Effects of male sweat on human physiology and behavior

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Content

Index of abbreviations

ANOVA	analysis of variance
AOB	accessory olfactory bulb
ß	response criterion
c	criterion
C	control flow
cm	centimeter
CO_2	carbon dioxide
CSERPs	chemosensory event-related potentials
d’	sensitivity
D	dilution flow
dB	decibel
EEG	electro encephalography
EMG	electromyography
fMRI	functional magnetic resonance imaging
GG	Grueneberg ganglion
HLA	human leukocyte antigene
Hz	Hertz
ISI	inter stimulus interval
l	litre
ME	main exhaust
min	minutes
MOE	main olfactory epithelium
ms	milliseconds
n.s.	not significant
O	odorant flow

OB	olfactory bulb
PET	positron emission tomography
SCR	skin conductance response
SD	standard deviation
SDT	signal detection theory
s	seconds
SO	septal organ of Masera
SPL	sound pressure level
STAI	Spielberger's state-trait anxiety inventory
VAS	visual analogue scale
VNO	vomeronasal organ

Index of tables

II. Chemosensory properties of human sweat

III. Effects of male anxiety chemosignals on the evaluation of happy facial expressions

IV. Sex-specific effects of male sweat on human skin conductance response

Index of figures

I. Introduction

II. Chemosensory properties of human sweat

III. Effects of male anxiety chemosignals on the evaluation of happy facial expressions

IV. Sex-specific effects of male sweat on human skin conductance response

I. Introduction

1. Human sweat

The central relevance of human sweat is thermoregulation, excretion of electrolytes and toxic substances, protection from environmental hazards including the hydrogenation of human skin and the maintenance of the acid mantle (Schaal and Porter 1991). Water, electrolytes, fatty acids, lactic acid, and nitrogen metabolites, such as ammonia, urea, and uric acid have been analyzed as the main constituents of human sweat (Emrich and Oelert 1966; Peter *et al.* 1970; Takemura *et al.* 1989; Bernier *et al.* 1999; Haze *et al.* 2001; Huang *et al.* 2002; Curran *et al.* 2005). Sweat is secreted by glands located in the dermal tissue which are distributed in high density especially in the axilla, palm of hand, root base, and nether regions. The hypothalamus constitutes the main thermoregulatory centre which innervates the sweat glands via tracts of the sympathic nervous system (see Figure 1).

Sweat, when it is secreted by axillary glands, is odorless, until skin bacteria generate the odoriferous principles from the scentless analogues (Shelley *et al.* 1953; Shehadeh and Kligman 1963; Leyden *et al.* 1981). Fatty acids make a major contribution to the odoriphores

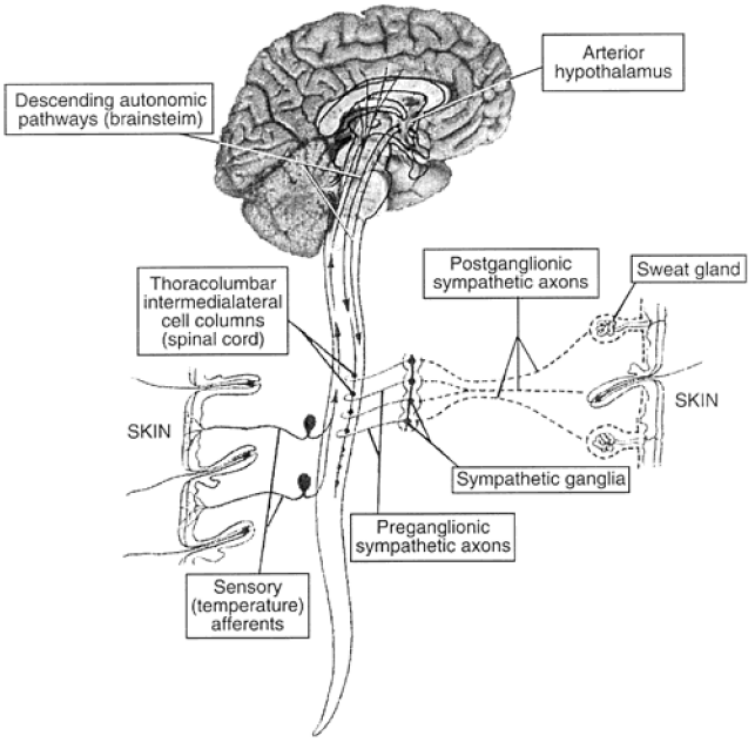


Figure 1 Functional anatomy of sudomotor pathways (Vetrugno *et al.* 2003).

(Zeng *et al.* 1991), but also the chemosignal androstenone is an odoriferous substance of human body odors (Claus and Alsing 1976). Androgen steroids have been determined as human sexual chemosignals existing in body odors (Nixon *et al.* 1988; Gower *et al.* 1994; Pause 2004) indicating that human secretions are also relevant for the communication between humans via chemosensory signals (e.g. McClintock 1971; Russell *et al.* 1980; Preti *et al.* 1986; e.g. Stern and McClintock 1998; Saxton *et al.* 2008). Recent studies give evidence for the presence of anxiety chemosignals comprised in sweat of emotional stress (Pause *et al.* 2004; Chen *et al.* 2006; Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009; Zhou and Chen 2009).

Therefore, the exploration of human secretions is not only relevant for medical objects including chemical analyses (Emrich and Oelert 1966; Peter *et al.* 1970; Takemura *et al.* 1989; Bernier *et al.* 1999; Haze *et al.* 2001; Huang *et al.* 2002; Curran *et al.* 2005) and medical treatment (Gelbard *et al.* 2008; Schlereth *et al.* 2009), but also for research in the field of chemical senses including related neural correlates, and behavioral and physiological responses mediated by human body odors. Various imaging techniques were used to investigate the neural correlates in response to body odors. Lundstrom *et al.* (2008) demonstrated in a positron emission tomography (PET) study that smelling a friend's body odor activates brain areas known to be associated with familiar stimuli, whereas smelling a stranger activates amygdala and insular regions akin to what has been demonstrated for fearful stimuli (Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009). To explore human autonomic nervous system under exposure of body odors experiments with electrophysiological techniques have been accomplished. Pause *et al.* (1998) demonstrated in an electroencephalogram (EEG) study that the central nervous processing of one's own body odor was faster than the processing of the chemosensory non-self signal, and that the chemosensory event-related potentials (CSERPs) appeared to be larger when subjects perceived their own body odor. Numerous behavioral experiments were conducted to explore various aspects of effects of human secretions on behavior, and related physiological objects including human communication of chemosensory signals comprised in body odors, and the

underlying mechanism of detection (e.g. McClintock 1971; Cutler *et al.* 1998; Pause *et al.* 1998; Chen and Haviland-Jones 1999; Preti *et al.* 2003; Havlicek *et al.* 2005; Zhou and Chen 2009).

2. Chemosensory signals

Chemosensory signals are social-environmental chemical stimuli which are produced by one individual and can be detected by another individual of the same species (Karlson and Luscher 1959). In mammals the phenomenon of the communication between conspecifics via chemosensory signals which elicit specific behavioral and physiological responses is well explored (Gosling and McKay 1990; Smith *et al.* 2001; Setchell *et al.* 2010). Chemosensory signals modulate reproductive states including hormonal changes and related behavior in receivers (Michael and Keverne 1968; Epple 1974; Coopersmith and Banks 1983; Albone 1984; Barrett *et al.* 1993; Tang-Matinez *et al.* 1993; Wyatt 2004). Evidence for the communication of alarm chemosignals has been demonstrated. Observations on mammalian receivers of alarm signals show increased arousal and alertness, increased motor activity, avoidance of the odor source, and withdrawal behavior (Abel 1991; Zalaquett and Thiessen 1991; Kiyokawa *et al.* 2004; Wyatt 2004; Kiyokawa *et al.* 2006; Inagaki *et al.* 2008).

The communication between humans via chemosensory signals seems to be less important as their communication is dominated by auditory and visual information. Nevertheless, an increasing number of studies suggest effects of chemosignals on human physiology and behavior. These effects are thought to arise from chemosensory signals comprised in human body odors which not only release behavioral responses in receivers (Havlicek *et al.* 2005; Zhou and Chen 2009) and modify mood (Chen and Haviland-Jones 1999; Preti *et al.* 2003), but also prime physiological reactions including neurological and endocrinological changes (Preti *et al.* 2003; Wyart *et al.* 2007; Miller and Maner 2009; Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009). Male axillary secretions have a calming effect on women by

modulating females mood with a reduction of tension and an increase of relaxation (Preti *et al.* 2003). Body odors alter the cycle length and the timing of the menstrual cycle in female recipients (McClintock 1971; Russell *et al.* 1980; Preti *et al.* 1986; Cutler *et al.* 1998). Furthermore, human secretions provide information about the signaller. Kin recognition and inbreeding avoidance is probably based on components of body odors which influence human mate choice (Wedekind *et al.* 1995; Wobst *et al.* 1998; Jacob *et al.* 2002; Weisfeld *et al.* 2003; Pause *et al.* 2006). Human emotional state is supposable contained within axillary secretions, and receivers can accurately infer the emotional state of the donor (Chen and Haviland-Jones 2000). Research about human communication via chemosignals has mainly focused on steroidal compounds present in human secretions, and their impact on reproduction (McClintock 1971; Russell *et al.* 1980; Preti *et al.* 1986; Stern and McClintock 1998; Wyart *et al.* 2007; Saxton *et al.* 2008), but also alarm signals comprised in axillary sweat of emotional stress have been explored in recent years (Pause *et al.* 2004; Chen *et al.* 2006; Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009; Zhou and Chen 2009). To date only a couple of studies give evidence for the communication of anxiety chemosignals. Effects of anxiety signals in humans have been shown based mainly on behavioral studies (Owen 1981; Chen and Haviland-Jones 2000; Ackerl *et al.* 2002; Pause *et al.* 2004; Chen *et al.* 2006; Zhou and Chen 2009), but also on data of electrophysiological (Prehn *et al.* 2006; Pause *et al.* 2009), and imaging techniques (Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009). Studies using functional magnetic resonance imaging (fMRI) showed amygdala activation (Mujica-Parodi *et al.* 2009), and activation in brain areas involved in the processing of emotional stimuli, and in the regulation of empathic feelings when subjects were exposed to chemosignals of anxiety (Prehn-Kristensen *et al.* 2009). Data of electromyography (EMG) demonstrated a higher startle reflex in response to auditory stimuli under influence of anxiety sweat compared to reference samples (Prehn *et al.* 2006; Pause *et al.* 2009). Behavioral studies give evidence for an effect of anxiety signals on the perception of emotional faces by enforcing and modulating the cognition of ambiguous facial stimuli (Pause *et al.* 2004; Mujica-Parodi *et al.* 2009; Zhou and Chen 2009). Chen *et al.* (2006) demonstrated that anxiety

chemosignals enhance recipients' cognitive performance in a word-association task. Furthermore, a few studies suggest that chemosensory alarm signals can be distinguished from chemosensory neutral odors when presented in a discrimination task (Chen and Haviland-Jones 2000; Ackerl *et al.* 2002), but have no influence on recipients' hedonic perception (Chen *et al.* 2006; Prehn *et al.* 2006; Mujica-Parodi *et al.* 2009; Pause *et al.* 2009; Prehn-Kristensen *et al.* 2009; Zhou and Chen 2009). Chemosensory signals exert their influence whether or not they are consciously detected by their receiver. Neuronal signals mediated by chemosignals are directly projected to the amygdala and the hypothalamus, and thus invoking behavioral and endocrine responses which do not necessarily include conscious perception (Savic *et al.* 2001; Sobel and Brown 2001; Savic 2002; Bhutta 2007).

3. Human sense of smell

In humans the physiological basis of the communication of chemosensory signals is still unclear. In contrast, in mammals the associated sensory system for detecting chemosignals is primarily assigned to the *vomeronasal organ* (VNO); axons project the electrical impulse to the *accessory olfactory bulb*, a posterior dorsal region of the main olfactory bulb (Rodriguez 2004; Tirindelli *et al.* 2009). The recognition of specific alarm signals in mice is attributed to the *Grueneberg ganglion* (Brechbuhl *et al.* 2008) (see Figure 2).

Due to the loss of some important elements of the VNO in humans the preponderance of the published data indicates that adult humans have no functional VNO (Trotier *et al.* 2000; Bhatnagar and Smith 2001; Knecht *et al.* 2003). Nevertheless, there is convincing evidence that both human physiology and behavior are influenced by chemosensory signals detected by specific receptors in the main olfactory epithelium. One superfamily of the *putative pheromone receptor genes* (V1R) which is attributed to express pheromone receptors in the functional VNO neuroepithelium of mammals (Boschat *et al.* 2002), is also expressed in cells of the human olfactory mucosa (Rodriguez *et al.* 2000).

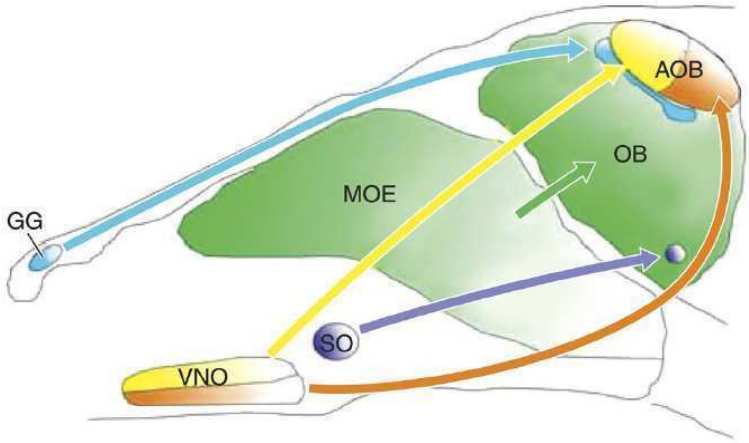


Figure 2 Anatomy of the vomeronasal organ in a generalized mammalian. Abbreviations: AOB, accessory olfactory bulb; GG, Grueneberg ganglion; MOE, main olfactory epithelium; OB, olfactory bulb; SO, septal organ of Masera; VNO, vomeronasal organ (Tirindelli *et al.* 2009).

The olfactory epithelium is located inside the nasal cavity, and is a specialized epithelial tissue consisting of olfactory sensory neurons for detecting volatile, airborne substances, supporting cells, and basal cells. The olfactory receptor cells are bipolar neurons in the nasal epithelium with *ciliae* on one pole which constitute the contact to the outside world, and olfactory axons on the opposite pole which project to the olfactory bulb. After an odor molecule is absorbed and transported via the mucus on the mucosa, the molecule binds to a specific olfactory receptor protein; thereby, a signal transduction cascade is activated which generates the excitation of olfactory neurons (see Figure 3). Signals are transmitted to the olfactory bulb and via the olfactory tract to higher cortical areas (Jones and Reed 1989; Tegoni *et al.* 2000; Albrecht and Wiesmann 2006).

Besides the olfactory system which is responsible for the perception of smelling volatile molecules, the trigeminal system is represented in the nasal cavity. The trigeminal nerve endings in the nasal mucosa detect irritants which are chemical substances that excite sensations like burning, stinging, warmth, coolness, or itching (Hummel 2000). Activations of the *nervus trigeminus* are projected to the thalamus, and are then transmitted to higher cortical areas, such as the somatosensory cortex. Most odorants activate both the olfactory and the trigeminal system which have a close anatomical as well as functional relationship;

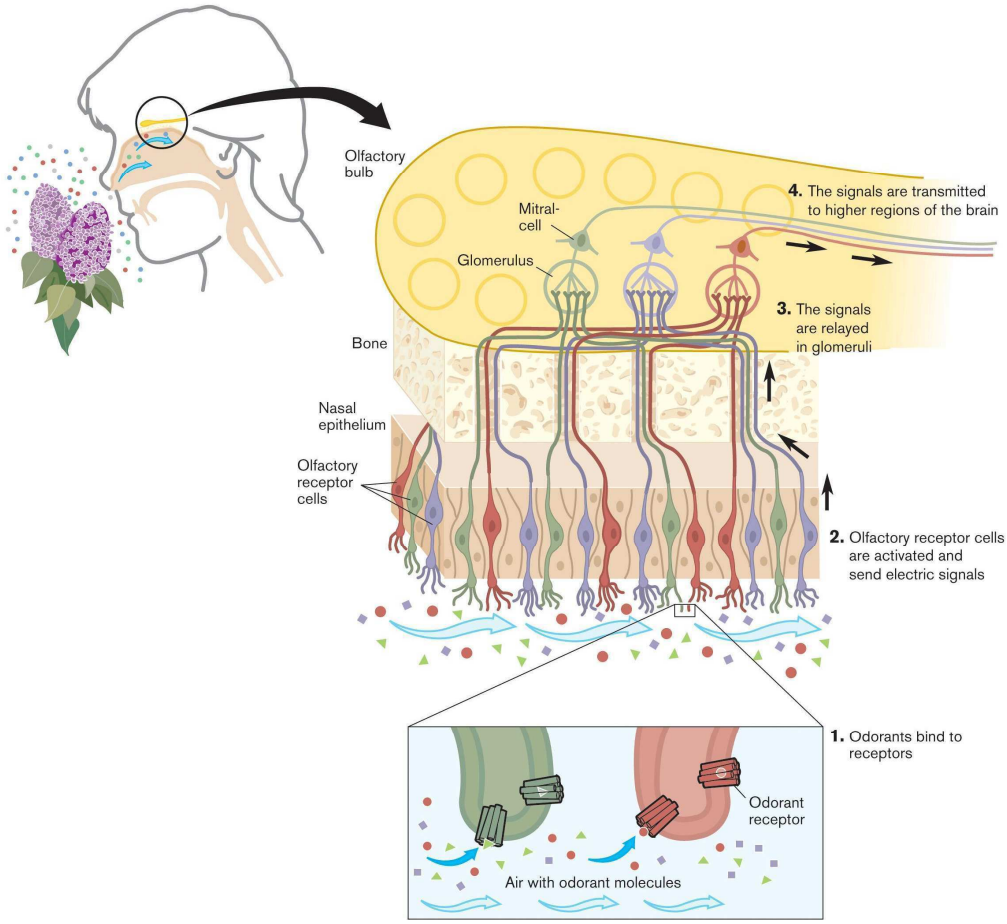


Figure 3 The organization of the olfactory system

(http://www.nobelprize.org/nobel_prizes/medicine/laureates/2004/press.html).

the two systems interact by suppressing and enhancing each other mutually (Doty *et al.* 1978; Cain and Murphy 1980; Livermore *et al.* 1992; Cashion *et al.* 2006). Most odorants activate the chemosensory system in a dose-dependent manner (Hummel *et al.* 1992; Boyle *et al.* 2006). At lower concentrations chemoreception will be mainly based on olfactory perception while at higher concentrations the trigeminal pathway will additionally contribute to the perception of the odorants. The interactions between the olfactory and the trigeminal system are especially important for the exploration of mixtures of odorants. Human sweat comprises substances with trigeminal as well as olfactory properties (Schneider and Schmidt 1967; Doty 1975; Doty *et al.* 1978). Some fatty acids comprised in human sweat (Peter *et al.* 1970; Takemura *et al.* 1989; Haze *et al.* 2001; Curran *et al.* 2005) activate the trigeminal system

when tested as monomolecular substances (Doty 1975; Doty *et al.* 1978), whereas decanoic acid, for example, excites exclusively the olfactory nerve structures (Doty 1975; Doty *et al.* 1978). Androstenone, a sexual chemosignal contained in body odors, produces a concentration-dependent degree of trigeminal stimulation (Boyle *et al.* 2006).

Humans can accurately allocate nasal stimuli to the right and left nostril only when the assessed odorant excites the trigeminal system. Pure odorants, which selectively stimulate the olfactory chemosensory system, cannot be localized (von Skramlik 1925; Kobal *et al.* 1989; Hummel *et al.* 2003; Wysocki *et al.* 2003; Frasnelli *et al.* 2009; Kleemann *et al.* 2009). Therefore, the trigeminal system is responsible for the orientation in space via chemosensation. Another responsibility of the trigeminal nerve endings in the nasal cavity is the recognition of dangerous substances. Carbon dioxide (CO_2) for example is an odorless and in higher concentrations a noxious substance which excites exclusively the trigeminal system (Boyle *et al.* 2007; Iannilli *et al.* 2008; Kleemann *et al.* 2009). CO_2 mediates pungent sensations inside the nose by activating the trigeminal system, and can thereby be consciously perceived, even though it is not detected by olfactory receptors. Similarly, the function of a warning system is also attributed to the olfactory system by inspecting food to its deleteriousness. Further functions of the sense of smell are localizing food sources, communication between humans, and affecting reproductive behavior by detecting chemosensory signals (Havlicek *et al.* 2005; Wyart *et al.* 2007).

4. Objectives

The present thesis work aimed at the investigation of the underlying mechanisms of sweat perception in humans. One objective was the exploration of which kind of receptors are activated by axillary odors assessed in concentration encountered in daily life, if human sweat activates the olfactory or additionally the trigeminal chemosensory system (cf. chapters II.). Further objectives were the investigation of the impact of chemosensory signals comprised in

axillary secretions on recipients' physiology and behavior, if human's perception of emotional faces are influenced by chemosensory stimuli (cf. chapters III.), and if human body odors influence human skin conductance response (cf. chapters IV.).

Previous studies are controversial regarding which intranasal nerve structures (olfactory versus trigeminal) are activated by human sweat. Therefore, one aim of the present work was to explore the chemosensory processing of human sweat based on psychophysical data of the so-called *detection* and *localization* experiments by applying the stimuli using an olfactometer (cf. chapter II.). Using these established methods we investigated if subjects are able to detect an odorant presented in a specific concentration (detection experiment), and if subjects are able to allocate these stimuli to the right and left nostril accurately (localization experiment). The results of the detection experiment ensure the conscious perception of the assessed stimuli, a precondition for an accurate accomplishment of the localization experiment. The results of the localization experiment indicate if the presented odorant concentration activates the olfactory system, or additionally the trigeminal system of the nasal mucosa. As human sweat is mostly applied in concentrations at or just above the olfactory threshold in scientific studies, the investigation of the chemosensory properties of human sweat were accordingly based on concentrations encountered in daily life. The sweat was collected from male donors during a bicycle workout.

In the experiments regarding human communication of chemosensory signals (cf. chapters III. and IV.) two kinds of axillary sweat were applied to recipients. Exercise sweat was collected during an ergometer training (exercise condition), and anxiety sweat was collected during a visit of a high rope course (anxiety condition). As previously published studies collected sweat of emotional stress during first tandem skydives, watching terrifying movies, and awaiting academic examinations, the present work established a new method of collecting anxiety chemosignals comprised in axillary sweat by inducing emotional stress in male sweat donors during a visit of a high rope course.

In previous studies using stress odors there was a tendency to use only female receivers. Therefore, in both experiments about chemosensory signals (cf. chapters III. and IV.) a

potential sex-related impact of chemosignals was considered by collecting sweat exclusively from male donors, and by regarding the differentiation between male and female receivers.

The objective of the second experiment about chemosensory signals (cf. chapters III.) was to investigate the effects of anxiety chemosignals on recipients' evaluation of emotional faces. Previous similar studies explored subjects' perception of pictures morphed with negative facial expressions by using an experimental design of a two forced-choice judgment during an emotion identification task. On the contrary, in the current study the impact of anxiety chemosignals was explored on the evaluation of morphed happy facial expressions by using visual analog scales. Participants were instructed to rate emotional male faces of different morphing levels (neutral - happy) under exposure of three different samples (exercise sweat, anxiety sweat, and control material). Therefore, the present study investigated if anxiety signals modulate the perception of happy male facial expressions, not only enforce the same emotion.

The aim of the third experiment about chemosensory signals (cf. chapter IV.) was to investigate the effects of two kinds of male sweat, exercise and anxiety sweat, on recipients' skin conductance response (SCR) elicited by acoustic startle stimuli. Previous studies explored the impact of androstadienone on human SCR, as well as the impact of anxiety chemosignals on human startle reflex, but no study has been published regarding the effects of axillary secretions on human SCR. Therefore, the purpose of the third study was to investigate possible sex-specific effects of exercise sweat which contains androgen steroids determined as human sexual chemosignals, and the effects of anxiety signals comprised in sweat of emotional stress as well as a control condition on recipients' autonomic skin conductance response. Additionally, the possible impact of different durations of sample exposure was investigated by measuring subjects' SCR at two different points in time.

II. Chemosensory properties of human sweat

The experiment about the chemosensory properties of human sweat examined which parts of the intranasal chemosensory system are involved in the neuronal processing of axillary secretions. Human sweat contains a mixture of various substances with trigeminal as well as olfactory properties. It has been shown that trigeminal perception is necessary to localize odors and that humans are not able to localize substances which only activate the olfactory system. By using established psychophysical methods the present experiment explored the activation of olfactory versus trigeminal receptors inside the nasal cavity. The so-called *localization experiment* investigated if subjects had the ability to localize the presented odorant to the accurate nostril. The *detection experiment* quantified human's olfactory perception; to assure that subjects perceived the presented stimuli consciously the sensitivity to the assessed odorant concentration had to be determined. For this purpose, human sweat was collected during a bicycle workout and was then applied to recipients by means of an olfactometer.

5. Material und methods

The entire study was approved by the local Medical Ethics Review Committee of our University and was conducted in accordance with the Declaration of Helsinki. All subjects provided their written informed consent.

5.1. Collection of sweat stimuli

5.1.1. Requirements to participate as sweat donor

To participate as sweat donor in the sweat sampling sessions subjects had to fulfill the following criteria:

- male
- age between 18 and 55 years
- mental and physical health

- no psychiatric or neurological diseases in anamnesis
- no metabolic diseases
- no use of any medications
- no use of any tobacco products

Furthermore, participants were screened regarding their sexual orientation by using a 7-point scale (0 = exclusively heterosexual, 6 = exclusively homosexual) (Kinsey *et al.* 1953). To preclude influences of toiletries and diet on donors' sweat subjects were required to undergo certain dietary and behavioral restrictions two days prior and on the day of the samplings. At least three days before odor sampling subjects received the following instructions, along with a scent-free shower gel (Balea, Ultra Sensitive, dm-dogerie markt, Karlsruhe, Germany). They were instructed not to use any perfumed toiletries (perfumes, deodorants/antiperspirants, aftershaves, perfumed body lotions, and shower gels), and to wash themselves only with the scent-free shower gel provided by the experimenters. Furthermore, they were requested not to visit a swimming pool due to the chlorine, and to refrain from eating garlic, onion, asparagus, spicy food, and from drinking alcohol. The evening before sampling, donors were instructed to take a shower and wash their bodies with the non-perfumed shower gel, and were asked to wear only loose and odorless clothes after that. On the sampling days the participants were required to wash their armpits exclusively with water.

5.1.2. Sweat donors

Twenty healthy male subjects between the ages of 21 and 52 years (mean age 27.2 years, SD 7.0 years) participated as sweat donors in an ergometer workout. All participants affirmed that they complied with the requirements and the instructions (cf. subsection 5.1.1.). All donors described themselves as exclusively heterosexual on a 7-point scale (mean 0.0, SD 0.0). The sweat samples were used in the odor perception experiment (cf. subsection 5.2.) within six weeks.

5.1.3. Sweat sampling procedure during exercise condition

For the odor perception experiment sweat was collected during a 20 min workout with an estimated power of 120 watt and 90 revolutions per minute on a bicycle ergometer in the Department of Physiotherapy of the Hospital Großhadern. Each participant accomplished this workout twice. Between both sessions participants rested for approximately 15 min.

During sweat sampling cotton pads (16 cm x 5.5 cm) were placed under the armpits, and participants wore tight, white long-sleeve cotton shirts to ensure a close fit of the pads in the armpits, and wore raincoats to increase participants' perspiration. To prevent any bacterial degradation participants' cotton pads were collected at the end of all sessions (after run 1 and run 2 of the ergometer training), and were immediately frozen using dry ice. The pads were cut into approximately 1 x 1 cm-sized pieces. Slices of all samples were pooled across all donors, and were stored all together in one big odorless freezer bag at -40°C until testing.

Clean, unused cotton pads served as a control stimulus. These pads were cut and stored in the same manner as described above until testing.

5.2. Detection and localization experiments of human sweat

The experiment about chemosensory properties of human sweat investigated which parts of the intranasal chemosensory system are involved in the neuronal processing of axillary secretions. To investigate subjects' sensitivity to the applied concentration of the sweat stimuli a *detection experiment* based on the Signal Detection Theory (SDT) was accomplished which is a reliable method for the quantification of human perception (Green and Swets 1966; Lloyd and Appel 1976). To explore if subjects have the ability to localize the presented sweat stimuli to the accurate nostril and therefore, which nerve structures (trigeminal versus olfactory) are activated, the so-called *localization experiment* was accomplished (Kobal *et al.* 1989; Hummel *et al.* 2003; Frasnelli *et al.* 2009; Kleemann *et al.* 2009).

5.2.1. Requirements to participate as recipient

To participate as recipient subjects had to fulfill the following criteria:

- age between 18 and 55 years
- mental and physical health
- no psychiatric or neurological diseases in anamnesis
- no metabolic diseases
- no acute or chronic dysfunction of the respiratory system
- no use of any medications
- no use of any tobacco products
- no use of hormonal contraceptives, no pregnancy and no lactation (females)

Furthermore, subjects were screened for normal olfactory function using the Sniffin' Sticks test battery (cf. subsection 5.2.2.) (Kobal *et al.* 1996; Hummel *et al.* 1997). To prevent any interference with sample perception subjects were instructed not to use any perfumed toiletries on the day of the experiment. The evening before and on the day of the experiment participants were asked to refrain from eating onion, garlic, and drinking alcohol. Two hours before data collection they were instructed to abstain from drinking coffee.

5.2.2. Olfactory screening

Olfactory testing was performed by means of the Sniffin' Sticks test battery (Burghart Instruments, Wedel, Germany) which comprises three subtests, namely odor threshold, odor discrimination, and odor identification (Kobal *et al.* 1996; Hummel *et al.* 1997) (see Figure 4). All tests were conducted with the Sniffin' Sticks testing software named Olaf (http://www.tu-dresden.de/medkhno/riechen_schmecken/download.htm).

Odor detection threshold concentrations provide a measure of the lower limits of olfactory detection and sensitivity of subjects. For threshold measurements the odorant *n*-butanol is assessed in different dilutions starting with the lowest concentration. The odorant is diluted in geometric series consisting of sixteen steps with a dilution ratio of 1:2; the highest administered concentration is 4 % v/v. Three sticks are presented in a randomized order; two

Figure 4 The Sniffin' Sticks (Burghart). Pictured is one of the three olfactory subtests of the test battery to test for olfactory function.

of them contain only the solvent (aqua conservata) serving as blanks and the third contains the odor at a certain dilution. Participants have to identify the odor-containing pen. Two successful identifications in a row or one unsuccessful identification trigger a reversal of the staircase to the next higher or next lower dilution, respectively. The threshold score is defined as the mean of the last four out of seven staircase reversals and range from 1 (lowest sensitivity) to 16 (highest sensitivity).

In the odor discrimination task, sixteen triplets of odorants are presented in randomized order. In each triplet two sticks contain the same odor and the third contains a different odor. Participants are required to determine which of the three odor-containing sticks smell different compared to the other two sticks. Resulting scores range from 0 (no correct discrimination) to 16 (perfect discrimination).

Odor identification is assessed by means of sixteen commonly known every day odorants in randomized order. Using a multiple choice task, identification of individual odors is performed from lists of four descriptors each. Resulting scores ranged from 0 (no correct identification) to 16 (perfect identification).

All subtests are assessed birhinally. During the threshold and discrimination tasks subjects are blindfolded to prevent any visual cues during testing.

From the results of the three olfactory subtest, a composite TDI score (sum of threshold, discrimination, and identification scores) is derived which is used to determine normal olfactory function. The TDI scores can range from 1 to 48. Normosmia is defined as a TDI

score of >30 (Hummel *et al.* 1997; Kobal *et al.* 2000; Wolfensberger *et al.* 2000; Hummel *et al.* 2007).

5.2.3. Recipients

Thirty-four healthy subjects (17 female and 17 male subjects; age range 20 – 48 years; mean age 31.2 years, SD 7.2 years) participated as recipients. All participants affirmed that they fulfill all requirements (cf. subsection 5.2.1.). Mean age did not differ significantly between male (mean age 32.7 years, SD 8.3 years) and female (mean age 29.8 years, SD 5.9 years) subjects (independent two-sample t-test; $t(32) = 1.20$, p = not significant [n.s.]). Olfactory screening revealed a mean TDI score of 35.70 (SD 2.88; range 32.00 - 43.75) indicating normal olfactory function.

5.2.4. Experimental procedure

The experiment about chemosensory properties of human sweat was divided into two sessions (detection and localization experiments). The detection experiment examined if subjects are able to detect the applied sweat concentration. In the localization experiment subjects were asked to localize the presented stimuli to the right and left nostril (Kobal *et al.* 1989; Hummel *et al.* 2003; Frasnelli *et al.* 2009; Kleemann *et al.* 2009). The order of both sessions was pseudo randomized. Olfactory stimuli were presented using a computer-controlled olfactometer (OM6b, Burghart Instruments, Wedel, Germany) (Kobal 1981; Kobal and Hummel 1988; Kobal *et al.* 1989) (see Figures 5 and 6; cf. subsection 5.2.5.). All stimuli were presented birhinally with applying a sweat stimulus to one nostril and a blank stimulus to the other nostril simultaneously to prevent any asymmetrical tactile stimulation. In both sessions stimuli were presented for 2000 ms each embedded in a constantly flowing airstream (4 l/min). The average inter stimulus interval (ISI) was set at 30 s (± 3 s). For odor presentation 13 grams of the sweat pads collected in the sampling session (cf. subsection 5.1.) were used in the detection as well as in the localization experiment, and 5 grams of the blank control.

Figure 5 The Olfactometer (OM6b, Burghart).

Figure 6 Recipient during stimulus presentation using an olfactometer.

During sample presentation subjects performed the technique of *velopharyngeal closure* (see Figure 7; cf. subsection 5.2.5.) (Kobal 1981). Subjects were laying in supine position, with their eyes closed (Wiesmann *et al.* 2006), and white noise of approximately 80 dB (SPL) was presented binaurally to prevent the subjects from hearing the switching valves of the olfactometer. In each experiment recipients were instructed to evaluate the assessed stimuli. They were asked to respond to an auditory signal presented 2 s after each stimulus by pressing either the right or the left mouse button (stimulus detected yes/no, localized to the right/left nostril). The response signal was recorded using LabView 7.0 software (National Instruments, Austin, Texas, USA). After each session subjects filled in a questionnaire. One run lasted about 17 min; both sessions were separated by a 30 min break to avoid olfactory adaptation effects. Subjects were not aware of the nature of the odorants. They were told that they would receive a mixture of different odorants.

5.2.5. Chemosensory stimulation

The computer-controlled olfactometer (OM6b, Burghart Instruments, Wedel, Germany; see Figure 5) (Kobal 1981; Kobal and Hummel 1988; Kobal *et al.* 1989) is a chemosensory

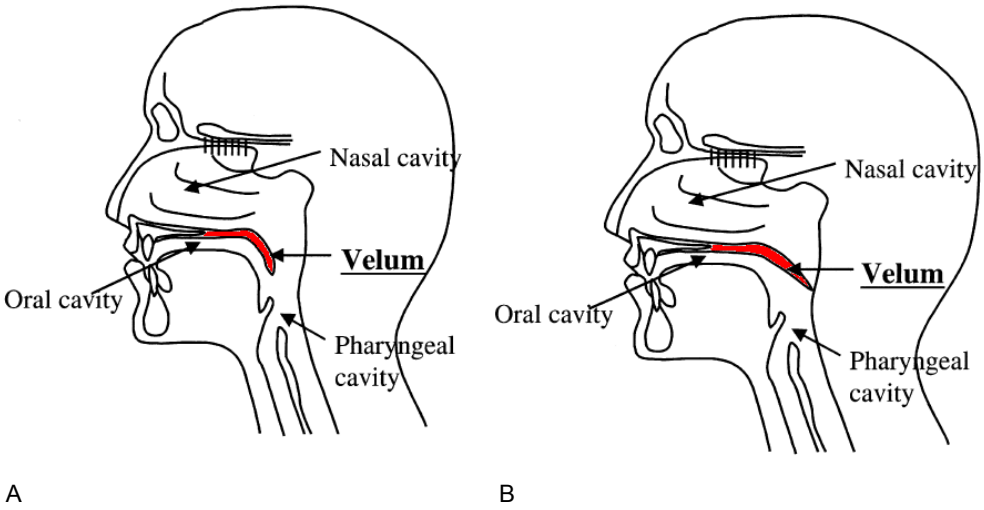
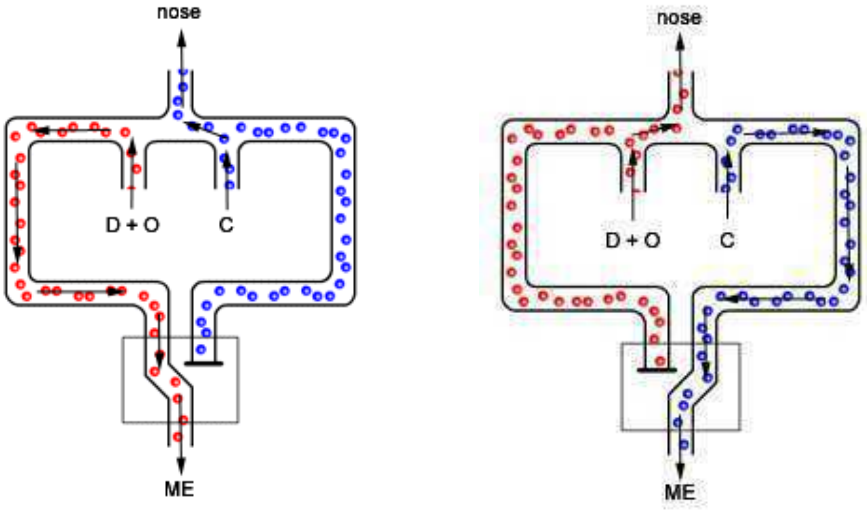


Figure 7 Velopharyngeal closure. Schematic drawing of human head showing velum position for A) natural breathing and B) velopharyngeal closure (Thesen and Murphy 2001).

stimulator which delivers odorants in a constantly flowing air stream.

In the current study the temperature (37°C) and the relative humidity (80%) of the airflow at the end of the olfactometer tube were controlled and kept constant. With this technique the chemosensors, but not the mechano- or thermosensors of the nasal mucosa are activated. The constant humidification prevents swelling of the nasal mucosa, mucus production, and pain. Irritations would interfere with the study design, especially in detection and localization experiments this would lead to false results.

For olfactory stimulation samples were sourced via internal chambers containing the sweat and control pads, respectively, inside the olfactometer. Air is continuously channelled through the chamber via a frit located at the chamber base. This odorous enriched air stream (*odorant flow, O*) is subsequently diluted with humidified, non-odorous *dilution air* (*D*) inside the switching device, and is lead towards the outlet port of the olfactometer. During olfactory stimulation this odorant/dilution flow reaches subjects' nose, whereas the *control flow* (*C*) is completely exhausted by means of a vacuum flow (*main exhaust, ME*). A constant flow continuously exits the outlet port, so that there is no noticeable change in the delivered flow other than the odor itself (C = O + D). During the inter stimulus interval (ISI) only the non-



A B

Figure 8 Stimulation principle of the nasal mucosa by using an olfactometer. A) Non-odorous control air (ISI), B) Olfactory stimulation. C = control flow; D = dilution flow, O = odorant flow, ME = main exhaust (modified from http://www.burghart-mt.de/texte/de/produkte/funktionsweise.php).

odorous control flow is send to the end of the olfactometer tube, the mixture of odorant and dilution is exhausted. A schematic simplified drawing of the stimulation principle is shown in Figure 8. Before using the olfactometer for measurements a calibration has to be accomplished. During chemosensory stimulation subjects performed the technique of *velopharyngeal closure* (see Figure 7). This technique avoids the flow of respiratory air within subjects' nasal cavities, and thereby, the amount of odorous molecules reaching the nasal mucosa can be experimentally determined (Kobal 1981). With this breathing technique the connection between the nasal and the oral cavity is closed by the elevation of the soft palate, and by the contraction of the posterior and lateral pharyngeal wall.

5.2.6. Detection experiment

Subjects' ability to detect the assessed concentration of the sweat stimuli were investigated by presenting a total of 20 stimuli and 10 blanks (control material) to either one nostril. While a stimulus was applied to one nostril, a blank was applied to the contralateral nostril

simultaneously. The stimulation of the left (10 stimuli) or right (10 stimuli) nostril or the presentation of blank stimulus followed a pseudo randomized sequence. After each stimulus (auditory signal) subjects made a two-alternative, forced-choice judgment. They were asked to separate the signal (sweat stimulus) from the noise (blank stimulus) by pressing either the right (signal) or left (noise) mouse button.

5.2.7. Localization experiment

To examine subjects' ability to localize the sweat stimuli to the accurate nostril an experimental paradigm comprising a total of 30 stimuli was used. Fifteen sweat stimuli were applied to the right, and fifteen stimuli to the left nostril. The order of nostril's stimulation was pseudo randomized. Olfactory stimuli were presented to either one of both nostrils; simultaneously a blank stimulus was applied to the contralateral nostril. After each stimulus subjects heard an auditory signal. They were instructed to provide a two-alternative, forced-choice judgment to localize the assessed odor to the right (right mouse button) or left (left mouse button) nostril.

5.2.8. Questionnaire

Two questionnaires, one for each experiment (detection and localization experiments) were employed to measure recipients' emotional states, their perceptions of the sweat stimuli, and their associations when smelling the odorant. After each testing session subjects rated their emotional valence (0 = negative, 100 = positive), arousal (0 = calm, 100 = aroused), alertness (0 = very inattentive, 100 = very attentive), as well as the dominance (0 = submissive, 100 = dominant), and the pleasantness (0 = pleasant, 100 = unpleasant) of the olfactory stimuli during the experiment, its familiarity (0 = not familiar, 100 = very familiar), its sexual attractiveness (0 = not appealing, 100 = very appealing), its masculinity/femininity (0 = masculine, 100 = feminine), and its intensity. After the detection experiment subjects rated the odor intensity (0 = very weak, 100 = very strong), and the variation in intensity between the stimuli (0 = little variation, 100 = strong variation). After the localization experiment subjects

rated the intensity of the olfactory stimuli (0 = very weak, 100 = very strong), and their variation in intensity (0 = little variation, 100 = strong variation) for the right and left nostril, separately.

The questions were answered by the participants using a visual analogue scale (VAS). They were trained to give a response by placing a mark on a 100-mm horizontal line. Visual analogue scales have been shown to measure even minor changes in affect with high reliability and validity (Aitken 1969; Folstein and Luria 1973).

5.2.9. Data analyses

The data of the detection experiment were analyzed based on the Signal Detection Theory (SDT). For any event four outcomes are possible: hit (correct detection of a presented signal), correct rejection (correct detection of an absent signal), miss (miss to detect a presented signal), false alarm (incorrect detection of an absent signal). Based on these outcomes the parameters sensitivity *d'* and response criterion *ß* were calculated. Sensitivity *d*' indicates the strength of the signal (relative to the background noise). The proportions of hits and false alarms reflect the ability to separate between signal (the relevant input event) from the noise (background activity or irrelevant inputs). $d' < 0.5$ corresponds to a low sensitivity, d' between 0.5 and 2 indicates a moderate sensitivity, $d' \geq 2$ corresponds to a high sensitivity. The response criterion *ß* reflects the subjects' strategy of response. $ß < 1$ corresponds to a low criterion, i.e. the subjects tend to answer with yes, $ß = 1$ means neutral criterion, $ß > 1$ corresponds to a high criterion, i.e. the subjects tend to answer with no.

Task performance of localization was calculated by adding up the number of correct localizations following the presentation of an odorant to either the left or right nostril (Kobal *et al.* 1989; Hummel *et al.* 2003; Frasnelli *et al.* 2009; Kleemann *et al.* 2009). To analyze the behavioral parameters of the localization experiment, for a left-sided stimulation four outcomes are possible: hit (answer left when stimulus was left), correct rejection (answer right when stimulus was right), miss (answer right when stimulus was left), false alarm (answer left when stimulus was right). Based on these outcomes the SDT parameters sensitivity *d'* and

criterion *c* were calculated as suggested by Macmillan & Creelman (2005). Specifically, the criterion *c* measures a leftward or rightward tendency in subjects' response. A criterion $c < 0$ implies a tendency to the right, a $c = 0$ signifies no tendency, and a $c > 0$ indicates a tendency to the left.

Statistical analyses were accomplished using SPSS 17.0 for Windows (SPSS Inc, Chicago, IL, USA). Normality of the data was tested using the Kolmogorov–Smirnov test. Normally distributed data (results of the localization experiment, criterion *c*, sensitivity *d'* of the localization experiment, valence, alertness, pleasantness, familiarity, masculinity/femininity, sexual attractiveness, intensity, variations in intensity) were submitted to Student's paired t-tests, not normally distributed data (sensitivity *d'* of the detection experiment, response criterion *ß*, arousal, dominance) were submitted to non-parametric Wilcoxon signed-rank tests to explore differences between the detection and the localization experiments concerning the ratings of the questionnaire, and to compare the results of the localization experiment regarding left versus right nostril. To examine if subjects were able to localize the presented stimuli above chance level we used one-sample t-tests. To analyze sex-differences data were submitted to independent two-sample t-tests (normally distributed data) or to Mann-Whitney U tests (not normally distributed data). P-values ≤ 0.05 were considered significant. Results of the questionnaire were corrected for multiple testing using the Bonferroni method.

6. Results

6.1. Detection experiment

Recipients detected the sweat stimuli with low to high sensitivity *d'* (mean 1.91; 14.4 ± 4.5 ≙ 72.2 % hits). For further analyses subjects were subdivided in three groups according to their sensitivity to the sweat samples: eleven of thirty-four subjects (four females) detected the applied sweat stimuli with a high sensitivity *d'* (sensitivity class 1: mean 4.04, range 2.48 –

Table 1 Results of the detection experiment. Reported are means ± standard deviations (sensitivity class 1: n = 11, class 2: n = 12, class 3: n = 11).

	Hit (maximum = 20)	**Miss** (maximum = 20)	**False alarm** (maximum = 10)	**Correct rejection** (maximum = 10)
All subjects	14.4 ± 4.5 (72.2 %)	5.6 ± 4.5 (27.8 %)	3.1 ± 2.8 (31.2 %)	6.9 ± 2.8 (68.8 %)
Sensitivity class 1	16.4 ± 5.1 (82.3 %)	3.6 ± 5.1 (17.7 %)	1.0 ± 1.3 (10.0 %)	9.0 ± 1.3 (90.0 %)
Sensitivity class 2	15.2 ± 3.0 (75.8 %)	4.8 ± 3.0 (24.2 %)	3.4 ± 2.9 (34.2 %)	6.6 ± 2.9 (65.8 %)
Sensitivity class 3	11.6 ± 4.3 (58.2 %)	8.4 ± 4.3 (41.8 %)	4.9 ± 2.4 (49.1 %)	5.1 ± 2.4 (50.9 %)

6.00; 16.4 ± 5.1 ≙ 82.3 % hits), twelve subjects (seven females) detected the stimuli with a moderate sensitivity (sensitivity class 2: mean 1.46, range 0.78 – 1.81; 15.2 ± 3.0 ≙ 75.8 % hits), and eleven subjects (six females) had a low sensitivity in response to the stimulation (sensitivity class 3: mean 0.27, range -0.17 – 0.42; 11.6 ± 4.3 ≙ 58.2 % hits) (see Table 2). Descriptive statistics are shown in Table 1. Results of the detection experiment (sensitivity *d'*, response criterion *ß*) revealed no significant differences between men and women, neither for subjects all together, nor for the three sensitivity classes analyzed separately (Mann-Whitney U Tests, p = n.s.). Means of the response criterion *ß* constituted data of *ß* > 1 indicating a conservative behavior during the decision (see Table 2). These findings applied to all sensitivity classes; class 1 revealed a high response criterion of 32.92 indicating that subjects tend to answer with no, whereas classes 2 and 3 were nearby the neutral criterion ($ß_{class2}$ = 1.20, $ß_{class3}$ = 1.02).

6.2. Localization experiment

Individuals failed to localize the sweat stimuli during the localization experiment (mean ± SD = 14.6 ± 2.4 ≙ 48.7 % correct assignment) (see Table 3) and showed no rise above chance

Table 2 Behavioral parameters of the detection and the localization experiments (sensitivity class 1: n = 11, class 2: n = 12, class 3: n = 11).

	Detection experiment		Localization experiment	
	Sensitivity *d'*	**Response criterion *ß***	**Sensitivity *d'***	**Criterion *c***
All subjects	1.91	11.40	- 0.07	- 0.08
Sensitivity class 1	4.04	32.92	- 0.11	- 0.14
Sensitivity class 2	1.46	1.20	- 0.14	- 0.04
Sensitivity class 3	0.27	1.02	- 0.05	- 0.06

level. This was true for the total group of subjects ($t(33) = 0.89$, p = n.s.), as well as when the three sensitivity classes were analyzed separately ($sensitivity_{class\ 1}$: $t(10) = 0.90$, p = n.s.; $sensitivity_{class\ 2}$: $t(11) = 1.20$, p = n.s.; $sensitivity_{class\ 3}$: $t(10) = 0.30$, p = n.s.). Based on a binomial distribution a subject is considered to perform above chance level if he/she scores 20 or more correct assignments out of 30. Thus, when data were analyzed separately for each individual subject only one male performed significantly above chance level; the participant had 22 (≙ 73.3 %) correct assignments. All other subjects showed scores below 20 correct assignments. There were no significant differences in the localization rate of the sweat stimuli between the right and left nostril (Student's paired t-tests, $t(33) = 1.48$, p = n.s.). Men could localize the applied stimuli to the accurate nostril better when compared to women (independent two sample t-test, $t(32) = 2.26$, $p = 0.031$). However, when data were analyzed separately, neither men nor women were able to localize the sweat stimuli above chance level (men: 15.5 ± SD 2.5 ≙ 51.8 % correct assignment, $t(16) = 0.87$, p = n.s.; women: 13.7 ± SD 2.2 ≙ 45.7 % correct assignment). The women's score was significantly below chance level ($t(16) = 2.42$, $p = 0.028$). The criterion *c* of the left-sided stimulation of the localization

Table 3 Results of the localization experiment. Reported are means ± standard deviations (sensitivity class 1: n = 11, class 2: n = 12, class 3: n = 11).

	Correct assignment (maximum = 30)	**Left** (maximum = 15)	**Right** (maximum = 15)	**Mistake** (maximum = 30)
All subjects	14.6 ± 2.4 (48.7 %)	7.8 ± 2.0 (53.6 %)	6.8 ± 2.4 (46.4 %)	15.4 ± 2.4 (51.3 %)
Sensitivity class 1	14.5 ± 2.0 (48.2 %)	8.0 ± 2.2 (55.7 %)	6.5 ± 2.4 (44.3 %)	15.5 ± 2.0 (51.8 %)
Sensitivity class 2	14.2 ± 2.4 (47.2 %)	7.3 ± 1.7 (52.9 %)	6.9 ± 2.8 (47.1 %)	15.8 ± 2.4 (52.8 %)
Sensitivity class 3	15.3 ± 3.1 (50.9 %)	8.0 ± 2.2 (52.3 %)	7.3 ± 2.0 (47.7 %)	14.7 ± 3.1 (49.1 %)

experiment revealed an almost neutral criterion ($c_{\text{all subjects}}$ = - 0.08, $c_{\text{class 2}}$ = - 0.04, $c_{\text{class 3}}$ = - 0.06); only class 1 showed a *c* value of - 0.14 indicating that subjects had a slight rightward tendency in their response (see Table 2). There were no significant differences between men and women regarding criterion *c*, neither for all subjects nor for the three sensitivity classes separately (independent two sample t-tests, p = n.s.). All subjects revealed a low sensitivity *d'* in the localization experiment; these data were determined for all subjects ($d_{\text{all subjects}}$ = - 0.07), as well as for the three sensitivity classes separately ($d_{\text{class 1}}$ = - 0.11, $d_{\text{class 2}}$ = - 0.14, $d_{\text{class 3}}$ = 0.05; see Table 2). There were no significant differences between men and women when data were analyzed for the three classes separately (independent two sample t-tests, p = n.s.); when data were analyzed for all subjects results revealed significant sex-differences (independent two sample t-test, t(32) = 2.17, p = 0.038).

6.3. Questionnaire

All parameters of the questionnaire revealed no significant differences between the two experiments (Student's paired t-tests or Wilcoxon signed-rank tests, p = n.s.). There were no significant differences in respect to the sex of the subjects (independent two-sample t-test or Mann-Whitney U test, p = n.s.) regarding the different questions. Descriptive statistics are

Table 4 Ratings of the questionnaire. Reported are means ± standard deviations of the detection and the localization experiment (n = 34).

	Detection experiment	Localization experiment
Valence	59.6 ± 26.6	57.3 ± 23.9
Arousal	17.7 ± 22.4	18.0 ± 17.2
Alertness	78.2 ± 11.3	73.3 ± 14.6
Dominance	44.3 ± 16.2	44.9 ± 16.1
Pleasantness	64.9 ± 18.9	67.0 ± 17.6
Familiarity	46.6 ± 25.8	44.4 ± 27.8
Masculinity/Femininity	32.1 ± 18.2	35.6 ± 20.0
Sexual attractiveness	23.4 ± 21.6	24.4 ± 22.9
Intensity	56.4 ± 23.0	
Intensity left nostril		58.5 ± 26.8
Intensity right nostril		55.8 ± 24.8
Variations in intensity	55.0 ± 21.3	
Variations in intensity left nostril		45.6 ± 26.8
Variations in intensity right nostril		42.5 ± 25.9

shown in Table 4. Subjects rated their emotional conditions (valence, arousal), as well as the dominance, familiarity, intensity, and the variations in intensity of the applied sweat stimuli as moderate, and administrated the odorant as unpleasant, not sexually attractive, and as masculine in both experiments.

7. Discussion

7.1. Detection and localization experiments

The study about chemosensory properties of human sweat revealed that although participants were able to detect the sweat stimuli consciously they were unable to localize the presented stimuli to the accurate nostril. It has been shown that trigeminal perception is necessary to localize odors and that humans are not able to localize substances which selectively activate the olfactory system (von Skramlik 1925; Kobal *et al.* 1989; Hummel *et al.* 2003; Wysocki *et al.* 2003; Frasnelli *et al.* 2009; Kleemann *et al.* 2009). Thus, the present results strongly suggest that human sweat predominately activates the olfactory chemosensory system, but not the trigeminal system.

Axillary sweat is a mixture of several components with trigeminal as well as olfactory properties (Emrich and Oelert 1966; Peter *et al.* 1970; Takemura *et al.* 1989; Bernier *et al.* 1999; Haze *et al.* 2001; Huang *et al.* 2002; Curran *et al.* 2005). The olfactory system is thought to be responsible for the perception of smelling volatile molecules, whereas the trigeminal nerve endings in the nasal mucosa contribute to detect irritants (Hummel 2000).

Sweat, when it is secreted by axillary glands, is odorless, until skin bacteria generate the odoriferous principles from the scentless analogues (Shelley *et al.* 1953; Shehadeh and Kligman 1963; Leyden *et al.* 1981). In the detection experiment these smelling volatiles were responsible for subjects' ability to consciously detect the applied sweat stimuli. Fatty acids make a major contribution of the odoriphores (Zeng *et al.* 1991), but also the pheromone androstenone is an odoriferous substance of human body odor (Claus and Alsing 1976).

Up to now, substances in human sweat have been investigated relative to chemosensory perception by using localization experiments only as individual components. The fatty acids comprised in human sweat (Peter *et al.* 1970; Takemura *et al.* 1989; Haze *et al.* 2001; Curran *et al.* 2005) could potentially activate the trigeminal system (Doty 1975; Doty *et al.* 1978). But against one's expectations not all fatty acids cause trigeminal activations; decanoic acid for example excites exclusively the olfactory nerve structures (Doty 1975; Doty *et al.* 1978).

Androstenone, a sexual pheromone consisting in human sweat, is an odorant that produces a concentration-dependent degree of trigeminal stimulation (Boyle *et al.* 2006). Lactic acid and ammonia also excite the trigeminal nerve structures when tested as monomolecular substances in previous studies (Emrich and Oelert 1966; Schneider and Schmidt 1967; van Thriel *et al.* 2006).

Thus, the results of subjects' inability of localizing human sweat to the accurate nostril, and therefore, the hypothesis that human body odor originating from a sport condition excites exclusively the olfactory chemosensory system might be surprising. However, there is a close relationship between the olfactory and the trigeminal system. The two systems interact by suppressing and enhancing each other mutually (Cain and Murphy 1980; Livermore *et al.* 1992; Cashion *et al.* 2006). Therefore, it is not a necessary consequence that human secretions activate the trigeminal nerve structures, although it comprises several trigeminal substances. The neuronal processing of mixtures of different odorants is complex, particularly if individual substances are represented in different concentrations in the compound.

Trigeminal perception is heavily dependent on the concentration of the tested substance. The absence of trigeminal excitations in the current study could be due to the low concentrations of the trigeminal components represented in human sweat. Van Thriel (2006) showed that there are different ranges from odor detection thresholds to irritation thresholds for each odorant. The chemosensory thresholds of ammonia, for example, are very far apart from each other. The trigeminal thresholds are typically well above olfactory thresholds (Cometto-Muniz *et al.* 1998; 2005). This indicates that most odorants activate the chemosensory system in a dose-dependent manner (Hummel *et al.* 1992; Boyle *et al.* 2006). At lower concentrations chemoreception will be mainly based on olfactory stimulation while at higher concentrations the trigeminal pathway will additionally contribute to the perception of the odorants. Therefore, stimulants at concentrations below the trigeminal threshold already elicit an odorous sensation, and the distinction between blank and stimulant is possible by the distinction between 'smell' and 'no smell' (Thurauf *et al.* 2002). This suggests that a vapor can only be localized via chemesthesis when it has reached the threshold of true trigeminal perception,

not only the olfactory detection threshold, even if it is a bimodal or an olfactory/trigeminal substance. In the present study subjects failed to localize the applied sweat stimuli to the accurate nostril, even if they detected the stimuli consciously. These findings indicate that the applied sweat concentration reached the olfactory detection threshold, but did not reach the irritation threshold.

Results revealed no sex-differences in the detection experiment. In the localization experiment men's performance in localizing the applied stimuli to the accurate nostril was significantly better when compared to women. However, male subjects were unable to localize the sweat stimuli above chance level. Women performed significantly below chance level in the localization experiment. This underperformance cannot be ascribed to outliers. Our results confirm previous reports in which subjects' performance of localization was significantly below chance level, when pure odorants were applied to subjects (Schneider and Schmidt 1967; Frasnelli *et al.* 2009).

Physiological parameters of the detection and the localization experiments revealed similar results for the response criterions *ß* and *c*, but different results for the sensitivity *d'*. Class 2 and 3 showed nearby neutral criterions, i.e. subjects did not tend to answer with yes or no in the detection experiment, and showed neither rightward nor leftward tendency in their response in the localization experiment. Class 1 revealed for both experiments a tendency to say no, and a slight tendency to allocate the stimuli to the contralateral nostril, respectively. The analysis of the sensitivity *d'* to the applied sweat stimuli revealed different results between the detection and the localization experiments. This might be surprising especially since the concentration of the stimuli was the same in both sessions. However, as the sensitivity is analyzed from hits and false alarms it is a necessary consequence that subjects showed a low sensitivity during the localization experiment. If one cannot localize a stimulus to the accurate nostril, the number of hits decreases and the number of false alarms increases which consequently leads to a low sensitivity. Therefore, the experimental design consisted of two different sessions to analyze subjects' sensitivity and their ability to localize the stimuli to the accurate nostril.

7.2. Questionnaire

The present study revealed no significant differences between men and women regarding the different parameters of the questionnaire. It is especially surprising that no significant differences in ratings of the sexual attractiveness of male sweat between men and women have been found. Previous studies showed that the hedonic perception of human sweat depends on components, such as human leukocyte antigene (HLA) histocompatibility genes (Wobst *et al.* 1998; Weisfeld *et al.* 2003; Pause *et al.* 2006). In the present study the sweat pads were homogenized, subjects received a mixture of donors' samples. Thus, potential preferences of the recipients for body odors of specific donors were prevented due to the application of pooled sweat samples.

7.3. Conclusion

The present study suggests that human axillary sweat does not cause a trigeminal percept, although the stimuli were consciously perceived. This might be attributed to the close relationship between the olfactory and the trigeminal chemosensory system and the interactions between each other, and to the concentration-dependent activation of the trigeminal system. Human axillary odor is a complex mixture of volatile organic compounds; some of them are represented in very low concentrations. The neuronal processing of mixtures, especially of human body odors, is hardly understood.

According to the literature some of the volatile organic compounds which are present in human sweat do possess trigeminal properties. Accordingly, it is clear that when sweat is applied in very high concentrations, trigeminal effects will occur. However, it is controversially discussed and therefore, of scientific interest whether human sweat activates the nasal trigeminal system, when it is applied in concentrations encountered in daily life. Recently, the effects of human sweat, especially with relevance to its potential behavioral influences on other humans, have been addressed in several scientific studies. In most of these studies stimulus concentrations were at or just above the olfactory threshold. This compares well with the current study design. Thus, the present findings are relevant for this emerging field of

research. Nevertheless, further investigations are needed to explore the chemosensory activities of human sweat in detail including various concentrations and imaging techniques.

III. Effects of male anxiety chemosignals on the evaluation of happy facial expressions

In humans the knowledge about the communication of chemosensory signals are based mainly on investigations regarding reproductive behavior, but also chemosignals of anxiety seem to be present in axillary odors of emotional stress and seem to influence human physiology and behavior. The current experiment examined if anxiety chemosignals affect subjects' visual perception of emotional faces and in specific consideration, if chemosignals are communicated between men. For this purpose, two kinds of male sweat were collected during a bicycle workout and during a visit of a high rope course, and were then applied to male recipients during an emotion evaluation task. Participants were instructed to rate emotional facial expressions of different morphing levels (neutral - happy) by using a visual analog scale under exposure of three different samples (exercise sweat, anxiety sweat, and control material).

8. Material und methods

The entire study was approved by the local Medical Ethics Review Committee of our University and was conducted in accordance with the Declaration of Helsinki. All subjects provided their written informed consent.

8.1. Collection of sweat stimuli

For sweat sampling donors participated in two different sessions (exercise and anxiety condition). During anxiety condition donors attended a high rope course, during the exercise condition donors performed an ergometer workout.

8.1.1. Anxiety assessment

To assess donors' anxiety the Spielberger's state-trait anxiety inventory (STAI X; Spielberger *et al.* 1970, German version by Laux *et al.* 1981) was used. This questionnaire consists of two distinct anxiety scales: the trait and the state scale. Both scales are composed of 20 items.

A B C

D E F

Figure 9 Exercises during the high rope course. A) beam, B) tremor bridge, C) double beam, D) flea jump, E) cargo net, F) pamper pole.

Participants evaluate how they feel in general (trait anxiety, STAI X2) and how they feel during a specific moment (state anxiety, STAI X1) by using a 4-point scale (1 = rare, 4 = often). Sweat donors were screened regarding their trait scale. Furthermore, participants completed the STAI X1 at specific points in time during the sweat collecting sessions (exercise and anxiety condition) to assess their state anxiety. Subjects were advised to fill in the STAI X1 before (t_0) the ergometer training and before the high rope course started, and at the end of both donation sessions (t_2). Furthermore, participants completed the questionnaire directly after each exercise (t_1; mean scores collected during run 1 and 2 of the ergometer training, and mean scores collected during the parcour and the pole of the high rope course) and were told to focus on the feelings they had during each session.

8.1.2. Sweat donors

Twenty-one healthy male subjects between the ages of 18 and 47 years (mean age 28.3 years, SD 7.9 years) participated as sweat donors. All subjects affirmed that they complied with the instructions as described in subsection 5.1.1. Donors were screened regarding their trait scale (cf. subsection 8.1.1.); participants' mean STAI X2 score was 31.7 (SD 6.2) indicating a normal anxiety level (Laux *et al.* 1981). All donors described themselves as exclusively heterosexual on a 7-point scale (mean 0.0, SD 0.0). The sweat samples were used in the odor perception experiment (cf. subsection 8.2.) within four months.

8.1.3. Sweat sampling procedure during exercise and anxiety conditions

The procedure of sweat sampling was identically conducted for both the exercise and the anxiety conditions. A detailed description is given in subsection 5.1.3.

Exercise sweat was collected during a 30 min workout with an estimated power of 120 watt and 90 revolutions per minute on a bicycle ergometer in the Department of Physiotherapy of the Hospital Großhadern. Each participant accomplished this workout twice. Between both sessions subjects rested for approximately 15 min.

Sweat of anxiety was collected during a visit of a high rope course (www.hochseilgartenundmehr.de) (see Figure 9). Donors were advised to complete a parcour and to climb on a pole. During the parcour participants were asked to do five different exercises in a height of nine meters. Participants had to walk along a *beam*, a *tremor bridge*, and a *double beam* freehand; they were instructed to do a *flea jump*, and to climb along a *cargo net*. Afterwards participants rested for approximately 15 min. In session two participants were requested to climb the so called *pamper pole*, a 7-meter high pole, and to stand on that pole without holding on to something and without seeing the security device on their back. After that subjects' task was to jump from that pole. Both sessions (parcour, pole) lasted about 30 min.

8.1.4. Data analyses

Statistical analyses were done using SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA). Normality of the data was tested using the Kolmogorov–Smirnov test. Donors' STAI X1 data were normally distributed, and were submitted to two-tailed Student's paired t-tests. The alpha level was set at 0.05.

8.2. Evaluation of emotional faces

In recipients the effects of anxiety sweat on subjects' perception of facial expressions was explored compared to exercise sweat and control material.

8.2.1. Recipients

Fifteen healthy male subjects (age range 21 – 50 years; mean age 33.8 years, SD 8.9 years) participated as sample recipients. Olfactory screening (cf. subsection 5.2.2.) revealed a mean TDI score of 35.6 (SD 2.3; range 30.8 - 39.5) indicating normal olfactory function. Recipients were screened regarding their trait anxiety (cf. subsection 8.1.1.); the mean STAI X2 score was 36.8 (SD 6.1) indicating a normal anxiety level (Laux *et al.* 1981).

8.2.2. D2 test of attention

Before the experiment started participants were instructed to accomplish the d2 test of attention to compare subjects' alertness across all testing days (Brickenkamp and Zillmer 1998). This test measures speed and quality of performance in crossing out "d" letters with two dashes in rows of similar letters. Measures of performance include the total number of items processed (TN), the total number of errors (E), the percentage of errors (E%), the total number of items minus errors (TN–E), and the concentration performance (CP) derived from the number of correctly crossed out items minus errors of commission. Since subjects completed the test three times (1 time/condition) the time for crossing out the "d" letters in each row was reduced from 20 to 15 s according to the instructions of the d2 test manual.

8.2.3. Experimental procedure

For odor presentation 0.5 grams of the sweat pads collected in the sampling sessions (exercise and anxiety condition; cf. subsection 8.1.) were used, and accordingly of the control material. After unfreezing olfactory stimuli of the respective condition were presented birhinally; pads were placed under recipients' nose using an odorless tea bag which was adjusted with an elastic strap. The order of the anxiety, exercise, and neutral conditions was pseudo randomized. To exclude adaptation effects and to prevent interferences with effects of the different samples three sessions were conducted on three different days (1 condition/day) within maximal three weeks. Each session was accomplished at approximately the same time of the day. Between the sessions a minimum interval of three days was maintained. On the days of the experiments subjects conducted the emotion evaluation task after approximately 30 min of sample exposure. They were asked to rate the emotion of the presented face by using a visual analog scale (VAS; 0 = happy, 100 = scary). They were trained to give a response by placing a mark on a 100-mm horizontal line. Visual analogue scales have been shown to measure even minor changes in affect with high reliability and validity (Aitken 1969; Folstein and Luria 1973). Testing was divided up into two runs per day with a break of approximately 10 min between both runs.

The experiment was conducted in a double blind design: participants were not aware of the nature of the chemosensory signals, and the experimenters were blinded regarding which kind of stimulus was presented.

8.2.4. Emotional faces

Visual stimuli were obtained from the pictures of Facial Affect (Paul Ekman Inc., Oakland CA; Ekman and Friesen 1976). To create ambiguity in facial emotions seven morphing levels were produced by using the freely available software MorphX, Version 2.9.5 (http://www.norrkross.com/software/morphx/download.php). Morphed images were equally distributed between the prototypical neutral (0%) and the prototypical happy (100%) expressions of three actors (EM, JJ, PE; all male) (see Figure 10). The resulting 27 different

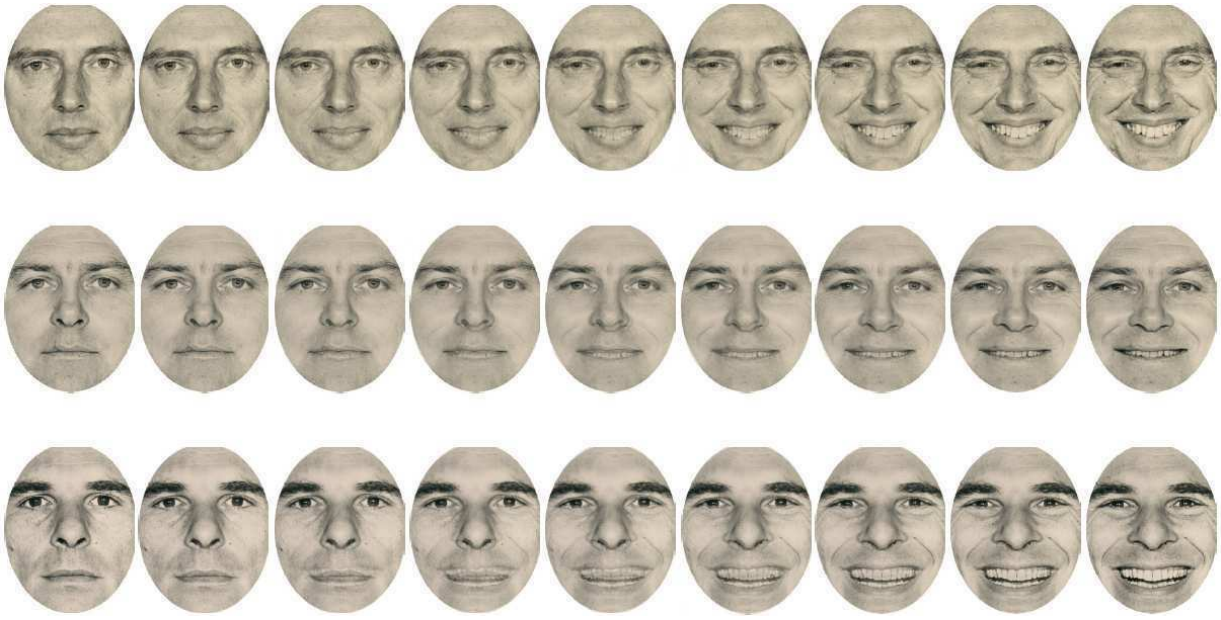


Figure 10 Morphed facial expressions between the emotions neutral and happy.

images were presented in each run in randomized sequence on a computer monitor situated approximately 50 cm in front of the participant. Each face was presented for 200 ms; between the pictures a black background was presented for 4800 ms. During this time subjects were instructed to rate the emotion of the currently presented face by using a VAS, and were instructed to respond as accurately as possible.

8.2.5. Questionnaire

After participants were exposed to the samples they were immediately asked to fill in a questionnaire about how they perceived and evaluated the respective scent. They rated the pleasantness (0 = unpleasant, 100 = pleasant), intensity (0 = weak, 100 = very intensive), familiarity (0 = not familiar, 100 = very familiar), masculinity/femininity (0 = very feminine, 100 = very masculine), sexual attractiveness (0 = not appealing, 100 = very appealing) of the assessed samples, and evaluated their emotional conditions (valence: 0 = negative, 100 =

positive; arousal: 0 = calm, 100 = aroused; dominance: 0 = submissive, 100 = dominant) during sample exposure. The questions were answered by the participants using a VAS.

8.2.6. Data analyses

Statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA). Normality of the data was tested using the Kolmogorov–Smirnov test. Results of recipients' d2 test of attention (normally distributed) were submitted to repeated measures analyses of variance (ANOVA). To compare subjects' ratings during exposure to the three different samples normally distributed data (results of the questionnaire) were submitted to repeated measures ANOVA using the general linear model, and were corrected for multiple testing using the Bonferroni method. Not normally distributed data (results of the emotion evaluation task) were submitted to Friedman tests and subsequent non-parametric Wilcoxon signed-rank tests to compare the differences of recipients' VAS ratings of the emotional faces. P-values ≤ 0.05 were considered significant.

9. Results

9.1. Anxiety assessment of the sweat donors

Sweat donors revealed significantly higher STAI X1 scores during the high rope course when compared to the scores rated during the ergometer training across all three points in time (t_0-t_2; Table 5). Anxiety increased during the exercises and decreased again at the end of the sessions. The highest scores were rated during the accomplishment of the exercises of the high rope course (t_1 anxiety condition: mean 50.2, SD 9.7) indicating an anxiety level above normal values. Descriptive statistics are shown in Table 5.

Table 5 Results of donors' state anxiety (STAI X1) measured before (t_0), during (t_1), and after (t_2) the bicycle workout (exercise condition), and the high rope course (anxiety condition) (n = 21). Reported are means ± standard deviations, and results of two-tailed Student's paired t-tests. * Significant with $p < 0.05$.

STAI X1	Anxiety condition	Exercise condition	Paired t-test
T_0	39.33 ± 11.71	32.62 ± 7.30	p = 0.032*
T_1	50.21 ± 8.45	36.14 ± 9.33	p < 0.001*
T_2	37.14 ± 11.26	30.95 ± 6.69	p = 0.018*

9.2. Recipients

9.2.1. D2 test of attention

Repeated measures ANOVA of the d2 test of attention revealed no significant differences between the three testing days regarding the different parameters (TN: $F(2,28) = 0.40$, E: $F(2,28) = 0.50$, E: $F(2,28) = 0.56$, TN-E: $F(2,28) = 0.37$, CP: $F(2,28) = 3.31$, each with p = n.s.) indicating that participants' attention was similar during each session. Descriptive statistics are shown in Table 6.

9.2.2. Emotion evaluation task

Results of the emotion evaluation task revealed a consistent decline of subjects' rating scores (i.e., they rated the faces as being more happy) with increasing morphing levels of the happy faces. The perception of the faces under the three conditions was similar for the neutral facial expression (morphing level 0) and predominantly neutral morphing levels (morphing levels 1 and 2; see Figure 11). Participants' evaluations diverged between the three stimuli with increasing ambiguity of facial expressions, and were again similar between the three conditions for the prototypic happy face (morphing level 8) and the preceding morphing level (morphing level 7). Subjects' ratings revealed significant differences only for morphing levels 5 and 6. Recipients evaluated these ambiguous emotional faces under chemosensory stimuli of anxiety as less happy when compared to the ratings collected under the influence of the

Table 6 Recipients' means ± standard deviations of the d2 test of attention measured before sample exposure of the anxiety, control, and exercise conditions (n = 15). Measures of attention included the total number of items processed (TN), the total number of errors (E), the percentage of errors (E%), the total number of items minus errors (TN-E), and the concentration performance (CP). Differences between conditions are not significant.

	Anxiety condition	**Exercise condition**	**Control condition**
TN	414.2 ± 87.3	422.2 ± 83.8	422.3 ± 76.3
E	29.1 ± 33.0	24.9 ± 34.7	23.4 ± 18.0
E%	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
TN-E	385.1 ± 78.5	397.3 ± 76.9	384.6 ± 101.5
CP	153.2 ± 40.2	166.4 ± 37.2	167.0 ± 37.3

exercise sweat (level $5_{\text{anxiety condition}}$: mean 23.23, SD 14.78; level $5_{\text{exercise condition}}$: mean 18.11, SD 12.03; Friedman test, p = n.s.; Wilcoxon signed-rank test, p = 0.017; level $6_{\text{anxiety condition}}$: mean 22.00, SD 14.62; level $6_{\text{exercise condition}}$: mean 17.16, SD 11.24; Friedman test, p = 0.023; Wilcoxon signed-rank test, p = 0.015). Additionally, data of morphing level 6 revealed significant differences between ratings made by the participants during anxiety versus control condition (Friedman test, p = 0.023; Wilcoxon signed-rank test, p = 0.021); subjects recognized the facial expression under exposure of control stimulus as more happy (level $6_{\text{control condition}}$: mean 19.17, SD 14.81) when compared to the evaluations made under exposure of anxiety sweat (level $6_{\text{anxiety condition}}$: mean 22.00, SD 14.62). The pairwise comparison of control versus sport condition revealed no significant difference (Wilcoxon signed-rank test, p = n.s.).

An analysis including all data independent of the morphing levels of the emotion evaluation task (morphing level 1 - 8) affirmed the results of the separately analyzed pictures (see Figure 12). Participants rated the facial expressions under exposure of anxiety sweat (mean 29.20, SD 17.08) as significantly less happy compared to the ratings made under exposure of exercise (mean 27.28, SD 16.91; Friedman test, p = 0.003; Wilcoxon signed-rank test, p =

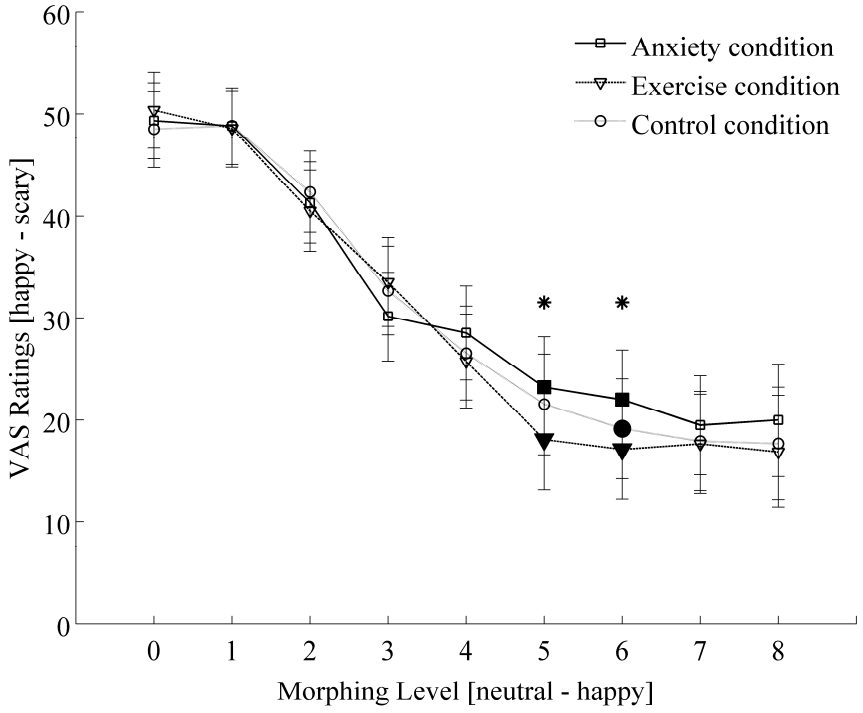


Figure 11 Results of the emotion evaluation task (n = 15). Shown are means and standard errors of the mean of recipients' ratings of morphed faces (0 - 8, neutral - happy) by using visual analog scales (VAS; 0 - 100, happy - scary). Significant differences are accentuated by bold signs. * Wilcoxon signed-rank test significant with $p \leq 0.02$.

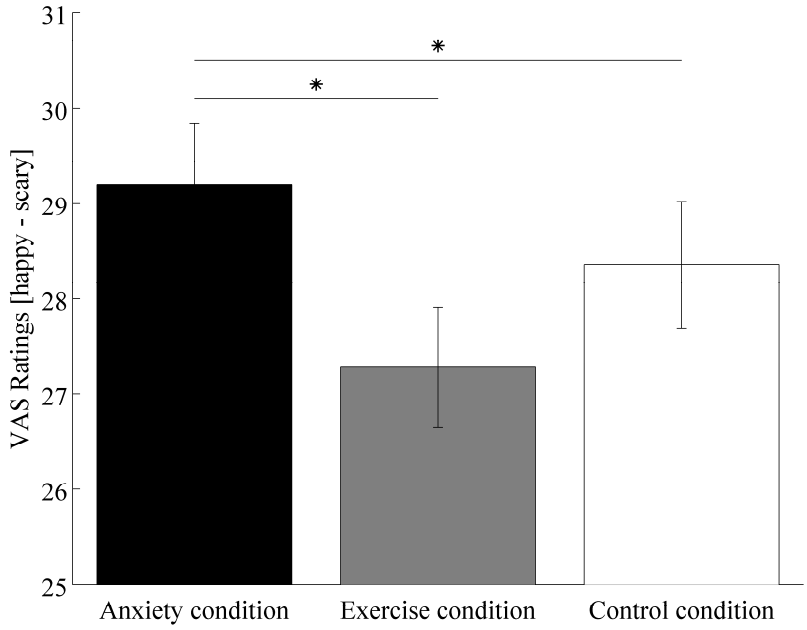


Figure 12 Results of the analysis over all data (including morphing levels 1 - 8) independent of the different morphing levels of the emotion evaluation task by using visual analog scales (VAS; 0 - 100, happy - scary) (n = 15). Shown are means and standard errors of the mean. * Wilcoxon signed-rank test significant with $p < 0.05$.

0.004) and control samples (mean 28.35, SD 17.91; Wilcoxon signed-rank test, p = 0.048). The pairwise comparison of control versus sport condition revealed no significant difference (Wilcoxon signed-rank test, p = n.s.).

9.2.3. Questionnaire

Results of the repeated measure ANOVA of recipients' ratings of the questionnaire revealed no significant differences regarding the different parameters (valence: F(2,28) = 1.12; arousal: F(2,28) = 0.33; dominance: F(2,28) = 0.06; intensity: F(2,28) = 4.01; pleasantness: F(2,28) = 6.71; femininity: F(2,28) = 0.72; sexual attractiveness: F(2,28) = 4.27; familiarity: F(2,28) = 3.26; each with p = n.s.; all pairwise comparisons, each with p = n.s.). Participants felt neutral to slightly positive, predominantly calm, and neither submissive nor dominant when smelling the different samples, and evaluated the stimuli as neutral to slightly unpleasant, and as not very intense. Subjects rated all samples as neither very masculine nor as distinctive feminine; all stimuli were perceived as not sexually attractive and as not familiar by trend (see Table 7).

Table 7 Results of the questionnaire. Reported are means ± standard deviations of recipients' perception of the different samples (n = 15). Differences between conditions are not significant.

	Anxiety condition	**Exercise condition**	**Control condition**
Pleasantness	43.80 ± 25.69	45.20 ± 30.80	52.40 ± 12.45
Intensity	32.00 ± 32.27	35.40 ± 41.78	6.80 ± 11.50
Familiarity	48.80 ± 30.56	25.07 ± 34.94	33.67 ± 29.72
Masculinity/Femininity	53.87 ± 23.23	46.47 ± 21.17	48.73 ± 16.47
Sexual attractiveness	20.87 ± 21.47	35.60 ± 21.06	37.20 ± 18.65
Valence	57.27 ± 33.55	58.27 ± 35.53	69.07 ± 24.60
Arousal	23.00 ± 20.05	29.33 ± 35.06	22.67 ± 26.20
Dominance	50.07 ± 6.71	50.07 ± 7.03	50.80 ± 12.21

10. Discussion

10.1. Anxiety assessment of the sweat donors

The self-report measurements of the sweat donors confirm that the paradigm of inducing emotional stress during the anxiety condition was successful. In contrast to previously published studies, which collected chemosignals of emotional stress during first tandem skydives (Mujica-Parodi *et al.* 2009), watching terrifying movies (Ackerl *et al.* 2002; Chen *et al.* 2006; Zhou and Chen 2009), and awaiting academic examinations (Pause *et al.* 2004; Prehn *et al.* 2006; Pause *et al.* 2009; Prehn-Kristensen *et al.* 2009), emotional stress was induced in participants during a visit of a high rope course. Donors' state anxiety increased during the accomplishment of the exercises in the high rope course, and was significantly higher compared to the ratings made during the ergometer training. Therefore, donors were more afraid during the sweat collecting session of anxiety versus sport indicating that the method of inducing emotional stress in donors was successful.

10.2. Emotion evaluation task

The present study provides further evidence for the hypothesis that humans communicate emotional stress via chemosensory signals. Our findings demonstrate an influence of anxiety signals on the perception of happy facial expressions. Interestingly, significant effects were observed only for ambiguous emotional faces. No significant differences between the conditions were observed for the prototypical pictures and the facial expressions morphed only slightly. These results are in line with previously published studies; alarm signals affected only the perception of facial expressions of a high ambiguity (Mujica-Parodi *et al.* 2009; Zhou and Chen 2009).

Mujica-Parodi *et al.* (2009) and Zhou & Chen (2009) demonstrated that participants were more likely to judge a face to be negative when exposed to anxiety sweat when compared to the reference condition. The current experiment revealed similar effects of chemosignals of anxiety; participants evaluated ambiguous happy faces as less happy under exposure of

anxiety sweat when compared to sport or control samples. Therefore, chemosignals of anxiety affect not only the perception of threatening (Mujica-Parodi *et al.* 2009) or fearful (Zhou and Chen 2009) facial expressions, but also diminish the evaluation of happy faces. Thus, alarm signals not only enforce the emotion-perception of negative cues, but also impair the perception of the contrary emotion. Similarly, Pause *et al.* (2004) demonstrated that the priming effect of happy faces was reduced in females when exposed to anxiety signals.

In previous studies using stress odors there was a tendency to use only female receivers (Ackerl *et al.* 2002; Chen *et al.* 2006; Zhou and Chen 2009). Therefore, little is known about potential sex-related different impacts of anxiety signals. To date there are only three studies investigating the influence of stress sweat on exclusively male participants, and they report contradictory findings. Pause *et al.* (2004) observed no effect on males, but found effect on females in an emotional priming task; in another study the authors (Pause *et al.* 2009) gave evidence for the communication of stress signals in men and women by augmentation of the acoustic startle reflex. Mujica-Parodi *et al.* (2009) did not observe any sex-specific interactions in males and females while investigating activations of the amygdala in response to male and female anxiety sweat in a functional magnetic resonance imaging study. Therefore, the present study extends the knowledge about the communication of anxiety chemosigals between men.

10.3. Questionnaire

A few studies suggest that chemosensory alarm signals can be distinguished from chemosensory neutral odors when presented in a discrimination task (Chen and Haviland-Jones 2000; Ackerl *et al.* 2002), but have no influence on recipients' hedonic perception (Chen *et al.* 2006; Prehn *et al.* 2006; Mujica-Parodi *et al.* 2009; Pause *et al.* 2009; Prehn-Kristensen *et al.* 2009; Zhou and Chen 2009). The present results of the questionnaire are in line with previously published studies about subjects' perception of the assessed stimuli; no significant differences were found between the three samples regarding the different questions. Although behavioral studies in mammals showed increased arousal when animals

were exposed to stress signals (Zalaquett and Thiessen 1991; Wyatt 2004), to date no experiment (Chen *et al.* 2006; Pause *et al.* 2009; Zhou and Chen 2009) yields any evidence of such a behavioral reaction mediated by anxiety signals in humans. Since subjects‘ ratings of their conscious percept of the stimuli did not differ between conditions, it is justified to postulate that the findings in the emotional evaluation task were affected by the unconscious percept of chemosensory alarm signals in human sweat, but not by hedonic effects or other aspects of the conscious percept of the stimuli. The finding of no differences in the conscious percept of the samples, but effects of alarm signals on human behavior, support the hypothesis that the neural signals of pheromonal chemosignals are projected to the amygdala and the hypothalamus not only in mammals (Keverne 1999), but also in humans, and thus invoking behavioral and endocrine responses (Savic *et al.* 2001; Sobel and Brown 2001; Savic 2002; Bhutta 2007) which do not necessarily include conscious perception.

10.4. Conclusion

The present study accounts for the communication of social information via chemosensory signals, and gives evidence that alarm substances comprised in male sweat modulate the perception of emotional facial expressions in male recipients. Results demonstrated that chemosignals of anxiety diminish men’s evaluation of ambiguous happy male faces in comparison to reference samples, but had no significant effect when the facial emotion was more discernible. As previous emotion identification tasks investigated the effects of anxiety signals on faces morphed with negative facial expressions, and as the present study was conducted with another experimental design, and explored specifically the male to male communication, our findings are new and extend the knowledge about chemosignals of emotional stress in humans. One limitation of our study design was that we conducted the experiment only with male recipients. The influence of alarm signals on women’s perception of happy facial expressions should be investigated in further studies to explore possible sex-specific interactions.

IV. Sex-specific effects of male sweat on human skin conductance response

Research about the communication between humans via chemosensory signals has mainly focused on behavioral studies; the impact of chemosignals on autonomic responses is hardly understood. The purpose of the present experiment was to investigate the effect of body odors on human skin conductance response (SCR). For this purpose, two kinds of human sweat were collected from male subjects during a bicycle workout (exercise condition) and during a visit of a high rope course (anxiety condition), and were then applied to male and female recipients. Subjects' skin conductance responses were recorded during the presentation of acoustic startle stimuli after 5 min and after 30 min of sample exposure. The experiment investigated the relationship between two kinds of axillary secretions and human SCR with regard to possible sex-specific effects and including possible interactions with the duration of sample exposure.

11. Material und methods

The entire study was approved by the local Medical Ethics Review Committee of our University and was conducted in accordance with the Declaration of Helsinki. All subjects provided their written informed consent.

11.1. Collection of sweat stimuli

For sweat sampling donors participated in two different sessions (exercise and anxiety condition). During anxiety condition donors attended a high rope course, during the exercise condition donors performed an ergometer workout. As the collection of the sweat stimuli was one part of a larger study on chemosensory signals, the sweat samples of the experiment about the effects of male anxiety chemosignals on the evaluation of happy facial expressions (cf. chapter III.) and the sweat samples of the current experiment were originated from the same donation sessions. Therefore, detailed descriptions about sweat donors, the sweat

sampling procedure, statistical analyses, and the results of their anxiety levels as well as a discussion of donors' results can be found in the subsections 8.1., 9.1., and 10.1.

11.2. Skin conductance response elicited by acoustic startle stimuli

The experiment explored the effects of two kinds of male sweat (exercise and anxiety sweat) on skin conductance response elicited by acoustic startle stimuli of male and female subjects. Additionally, the experiment was accomplished with unused pads (control condition).

11.2.1. Recipients

Thirty-one healthy, dextral subjects participated as recipients. Due to technical problems during the recording of the skin conductance response (SCR) the data of one male subject was excluded from statistical analyses (14 males, 16 females; age range 18 – 50 years; mean age 32.1 years, SD 8.4 years). Age did not differ significantly between male and female subjects (independent two sample t-test, $t(28) = 1.35$, p = n.s.). All participants affirmed that they complied with the requirements and instructions (cf. subsection 5.2.1.). Olfactory screening (cf. subsection 5.2.2.) revealed a mean TDI score of 35.6 (SD 2.3; range 30.8 - 39.5) indicating normal olfactory function. There were no significant differences between male and female subjects regarding their TDI scores (independent two sample t-test, $t(28) = 0.23$, p = n.s.). Recipients were screened regarding their trait anxiety (cf. subsection 8.1.1.); the mean STAI X2 score was 37.4 (SD 8.1) indicating a normal anxiety level (Laux *et al.* 1981). Trait anxiety did not differ significantly between male and female participants (independent two sample t-test, $t(28) = 1.00$, p = n.s.).

11.2.2. SCR recording and acoustic startle stimuli

Skin conductance response (SCR) was recorded using the software Biopac Student Lab PRO 3.7. (Biopac Systems, Inc.; see Figure 13), and was obtained in micro Siemens (µS) with two circular 8-mm Ag/AgCl electrodes. The two electrodes were attached to the inner palm surface of the left (non-dominant) hand. For grounding one 8-mm Ag/AgCl electrode was

attached above the ankle of the left foot. Sampling rate was set at 500 Hz. SCR was recorded after 5 min and after 30 min of sample exposure per condition, and in each case for a period of 5 min while ten auditory stimuli were presented binaurally via headphones. The acoustic startle stimulus consisted of a 100 dB beep of 440 Hz and 50 ms duration. Ten auditory stimuli were distributed within 5 min with pseudo randomized durations of interstimulus intervals; between the stimuli a minimal interstimulus interval of 15 s was maintained. Skin conductance responses were identified as elicited when they occurred within a time window of 0.5 - 4.5 s after stimulus presentation. After each stimulus the SCR amplitude within this time window of 4 s was calculated. The boundary between response and non-response was set at ≥ 0.05 µS (Cacioppo *et al.* 2007). For each condition (anxiety, exercise, and control condition) mean SCR amplitudes were calculated for the time points 5 min and 30 min of sample exposure.

11.2.3. Experimental procedure

After the electrodes were attached subjects were instructed to sit quietly and relax for approximately 5 min. Subsequently, an amount of 0.5 grams of the respective sample (exercise sweat, anxiety sweat, or control material; cf. subsection 8.1.) was placed under recipients' nose using an odorless tea bag which was adjusted with an elastic strap (see Figure 14). Afterwards, subjects were immediately asked to fill in a questionnaire about how they perceived the samples. To test subjects' skin conductance responses dependent on the different samples, participants' SCR was recorded after 5 min and after 30 min of sample exposure while presenting acoustic startle stimuli. To exclude adaptation effects and to prevent interferences with effects of the different samples we conducted three sessions on three different days (1 condition/day) within maximal three weeks; each session was accomplished at approximately the same time of the day. Between the sessions a minimum interval of three days was maintained. The order of the anxiety, exercise, and neutral condition was pseudo randomized. The experiment was conducted in a double blind design; subjects were not aware of the nature of the chemosensory signals, and the experimenters were blinded regarding which kind of stimulus was presented. Subjects were told that they

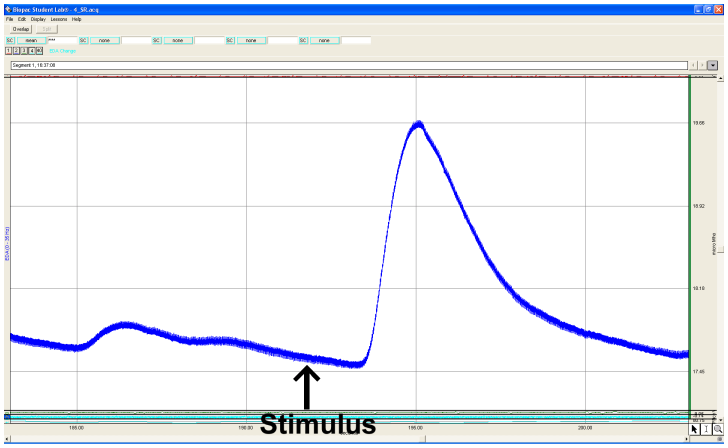


Figure 13 Example of SCR recording during an acoustic startle stimulus using the BioPac software.

Figure 14 Recipient during experiment.

would receive different odorants while their SCR was recorded.

11.2.4. Questionnaire

After subjects were exposed to the odor samples they were immediately asked to fill in a questionnaire about how they perceived the respective scent. They rated the pleasantness (0 = unpleasant, 100 = pleasant), intensity (0 = weak, 100 = very intensive), familiarity (0 = not familiar, 100 = very familiar), masculinity/femininity (0 = very feminine, 100 = very masculine), sexual attractiveness (0 = not appealing, 100 = very appealing) of the assessed samples, and evaluated their emotional conditions (valence: 0 = negative, 100 = positive; arousal: 0 = calm, 100 = aroused; dominance: 0 = submissive, 100 = dominant) during sample exposure. The questions were answered by the participants using a 100-mm visual analogue scale (VAS; Aitken 1969; Folstein and Luria 1973).

11.2.5. Data analyses

Statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA). Normality of the data was tested using the Kolmogorov–Smirnov test. To compare subjects' ratings of the three different samples (results of the questionnaire, normally

distributed) data were submitted to repeated measures analyses of variance (ANOVA) using the general linear model, and were corrected for multiple testing using the Bonferroni method. Not normally distributed data (results of the SCR recordings) were submitted to Friedman tests, and subsequent to non-parametric Wilcoxon signed-rank tests to compare the differences of recipients' SCR amplitudes in respect of the three different conditions. Differences between men and women regarding the SCR amplitudes were tested using Mann Whitney U tests. P-values ≤ 0.05 were considered significant.

12. Results

12.1. Skin conductance response of male recipients

Analyses of men's skin conductance response elicited by acoustic startle stimuli revealed significant results between anxiety versus exercise condition after 5 min and after 30 min of sample exposure (Friedman tests, 5 min: $p = 0.009$, 30 min: $p = 0.010$; Wilcoxon signed-rank tests, $5\ min_{anxiety\ vs.\ exercise\ condition}$: $p = 0.028$, $30\ min_{anxiety\ vs.\ exercise\ condition}$: $p = 0.040$; see Figure 15). SCR amplitudes after 5 min were significantly higher measured during the exercise condition when compared to the measurements during the anxiety condition, whereas the SCR after 30 min was significantly higher during the anxiety condition compared to the exercise condition. The SCR amplitudes measured during anxiety condition were higher than the SCR amplitudes during control condition by trend, both for the measurements after 5 min and after 30 min of sample exposure, but no significant differences were observed (Wilcoxon signed-rank tests, $5\ min_{anxiety\ vs.\ control\ condition}$: $p = n.s.$, $30\ min_{anxiety\ vs.\ control\ condition}$: $p = n.s.$). The SCR amplitudes of the exercise condition measured after 5 min showed significantly higher values when compared to the amplitudes of the control condition; the comparison of the SCR amplitudes after 30 min was not significant (Wilcoxon signed-rank tests, $5\ min_{exercise\ vs.\ control\ condition}$: $p = 0.022$, $30\ min_{exercise\ vs.\ control\ condition}$: $p = n.s.$). The comparison between the

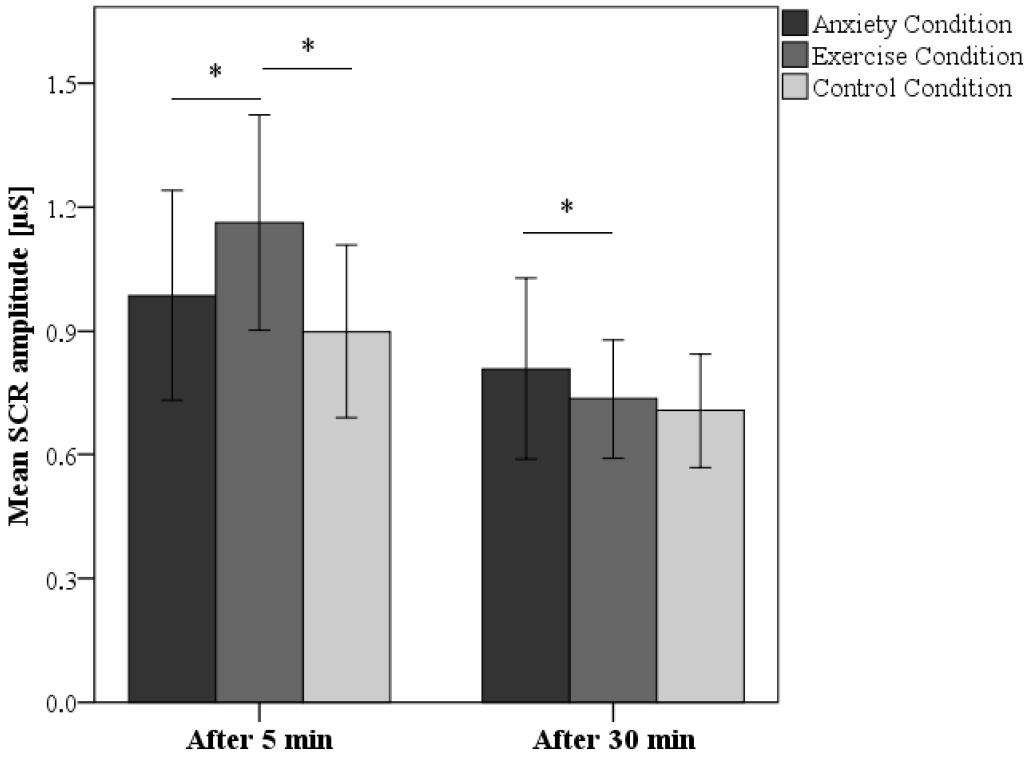


Figure 15 Results of male subjects (n = 14). Shown are means and standard errors of the mean of the skin conductance response (SCR) amplitudes after 5 min and after 30 min of sample exposure (anxiety, exercise, control condition). * Friedman tests, significant with $p \leq 0.01$.

amplitudes after 5 min versus 30 min revealed significant differences for male subjects only for the exercise condition (Wilcoxon signed-rank tests, anxiety condition$_{5 \text{ min vs. } 30 \text{ min}}$: p = n.s., exercise condition$_{5 \text{ min vs. } 30 \text{ min}}$: p = 0.005, control condition$_{5 \text{ min vs. } 30 \text{ min}}$: p = n.s.); SCR revealed significantly higher amplitudes after 5 min than after 30 min of sample exposure.

12.2. Skin conductance response of female recipients

Analyses of women's SCR amplitudes showed significantly higher scores of the anxiety versus exercise condition, both after 5 min and after 30 min of sample exposure (Friedman tests, 5 min: $p < 0.001$, 30 min: $p < 0.001$; Wilcoxon signed-rank tests, 5 min$_{\text{anxiety vs. exercise condition}}$: p = 0.028, 30 min$_{\text{anxiety vs. exercise condition}}$: p = 0.005; see Figure 16). The comparison between the SCR amplitudes of anxiety versus control condition showed significant results

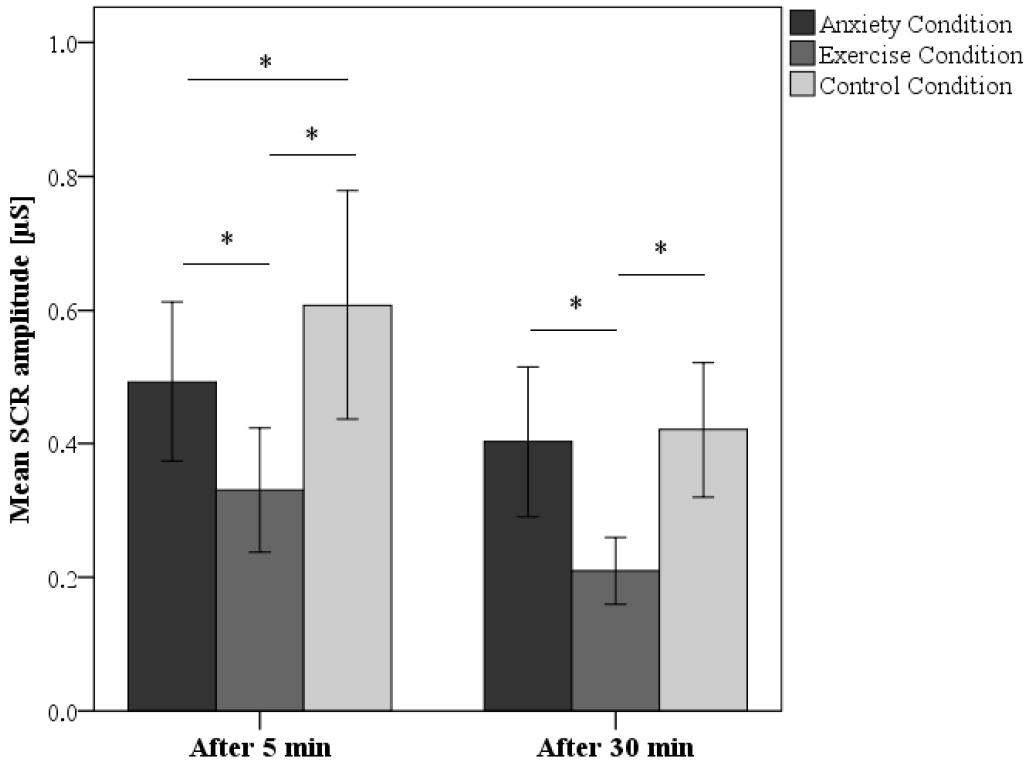


Figure 16 Results of female subjects (n = 16). Shown are means and standard errors of the mean of the skin conductance response (SCR) amplitudes after 5 min and after 30 min of sample exposure (anxiety, exercise, control condition). * Friedman tests, significant with $p < 0.001$.

after 5 min (Wilcoxon signed-rank test, 5 $min_{\text{anxiety vs. control condition}}$: $p = 0.023$), but no significant differences after 30 min of sample exposure (Wilcoxon signed-rank test, 30 $min_{\text{anxiety vs. control condition}}$: p = n.s.); females' SCR amplitudes after 5 min were significantly higher during control condition than during anxiety condition. The SCR amplitudes of the control condition showed significantly higher scores when compared to the amplitudes of the exercise condition, both after 5 min (Wilcoxon signed-rank test, 5 $min_{\text{exercise vs. control condition}}$: $p < 0.001$) and after 30 min of sample exposure (Wilcoxon signed-rank test, 30 $min_{\text{exercise vs. control condition}}$: $p < 0.001$). The comparison between the amplitudes after 5 min versus 30 min revealed significant differences for female subjects for the anxiety and the control conditions (Wilcoxon signed-rank tests, anxiety $condition_{5 \text{ min vs. } 30 \text{ min}}$: $p = 0.032$, exercise $condition_{5 \text{ min vs. } 30 \text{ min}}$: p = n.s., control

condition$_{5\ min\ vs.\ 30\ min}$: p = 0.015). SCR revealed significantly higher amplitudes after 5 min than after 30 min of sample exposure, both during the anxiety and the control condition.

12.3. Skin conductance response of male versus female recipients

Statistical analyses of the SCR amplitudes of men versus women revealed significant differences for all measurements. Males showed significantly higher SCR amplitudes than females (Mann Whitney U tests, anxiety condition$_{5\ min}$: p = 0.001, anxiety condition$_{30\ min}$: p < 0.001, exercise condition$_{5\ min}$: p < 0.001, exercise condition$_{30\ min}$: p < 0.001, control condition $_{5\ min}$: p = 0.009, control condition$_{30\ min}$: p < 0.001).

12.4. Questionnaire

Descriptive statistics of the questionnaire are shown in Table 8. Results of the repeated measure analyses of variance analyzed for male subjects revealed no significant differences between the three conditions for all ratings (pleasantness, F(2,26) = 0.55; intensity, F(2,26) = 3.88; familiarity, F(2,26) = 5.54; masculinity/femininity, F(2,26) = 0.72; sexual attractiveness, F(2,26) = 4.09; valence, F(2,26) = 0.68; arousal, F(2,26) = 0.06; dominance, F(2,26) = 0.23; each with p = n.s.). When data were analyzed for female subjects results of the parameters dominance (F(2,30) = 0.44, p = n.s.), arousal (F(2,30) = 4.29, p = n.s.), sexual attractiveness (F(2,30) = 1.76, p = n.s.), and familiarity (F(2,30) = 2.81, p = n.s.) showed no significant differences between the three conditions. Results of the parameters valence (F(2,30) = 10.22, p < 0.001), masculinity/femininity (F(2,30) = 6.98, p = 0.003), and intensity (F(2,30) = 11.56, p < 0.001) revealed significant differences; women felt significantly more positive when exposed to control material compared to anxiety sweat (pairwise comparisons: anxiety vs. control condition, p = 0.003; exercise vs. control condition, p = n.s.; anxiety vs. exercise condition, p = n.s.), and rated the control pads as more feminine (pairwise comparisons: anxiety vs. control condition, p = 0.004; exercise vs. control condition, p = n.s.; anxiety vs. exercise condition, p = n.s.), and less intense when compared to the anxiety condition (pairwise comparisons: anxiety vs. control condition, p < 0.001; exercise vs. control condition, p = n.s.; anxiety vs. exercise

Table 8 Results of the questionnaire. Reported are means and standard errors of the mean of recipients' perception of the different samples (males: n = 14, females: n = 16).

	Anxiety condition		Exercise condition		Control condition	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Pleasantness	43.5 ± 7.1	29.6 ± 5.5	48.4 ± 7.8	29.0 ± 4.1	51.8 ± 3.4	66.6 ± 5.6
Intensity	34.3 ± 8.6	48.8 ± 7.3	30.8 ± 10.5	39.8 ± 8.4	7.3 ± 3.2	8.7 ± 4.2
Familiarity	48.6 ± 8.5	47.4 ± 5.9	19.7 ± 7.8	38.1 ± 5.3	34.8 ± 8.2	54.4 ± 6.8
Masculinity/Femininity	53.9 ± 6.4	55.4 ± 4.5	46.1 ± 5.9	55.3 ± 5.7	48.4 ± 4.5	37.4 ± 5.5
Sexual attractiveness	22.4 ± 5.7	23.6 ± 5.9	38.1 ± 5.2	19.8 ± 6.0	39.1 ± 4.8	34.0 ± 5.3
Valence	57.9 ± 9.3	32.0 ± 6.1	62.4 ± 8.8	39.7 ± 5.9	67.6 ± 6.7	67.7 ± 6.7
Arousal	21.4 ± 5.3	32.3 ± 6.1	24.3 ± 8.1	34.8 ± 5.6	23.5 ± 7.2	14.8 ± 5.0
Dominance	50.2 ± 1.9	45.0 ± 3.6	49.1 ± 1.7	50.6 ± 3.5	50.8 ± 3.4	46.0 ± 3.2

condition, p = n.s.). Females evaluated the control material as significantly more pleasant when compared to both kinds of sweat, whereas the comparison of anxiety versus exercise condition revealed no significant differences (pairwise comparisons: anxiety vs. control condition, p = 0.004; exercise vs. control condition, p < 0.001; anxiety vs. exercise condition, p = n.s.).

13. Discussion

13.1. Skin conductance response elicited by acoustic startle stimuli

The present study suggests sex-specific effects of male sweat on subjects' skin conductance response with significantly higher SCR amplitudes in men when compared to women during presentation of acoustic startle stimuli. Male subjects showed the highest skin conductance

amplitudes during exposure of male sweat, whereas females' SCR revealed the highest amplitudes during control condition. These findings indicate that male sweat has an arousing effect on men, but a calming effect on women. Similar findings were reported by Preti *et al.* (2003) who demonstrated that women's mood was modulated by male axillary secretions with a reduction of tension and an increase of relaxation. Grosser *et al.* (2000) showed a significant decrease in female's skin conductance response during exposure of androstadienone (AND), the most prominent androstene present in the male axilla (Nixon *et al.* 1988; Pause 2004). In the current study the calming effect of exercise sweat on women was significantly higher than the effects of anxiety sweat demonstrated for both points in time, whereas men's amplitudes of anxiety and exercise conditions reversed. After 5 min males' SCR was higher during exercise sweat compared to anxiety sweat, and vice versa after 30 min of sample exposure. These observations suggest that men might react faster on signals comprised in exercise sweat. The sexual chemosignal androstadienone is supposed to provoke mediate aversive behavior in men, which leads to higher SCR amplitudes. These arousing effects by exercise sweat in men significantly decreased over time, which might be attributed to the lack of a real potential counterpart, whereas SCR of anxiety and control condition did not significantly change over time.

The present findings of higher SCR amplitudes during anxiety than during exercise condition after 30 min of sample exposure suggest that constituents comprised in anxiety sweat increase the activity of the autonomic nervous system. Previously published evidence for an impact of alarm signals on human physiology have been demonstrated in studies by using means of functional magnetic resonance imaging (Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009) and electromyography (Prehn *et al.* 2006; Pause *et al.* 2009). The results of the present study are comparable to previous studies which observed higher startle reflex responses during exposure of alarm signals compared to a reference sample (Prehn *et al.* 2006; Pause *et al.* 2009). Both, those and our results suggest a higher fright response elicited by acoustic startle stimuli under the influence of alarm signals. In mammals increased startle reflex responses and arousal, avoidance of the odor source, and withdrawal behavior have

been demonstrated in receivers of stress signals (Zalaquett and Thiessen 1991; Kiyokawa *et al.* 2006; Inagaki *et al.* 2008). The communication of danger via chemosensory alarm signals appears to have the implication of inducing appropriate behavioral and physiological responses in conspecifics to escape from a threatening situation. The development of this early-warning system seems to be evolutionarily advantageous, and seems to be still present in humans. In the current study the higher SCR amplitudes during anxiety condition compared to control condition in men could only be demonstrated by trend, but not with significant differences. This fact could have been caused by an insufficient number of tested subjects. Nevertheless, male's SCR showed significantly higher amplitudes after 30 min of anxiety versus exercise sweat. In women the SCR amplitudes were higher during control versus anxiety condition; this fact could be attributed to the calming effect of male sweat, whether collected during a sport or anxiety condition. Nevertheless, skin conductance response was significantly higher during anxiety versus exercise condition, both after 5 min and after 30 min of sample exposure, indicating an arousing effect of anxiety sweat compared to exercise sweat, and the presence of alarm signals in sweat collected during a visit of a high rope course.

It might be surprising, that the current study revealed on the one side a calming effect of male exercise sweat on female subjects, and on the other side an arousing effect of male anxiety sweat. Previous studies explored anxiety signals compared to exercise sweat without an additional control condition as reference (Pause *et al.* 2004; Mujica-Parodi *et al.* 2009; Prehn-Kristensen *et al.* 2009), or explored anxiety signals without sex-specific differentiation (Prehn *et al.* 2006) and only in female subjects (Chen *et al.* 2006; Zhou and Chen 2009), respectively. To date only one study investigated the effects of anxiety signals with regard to three different conditions (anxiety, exercise, and control condition) and in respect to sex-specific differentiation (Pause *et al.* 2009). Therefore, the present study extends the knowledge about chemosensory signals regarding sex and different chemosignals comprised in different kinds of sweat. The study of Pause *et al.* (2009) about startle reflex response demonstrated no sex-differences between receivers. As in their study the differences between conditions were

observed only for exercise versus anxiety sweat and only for highly anxious participants, but not for non-anxious participants and not compared to control condition, their results are not contradictory to the present results. The current study demonstrated sex-specific effects of chemosignals on skin conductance response of healthy subjects. Therefore, we suggest that future studies of anxiety signals should be accomplished invariably with three conditions to examine possible effects of chemosignals comprised in exercise sweat compared to anxiety and control conditions, and suggest that future studies should clearly differentiate between sex, both in sweat donors and in recipients.

13.2. Questionnaire

Since the results of recipients' questionnaire revealed no significant differences between the three conditions for male subjects, the present findings suggest that men's skin conductance was not affected by their hedonic perception of the samples, but only by the preconscious percept of chemosensory signals in human sweat. On the contrary, women's SCR might have been affected by their partial consciously perceived differences; but as the differences were only related to control and sweat samples, possible hedonic effects could only have influenced the SCR amplitudes during control versus sweat material, but not between both kinds of sweat. Therefore, our findings of generally higher SCR amplitudes during control condition in females could be attributed to their ratings of the samples. Nevertheless, previous studies do not demonstrate consistent findings regarding the relationship between the hedonic perception of odorants and the related skin conductance response (Brand *et al.* 2000; Moller and Dijksterhuis 2003). In addition to the inconsistency of these findings, the hypothesis of these studies was that unpleasant odorants in general produce larger SCR than pleasant odorants. This assumption would have lead to converse results in the current study that showed higher SCR during control material, which females rated as more pleasant than the sweat samples. Therefore, it is justified to postulate that the higher SCR amplitudes during control condition in women were caused by the calming effects of male sweat, whether

exercise or anxiety sweat, but not by differences in hedonic perception compared to the sweat samples.

13.3. Conclusion

The present study confirms the hypothesis that male sweat has a calming effect on women, but an arousing effect on men. We could demonstrate that these assumptions are valid for two kinds of male sweat, both for anxiety sweat collected during a visit of a high rope course, and for exercise sweat collected during ergometer training. Furthermore, the current study gives evidence that alarm signals are communicated between humans, and affect the autonomic system of subjects. The arousing effect of anxiety signals on human skin conductance response compared to exercise sweat was demonstrated for male subjects after 30 min of sample exposure, for female subjects both after 5 min and after 30 min. Since we found those sex-differences regarding the timing of chemosensory communication we suggest that future studies should implement this knowledge, and should further explore the effects of chemosensory signals over time with sex-specific differentiation.

V. Conclusion and outlook

The present thesis work about the effects of male sweat on human physiology and behavior extends the knowledge about body odors, more precisely what kind of receptors are activated when sweat stimuli are applied to the nasal mucosa, how the visual perception of emotional pictures are affected by chemosensory signals comprised in anxiety sweat, and how axillary secretions affect human skin conductance response. The method of collecting anxiety sweat during a visit of a high rope course was introduced and established by means of the STAI X questionnaire and by the shown effects on the recipients. All studies were accomplished with sweat samples of exclusively male subjects and additionally with control material. In recipients the parameter sex was statistically analyzed to explore possible sex-specific differences. Most previous studies about chemosensory signals were restricted regarding the parameter sex by either accomplishing investigations without a sex-specific differentiation in sweat donors and recipients, respectively, or by only accomplishing investigations in female recipients. Furthermore, previous studies about anxiety chemosensory signals often used only exercise sweat as reference, but no additional control material. Therefore, future studies should be executed with three conditions, and should clearly differentiate between sex, both in sweat donors and in recipients.

The study about chemosensory properties of axillary sweat was the first experiment which examined the activation of olfactory versus trigeminal receptors based on psychophysical methods. One limitation was that the experiment was conducted only with one concentration of sweat. Future studies about the chemosensory activities of body odors are needed to explore detection thresholds of various sweat concentrations based on sensations of the olfactory chemosensory system, to explore possible thresholds of true pungency based on sensations of the trigeminal system, and to determine the range between olfactory and irritation thresholds in respect to stimuli concentration.

The experiment about the relevance of anxiety chemosignals on the perception of emotional facial expressions specifically extends the knowledge about male to male communication and demonstrates that anxiety signals enforce not only the same emotion, but also modulate the perception of pictures of the opposite emotion. Future investigations are needed to

understand the different impact of chemosensory signals on male and female subjects; especially the communication via chemosignals comprised in female sweat is nearly unexplored.

The study about the impact of chemosensory signals on human skin conductance response was the first experiment discovering the relationship between two kinds of axillary secretions and the SCR of males and females including possible interactions with the duration of sample exposure. Further investigations are needed to determine the effects of chemosignals over time in respect to the parameter sex, and to examine the effects of chemosignals on autonomic responses using various methods and study designs. As anxiety can induce defensive or offensive behavior the relevance of anxiety signals on risky behavior and decision making displays another open field of research which should be investigated.

Summary

Human sweat is object of a lot of scientific studies including investigations of neuronal correlates, and human communication of chemosensory signals comprised in body odors. However, little is known about the combination of receptors activated by the application of this mixture of odorants; the effects of chemosignals on human physiology and behavior are hardly understood.

Human sweat contains a mixture of odorants with trigeminal as well as olfactory properties. It has been shown that trigeminal perception is necessary to localize odors, i.e. to accurately allocate olfactory stimuli to the right and left nostril, and that humans are not able to localize substances which only activate the olfactory system. To analyze the chemosensory properties of human sweat humans' ability to localize sweat stimuli to the different nostrils were studied (cf. chapter II.). Human sweat was collected during a bicycle workout (20 males), and was then applied to thirty-four different subjects (17 females) during odor detection and localization experiments by using an olfactometer. During the detection experiment subjects were instructed to discriminate between sweat stimuli (20) and blanks (10). During the localization experiment they were assigned to allocate the stimuli to either the right (15) or the left nostril (15). Subjects were able to detect the sweat stimuli with moderate to high sensitivity. However, they failed to localize the sweat stimuli to the accurate nostril above chance level. In conclusion, due to the inability to localize the stimuli human sweat does not activate the intranasal trigeminal system, but only the olfactory system when presented in concentration encountered in daily life.

The communication of chemosensory signals is well explored in mammals. In humans the effects of chemosignals seem to be less important due to their high-developed visual system, and their sophisticated ability to communicate via speech and body language. Nevertheless, an increasing number of studies suggest an effect of chemosensory signals on human physiology and behavior. Research about the communication between humans via chemosensory signals has mainly focused on sexual behavior, but also anxiety signals have been investigated in recent years. The effects of human sweat on autonomic responses are

hardly understood. To date no experiment about human skin conductance response (SCR) regarding body odors has been published.

For the present studies about chemosensory signals two kinds of human sweat were collected from twenty-one males during a bicycle workout (exercise condition) and during a visit of a high rope course (anxiety condition), and were then applied to recipients in two different experiments to investigate the effects of chemosignals comprised in axillary secretions.

In the study about anxiety signals on men's perception of emotional faces (cf. chapter III.) sweat samples were applied to fifteen different healthy male participants during an emotion evaluation task. Participants were instructed to rate emotional male faces of different morphing levels (neutral - happy) by using a visual analog scale under exposure of three different samples (exercise, anxiety, and control condition). The study revealed that subjects rated happy faces as less happy under the influence of anxiety sweat compared to the exercise and the control conditions; significant differences were demonstrated only for ambiguous emotional faces. In conclusion, chemosignals of anxiety comprised in human sweat are communicated between males; they diminish the evaluation of ambiguous happy male facial expressions in men and thereby influence the perception of emotional faces.

In the study about the effects of male sweat on human skin conductance response (SCR) (cf. chapter IV.) two kinds of human sweat were applied to thirty-one healthy subjects (16 females). In addition unused pads were used as control condition. Subjects' SCR was recorded during the presentation of acoustic startle stimuli after 5 min and after 30 min of sample exposure. The study revealed that male sweat had a calming effect on women by diminishing the SCR amplitudes compared to control material, but an arousing effect on men; male subjects revealed higher SCR scores during exposure of male sweat compared to control material. Anxiety sweat increased the SCR amplitudes compared to exercise sweat in males and females after 30 min of sample exposure; in females this effect was additionally observed after 5 min of sample exposure. In conclusion, anxiety and exercise sweat collected from males comprise different chemosensory signals which are communicated between humans with a sex-specific differentiation.

The present work extends the knowledge about human sweat, its underlying mechanisms of detection, and gives evidence for the communication of chemosensory signals comprised in male axillary secretions.

Zusammenfassung

Menschlicher Schweiß ist Gegenstand vieler wissenschaftlicher Studien, einschließlich der Erforschung der neuronalen Korrelate und der Kommunikation zwischen Menschen via chemosensorischer Signale, welche in Körpersekreten enthalten sind. Welche Kombination von Rezeptoren in der Nasenschleimhaut bei der Applikation von Schweiß aktiviert wird, ist weitestgehend noch ungeklärt; die Erforschung von Chemosignalen und deren Einfluss auf die Physiologie und das Verhalten des Menschen steht noch am Anfang.

Menschlicher Schweiß ist ein Gemisch verschiedenster Substanzen mit trigeminalen als auch mit olfaktorischen Eigenschaften. Studien haben gezeigt, dass trigeminale Perzeption Voraussetzug ist, um einen Geruch lokalisieren zu können, das heißt Geruchsstimuli dem rechten und linken Nasenloch richtig zuzuordnen. Substanzen, die nur das olfaktorische System aktivieren, können hingegen von Menschen nicht lokalisiert werden.

Um die chemosensorischen Eigenschaften von menschlichem Schweiß zu untersuchen, wurden Probanden auf ihre Fähigkeit, Schweißstimuli richtig zu lokalisieren, getestet (vgl. Kapitel II.). Dafür wurde Achselschweiß von zwanzig gesunden Männern während eines Ergometertrainings gesammelt und anschließend vierunddreißig verschiedenen Rezipienten (17 Frauen) während eines Detektions- und eines Lokalisationsexperimentes präsentiert. Die Applikation der Stimuli erfolgte mittels eines Olfaktometers. Während des Detektionsexperimentes wurden die Probanden aufgefordert, zwischen Schweißstimuli (20) und Leerstimuli (10) zu diskriminieren. Während des Lokalisationsexperimentes wurden sie dazu angehalten, die Stimuli dem rechten (15) und dem linken (15) Nasenloch zuzuordnen. Der Versuch zeigte, dass Probanden die Schweißstimuli mit moderater bis hoher Sensitivität detektieren konnten, wohingegen ihre Lokalisationsrate nicht über der Ratewahrscheinlichkeit lag. Aufgrund dieses Unvermögens die Stimuli dem richtigen Nasenloch zuzuordnen, kann gefolgert werden, dass menschlicher Schweiß nicht das intranasale trigeminale System, sondern nur das olfaktorische System aktiviert, wenn Schweiß in Konzentrationen, wie sie im täglichen Leben anzutreffen sind, appliziert wird.

Die Kommunikation von chemosensorischen Signalen ist bei Säugetieren gut erforscht. Bei Menschen scheint die Kommunikation via Chemosignale weniger bedeutend zu sein aufgrund

ihres hochentwickelten visuellen Systems und ihrer Fähigkeit mit Laut- und Körpersprache zu kommunizieren. Nichtsdestotrotz weist eine ansteigende Studienanzahl auf Effekte von chemosensorischen Signalen auf die Physiologie und das Verhalten des Menschen hin. Die Kommunikation zwischen Menschen mittels chemosensorischer Signale wurde vor allem bezüglich des Sexualverhaltens erforscht, doch auch der Einfluss von Angstsignalen wurde in den letzten Jahren untersucht. Die Effekte von menschlichem Schweiß auf das autonome Nervensystem sind noch kaum erforscht. Bislang wurde keine Studie über die Auswirkung von Körperschweiß auf die Hautleitfähigkeit (skin conductance response, SCR) des Menschen veröffentlicht.

Für die gegenwärtigen Studien über den Einfluss von chemosensorischen Signalen wurden zwei verschiedene Arten von Männerschweiß gesammelt, zum einen während eines Fahrradtrainings (Sportbedingung) und zum anderen während eines Besuchs in einem Hochseilgarten (Angstbedingung). Anschließend wurden diese Proben Rezipienten in zwei verschiedenen Experimenten appliziert, um die Effekte von Chemosignalen in Achselschweiß auf den Menschen zu untersuchen.

In der Studie über die Auswirkungen von Angstsignalen auf die Wahrnehmung von emotionalen Gesichtsausdrücken (vgl. Kapitel III.) wurden die Schweißproben fünfzehn verschiedenen, gesunden, männlichen Probanden appliziert. Die Probanden wurden instruiert emotionale männliche Gesichter verschiedener Morph-Level (neutral - fröhlich) mithilfe einer visuellen Analogskala unter drei verschiedenen Bedingungen (Sportschweiß, Angstschweiß und Leerprobe) zu bewerten. Die Studie zeigte, dass Männer unter Einfluss von Angstschweiß fröhliche Gesichter weniger fröhlich einschätzten, verglichen mit den Ergebnissen der Sport- und Kontrollbedingung; signifikante Unterschiede konnten nur für nicht eindeutige, emotionale Gesichter gezeigt werden. Angstsignale, die mit Männerschweiß sezerniert werden, werden demnach zwischen Männern kommuniziert; sie führen bei männlichen Versuchspersonen zu einer negativeren Bewertung von nicht eindeutig fröhlichen, männlichen Gesichtsausdrücken und beeinflussen dabei die Wahrnehmung von emotionalen Gesichtern.

In der Studie über den Einfluss von Männerschweiß auf die Hautleitfähigkeit (SCR) von Menschen (vgl. Kapitel IV.) wurden zwei Arten von menschlichem Schweiß (Angst- und Sportschweiß) einunddreißig Rezipienten (16 Frauen) appliziert. Zusätzlich wurden Leerproben als Kontrollbedingung genutzt. Die SCR der Probanden wurde während der Präsentation von akustischen Schreckstimuli nach 5 min und nach 30 min Probenexposition aufgezeichnet. Die Studie zeigte, dass Männerschweiß einen beruhigenden Effekt auf Frauen hatte, da die SCR geringere Amplituden im Vergleich zur Kontrollbedingung aufwies, aber einen erregenden Effekt auf Männer. Männliche Probanden zeigten unter Einfluss von Männerschweiß höhere SCR Amplituden verglichen zur Kontrollbedingung. Angstschweiß erhöhte die SCR Amplituden bei Männern und Frauen im Vergleich zu Sportschweiß nach 30 min Probenexposition; bei Frauen war dies zusätzlich nach 5 min Exposition zu beobachten. Angst- und Sportschweiß von Männern beinhalten demnach verschiedene chemosensorische Signale, welche zwischen Menschen mit geschlechtsspezifischen Unterschieden kommuniziert werden.

Die gegenwärtige Arbeit erweitert das Wissen über menschlichen Achselschweiß, die grundlegenden Mechanismen der Wahrnehmung, und liefert Beweise für die Kommunikation zwischen Menschen mittels chemosensorischer Signale, welche in Männerschweiß enthalten sind.

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