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ORIGINAL RESEARCH REPORT

Stress lowers the detection threshold for foul-smelling 2-mercaptoethanol

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Abstract

Previous studies have reported enhanced vigilance for threat-related information in response to acute stress. While it is known that acute stress modulates sensory systems in humans, its impact on olfaction and the olfactory detection of potential threats is less clear. Two psychophysical experiments examined, if acute stress lowers the detection threshold for foul-smelling 2-mercaptoethanol. Participants in Experiment 1 ($N = 30$) and Experiment 2 ($N = 32$) were randomly allocated to a control group or a stress group. Participants in the stress group underwent a purely psychosocial stressor (public mental arithmetic) in Experiment 1 and a stressor that combined a physically demanding task with social-evaluative threat in Experiment 2 (socially evaluated cold-pressor test). In both experiments, olfactory detection thresholds were repeatedly assessed by means of dynamic dilution olfactometry. Each threshold measurement consisted of three trials conducted using an ascending method of limits. Participants in the stress groups showed the expected changes in heart rate, salivary cortisol, and mood measures in response to stress. About 20 min after the stressor, participants in the stress groups could detect 2-mercaptoethanol at a lower concentration than participants in the corresponding control groups. Our results show that acute stress lowers the detection threshold for a malodor.

Keywords

Cortisol, olfaction, psychophysics, sensory hypervigilance, socially evaluated cold-pressor test, adapted Trier Social Stress Test for Groups

History

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Introduction

Acute stress can affect taste, pain, and auditory thresholds in humans (Crettaz et al., 2013; Fehm-Wolfsdorf et al., 1993; Ileri-Gurel et al., 2013). Inconsistencies of effect direction within a sensory modality have been attributed to differences in testing procedures (Ileri-Gurel et al., 2013), laboratory stressor applied or magnitude of stressor appraisal (Geva et al., 2014). It is unclear, if acute stress influences the nasal chemosensory system, which is composed of the olfactory and trigeminal pathway (Hummel & Livermore, 2002).

While higher stimulus concentrations are usually needed to provoke trigeminal chemoreception, olfactory sensations can be evoked relatively fast and at very low concentrations (Hummel & Livermore, 2002). The olfactory system is closely linked to emotion processing (Adolph & Pause, 2012; Krusemark et al., 2013) and one of its key functions is threat detection (Stevenson, 2010). Unpleasant odors can fulfill their warning function by evoking negative emotions such as fear or disgust (Croy et al., 2013; Stevenson, 2010).

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In response to stress, attention regulation switches from a task-directed mode governed by the prefrontal cortex to a sensory-vigilance mode governed by the sensory cortices and the amygdala (Arnsten, 2009; Shackman et al., 2011). This switch to bottom-up control is thought to be mediated in part by catecholamines, their impact on the amygdala and on the signal-to-noise ratio in the primary sensory cortices (Arnsten, 2009). When stressed, humans allocate more processing resources to threat information (Mogg et al., 1990). It has been hypothesized that the enhanced vigilance in response to stress leads to improved detection of threatening or generally salient events (van Marle et al., 2009). This represents a survival value for the organism in adverse situations where the failure to detect threat might result in serious harm.

Taken together, the above findings suggest that acute stress could improve olfactory detection through sensory hypervigilance. We tested this hypothesis in two laboratory experiments. Stress protocols differ in the likelihood and magnitude of eliciting physiological and psychological stress responses (Skoluda et al., 2015). Therefore, two different stress protocols were chosen to investigate the effect of acute stress on olfactory detection threshold.

For both experiments short stress protocols were selected that were compatible with psychophysical threshold testing. For Experiment 1, a stress protocol based on the mental

Table 1. Sample characteristics for Experiment 1 and Experiment 2.

	Experiment 1		Experiment 2	
	Control group	Stress group	Control group	Stress group
Women/men	9/7	9/5	8/8	8/8
Age	25.6 ± 1.2	23.8 ± 0.7	23.8 ± 0.8	23.4 ± 0.9
Body-mass index, kg/m^2	–	–	23.4 ± 0.9	24.6 ± 1.0
Personality				
Neuroticism	17.9 ± 1.8	19.9 ± 2.2	–	–
Trait anxiety	–	–	39.6 ± 2.1	39.2 ± 1.5
Olfactory function[a]				
n-Butanol threshold, ppb	55.8 ± 9.3	58.7 ± 13.5	–	–
TDI	–	–	35.4 ± 0.9	35.9 ± 0.9

Mean ± SEM are shown. TDI, Sniffin' Sticks total score.
[a]Sex differences in olfactory function tests were not significant, all $p > 0.10$.

arithmetic subtask of the Trier Social Stress Test for Groups (TSST-G, von Dawans et al., 2011) was selected combining a motivated performance task with social evaluative threat and uncontrollability. The stress protocol in Experiment 2 combined a physically demanding task with social-evaluative threat (socially evaluated cold-pressor test, SECPT, Schwabe et al., 2008). In both experiments olfactory detection thresholds were determined for 2-mercaptoethanol, which has a sewage-like, foul odor. In accordance with a sensory hypervigilance account of stress it was hypothesized that stress would lower the olfactory detection threshold in both experiments.

Methods

Participants

There was no overlap between participants of Experiment 1 and Experiment 2.

Experiment 1

Thirty-six nonsmoking participants were recruited for participation in Experiment 1. Exclusion criteria included pregnancy, asthma, and acute or chronic upper airway diseases. Participants were advised to refrain from alcohol, caffeine, drugs, and physical exercise on the test day. Participants were instructed not to eat and to drink only water 1 h before the test session.

Six participants were excluded from the statistical analyses: two participants had abnormally high cortisol levels at study onset (>28.4 nmol/l, see Westermann et al., 2004), three participants failed screening for olfactory function, and one participant had increased false alarm rates during all 2-mercaptoethanol threshold measurements (more than 20% false alarms). The latter two exclusions were performed to avoid biased odor thresholds.

Thus, the final sample consisted of 16 participants in the control group and 14 participants in the stress group. Sample characteristics are provided in Table 1. There were no significant differences between the two groups with respect to age, threshold for n-butanol or neuroticism scores (all independent sample *t*-tests $p > 0.10$). While large epidemiological studies indicate that women have lower olfactory thresholds than men (Doty & Cameron, 2009), sex differences with regard to n-butanol thresholds were not significant for the investigated sample, $t(28) = 1.2$, $p = 0.231$.

Experiment 2

Thirty-six nonsmoking participants were recruited for participation in Experiment 2. In accordance with the SECPT study protocol the exclusion criteria of Experiment 1 were extended to regular intake of medicine, use of hormonal contraceptives, drug use, any chronic or acute illnesses, and current medical or psychological treatment.

As in Experiment 1, participants were advised to refrain from alcohol, caffeine, and physical exercise on the test day and to drink only water 1 h before the start of the test session. Four participants were excluded from data analyses due to increased false alarm rates during the threshold measurements (>20% false alarms) to avoid biased odor detection thresholds. The final sample comprised 32 participants.

Sample characteristics are summarized in Table 1. Participants in the stress and the control group had similar age, body mass index, trait anxiety and Sniffin' Sticks total score (TDI, all independent sample *t*-tests $p > 0.10$). All participants passed screening for anosmia (TDI > 16.5, see Hummel et al., 2007). As in Experiment 1, sex differences with regard to olfactory function were not significant, $t(30) = 0.4$, $p = 0.664$.

Procedure

The study protocols were approved by the local ethics committee. All participants provided written informed consent prior to study participation. After participation, participants were debriefed about the goals of the stress induction.

Experiment 1

Gender-mixed groups of three participants were tested between 2:00 and 4:30 p.m. to control for diurnal cycle of cortisol. Participants were randomly assigned to either the control or the stress group. In a first step they answered a demographic questionnaire and a Big Five personality inventory to assess neuroticism (NEO-FFI, Borkenau & Ostendorf, 2008).

Next, participants were screened for olfactory function with a threshold test for n-butanol (dynamic dilution

olfactometer TO 7, ECOMA GmbH, Kiel, Germany). The standard procedure of ascending method of limits was used (Kleinbeck et al., 2011). Participants were excluded if they were unable to detect a concentration of 110 ppb n-butanol in three consecutive trials.

Subsequently, the detection threshold for 2-mercaptoethanol was assessed three times intermittently by either stress or control blocks (TO 7, ECOMA GmbH, Kiel, Germany). Each stress or control block lasted 8 min. After the first threshold measurement, the first stress block for the stress group or the first control block for the control group followed. Next, the detection threshold was assessed again, followed by another stress block or control block. The detection threshold was assessed for a third time 5 min after the end of the second stress or control block (21 min after the end of the first stress or control block).

Stress blocks were based on a subtask of the Trier Social Stress Test for Groups, which can be described as a standardized motivated performance task protocol that combines high levels of socio-evaluative threat and uncontrollability in a group format (von Dawans et al., 2011). During each stress block, participants were asked to serially subtract a two-digit number (13, 14, 16, or 17) from a given four-digit number as quickly and accurately as possible. Participants were interrupted and asked to calculate four times in each block resulting in 120 s of calculating for each participant per block. To increase the feeling of uncontrollability, the order in which participants were asked to calculate was random and minuends and subtrahends were different for each participant every time. If the participants made a mistake, they had to restart at the beginning with one experimenter interrupting, ''Incorrect. Start again.'' To further enhance the feeling of social evaluative threat, the participants had to stand up and face one reserved experimenter, who monitored and videotaped them during the task. They were told that, on top of their performance, their facial expressions and body language would be continuously monitored and evaluated.

Participants in the control group had to read magazine articles during the control blocks.

Experiment 2

Experimental sessions were run between 1:00 and 4:30 p.m. to control for diurnal cycle of cortisol. To control for potential effects of time of day on threshold measurements between the two experiments, the first threshold measurement of Experiment 2 was scheduled to coincide with that of Experiment 1 (3:10 p.m.).

Participants were randomly assigned to either the control or the stress group. First, they answered a demographics questionnaire and the trait version of the state-trait-anxiety inventory (Spielberger, 1983). Then, participants were screened for anosmia with the help of the Sniffin' Sticks test (Burghart, Wedel, Germany) and nasal congestion was checked (computer-aided anterior rhinomanometry, CAAR, Atmos Inc., Lenzkirch, Germany).

Next, the detection threshold for 2-mercaptoethanol was determined using a dynamic dilution olfactometer (TO 8, ECOMA GmbH, Kiel, Germany). Subsequently, the socially evaluated cold-pressor test (SECPT) as described in Schwabe et al. (2008) was applied in the stress group. In short, participants were asked to immerse their hand into ice water (0–3 °C) for 3 min while being videotaped and monitored by a reserved experimenter. In the control group, participants immersed their hand into warm water (36–38 °C) for 3 min without being videotaped or monitored.

Immediately afterwards, participants were screened for nasal congestion for a second time. Twenty minutes after the end of the SECPT or the control procedure the detection threshold for 2-mercaptoethanol was determined again. Nasal congestion was checked directly afterwards.

Measurement of the stress response

Heart rate and respiratory rate

Heart rate was assessed as a marker of autonomic nervous system (ANS) activity in both experiments. Respiratory rate was assessed additionally in Experiment 2.

Experiment 1. An electrocardiogram (ECG) was continuously recorded from the onset of the first threshold measurement until 3 min after the end of the third threshold measurement using the Varioport system (Becker Meditec, Karlsruhe, Germany). The ECG was sampled at 512 Hz. Beat detection and artifact control was performed offline using PhysioToolkit software (Goldberger et al., 2000). For analysis, the mean heart rate for every minute of the recording period was calculated.

Experiment 2. Heart rate and respiratory rate were assessed using the BioHarness system (Zephyr Technology Corp., Annapolis, MD, USA). Sampling frequencies for heart rate and respiratory rate were 250 and 25 Hz respectively. Recordings were made from 3 min prior to each threshold measurement to 3 min after the end of each threshold measurement, as well as from the onset of the hand immersion in cold/warm water until 3 min after the end of the hand immersion in cold/warm water. Artifact control, as well as calculation of heart rate and respiratory rate, was performed using the BioHarness software. For analysis, the mean heart and respiratory rates over 3-min intervals were calculated.

Saliva sampling and analysis

Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany). The samples were immediately stored at −20 °C until biochemical analysis took place at the central scientific unit Analytical Chemistry of the Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany.

As a marker of hypothalamic–pituitary–adrenal (HPA) axis activity, cortisol was analyzed in saliva samples in both experiments. As an additional marker of ANS activity, alpha-amylase was analyzed in saliva samples in Experiment 2. Commercial assays were used for all analyses (ELISA, IBL International, Hamburg, Germany). Inter- and intra-assay variabilities were below 10%.

Experiment 1. Four saliva samples were collected per session and participant: 35 and 15 min prior to the onset of the first stress block, and 10 and 30 min after the end of the first stress block.

Experiment 2. Five saliva samples were collected per participant: 20 and 10 min prior to the onset of the SECPT, immediately after, and again 20 and 30 min after the end of the SECPT. Saliva from one participant in the stress group and one participant in the control group could not be analyzed for alpha-amylase due to an insufficient amount of donated saliva. Thus, the two participants could not be included in the analysis of alpha-amylase data.

Mood and subjective stress response

Experiment 1. The state version of the Positive and Negative Affect Schedule (PANAS, Watson et al., 1988) was used to assess current mood at four time points: 35 and 15 min prior to the onset of the first stress block, and 10 and 30 min after the end of the first stress block.

Experiment 2. Directly after the control or stress procedure, participants rated how stressful, painful, and unpleasant they had felt during the procedure on scales from 0 (*not at all*) to 100 (*very much*). This method was adapted from Schwabe et al. (2008).

Nasal congestion

Computer-aided anterior active rhinomanometry (CAAR, Atmos Inc., Lenzkirch, Germany) was used to monitor nasal congestion as a potential physiological confound at three time points during Experiment 2: 40 min prior to the first 2-mercaptoethanol threshold measurement, 10 min before the second threshold measurement, and 10 min after the second threshold measurement.

Anterior active rhinomanometry measures nasal airflow and the transnasal pressure gradient between the nostrils and the epipharynx. Nasal congestion leads to increased nasal resistance and lower nasal airflow. During each measurement left and right nostrils were measured separately. Flow values at transnasal pressures of 75, 150, and 300 Pa were calculated from the flow curves and only values fulfilling the CAAR criteria were analyzed. For data analyses, nasal airflow at a transnasal pressure of 150 Pa was analyzed.

Measurement of olfactory detection thresholds

A dynamic dilution olfactometer TO 7 (ECOMA GmbH, Kiel, Germany) was used in Experiment 1 and a dynamic dilution olfactometer TO 8 (ECOMA GmbH, Kiel, Germany) in Experiment 2. 2-Mercaptoethanol (CAS: 60-24-2, Sigma Aldrich, Seelze, Germany, >99% pure) was injected into 25 L Tedlar-bags filled with nitrogen. The mixture was homogenized by heating and rotation of the bag.

The standard procedure of ascending method of limits with a 2-fold geometric dilution series was used to assess detection thresholds (Kleinbeck et al., 2011; Smeets et al., 2007). Compared to static olfactometry, this method yields more reliable estimates for odor detection thresholds (Smeets et al., 2007).

One threshold measurement consisted of three trials. In each trial, increasing concentration steps of 2-mercaptoethanol were presented interspersed with blank samples. Participants were asked to press a button whenever they thought they detected an odor. The lower of two subsequent correctly identified concentration steps was used as an estimate of reliable olfactory detection in that trial. The geometric mean of the three trial estimates was calculated and represented the individual detection threshold (Kleinbeck et al., 2011; Smeets et al., 2007). Detection thresholds were subjected to a log-transformation before data analysis (Smeets et al., 2007).

In both experiments, nine different concentrations of 2-mercaptoethanol were generated by means of the olfactometer's dilution unit. Comparable concentration steps were presented in both experiments (deviation per step $<0.01\%$), although two different olfactometers were used.

Experiment 1

In Experiment 1, an initial concentration of 13.8 ppm in the Tedlar-bag was used and a geometric dilution series with dilutions from 1/250 (corresponding to a concentration of 0.0552 ppm) to 1/64,000 (corresponding to a concentration of 0.0002 ppm) was generated. A detection threshold measurement lasted approximately 8 min.

Experiment 2

In Experiment 2, an initial concentration of 3.53 ppm in the Tedlar-bag was used. This yielded a concentration range from 0.0002 to 0.0552 ppm. A detection threshold measurement lasted approximately 9 min due to slightly longer breaks between the three trials.

Statistics

Analyses were carried out in SPSS 22 (IBM Corp.). The level of significance for all statistical tests was set to 0.05. Data were analyzed using *t*-tests and repeated measures analysis of variance (ANOVA) where appropriate. If the assumption of sphericity in repeated measures analysis of variance was violated, Greenhouse–Geisser corrected degrees of freedom were used. Significant interaction effects were further analyzed by Bonferroni-adjusted *post hoc* tests.

As no significant sex differences emerged during the olfactory screening of participants in both experiments (see Table 1), sex was not expected to impact olfactory thresholds for 2-mercaptoethanol. Cortisol stress responses are influenced by hormonal contraceptive intake (Kirschbaum et al., 1999). Contraceptive intake was not assessed in Experiment 1. In order to account for differences in cortisol reactivity between men and women, sex was included as a between-subject confound in all statistical analyses of Experiment 1. As it can be hypothesized that acute physiological responses to the SECPT in Experiment 2 are dependent on the time the hand is kept in the cold water, time of hand immersion was included as a covariate in the statistical analyses of heart and respiratory rate in Experiment 2.

Results

Stress response

Results confirmed that the experimental manipulations in Experiment 1 and Experiment 2 successfully elicited stress responses in the participants of the stress groups.

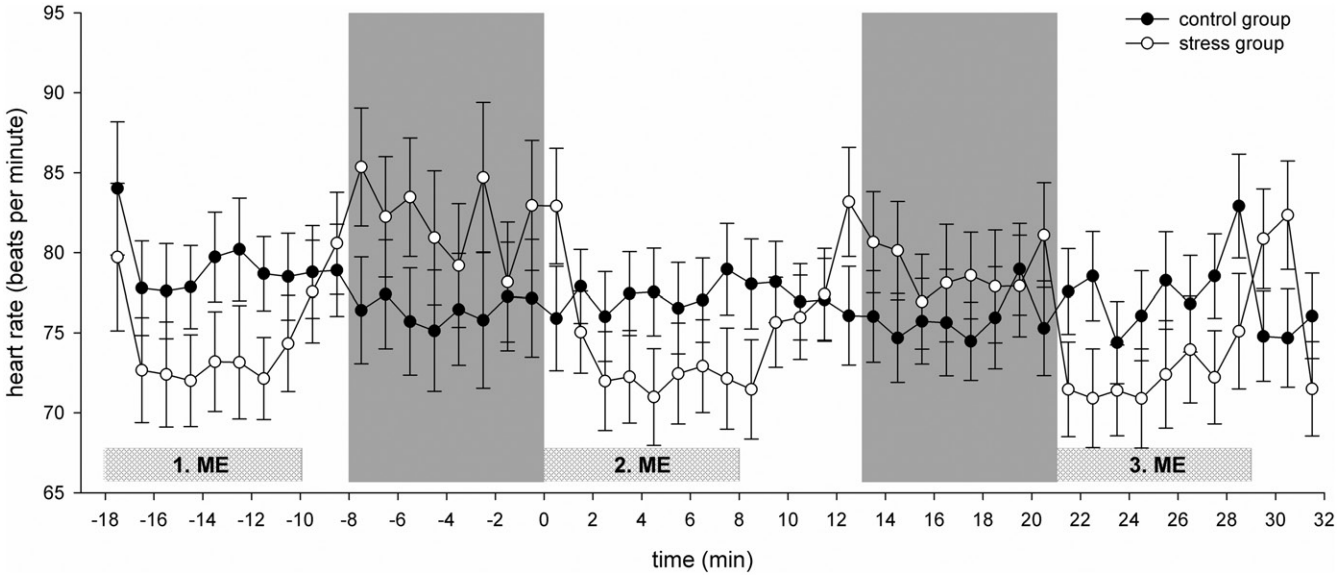

Figure 1. Time course of the heart rates (mean ± *SEM*) in the stress group and in the control group (Experiment 1). Shaded areas depict the time of the stress blocks. 1. ME = first threshold measurement for 2-mercaptoethanol; 2. ME = second threshold measurement for 2-mercaptoethanol; 3. ME = third threshold measurement for 2-mercaptoethanol.

Experiment 1

Heart rate. While the control group had an overall constant heart rate, stress-related increases of heart rate were observed in the stress group (Figure 1). This is reflected by a significant Group × Time interaction, $F(9, 223) = 4.8$, $p < 0.001$, and a significant main effect of time, $F(9, 223) = 3.1$, $p = 0.002$, on heart rate.

The stress group exhibited a stronger increase in heart rate during the stress blocks than the control group during the control blocks: during the first threshold assessment mean heart rate was comparable between the stress group ($M = 75.2$ bpm, $SEM = 2.9$ bpm) and the control group ($M = 78.7$, $SEM = 2.8$ bpm), $t(28) = 0.9$, $p = 0.399$. During the stress blocks mean increase in heart rate was 24.7 bpm ($SEM = 3.6$ bpm) for the stress group compared to 4.7 bpm ($SEM = 1.3$ bpm) during the control blocks for the control group, $t(17) = 5.2$, $p < 0.001$. Individual increases in heart rate were calculated as the difference between baseline heart rate during first threshold measurement and the individual maximum heart rate during stress or control blocks (von Dawans et al., 2011).

Salivary cortisol. Overall, a significant decrease in salivary cortisol was observed over the course of the experimental session as reflected by a main effect of time, $F(2, 52) = 7.1$, $p = 0.002$. However, the stress protocol affected salivary cortisol concentration (see Table 2): this is reflected by a significant Group × Time interaction, $F(2, 52) = 5.9$, $p = 0.005$. Participants in the stress group had higher salivary cortisol than participants in the control group 30 min after the end of the first stress block as reflected by a significant Bonferroni-adjusted *post hoc* test for this time point, $F(1, 26) = 6.9$, $p = 0.015$.

Mood. Stress did not affect mood as reflected by a nonsignificant interaction effect Group × Time on negative affectivity, $F(2, 49) = 1.2$, $p = 0.306$, and on positive affectivity, $F(3, 78) = 2.3$, $p = 0.084$. Pre-stress levels of positive and negative affectivity were comparable between the two groups as reflected by nonsignificant Bonferroni-adjusted comparisons for levels of negative and positive affectivity 35 and 15 min before stress onset, all $p > 0.10$. A main effect of time emerged with regard to positive affectivity suggesting that positive affectivity decreased in both groups over the course of the experimental session, $F(3, 78) = 27.1$, $p < 0.001$. Descriptive statistics for negative and positive affectivity over the course of Experiment 1 are provided in Table 2.

Table 2. Experiment 1: salivary cortisol concentrations (nmol/l), negative affectivity, and positive affectivity in the control group and in the stress group.

	Control group	Stress group
Salivary cortisol (*nmol/l*)		
35 min before stress[a] onset	7.6 ± 1.0	6.6 ± 1.2
15 min before stress[a] onset	6.2 ± 0.8	5.9 ± 1.2
10 min after stress[a] offset	4.9 ± 0.7	6.3 ± 1.2
30 min after stress[a] offset	4.0 ± 0.5	6.0 ± 1.2*
Negative affectivity (*PANAS*)		
35 min before stress[a] onset	11.6 ± 0.6	12.8 ± 0.6
15 min before stress[a] onset	11.3 ± 0.4	11.6 ± 0.5
10 min after stress[a] offset	10.8 ± 0.3	12.2 ± 0.7
30 min after stress[a] offset	10.6 ± 0.2	12.1 ± 0.8
Positive affectivity (*PANAS*)		
35 min before stress[a] onset	28.4 ± 1.3	26.9 ± 1.3
15 min before stress[a] onset	26.4 ± 1.5	23.4 ± 1.7
10 min after stress[a] offset	23.4 ± 1.4	21.9 ± 1.9
30 min after stress[a] offset	21.4 ± 1.7	22.7 ± 1.3

Mean ± *SEM* are shown. PANAS, Positive and Negative Affect Schedule.
[a]Referring to the first stress block.

Experiment 2

Mean duration of hand immersion into cold water (stress group) and warm water (control group) was not significantly different ($M_{control} = 180$ s, $SEM = 0$ s, and $M_{stress} = 158$ s, $SEM = 12$ s; $t(15) = 1.9$, $p = 0.083$).

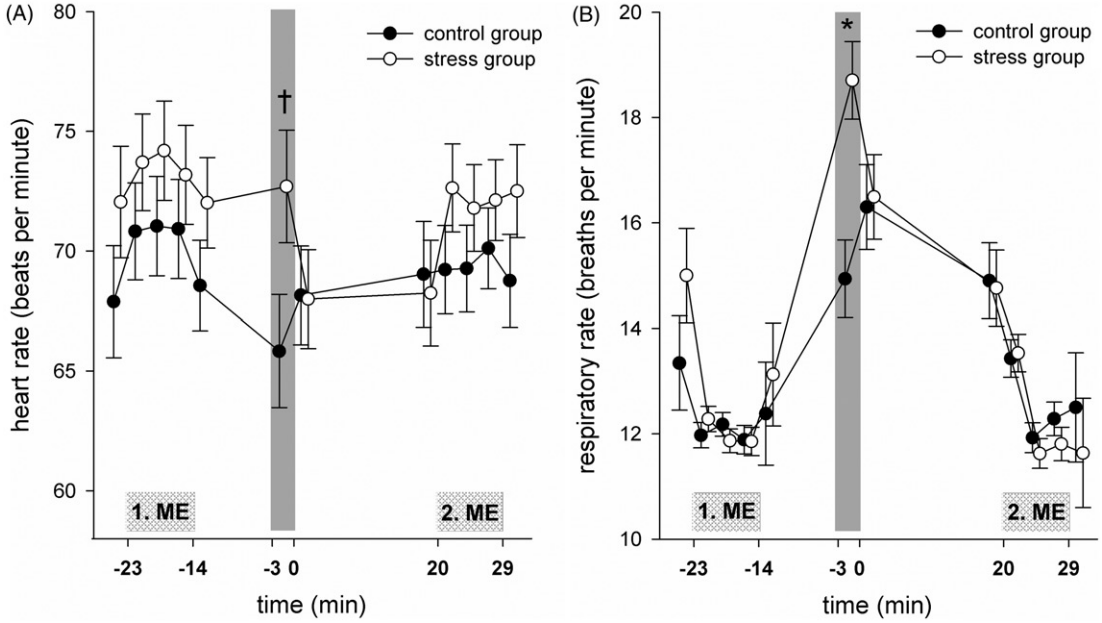

Figure 2. Time courses of heart (A) and respiratory rates (B) in the stress group and in the control group (Experiment 2). Shown are mean ± *SEM* corrected for the covariate (time of water immersion). The shaded area represents the time of the SECPT. 1. ME = first threshold measurement for 2-mercaptoethanol; 2. ME = second threshold measurement for 2-mercaptoethanol. *$p \leq 0.05$ Bonferroni-adjusted *post hoc* test. †$p = 0.054$ Bonferroni-adjusted *post hoc* test.

Heart rate. There was a significant Group × Time interaction on heart rate, $F(5, 153) = 2.9$, $p = 0.015$ (Figure 2A). Bonferroni-adjusted *post hoc* tests revealed no significant difference between control and stress group at any time point (during water immersion: $F(1, 29) = 4.0$, $p = 0.054$).

Respiratory rate. The SECPT affected respiratory rate (Figure 2B): this is reflected by a significant Group × Time interaction on respiratory rate, $F(5, 152) = 2.3$, $p = 0.042$. Bonferroni-adjusted *post hoc* tests revealed a significant difference between the stress group and the control group during the water immersion, $F(1, 29) = 12.4$, $p = 0.001$. The stress group had a significantly higher mean respiratory rate during the water immersion than the control group.

Salivary cortisol and alpha-amylase. In response to the SECPT, the stress group showed an increase in salivary cortisol concentration (see Table 3), as reflected by a significant Group × Time interaction, $F(2, 62) = 8.6$, $p < 0.001$. Bonferroni-adjusted *post hoc* tests indicated that participants in the stress group had significantly higher salivary cortisol than participants in the control group 20 min, $F(1, 30) = 7.1$, $p = 0.012$, and 30 min, $F(1, 30) = 4.9$, $p = 0.035$, after stress offset. Overall, a significant main effect of time, $F(2, 62) = 7.0$, $p = 0.002$, on salivary cortisol emerged.

Data for salivary alpha-amylase were characterized by large variance and skewness (see Table 3). The ANOVA analysis revealed that the SECPT did not affect salivary alpha-amylase as reflected by a nonsignificant Group × Time interaction, $F(3, 78) = 0.9$, $p = 0.444$. The application of standard data processing techniques such as log-transformation or baseline correction to raw data did not change results.

Subjective stress ratings. Participants in the stress group experienced the hand immersion as significantly more stressful ($M_{control} = 1.9$, $SEM = 1.0$, and $M_{stress} = 70.6$, $SEM = 6.9$; $t(16) = 9.8$, $p < 0.001$), painful ($M_{control} = 1.9$, $SEM = 1.4$, and $M_{stress} = 76.9$, $SEM = 5.2$; $t(17) = 13.9$, $p < 0.001$), and unpleasant ($M_{control} = 5.6$, $SEM = 3.2$, and $M_{stress} = 77.5$, $SEM = 5.4$; $t(30) = 11.6$, $p < 0.001$) than the control group.

Nasal congestion. Nasal congestion was not influenced by the experimental stress induction as reflected by a nonsignificant Group × Time interaction, $F(4, 120) = 2.0$, $p = 0.100$. Data are not shown.

Olfactory detection thresholds

Experiment 1

Stress influenced detection thresholds (Figure 3) as reflected by a significant Group × Time interaction effect, $F(2, 43) = 5.4$, $p = 0.012$. During the first threshold measurement at baseline, odor thresholds were comparable for the two groups. Stress preserved olfactory sensitivity in the stress group compared to the control group: while the difference between control and stress group was not statistically significant during the second threshold measurement, $F(1, 26) = 2.1$, $p = 0.156$, participants in the stress group could detect 2-mercaptoethanol at a lower concentration than participants in the control group during the third threshold measurement. This is reflected by a significant Bonferroni-adjusted pairwise comparison for the third threshold measurement, $F(1, 26) = 8.8$, $p = 0.006$.

There were no sex differences with regard to olfactory detection thresholds for 2-mercaptoethanol, as reflected by a nonsignificant main effect of sex, $F(1, 26) = 0.01$, $p = 0.907$, and nonsignificant interaction effects between sex and the other factors, all $p > 0.10$.

The number of false alarms during the threshold measurements was unaffected by stress: this is reflected by a nonsignificant interaction effect Group × Time, $F(2, 52) = 0.5$, $p = 0.609$. Moreover, there was a main effect

Table 3. Experiment 2: salivary cortisol concentrations (nmol/l) and alpha-amylase (U/ml) concentrations in the control group and in the stress group.

	Cortisol (nmol/l)		Alpha-amylase (U/ml)	
Time	Control group	Stress group	Control group	Stress group
20 min before stress onset	5.7 ± 0.6	4.7 ± 0.5	193.6 ± 36.7	136.2 ± 26.8
10 min before stress onset	6.9 ± 1.6	5.0 ± 0.7	158.1 ± 26.7	112.6 ± 24.1
Immediately after stress	6.1 ± 0.8	5.4 ± 0.9	156.8 ± 41.4	159.9 ± 38.9
20 min after stress offset	5.8 ± 0.6	11.1 ± 1.9*	153.0 ± 28.2	165.5 ± 33.9
30 min after stress offset	6.1 ± 0.8	9.6 ± 1.3*	127.3 ± 23.1	104.9 ± 26.9

Mean ± SEM are shown.
*$p \leq 0.05$. Bonferroni-adjusted *post hoc* test.

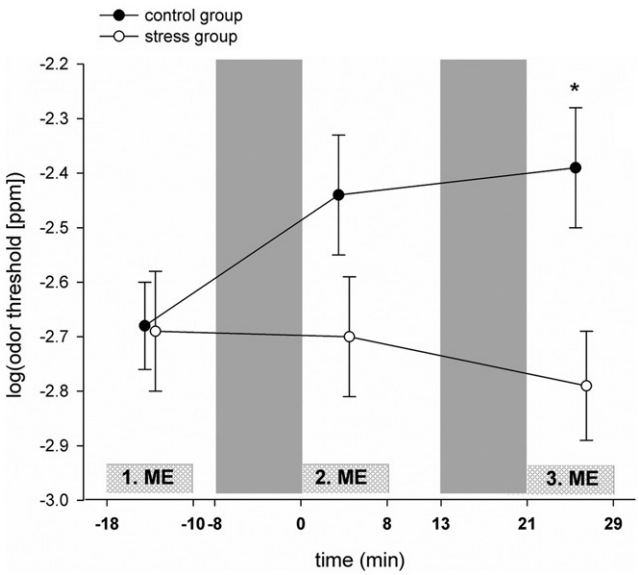

Figure 3. Time course of the log-transformed odor detection thresholds for 2-mercaptoethanol (mean ± *SEM*) in the stress and the control group (Experiment 1). Shaded areas depict the time of the stress blocks. 1. ME = first threshold measurement for 2-mercaptoethanol; 2. ME = second threshold measurement for 2-mercaptoethanol; 3. ME = third threshold measurement for 2-mercaptoethanol. *$p \leq 0.05$ Bonferroni-adjusted *post hoc* test.

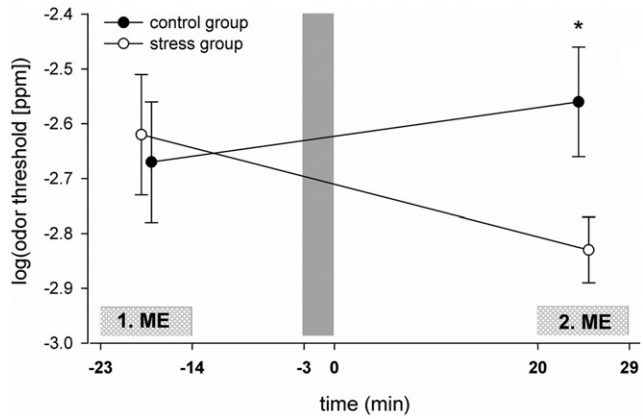

Figure 4. Time course of the log-transformed odor detection thresholds for 2-mercaptoethanol (mean ± *SEM*) in the stress group and in the control group (Experiment 2). The shaded area represents the time of the SECPT. 1. ME = first threshold measurement for 2-mercaptoethanol; 2. ME = second threshold measurement for 2-mercaptoethanol. *$p \leq 0.05$ Bonferroni-adjusted *post hoc* test.

of time, $F(2, 52) = 5.2$, $p = 0.011$, suggesting improved accuracy in both groups during the third threshold measurement: Bonferroni-corrected comparisons indicated that during the third threshold measurement fewer false alarms were made than during the first ($M_{\text{first threshold}} = 2.0$, $SEM = 0.4$, and $M_{\text{third threshold}} = 1.0$, $SEM = 0.3$; $F(1, 26) = 9.1$, $p = 0.006$) or second threshold measurement ($M_{\text{second threshold}} = 2.2$, $SEM = 0.5$, and $M_{\text{third threshold}} = 1.0$, $SEM = 0.3$; $F(1, 26) = 7.9$, $p = 0.009$).

Experiment 2

Stress influenced detection thresholds (see Figure 4), as reflected by a significant Group × Time interaction, $F(1, 30) = 7.5$, $p = 0.010$. Bonferroni-adjusted pairwise comparisons indicated that during the second threshold measurement, the stress group could detect 2-mercaptoethanol at a significantly lower concentration than the control group, $F(1, 30) = 4.6$, $p = 0.039$.

Exploratory analyses revealed that there were no sex differences with regard to olfactory detection thresholds, as reflected by a nonsignificant main effect of sex, $F(1, 28) = 0.1$, $p = 0.476$, and nonsignificant interaction effects between sex and the other factors, all $p > 0.10$. Acute stress did not affect the number of false alarms during the threshold measurement. This is reflected by a nonsignificant interaction effect Group × Time on the number of false alarms, $F(1, 30) = 1.6$, $p = 0.210$. Overall 1.1 ($SEM = 0.2$) false alarms were made during each threshold measurement.

Discussion

The results of two experiments show that acute stress affects olfactory thresholds: stress seems to preserve or lower the detection threshold for a malodor. The results are consistent with sensory hypervigilance due to stress. Under conditions of increased arousal a heightened sensory awareness of threat-signaling malodors might be adaptive.

Lower olfactory thresholds during stress could explain why odors are potent retrieval cues for stressful episodes in healthy individuals (Wiemers et al., 2014), as well as in patients with post-traumatic stress disorder (Vermetten et al., 2007). Within a symptom-learning account of idiopathic environmental intolerance (Meulders et al., 2010), lower olfactory thresholds during stress could facilitate the conditioning of symptoms to odors.

Which biological mechanisms might be involved?

Stress can induce a state of sensory hypervigilance and threat hyper-responsivity similar to anxiety (Arnsten, 2009; Mogg

et al., 1990): vigilance is thought to be up-regulated by fast-acting catecholamines that increase excitability in the amygdala and signal-to-noise ratio in primary sensory cortices. The ANS is responsible for the release of catecholamines such as noradrenaline and adrenaline during stress (Schommer et al., 2003). It was activated in participants of the stress group in both experiments as reflected in the increase in heart rate and respiratory rate. Thus, it may have contributed to the effect on detection thresholds through central as well as peripheral processes that influence the olfactory signal-to-noise ratio.

Behavioral responses to near threshold odorants can be improved by infusions of noradrenaline into the olfactory bulb of rats (Escanilla et al., 2012). Hence, Escanilla et al. (2012) argued that the noradrenergic system is specifically activated when signal-to-noise ratios have to be modulated. Furthermore, adrenaline produces a steeper dose–response relationship in odorant receptors *in vitro* via phosphorylation-dependent changes in the activities of several ion channels (Firestein & Menini, 1999). Thus, adrenaline increases the odorant receptors' sensitivity for the difference between the presence of an odor and its absence (Kawai et al., 1999). Kawai (1999) argued that under ''natural conditions'' this would improve the odorant receptors' ability to identify the presence of an odorant.

Besides the fast-acting response of the ANS, stress also activates the slower-acting HPA axis. An established biomarker of HPA activity is salivary cortisol (Kirschbaum & Hellhammer, 1994). Our results suggest that salivary cortisol was affected by stress in both experiments.

Glucocorticoid receptor mRNA and protein as well as corticosteroid binding globulin are expressed in the olfactory mucosa (Dölz et al., 2013; Robinson et al., 1998). Beneficial effects of corticoids on olfaction are found in the treatment of anosmia or hyposmia: oral and intranasally applied corticoids have been shown to improve olfaction, most likely through a reduction of nasal congestion and inflammation (Alobid et al., 2014). Nasal congestion in Experiment 2 was unaffected by acute stress indicating that stress-induced increases in nasal cortisol were not sufficient to impact nasal congestion.

It has been suggested that cortisol increases the threshold for the perception of stimuli in all sensory modalities via effects on the central nervous system (Fehm-Wolfsdorf & Nagel, 1996). This hypothesis is primarily based on studies investigating the intake of exogenous corticoids by volunteers (Fehm-Wolfsdorf & Nagel, 1996; Fehm-Wolfsdorf et al., 1989) or on studies investigating patients with an endogenous lack of corticoids (Henkin & Bartter, 1966). However, there are several confounds in the aforementioned patient studies such as nasal swelling as criticized by McClintock (2002). Furthermore, Pause et al. (1996) found that in healthy women increased salivary cortisol levels are associated with improved odor detection ability. Thus, it is uncertain, if the hypothesis holds for stress-induced, transitory increases in cortisol as they occur in the daily life of healthy individuals.

Odors are processed differently in the human brain depending on their valence (Croy et al., 2013). From an ecological point of view, unpleasant olfactory stimuli can serve as threat signals (Stevenson, 2010). During stress, the brain's response patterns shift from slow, thoughtful regulation by the prefrontal cortex to reflexive and emotional responses mainly orchestrated by the amygdala (Arnsten, 2009). The amygdala also plays an important role in olfaction (Buchanan et al., 2003; Winston et al., 2005), in particular in the processing of unpleasant olfactory stimuli, which are emotionally salient (Vermetten et al., 2007). It has been reported that the amygdala can be influenced by the interaction of endogenous cortisol and emotional arousal-induced noradrenergic activation (van Stegeren et al., 2007). Cortisol increases after a laboratory stressor has been linked to enhanced vigilance for threat cues in a shoot/don't shoot video simulation (Akinola & Mendes, 2012). Thus, the observed effect in our experiments might not generalize to pleasant odors as they are differently processed in the human brain compared to threat-signaling unpleasant odors.

Methodological considerations regarding the two experiments

Different stress protocols were used in the two experiments to probe the generalizability of the effect. While in both experiments distinct physiological stress responses were elicited, the elicited stress responses differed in magnitude.

The stress response to the social-evaluative stressor in Experiment 1 was mild: the stress protocol successfully affected heart rate and salivary cortisol but did not affect mood. With regard to cortisol the diurnal decrease in salivary cortisol was attenuated in the stress group compared to the control group. Mild stress preserved olfactory sensitivity in the stress group, while olfactory sensitivity in the control group decreased over the three threshold measurements.

The SECPT protocol, that combines social-evaluative threat with a physically demanding task, has been optimized to reliably induce strong HPA axis responses (Schwabe et al., 2008). Subjective and physiological data for Experiment 2 confirmed that the SECPT successfully elicited stress. Stress lowered olfactory detection thresholds: participants in the stress group could detect 2-mercaptoethanol at a lower concentration than participants in the control group 20 min after the end of the stress protocol.

The two experiments did not only differ in the applied stress protocols, but also partly in inclusion and exclusion criteria, screening for olfactory function, assessment of personality factors and subjective stress response, as well as in time schedule for the collection of saliva samples. Inclusion criteria were stricter in Experiment 2 compared to Experiment 1 taking into account the effect of hormonal contraceptive intake on cortisol reactivity (Kirschbaum et al., 1999). Additionally, a standardized test for olfactory function was used in Experiment 2 (Sniffin' Sticks), as well as a shorter questionnaire to assess anxiety to not prolong the experiment. Furthermore, a more specific method to assess the subjective stress response was used (Schwabe et al., 2008). Due to the use of an established stress protocol in Experiment 2 (Schwabe et al., 2008), a different time schedule was adopted for the collection of saliva samples. Respiratory rate and nasal congestion were monitored in Experiment 2 in contrast to Experiment 1 to better characterize the test groups and the response to the stressor.

Limitations

Activation of the trigeminal system can be excluded as a potential confound in the presented experiments: trigeminal

activation by 2-mercaptoethanol occurs at concentrations above 0.24 ppm, if dilution olfactometry is used as a vapor delivery method (Kleinbeck et al., 2012).

While repeated exposure to an odorant can lead to peripheral adaptation (Dalton, 2000), repeated exposure to the same laboratory stressor can lead to habituation of physiological stress response systems (Schommer et al., 2003). It can be assumed that both physiological processes occurred to a greater degree in Experiment 1 compared to Experiment 2, as the protocol of Experiment 1 relied more heavily on repeated testing. Thus, the generally adaptive processes habituation and adaptation might have influenced the results of the two experiments to a different degree.

While large epidemiological and experimental studies report sex differences in olfactory function and stress responses (Doty & Cameron, 2009; Kirschbaum et al., 1999), no evidence for sex differences was found in the presented experiments. Sample characteristics at study onset (Table 1, olfactory screening) and methodological factors (e.g. small sample size) are possible explanations. Still, given sex differences in stress responses and olfactory detection thresholds in the literature, future studies should explore whether sex modulates the effect of stress on olfactory detection thresholds.

Conclusions

Despite differences in stress protocols and magnitude of elicited stress responses, the two presented experiments produced consistent results: both experiments suggest that stress can affect the olfactory detection threshold for a malodor. The results are not only consistent with a sensory hypervigilance account of stress, but they also expand upon this account by demonstrating the impact of stress on the olfactory system.

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Declaration of interest

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References

Adolph D, Pause BM. (2012). Different time course of emotion regulation towards odors and pictures: are odors more potent than pictures? Biol Psychol 91:65–73.

Akinola M, Mendes WB. (2012). Stress-induced cortisol facilitates threat-related decision making among police officers. Behav Neurosci 126:167–74.

Alobid I, Benitez P, Cardelus S, de Borja Callejas F, Lehrer-Coriat E, Pujols L, Picado C, Mullol J. (2014). Oral plus nasal corticosteroids improve smell, nasal congestion, and inflammation in sino-nasal polyposis. Laryngoscope 124:50–6.

Arnsten AF. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci 10:410–22.

Borkenau P, Ostendorf F. (2008). NEO-FFI: NEO-Fünf-Faktoren-Inventar nach Costa und McCrae, Manual [NEO-FFI: NEO big five questionnaire according to Costa and McCrae, manual]. 2nd ed. Göttingen: Hogrefe.

Buchanan TW, Tranel D, Adolphs R. (2003). A specific role for the human amygdala in olfactory memory. Learn Mem 10:319–25.

Crettaz B, Marziniak M, Willeke P, Young P, Hellhammer D, Stumpf A, Burgmer M. (2013). Stress-induced allodynia-evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. PLoS One 8:e69460.

Croy I, Maboshe W, Hummel T. (2013). Habituation effects of pleasant and unpleasant odors. Int J Psychophysiol 88:104–8.

Dalton P. (2000). Psychophysical and behavioral characteristics of olfactory adaptation. Chem Senses 25:487–92.

Dölz W, Eitner A, Caldwell JD, Jirikowski GF. (2013). Expression of corticosteroid binding globulin in the rat olfactory system. Acta Histochem 115:376–81.

Doty RL, Cameron EL. (2009). Sex differences and reproductive hormone influences on human odor perception. Physiol Behav 97: 213–28.

Escanilla O, Alperin S, Youssef M, Ennis M, Linster C. (2012). Noradrenergic but not cholinergic modulation of olfactory bulb during processing of near threshold concentration stimuli. Behav Neurosci 126:720–8.

Fehm-Wolfsdorf G, Nagel D. (1996). Differential effects of glucocorticoids on human auditory perception. Biol Psychol 42:117–30.

Fehm-Wolfsdorf G, Scheible E, Zenz H, Born J, Fehm HL. (1989). Taste thresholds in man are differentially influenced by hydrocortisone and dexamethasone. Psychoneuroendocrinology 14:433–40.

Fehm-Wolfsdorf G, Soherr U, Arndt R, Kern W, Fehm HL, Nagel D. (1993). Auditory reflex thresholds elevated by stress-induced cortisol secretion. Psychoneuroendocrinology 18:579–89.

Firestein S, Menini A. (1999). The smell of adrenaline. Nat Neurosci 2: 106–8.

Geva N, Pruessner J, Defrin R. (2014). Acute psychosocial stress reduces pain modulation capabilities in healthy men. Pain 155:2418–25.

Goldberger AL, Amaral LA, Glass L, Hausdorff JM, Ivanov PC, Mark RG, Mietus JE, et al. (2000). PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals. Circulation 101:E215–20.

Henkin RI, Bartter FC. (1966). Studies on olfactory thresholds in normal man and in patients with adrenal cortical insufficiency: the role of adrenal cortical steroids and of serum sodium concentration. J Clin Invest 45:1631–9.

Hummel T, Kobal G, Gudziol H, Mackay-Sim A. (2007). Normative data for the ''Sniffin' Sticks'' including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3000 subjects. Eur Arch Otorhinolaryngol 264:237–43.

Hummel T, Livermore A. (2002). Intranasal chemosensory function of the trigeminal nerve and aspects of its relation to olfaction. Int Arch Occup Environ Health 75:305–13.

Ileri-Gurel E, Pehlivanoglu B, Dogan M. (2013). Effect of acute stress on taste perception: in relation with baseline anxiety level and body weight. Chem Senses 38:27–34.

Kawai F. (1999). Simulation analysis of effects of adrenaline on spike generation in olfactory receptor cells. Chem Senses 24:701–4.

Kawai F, Kurahashi T, Kaneko A. (1999). Adrenaline enhances odorant contrast by modulating signal encoding in olfactory receptor cells. Nat Neurosci 2:133–8.

Kirschbaum C, Hellhammer DH. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 19:313–33.

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom Med 61:154–62.

Kleinbeck S, Riether N, Schäper M, van Thriel C. (2012). Risikoeinschätzung von stinkenden Arbeitsstoffen: Subjektive und objektive Indikatoren [Risk assessment for malodorous chemicals in

the working environment: Subjective and objective indicators]. Arbeitsmedizin Sozialmedizin Umweltmedizin 47:168.

Kleinbeck S, Schäper M, Juran SA, Kiesswetter E, Blaszkewicz M, Golka K, Zimmermann A, et al. (2011). Odor thresholds and breathing changes of human volunteers as consequences of sulphur dioxide exposure considering individual factors. Saf Health Work 2: 355–64.

Krusemark EA, Novak LR, Gitelman DR, Li W. (2013). When the sense of smell meets emotion: anxiety-state-dependent olfactory processing and neural circuitry adaptation. J Neurosci 33:15324–32.

McClintock MK. (2002). Pheromones, odors, and vasanas: the neuroendocrinology of social chemosignals in humans and animals. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, editors. Hormones, brain and behavior. San Diego, CA: Elsevier Science. p. 797–870.

Meulders A, Fannes S, van Diest I, de Peuter S, Vansteenwegen D, van den Bergh O. (2010). Resistance to extinction in an odor–20% CO2 inhalation paradigm: further evidence for a symptom learning account of multiple chemical sensitivity. J Psychosom Res 68:47–56.

Mogg K, Mathews A, Bird C, Macgregor-Morris R. (1990). Effects of stress and anxiety on the processing of threat stimuli. J Pers Soc Psychol 59:1230–7.

Pause BM, Sojka B, Krauel K, Fehm-Wolfsdorf G, Ferstl R. (1996). Olfactory information processing during the course of the menstrual cycle. Biol Psychol 44:31–54.

Robinson AM, Kern RC, Foster JD, Fong KJ, Pitovski DZ. (1998). Expression of glucocorticoid receptor mRNA and protein in the olfactory mucosa: physiologic and pathophysiologic implications. Laryngoscope 108:1238–42.

Schommer NC, Hellhammer DH, Kirschbaum C. (2003). Dissociation between reactivity of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. Psychosom Med 65:450–60.

Schwabe L, Haddad L, Schachinger H. (2008). HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology 33: 890–5.

Shackman AJ, Maxwell JS, McMenamin BW, Greischar LL, Davidson RJ. (2011). Stress potentiates early and attenuates late stages of visual processing. J Neurosci 31:1156–61.

Skoluda N, Strahler J, Schlotz W, Niederberger L, Marques S, Fischer S, Thoma MV, et al. (2015). Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. Psychoneuroendocrinology 51:227–36.

Smeets MA, Bulsing PJ, van Rooden S, Steinmann R, de Ru JA, Ogink NW, van Thriel C, Dalton PH. (2007). Odor and irritation thresholds for ammonia: a comparison between static and dynamic olfactometry. Chem Senses 32:11–20.

Spielberger CD. (1983). Manual for the state-trait anxiety inventory. Palo Alto, CA: Consulting Psychologists Press.

Stevenson RJ. (2010). An initial evaluation of the functions of human olfaction. Chem Senses 35:3–20.

van Marle HJ, Hermans EJ, Qin S, Fernandez G. (2009). From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. Biol Psychiatry 66:649–55.

van Stegeren AH, Wolf OT, Everaerd W, Scheltens P, Barkhof F, Rombouts SA. (2007). Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. Neurobiol Learn Mem 87:57–66.

Vermetten E, Schmahl C, Southwick SM, Bremner JD. (2007). Positron tomographic emission study of olfactory induced emotional recall in veterans with and without combat-related posttraumatic stress disorder. Psychopharmacol Bull 40:8–30.

von Dawans B, Kirschbaum C, Heinrichs M. (2011). The Trier Social Stress Test for Groups (TSST-G): a new research tool for controlled simultaneous social stress exposure in a group format. Psychoneuroendocrinology 36:514–22.

Watson D, Clark LA, Carey G. (1988). Positive and negative affectivity and their relation to anxiety and depressive disorders. J Abnorm Psychol 97:346–53.

Westermann J, Demir A, Herbst V. (2004). Determination of cortisol in saliva and serum by a luminescence-enhanced enzyme immunoassay. Clin Lab 50:11–24.

Wiemers US, Sauvage MM, Wolf OT. (2014). Odors as effective retrieval cues for stressful episodes. Neurobiol Learn Mem 112:230–6.

Winston JS, Gottfried JA, Kilner JM, Dolan RJ. (2005). Integrated neural representations of odor intensity and affective valence in human amygdala. J Neurosci 25:8903–7.