

Chemosensory Properties of Human Sweat

R. Zerneck¹, A.M. Kleemann¹, K. Haegler¹, J. Albrecht^{1,2}, B. Vollmer¹, J. Linn¹, H. Brückmann¹ and M. Wiesmann^{1,3}

¹Department of Neuroradiology, Ludwig-Maximilians-University of Munich, Marchioninistrasse 15, 81377 Munich, Germany, ²Cognitive Neuroimaging Laboratory, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA and ³Department of Radiology and Neuroradiology, Helios Kliniken Schwerin, Wismarsche Straße 393-397, 19047 Schwerin, Germany

Correspondence to be sent to: Rebekka Zerneck, Department of Neuroradiology, Ludwig-Maximilians-University of Munich, Marchioninistrasse 15, 81377 Munich, Germany. e-mail: rebekka.zerneck@med.uni-muenchen.de

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Abstract

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 and that humans are not able to localize substances that only activate the olfactory system. To analyze the chemosensory properties of human sweat, we studied humans' ability to localize sweat stimuli to the different nostrils.

Human sweat was collected during a bicycle workout (20 males) and was then applied to 34 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).

We found that 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. Due to this inability to localize the stimuli, we conclude that human sweat does not activate the intranasal trigeminal system but only the olfactory system.

Key words: body odors, detection, localization, olfactory, trigeminal

Introduction

Water, electrolytes, fatty acids, lactic acid, and nitrogen metabolites, such as ammonia, urea, and uric acid have been analyzed as the main constituents of sweat (Emrich and Oelert 1966; Peter et al. 1970; Takemura et al. 1989; Zeng et al. 1991; Bernier et al. 1999; Haze et al. 2001; Huang et al. 2002; Curran et al. 2005), substances with trigeminal as well as olfactory properties (Schneider and Schmidt 1967; Doty 1975; Doty et al. 1978). Androgen steroids have been determined as human sexual pheromones existing in body odors (Pause 2004). Kin recognition and inbreeding avoidance are probably based on components of human secretions (Wobst et al. 1998; Weisfeld et al. 2003; Pause et al. 2006), and various imaging techniques have been used to investigate the neuronal correlates in response to axillary odors (Pause et al. 1998; Lundstrom et al. 2008; Mujica-Parodi et al. 2009; Prehn-Kristensen et al. 2009). However, little is known about the combination of receptors activated

through the application of this mixture of odorants. Previous studies are controversial regarding which intranasal nerve structures (olfactory vs. trigeminal) are activated by human sweat. Pause et al. (1998) assumed that the body odor mixture is dominated by olfactory substances rather than by trigeminal substances based on the N1 amplitude of electroencephalogram data. Controversial to this, Lundstrom et al. (2008) suggested that sweat preferentially activates trigeminal nerve structures due to the specific activations in the postcentral gyrus of a positron emission tomography study that investigated the neural correlates of body odors. To our knowledge, no experiment about the chemosensory processing of human body odors based on psychophysical data of a localization experiment has been published yet.

In the present study, we used human sweat to test which parts of the intranasal chemosensory system are involved

in the neuronal processing of axillary secretions. To investigate the influence of odorants on the olfactory or trigeminal chemosensory system, the so-called “localization experiment” has become an established method (Kobal et al. 1989; Hummel et al. 2003; Frasnelli et al. 2009; Kleemann et al. 2009). This experiment investigates if subjects have the ability to localize a presented odorant to the accurate nostril and therefore which nerve structures are activated. There is a strong consensus that humans can localize only odorants that excite additionally the trigeminal system. Pure odorants, which stimulate the olfactory chemosensory system selectively, 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).

A necessary requirement for an accurate accomplishment of the localization experiment is to assure that subjects perceive the presented stimuli consciously. A reliable method for the quantification of human perception has become the “detection experiment” which is based on the signal detection theory (SDT; Green and Swets 1966; Lloyd and Appel 1976). The detection experiment determines the human sensitivity d' to the assessed odor, which enables to separate the signal (the relevant input event) from the noise (background activity or irrelevant inputs), and analyzes the subjects' tendency to report that a given event has occurred (response criterion β).

In the present study, we first collected human sweat in a bicycle workout and then conducted 2 experiments to analyze the neuronal processing of human axillary secretions. To investigate the sensitivity of subjects to the applied concentration of the sweat stimuli, we conducted the detection experiment based on the SDT. To determine whether human sweat activates the olfactory or the trigeminal chemosensory system, we examined if subjects are able to localize the sweat stimuli to the right and left nostril accurately.

Materials and methods

The entire study was approved by the local Medical Ethics Review Committee of our University. All subjects provided their written informed consent.

Part I: collection of sweat stimuli

Sweat donors

Twenty healthy male subjects between the ages of 21 and 52 years (mean age: 27.2 years, standard deviation [SD]: 7.0 years) participated as sweat donors. All participants described themselves as exclusively heterosexual on a 7-point scale (mean: 0.00, SD: 0.00; Kinsey et al. 1953). All of them were nonsmokers and were not taking any medication.

At least 3 days before odor sampling, each participant received instructions along with a scent-free shower gel. The donors were required to undergo certain dietary and behavioral restrictions 2 days prior and on the day of the sampling.

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 (Balea, Ultra Sensitive, dm-dogerie markt) 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, hot-spiced food, and from drinking alcohol. The evening before sampling, donors were instructed to take a shower with the nonperfumed shower gel and were asked to wear only loose and odorless clothes after that. On the sampling day, the participants were required to wash their armpits exclusively with water.

Sweat sampling procedure

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 our institution. Each participant accomplished this workout twice. Between both sessions, subjects rested for approximately 15 min. During the 2 donation sessions, cotton pads (16 × 5.5 cm) were placed under the armpits, and subjects wore tight, white cotton long sleeve shirts and raincoats. The shirts were used to ensure a close fit of the pads in the armpits, the raincoats assured to increase subjects' perspiration. At the end of both sessions, pads were collected and immediately frozen using dry ice. The pads were cut into approximately 1 × 1 cm sized pieces. Slices of all samples were mixed, and pooled across all 20 donors, and were stored all together in one big odor-free freezer bag at −40 °C until testing. The sweat samples were used in the odor perception experiments (Part II) within 6 weeks.

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

Part II: detection and localization experiment

Sweat recipients

Thirty-four healthy subjects (17 females and 17 male subjects; age range: 20–48 years; mean age: 31.2 years, SD: 7.2 years) participated as recipients of the sweat stimuli. 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 2-sample t -test; $t_{32} = 1.20$, $P = 0.24$). Subjects did not suffer from any acute or chronic dysfunction of the respiratory system. They were screened for normal olfactory function using the Sniffin' Sticks test battery (Kobal et al. 1996, 2000; Hummel et al. 1997; mean threshold discrimination identification score: 35.70, SD: 2.88; range: 32.00–43.75). All subjects reported not using any tobacco products and were not taking any medication known to interfere with sensory perception (Frye et al. 1990; Schiffman 1994; Doty and Bromley 2004).

Women were neither taking hormonal contraceptives nor reporting to be pregnant. 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. Between the 2 testing sessions, they were allowed only to drink water.

Subjects were not aware of the nature of the odorants. They were told that they would receive a mixture of different odorants.

Experimental procedure

The experiment was divided into 2 sessions. During session 1, we examined if subjects are able to detect the applied sweat concentration (detection experiment). During session 2, subjects were asked to localize the presented stimuli to the right and left nostril (localization experiment; Kobal et al. 1989; Hummel et al. 2003; Frasnelli et al. 2009; Kleemann et al. 2009). The order of both sessions was pseudorandomized. The olfactory stimuli were presented using a computer-controlled olfactometer (OM6b, Burghart Instruments; Kobal 1981; Kobal et al. 1989). 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 mechanosensors or thermosensors of the nasal mucosa are activated that could interfere with the subjects' ability to localize the odor. Olfactory stimuli were presented binorally 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 interstimulus interval was set at 30 s (± 3 s). For odor presentation in the detection as well as in the localization experiment, we used 13 g of the sweat pads collected in the sampling session (Part I) and 5 g of the blank control. Each portion of 13 g of the sweat pads was taken out of the bag containing the mixed samples of all 20 donors.

During odor presentation, subjects performed the technique of velopharyngeal closure to avoid the flow of respiratory air within the subjects' nasal cavities (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, odor 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). 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.

Detection experiment

We investigated subjects' ability to detect the assessed concentration of the sweat stimuli by presenting a total of 20 stimuli and 10 blanks (control material) to either one nostril. Whereas 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 pseudorandomized sequence. After each stimulus (auditory signal), subjects made a 2-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.

Localization experiment

To examine subjects' ability to localize the sweat stimuli to the accurate nostril, we used an experimental paradigm comprising a total of 30 stimuli. Fifteen sweat stimuli were applied to the right and 15 stimuli to the left nostril. The order of nostril's stimulation was pseudorandomized. 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 2-alternative, forced-choice judgment to localize the assessed odor to the right (right mouse button) or left (left mouse button) nostril.

Questionnaire

Two questionnaires, one for each experiment (detection and localization), were employed to measure the recipients' emotional states, their perceptions of the sweat stimuli, and their associations when smelling this 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 variations in intensity between the stimuli (0 = little variations, 100 = strong variations). After the localization experiment, subjects rated the intensity of the olfactory stimuli (0 = very weak, 100 = very strong) and their variations in intensity (0 = little variations, 100 = strong variations) for the right and left nostril, separately.

The questions were answered by the participants using a visual analog scale (VAS). They were trained to give

a response by placing a mark on a 100-mm horizontal line. VASs have been shown to measure even minor changes in affect with high reliability and validity (Aitken 1969; Folstein and Luria 1973).

Data analysis

The data of the detection experiment were analyzed based on the SDT. For any event, 4 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), and false alarm (incorrect detection of an absent signal). Based on these outcomes, the parameters sensitivity d' and response criterion β 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 and noise. $d' < 0.5$ corresponds to a low sensitivity, d' between 0.5 and 2 indicates a moderate sensitivity, and $d' \geq 2$ corresponds to a high sensitivity. The response criterion β reflects the subjects' strategy of response. $\beta < 1$ corresponds to a low criterion, that is, the subjects tend to answer with yes, $\beta = 1$ means neutral criterion, $\beta > 1$ corresponds to a high criterion, that is, 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 4 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), and 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 and 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 done using SPSS 17.0 for Windows (SPSS Inc.). 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, maleness/femaleness, sexual attractiveness, intensity, variations in intensity) were submitted to Student's paired t -tests, and not normally distributed data (sensitivity d' of the detection experiment, response criterion β , arousal, dominance) were submitted to nonparametric Wilcoxon signed-rank tests to explore differences between the detection and the localization experiment 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 1-sample t -tests. To analyze gender differences, data were submitted to independent 2-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.

Results

Detection experiment

Overall subjects detected the sweat stimuli with low to high sensitivity d' (mean: 1.91; $14.4 \pm 4.5 \triangle 72.2\%$ hits). For further analysis, we subdivided the subjects into 3 groups according to their sensitivities to the sweat samples: Eleven of 34 subjects (4 females) detected the applied sweat stimuli with a high sensitivity d' (sensitivity class 1: mean: 4.04, range: 2.48–6.00; $16.4 \pm 5.1 \triangle 82.3\%$ hits), 12 subjects (7 females) detected the stimuli with a moderate sensitivity (sensitivity class 2: mean: 1.46, range: 0.78–1.81; $15.2 \pm 3.0 \triangle 75.8\%$ hits), and 11 subjects (6 females) had a low sensitivity in response to the stimulation (sensitivity class 3: mean: 0.27, range: –0.17 to 0.42; $11.6 \pm 4.3 \triangle 58.2\%$ hits; Table 2). Descriptive statistics are shown in Table 1. Results of the detection experiment (sensitivity d' , response criterion β) revealed no significant differences between men and women, neither for subjects all together nor for the 3 sensitivity classes separately (Mann–Whitney U Tests, P = not significant [n.s.]). Means of the response criterion β constituted data of $\beta > 1$ indicating a conservative behavior during the decision (Table 2). These findings applied to all sensitivity classes; class 1

Table 1 Results of the detection experiment

	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%)

Reported are means ± SDs (sensitivity class 1: $n = 11$, class 2: $n = 12$, class 3: $n = 11$).

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 ($\beta_{\text{class}2} = 1.20$, $\beta_{\text{class}3} = 1.02$).

Localization experiment

Individuals failed to localize the sweat stimuli during the localization experiment (mean \pm SD = $14.6 \pm 2.4 \triangle 48.7\%$ correct assignment; Table 3) and showed no rise above chance level. This was true for the total group of subjects ($t_{33} = 0.892$, $P = 0.379$), as well as when the 3 sensitivity classes were analyzed separately (sensitivity class 1: $t_{10} = 0.896$, $P = 0.391$; sensitivity class 2: $t_{11} = 1.20$, $P = 0.255$; sensitivity class 3: $t_{10} = 0.295$, $P = 0.774$). 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 ($\triangle 73.3\%$) 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, $t_{33} = 1.478$, $P = 0.149$). Men could localize the applied stimuli to the accurate nostril better when compared with women (independent 2 sample t -test, $t_{32} = 2.256$, $P = 0.031$). However, when data were analyzed separately, neither men nor women were able to localize the sweat stimuli above chance level (men: $15.5 \pm \text{SD } 2.5 \triangle 51.8\%$ correct assignment, $t_{16} = 0.872$, $P = 0.396$; women: $13.7 \pm \text{SD } 2.2 \triangle 45.7\%$ correct assignment). The women's score was significantly below chance level ($t_{16} =$

2.424, $P = 0.028$). The criterion c of the left-sided stimulation of the localization 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 (Table 2). There were no significant differences between men and women regarding criterion c , neither for all subjects nor for the 3 sensitivity classes separately (independent 2 sample t -tests, $P = \text{n.s.}$). 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 3 sensitivity classes separately ($d'_{\text{class } 1} = -0.11$, $d'_{\text{class } 2} = -0.14$, $d'_{\text{class } 3} = 0.05$; Table 2). There were no significant differences between men and women when data were analyzed for the 3 classes separately (independent 2 sample t -tests, $P = \text{n.s.}$), when analyzed for all subjects data revealed significant differences between both genders (independent 2 sample t -test, $t_{32} = 2.165$, $P = 0.038$).

Questionnaire

All parameters of the questionnaire revealed no significant differences between the 2 experiments (Student's paired t -tests or Wilcoxon signed-rank tests, $P = \text{n.s.}$). There were no significant differences in respect to the factor gender (independent 2-sample t -test or Mann-Whitney U test, $P = \text{n.s.}$) regarding the different questions. Descriptive statistics are 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.

Discussion

Our study reveals 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 that 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, our results strongly suggest

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 B	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

Table 3 Results of the localization experiment

	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%)

Reported are means \pm SDs (sensitivity class 1: $n = 11$, class 2: $n = 12$, class 3: $n = 11$).

Table 4 Ratings of the questionnaire

	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

Reported are means ± SDs of the detection and the localization experiment ($n = 34$).

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; Zeng et al. 1991; 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, as it is secreted by axillary glands, is odorless until skin bacteria generate the odoriferous principles from the scentless analogs (Shelley et al. 1953; Shehadeh and Kligman 1963; Leyden et al. 1981). In our detection experiment, these smelling volatiles were responsible for the 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, exclusively excites 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, our results of the 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 2 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 et al. (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; Cometto-Muniz 2001; Boyle et al. 2006). At lower concentrations, chemoreception will be mainly based on olfactory stimulation whereas 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.

Our study revealed that women performed significantly below chance level in the localization experiment. This underperformance cannot be ascribed to outliers. Our results confirm previous reports in which participants' 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 criteria β and c but different results for the sensitivity d' . Class 2 and 3 showed nearby neutral criteria, that is, 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 because 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, we conducted the experiment in 2 different sessions to analyze subjects' sensitivity and their ability to localize the stimuli to the accurate nostril.

There were 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 antigen histocompatibility genes (Weisfeld et al. 2003; Pause et al. 2006). In our study, the sweat pads were homogenized; subjects received a mixture of donors' samples. Thus, potential preferences of the recipients for human body odors of specific donors were prevented due to the application of pooled sweat samples.

Our 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 contains a complex mixture of volatile organic compounds, some of them in very low concentrations. The neuronal processing of mixtures, especially of human body odors, is hardly understood.

According to the literature, it may be assumed that some of the volatile organic compounds that are present in human sweat do possess trigeminal properties. Accordingly, it is clear that when 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 our study design. Thus, we are confident that our findings are of relevance to 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.

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References

- Aitken RC. 1969. Measurement of feelings using visual analogue scales. *Proc R Soc Med.* 62(10):989–993.
- Bernier UR, Booth MM, Yost RA. 1999. Analysis of human skin emanations by gas chromatography/mass spectrometry. 1. Thermal desorption of attractants for the yellow fever mosquito (*Aedes aegypti*) from handled glass beads. *Anal Chem.* 71(1):1–7.
- Boyle JA, Lundstrom JN, Knecht M, Jones-Gotman M, Schaal B, Hummel T. 2006. On the trigeminal percept of androstenone and its implications on the rate of specific anosmia. *J Neurobiol.* 66(13):1501–1510.
- Cain WS, Murphy CL. 1980. Interaction between chemoreceptive modalities of odour and irritation. *Nature.* 284(5753):255–257.
- Cashion L, Livermore A, Hummel T. 2006. Odour suppression in binary mixtures. *Biol Psychol.* 73:288–297.
- Claus R, Alsing W. 1976. Occurrence of 5 α -androst-16en-3-one, a boar pheromone, in man and its relationship to testosterone. *J Endocrinol.* 68:483–484.
- Cometto-Muniz JE. 2001. Physicochemical basis for odor and irritation potency of VOCs. In: Spengler JD, Samet JM, McCarthy JF, editors. *Indoor air quality handbook*. New York: McGraw-Hill. p. 20.1–20.21.
- Cometto-Muniz JE, Cain WS, Abraham MH. 1998. Nasal pungency and odor of homologous aldehydes and carboxylic acids. *Exp Brain Res.* 118(2):180–188.
- Cometto-Muniz JE, Cain WS, Abraham MH. 2005. Determinants for nasal trigeminal detection of volatile organic compounds. *Chem Senses.* 30(8):627–642.
- Curran AM, Rabin SI, Prada PA, Furton KG. 2005. Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. *J Chem Ecol.* 31(7):1607–1619.
- Doty RL. 1975. Intranasal trigeminal detection of chemical vapors by humans. *Physiol Behav.* 14(6):855–859.
- Doty RL, Bromley SM. 2004. Effects of drugs on olfaction and taste. *Otolaryngol Clin North Am.* 37(6):1229–1254.
- Doty RL, Brugger WE, Jurs PC, Orndorff MA, Snyder PJ, Lowry LD. 1978. Intranasal trigeminal stimulation from odorous volatiles: psychometric responses from anosmic and normal humans. *Physiol Behav.* 20(2):175–185.
- Emrich HM, Oelert H. 1966. [pH value and total ammonia in human sweat]. *Pflügers Arch Gesamte Physiol Menschen Tiere.* 290(4):311–314.

- Folstein MF, Luria R. 1973. Reliability, validity, and clinical application of the Visual Analogue Mood Scale. *Psychol Med.* 3(4):479–486.
- Frasnelli J, Charbonneau G, Collignon O, Lepore F. 2009. Odor localization and sniffing. *Chem Senses.* 34(2):139–144.
- Frye RE, Schwartz BS, Doty RL. 1990. Dose-related effects of cigarette smoking on olfactory function. *JAMA.* 263(9):1233–1236.
- Green DM, Swets JA. 1966. Signal detection theory and psychophysics. New York: Wiley.
- Haze S, Gozu Y, Nakamura S, Kohno Y, Sawano K, Ohta H, Yamazaki K. 2001. 2-Nonenal newly found in human body odor tends to increase with aging. *J Invest Dermatol.* 116(4):520–524.
- Huang CT, Chen ML, Huang LL, Mao IF. 2002. Uric acid and urea in human sweat. *Chin J Physiol.* 45(3):109–115.
- Hummel T. 2000. Assessment of intranasal trigeminal function. *Int J Psychophysiol.* 36(2):147–155.
- Hummel T, Futschik T, Frasnelli J, Huttenbrink KB. 2003. Effects of olfactory function, age, and gender on trigeminally mediated sensations: a study based on the lateralization of chemosensory stimuli. *Toxicol Lett.* 140–141:273–280.
- Hummel T, Livermore A, Hummel C, Kobal G. 1992. Chemosensory event-related potentials in man: relation to olfactory and painful sensations elicited by nicotine. *Electroencephalogr Clin Neurophysiol.* 84(2):192–195.
- Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 1997. 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem Senses.* 22(1):39–52.
- Kinsey C, Pomeroy WB, Martin CE, Gebhard PH. 1953. Sexual behaviour in the human female. Philadelphia (PA): Saunders.
- Kleemann AM, Albrecht J, Schöpf V, Haegler K, Kopietz R, Hempel JM, Linn J, Flanagan VL, Fesl G, Wiesmann M. 2009. Trigeminal perception is necessary to localize odors. *Physiol Behav.* 97(3–4):401–405.
- Kobal G. 1981. Elektrophysiologische Untersuchungen des menschlichen Geruchssinns. Stuttgart (Germany): Thieme Verlag.
- Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S. 1996. "Sniffin' sticks": screening of olfactory performance. *Rhinology.* 34(4):222–226.
- Kobal G, Klimek L, Wolfensberger M, Gudziol H, Temmel A, Owen CM, Seeber H, Pauli E, Hummel T. 2000. Multicenter investigation of 1,036 subjects using a standardized method for the assessment of olfactory function combining tests of odor identification, odor discrimination, and olfactory thresholds. *Eur Arch Otorhinolaryngol.* 257(4):205–211.
- Kobal G, Van Toller S, Hummel T. 1989. Is there directional smelling? *Experientia.* 45(2):130–132.
- Leyden JJ, McGinley KJ, Holzle E, Labows JN, Kligman AM. 1981. The microbiology of the human axilla and its relationship to axillary odor. *J Invest Dermatol.* 77:413–416.
- Livermore A, Hummel T, Kobal G. 1992. Chemosensory event-related potentials in the investigation of interactions between the olfactory and the somatosensory (trigeminal) systems. *Electroencephalogr Clin Neurophysiol.* 83(3):201–210.
- Lloyd MA, Appel JB. 1976. Signal detection theory and the psychophysics of pain: an introduction and review. *Psychosom Med.* 38(2):79–94.
- Lundstrom JN, Boyle JA, Zatorre RJ, Jones-Gotman M. 2008. Functional neuronal processing of body odors differs from that of similar common odors. *Cereb Cortex.* 18(6):1466–1474.
- Macmillan NA, Creelman CD. 2005. Detection theory: a user's guide. Mahwah (NJ): Lawrence Erlbaum Associates.
- Mujica-Parodi LR, Strey HH, Frederick B, Savoy R, Cox D, Botanov Y, Tolkunov D, Rubin D, Weber J. 2009. Chemosensory cues to conspecific emotional stress activate amygdala in humans. *PLoS One.* 4(7):e6415.
- Pause BM. 2004. Are androgen steroids acting as pheromones in humans? *Physiol Behav.* 83(1):21–29.
- Pause BM, Krauel K, Schrader C, Sojka B, Westphal E, Muller-Ruchholtz W, Ferstl R. 2006. The human brain is a detector of chemosensorily transmitted HLA-class I-similarity in same- and opposite-sex relations. *Proc Biol Sci.* 273(1585):471–478.
- Pause BM, Krauel K, Sojka B, Ferstl R. 1998. Body odor evoked potentials: a new method to study the chemosensory perception of self and non-self in humans. *Genetica.* 104(3):285–294.
- Peter G, Schropf F, Feisel HG, Thurauf W. 1970. [Gaschromatographic investigations of free and esterified fatty acids in human eccrine sweat]. *Arch Klin Exp Dermatol.* 238(2):154–159.
- Prehn-Kristensen A, Wiesner C, Bergmann TO, Wolff S, Jansen O, Mehdorn HM, Ferstl R, Pause BM. 2009. Induction of empathy by the smell of anxiety. *PLoS One.* 4(6):e5987.
- Schiffman S. 1994. Changes in taste and smell: drug interactions and food preferences. *Nutr Rev.* 52(8 Pt 2):11–14.
- Schneider RA, Schmidt CE. 1967. Dependency of olfactory localization on non-olfactory cues. *Physiol Behav.* 2:305–309.
- Shehadeh N, Kligman AM. 1963. The bacteria responsible for axillary odor. II. *J Invest Dermatol.* 41:39–43.
- Shelley WB, Hurley HJ Jr, Nichols AC. 1953. Axillary odor; experimental study of the role of bacteria, apocrine sweat, and deodorants. *AMA Arch Derm Syphilol.* 68:430–446.
- Takemura T, Wertz PW, Sato K. 1989. Free fatty acids and sterols in human eccrine sweat. *Br J Dermatol.* 120(1):43–47.
- Thurauf N, Gunther M, Pauli E, Kobal G. 2002. Sensitivity of the negative mucosal potential to the trigeminal target stimulus CO(2). *Brain Res.* 942(1–2):79–86.
- van Thriel C, Schaper M, Kiesswetter E, Kleinbeck S, Juran S, Blaszkewicz M, Fricke HH, Altmann L, Berresheim H, Bruning T. 2006. From chemosensory thresholds to whole body exposures-experimental approaches evaluating chemosensory effects of chemicals. *Int Arch Occup Environ Health.* 79(4):308–321.
- Von Skramlik E. 1925. Über die Lokalisation der Empfindungen bei den niederen Sinnen. *Zeitschrift für Sinnesphysiologie.* 56:69–140.
- Weisfeld GE, Czilli T, Phillips KA, Gall JA, Lichtman CM. 2003. Possible olfaction-based mechanisms in human kin recognition and inbreeding avoidance. *J Exp Child Psychol.* 85(3):279–295.
- Wiesmann M, Kopietz R, Albrecht J, Linn J, Reime U, Kara E, Pollatos O, Sakar V, Anzinger A, Fesl G, et al. 2006. Eye closure in darkness animates olfactory and gustatory cortical areas. *Neuroimage.* 32(1):293–300.
- Wobst B, Zavazava N, Luszyk D, Lange K, Ussat S, Eggert F, Ferstl R, Muller-Ruchholtz W. 1998. Molecular forms of soluble HLA in body fluids: potential determinants of body odor cues. *Genetica.* 104(3):275–283.
- Wysocki CJ, Cowart BJ, Radil T. 2003. Nasal trigeminal chemosensitivity across the adult life span. *Percept Psychophys.* 65(1):115–122.
- Zeng X-N, Leyden JJ, Lawley HJ, Sawano K, Nohara I, Preti G. 1991. Analysis of characteristic odors from human male axillae. *J Chem Ecol.* 17(7):1469–1492.