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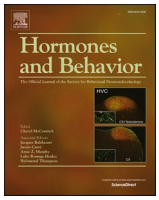

Increased sensitivity to unpleasant odor following acute psychological stress

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ABSTRACT

Previous studies have reported increased sensitivity to malodor after acute stress in humans. However, it is unclear whether stress-related "hypersensitivity" to odors depends on odor pleasantness. Forty participants (mean age 19.13 ± 1.14 years, 21 men and 19 women) completed a stress (Trier Social Stress Test, TSST) and a control session in randomized order. Detection threshold to three odors varying in pleasantness (pleasant: β-Citronellol; neutral: 2-Heptanol; unpleasant: 4-Methylpentanoic acid), odor discrimination, odor identification, sensitivity to trigeminal odor, and suprathreshold odor perception were assessed after participants' completion of the stress or the control tasks. Salivary cortisol, subjective stress, and heart rate were assessed throughout the experiment. After TSST, participants showed an increased sensitivity for the unpleasant odor. Moreover, there were correlations between stress-related salivary cortisol and the increased sensitivity for the unpleasant odor ($r = 0.32$, $p = 0.05$) and the neutral odor ($r = 0.34$, $p < 0.05$). Besides, salivary cortisol response was correlated to the increased odor discrimination performance (Δ stress - control) ($r = 0.34$, $p < 0.05$). The post-TSST perceived stress was correlated with decreased odor identification and decreased sensitivity to the unpleasant odor. After stress, participants rated lower pleasantness for β-Citronellol than the control condition. Overall, these results suggest the impact of acute psychological stress on odor sensitivity depends on the odor valence, and that the stress-related cortisol responses may play an important role in this effect.

1. Introduction

Olfaction serves as a warning system to detect potential harmful or threatening substance in our food, environment, and social interactions (Stevenson, 2010). Acute stress is defined as a bodily response to physical or emotional threats, which results in temporal changes of certain physiological functions, such as cognitive functions, and changes in the endocrine system, and the peripheral and central nervous system (O'Connor et al., 2021).

The human olfactory cortex is extensively connected to the limbic system, which is largely affected by stress exposure (Berretz et al., 2021). Odor perceptions can be affected by stress and stress-related emotional states (Bombail, 2019). A few previous studies investigated the impact of acute stress on human odor perception (Hoenen et al., 2017; Pacharra et al., 2016). Pacharra et al. (2016) found the detection threshold for an unpleasant odor is reduced following acute stress. Hoenen et al. (2017) showed that the subjective anger level after acute stress is associated with decreased odor identification performances. In contrast, a more recent study reported no significant impact of acute psychological stress exposure on odor sensitivity (Cortese et al., 2022). Although it may be argued from existing literature that acute stress could enhance odor detection, whether stress affects the sensitivity to different odors in different ways is unknown. Odor pleasantness is the primary axis of olfactory perception. The perception of odor pleasantness is innate and odor pleasantness is, to some degree, shared across cultures (Arshamian et al., 2022; Yeshurun and Sobel, 2010). The pleasantness and unpleasantness of odor stimuli are processed differently in the olfactory cortex, both concerning early detection and later cognitive processing (Kato et al., 2022). Moreover, previous research investigating the effect of acute stress on olfaction did not cover certain aspects of odor perception. For example, some core executive functions (e.g. working memory and cognitive flexibility), which are affected by acute stress (Shields et al., 2016), are important for discriminating odors (Hedner et al., 2010).

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Stress-induced physiological or emotional responses have effects on the olfactory system. Activation of the hypothalamic-pituitary-adrenal (HPA) axis following acute stress leads to the secretion of cortisol. The effect of cortisol on olfaction has been shown in animals, with increased reactivity to complex odors after glucocorticoid treatment (Meunier et al., 2020). In humans, early research reported a correlation between cortisol level and superior olfactory sensitivity (Pause et al., 1996), and better recognition of infants' odors (Fleming et al., 1997). Besides, acute stress-related cortisol is associated with elevated odor recognition ability and higher perceived odor intensity (Hoenen et al., 2017). Cortisol receptors are expressed in multiple sites of the olfactory system, including the mucosa, the olfactory bulb (Dolz et al., 2013; Meunier et al., 2020), and multiple brain regions such as the amygdala, orbitofrontal cortex, and hippocampus (Morimoto et al., 1996; Robinson et al., 1998). In addition, previous research found that the induction of a negative emotional state reduces olfactory sensitivity (Pollatos et al., 2007), and decreased odor pleasantness (Krusemark et al., 2013).

The current study was aimed to examine the effect of acute psychological stress and stress-related cortisol responses on olfactory perception. Specifically, in a within-subject design, detection threshold to three odors with varied pleasantness (β-Citronellol, 2-Heptanol, 4-Methylpentanoic acid), odor discrimination and identification, trigeminal odor sensitivity, and suprathreshold odor perceptions were assessed after a psychological stress or a control treatment. We hypothesized that acute stress would increase olfactory sensitivity for the unpleasant odor, but exert little influence on the sensitivity for pleasant or neutral odors. In addition, we hypothesized the levels of stress-related cortisol response correlate to the alteration in olfactory sensitivity. Additionally, women have better olfactory perception than do men (Doty and Cameron, 2009). Men have higher stress-induced salivary cortisol levels than women (Kudielka and Kirschbaum, 2005) and cortisol elevations are differently associated with stress-related brain responses depending on sex (Henze et al., 2021). Therefore, the current study also aimed to explore potential gender differences regarding the impact of acute stress on odor perception.

2. Materials and methods

2.1. Participants

Participants were recruited from the Southwest University students. According to a previous study with similar within-subject design, we set a target sample size of 40 participants, which could reach a comparable effect size (Al'Absi et al., 2012). Given that the menstrual cycle had an influence on cortisol reactivity after acute stress (Kirschbaum et al., 1993), women participants were included only when the prior three menstrual periods had been constant and all tests were conducted during the luteal phase (from day 20 of the menstrual cycle to the start of the next cycle). In addition, in order to ensure the effectiveness of stress manipulation (reduce the impact of job interview experience), we mainly recruited freshman and sophomore students. Participants were excluded if they met any of the following: 1) self-reported acute or chronic upper airway diseases or smell dysfunction, 2) cardiovascular disease, diabetes, depression, or high trait anxiety level, 3) currently taking psychoactive medications, 4) regular smokers or drinkers. The severity of depression and trait anxiety symptoms was assessed by means of the Beck Depression Inventory (BDI) (Beck and Steer, 1996), and the State and Trait Anxiety Inventory (STAI) (Spielberger, 1970), respectively. We excluded participants with indicative severe depression (BDI $>$ 13 points) or trait anxiety (STAI $>$ 56 points) to avoid extreme acute stress reactions. All participants reached at least 4 points using the five items "Sniffin' Sticks" identification test kit (Mueller and Renner, 2006), indicating the absence of general anosmia. Twenty-one men (mean age $=$ 19.33 $\pm$ 1.11 years; BMI $=$ 20.49 $\pm$ 2.27 kg/m^2) and nineteen women (mean age $=$ 18.89 $\pm$ 1.15 years; BMI $=$ 20.14 $\pm$ 2.30 kg/m^2) participants took part into the study. Men and women participants did not differ in terms of chronic stress, trait anxiety or depression (Supplementary Results Table S5). The study was approved by the Ethics Committee, Faculty of Psychology, Southwest University (Approval No. H21008) and in compliance with the Declaration of Helsinki. All participants signed the consent form prior to the experiment and received financial compensation at the end of the study.

2.2. Experimental procedure

The study adopted a within-subject design in which participants served as their own controls. Each participant attended a stress and a control experimental session at the same time on two days (e.g., a participant's stress condition and control condition both started at 13: 30) separated by a one-week interval. The order of the two conditions was counterbalanced between participants. The experiment was conducted between 13:30 and 18:00 to control for diurnal cortisol variations.

Participants refrained from taking any hormone medications, consuming any alcohol or coffee 24 h prior to the study, or staying up late. On the day of testing, participants were instructed to avoid exercising strenuously, eating, chewing gums, or drinking (except water) 2 h prior to the study. The experimenter obtained verbal confirmation that participants followed all testing day requirements. Upon arrival, participants received the 5-item "Sniffin' Sticks" odor identification test to ensure their normal smell functions. Then participants were equipped with a heart-rate monitor and rested quietly for 15 min prior to the stress or control session. During this time, participants rated their hunger, satiety, and desire to eat on 100-mm visual analogue scales (VAS) from 0 (none) to 100 (most imaginable). Participants also reported their subjective stress and painful feelings (100-mm VAS), their valence and arousal (9-point Likert-type scales), and their state anxiety levels (6-item State-Trait Anxiety Inventory [STAI-state]) (Marteau and Bekker, 1992). Hereafter, they underwent the stress or the control session. After a 5-min rest, participants were indicated to perform the olfactory tests, with the order of 1) suprathreshold odor perceptions including pleasantness, intensity, familiarity, and pungency 2) odor threshold test, 3) odor identification test, 4) odor discrimination test, 5) nasal trigeminal sensitivity test. At the end of the whole experiment, participants were told the general research purpose, and the experimenters then answered all queries from participants. The whole experimental procedure lasted about 1.5 h. The experiment flow and tests are shown in Fig. 1.

2.3. Acute stress manipulation

The Trier Social Stress Test (Kirschbaum et al., 1993) is a stress paradigm that reliably activates emotional, endocrine, and cardiovascular responses. The task includes four 5-min stressful periods: (1) introduction to two "experts" and receiving instructions for the task, (2) preparation for a 5 min speech on "applying for an ideal job", (3) delivering the speech, and (4) performing a challenging serial subtraction task. The details of the TSST procedure can be found in the Supplementary Methods. Participants were asked to attend a control session. During the time, they performed no subsequent test to maintain a state of calm as a control.

2.4. Physiological stress assessment

Heart rate was monitored with the Biopac MPI 150 system. Specifically, participants' cardiovascular activity was recorded using an electrocardiogram (ECG) amplifier module and three disposable electrodes positioned on the chest, left armpit, and abdomen. Heart rate was reported in beats per minute (bpm). The task of examining heart rate did not commence until a clear and accurate ECG recording was obtained. Participants' successive heart rates were extracted and analyzed using the AcqKnowledge software package (Biopic Systems, Goleta, CA). Heart rate data were collected at 6 time points (T1: 0 min; between T1 and T2: +15 min; T2: +20 min; T3: +35 min; T4: +60 min; T5: +80 min)

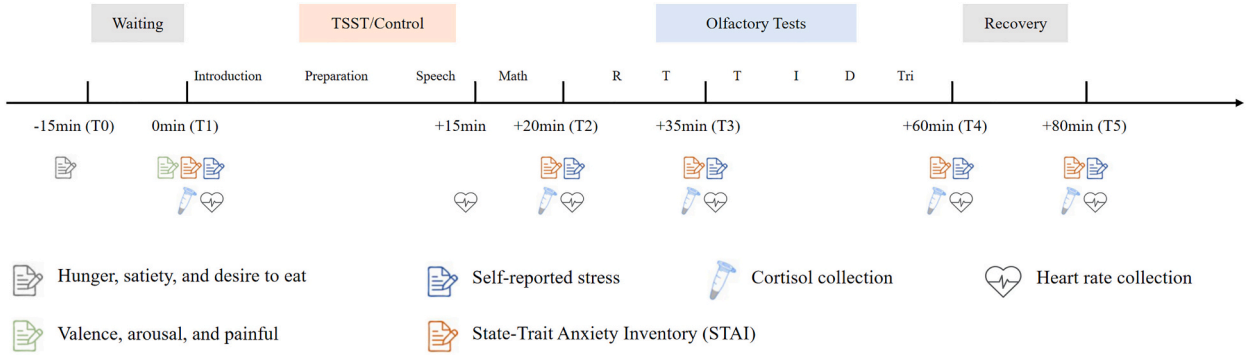

Fig. 1. Schematic description of the laboratory sessions. R, Subjective rating; T, Threshold; I, Identification; D, Discrimination; Tri, Trigeminal sensitivity; TSST, Trier Social Stress Test.

(Fig. 1). We took 2 min of heart rate averages at each of the 6 time points for analysis.

Saliva samples were collected using Salivettes® (SARSTEDT, Germany). Participants were asked to put a swab into their mouth, keeping it on the tongue for 2 min, and then spit it back into the collector. During the process, participants were instructed to refrain from touching the swab with hands to avoid contamination. Five saliva samples (T1: 0 min; T2: +20 min; T3: +35 min; T4: +60 min; T5: +80 min) were collected from each participant at each session (Fig. 1). All the samples were frozen at −20 °C until assayed (interval between assessment and measurement: about 6 months). Cortisol levels were analyzed using an enzyme-linked immunosorbent assay (IBL-Hamburg, Germany) following the manufacturer's instructions. The sensitivity of the assay was 0.005 μg/dL and the inter- and intra-assay variations for the cortisol assays were 13.2 % and 4.3 %, respectively.

2.5. Subjective stress

Participants indicated their subjective stressful level during the experiment via 100-mm VAS. The state anxiety portion of the 6-item State-Trait Anxiety Inventory (STAI) (Marteau and Bekker, 1992) was used to assess self-reported situational anxiety levels which describes how an individual may feel at the present moment. Participants rated how they currently feel on a scale from 1 (not at all) to 4 (very much so) and higher scores indicate greater levels of state anxiety. For stress-induced changes of subjective stress and state anxiety, we calculated the area under the curve with respect to increase (AUC_i, reflected the changes over time) (Luettgau et al., 2018). Participants reported momentary stressful feelings and state anxiety levels at five time points (T1: 0 min; T2: +20 min; T3: +35 min; T4: +60 min; T5: +80 min) (Fig. 1) that correspond to the saliva collection.

2.6. Other psychometric measures

Trait anxiety was measured using the Spielberger State-Trait Anxiety Inventory (STAI-trait) (Spielberger, 1970). It contains 20 statements describing how an individual may feel generally. Participants rated these statements on a scale from 1 (not at all) to 4 (very much so), with higher scores indicating greater levels of trait anxiety. Depression symptoms were assessed using the Beck Depression Inventory (BDI) (Beck and Steer, 1996). Each of the 13 forced-choice questions has a set of at least four possible answer choices, with increasing severity of depressive symptoms from 0 to 3. Levels of perceived life psychological stress were measured using the 10-item Perceived Stress Scale (PSS-10) (Cohen et al., 1983). The PSS-10 assesses how unpredictable, uncontrollable, and overloaded respondents view their lives, and directly inquire about levels of experienced stress in the past month with answer choices ranging from 0 (never) to 4 (very often). Scores range from 0 to 40 and higher scores indicated greater perceived life stress.

2.7. Olfaction measures

2.7.1. Odor selection

Three odorants varied in pleasantness were selected for the current study: β-Citronellol (CAS 7540-51-4, Macklin, China, "lemongrass, rose"), 2-Heptanol (CAS 543-49-7, Bide Pharm, China, "earthy, oily"), and 4-Methylpentanoic acid (CAS 646-07-1, Energy Chemical, China, "sweaty socks"). Pilot study results showed that the three odors differ in terms of perceived pleasantness, but not familiarity. All three odors are rated as low-to-medium in terms of familiarity (Supplementary Methods and Table S1).

2.7.2. Odor sensitivity

The odor threshold test followed a single staircase, three alternative forced-choice procedure. Sixteen concentrations for each odorant, each in 30 mL, were prepared into 100 mL brown glass vials using the 1.5 or 2-fold serial dilutions method. The odorants were first dissolved in 3 mL Propylene glycol (CAS 57-55-6, Sigma Aldrich, Germany), then diluted in distilled water (Pino and Mesa, 2006). In the experiment, 0.0003125–5.76 ppm for β-Citronellol, 0.000625–20.48 ppm for 2-Heptanol, and 0.003125–43.2 ppm for 4-Methylpentanoic acid were finally used. The specific concentration gradient was provided in the Supplementary Methods Table S2. Testing orders of the three odors were randomized across participants using a Latin Square design. The procedure of odor sensitivity test was detailed in the Supplementary Methods.

2.7.3. Suprathreshold odor perception

Participants rated the pleasantness (1 = very unpleasant, 9 = very pleasant), intensity (1 = not perceivable, 9 = extremely strong), familiarity (1 = very unfamiliar, 9 = very familiar), and pungency (1 = not pungency, 9 = very pungency) of each odor using 9-point Likert-type scales. The odor concentrations were 0.0001 v/v for β-Citronellol, 0.0001 v/v for 2-Heptanol, and 0.0002 v/v for 4-Methylpentanoic acid. These concentrations were twice as much as were used in the pilot test, so that all participants could rate based on their clear perception. The three odors were presented at an interval of approximately 60 s. The order of presentation of the three odors (six different sequences in total) was largely balanced among subjects, while the order of odor presentation remained consistent between subjects in the stress and control conditions.

2.7.4. Odor discrimination

Odor discrimination was assessed using the standard "Sniffin' Sticks" test battery (Hummel et al., 1997). In total 16 triplets were tested and the correct answers were summed and expressed as the discrimination score. The procedure of odor discrimination test was detailed in the Supplementary Methods.

2.7.5. Odor identification

In light of a previous study (Feng et al., 2019; Freiherr et al., 2012; Haehner et al., 2009; Sorokowska et al., 2015), we developed a 32-item odor identification test based on the "Sniffin' Sticks" odor identification test for the use of the current study. The familiarity of certain odors and the test difficulty (too low for potential ceiling effect), as well as the number of the tests were considered and optimized in our test (see Supplementary Methods for details, Table S3). The test was split into two subsets with each contains 16 odors. The number of times the two odor groups were used in the stress condition or control condition was counterbalanced among participants. For each odor stimuli, participants were asked to select one of four possible descriptors. The summed number of correctly identified odors was taken as the identification score.

2.7.6. Nasal trigeminal sensitivity

The intranasal trigeminal sensitivity for L-menthol (CAS 2216-51-5, Sigma Aldrich, Germany) was assessed using the odor lateralization test (Frasnelli et al., 2011b). The test device consists of two identical and squeezable bottles (250 mL in total volume). Each bottle has a spout that was placed into the participants' each nostril. One bottle was filled with 30 mL of the test odors: 0.18 g/mL L-menthol solution dissolved in propylene glycol (Frasnelli et al., 2011a), or 50 % v/v rose-like odor 2-Phenylethanol (PEA; CAS 60-12-8, Shanghai Yuan-Ye, China), and the other bottle was filled with odorless 30 mL propylene glycol. During the test, participants were blindfolded and were instructed to briefly hold their breath when the experimenter squeezed two bottles and 15 mL of air was presented simultaneously to each nostril. Each odor was tested 20 times with a 30 s interval between two adjacent tests. Stimuli were presented to the left and right nostrils in a pseudo-random fashion. After presenting each stimulation, participants had to determine which side of the nostril received odor stimulation. The sum of correct identifications was used for further statistical analyses. The sequence of the tests for L-menthol or PEA was balanced between participants.

2.8. Statistical analyses

Two participants provided an insufficient amount of saliva for most time points, and one participant presented a very high initial cortisol concentration (>20 nmol/L at T1 in the stress condition), thus were excluded from cortisol-related analyses. Other cortisol missing data (5.7 %) were replaced by using multiple imputations (Sterne et al., 2009) (see Supplementary Results Tables S6 and S7). Cortisol reactivity was operationalized using areas under the curve with respect to ground (AUC_g, reflected the total amount of hormonal output) from stressor onset (0 min) to the end of recovery (80 min) (Pruessner et al., 2003). For the stress manipulation check, the three-way mixed ANOVAs with stress (stress, control) and time points (5 time points for subjective stress and salivary cortisol; 6 time points for heart rates) as within-subject factors, and sex as a between-subject factor were used. *P*-values of the follow-up simple effects tests at each time point were Bonferroni corrected for the number of comparisons (i.e., $\alpha = 0.05/5$ for subjective stress and salivary cortisol; $\alpha = 0.05/6$ for heart rates).

To test our hypothesis regarding the effect of acute stress on odor sensitivity, the within-subject repeated-measure ANOVAs were applied for each odor separately, with sex as a between-subject factor. Participants' baseline hunger level and self-reported stress (Δ stress - control) were included as covariables of no interest. Similar analyses were performed to explore the effect of stress on odor discrimination, identification, lateralization, and subjective ratings (pleasantness, familiarity, intensity, and pungency) for each odorant, taking sex as a between-subject factor. In addition, the Pearson's correlation (two-tailed) was used to investigate associations between cortisol responses ($AUC_{g\text{-}stress}$) or subjective stress and olfactory measures. The difference between correlation coefficients in male and female participants were assessed by Fisher's r to z transformation using the formula $z = \frac{Z_{r(men)} - Z_{r(women)}}{\sqrt{\frac{1}{n_{(men)}-3} + \frac{1}{n_{(women)}-3}}}$.

Data for both olfactory tests are presented as means with 95 % confidence intervals (CI 95 %). All post-hoc comparisons were Bonferroni corrected and Greenhouse-Geisser values were reported when the sphericity assumption was violated (Mauchly's Test of Sphericity, $p > 0.05$). The statistical analysis was performed using IBM SPSS Statistics 24 and GraphPad Prims 8.3.0.

3. Results

3.1. Stress manipulation check

There were significant main effects of stress [$F_{(1,35)} = 16.85$, $p < 0.001$, $\eta^2 = 0.33$], time points [$F_{(4,140)} = 23.38$, $p < 0.001$, $\eta^2 = 0.40$], and a stress × time points interaction [$F_{(4,140)} = 14.41$, $p < 0.001$, $\eta^2 = 0.29$] on salivary cortisol levels. There was no stress × sex [$F_{(1,35)} = 2.24$, $p = 0.14$] or sex × time points interaction [$F_{(4,140)} = 1.46$, $p = 0.22$] on salivary cortisol levels. Cortisol levels did not differ between the stress and control conditions at baseline ($t = -0.61$, $p > 0.01$) and T2 ($t = 2.49$, $p > 0.01$), but was significantly higher in the stress compared to the control condition at T3 ($t = 5.74$, $p < 0.0002$), T4 ($t = 4.57$, $p < 0.0002$), and T5 ($t = 3.40$, $p < 0.002$) (Fig. 2a).

For heart rates, there were significant main effects of stress [$F_{(1,38)} = 5.96$, $p < 0.05$, $\eta^2 = 0.14$], time points [$F_{(5,190)} = 93.61$, $p < 0.001$, $\eta^2 = 0.71$], and a stress × time points interaction [$F_{(5,190)} = 45.35$, $p < 0.001$, $\eta^2 = 0.54$]. There was no stress × sex [$F_{(1,38)} = 0.44$, $p = 0.51$] or sex × time points interaction [$F_{(5,190)} = 1.10$, $p = 0.36$] on heart rates. Heart rate was significantly higher during the stress than the control condition ($t = 7.74$, $p < 0.0002$) (Fig. 2b).

For subjective stress, there were significant main effects of stress [$F_{(1,38)} = 31.29$, $p < 0.001$, $\eta^2 = 0.45$], time points [$F_{(4,152)} = 25.45$, $p < 0.001$, $\eta^2 = 0.40$], and a stress × time points interaction [$F_{(4,152)} = 43.52$, $p < 0.001$, $\eta^2 = 0.53$]. There was no stress × sex [$F_{(1,38)} = 2.23$, $p = 0.14$] or sex × time points interaction [$F_{(4,152)} = 0.42$, $p = 0.79$] on subjective stress levels. Subjective stress was significantly higher in the stress than control condition at T2 ($t = 10.57$, $p < 0.0002$), and T3 ($t = 3.14$, $p < 0.01$) (Fig. 2c). Regarding state anxiety, it revealed significant main effects of stress [$F_{(1,38)} = 22.20$, $p < 0.001$, $\eta^2 = 0.37$], time points [$F_{(4,152)} = 17.74$, $p < 0.001$, $\eta^2 = 0.32$], and a stress × time points interaction [$F_{(4,152)} = 26.18$, $p < 0.001$, $\eta^2 = 0.41$]. There was no stress × sex [$F_{(1,38)} = 0.24$, $p = 0.63$] or sex × time points interaction [$F_{(4,152)} = 0.14$, $p = 0.97$] on state anxiety. State anxiety was significantly higher in the stress than control condition at T2 ($t = 9.11$, $p < 0.0002$). The results of self-reported perceived stress and state anxiety from T1 to T5 are shown in the Supplementary Results (Table S8).

3.2. Odor sensitivity

There was a significant effect of stress on odor sensitivity to the unpleasant odor [$F_{(1,36)} = 5.94$, $p = 0.02$, $\eta^2 = 0.14$], with an increase of sensitivity to 4-Methylpentanoic acid after stress compared to control condition was observed [stress $M = 6.54$, SD = 2.85; control $M = 5.49$, SD = 2.40] (Fig. 3). However, there was no significant effect of stress on sensitivity to the pleasant [$F_{(1,36)} = 0.07$, $p = 0.79$], or the neutral [$F_{(1,36)} = 0.02$, $p = 0.88$] odors (Fig. 3). Besides, there was no significant effect of stress × sex interaction on individuals' sensitivity to the unpleasant [$F_{(1,36)} = 0.89$, $p = 0.35$], the pleasant [$F_{(1,36)} = 0.57$, $p = 0.45$], or the neutral [$F_{(1,36)} = 2.87$, $p = 0.10$] odors. Odor sensitivity for three odors in male and female participants were summarized in the Supplementary Results (Table S4).

3.3. Odor discrimination, odor identification and trigeminal lateralization

There was no significant effect of stress [stress $M = 9.58 \pm 2.37$,

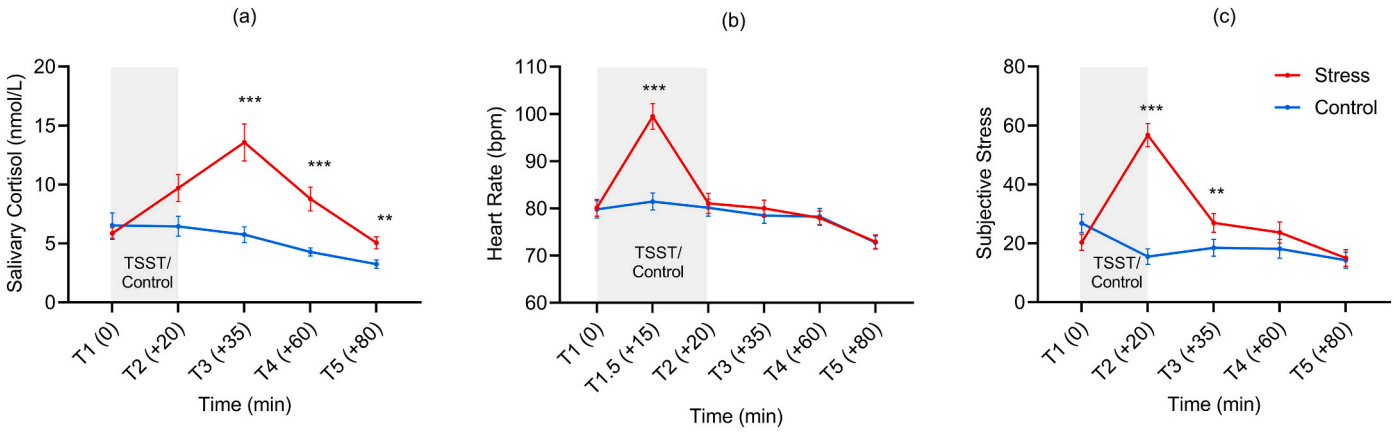

Fig. 2. Salivary cortisol, heart rate, and subjective stress response over the course of the experimental sessions. (a) Mean salivary cortisol (nmol/L ± SEM) in $N = 37$ participants, (b) mean heart rate responses (bpm ± SEM) in $N = 40$ participants, and (c) mean subjective stress ratings on visual analogue scales in $N = 40$ participants during the stress condition and control condition. Note the alpha level was adjusted with Bonferroni correction for multiple comparisons. TSST, Trier Social Stress Test. *significant at $p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Bonferroni-adjusted *post hoc* test.

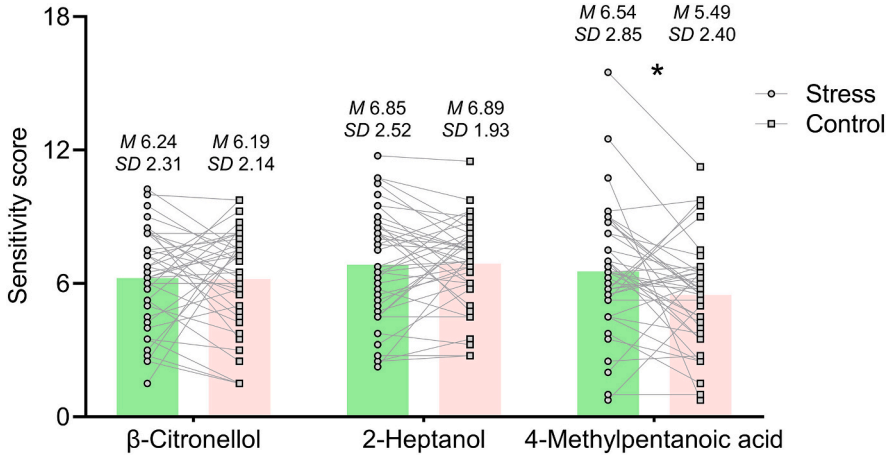

Fig. 3. Difference of sensitivity (detection threshold) for three odors after acute stress or control in all participants ($N = 40$). *significant at $p < 0.05$; *M*, Mean value; *SD*, Standard Deviation.

control $M = 9.88 \pm 2.49$; $F_{(1,38)} = 0.26$, $p = 0.62$] or stress × sex interaction [$F_{(1,38)} = 1.70$, $p = 0.20$] on odor identification, and no significant effect of stress [stress $M = 10.30 \pm 1.91$, control $M = 10.85 \pm 1.94$; $F_{(1,38)} = 2.07$, $p = 0.16$] or stress × sex interaction [$F_{(1,38)} = 0.21$, $p = 0.65$] on odor discrimination. Odor lateralization score for L-menthol was significantly higher than chance level in both the stress ($t = 5.85$, $p < 0.05$) and control conditions ($t = 7.71$, $p < 0.05$), while the lateralization scores for PEA were close to chance level in both stress and control condition (all *p*s > 0.10). However, there was no effect of stress [stress $M = 13.64 \pm 3.89$, control $M = 14.03 \pm 3.37$; $F_{(1,38)} = 0.23$, $p = 0.63$] or stress × sex interactions [$F_{(1,38)} = 0.12$, $p = 0.73$] on the lateralization score for L-menthol, and no effect of stress [stress $M = 9.38 \pm 2.59$, control $M = 8.92 \pm 2.78$; $F_{(1,38)} = 0.67$, $p = 0.42$] or stress × sex interactions [$F_{(1,38)} = 0.06$, $p = 0.80$] on the lateralization score for PEA.

3.4. Odor pleasantness, intensity, familiarity and pungency

There was a significant main effect of stress on pleasantness ratings for pleasant odor [$F_{(1,38)} = 10.18$, $p < 0.05$, $\eta^2 = 0.21$]. The pleasantness for the pleasant odor was significantly decreased in the stress compared to the control conditions, while there was no significant difference for the unpleasant or the neutral odors. Regarding odor intensity, familiarity, and pungency, there was no main effect of stress condition or interaction effect of stress by sex for each odor (all *p*s > 0.05). Among these, we found a trend of decreased pungency for the unpleasant odor after stress ($p = 0.06$). Table 1 summarizes the subjective ratings for all three odors in the stress and control conditions.

3.5. Correlations

3.5.1. Correlation between salivary cortisol reactivity and olfactory measures

For participants with valid cortisol measures ($N = 37$), the cortisol $AUC_{g\text{-}stress}$ was positively correlated with the increased sensitivity from control to stress conditions to the unpleasant ($r = 0.32$, $p = 0.05$; Fig. 4a) and neutral ($r = 0.34$, $p = 0.04$; Fig. 4b) odors. That is to say, a stronger cortisol reactivity was associated with higher sensitivity to 2-Heptanol and 4-Methylpentanoic acid. The correlations between the cortisol $AUC_{g\text{-}stress}$ and odor sensitivity did not differ between male and female participants to the unpleasant (men $r = 0.30$, $p = 0.21$; women $r = 0.33$, $p = 0.18$; $z = -0.09$, $p > 0.05$) or neutral odors (men $r = 0.29$, $p = 0.24$, women $r = 0.41$, $p = 0.09$; $z = -0.38$, $p > 0.05$).

Additionally, cortisol $AUC_{g\text{-}stress}$ was positively correlated to the increased odor discrimination score from control to stress conditions ($r = 0.34$, $p = 0.04$) (Fig. 4c). There was no significant gender difference in the association of changed odor discrimination score from control to stress conditions with cortisol $AUC_{g\text{-}stress}$ (men $r = 0.46$, $p < 0.05$,

Table 1
Mean (standard deviation) scores on pleasantness, intensity, familiarity and pungency ratings for three odors in the stress and control conditions.

	Odor	Stress	Control	F	*p*
Pleasantness	4-Methylpentanoic acid	2.40 (1.45)	2.45 (1.63)	0.05	0.83
	β-Citronellol	4.43 (2.24)	5.58 (2.30)	10.18	0.003
	2-Heptanol	4.08 (1.93)	4.63 (1.86)	3.15	0.08
Intensity	4-Methylpentanoic acid	5.05 (2.06)	5.35 (1.64)	0.74	0.40
	β-Citronellol	4.83 (1.85)	4.98 (1.73)	0.29	0.59
	2-Heptanol	4.18 (2.00)	4.33 (1.83)	0.32	0.58
Familiarity	4-Methylpentanoic acid	3.08 (1.86)	3.60 (2.17)	2.12	0.15
	β-Citronellol	4.60 (2.12)	5.28 (2.03)	2.92	0.10
	2-Heptanol	3.98 (2.09)	4.43 (2.43)	1.33	0.26
Pungency	4-Methylpentanoic acid	4.13 (2.37)	4.95 (2.16)	3.67	0.06
	β-Citronellol	3.18 (2.04)	2.85 (1.63)	0.56	0.46
	2-Heptanol	3.05 (1.96)	2.95 (1.90)	0.12	0.73

women $r = 0.10$, $p = 0.69$; z = 1.10, $p > 0.05$). The cortisol $AUC_{g\text{-}stress}$ was not correlated to the changed odor intensity, pleasantness, pungency, or familiarity ratings between control and stress conditions (all *p*s > 0.10).

3.5.2. *Correlation between stress-related subjective feelings and olfactory measures*

There was a significant negative correlation between the $AUC_{i\text{-}stress}$ of subjective stress level and changed sensitivity for the unpleasant odor ($r = -0.33$, $p = 0.04$), and changed odor identification score ($r = -0.32$, $p = 0.045$) from the control to the stress condition. In other words, a poor odor identification performance and lower odor sensitivity for 4-Methylpentanoic acid were observed with increased stressful feelings. No gender differences in the association of changed sensitivity for the unpleasant odor (men r = −0.33, $p = 0.15$; women $r = -0.35$, $p = 0.14$; z = 0.06, $p > 0.05$) or odor identification (men $r = -0.29$, $p = 0.21$; women $r = -0.38$, $p = 0.11$; z = 0.29, $p > 0.05$) with the $AUC_{i\text{-}stress}$ of subjective stress level.

There were negative correlations between the $AUC_{i\text{-}stress}$ of state anxiety and the changed odor pleasantness rating for 4-Methylpentanoic acid ($r = -0.31$, $p = 0.054$), and 2-Heptanol ($r = -0.37$, $p = 0.02$) from the control to the stress condition. It suggested participants rated the two odors more unpleasant with increased anxiety level. No gender differences in the association of changed odor pleasantness from control to stress conditions for the unpleasant odor (men $r = -0.45$, $p = 0.04$; women $r = -0.19$, $p = 0.43$; z = −0.85, $p > 0.05$) or neutral odor (men $r = -0.55$, $p = 0.009$; women $r = -0.21$, $p = 0.39$; z = −1.18, $p > 0.05$) with the $AUC_{i\text{-}stress}$ of state anxiety.

4. Discussion

The present study found increased sensitivity to an unpleasant odor 4-Methylpentanoic acid after exposure to acute psychological stress. This finding is in accord with a previous study which showed stress-related increased sensitivity for a foul-smelling odor 2-mercaptoethanol (Pacharra et al., 2016). Moreover, our results further revealed that the effect of acute stress on odor sensitivity was restricted to a negatively valenced odor, but not the neutral or the pleasant odors. Acute stress leads to increased attentional bias selectively towards threatening stimuli (Mogg et al., 1990). An unpleasant odor directly catches more attention than pleasant odors and can fulfill their warning function (Croy et al., 2013). However, a recent study found no significant altered sensitivity to a smoke-like trigeminal odor (guaiacol) or a rose-like odor (PEA) in anxiety youth after TSST. Acute stress merely amplified the bias in odor sensitivity to guaiacol and PEA at baseline in high anxious youth (Cortese et al., 2022). One possible explanation could be that the odor stimuli (phenyl ethyl alanine and guaiacol) are not perceived as unpleasant. Our study, together with previous literature, suggests that acute stress exposure leads to a state of hypervigilance and promotes processing of aversive olfactory stimuli.

We found the stress-related salivary cortisol response was associated with increased odor sensitivity (Δ stress - control) for the unpleasant and the neutral odors. In rats, glucocorticoid receptor as well as corticosteroid binding globulin are expressed in the olfactory mucosa (Dolz et al., 2013), and multiple brain structures including olfactory cortex, amygdala, hippocampus (Morimoto et al., 1996). Animal electrophysiological studies suggested the boosting effect of glucocorticoids on odor detection, starting at the first steps of olfactory detection, such as the olfactory mucosa (Meunier et al., 2020). In humans, an early study investigating the link between cortisol level and olfactory function found that increased cortisol is associated with improved odor detection abilities in women (Pause et al., 1996). 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 and Nagel, 1996). Moreover, high corticosteroid levels significantly increase emotional interference and facilitate to detect threats, which contributes to this state of hypervigilance (Henckens et al., 2012). Cortisol elevations lead to heightened arousal in response to objectively nonarousing neutral stimuli (Abercrombie et al., 2005). Additionally, a lower olfactory sensitivity for the unpleasant odor was observed with increased stressful feelings after stress in our study. This is correspondent to the study that inducing a negative emotional state reduces olfactory

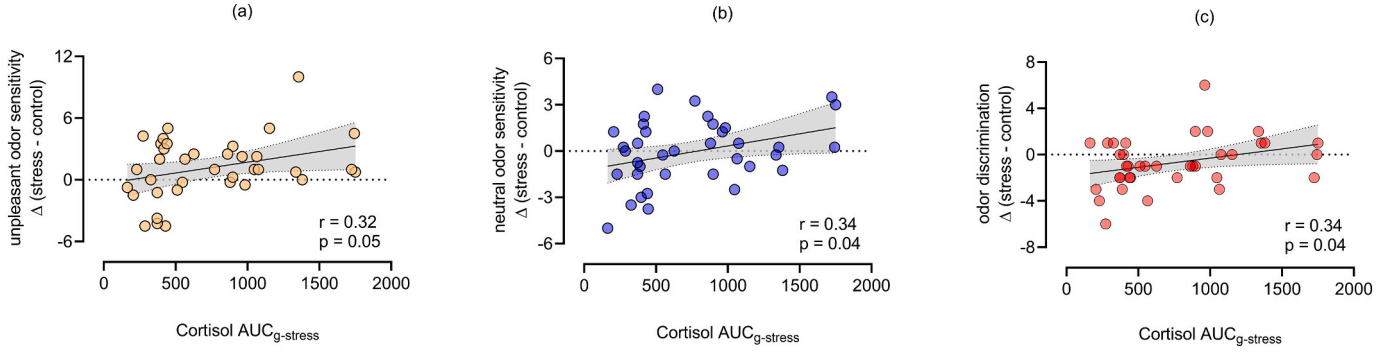

Fig. 4. Scatter plots describe the relationship between salivary cortisol after stress ($AUC_{g\text{-}stress}$) and the change of odor sensitivity to unpleasant odor (a), neutral odor (b), and odor discrimination (c) from the control to the stress condition.

sensitivity (Pollatos et al., 2007). Taken together, it is possible that stress-related cortisol acts in concert with psychological effects to alter sensitivity for unpleasant odors.

We found the pleasantness of an initially pleasant odor (β-Citronellol) decreased significantly after stress and became near neutral. The changed pleasantness for 4-Methylpentanoic acid and 2-Heptanol were negatively correlated with state anxiety. This is associated with the view that mood induction influences odor perception (Shanahan and Kahnt, 2022). This result is in line with a previous study showing that an initially neutral odor becomes unpleasant following acute induction of anxiety (Krusemark et al., 2013). Participants rate odors as less pleasant following a negative mood induction using unpleasant picture and rate as more pleasant following a positive mood induction using a pleasant picture (Pollatos et al., 2007).

The cortisol response ($AUC_{g\text{-}stress}$) was correlated with the increased odor discrimination ability from control to stress conditions. Unlike odor detection, discriminating odors requires higher-order executive functions, such as short-term working memory (Jonsson et al., 2011; Plailly et al., 2007) and executive functioning (Hedner et al., 2010). One recent study found higher cortisol reactivity after acute stress is related to better working memory performances in the N-back task (Lin et al., 2020) (also see Luers et al. (2020) showing an opposite effect of acute stress on working memory in men and women). Therefore, acute stress may act in a complex way to influence cognitive performances (Shields et al., 2016). Additionally, it is suggested that odor pleasantness is the primary aspect of odor spontaneously used by participants in olfactory discrimination tasks (Schiffman, 1974). High state anxiety level is positively associated with negative odor discrimination accuracy (Krusemark and Li, 2012). For odor identification, Hoenen et al. (2017) found that post-stress increased anger is correlated with lower odor identification scores. Although we did not assess the anger emotion, our result found an association between the increased stressful feeling ($AUC_{i\text{-}stress}$) and a worse odor identification performance. Taken together, the stress-related cortisol may work through mechanisms aside from or in addition to stress per se to influence olfaction that involved in higher-order cognitive produce a state characterized by improved olfactory perception.

We did not find gender differences for the effect of acute stress on olfaction. However, a previous study reported differences between men and women in terms of stress-related cortisol responses and brain activities (Henze et al., 2021). The stress-induced cortisol responses were stronger in men than in women, and stress-related cortisol was associated with limbic brain activation in men but correlated to deactivation of the same brain regions in women (Henze et al., 2021). Although no significant gender differences were obtained in our statistical analysis, we propose that future research with a larger sample size are necessary to conclude whether sex plays a key role in the effect of acute stress on olfaction.

5. Strengths and limitations

Strengths of the current study include the use of validated olfactory tests, and a randomized within-subject experimental design. Potential confounders were adjusted in the analyses, including baseline hunger and stress feeling. Still, our study had several limitations. *Firstly*, only one odorant was selected for each valence category, thus it is problematic to generalize the present findings. *Secondly*, a more appropriate olfaction screening method should be considered and the individual threshold variation should be taken into consideration in future research. *Thirdly*, although participants were allowed to rest after suprathreshold tests, potential impact from odor pre-exposure on the subsequent sensitivity test cannot be ruled out. *In addition*, missing salivary cortisol data decreased the statistical power of the findings, which may be addressed by including a larger sample size in future experiments. *Finally*, our participants were mostly freshmen and sophomores, so whether the current findings can be generalized to participants of other ages remains open.

6. Conclusion

To conclude, the present study found that acute psychological stress increased sensitivity for a negative-valenced odor 4-Methylpentanoic acid, but not for a positive- (β-Citronellol) or a neutral-valenced (2-Heptanol) odor. Salivary cortisol reactivity was correlated with the stress-related increased sensitivity to the unpleasant or the neutral odors, and also correlated with increased odor discrimination performance. These findings indicated a state of hypervigilance after exposure to acute psychological stress may increase the sensitivity of the olfactory system to detection of potentially threatening stimuli, which is influenced by stress-related cortisol reactivity.

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CRediT authorship contribution statement

Yun Ai: Investigation, Data curation, Formal analysis, Writing – original draft, Visualization, Project administration. **Juan Yang:** Writing – review & editing, Methodology. **Haoyu Nie:** Investigation, Data curation. **Thomas Hummel:** Writing – review & editing, Methodology. **Pengfei Han:** Writing – review & editing, Resources, Supervision, Funding acquisition, Conceptualization, Methodology.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2023.105325.

References

Abercrombie, H.C., Kalin, N.H., Davidson, R.J., 2005. Acute cortisol elevations cause heightened arousal ratings of objectively nonarousing stimuli. Emotion 5, 354–359.
Al'Absi, M., Nakajima, M., Hooker, S., Wittmers, L., Cragin, T., 2012. Exposure to acute stress is associated with attenuated sweet taste. Psychophysiology 49, 96–103.
Arshamian, A., Gerkin, R.C., Kruspe, N., Wnuk, E., Floyd, S., O'Meara, C., Garrido Rodriguez, G., Lundstrom, J.N., Mainland, J.D., Majid, A., 2022. The perception of odor pleasantness is shared across cultures. Curr. Biol. 32 (9), 2061–2066.
Beck, A.T., Steer, R.A., 1996. Beck Depression Inventory: Manual. The Psychological Corporation Harcourt Brace Jovanovich, San Antonio.
Berretz, G., Packheiser, J., Kumsta, R., Wolf, O.T., Ocklenburg, S., 2021. The brain under stress-a systematic review and activation likelihood estimation meta-analysis of changes in BOLD signal associated with acute stress exposure. Neurosci. Biobehav. Rev. 124, 89–99.
Bombail, V., 2019. Perception and emotions: on the relationships between stress and olfaction. Appl. Anim. Behav. Sci. 212, 98–108.
Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385–396.
Cortese, B.M., Uhde, T.W., Schumann, A.Y., McTeague, L.M., Sege, C.T., Calhoun, C.D., Danielson, C.K., 2022. Anxiety-related shifts in smell function in children and adolescents. Chem. Senses 46.
Croy, I., Maboshe, W., Hummel, T., 2013. Habituation effects of pleasant and unpleasant odors. Int. J. Psychophysiol. 88, 104–108.
Dolz, W., Eitner, A., Caldwell, J.D., Jirikowski, G.F., 2013. Expression of corticosteroid binding globulin in the rat olfactory system. Acta Histochem. 115, 376–381.

Doty, R.L., Cameron, E.L., 2009. Sex differences and reproductive hormone influences on human odor perception. Physiol. Behav. 97, 213–228.
Fehm-Wolfsdorf, G., Nagel, D., 1996. Differential effects of glucocorticoids on human auditory perception. Biol. Psychol. 42, 117–130.
Feng, G., Zhuang, Y., Yao, F., Ye, Y., Wan, Q., Zhou, W., 2019. Development of the Chinese smell identification test. Chem. Senses 44, 189–195.
Fleming, A.S., Steiner, M., Corter, C., 1997. Cortisol, hedonics, and maternal responsiveness in human mothers. Horm. Behav. 32, 85–98.
Frasnelli, J., Albrecht, J., Bryant, B., Lundstrom, J.N., 2011a. Perception of specific trigeminal chemosensory agonists. Neuroscience 189, 377–383.
Frasnelli, J., Hummel, T., Berg, J., Huang, G., Doty, R.L., 2011b. Intranasal localizability of odorants: influence of stimulus volume. Chem. Senses 36, 405–410.
Freiherr, J., Gordon, A.R., Alden, E.C., Ponting, A.L., Hernandez, M.F., Boesveldt, S., Lundstrom, J.N., 2012. The 40-item Monell extended sniffin' sticks identification test (MONEX-40). J. Neurosci. Methods 205, 10–16.
Haehner, A., Mayer, A.M., Landis, B.N., Pournaras, I., Lill, K., Gudziol, V., Hummel, T., 2009. High test-retest reliability of the extended version of the "Sniffin' Sticks" test. Chem. Senses 34, 705–711.
Hedner, M., Larsson, M., Arnold, N., Zucco, G.M., Hummel, T., 2010. Cognitive factors in odor detection, odor discrimination, and odor identification tasks. J. Clin. Exp. Neuropsychol. 32, 1062–1067.
Henckens, M.J., van Wingen, G.A., Joels, M., Fernandez, G., 2012. Time-dependent effects of cortisol on selective attention and emotional interference: a functional MRI study. Front. Integr. Neurosci. 6, 66.
Henze, G.I., Konzok, J., Kreuzpointner, L., Bartl, C., Giglberger, M., Peter, H., Streit, F., Kudielka, B.M., Kirsch, P., Wust, S., 2021. Sex-specific interaction between cortisol and striato-limbic responses to psychosocial stress. Soc. Cogn. Affect. Neurosci. 16, 972–984.
Hoenen, M., Wolf, O.T., Pause, B.M., 2017. The impact of stress on odor perception. Perception 46, 366–376.
Hummel, T., Sekinger, B., Wolf, S.R., 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, 39–52.
Jonsson, F.U., Moller, P., Olsson, M.J., 2011. Olfactory working memory: effects of verbalization on the 2-back task. Mem. Cogn. 39, 1023–1032.
Kato, M., Okumura, T., Tsubo, Y., Honda, J., Sugiyama, M., Touhara, K., Okamoto, M., 2022. Spatiotemporal dynamics of odor representations in the human brain revealed by EEG decoding. Proc. Natl. Acad. Sci. U. S. A. 119, e2114966119.
Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.
Krusemark, E.A., Li, W., 2012. Enhanced olfactory sensory perception of threat in anxiety: an event-related fMRI study. Chemosens. Percept. 5, 37–45.
Krusemark, E.A., Novak, L.R., Gitelman, D.R., Li, W., 2013. When the sense of smell meets emotion: anxiety-state-dependent olfactory processing and neural circuitry adaptation. J. Neurosci. 33, 15324–15332.
Kudielka, B.M., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. Biol. Psychol. 69, 113–132.
Lin, L., Leung, A.W.S., Wu, J., Zhang, L., 2020. Individual differences under acute stress: higher cortisol responders performs better on N-back task in young men. Int. J. Psychophysiol. 150, 20–28.
Luers, P., Schloeffel, M., Prussner, J.C., 2020. Working memory performance under stress. Exp. Psychol. 67, 132–139.
Luettgau, L., Schlagenhauf, F., Sjoerds, Z., 2018. Acute and past subjective stress influence working memory and related neural substrates. Psychoneuroendocrinology 96, 25–34.
Marteau, T.M., Bekker, H., 1992. The development of a six-item short-form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI). Br. J. Clin. Psychol. 31, 301–306.
Meunier, N., Raynaud, A., Le Bourhis, M., Grebert, D., Dewaele, A., Acquistapace, A., Bombail, V., 2020. The olfactory mucosa, first actor of olfactory detection, is sensitive to glucocorticoid hormone. Eur. J. Neurosci. 51, 1403–1418.
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, 1230–1237.
Morimoto, M., Morita, N., Ozawa, H., Yokoyama, K., Kawata, M., 1996. Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. Neurosci. Res. 26, 235–269.
Mueller, C., Renner, B., 2006. A new procedure for the short screening of olfactory function using five items from the "Sniffin' Sticks" identification test kit. Am. J. Rhinol. 20, 113–116.
O'Connor, D.B., Thayer, J.F., Vedhara, K., 2021. Stress and health: a review of psychobiological processes. Annu. Rev. Psychol. 72, 663–688.
Pacharra, M., Schaper, M., Kleinbeck, S., Blaszkewicz, M., Wolf, O.T., van Thriel, C., 2016. Stress lowers the detection threshold for foul-smelling 2-mercaptoethanol. Stress 19, 18–27.
Pause, B.M., 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.
Pino, J.A., Mesa, J., 2006. Contribution of volatile compounds to mango (*Mangifera indica* L.) aroma. Flavour Frag. J. 21, 207–213.
Plailly, J., Radnovich, A.J., Sabri, M., Royet, J.P., Kareken, D.A., 2007. Involvement of the left anterior insula and frontopolar gyrus in odor discrimination. Hum. Brain Mapp. 28, 363–372.
Pollatos, O., Kopietz, R., Linn, J., Albrecht, J., Sakar, V., Anzinger, A., Schandry, R., Wiesmann, M., 2007. Emotional stimulation alters olfactory sensitivity and odor judgment. Chem. Senses 32, 583–589.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.
Robinson, A.M., Kern, R.C., Foster, J.D., Fong, K.J., Pitovski, D.Z., 1998. Expression of glucocorticoid receptor mRNA and protein in the olfactory mucosa: physiologic and pathophysiologic implications. Laryngoscope 108, 1238–1242.
Schiffman, S.S., 1974. Physicochemical correlates of olfactory quality: a series of physicochemical variables are weighted mathematically to predict olfactory quality. Science 185, 112–117.
Shanahan, L.K., Kahnt, T., 2022. On the state-dependent nature of odor perception. Front. Neurosci. 16, 964742.
Shields, G.S., Sazma, M.A., Yonelinas, A.P., 2016. The effects of acute stress on core executive functions: a meta-analysis and comparison with cortisol. Neurosci. Biobehav. Rev. 68, 651–668.
Sorokowska, A., Albrecht, E., Haehner, A., Hummel, T., 2015. Extended version of the "Sniffin' Sticks" identification test: test–retest reliability and validity. J. Neurosci. Methods 243, 111–114.
Spielberger, C.D., 1970. Manual for the State-Trait Anxiety Inventory. Self Evaluation Questionnaire.
Sterne, J.A., White, I.R., Carlin, J.B., Spratt, M., Royston, P., Kenward, M.G., Wood, A.M., Carpenter, J.R., 2009. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. Bmj 338, b2393.
Stevenson, R.J., 2010. An initial evaluation of the functions of human olfaction. Chem. Senses 35, 3–20.
Yeshurun, Y., Sobel, N., 2010. An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. Annu. Rev. Psychol. 61 (219–241), C211–C215.

Supplementary Materials

Increased sensitivity to unpleasant odor following acute psychological stress

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Supplementary Methods

The Trier Social Stress Test procedure

During the introduction phase, the researcher informed the participants that they need to give a speech that will be audio- and video-recorded for later analysis. Participants were then introduced to a male and a female 'expert' composed of two research assistants wearing white laboratory coats who would be evaluating their speech. The researcher then asked participants to imagine that they were applying for their ideal job and to take 5 min to prepare their speech describing why they should be the ideal candidate for the position. During the speech period, the participants were instructed to deliver their speech and the experts kept neutral facial expressions. If the participant finished before 5 min, the experts responded in a standardized way by firstly keeping silent for 20 s and then asking the participant to continue. If necessary, the experts asked prepared questions to ensure that participants spoke for the entire period. Finally, the researcher asked the participants to perform mental math for 5 min by serially subtracting 13 from 1022 aloud as quickly and accurately as possible. Their progress was monitored, and when an error was made, the experimenter told the participants to start over from the beginning.

Pilot study of odor selection

Given the association between odor intensity (concentration) and pleasantness, 7 participants (age Mean = 23.43 ± 0.98 years, 3 male and 4 female) judged the pleasantness and familiarity of each odor at two concentrations (β-Citronellol 5.76 ppm and 50 ppm; 4-Methylpentanoic acid 10.8 ppm and 100 ppm; 2-Heptanol 5.12 ppm and 50 ppm) using the 9-point Likert-type scale.

The pleasantness ratings of the three odors at the medium-high concentrations were summarized in Table S1. A 3 (odor type) × 2 (odor concentration) within-subject repeated-measure ANOVA showed a significant effect of odor type on rated pleasantness ($F = 16.09$, $p < 0.005$, $\eta^2 = 0.73$), pairwise comparisons indicate that the pleasantness ratings for 3 odors were significantly different from each other. However, there was no significant effect of odor concentration ($F = 1.00$, $p = 0.36$), or odor × concentration interaction ($F = 2.03$, $p = 0.17$) on pleasantness ratings, indicating that the pleasantness for each odor at high or low concentrations were consistent.

Table S1 Pleasantness and familiarity of odors ($N = 7$)

	β-Citronellol		4-Methylpentanoic acid		2-Heptanol	
	5.76 ppm	50 ppm	10.8 ppm	100 ppm	5.12 ppm	50 ppm
Pleasantness	6.00 (1.29)	5.86 (1.35)	3.14 (1.95)	2.14 (1.07)	4.29 (0.76)	4.71 (1.11)
Familiarity	4.29 (2.29)	4.86 (2.04)	4.57 (2.76)	4.71 (2.56)	5.14 (1.68)	5.29 (1.60)

Ratings on 9-point Likert scale; Data shown as Mean (Standard Deviation)

Odor sensitivity test

Three bottles were required for each test, two containing the solvent and one containing solutions of the odor stimuli, which were presented in a randomized order. Participants were required to identify the bottle with the specific odor stimuli. During the evaluation, the bottle was placed approximately 2 cm from the participant's nose for around 3 s. Triplets were presented at intervals of approximately 30 s. The participants were blindfolded to prevent visual identification of the bottle containing the odor stimuli. The reversal of the staircase was triggered when the odor was correctly identified in two successive trials. The detection threshold was determined from the geometric mean of the last four (from seven) reversal points and expressed as the threshold score.

Table S2 Solution concentrations (ppm) for odor threshold test

	β-Citronellol	4-Methylpentanoic acid	2-Heptanol
1	0.0003125	0.003125	0.000625
2	0.000625	0.00625	0.00125
3	0.00125	0.0125	0.0025
4	0.0025	0.025	0.005
5	0.005	0.05	0.01
6	0.01	0.1	0.02
7	0.02	0.2	0.04
8	0.04	0.4	0.08
9	0.08	0.6	0.16
10	0.16	0.9	0.32
11	0.32	1.35	0.64
12	0.64	2.7	1.28
13	0.96	5.4	2.56
14	1.44	10.8	5.12
15	2.88	21.6	10.24
16	5.76	43.2	20.48

Sniffin' Sticks odor discrimination test

Triplets of pens were presented in a randomized order, with two containing the same and one a different odorant. Participants had to determine which of three pens in 16 triplets was different in odor. The participants were blindfolded to prevent visual identification of the pen containing a different odor. The pens were presented under the nose at a distance of about 2 cm for about 3 s. The triplets were presented at an interval of approximately 30 s. The interval between presentations of individual pens was approximately 5 s.

32-item odor identification test

Since the original "Sniffin' Sticks" odor identification test was developed to determine olfactory dysfunction in clinical settings which is not suitable for assessing variability in odor identification in young healthy participants (Freiherr et al., 2012). Further, a larger number of odor items is necessary to allow for a split into two subtests to avoid practice effect in a within-subject study design. In addition, due to the desire to reduce the impact of smells and descriptors unfamiliar to Chinese participants on the accuracy. Five odors (cinnamon, liquorice, turpentine, leather, and cloves) with low familiarity to the Chinese population from the original Sniffin' Sticks odor identification test were replaced by medium-high familiarity from the Chinese Smell Identification Test (Feng et al., 2019). In addition, to avoid the ceiling effect, the options were modified by adding odor descriptors with moderate similarity to the target odor to increase difficulty (Freiherr et al., 2012). Therefore, a total of 32 odors were used for the test of odor identification, with 16 odors used after the stress session and 16 odors used after the control session. A description of the odors and distractors included in the final identification task was listed in the **Table S3**. The final task was evaluated with 8 participants who did not participate in the main study. Result showed comparable scores between the two sub-tests (set A: 9.75 ± 1.75, set B: 9.50 ± 1.31; $t = 0.39$, $p = 0.71$). The number of times the two odor groups were used in the stress condition or control condition was counterbalanced among participants.

Table S3 Odor descriptors of odor identification test: group A and group B consist of 16 items each (bold font indicates the correct answer)

Set	Item	Descriptors			
A	1	**lemon**	apple	grapefruit	bayberry
	2	celery	**lavender**	wood	cabbage
	3	cantaloupe	plum	**pear**	watermelon
	4	bread	ham	cheese	**fish**
	5	blueberry	lime	pineapple	**grape**
	6	soy milk	sesame	**peanut**	ham
	7	black pepper	**garlic**	pickle	onion
	8	**mango**	orange	yogurt	peach
	9	**almond**	rubber	chocolate	walnut
	10	bayberry	**strawberry**	orange	blueberry
	11	dried dates	litchi	**dried longan**	white sugar
	12	mosquito coil	camphor	osmanthus	**sandalwood**
	13	cigarette	black tea	wine	**coffee**
	14	cantaloupe	peach	**apple**	orange
	15	peanut	**sesame oil**	butter	mushroom
	16	**grapefruit**	strawberry	grape	pear
B	1	**coconut**	sesame oil	osmanthus	oatmeal
	2	carambola	**rose**	lavender	mango
	3	honey	dried longan	**dried dates**	roasted sweet potato
	4	gasoline	butter	ham	**mushroom**
	5	watermelon	tomato	peach	**pineapple**
	6	bamboo	grass	**green tea**	carrot
	7	almond	**honey**	dried dates	corn
	8	**chocolate**	coffee	rubber	caramel
	9	**kiwi**	lime	grape	strawberry
	10	cherry	**hawthorn**	bayberry	tomato
	11	longan	grapefruit	**orange**	pineapple
	12	peanut	leather	almond	**walnut**
	13	lavender	cabbage	rose	**grass**
	14	apple	cucumber	**cantaloupe**	plum
	15	cumin	**anise**	wood	tangerine peel
	16	**banana**	pear	osmanthus	peach

Supplementary Results

Table S4 Mean (standard deviation) scores on odor detection threshold for three odorants

		Stress	Control
All	4-Methylpentanoic acid	6.54 (2.85)	5.49 (2.40)
	β-Citronellol	6.24 (2.31)	6.19 (2.41)
	2-heptanol	6.85 (2.52)	6.89 (1.93)
Men	4-Methylpentanoic acid	6.43 (3.72)	4.86 (2.78)
	β-Citronellol	6.01 (2.16)	5.63 (2.19)
	2-heptanol	7.66 (2.21)	7.13 (1.76)
Women	4-Methylpentanoic acid	6.66 (1.48)	6.18 (1.70)
	β-Citronellol	6.50 (2.49)	6.82 (2.55)
	2-heptanol	5.96 (2.60)	6.62 (2.13)

Table S5 Demographic characteristics of participants included in the final analysis

	Male $N = 21$	Female $N = 19$	p
Age in year	19.33 (1.11)	18.89 (1.15)	0.23
BMI	20.49 (2.27)	20.14 (2.30)	0.63
Depression	2.48 (2.87)	3.00 (2.79)	0.56
Perceived Life Stress	13.43 (5.43)	13.63 (3.85)	0.89
Trait Anxiety	38.52 (8.59)	39.47 (7.36)	0.71

Data presented as Mean (Standard Deviation); BMI: Body Mass Index.

Table S6 Raw data of salivary cortisol (nmol/L)										
ID	**Stress-T1**	**Stress-T2**	**Stress-T3**	**Stress-T4**	**Stress-T5**	**Control-T1**	**Control-T2**	**Control-T3**	**Control-T4**	**Control-T5**
1	14.218	23.467	33.686	19.616	8.269	8.072	9.944	17.560	6.592	3.950
2	5.482	6.092	5.420	4.560	2.486	5.102	4.290	2.405	3.040	3.606
3	4.818	5.358	6.249	5.089	3.125	2.040	2.212	2.212	1.979	1.854
4	4.547		18.002	13.766	6.249	5.369	8.542	5.028	2.222	5.065
5	4.696		31.969	25.685	14.202	11.870	16.209	11.870	5.177	1.813
6	11.935	27.558	32.753	18.186	5.798	3.995	2.841	4.973	2.395	1.750
7	10.383	27.558	23.350	0.335	9.232	2.785	3.211	1.750	3.004	3.634
8	3.088	3.404	4.757	3.140	1.646	2.910	1.469	0.958	4.900	4.829
9	2.212	6.660	8.618	3.839	3.167	1.726	1.533	1.422		1.022
10	2.093	7.506	11.064	6.130	3.567	1.829	2.326	1.773	1.703	1.821
11		12.472	11.173		5.798	2.819	4.056	3.430	2.269	1.612
12	10.562	15.984	19.852	19.604	13.227	22.596	21.199	14.705	7.029	6.269
13	3.247		5.653	5.769	4.708	1.511	3.174	4.290	2.992	0.675
14	1.845	18.599	32.228	15.066	5.413	6.114	8.913	6.831	3.524	1.303
15	4.086	2.632	2.268	2.233	2.254	4.743	6.704	5.473	3.496	2.888
16	5.935	13.123	16.402	9.304	5.275	5.874	3.566	3.761	3.476	2.931
17	5.693	5.648	7.761	3.408	3.054		3.293	0.896	7.095	1.200
18	4.520	4.264	7.059	4.403	2.884	8.530	15.490	9.130	7.019	3.624
19	3.193	1.787	3.614	3.154		4.009	3.507	3.362	3.539	2.001
20		3.888	2.983	1.948	3.340	4.386		5.083		1.760
21	6.236	15.596	20.195	12.365	4.781	4.131	4.642	3.709	2.787	1.967
22	2.326	3.888	9.447	5.663	3.792	1.545	1.929	2.073	2.326	1.485
23	4.730	9.116	18.861	10.437	7.921	5.110	6.618	6.791	5.444	3.803
24	5.516	6.301	5.920	5.124	4.120	1.568	2.334	2.491	2.445	6.739
25		13.776	18.970		7.657	3.183	6.363	11.442	6.789	11.830
26	11.407	15.396	24.303	17.373	7.532	10.023	0.001	6.495	5.356	6.019
27	6.232	10.307	6.429	6.789	4.081	3.304	3.656	2.625	2.251	1.291
28	4.034	5.203	21.044	13.655	3.715	2.477	4.160	6.429	4.661	2.048
29		1.211	2.322	1.905	1.252					
30	8.123	15.940	14.781	7.965	2.660	9.062	7.939	6.495	5.203	2.029
31		1.697	3.118	8.582	1.014	3.456	4.356		2.876	3.263
32	3.304	8.176	18.337	15.620	8.862	7.836	7.733	6.675	5.670	3.428
33	4.592	5.751	4.927	4.144	3.277	3.345	3.372	4.783	3.498	1.672
34	2.911	5.904	15.536	6.503	3.701	34.772	21.337	13.567	9.053	5.297
35	11.469	6.267	6.411	5.467	3.528		7.881	6.006	4.081	2.575
36	6.689	10.435	21.950	12.799	9.942	8.850	9.027	7.926	7.139	4.494
37	6.057	5.405	4.573	4.190	3.106	17.254	11.502	9.636	8.458	5.104

Table S7 Salivary cortisol after supplementing using multiple imputation (nmol/L)										
ID	**Stress-T1**	**Stress-T2**	**Stress-T3**	**Stress-T4**	**Stress-T5**	**Control-T1**	**Control-T2**	**Control-T3**	**Control-T4**	**Control-T5**
1	14.218	23.467	33.686	19.616	8.269	8.072	9.944	17.560	6.592	3.950
2	5.483	6.092	5.420	4.560	2.486	5.102	4.290	2.405	3.040	3.606
3	4.818	5.359	6.250	5.089	3.125	2.040	2.212	2.212	1.979	1.854
4	4.547	10.528	18.002	13.767	6.250	5.369	8.542	5.028	2.222	5.065
5	4.696	19.239	31.969	25.685	14.202	11.870	16.209	11.870	5.177	1.813
6	11.935	27.558	32.753	18.186	5.798	3.995	2.841	4.973	2.395	1.750
7	10.383	27.558	23.350	0.335	9.232	2.785	3.211	1.750	3.004	3.634
8	3.088	3.404	4.757	3.140	1.646	2.910	1.469	0.958	4.900	4.829
9	2.212	6.660	8.618	3.839	3.167	1.726	1.533	1.422	0.057	1.022
10	2.093	7.506	11.064	6.130	3.567	1.829	2.327	1.773	1.703	1.821
11	9.216	12.472	11.173	7.946	5.798	2.819	4.056	3.430	2.269	1.613
12	10.562	15.984	19.852	19.604	13.227	22.596	21.199	14.705	7.029	6.269
13	3.248	2.826	5.653	5.769	4.708	1.511	3.174	4.290	2.992	0.675
14	1.845	18.600	32.228	15.066	5.413	6.114	8.913	6.831	3.524	1.304
15	4.086	2.632	2.268	2.233	2.254	4.743	6.704	5.473	3.496	2.888
16	5.935	13.123	16.402	9.304	5.275	5.874	3.566	3.761	3.476	2.931
17	5.693	5.648	7.761	3.408	3.054	3.831	3.293	0.896	7.095	1.200
18	4.520	4.264	7.059	4.404	2.884	8.530	15.490	9.130	7.019	3.624
19	3.193	1.787	3.614	3.154	2.292	4.009	3.507	3.362	3.539	2.001
20	8.147	3.888	2.983	1.948	3.340	4.386	2.628	5.083	4.338	1.760
21	6.236	15.596	20.195	12.365	4.781	4.131	4.642	3.709	2.787	1.967
22	2.326	3.888	9.447	5.663	3.792	1.545	1.929	2.074	2.326	1.485
23	4.730	9.116	18.861	10.437	7.921	5.110	6.618	6.791	5.444	3.803
24	5.516	6.301	5.920	5.124	4.120	1.568	2.334	2.491	2.445	6.739
25	5.689	13.776	18.970	13.024	7.657	3.183	6.363	11.442	6.789	11.830
26	11.407	15.396	24.303	17.373	7.532	10.023	4.332	6.495	5.356	6.019
27	6.232	10.307	6.429	6.789	4.081	3.304	3.656	2.625	2.251	1.291
28	4.034	5.204	21.044	13.655	3.715	2.477	4.160	6.429	4.661	2.048
29	4.042	1.211	2.322	1.905	1.252	6.484	6.425	5.696	4.257	3.230
30	8.123	15.940	14.781	7.965	2.660	9.062	7.939	6.495	5.204	2.029
31	3.166	1.697	3.118	8.582	1.014	3.456	4.356	2.821	2.876	3.263
32	3.304	8.176	18.337	15.620	8.862	7.836	7.733	6.675	5.670	3.428
33	4.592	5.751	4.927	4.144	3.277	3.345	3.372	4.783	3.498	1.672
34	2.911	5.904	15.536	6.503	3.701	34.773	21.337	13.567	9.053	5.297
35	11.469	6.267	6.411	5.467	3.528	9.055	7.881	6.006	4.081	2.575
36	6.689	10.435	21.950	12.799	9.942	8.850	9.027	7.926	7.139	4.494
37	6.057	5.405	4.573	4.190	3.106	17.254	11.502	9.636	8.458	5.104

Table S8 Mean (Standard Deviation) scores of perceived stress, and state anxiety from T1 to T5 for acute stress condition and control condition

		T1 (0) Baseline	T2 (+ 20) Post-stress	T3 (+ 35) Post-stress	T4 (+ 60) Post-stress	T5 (+ 80) Post-stress
Perceived stress	TSST	20.31	56.73	26.93	23.66	14.99
		(16.82)	(24.80)	(20.15)	(22.50)	(18.02)
	Control	26.76	15.48	18.44	18.13	14.25
		(19.75)	(16.77)	(18.07)	(20.24)	(17.23)
State anxiety	TSST	10.88	16.15	12.38	11.78	10.25
		(2.97)	(3.58)	(3.02)	(3.08)	(2.22)
	Control	10.98	10.53	11.30	11.48	10.60
		(2.63)	(2.42)	(3.07)	(3.24)	(3.19)

References

Feng, G., Zhuang, Y., Yao, F., Ye, Y., Wan, Q., Zhou, W., 2019. Development of the Chinese Smell Identification Test. Chemical senses 44, 189-195.

Freiherr, J., Gordon, A.R., Alden, E.C., Ponting, A.L., Hernandez, M.F., Boesveldt, S., Lundstrom, J.N., 2012. The 40-item Monell Extended Sniffin' Sticks Identification Test (MONEX-40). J Neurosci Meth 205, 10-16.

Haehner, A., Mayer, A.M., Landis, B.N., Pournaras, I., Lill, K., Gudziol, V., Hummel, T., 2009. High test-retest reliability of the extended version of the "Sniffin' Sticks" test. Chemical senses 34, 705-711.

Sorokowska, A., Albrecht, E., Haehner, A., Hummel, T., 2015. Extended version of the “Sniffin’Sticks” identification test: Test–retest reliability and validity. J Neurosci Meth 243, 111-114.