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# Smelling Chemosensory Signals of Males in Anxious Versus Nonanxious Condition Increases State Anxiety of Female Subjects

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## Abstract

The hypothesis of this experiment was that humans in an anxious state compared with a nonanxious state are able to increase anxiety levels in other humans via their body odors. Specifically, we hypothesized that male chemosensory anxiety signals compared with neutral chemosignals increase state anxiety of female subjects. Thirteen male subjects participated in 2 different sweat donation sessions: chemosignals were collected during participation in a high rope course (anxiety condition) and in an ergometer workout (neutral condition). State and trait anxiety were evaluated in 20 female odor recipients using Spielberger's state-trait anxiety inventory in a double-blind design. Comparison of state anxiety of odor donors between control and anxiety condition differed significantly indicating that our model of anxiety induction successfully led to the expected change in emotion. Comparison of state anxiety of odor recipients showed a trend toward higher state anxiety in the anxiety condition compared with the neutral condition after 5 min of odor exposure. After 20 min of odor exposure, state anxiety of female subjects was significantly higher during the perception of sweat collected during the anxiety condition in comparison with the perception of sweat collected during the neutral condition. This experiment gives evidence that male anxiety chemosignals compared with neutral chemosignals are capable of inducing an increased state anxiety in female subjects.

**Key words:** fear, odor, olfaction, sweat, VNO

## Introduction

It is very well known that animals are able to communicate affective states like stress, alarm, fear, or anxiety utilizing an alteration in their body odor (Kiyokawa et al. 2004; 2006) and that these signals are conveyed by the vomeronasal organ (VNO) (Kiyokawa et al. 2007). In humans, an antomically similar structure is existent, however, it lacks its complexity (Witt and Wozniak 2006) as well as its function during the perception of chemosensory signals (Frasnelli et al. 2010). It is suggested that besides olfactory receptors (Kiyokawa et al. 2009; Savic et al. 2009) another group of chemosensory receptors, the so called trace amine-associated receptors are used to sense chemosensory signals (Liberles and Buck 2006).

The first evidence of chemosensory communication of anxiety signals in humans originates from a study by Owen (1981). The author demonstrated that male subjects were able to detect the anxiety signal in comparison with a relaxation and sexual arousal signal during an unpleasantness rating and a free imagery task. Further evidence was given by Chen and Haviland-Jones (2000), who proofed that male and female subjects were able to choose the male anxiety signal in a 3- or 6-choice task. Ackerl et al. (2002) ascertained the ability of female subjects to discriminate between fear and nonfear axillary pads of female donors. The authors of the mentioned studies used images or movies as a means for emotion induction. Utilizing the situation of waiting for

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an academic examination, Pause and colleagues (Pause et al. 2004, 2009; Prehn et al. 2006) introduced a new method of anxiety induction. They demonstrated a change in subliminal face perception during the perception of chemosensory anxiety signals. Axillary sweat samples of male subjects containing anxiety signals in comparison with those containing exercise body odors diminished the positive emotional priming of facial affect perception in female subjects (Pause et al. 2004). Furthermore, the startle reflex amplitude of male and female subjects to auditory stimuli recorded in the context of chemosensory anxiety signals was increased in comparison with the amplitude recorded during a condition of receiving stimuli originating from exercise or from an unused pad (Prehn et al. 2006). The results of a recent follow-up study showed that this startle response potentiation especially occurs in socially anxious individuals (Pause et al. 2009). Another study indicated that anxiety chemosignals are capable of enhancing cognitive performance (Chen et al. 2006). The authors discovered that female subjects performed more accurately during a word-association task when exposed to the anxiety condition in comparison with the neutral and control odor carrier condition. In a recent study, Zhou and Chen (2009) were able to verify that male chemosensory anxiety signals biased women toward interpreting ambiguous facial expressions as more fearful but had no effect when the facial expression was more discernable.

In the first neuroimaging study about the neuronal correlates of smelling human alarm signals, Mujica-Parodi et al. (2009) showed activation of the amygdala following smelling male sweat samples collected during a first time tandem skydive compared with a male sweat sample collected during a control condition (running on a treadmill). This finding was confirmed by Prehn-Kristensen et al. (2009), who compared brain activation due to chemosensory anxiety signals (subjects awaiting an academic examination) with control stimuli (sport condition). In addition to the insula activation, the authors obtained activation in fusiform gyrus, precuneus, cingulate cortex, thalamus, prefrontal cortex, and cerebellum in response to the chemosensory perception of anxiety. These areas are interpreted to belong to an empathy-processing network.

With the current study, we introduce a new method of anxiety induction in humans: subjects visiting a high rope course. Compared with other anxiety induction approaches, our anxiety induction method as well as the method presented by Mujica-Parodi et al. (2009) are free of any social anxiety aspects. Our approach to induce anxiety in humans has several advantages compared with the method of Mujica-Parodi et al. (2009): it is less cost- and time-consuming, it is accessible for research groups, and it involves a certain amount of physical activity during the anxiety induction, nevertheless, guarantees a high nonsocial anxiety level in the subjects. The results of the behavioral studies mentioned above demonstrate that chemosensory anxiety signals sharpen sensory and emotional processing as well as cognitive performance in order to react faster and with more cautioness. Therefore the hypothesis of this study was that sweat originating from the anxiety condition compared with the nonanxiety condition increases subjective perception of anxiety in odor recipients. This one-tailed hypothesis was tested using the established method of the Spielberger's state-trait anxiety inventory (STAI) in a double-blind design. In order to exclude general effects of testing time in odor recipients, we decided to control for subjects' alertness using the d2 Test of Attention (Brickenkamp and Zillmer 1998).

## Materials and methods

This study was conducted according to the Declaration of Helsinki and was approved by the Institutional Review Board of the Ludwig-Maximilians-University Munich. All subjects provided written informed consent for the collection and application of samples as well as subsequent analysis.

### Part I: Body odor donation

#### *Participants*

Thirteen healthy male subjects between the ages of 23 and 33 years (mean age 27.6 years, standard deviation [SD] 2.9 years) participated in the sweat odor donation sessions. It is known that sexual orientation influences perception and hedonics (Martins et al. 2005), as well as cerebral responses related to chemosensory signals (Savic et al. 2005; Berglund et al. 2006; Savic and Lindstrom 2008). Therefore, we controlled for sexual orientation of the subjects. All participants described themselves as having exclusively heterosexual contacts on a 7-point scale (Kinsey et al. 1953) (mean 0.0, SD 0.0).

#### *Collection of chemosensory samples*

Subjects were advised to abstain from smoking, heavily flavored foods, garlic, onions, asparagus, and not to use deodorants/antiperspirants, perfumed body lotion, or perfumes 2 days before the sweat donation session until the session was over. Donors were instructed to use a scent-free shower gel (Balea Med Ultra Sensitive; dm-Markt GmbH & Co. KG) provided by the experimenters and to take a shower the evening before the donation session. They were asked to wear only loose and odorless clothes after this shower and to wash their armpits exclusively with water in the morning of the experiment. An 18.0 × 5.5 cm pad (polypropylene-fleece and cellulose near the body, polyethylene film at the outside) was placed under the armpits using plaster for sensitive skin (Leukosilk; BSN Medical GmbH) during the donation sessions. Each donation session documented a different emotional state (anxiety state, neutral state). During these sessions, subjects wore tight cotton t-shirts and raincoats, both provided by the experimenters. Pads were collected and immediately cooled down by dry ice. After the transfer to the hospital of the Ludwig-Maximilians-University

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Munich, the pads were cut into 1 × 1 cm pieces using aseptic scissors. In order to mix samples across odor donors, they were put into one big plastic bag and stored at –40 °C until the testing session.

To avoid contamination with the experimenters' bacterial skin flora a hygenical hand disinfection was conducted using isopropanol (70% v/v isopropanol, local pharmacy) and scent-free shower gel (Balea Med Ultra Sensitive; dm-Markt GmbH & Co. KG) before the pads were touched.

### *Anxiety induction*

During the first session, emotion of anxiety was induced by an exercise during a high rope course. The subjects were advised to climb a 7-m high pole and to stand on that pole without seeing the securing device that was fixed at the subjects' back (Figure 1). After evaluation of anxiety and measurement of blood pressure and heart rate, the subjects' task was to jump from the pole. During the anxiety induction session, the participants wore the pads for body odor collection for approximately 20 min.

The control samples were collected during a 20 min ergometer workout in the hospital of the Ludwig-Maximilians-University Munich on a different day. The subject's task was to workout with an estimated power of 110 W/h.

**Figure 1** Subject during anxiety odor donation session.

### *Anxiety assessment*

For anxiety assessment, the STAI X (Spielberger et al. 1970; german version by Laux et al. (1981)) was used. This inventory consists of 2 distinct anxiety scales: the trait scale (trait anxiety) and the state scale (state anxiety). Both scales are composed of 20 items and require that the subjects evaluate how they feel in general (trait scale, STAI X2) and how they feel during a specific moment (state anxiety, STAI X1). Individual responses to each item were obtained on a 4-point scale (1 = rare, 4 = often). Normative values of the trait anxiety (STAI X2) of 34.5 (SD 8.8) for male subjects ($n$ = 1107) and 37.0 (SD 10.0) for female subjects ($n$ = 1278) were reported previously (Laux et al. 1981).

Odor donors were screened regarding their trait anxiety (STAI X2) at the beginning of the experiment. The body odor sample of one subject who scored too high (49) was excluded from further investigation. The mean STAI X2 score (trait anxiety) of the remaining odor donors at the beginning of the experiment was 32.8 (SD 6.2, range: 22–44), indicating a normal anxiety level.

Subjects were advised to evaluate their subjective anxiety on the state scale (STAI X1) at the beginning of each donation session. Besides this, subjects were asked to self-evaluate the perceived anxiety during both donation sessions on a 7-point scale (0 = not anxious at all, 6 = very anxious) representing a part of the Profile of Mood States (POMS) questionnaire (McNair and Lorr 1964). These ratings were acquired on demand and written down by the experimenter. Furthermore, subjects completed the state scale (STAI X1) directly after finishing the tasks of each condition and were told to focus on the feelings they had during each condition.

### *Physiological recordings*

Blood pressure and heart rate of the subjects were measured utilizing blood pressure monitors (Omron 637IT; Omron Matsusaka Co., Ltd.). Three measurements of subjects' heart rate and one measurement of their blood pressure were recorded before and during the donation sessions. During both conditions, heart rate and blood pressure measurements were obtained on demand and written down by the experimenter. Means of the measured heart rate and blood pressure are reported.

### *Statistics*

Statistical analyses were conducted using SPSS 16.0 (SPSS Inc.). Normal distribution of the data was tested using the Shapiro–Wilk test. Normally distributed data (POMS anxiety and physiological recordings) were submitted to parametric $t$-tests for paired samples. Not normally distributed data (STAI X1) were submitted to nonparametric tests for paired samples (Wilcoxon signed ranks test). $P$ values $\leq 0.05$ were considered significant.

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### Part II: Examination of the effect of emotional chemosignals

#### *Participants*

Twenty healthy female subjects between the ages of 20 and 33 years (mean age 24.7 years, SD 3.3 years) participated as recipients of the chemosignals. The subjects were screened for normal olfactory function using the Sniffin' Sticks test battery (Kobal et al. 1996; Hummel et al. 1997). A mean olfactory TDI (Threshold, Discrimination, Identification) score of 37.6 (SD 3.5) indicated normal olfactory function for all tested subjects (Kobal et al. 2000; Hummel et al. 2007). All subjects were nonsmokers. None of them was using hormonal contraceptives at the time point of the experiment. All odor recipients described themselves as exclusively heterosexual (Kinsey et al. 1953) (mean 0.0, SD 0.0). They were not taking any medication known to interfere with sensory perception (Doty and Bromley 2004).

Subjects were not aware of the nature of the odorants nor of the hypothesis of this experiment. They were told that they would receive low intense odor stimuli and that the aim of the study was to examine odor sensitivity throughout the menstrual cycle.

Odor recipients were screened regarding their trait anxiety (STAI X2). The mean STAI X2 score (trait anxiety) of the odor recipients at the beginning of the experiment was 32.4 (SD 6.8, range: 21–46), indicating a normal anxiety level.

#### *Stimulus material*

Approximately 5 pieces of the pads were taken out of the freezer 20 min before testing. The pads were put into a paper tea bag which was placed under the nose of the recipients using an elastic strap. The order of the anxiety and neutral condition was pseudorandomized. To exclude adaptation effects, we conducted 2 sessions on 2 different days (interval: mean = 10.7 days, SD = 7.6 days). The experimenters were not aware regarding the condition (anxiety or neutral condition) they tested.

#### *Psychometric measures*

Subjects were advised to evaluate their subjective anxiety on the state scale (STAI X1) 5 and 20 min after the odor pads were placed under their noses.

To compare the attention of the subjects during both conditions the d2 Test of Attention (Brickenkamp and Zillmer 1998) was applied after subjects had completed the first STAI X1 questionnaire. This test measures speed and quality of performance in crossing out "d" letters with 2 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 error scores (TN – *E*), and the concentration performance (CP) derived from the number of correctly crossed out items minus errors of commission. Because each subject had to complete the test twice (during both conditions), the time permitted for crossing out the "d" letters with 2 dashes in each row of letters was shortened from 20 s to 15 s according to the instructions in the d2 test manual (Brickenkamp and Zillmer 1998). In doing so, ceiling effects were diminished.

At the end of the testing sessions, subjects were asked to complete a questionnaire regarding the pleasantness (0 = very unpleasant, 100 = very pleasant), intensity (0 = perceived no smell, 100 = perceived a very strong smell), familiarity (0 = not familiar, 100 = very familiar), masculinity/femininity (0 = very masculine, 100 = very feminine), and sexual attractiveness of the odor (0 = very sexually attractive, 100 = very sexually unattractive) on a visual analogue scale (Aitken 1969). Additionally, subjects were asked to evaluate their emotional valence (0 = negative, 100 = positive), arousal (0 = calm, 100 = aroused), and dominance (0 = submissive, 100 = dominant) while smelling the odor.

#### *Physiological recordings*

Blood pressure and heart rate of the subjects were measured utilizing blood pressure monitors (Omron 637IT; Omron Matsusaka Co., Ltd.). Three measurements of subjects' heart rate and one measurement of their blood pressure were recorded before the first and before and after the second time they completed the STAI X1 scale. Means of heart rate and blood pressure are reported.

#### *Statistics*

Statistical analyses were conducted using SPSS 16.0 (SPSS Inc.). Normal distribution of the data was tested using the Shapiro–Wilk test. Our data (psychometric measures and physiological recordings) were not normally distributed and were therefore submitted to nonparametric tests for paired samples (Wilcoxon signed ranks test/Friedman test). Because the hypothesis of this study was unidirectional, we used a one-tailed Wilcoxon signed ranks test for the comparison of anxiety ratings. $P$ values $\leq 0.05$ were considered significant.

## Results

### Part I: Body odor donation

The results of the state anxiety scale (STAI X1) demonstrate that the emotion induction method successfully produced anxiety and neutral affective states in the donors. Sweat donors differed significantly regarding their state anxiety between neutral and anxiety condition before (anxiety condition: mean 33.4, SD 9.8; neutral condition: mean 28.9, SD 5.3; $t_{1,12} = 1.9$, $P = 0.04$, Wilcoxon signed ranks test) as well as during each of the sessions (anxiety condition: mean 57.2, SD 9.4; neutral condition: mean 34.5, SD 7.9; $t_{1,12} = 2.9$, $P = 0.021$; $t_{1,12} = 7.9$, $P = 0.001$, Wilcoxon signed ranks test). Additionally, odor donors self-evaluated their anxiety as significantly higher during the anxiety condition (mean 5.5,

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**Table 1** Systolic and diastolic blood pressure and heart rate of odor donors before and during the anxiety condition as well as before and during the neutral condition ($n$ = 13)

| | Donors' anxiety condition | | |
|---|---|---|---|
| | Before | During | $t$-test |
| Systolic blood pressure | 120.8 (15.4) | 123.0 (13.8) | $P$ = 0.66 |
| Diastolic blood pressure | 79.9 (12.5) | 83.0 (10.8) | $P$ = 0.33 |
| Heart rate | 90.5 (12.6) | 112.0 (22.6) | $P$ = 0.001 |
| | Donors' neutral condition | | |
| | Before | During | $t$-test |
| Systolic blood pressure | 116.3 (10.3) | 120.8 (18.9) | $P$ = 0.30 |
| Diastolic blood pressure | 75.6 (9.3) | 71.0 (22.8) | $P$ = 0.49 |
| Heart rate | 87.3 (11.8) | 126.5 (28.9) | $P$ < 0.001 |

Reported are means, standard deviations, and results of the paired $t$-tests.

SD 0.7) compared with the neutral condition (mean 0.0, SD 0.0; $t_{1,12}$ = 29.8, $P$ < 0.001) answering one question of the POMS questionnaire.

Blood pressure and heart rate values of odor donors are reported in Table 1. Measurements before the respective condition show that blood pressure and heart rate of the subjects did not differ significantly between neutral and anxiety condition (systolic: $t_{1,12}$ = 1.7, $P$ = not significant [ns]; diastolic: $t_{1,12}$ = 1.2, $P$ = ns; heart rate: $t_{1,12}$ = 1.6, $P$ = ns). Measurements during the respective condition show that blood pressure values were significantly different (systolic: $t_{1,12}$ = 2.6, $P$ = 0.02; diastolic: $t_{1,12}$ = 4.8, $P$ < 0.001), whereas heart rate values were not ($t_{1,12}$ = 2.0, $P$ = ns). Systolic and diastolic blood pressure did not significantly differ before versus after the anxiety or neutral condition, whereas heart rate did (anxiety condition: $t_{1,12}$ = 4.67, $P$ = 0.001, neutral condition: $t_{1,12}$ = 6.31, $P$ < 0.001).

### Part II: Examination of the effect of emotional chemosignals

Attention of the subjects did not differ significantly between neutral and anxiety condition. These results are shown in Table 2. Additionally, we found no significant differences in subjective evaluation of the odors during both conditions (Table 3).

We obtained a trend of higher state anxiety of the subjects during the anxiety (mean 35.2, SD 9.3) compared with the neutral condition (mean 33.6, SD 6.4) after 5 min of exposure; the difference was not statistically significant ($t_{1,19}$ = 1.3, $P$ = ns, one-tailed Wilcoxon signed ranks test). However, the recipients' state anxiety differed significantly between neutral (mean 32.8, SD 7.7) and anxiety condition (mean 36.3, SD 9.3) after 20 min of odor exposure ($t_{1,19}$ = 1.9, $P$ = 0.035, one-tailed Wilcoxon signed ranks test) (Figure 2).

Blood pressure and heart rate of the odor recipients did not significantly differ between neutral and anxiety condition (Table 4). Blood pressure values did also not significantly differ before the first (5 min of odor exposure) versus before or after the second anxiety evaluation (20 min of odor exposure). However, the heart rate values differed significantly between these 3 time points during anxiety ($P$ = 0.04 Friedman test) as well as during neutral condition ($P$ < 0.001 Friedman test) indicating a slight decrease of heart rate over time.

**Table 2** Attention of odor recipients during anxiety and neutral condition ($n$ = 20)

| | Recipients' anxiety condition | Recipients' neutral condition | Wilcoxon signed ranks test |
|---|---|---|---|
| TN | 412.3 (68.5) | 422.2 (71.4) | $P$ = 0.30 |
| $E$ | 9.8 (7.3) | 10.1 (8.7) | $P$ = 0.92 |
| $E$% | 2.6 (2.4) | 2.5 (2.2) | $P$ = 0.88 |
| TN − $E$ | 402.5 (71.0) | 407.1 (66.0) | $P$ = 0.79 |
| CP | 172.2 (26.4) | 178.0 (29.6) | $P$ = 0.12 |

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 error scores (TN − $E$), and the concentration performance (CP) derived from the number of correctly crossed out items minus errors of commission. Reported are means, standard deviations, and results of the Wilcoxon signed ranks tests.

**Table 3** Subjective evaluation of the odors as well as emotional ratings while smelling the odors during anxiety and neutral condition (n = 20)

| | Recipients' anxiety condition | Recipients' neutral condition | Wilcoxon signed ranks test |
|---|---|---|---|
| Pleasantness | 48.7 (26.0) | 57.3 (21.6) | $P$ = 0.10 |
| Intensity | 33.2 (30.9) | 30.3 (25.5) | $P$ = 0.82 |
| Familiarity | 46.6 (24.0) | 34.1 (23.7) | $P$ = 0.12 |
| Masculinity/feminity | 45.1 (16.9) | 43.7 (14.6) | $P$ = 0.56 |
| Sexual attractiveness | 34.3 (24.5) | 37.1 (19.7) | $P$ = 0.49 |
| Emotional valence | 53.4 (14.6) | 55.0 (16.6) | $P$ = 0.57 |
| Emotional arousal | 27.9 (23.7) | 21.9 (15.4) | $P$ = 0.25 |
| Dominance | 49.3 (15.3) | 51.6 (16.4) | $P$ = 0.74 |

Odor evaluation: pleasantness (0 = very unpleasant, 100 = very pleasant), intensity (0 = perceived no smell, 100 = perceived a very strong smell), familiarity (0 = not familiar, 100 = very familiar), masculinity/femininity (0 = very masculine, 100 = very feminine), sexual attractiveness of the odors (0 = very sexually attractive, 100 = very sexually unattractive). Emotional ratings: Emotional valence (0 = negative, 100 = positive), emotional arousal (0 = calm, 100 = aroused), and dominance (0 = submissive, 100 = dominant). Reported are means, standard deviations, and results of the Wilcoxon signed ranks tests.

## Discussion

Our results indicate that male sweat collected during an anxiety condition compared with male sweat collected during

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an emotionally neutral sport condition is capable of inducing anxiety in female odor recipients, thereby providing validity of our new anxiety induction method as well as evidence that anxiety chemosignals are transferred between human subjects. In the past, a few studies verified that chemosensory anxiety signals can be distinguished from chemosensory neutral odors, thereby providing evidence that humans are able to detect chemosensory anxiety signals in sweat samples (Owen 1981; Chen and Haviland-Jones 2000; Ackerl et al. 2002). The results of other studies show that chemosensory anxiety signals influence facial affect perception (Pause et al. 2004; Zhou and Chen 2009), startle reflex in response to auditory signals (Prehn et al. 2006; Pause et al. 2009), and cognitive performance during a word-association task (Chen et al. 2006), thereby proofing the effects of chemosensory anxiety signals. To date 2 studies provided knowledge about the neural correlates of the perception of chemosensory anxiety signals (Mujica-Parodi et al. 2009; Prehn-Kristensen et al. 2009). However, to our knowledge, it has never been proven that male chemosensory anxiety signals in comparison with nonanxiety signals increase subjective perception of anxiety in female subjects utilizing the validated method of the Spielberger's state-trait anxiety inventory in a double-blind experimental design.

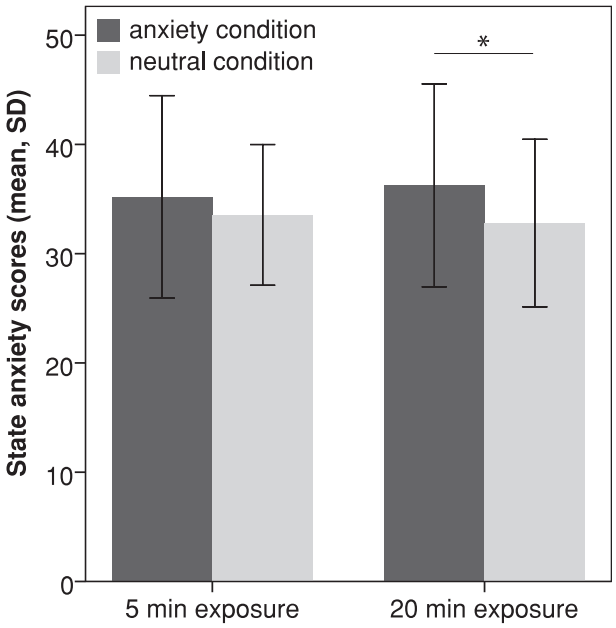


**Figure 2** State anxiety scores during neutral and anxiety condition after 5 and 20 min of odor exposure ($n$ = 20). State anxiety differed significantly between neutral and anxiety condition after 20 min but not after 5 min of odor exposure (*$P$ < 0.05).

We decided to solely test the effects of male sweat samples on female odor recipients because it has been demonstrated that the largest effects are expected to occur if male sweat samples are presented to female recipients (Chen and Haviland-Jones 2000; Prehn et al. 2006; Lenochova et al. 2009; Zhou and Chen 2009). Besides this, it has been shown that females show a higher sensitivity to aversive stimulation in general (Wrase et al. 2003). Moreover, we assured to include only heterosexual subjects because it has been shown that homo- and heterosexual subjects differ in their preference for body odors (Martins et al. 2005) and cortical processing of chemosensory signals (Savic et al. 2005; Berglund et al. 2006; Savic and Lindstrom 2008).

Odor donors differed in state anxiety, heart rate, and blood pressure between anxiety and neutral condition. The fact that they differed even before the beginning of the sweat donation sessions can be attributed to their knowledge about the purpose of the study. Additionally, odor donors completed the STAI X questionnaire and measurements of heart rate and

**Table 4** Systolic and diastolic blood pressure and heart rate of odor recipients before the first (5 min of odor exposure) and before and after the second anxiety evaluation (20 min of odor exposure) during neutral and anxiety condition ($n$ = 20)

| | Recipients' anxiety condition | | | |
|---|---|---|---|---|
| | Before first | Before second | After second | |
| | Anxiety evaluation | | | Friedman test |
| Systolic blood pressure | 111.4 (8.7) | 110.8 (8.4) | 110.7 (9.1) | $P$ = 0.57 |
| Diastolic blood pressure | 70.0 (7.2) | 70.0 (7.1) | 70.6 (8.9) | $P$ = 0.84 |
| Heart rate | 74.8 (9.0) | 71.3 (7.9) | 71.2 (7.9) | $P$ = 0.04 |
| | Recipients' neutral condition | | | |
| | Before first | Before second | After second | |
| | Anxiety evaluation | | | Friedman test |
| Systolic blood pressure | 112.1 (11.2) | 113.4 (13.1) | 112.1 (12.3) | $P$ = 0.69 |
| Diastolic blood pressure | 72.0 (9.5) | 71.1 (9.0) | 73.0 (11.0) | $P$ = 0.53 |
| Heart rate | 73.7 (8.4) | 71.6 (7.5) | 68.2 (10.5) | $P$ < 0.001 |

Reported are means, standard deviations, and results of the Friedman tests.

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blood pressure before the sweat donation started, however, while they already faced the high rope course. Both facts could have resulted in priming that led to an increased anxiety at the beginning of the anxiety sweat sampling condition.

In odor recipients, a trend of higher state anxiety after 5 min of odor exposure was obtained; however, this difference was not statistically significant if compared with the neutral condition. After 20 min of odor exposure, the difference in state anxiety of both conditions was statistically significant. We suppose that this temporal delay points out that a certain amount of time is needed to transfer the emotion of anxiety. Further research is needed to investigate the time course of the transfer of anxiety information mediated by chemosensory stimuli.

The hypothesis of this study was that male chemosensory anxiety signals in comparison with neutral chemosensory signals increase state anxiety in female odor recipients. Because there are many references outlining the existence of chemosensory anxiety signals in humans and the capability of these signals to modulate human behavior in the way that humans act with more cautiousness, we think that the one-tailed statistical approach is appropriate. If this hypothesis would not have existed, we should have utilized a 2-tailed statistical test, which probably would not have led to a significant result due to the relatively small sample of subjects. A further limitation of this study is that we did not measure baseline levels of recipients' anxiety before applying the stimuli. Nonetheless, we claim that the sweat donated during a nonanxious state (neutral condition) can be used as a valid control or baseline condition. Another limitation of our study is that we did not use empty pads as an additional control condition. Because our subjects were able to detect the smell of the sweat samples, this would not have been in line with the double-blind design of our study. Our data may additionally support the hypothesis that male sweat originating from an exercise condition has a calming effect on women as suggested by others (Grosser et al. 2000; Preti et al. 2003). However, Mujica-Parodi et al. (2009) did not obtain any sex-specific interactions in male or female subjects while investigating activation of the amygdala in response to male and female anxiety and exercise sweat. This result supports the notion that the effect described in our study is not related to a calming effect of male reproductive chemosensory signals on female subjects. In future studies, this issue should be addressed by including an appropriate control condition and investigating the effects of female and male chemosensory signals on an equal distribution of female and male subjects in order to demonstrate that there are no donor–recipient sex interactions.

We acknowledge that using the current study design, we might have compared the effects of secretions of 2 different gland types of the human skin. Sweat collected during the exercise or neutral condition—a task that is high in physical activity but low in emotion—most likely originates from eccrine glands, known to produce a clear fluid that mainly serves thermoregulation (Schaal and Porter 1991). On the other hand, sweat collected during the anxiety induction—a task presumably being high in emotion but low in physical activity—mainly originates from apocrine glands, which are known for secretion of a complex mixture containing the precursors of odorants. It is also known that apocrine glands commonly react to psychological stimuli (Schaal and Porter 1991). Subsequently, one would expect that during the comparison of the 2 different emotional states, we find different amounts of sweat in the pads and therefore not equally intense stimulation samples. However, the subjects in this study rated both samples as equally intense. We claim that our anxiety task was lower in physical activity compared with the neutral condition, however, still involved a certain amount of physical activity during climbing the high rope course. We assume that the neutral pads contained exercise sweat only (secreted from the eccrine glands), whereas the pads of the anxiety condition contained exercise sweat plus the anxiety signal (secreted by the eccrine and apocrine glands), rendering the comparison between both conditions suitable.

Our results that odor recipients' alertness, the subjective ratings of the odorants (pleasantness, intensity, familiarity, masculinity, and sexual attractiveness), as well as ratings regarding emotional valence, arousal, and dominance did not significantly differ between the anxiety and control condition supports the results of other experiments (Chen et al. 2006; Prehn et al. 2006; Zhou and Chen 2009). Thereby evidence is provided that chemosensory anxiety signals are conveyed unconsciously, which is in concordance with other studies (Lundstrom et al. 2008a, 2008b; Mujica-Parodi et al. 2009; Zhou and Chen 2009). The slight trend toward increased pleasantness ratings of the odor recipients during the neutral compared with the anxiety condition ($P$ = 0.10) again raises the question if the difference between the conditions is driven by the exercise sweat's positive effect or the anxiety sweat's negative effect on perceived anxiety level. Heart rates of odor recipients significantly decreased as a function of odor exposure. However, this decrease is existent in both conditions, neutral as well as anxiety condition, whereas anxiety ratings only change significantly during exposure to the chemosensory anxiety signal. This fact assures that our results account for the negative effect of the anxiety sweat. The slight decrease of subjects' heart rate over time could be attributed to a general familiarization and thereby habituation of the subjects to the task (anxiety evaluation). Because we did not measure the blood pressure and heart rate values before attaching the bag with the chemosensory stimuli, the difference between heart rate values during exposure to chemosignals and baseline values remains unknown. It is likely that heart rate values during both conditions are higher during the first anxiety evaluation and return to baseline level after the second anxiety evaluation. Because this is true for both conditions, the fact that higher heart rates measured before the first anxiety

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evaluation are caused by the task rather than by the chemosignals is supported. Another possibility is that the heart rate values during anxiety condition peaked in between our measurement time points and we therefore were not able to detect. Additionally, it is possible that internal measures like heart rate possess slightly different temporal properties than subjective ratings. In concordance with our results, none of the previous studies about chemosensory anxiety signals have shown an increase of heart rate in response to chemosensory anxiety signals. To get a better understanding, future studies should aim to measure baseline heart rate as well as blood pressure and continuously acquire both parameters over time.

To conclude, this study is the first study that provides direct evidence that males are able to communicate chemosensory anxiety signals via the sense of smell resulting in an increased state anxiety in females. This behavioral aspect is of high evolutionary importance for alarm reaction and danger avoidance. Further research should provide deeper insights into brain function during the perception of chemosensory anxiety signals.

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