION II**

### **EXPERIMENTAL STUDIES**

#### **GENERAL INTRODUCTION**

To maximize the chances of survival throughout generations, it is crucial for each organism to accurately observe its environment. Thus, the brain extracts necessary information gathered from the environment and uses this information to generate behavior and to regulate physiology (Cosmides & Tooby, 2013). In order to judge whether a stimulus is relevant - in positive or negative terms - while it requires us to further react to it, it is necessary to have an intact *perceptual system*, able to detect stimuli in different modalities, a functioning emotional system, apt to evaluate the affective tone of the stimulus (e.g., safe vs. dangerous) and a preserved motor system capable of promptly reacting in accordance with the evaluation of the stimulus (e.g., approach vs. avoidance). Many are the sensory stimuli present in the environment. However, visual stimuli are deemed to be the gold standard of sensory information in humans. Faces are probably the most important visual stimuli, not only because they serve as a means of individual recognition, but also because they provide rich sources of visual information with social significance (Slater & Quinn, 2001). It is therefore not surprising that faces are perhaps the most prominent visual stimuli in infants (Slater & Quinn, 2001). Faces can become even more informative when they show a particular facial expression as they can communicate the emotional state as well as behavioral intentions of individuals (Horstmann, 2003).

However, other sensory modalities have demonstrated to have the ability to communicate relevant messages. In this respect, chemosensory stimuli seem to provide

relevant information about the environment often without our awareness. It might therefore be surprising that our ability to adjust to chemical signals of a social and nonsocial nature is thought to be one of the main forces for the brain development and that social chemical communication is still considered as playing an important role for the human survival and development (Pause, 2012). Unlike vision, humans do not rely on olfaction as their primary source of information. However, research confirmed that we are accurate in detecting the presence of an odor (Miyazawa et al., 2009), we can easily attribute odors with an affective evaluation (Winston et al., 2005) and we are able to tune our actions towards objects according to odor cues (Castiello et al., 2006; Hedner et al., 2010; Tubaldi et al., 2008).

Sensory stimuli, although often studied in isolation, are simultaneously present in the environment and our perceptual system is structured to merge the information provided by different sensory modalities at once (Calvert et al., 2004). A signal can be of a unimodal kind (e.g. visual signal or olfactory signal independently) or of a multimodal kind (e.g. visuo-olfactory signal), which more likely represents the natural environment. However, simply perceiving multimodal stimuli does not guarantee better chances at survival. In fact, each stimulus can elicit complex patterns of reactions in an organism (Osgood, 1952). What becomes crucial is the ability to distinguish between "good" and "bad" stimuli and to accordingly react to them in order to apply the best strategy to survive. Adding an emotional evaluation to various sensory stimuli is an important mechanism as it helps in communicating information on possible dangers in the surrounding environment. As an example, we tend to fear events that are considered universally life-threatening (e.g. facial expressions of conspecifics as they can indicate

the emotional state or underlying intentions of the poser (Horstmann, 2003), or such as the odor of rotten food indicating the possibility of food poisoning (Stevenson, 2010; Boesveldt et al., 2010). In visual terms, a potential threat could be an angry face, which, according to Marsh and colleagues (Marsh et al., 2005), might signal the presence of a direct danger for the perceiver. This, as a consequence, might lead the perceiver to automatically avoid the stimulus (Roelofs et al., 2010; Seidel et al., 2010; Stins et al., 2011). A happy face, conversely, gives a positive sign and probably leads to an approach reaction (Seidel et al., 2010). Finally, a neutral face, due to its unclear expressivity, has been linked to neither approach nor avoidance tendency (Heuer et al., 2007).

A similar evaluation can be pictured for odors. Common odors, which differ in valence, can elicit different behavioral reactions in the perceiver. For instance, a study using isointense negative and positive olfactory stimuli with no social component showed significantly increased startle-reflex amplitudes for the negative and decreased amplitudes for the positive stimulus indicating that unpleasant odors induce a psychophysiological arousal similar to that experienced during a stressful/ negative situation (Miltner et al., 2007). Thus, it appears evident that olfactory-mediated motor responses such as the startle reflex are influenced by the valence of a common odor. Body odors can tell a similar story. In fact, it has been shown that body odors produced by a stranger can activate the same cerebral regions as threatening stimuli (Lundström et al., 2008). Keeping this in mind, it is plausible that a stranger's body odor might induce an avoidance reaction, which is detectable at the behavioral level.

Once the sensory and affective evaluation of the stimuli in the environment has been accomplished, it is necessary to appropriately react to them. Thus, the motor system is activated favoring two basic motivational tendencies: approach and avoidance (Bargh, 1997, Cacioppo et al., 1993, Chen & Bargh, 1999, Davidson et al., 1990, Gray, 1994 and Lang et al., 1990). Generally speaking, the term "avoidance" immediately recalls the action of "turning away from" while the term "appetitive" reminds of the action of "going towards, approaching" an object or a person but does not necessarily mean "appealing". In other words, an automatic evaluative process unconsciously drives an individual's behavior toward positive objects and away from negative ones (Bargh & Chartrand, 1999). Thus, it leads to an approach reaction towards potentially good stimuli and an avoidance reaction towards potentially dangerous stimuli. Although not all aversive stimuli are threatening (some may be simply disgusting or conveying a sense of sadness instead), threatening stimuli are – by definition – aversive (Ohman & Mineka, 2001). A wide variety of studies shows that the perception of potentially threatening sensory stimuli seems to be associated with avoidance of the source (Mackaysim & Laing, 1981; Zalaquett & Thiessen, 1991). Therefore, any stimulus signaling potential threat is expected to activate avoidant mechanisms such as withdrawal (Cacioppo & Berntson, 1994; Lang, 1997).

At the behavioral level these types of motivational tendencies can be reflected in terms of avoidant and approach motor responses (Cacioppo & Berntson, 1994; Marsh et al., 2005; Schupp et al., 1997). Several studies showed that a stimulus evaluated as good facilitates arm muscles involved in pulling, whereas a negative evaluation facilitates the extensor arm muscles and therefore pushing (Chen & Bargh, 1999;

Solarz, 1960). Moreover, it has been demonstrated that perceivers push a lever (extension) in response to aversive stimuli faster than they pull (flexion) it in the same circumstances, but they pull a lever faster in response to appetitive stimuli than they push it (Chen & Bargh, 1999; Dagloria et al., 1994; Forster, Higgins & Idson, 1998; Solarz, 1960). This pattern of results reveals an affective stimulus evaluation – arm movement congruency (Chen & Bargh, 1999).

A paradigm apt to measure the approach and avoidance tendencies to affective stimuli by analyzing the reaction time of affective congruent and incongruent movements is the approach-avoidance task (AAT; Rinck & Becker, 2007). In this task, the reaction times (RTs) are recorded while participants are asked to either pull or push a joystick lever as fast and as accurately as possible when a visual stimulus is presented on a computer screen. In order to quantify the strength of both – approach and avoidance tendencies – an AAT bias score is calculated (Cousijn et al., 2011). This score is calculated by subtracting the median RT when pulling to a visual stimulus from the median RT when pushing to the same visual stimulus. Median RTs were considered, because of their lower sensitivity to outliers when compared to mean RTs (Rinck & Becker, 2007). Thus, a negative AAT bias score indicates a faster pushing response and, in consequence, avoidance. Correspondingly, a positive AAT score reveals a faster pulling response and therefore approach. This paradigm has been applied with several different categories of visual stimuli, such as visual stimuli showing emotional faces (Heuer et al., 2007; Roelofs et al., 2010; Seidel et al., 2010), potentially threatening animals (Rinck & Becker, 2007), non-social objects (Ernst et al., 2013; Najmi et al., 2010) or written words (Eder & Rothermund, 2008). Considering the fact that this task

serves as a suitable tool for measuring implicit avoidance behavior, it is surprising that so far it has only been used in a unimodal context. Hence, the intention was to bring the current literature on the topic a step forward by applying the AAT in a multimodal perspective, which would better reflect the natural circumstances. As a matter of fact, one is very unlikely to encounter an isolated stimulus outside of the lab environment. Thus, we face potentially threatening stimuli on different levels and only the ability to combine and integrate the information gathered from our different modalities enhances our chances of survival.

In order to shed light on this topic, three experiments using the AAT under the presentation of visual alone and both visual and olfactory stimuli presented simultaneously were conducted. The visual stimuli were photographs of faces showing angry, happy or neutral facial expressions and were used for all three experiments. The olfactory stimuli were clean air – serving as control condition, common odors or body odors for Experiments 1, 2 and 3, respectively.

In Experiment 1, faster responses to angry facial expressions in general compared to happy or neutral facial expressions were expected as previous studies have shown their evolutionary higher significance for humans (Palermo & Rhodes, 2007; Rotteveel & Phaf, 2004). Further, slower responses to neutral facial expressions were expected, as it takes longer to estimate underlying intentions when the shown emotion is ambiguous. For Experiment 2, it was hypothesized that smelling an unpleasant odor while looking at a facial stimulus would lead to faster push-responses and therefore a negative bias score, compared to smelling a pleasant odor. For Experiment 3, the use of body odors was expected to magnify the motivational tendencies. In other words, it is

foreseen that a stranger body odor will increase the reaction to a negative visual stimulus as compared to the effect of a common odor on the reaction to the same stimulus.

In the following chapters, a brief introduction explaining the specific aim of each study, the methods used and the exact procedure for each experiment will be provided.

## 3. APPROACH/AVOIDANCE BEHAVIOR TO EMOTIONAL VISUAL STIMULI

#### **3.1 INTRODUCTION**

Many studies using the AAT have been conducted in socially anxious individuals (Heuer et al., 2007; Lange et al., 2008; Roelofs et al., 2010; Taylor & Amir, 2012) and patients diagnosed with other mental disorders (Louise von Borries et al., 2012; Najmi et al., 2010; Slater & Quinn, 2001; Wiers et al., 2010). Their outcomes, as shown above, mainly showed a faster motor response in affective congruent situations as well as faster responses to possibly threatening stimuli. The aim of this study was to apply the paradigm to replicate the findings in healthy individuals when using human faces depicting emotional facial expressions as visual stimuli. This unimodal approach should serve as a baseline in a series of the subsequent multimodal investigations.

#### **3.2 MATERIALS AND METHODS**

#### 3.2.1 PARTICIPANTS

Ten healthy, right handed participants (five males, five females) reporting normal or corrected-to-normal vision and normal smell abilities working at Monell Chemical Senses Center were asked to respond to visual stimuli by pushing or pulling a joystick lever while smelling no odor (clean air). All participants were naïve as to the purpose of the experiment. Detailed written informed consent was obtained from all participants. The

experimental procedures were approved by the Institutional Review Board and were in accordance with the principles of the Declaration of Helsinki. One session lasted approximately 1.5 hours and participants were rewarded upon completion of the study with a total of \$20.

#### 3.2.2 STIMULI

Seventy-eight different visual stimuli were used for the experiment – standardized pictures of 26 Caucasian individuals (13 males, 13 females) each showing angry, happy and neutral facial expressions, respectively. The pictures were taken from the Karolinska Directed Emotional Faces (KDEF; Lundquvist et al., 1998) database after careful evaluation (see Pilot 1 – Appendix I). Each stimulus was presented four times throughout the whole experiment. While the participants were shown the stimuli, they were also presented with a clean air odor that was delivered through an olfactometer (Lundström et al., 2010).

#### 3.2.3 APPARATUS

All used instruments were synchronized via Eprime (E-Studio 2.0). A joystick (Logitech 3 Attack) was fixed on a board in front of the screen, for measuring the reaction time of the participants as a response to simultaneously presented visual and olfactory stimuli. The visual stimuli were presented on a screen via Eprime (E-Studio 2.0).

A Nalgene odor jar (4 oz; 74 mm x 64 mm x 64 mm) containing a clean pad quadrant (Ultra-Thin Nursing Pads, Gerber Inc., ON, Canada) served as the olfactory stimulus 'clean air' (Figure 4).



Figure 4: 4 oz Nalgene odor jar with pad quadrant

The jar was connected with an olfactometer (Lundström et al., 2010; Figure 5).



Figure 5: Olfactometer (Lundström et al., 2010)

The olfactometer delivered the odors through tubes leading into a nasal manifold and eventually into participant's both nostrils (Figure 6).

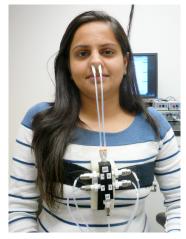


Figure 6: Participant carrying nasal manifold

The flow-rate was calibrated to 1.5 liters/minute, which was decided upon due to a prior pilot study (Pilot 2 – Appendix I). The calibration was performed by using a 'Gilian 2 Primary Flow Calibrator'.

#### 3.2.4 PROCEDURE

A within-subjects study design was chosen for the experiment. Prior to the experiment, participants' olfactory identification abilities were measured using the Sniffin' Sticks 16items Odor Identification test (Hummel et al., 2007). Participants with a score of 10 or lower were disqualified for the experiment due to potential hyposmia. Each participant was positioned 50 cm in front of a screen and was given a nasal manifold to carry the olfactory stimulus from an olfactometer to both nostrils. A board with a joystick was fixed on a table between the participant and the screen (Figure 7).

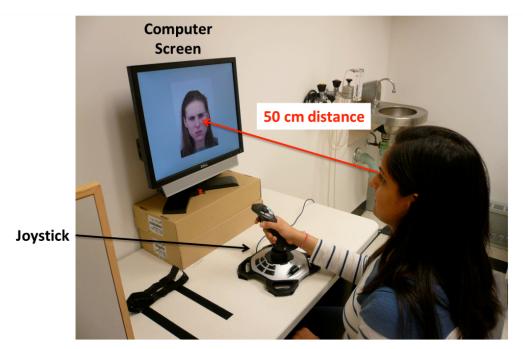


Figure 7: Experimental Setup - Participant seated 50 cm from computer screen with joystick in between

Before the experiment, participants were asked to answer the State subpart of the standardized psychometric State Trait Anxiety Inventory (STAI-S; Spielberger, 1983) and a behavioral questionnaire (Appendix II), after the experiment they were asked to answer the State and Trait subpart of the STAI (STAI-S and STAI-T). The completion of the STAI was administered to measure the situational and general anxiety, respectively (Spielberger, 1983). Then the participants were asked to do a training session to familiarize with the task. The training session was comparable to the actual experiment with only a limited number of stimuli. After successful completion, the participants could start with the experiment. The experiment was divided into six blocks. In each block participants were shown 78 visual stimuli showing a combination of angry and happy, angry and neutral or happy and neutral facial expressions. The participants were

instructed to respond to the visual stimulus by moving the lever of the joystick as quickly as possible directly forward or backward according to the instructions on the screen. As an example in one block participants were asked to push the lever when they saw a happy face and to pull when they saw an angry face; in another block they were asked to pull when they saw a happy face and to push when they saw an angry face. The order of the blocks was randomized across participants. Each stimulation started with a blank slide followed by a black fixation cross for an average of 1723 ms, which indicated the arrival of the visual and the olfactory stimulus. The olfactory stimulus was presented throughout the experiment (please note that it was clean air only), the visual stimulus lasted on the screen until the participants inclined the joystick either backward of forward for approximately 30° and the response was recorded. After three blocks participants were shown four cartoon faces representing the moods angry, happy, scared and neutral and were asked to rate on visual analogous scales (VAS) how well the faces described their own mood. Then they were asked to rate intensity, pleasantness and familiarity of the olfactory stimulus on visual analogous scales. All VAS were labeled from 'not at all' anchored on the left to 'very much' anchored on the right. During the whole experiment, participants were listening to white-noise to avoid environmental distractions. The experimenter was present throughout the duration of the experiment.

#### 3.2.5 STATISTICAL ANALYSES

The raw-data were obtained via EPrime (2.0) and the analyses were conducted via SPSS (17.0). To test for possible differences in the median RT as a function of experimental conditions Analysis of Variance (ANOVA) for the reaction times (RT) with

'facial expression' (angry,happy, neutral) as within subject factors was performed. To ascertain the approach-avoidance median RT the following steps were followed: (i) inaccurate responses, such as a misunderstanding of instructions (pushing while instructed to pull) were excluded, (ii) RTs differing more than two or minus two SD from the median RTs were removed, (iii) response latencies below 200 ms and above 2,000 ms were discarded. These cutoff criteria were shown to yield the greatest sensitivity of approach–avoidance measures (Krieglmeyer & Deutsch, 2010). The AAT bias score for the median RTs was calculated as described in the general introduction (see page 0). Bonferroni corrections ( $\alpha$ -level: p < 0.05) were applied, when required.

#### 3.2.6 RESULTS

#### **Reaction Times**

A main effect of 'facial expression' was found [F (1, 9) = 5.669, p = 0.029]. Post-hoc contrasts revealed that participants pushed significantly faster to angry faces as compared to happy faces, which is clearly visible, when comparing the AAT bias scores (-51 ms vs. 8 ms). This reveals a significant tendency to avoid angry faces as compared to the attempt to approach happy faces. Although not significant, the neutral faces show a marginal avoidance tendency (AAT bias score: - 4 ms).

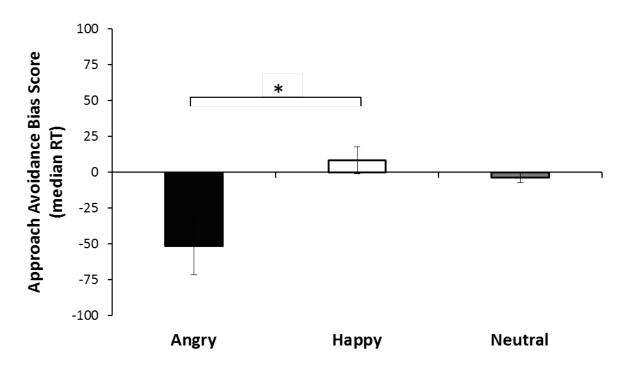


Figure 8: AAT median bias scores for Experiment 1

#### 3.2.7 DISCUSSION

The aim of the present study was to test the AAT in a unimodal context to social visual stimuli with healthy participants, in order to replicate previous findings. In addition, given our interest in multimodal integration, clean air was delivered during the task. It constitutes a non-informative olfactory stimulation useful in creating a baseline for further visuo-olfactory investigations. Experiment 1 confirms the affective arm movement congruency effect previously shown (Chen & Bargh, 1999 and Solarz, 1960). Angry as compared to happy faces facilitated arm extension, indicating an avoidance tendency. These findings confirm the notion that a potential threat – such as an angry face – has a negative connotation, which prompts an automatic avoidance reaction. As expected, the motivational tendency associated with happy faces is of approach. Interestingly, the

neutral face is considered as a stimulus to be potentially avoided. A possible explanation for this result is that the neutral face represents an emotionally ambiguous stimulus, which is known to be able to potentiate fear responses (Whalen et al., 2009).

In conclusion the present experiment allowed to replicate previously published findings and confirms the reliability of the present AAT setup, opening the possibility of studying approach and avoidance tendencies in a multimodal context.

# 4. APPROACH/AVOIDANCE BEHAVIOR TO EMOTIONAL VISUAL STIMULI UNDER THE EXPOSURE TO COMMON ODORS DIFFERENT IN VALENCE

#### **4.1** INTRODUCTION

The aim of the present study was to evaluate for the first time approach and avoidance tendencies via the AAT in a multimodal (visuo-olfactory) context. The same emotional visual stimuli as in the first experiment were used and additionally odors with differing valence – namely, a neutral, a pleasant and an unpleasant common odor – were presented.

#### **4.2 MATERIALS AND METHODS**

#### 4.2.1. PARTICIPANTS

Twenty-one participants (eleven males, ten females) reporting normal or corrected-tonormal vision and normal smell abilities were recruited via public advertisements and asked to respond to visual stimuli by pushing or pulling a joystick while smelling a series of olfactory stimuli differing in valence. All participants were naïve as to the purpose of the experiment. Two datasets of male participants had to be excluded from the final analyses: one participant did not finish the experiment due to fatigue; another participant did not follow the given instructions. Detailed written informed consent was obtained from all participants. The experimental procedures were approved by the Institutional Review Board of the University of Pennsylvania and Monell Chemical Senses Center and were in accordance with the principles of the Declaration of Helsinki. One session lasted approximately two hours and participants were rewarded upon completion of the study with a total of \$40.

#### 4.2.2. STIMULI

The same visual stimuli as reported in Chapter 3.2.2 were used (see page 33). Each stimulus was presented four times throughout the whole experiment. Three different olfactory stimuli were presented: Myrrh resoid 100 % (CAS-Nr. 8016-37-3), Gardenia (CAS-Nr. 5182-36-5) 6.25 % in Propylenglycole (PG) and Butyric Acid (CAS-Nr. 107-92-6) 6.25 % in PG. Four drops (200  $\mu$ l) of each odor solution were pipetted on individual nursing pad quadrants (Ultra-Thin Nursing Pads, Gerber Inc., ON, Canada). The quadrants imbued with one of the three odors were placed in a single Nalgene odor jar (4 oz; 74 mm x 64 mm x 64 mm) that was connected to a specific odor line to the olfactometer (Lundström et al., 2010). This equipment allowed the delivery of temporally precise, uncontaminated and fixed concentrations of odors to human subjects.

#### 4.2.3. APPARATUS

The same experimental setup was used as described in Chapter 3.2.3 (see page 33) with the only exception that the flow-rate for all olfactory stimuli was calibrated to 3.5 liters/minute. The decision to elevate the flow rate was made, as it is a common calibration, widely used in earlier studies in which actual odors were delivered.

#### 4.2.4. PROCEDURE

The experimental procedure was similar to the one described in Chapter 3.2.4 (see page 35), modifications to the procedure will be explained in detail below. Instead of the STAI, participants were asked to answer the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA; Gros et al., 2007). The decision of using the STICSA instead of the STAI was made because the STICSA better differentiates between cognitive and somatic anxiety symptoms (Elwood et al., 2012). As before, the experiment was divided into six blocks and the order of the blocks was randomized across participants. Each block started and ended with the evaluation of the three different odors in terms of intensity on visual analogous scales (VAS). In each block participants were shown 78 different visual stimuli showing a combination of angry and happy, angry and neutral or happy and neutral facial expressions. The participants were instructed to respond to the visual stimulus by moving the lever of the joystick as quickly as possible directly forward or backward according to the instructions on the screen (see Chapter 3.2.4, page 35). Each block was divided into 24 trials in which two, three or four pictures were presented with one olfactory stimulus. The presentation of the olfactory stimuli was randomized for each trial. A blank slide was followed by a black fixation cross. The black fixation cross turned green to indicate the arrival of the visual and olfactory stimulus at the beginning of each trial. The olfactory stimulus was presented for approximately 7 to 10 s, depending on the number of faces presented within each trial (range 2-4 faces). Each visual stimulus lasted on the screen until the participants moved the joystick either backward or forward for approximately 30° which recorded their response. Between the

visual stimuli of each trial, a black fixation cross was shown for 845 ms on average. Each trial ended with the evaluation of the presented odor in terms of pleasantness on VAS. The order of both the trials and the blocks was randomized. All VAS were labeled from 'not at all' anchored on the left to 'very much' anchored on the right of a 10 cm line. Throughout the whole experiment, participants were listening to white-noise to avoid environmental distractions. The experimenter was present throughout the duration of the experiment.

#### 4.2.5. STATISTICAL ANALYSES

The raw-data were recorded via EPrime (2.0) and the statistical analysis was conducted via SPSS (17.0). To evaluate odor intensity ratings a 3 X 2 X 6 repeated measures ANOVA with 'odor' (Myrrh, Gardenia, Butyric Acid), 'rating order' (before block, after block) and 'block' (AH, AN, HN push – pull and vice versa) as within subject factors was performed. To assess pleasantness ratings a 3 X 6 repeated measures ANOVA for the odor pleasantness ratings with 'odor' (Myrrh, Gardenia, Butyric Acid) and 'order of block presentation' (1<sup>st</sup> block to 6<sup>th</sup> block) as within subject factors was performed. The randomization of the block order was considered and the sequence in which the blocks were presented was kept. Thus, the evaluation was not conducted for the task for each block (AH, AN, HN), but for the block order, which differed for each participant. To ascertain the approach avoidance RTs the following steps were followed: (i) inaccurate responses, such as a misunderstanding of instructions (pushing while instructed to pull) were excluded, (ii) RTs differing more than two or minus two SD from the median RTs were removed, (iii) response latencies below 200 ms and above 2,000 ms were

discarded. These cutoff criteria were shown to yield the greatest sensitivity of approachavoidance measures (Krieglmeyer & Deutsch, 2010). One participant was not included in the final analysis due to the fact that he misunderstood the instructions to one block and therefore the percentage of correct responses was lower than 10%. A 3 X 3 repeated measures ANOVA for the RT with 'odor' (Myrrh, Gardenia, Butyric Acid) and 'facial expression' (angry, happy, neutral) as within subject factors was performed. The bias score was calculated for each condition on the base of median RTs (see General Introduction, page 25). Bonferroni corrections ( $\alpha$ -level: p < 0.05) were applied, when required.

#### 4.2.6. RESULTS

#### Intensity

The analysis revealed no main effect for 'odor' [F(2, 10) = 1.773, p = 0.226]. No two-way interaction was shown for 'odor' and 'rating order' [F(2, 10) = 0.865, p = 0.450], 'odor' and 'block' [F(10, 50) = 1.057, p = 0.412] or 'rating order' and 'block' [F(5, 25) = 1.413, p = 0.254]. Similarly, no three-way interaction for 'odor', 'rating order' and 'block', [F(10, 50) = 1.421, p = 0.199] was shown.

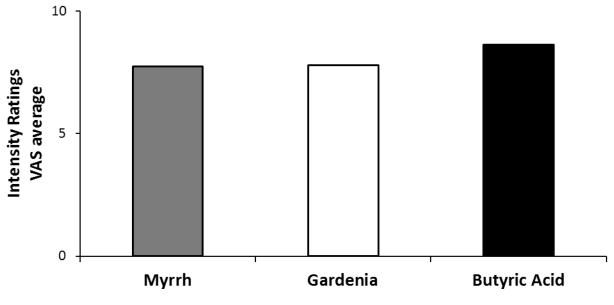


Figure 9: Intensity Ratings for Myrrh, Gardenia, Butyric Acid

#### Pleasantness

The results show a main effect of 'odor' [F(2, 38) = 62.419, p = 0.000]. Post hoc contrasts reveal that Gardenia was significantly more pleasant than Myrrh (neutral odor) and Butyric Acid (unpleasant odor; ratings: 7.83 vs. 5.00 vs. 3.66), respectively and that Myrrh was significantly more pleasant than Butyric Acid (ratings: 5.00 vs. 3.66). No main effect was found for 'order of block presentation' [F(5, 95) = 0.404, p = 0.845] or the two-way interaction of 'odor' and 'block' [F(10, 190) = 1.547, p = 0.125]. Thus, the participants rated the pleasantness of the olfactory stimuli similarly in each block.

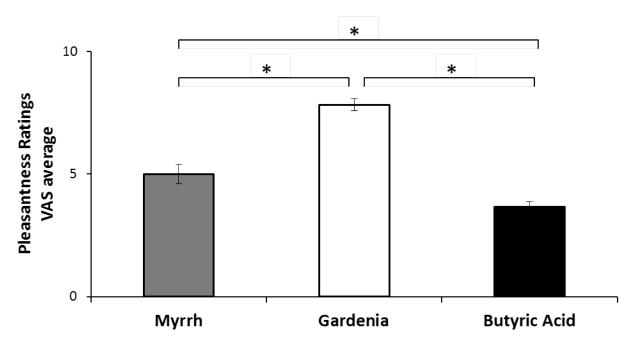


Figure 10: Pleasantness ratings for Myrrh, Gardenia, Butyric Acid

#### Reaction Times

The analyses revealed that no main effect of 'odor' [F(2, 38) = 0.571, p = 0.569] or 'facial expression' [F(2, 38) = 1.956, p = 0.155] was found. In addition the interaction 'odor by facial expression', yielded no significant effect [F(4, 76) = 2.040, p = 0.097].

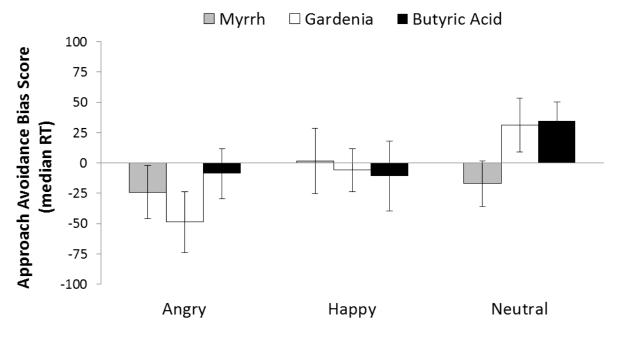


Figure 11: AAT median bias scores for Experiment 2

#### 4.2.7. DISCUSSION

The aim of the present study was to evaluate whether approach and avoidance motor tendencies to emotional visual stimuli (measured via the AAT) can be influenced by common olfactory stimuli differing in pleasantness. In order to avoid confounding effects on the odor pleasantness (Moskowitz et al., 1974), the olfactory stimuli should be isointense but, for the sake of the present experiment, different in valence. As the results show, the participants rated the odors to be isointense (Figure 9). Further, participants rated the odors to be significantly different in their pleasantness (Figure 10). On average, all participants rated the three odors Myrrh, Gardenia and Butyric acid as neutral, pleasant and unpleasant, respectively. It is also worth noting that these ratings remained constant throughout the experiment.

The outcomes from the repeated measure ANOVA of the AAT bias scores revealed no effect of the used odors on the behavioral approach avoidance reaction time response. Thus, this seems to indicate that this type of motivational motor response is not influenced by a 'common odor' conveying a positive, a negative or a neutral connotation.

These outcomes show that a difference in odor valence does not influence the approach-avoidance responses to social emotional visual stimuli such as angry, happy and neutral human faces. Thus, using a social visual and a non-social olfactory stimulus does not influence our motivational behavior as measured via the AAT paradigm. However, it might be the case that this lack of a significant result is due to the fact that the olfactory stimulus does not signal any social property. What happens when an emotional visual stimulus is presented with a social olfactory signal in the form of a body odor is the object of the next experiment.

# 5. APPROACH/AVOIDANCE BEHAVIOR TO EMOTIONAL VISUAL STIMULI UNDER THE EXPOSURE TO COMMON AND BODY ODORS

#### **5.1** INTRODUCTION

The aim of this study was to introduce a social signal, namely a body odor in the previously used paradigm in order to evaluate whether a social chemosignal can alter the approach avoidance reaction to social visual stimuli with emotional valence. As discussed in Chapter 1.3 (see page 20), body odors can highly influence our behavior in many different ways and they have to be distinguished when evaluating common odors (Lundström et al., 2008). Even though research has helped to conspicuously increase our knowledge in the field of body odor processing, the effect of body odors on our motor behavior regarding approach and avoidance tendencies has received no scientific attention, so far. Therefore, the aim is to provide preliminary information in this direction.

#### **5.2 MATERIALS AND METHODS**

#### 5.2.1 PARTICIPANTS

Preliminary data from a pilot study on four participants (two males, two females) were included. Participants were all working at Monell Chemical Senses Center at the time of the study. They reported normal or corrected-to-normal vision and normal smell abilities and they were asked to respond to visual stimuli by pushing or pulling a joystick while smelling different olfactory stimuli: Clean air with Myrrh (CAM), and Body Odor with Myrrh (BOM). The participants were naïve as to the purpose of the experiment and participated voluntarily.

#### 5.2.2 STIMULI

We used the same visual stimuli as described in Chapters 3.2.2 and 4.2.2 (see pages 33 and 42). Two different olfactory stimuli were used for the experiment: CAM, BOM. CAM stimuli were different for each participant given that each stimulus consisted of a supra-donor stimulus of four same-sex stranger individuals. In other words, the body odor collected from 4 different male donors were merged and presented to an unknown male participant. The same procedure was followed for female CAM stimuli. The body odor collection was conducted using an existing procedure previously used in the laboratory (Mitro et al., 2012). Each participant was presented with a different supradonor stimulus to avoid potential effects mediated by individual odor donors. One clean pad quadrant (Ultra-Thin Nursing Pads, Gerber Inc., ON, Canada) was placed in each of the different Nalgene odor jars (4 oz; 74 mm x 64 mm x 64 mm; see Figure 4, page 34). For the CAM condition, four additional clean pad guadrants were placed along the wall of the jar. For the BOM condition the four pads generating the supra-donor stimulus were placed like the ones in the CAM condition. For both conditions, CAM and BOM additionally 200 µl of Myrrh resoid 100 % IFF (CAS-Nr. 8016-37-3) were pipetted on the clean pad at the bottom of each jar. The two jars were connected with an olfactometer (see Figure 5, page 34) as to allow a temporally accurate and stable presentation of the stimulus throughout the experiment (Lundström et al., 2010).

#### 5.2.3 APPARATUS

The same experimental setup was used as described in 4.2.3. (see page Seite 42).

#### 5.2.4 PROCEDURE

The experimental procedure was similar to the one described in 4.2.4 (see page 43), modifications to the procedure will be explained in detail. Before and after the experimental motor task, participants had to discriminate the odors in a discrimination test (3 alternative forced-choice, 3 AFC; Swets, 1964). In this test, participants had to discriminate CAM and BOM. Each test consisted of eleven randomized trials. In each trial, participants had to sniff three odors, two of which were different, and had to indicate, which was the different one.

#### 5.2.5 STATISTICAL ANALYSES

The raw-data were obtained via EPrime (E-Studio 2.0) and the statistical analysis was conducted via SPSS (17.0). To evaluate the possibility to discriminate BOM from CAM, the number of times the participant correctly identified BOM as the different odor was calculated. A percentage value was subsequently calculated. To assess odor intensity and odor pleasantness, within-group comparisons for 'odor' (CAM, BOM), 'rating order' (before and after block) and 'block' (AH, AN, HN – push pull and vice versa) were made with a non-parametric Friedman's test. The randomization of the block order was considered and the sequence in which the blocks were presented was kept. Thus, the

evaluation was not conducted for the task for each block (AH, AN, HN), but for the block order, which differed for each participant. Within-group comparisons for the median RTs were made with Friedman's test for 'odor' (CAM, BOM) and 'facial expression' (angry, happy, neutral). To ascertain the approach avoidance RT the following steps were followed: (i) inaccurate responses, such as a misunderstanding of instructions (pushing while instructed to pull) were excluded, (ii) RTs differing more than two or minus two SD from the median RTs were removed, (iii) response latencies below 200 ms and above 2,000 ms were discarded. These cutoff criteria were shown to yield the greatest sensitivity of approach–avoidance measures (Krieglmeyer & Deutsch, 2010). The AAT bias score for the median RTs was calculated as described in the general introduction (see page 25). The significance level was set at p > 0.05.

#### 5.2.6 RESULTS

#### Discrimination test

On average, all participants could discriminate the odors correctly in 45.45 % of the trials before the experimental motor task and in 22.73 % after the experimental motor task  $[\chi^2(3) = 6.568, p = ns]$ . The results show, that the participants could not discriminate the BOM from the CAM odor above chance either before  $[x^2(3) = 2.750, p = ns]$  or after  $[x^2(3) = 2.138, p = ns]$  the main experiment. The percentage for the trials after the main experiment is not significantly different from the chance level (33%). Thus, participants were unable to discriminate the two odors across the experiment.

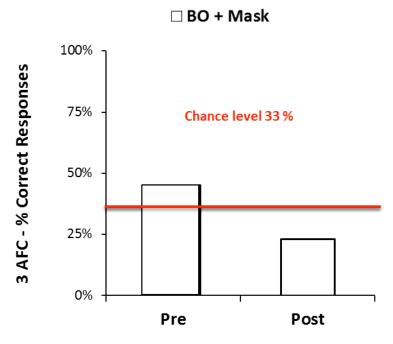


Figure 12: 3 Alternative Forced Choice correct responses

#### Intensity

The analysis yielded to no significant effect for 'odor' [ $x^2$  (4) = 26.122, p = 0.295], thus the odors can be considered as being isointense.

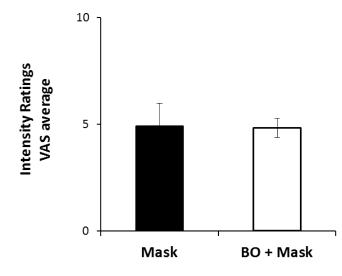


Figure 13: Intensity Ratings for CAM, BOM

#### Pleasantness

The analysis show no significant effect,  $x^2(4) = 7.955$ , p = 0.717, and indicate that the odors are similar in pleasantness.

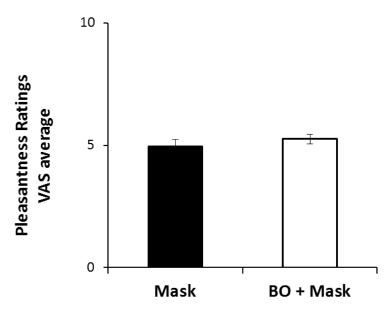


Figure 14: Pleasantness Ratings for CAM, BOM

#### **Reaction Times**

No significant effect for 'odor' and 'emotion' was found, similarly, no interaction for 'odor by facial expression' was found,  $x^2(4) = 4.571$ , p = 0.470. This is not surprising given the limited number of participants. However, an interesting trend can be highlighted: while the bias scores in the CAM condition were relatively similar among the different facial expressions, in the BOM condition, the bias score for an angry facial expression was negative, for the happy facial expression positive and for the neutral facial expression slightly negative.

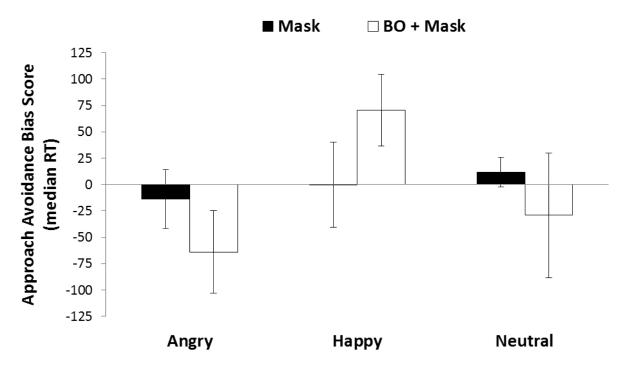


Figure 15: AAT median bias scores for Experiment 3

#### 5.2.7 DISCUSSION

The aim of this pilot study was to use the paradigm described in Chapter 4 (see page 41) and add a social component to the olfactory stimuli. Thus, body odor masked with a common odor (BOM) and the same common odor without body odor (CAM) as control condition were used. Considering the results of the discrimination test, it is clear that participants could not discriminate between the BOM and CAM odors at a conscious level. Similarly, both the intensity and the pleasantness ratings appear to be equivalent for the two conditions. Therefore, the two odors can be considered equal in terms of conscious perception. Although the data analysis revealed no significant comparison – most likely due to the limited number of participants tested – an interesting pattern is

discernible. The behavioral motor response to a social visual stimulus is not influenced by a non-social olfactory stimulus. The interaction for odor and facial expression is not significant; however, the median AAT bias scores show a different pattern in the two conditions. While in the CAM condition, the bias scores were rather similar for all three facial expressions. Participants tend to be faster in the BOM condition when avoiding angry or neutral facial expressions and when approaching happy facial expressions. This seems to indicate, that even though the odors are consciously not discriminable, the chemosensory signals elicited from the body odors have a dissociative influence on the behavioral response in the AAT. An explanation could be that we tend to avoid potential threatening stimuli more when we perceive body odor from a strange, and therefore potentially threatening individual. Because a neutral facial expression is ambiguous, it might be subconsciously regarded as negative and thus elicit avoidance behavior. The small sample size can only show tendencies and therefore no definitive conclusions can be drawn. However, the outcomes revealed interesting aspects and by administering this paradigm to a higher number of participants, significant results showing the influence of chemosensory signals on our evaluative behavior are expected.

#### **GENERAL CONCLUSIONS**

The large variety of stimuli we encounter in our daily environment contributes to our effective navigation of the world. Accurately perceiving the stimuli, integrating their information and correctly evaluating them in accordance to their positive or negative potential allows an individual to appropriately act in the environment and therefore to maximize its chances of survival (Darwin, 1872/1998 and Lang et al., 1990). Motor acts can be organized on the basis of two basic motivational tendencies, such as approach or avoidance behaviors (Bargh, 1997, Cacioppo et al., 1993, Chen & Bargh, 1999, Davidson et al., 1990, Gray, 1994 and Lang et al., 1990).

In general, people tend to approach positively evaluated stimuli and to avoid negatively evaluated ones (Chen & Bargh, 1999, Rotteveel & Phaf, 2004 and Solarz, 1960). In the lab, a way to investigate approach–avoidance motor tendencies in humans is offered by the approach avoidance task (Rinck & Becker, 2007), an experimental paradigm measuring the reaction time of arm movements used to evaluate the emotional valence of affective stimuli.

Capitalizing on this paradigm, the present project aimed at investigating whether and how common odors or social chemosignals can influence our evaluative motor behavior when reacting to emotional positive and negative visual stimuli with a social connotation.

The same visual stimuli – human faces, showing angry, happy or neutral facial expressions (Lundquvist et al., 1998) – were used for all three experiments, thus

enabling us to make direct comparisons between the different experimental outcomes. Although aware of the fact that emotions cannot be uniquely reduced to negative, positive and neutral, angry, happy and neutral facial expressions, as they are – especially angry and happy – probably amongst the most meaningful and most directly assessed human emotions, were used. While the visual stimuli remained the same, the odors were changed intentionally in each experiment. Whereas in Experiment 1 only clean air was presented as means to reproduce earlier findings and to test the paradigm, non-social common odors were used in Experiment 2 to evaluate how odor valence influences the evaluative behavior. Finally, in Experiment 3 a combination of non-social common and social odors was presented to evaluate the subconscious influence of social odors on our evaluative behavior.

The results in Experiment 1 confirmed what other studies had shown before: we avoid negative stimuli and approach positive ones (Chen & Bargh, 1999, Rotteveel & Phaf, 2004 and Solarz, 1960). The slightly negative AAT bias score (Cousijn et al., 2011) for the neutral faces indicates that we tend to avoid them. A possible explanation for this might be that we are unsure about an ambiguous face and prefer to avoid it, unless we know what the exact intention of the individual representing the face.

With the results confirming that the experimental setup works, common olfactory stimuli were added in Experiment 2. Here it was demonstrated that common non-social odors differing in valence do not influence the evaluative behavior significantly. The AAT bias scores were similar for each facial expression among the different odors. In conclusion, it can be said that odor valence in its isolated form is not a variable that influences evaluative behavior. However, it should be noted that the odors used had no

direct relation to the visual stimuli as a social visual stimulus was paired with a nonsocial olfactory stimulus.

This, in consequence, aroused the interest to examine what happens in the AAT with a masked social odor when showing social visual stimuli. Therefore, in Experiment 3 a social odor masked with a non-social neutral common odor and the common odor alone were used with the paradigm from Experiments 1 and 2. Participants were not able to discriminate the two presented odors from each other; thus, a difference in the response would indicate a behavioral influence of the body odor at a subconscious level. Due to the small sample size, no significant results could be retrieved, but a different pattern in the bias scores for each condition emerged. Results indicate that body odor might have an influence on the motor evaluative behavior. In the CAM condition (odor only), the AAT bias scores were slightly negative for angry and happy facial expressions and slightly positive for neutral ones. Conversely, in the BOM condition, the bias scores were negative for angry and neutral and positive for happy facial expressions. In general, the BOM bias scores were considerably higher than the CAM ones. In conclusion, it can be hypothesized that body odor influences the evaluative behavior but it is highly recommended to further investigate this topic as the results are, although not significant, at least promising.

The findings show how common non-social odors and social odors can influence our evaluative behavior as expressed in motor terms. However, the experiments open new questions that would be interesting to investigate in the future. A few ideas for future experiments examining the evaluative behavior using the AAT paradigm could be (1) to use non-social visual stimuli with corresponding odors (e.g. floral images with a

floral odor vs images of rotten food with a malodor), (2) to use social visual emotional stimuli showing expressions such as fear or disgust with social odors or (3) to use social visual emotional stimuli with different social olfactory stimuli e.g. stressed vs. relaxed body odor, kin vs. non-kin body odor, self vs. stranger body odor.

## SECTION III

### **A**PPENDIX

### **APPENDIX** I

In order to achieve outcomes of a high value in our main experiment, certain criteria had to be clarified prior to it. Therefore, several different pilot studies were conducted, two of which will be explained in detail below. In the first experiment the focus lay on the selection of suitable facial expressions (Pilot 1). The aim of the second pilot study (Pilot 2) was to find the best flow rate for the presentation of the different olfactory stimuli.

#### 1. PILOT 1 – SELECTING ANGRY, ANGRY AND NEUTRAL FACES

#### 1.1.1. PARTICIPANTS

Nine participants (three males, six females) working at Monell Chemical Senses Center were asked to rate visual stimuli on different dimensions.

#### 1.1.2. STIMULI

For selecting the visual stimuli five different databases were evaluated

- FRI Computer Vision Laboratory Face Database (Solina et al., 2003)
- Karolinska Detected Emotional Faces (KDEF) (Lundqvist et al., 1998)
- NimStim Set of Facial Expressions (Tottenham et al., 2009)
- 2D facial emotional Stimuli Upenn (Gur et al., 2002)
- AR Face Database (http://www.ece.osu.edu/~aleix/ARdatabase.html)

After having evaluated all the databases, several reasons led to the choice of the KDEF. First, the facial expressions of interest for the present study (e.g. happy, angry, neutral) were displayed in separate captions by each of the models recruited. Second, images were collected under the same lightning conditions and they were saved in a high-resolution format. This constituted an advantage when comparing the KDEF to most of the other databases retrieved. Third, the model's T-shirts had a uniform color; the faces were centered and positioned in fixed image coordinates. This feature was considered as being important as it would not distract the participant from the facial expression shown and it would reduce the variability accounted for inter-individual differences. Only pictures taken from a frontal angle were selected to maximally ensure the conformity among pictures. The KDEF was also selected because only Caucasian models are included. This reduces the possible variability resulting from a mismatch between the participant and the model's race, as previously reported in the literature (Cunningham et al., 2001; Hendricks & Bootzin, 1976). The aim of this pilot study was to select from the KDEF database those pictures that best showed the facial expressions of interest.

#### 1.1.3. PROCEDURE

In our pilot study, each of the resulting 201 stimuli was shown once. Participants were presented on a screen with all the stimuli in a random order and asked to rate each stimulus on four dimensions: degree of attractiveness (Figure 15), happiness, anger and, if the picture caused a feeling of strangeness (Figure 16) to them. The presentation of the images and the ratings were conducted via EPrime. The visual analogous rating scales for attractiveness, happiness and anger were labeled from an extremely negative pole to an extremely positive pole, considering one dimension at a time. As an example, when evaluating the attractiveness of the face, the participants were asked to provide

their answer on a scale anchored to "Extremely unattractive" on the left side and "Extremely attractive" on the right side (Figure 15). The participants were asked to tag on the scale via a mouse click. Finally, the participants were asked to rate whether the face was 'strange' or not (Figure 16). The concept of 'strange' was clarified in the instructions as something in the face providing an uneasy feeling. The task lasted approximately 30 minutes per participant.

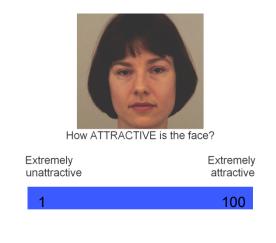


Figure 15: Rating for attractiveness of presented pictures on VAS



Is there something STRANGE about this face?



Figure 16: Rating for strangeness of presented pictures

### 1.1.4. STATISTICAL ANALYSES

The raw-data were obtained via EPrime and were exported into Excel where the ratings were evaluated. The score of the happiness, anger, and attractiveness ratings ranged from 0 to 100. For the attractiveness rating, 0 meant "Extremely attractive" and 100 "Extremely unattractive". For the strangeness ratings the score was either one for a positive strangeness rating, or two for a negative one.

#### 1.1.5. RESULTS

We started with evaluating the responses to the strangeness rating. This was considered as the most important criterion to exclude an image, as participants might get distracted from strange faces and, therefore, they could possibly not focus on the facial expression, which could influence the outcome of our future experiment. The result for the strangeness rating was either one or two, where one corresponded to "yes" and therefore meant, that the presented face was strange and two corresponded to "no" and indicated no strangeness for the participant. The three-picture-sets were excluded when either one alone, two, or all three pictures of the set together received five or more positive 'strange' ratings by the participants. This led to the exclusion of 37 picture-sets (16 female, 19 male). The next step was the evaluation for the ratings of attractiveness. Only pictures of people considered attractive 'on average' were included, as both extremes (extremely attractive and extremely unattractive) might lead the participants to being distracted from the facial expression. As all of the outcomes of the attractiveness rating ranged between slightly unattractive and average attractive (37-57 for women, 33-51 for men), this parameter did not lead to an exclusion of any picture-sets. Thereafter, the ratings of happiness were evaluated. These evaluations were of special importance as the outcome would have a significant meaning for the main experiment. If the facial expressions could not get discriminated appropriately, the approaching behavior to the picture, which is the main parameter of the experiment, could not be representative. Thus, it is essential that the facial expressions shown by the images can get recognized unambiguously. For the happiness rating, 0 corresponded to "Very unhappy" and 100 to "Very happy", faces with an average rating below 74.8 for women (average rating =

76.22, sd = 7.59) and 59.4 for men (average = 71.79, sd = 7.09) were excluded. This led to exclusion of 6 sets showing female models and of 1 set showing a male model. Then the ratings for anger were evaluated. In the evaluation of these scales, 0 corresponded to "Not angry at all" and 100 to "Very angry". The image sets were excluded, when the average rating was below 46.2 for women (average = 67.84, sd = 8.86) and 63.5 for men (average = 74.06, sd = 7.14). After the rating evaluations, 78 pictures (sets of three images of 13 males and 13 females) matched the desired criteria for the main experiment.

### 2. PILOT 2 – SELECTING THE AIRFLOW FOR THE OLFACTOMETER

#### 2.2.1. PARTICIPANTS

Four participants (all female) were presented with three different odor stimulations in two different airflow conditions. All of them were working at Monell Chemical Senses Center and participated voluntarily.

### 2.2.2. STIMULI

The participants were presented with three different odor stimulations (clean air, clean Tshirt, neutral body odor) under two different air-flow-conditions (1 liter/ minute and 1.5 liters/ minute) via an olfactometer (Lundström et al., 2010). Our aim was to find out, which of the two airflow conditions would lead to a better perception of the presented odors. As the flow-rate can have a high influence on the perception of an odor, this was considered as being important for our main experiment.

#### 2.2.3. PROCEDURE

First, three different Nalgene odor jars (4 oz; 74 mm x 64 mm x 64 mm) were prepared. The first jar was empty and it constituted the clean air stimulus. The second jar contained small pieces of a clean T-shirt (5 x 5 cm), comparable in texture and seize to the ones used for the body odor collection. The third jar contained small pieces of a tshirt impregnated with neutral body odor. Particular attention was paid on cutting and arranging the pieces of the clean and contaminated T-shirts as similar as possible.

After putting the jars in the olfactometer, the airflow for each jar had to be calibrated using a 'Gilian 2 Primary Flow Calibrator'. After having calibrated the flow for the three different odor jars, the first participant was tested. Between the two different blocks, the airflow for the three different jars had to be recalibrated each time. Each condition, which consisted of 15 randomized presentations of the three odors mentioned above, was presented once and lasted approximately ten minutes. The odorpresentation was obtained using a nasal manifold, which led the odor directly to both nostrils. The manifold was attached to the participant's chest with a chest-strap and received the odors from the olfactometer. The participants were asked to make their ratings on a computer using the program Eprime (E-Studio 2.0). After brief introductory instructions on the screen, the participants were asked to concentrate on a black fixation-cross in the middle of the screen. The fixation-cross turning green indicated that the odor was about to be presented. After the green fixation-cross disappeared, the instruction "please sniff" appeared on the screen. The odor-presentation lasted two seconds and afterwards the participants were asked to make their ratings concerning their ability to detect the odor, degree of intensity and pleasantness, and their ability to

identify the odor. First, participants were asked, if they were able to smell anything, or not. For the evaluation, the ratings of participants recording, that they did not smell anything were considered. The reason for including those ratings, was that the odor might induce an unconscious reaction. Then, the participants were asked to rate the intensity and pleasantness of the odor on VAS. The scales were labeled "Very weak" respectively "Very pleasant" on the left pole and "Very strong" respectively "Very unpleasant" on the right pole. Finally, the participants were asked, if they were able to identify the presented odor. Their responses were recorded on paper.

#### 2.2.4. ANALYSIS

The raw-data were obtained in Eprime (2.0) and were analyzed in Excel.

#### 2.2.5. RESULTS

For both ratings, pleasantness and intensity, zero corresponded to either "Very weak" or "Very unpleasant" and ten to "Very strong" or "Very pleasant". Only ratings were considered, where the participants confirmed their given answers by a mouse-click.

First, the ratings for "Intensity" for each of the three odors were compared. Surprisingly, the participants on average rated the intensity of clean air and the clean Tshirt higher for the 1-liter-per-minute-condition, than for the 1.5-liters-per-minutecondition (clean air: 3.2 compared to 2.4, clean T-shirt: 2.4 compared to 1.6). For the body-odor, the 1.5-liters-per-minute flowrate condition of the "Intensity" was rated higher (3.4 compared to 3.0).

The outcomes for the pleasantness rating under the 1-liter-per-minute-condition were 5.4 for clean air, 5.5 for clean T-shirt and 5.3 for body odor. For the 1.5-liters-per-minute-condition it was 5.8, 5.6 and 4.9, correspondingly. Thus, participants found the 1-liter-per-minute-condition less pleasant for both, clean air and clean T-shirt, while it was reversed for the body odor. As the pleasantness of the clean-T-shirt-odor in the 1.5-liters-per-minute-condition was more similar to the one of the T-shirt containing body odor compared to clean air, this encouraged us in our intention to take the clean T-shirt as a control-condition.

# **APPENDIX II:**

### 1. STICSA (GROS ET AL., 2007)

### STICSA-S STICSA: Your Mood at this Moment

Below is a list of statements, which can be used to describe how people feel. Beside each statement are four numbers, which indicate the degree with which each statement is self-descriptive of mood at this moment (e.g., 1 not at all, 4 very much so). Please read each statement carefully and circle the number which best indicates how you feel right now, at this very moment, even if this is not how you usually feel.

		Not at all	Somewhat	Moderately	Very much so
1.)	My heart beats fast.	1	2	3	4
2.)	My muscles are tense.	1	2	3	4
3.)	I feel agonized over my problems.	1	2	3	4
4.)	I think that others won't approve of me.	1	2	3	4
5.)	I feel like I'm missing out on things because I can't make	1	2	3	4
	up my mind soon enough.				
6.)	I feel dizzy.	1	2	3	4
7.)	My muscles feel weak.	1	2	3	4
8.)	I feel trembly and shaky.	1	2	3	4
9.)	I picture some future misfortune.	1	2	3	4

10.) I can't get some thought out of my mind.	1	2	3	4
11.) I have trouble remembering things.	1	2	3	4
12.) My face feels hot.	1	2	3	4
13.) I think that the worst will happen.	1	2	3	4
14.) My arms and legs feel stiff.	1	2	3	4
15.) My throat feels dry.	1	2	3	4
16.) I keep busy to avoid uncomfortable thoughts.	1	2	3	4
17.) I cannot concentrate without irrelevant thoughts intruding.	1	2	3	4
18.) My breathing is fast and shallow.	1	2	3	4
<ol> <li>I worry that I cannot control my thoughts as well as I would like to.</li> </ol>	1	2	3	4
20.) I have butterflies in the stomach.	1	2	3	4

### STICSA-T STICSA: Your General Mood State

Below is a list of statements, which can be used to describe how people feel.

Beside each statement are four numbers, which indicate how often each statement is true of you (e.g., 1 not at all, 4 very much so). Please read each statement carefully and circle the number which, best indicates how often, in general, the statement is true of you.

Not at all	Somewhat	Moderately	Very much so
1	2	3	4

### 1. My heart beats fast.

2.	My muscles are tense.	1	2	3	4
3.	I feel agonized over my problems.	1	2	3	4
4.	I think that others won't approve of me.	1	2	3	4
5.	I feel like I'm missing out on things because I can't make up my mind soon enough.	1	2	3	4
6.	I feel dizzy.	1	2	3	4
7.	My muscles feel weak.	1	2	3	4
8.	I feel trembly and shaky.	1	2	3	4
9.	I picture some future misfortune.	1	2	3	4
10	I can't get some thought out of my mind.	1	2	3	4
11	. I have trouble remembering things.	1	2	3	4
12	. My face feels hot.	1	2	3	4
13	. I think that the worst will happen.	1	2	3	4
14	. My arms and legs feel stiff.	1	2	3	4
15	. My throat feels dry.	1	2	3	4
16	I keep busy to avoid uncomfortable thoughts.	1	2	3	4
17	I cannot concentrate without irrelevant thoughts intruding.	1	2	3	4
18	. My breathing is fast and shallow.	1	2	3	4
19	I worry that I cannot control my thoughts as well as I would like to.	1	2	3	4
20	I have butterflies in the stomach.	1	2	3	4

### 2. STAI (SPIELBERGER, 1984)

### STAI-S

A number of statements which people have used to describe themselves are given below. Read each statement and then indicate on the right how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately	Very much so
1.) I feel calm	1	2	3	4
2.) I feel secure.	1	2	3	4
3.) I feel tense.	1	2	3	4
4.) I feel regretful.	1	2	3	4
5.) I feel at ease.	1	2	3	4
6.) I feel upset.	1	2	3	4
7.) I am presently worrying over future misfortune.	1	2	3	4
8.) I feel restless.	1	2	3	4
9.) I feel anxious.	1	2	3	4
10.) I feel comfortable.	1	2	3	4
11.) I feel self-confident.	1	2	3	4
12.) I feel nervous.	1	2	3	4
13.) I feel jittery.	1	2	3	4
14.) I feel high-strung.	1	2	3	4

15.) I feel relaxed.	1	2	3	4
16.) I feel content.	1	2	3	4
17.) I feel worried.	1	2	3	4
18.) I feel over-excited and rattled	1	2	3	4
19.) I feel joyful.	1	2	3	4
20.) I feel pleasant.	1	2	3	4

### STAI-T

A number of statements which people have used to describe themselves are given below. Read each statement and then indicate on the right how generally feel. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which seems to describe your present feelings best.

1.)	I feel pleasant	<ul> <li>Not at all</li> </ul>	Somewhat	ა Moderately	A Very much so
,	I tire quickly.	1	2	3	4
2.)	The quickly.	1	2	5	4
3.)	I feel like crying.	1	2	3	4
4.)	I wish I could be as happy as others seem to be.	1	2	3	4
5.)	I am losing up on things because I can't make up my mind soon enough.	1	2	3	4
6.)	I feel rested.	1	2	3	4
7.)	I am 'calm, cool and collected'.	1	2	3	4
8.)	I feel that difficulties are coming up so I cannot overcome them.	1	2	3	4

9.) I worry too much over something that really doesn't	1	2	3	4
matter				
10.) I am happy	1	2	3	4
11.) I am inclined to take things hard.	1	2	3	4
12.) I lack self-confidence.	1	2	3	4
13.) I feel secure.	1	2	3	4
14.) I try to avoid facing a crisis or difficulty.	1	2	3	4
15.) I feel blue.	1	2	3	4
16.) I am content.	1	2	3	4
17.) Some unimportant thought runs through my mind and	1	2	3	4
bothers me.				
18.) I take disappointments so keenly that I cannot overcome	1	2	3	4
them.				
19.) I am a steady person.	1	2	3	4
<ol> <li>I get in a state of tension or turmoil as I think over my recent concerns and interests.</li> </ol>	1	2	3	4

### 3. BEHAVIORAL QUESTIONNAIRE

### Instructions

These questions are about the kind of person you generally are: that is, how you usually have felt or behaved over the past several years.

- Fill in "Yes" or "No". When your answer is "Yes", please give a brief description in the space provided.
- Answer all questions.

- If a question is unclear, pick the answer that best describes the kind of person that you are. Consider each question and answer with a "Yes" or "No".
- Answer as fast as possible. Usually, the first impression better reflects your answer.
- Have you ever experienced sudden and physically inexplicable heart palpations, sweating, felt that it was hard to breathe, chills, dizzy/ light-headedness, chest pain, and/ or nausea?
- 2. Have you ever felt fatigued and had difficulty concentrating over an extended period of time?
- 3. Have you ever felt that you couldn't and/ or didn't need to sleep for an extended period of time?
- 4. Have you ever felt irritable and easily distracted, with thoughts of hopelessness racing through your mind?
- 5. Have you ever made rash decisions to do pleasurable things that you knew would have painful consequences?
- 6. Do you ever feel afraid that you'll be rejected by others, or that others are judging and disapproving of you? If so, how often?
- 7. Do you ever feel socially inhibited or inadequate?
- 8. Do you ever feel as if everyone is criticizing you?
- 9. Do you ever feel suddenly and inexplicably angry or impulsive?
- 10. Do you ever fear that people are abandoning you?

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# **CURRICULUM VITAE**



## LAURA HACKL

EDUCATION

### UNIVERSITY OF VIENNA Diploma Study of Pharmacy

VIENNA, AUSTRIA October 2006 – March 2013

### PROFESSIONAL EXPERIENCE – HIGHLIGHTS

#### MONELL CHEMICAL SENSES CENTER

Visiting scholar

- Composing of the Master's thesis 'Multisensory Integration of Natural Volatile Stimuli'
- Planning and conducting behavioral experiments
- Miscellaneous Works

### UNIVERSITY OF VIENNA

Teaching Assistant

- Assistance for lab-class "Quantitative Pharmaceutical Analytics"
- Helping students with practical work

### HOFSTÄTTER PHARMACY

### Trainee

- Working in the Formulation
- Miscellaneous Works

### CENTRE HOSPITALIER LUKUNGA, MALWEKA

#### Intern

- Assistance for Receptionist
- Assistance for Medical Staff
- Miscellaneous Works

### HOLLER PHARMACY

TRAUN, AUSTRIA

KINSHASA, DR CONGO

July 2010 – September 2010

Philadelphia, usa

*May 2012 – February 2013* 

VIENNA, AUSTRIA

March 2011 – March 2012

LINZ, AUSTRIA July 2011

<ul><li><i>Trainee</i></li><li>Working in the Formulation</li><li><i>Miscellaneous Works</i></li></ul>	August 2007 August 2009
<b>Retirement Home Traun</b>	Traun, Austria
Trainee	August 2004

- Personal Assistance for the Clients •
- Miscellaneous Works

#### **OTHERS**

### Participant

Solidarity Trip to the Province of Aurora, Philippines ٠

September 2009

August 2012

TCM Summer School in Cheng Du, PR China ٠

LANGUAGES AND TECHNICAL SKILLS

- German Native Language; Fluent in English Spoken and Written; Knowledge in • French; Basic Knowledge in Spanish
- Expert in Mac OS, Microsoft Excel, Word and PowerPoint •