



Emotional memory in the lab: Using the Trier Social Stress Test to induce a sensory-rich and personally meaningful episodic experience

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ABSTRACT

A myriad of clinical theories places emotional memory or mental representations at the root of mental disorders. Various cognitive-behavioural interventions are based on the assumption that targeting the underlying emotional memory is the working mechanism of treatment efficacy. To test the assumptions about the role of emotional memory in the development, maintenance, and treatment of mental disorders, we first need to establish ecologically valid paradigms that can induce emotional memory in the lab. For this, we used the Trier Social Stress Test (TSST), a standardized protocol to elicit social distress, paired with a neutral unfamiliar ambient odour, to create a sensory-rich and personally meaningful episodic experience. Seven days later, participants (N = 132) reactivated the memory of the TSST with the aid of auditory, olfactory, and visual retrieval cues, during which their heart rate and self-reported affective responses were collected. Although increases in heart rate were only observed during encoding, and not at retrieval, self-report ratings showed that cues which directly referred to the aversive experience evoked more negative valence, arousal, and feelings of lack of control during memory reactivation compared to control cues across sensory modalities. These findings are indicative of successful memory induction and corroborate the utility of ambient odours as retrieval aids. Moreover, the self-reported response to the reactivated emotional memory correlated with individual differences in indices of (social) anxiety and depression. Thereby, we provide preliminary evidence of the translational significance of this paradigm that offers potential for being a model to induce ecologically valid emotional memory in the lab.

1. Introduction

Many psychological interventions are based on the assumption that emotional memory lies at the root of psychological disorders and that targeting it leads to symptom reduction. Emotional memory may be defined “as an umbrella term for any construct that refers to a mental representation (see [Arntz, 2020](#) for review) of one, or a series of experiences (imagined or real), that carries an affective load, and that shapes the way one perceives and makes predictions about the self, others, and the world” ([Freund et al., 2022](#)). While not all treatment approaches explicitly conceptualize the role of memory in symptom development, many techniques used in cognitive behavioural therapy at least implicitly aim to target (aspects of) memory (e.g., a schema or belief) that is deemed maladaptive. Such treatments have shown to be successful at symptom reduction across a wide range of psychiatric disorders ([Butler et al., 2006](#); [Hofmann et al., 2012](#)). However, to date, there is little evidence indicating whether treatment response is indeed achieved by altering emotional memory. Instead of being the proposed

mechanism of action, emotional memory is perhaps solely a theoretical construct that provides a convincing narrative in psychoeducation. Clarifying the exact role of emotional memory in mental disorders through basic translational research is thus necessary for developing future clinical interventions ([Elsej and Kindt, 2017](#); [Holmes et al., 2018](#); [Visser et al., 2018](#)). Yet, before we can investigate whether emotional memory is a critical therapeutic target, we first need to develop paradigms that allow us to create sensory-rich emotional memories in a controlled but ecologically valid manner.

Experimental paradigms such as Pavlovian fear conditioning and the trauma film paradigm provide a means to induce emotional memory, and prospectively assess the consequent behaviour change in a laboratory setting ([Fullana et al., 2020](#); [Holmes and Bourne, 2008](#); [James et al., 2016](#)). While these models have clear translational value in the study of prominent symptoms in stress- and anxiety disorders, a disadvantage of these paradigms is that the memories are relatively simplistic compared to “real-life” memories, due to, for example, the lack of a multi-modal, sensory-rich context during encoding, and – in the case of Pavlovian

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conditioning – the unidimensional association between a conditional and unconditional stimulus. Moreover, participants often remain passive bystanders (i.e., of aversive scenes in the trauma film paradigm), thereby not capturing an important dimension of autobiographical memories, being its relation to the self. Here, we tested the utility of an established paradigm for inducing psychosocial stress, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), for creating a memory that is negative in valence, sensory-rich, and personally meaningful. In the TSST, participants undergo a surprise mental arithmetic task and a mock interview with an unresponsive panel. This paradigm has been used in memory research, albeit mostly for studying the effect of psychosocial stress on semantically unrelated memory performance (e.g., Cornelisse et al., 2011; Debeer et al., 2012; Luethi et al., 2009; Schultebrucks et al., 2019). Only recently has the TSST been conducted to investigate memory accuracy of the stressful episode itself (Bierbrauer et al., 2021; Herten, Otto et al., 2017; Herten, Pomrehn et al., 2017; Wiemers et al., 2014; Wiemers, Sauvage et al., 2013), indicating that it can be used to create a memory. While relevant for understanding how emotion interacts with basic memory processes, the focus in these studies was on the accuracy of the newly created memory and other aspects such as associative strength between central and peripheral details. In contrast, we are specifically interested in the memory's emotionality. Thus, the first aim of this study was to adapt the TSST to induce a sensory-rich emotional memory in a controlled yet ecologically valid manner. Memory induction was considered successful if the memory and related emotions could be reactivated seven days later.

Although memory reactivation appears to be paramount for psychotherapy and for measuring emotional memory, literature on what is an effective stimulus for reactivation is sparse. Most in-vivo exposure therapies rely on visual and/or auditory cues associated with the emotional memory. Less commonly, odour cues are used as retrieval aids in combination with other sensory cues in exposure therapy (e.g., Rizzo et al., 2006; Roy et al., 2010). The limited use of odours is surprising given that odours have been known to spontaneously evoke autobiographical memories with a strong emotive connotation, known as the 'Proust phenomenon' (Chu and Downes, 2002; Hackländer et al., 2019; Hakim et al., 2019; Herz, 2016 for reviews). Brain areas supporting olfaction are the only sensory structures that directly relay to the amygdala and entorhinal cortex, involved in emotion processing and memory (Doty, 2001; Wilson et al., 2004), suggesting that there may be a neurobiological explanation for why odours could be especially powerful cues for retrieving emotional memories. Indeed, some studies have shown that odour-evoked memories are experienced as more emotional (Arshamian et al., 2013; Chu and Downes, 2002; Herz, 2004; Herz et al., 2004; Herz and Schooler, 2002; Willander and Larsson, 2007) compared to those evoked by verbal and/or visual cues. However, findings are inconsistent as in other studies odour cues have shown to be outperformed (Miles and Berntsen, 2011; Toffolo et al., 2012; Willander et al., 2015; Willander and Larsson, 2006). Without aiming to compare the different modalities, in this study we harnessed multiple modalities to reactivate the memory, i.e., using auditory, olfactory, and visual retrieval cues, to capture the richness of the memory.

Most studies that investigated the effects of different sensory modalities on memory retrieval, focused on existing autobiographical memories, thereby not having control over encoding, which results in memories that differ in content, age, and valence. Rather than examining previously formed autobiographical memories, we prioritised having control over memory encoding. Furthermore, the extent to which odour plays a role during encoding may vary across experiences: in most naturalistic settings, odour is a peripheral or ambient cue (e.g., room fragrance in conference room), however, the odour may also be central to the event (e.g., actively smelling someone's aftershave). To simulate a real-life experience and to not deviate too much from the original TSST, we tested a peripheral odour cue. Additionally, most previous studies tested familiar odours (e.g., the smell of popcorn or coffee) that may have (multiple) pre-existing associations, such that one odour could be

associated with various memories of different emotional tones. The second aim of the study was to test the potency of an unfamiliar neutral odour for retrieving a lab-induced episodic memory of a negative event.

In the current multi-day study, participants underwent a TSST while being ambly exposed to an unfamiliar neutral odour. Seven days after encoding, the memory was reactivated with the help of TSST-related auditory, olfactory, and visual cues. The subjective and physiological (i.e., heart rate) emotional response acted as readouts of the memory. A stronger self-reported and physiological emotional response to the reactivated memory with the aid of TSST-related cues compared to control cues was indicative of successful memory induction. Lastly, our third aim was to investigate the translational significance of this experimental paradigm for clinical practice, exploring the extent to which individual traits, including indicators of depression, (social) anxiety, and stress response may predict a stronger emotional memory (i.e., a larger difference in emotional response to the TSST vs control cues).

2. Methods

2.1. Participants

A total of 140 participants were recruited via the university website and included in the study if they were between 18 and 65 years of age and proficient in English. The study included a mobile app to collect ecological momentary assessment data on mood states and intrusive memories for a duration of 14 days. The sample size was based on the requirements for building reliable network models with the ecological momentary assessment. However, due to a server outage of the mobile app, a substantial portion of the data was not collected, and these could therefore not be analysed. Due to the olfactory stimuli, participants were excluded if they presented cold symptoms and were instructed to not use fragrant cosmetics on testing days. Participants were asked to refrain from smoking, eating, drinking, and exercising two hours prior to the experimental sessions due to possible influences on cortisol. Participation was reimbursed with course credits or money (€10/hr). Prior to participation, participants provided written informed consent. The experiment received ethical approval by the University of Amsterdam's ethics committee (2021-CP-14026). Five participants dropped out of the study during the TSST and for three participants the TSST procedure was flawed (e.g., incomplete panel or TSST session). The resulting sample comprised 132 participants (104 women (sex assigned at birth) and 28 men (sex assigned at birth), M age = 20.41, SD = \pm 2.84 years, age range = 18–34 years). Yet, the exact sample size differed per analysis considering additional exclusions: four participants were excluded from the TSST heart rate analysis due to incomplete or noisy data. For the cortisol analyses, 27 participants had to be excluded because of incomplete salivary samples. A total of five additional participants were excluded from the memory reactivation analyses because they did not complete the last experimental session within the 14-day timeframe (n = 4) or had incomplete heart rate data (n = 1). Data on oral contraception was missing for six participants.

2.2. Design

The experimental paradigm consisted of a within-subject design. Participants attended three in-person lab sessions, with seven days between each session, see Fig. 1A for a schematic overview. All sessions were scheduled between 8:45 and 19:30. Besides completing baseline questionnaires on day 0 (D0), participants were instructed to use an app to record their daily mood states and intrusive memories for a duration of 14 days. However, we were unable to collect sufficient data because of a server outage of the app. Therefore, this part of the study was excluded in this article. Seven days after the first session (D7), participants returned to the lab for memory induction by means of a slightly adapted version of the TSST, described in Section 2.3.1. On day 14 (D14),

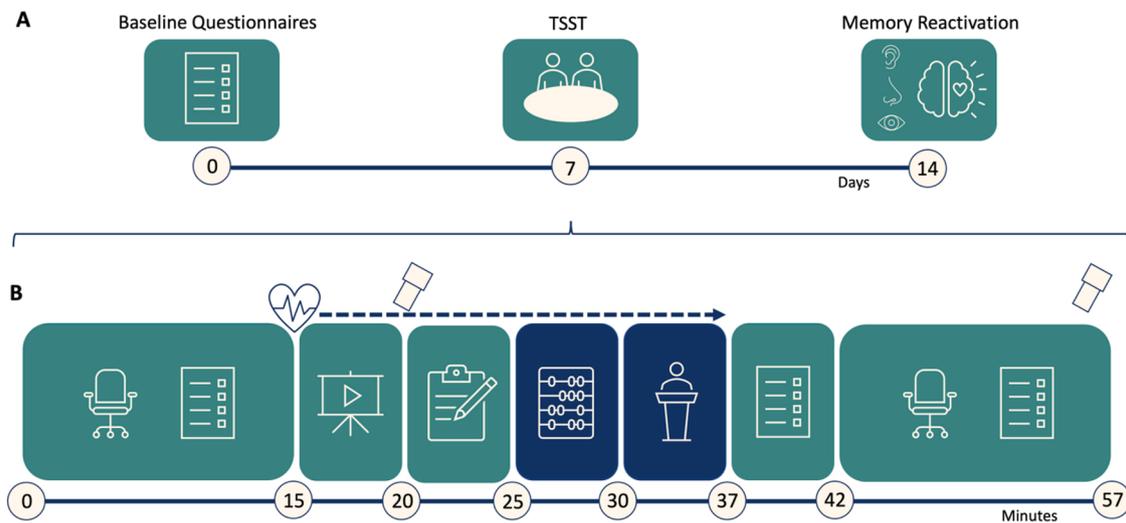


Fig. 1. Overview of experimental paradigm. *Note:* A Timeline of the three-day experiment. On day 0 (D0) participants completed baseline questionnaires, on day 7 (D7) they underwent the Trier Social Stress Test (TSST), and on day 14 (D14) they underwent memory reactivation with the aid of auditory, olfactory, and visual retrieval cues. B Schematic overview of the TSST on D7. During 15 min of baseline rest, participants waited 5 min in the waiting room and were brought to the laboratory where they assessed baseline state anxiety (pre-TSST). Subsequently, the heart rate monitor was attached for continuous measurement until the participant exited the panel room. For a baseline heart rate measurement, participants watched a 5 min calming art video, after which they provided the baseline salivary cortisol sample. Participants were then instructed about the mock interview. A sham microphone was attached to their shirt, followed by 5 min of speech preparation. They entered the odour-infused panel room for a 5 min mental arithmetic task, delivered their speech, and answered at least one interview-style question. Upon returning to the experiment room (post1) they rated their state anxiety. Subsequently participants were moved to a recovery room, where the last state anxiety assessment and cortisol sample were collected 20 min after exiting the panel room (post2).

participants returned for memory reactivation, which consisted of a 2 (cue condition: TSST vs. control) \times 3 (cue modality: auditory, olfactory, visual) within-subjects design. D0 and D14 were conducted in laboratory cubicles, whereas D7 took place in a different part of the building.

2.3. Materials

2.3.1. Trier Social Stress Test (TSST)

On D7 participants underwent an adapted version of the TSST. Before any mention of the upcoming interview, participants watched a 5 min art video (Bob Ross episode) to measure pre-TSST self-reported state anxiety, heart rate, and salivary cortisol at baseline. Afterwards, participants were instructed about undergoing a mock job interview with a panel, during which they would be filmed, and audio recorded for further analysis. A sham microphone was attached to the participants' upper body. While participants were indeed videotaped in the panel room, the sham microphone was solely used for odour dissemination. Participants had 5 min to prepare a speech about their suitability for their dream job. Then, they were brought to the panel, which consisted of a woman and a man dressed in white lab coats, who were trained to provide standardized verbal instructions and to restrict any verbal and non-verbal feedback. In the panel room, participants underwent a 5 min surprise mental arithmetic task (i.e., subtracting 13 from 1022), delivered their 5 min speech, and answered at least one interview-style question from the panel. We changed the order of the original TSST, starting with the mental arithmetic task rather than the speech to avoid any disruption of the perceptual-sensory memory of the mock interview due to possible cognitive interference of the arithmetic task (Hagenaars et al., 2017; Iyadurai et al., 2019; Kessler et al., 2018).

2.3.2. Memory reactivation

On D14 participants returned to the lab for the memory reactivation task, which was performed using *Presentation*[®] software (Version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA) on a Dell OptiPlex 9010 desktop computer with headphones, keyboard, and mouse. We presented retrieval cues of auditory (\pm 5 s), olfactory (\pm 3 s), and visual sensory modality (8 s). In line with exposure therapy, the retrieval cues

were used as aids for retrieving the newly induced memory. Upon cue presentation, participants were asked to retrieve the memory of the TSST (referred to as the 'job interview') for 15 s, and then rate the memory on valence, arousal, and feelings of control and lastly on cue familiarity (see Fig. 2). To assess the effect of condition, we used two cues per modality, namely a TSST-associated and a control cue. Retrieval cues were presented semi-randomly with 20 s inter-stimulus intervals; no TSST-associated and control cues of the same modality followed each other. It should be noted that the control cues differ in the degree to which they are truly control cues: while the odour control cue is not related to the TSST experience at all, the auditory and visual control cues still refer to the TSST (albeit to a lesser extent than their TSST-associated counterparts).

2.3.2.1. Auditory cues. The TSST-associated auditory cue was a voice recording of the panel member repeating a phrase from the arithmetic task ("that was incorrect, please start again from 1022"). As control the same sentence was played but in a voice that was unfamiliar to the particular participant (i.e., a panel member for other participants).

2.3.2.2. Olfactory cues. As we aimed to study the acquired affective associations between the odour and the TSST, we eliminated the existence of prior associations by using two neutral, unfamiliar odours, Indol 10 % in DPG and Isobutyl Quinoline (Sym), as olfactory cues. The two odours were selected in consultation with a perfumer and rated as most neutral and least familiar compared to two other odour samples that were assessed in a pilot study (N = 10). One of the two odours was paired with the TSST, namely by exposing participants to the odour through an infused sham microphone and a scented tissue box in the panel room. To simulate a naturalistic setting and to avoid deviating too much from the original TSST, the odour was peripheral to the TSST, and no attention was drawn to the odour. The other odour was never presented and served as a control cue for memory reactivation. The odour paired with the TSST was alternated per testing week to have a similar number of participants in each odour condition (Indol 10 % in DPG = 62, Isobutyl Quinoline (Sym) = 70). During memory reactivation the odours were presented in two bottles, which participants were prompted

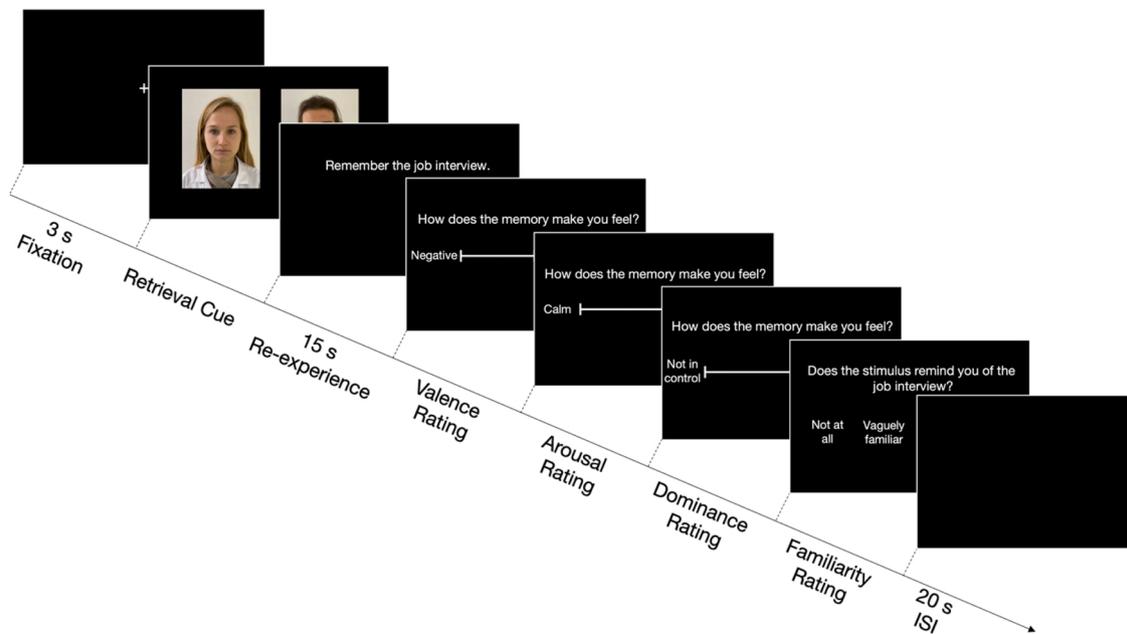


Fig. 2. Visualisation of memory reactivation. *Note:* Prior to the cue presentation, a centrally displayed fixation cross was shown. After 3 s, a cue was displayed, in this example a visual cue. Exposure to retrieval cue differed per modality (audition: \pm 5 s, olfaction: \pm 3 s, and vision: 8 s). Participants were instructed to think back of the memory of the job interview regardless of the cue. Next, valence, arousal, and feelings of control visual analogue scales were depicted consecutively for 8 s each, followed by a question relating to the familiarity of the cue and an inter-stimulus interval (ISI) of 20 s. Image not to scale.

to open and smell.

2.3.2.3. Visual cues. Portrait photographs of both panel members in white lab coats were presented during the memory reactivation task, see example in Fig. 2. The control cue was the same type of photograph but depicting a woman and man who were unfamiliar to the particular participant (i.e., who served as panel members for other participants), thereby controlling for general emotions associated with stern faces.

2.4. Measures

2.4.1. Manipulation checks

2.4.1.1. State anxiety. Participants assessed their state anxiety on the Spielberger State-Trait Anxiety Inventory (STAI-S; Spielberger et al., 1983), which was a back translation from the Dutch scale. State anxiety was measured pre-TSST (i.e., before hearing the speech instructions), directly (post1) and 20 min after exiting the panel room (post2). This is a 20-item inventory, rated on a four-point Likert scale (1 = *almost never* to 4 = *almost always*), resulting in a score between 20 and 80.

2.4.1.2. Salivary cortisol. Salivary cortisol was collected with salivette sampling devices (Sarstedt, Nümbrecht, Germany) pre-TSST and 20 min after exiting the TSST room, as peak cortisol response is best captured 30–45 min after TSST onset (Goodman et al., 2017). Subsequently, samples were stored at -20°C until analysis. Cortisol concentrations were determined by Dresden LabService GmbH. The samples were centrifuged at 3000 rpm for 5 min and the concentrations were obtained using chemiluminescence immunoassay (Tecan - IBL International, Hamburg, Germany; catalogue number R62111).

2.4.1.3. Heart rate. As the participants needed to walk and move freely during the stress induction, we required a mobile physiological measure. A Polar H10 chest strap, shown to be in satisfactory agreement with ECG (Plews et al., 2017), enabled us to continuously measure heart rate, while participants sat, stood, and walked between rooms. VSRP98 (Versatile Stimulus Response Registration Program) Version 12.1, June

2022, an inhouse programme of the University of Amsterdam running on Matlab, was used for data acquisition and reduction. Average heart rate was assessed per intervals: 5 min baseline, instructions and 5 min speech preparation, and the TSST itself (5 min arithmetic task, 5 min speech including the interview question(s)). For the baseline assessment participants watched a 5 min segment of an art video.

2.4.2. Emotional memory readouts

2.4.2.1. Self-report measures. After cue exposure and being asked to remember the job interview on D14, the affective component of the TSST-memory was assessed using visual analogue scales on valence (0 = *negative* to 100 = *positive*), arousal (0 = *calm* to 100 = *excited*), and feelings of control (0 = *not in control* to 100 = *completely in control*). Whether the retrieval cue was reminding the participant of the TSST was rated on a scale from 1 = *not at all* to 4 = *very vividly*. To improve interpretation such that a high score indicates a higher negative emotionality, valence and feelings of control were reverse coded, i.e., resulting in negative valence (0 = *positive* to 100 = *negative*) and feelings of lack of control (0 = *completely in control* to 100 = *not in control*).

2.4.2.2. Physiological measure. As a physiological measure of the response to the reactivated emotional memory, we collected heart rate data during the memory reactivation task with the same equipment as described above in Section 2.4.1.3. The physiological readout of emotional memory was the change in heart rate during retrieval, for which the average heart rate during the preceding inter-stimulus interval (20 s) was subtracted from the heart rate during memory retrieval (15 s).

2.4.3. Individual traits

To explore the relationship between the emotional memory response and individual differences, we assessed various individual traits, most of which were measured on D0. Trait anxiety was assessed using the Spielberger State-Trait Anxiety Inventory (STAI-T; Spielberger et al., 1983), which was an English back translation from the Dutch version. The STAI-T is a 20-item inventory, rated on a four-point Likert scale (1 =

almost never to 4 = almost always) with a range of scores from 20 to 80.

The Depression, Anxiety and Stress Scales (DASS-21; Lovibond and Lovibond, 1995) was used to capture symptoms of depression, anxiety, and stress. The DASS-21 consists of three scales, each consisting of seven symptoms that are assessed on how frequently they occur using a four-point Likert Scale, resulting in a score ranging from zero to 21.

As the TSST is a social stressor, we assessed social anxiety with the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987). The LSAS presents 24 different situations that are rated on a four-point Likert scale on both fear and avoidance, with scores ranging from zero to 144.

Stress sensitivity was assessed with the change in self-reported state anxiety (STAI-S) from pre-TSST to post1 (post1 – pre-TSST). To measure resilience, the ability to recover from stress, we calculated the difference in state anxiety (STAI-S) during recovery (post1 – post2).

As a physiological measure of stress regulation (i.e., autonomic arousal), heart rate variability (HRV) was additionally included as a trait (Appelhans and Luecken, 2006; Balzarotti et al., 2017). From the 5 min seated baseline on D7, HRV was calculated in the time domain using the root mean square of successive differences between normal heart beats (RMSSD).

2.5. Statistical analyses

As manipulation check, we used paired *t* tests to verify whether self-reported state anxiety (STAI-S) increased from pre-TSST to post1 and salivary cortisol increased from pre-TSST to post2. A repeated measures Analysis of Variance (ANOVA) was used to confirm an increase in heart rate from baseline, to speech instructions and preparation, and to the mock interview with the panels. Explicit knowledge of the cues' association with the TSST was inspected with a frequency table of the familiarity ratings.

Regarding our first research question, we tested whether the TSST-associated retrieval cues aided in achieving a stronger memory reactivation, operationalised as stronger subjective and physiological response upon memory reactivation, in contrast to the control cues. We conducted 2 (cue condition: TSST-related vs control) \times 3 (cue modality: auditory, olfactory, visual) repeated measures ANOVAs per outcome measure (negative valence, lack of control, arousal, and heart rate). Post-hoc pairwise comparisons tests with Bonferroni correction were used to determine the difference in condition. The effect of modality was controlled for, but not assessed, due to the differing degrees of relatedness to the TSST of the retrieval cues across modalities.

We explored whether the three self-reported measures of emotional reactivity (negative valence, arousal, lack of control) at memory reactivation could be condensed to reduce the number of tests for the exploratory analyses. For this we conducted a Bartlett's test of sphericity to test for redundancy between variables that can be summarised with fewer factors. A Kaiser-Meyer Olkin (KMO) test was employed to check the data's suitability for a factor analysis, for which a score between 0.7 and 0.8 was considered to be an indication of good factorability (Hutcheson and Sofroniou, 1999). In the case of significant findings, a principal component analysis was conducted to determine the number of factors our measures of negative valence, arousal, and lack of control, across the three modalities per condition could be reduced to. For the exploratory analyses, we conducted correlation analyses to identify to what extent the emotional response to the memory between TSST-associated and control cues may vary with individual traits.

To look at the potential effects of age, sex assigned at birth, and oral contraception we conducted post-hoc exploratory analyses. If a significant effect on the stress induction was found, we additionally checked for possible influences on the memory reactivation task. Spearman's rank correlations were computed to test the relation between age and stress responses (i.e., STAI-S, salivary cortisol, heart rate). We used mixed-measures ANOVAs with sex (woman vs man) as a between-subjects factor to test for any sex effects on the three stress measures. To explore possible effects of oral contraception use on stress induction,

we repeated the analyses of the manipulation check but excluding those participants that reported taking oral contraception.

Analyses were conducted using R version 4.0.3, RStudio version 1.3.1093. For significant results, Cohen's *d* or partial eta squared are reported as a measure of effect size. All analyses were two-sided with a significance level of $\alpha = 0.05$. In case of extreme outliers, data were log-transformed.

3. Results

3.1. Manipulation check

To confirm that our experimental manipulation was perceived as distressing, we compared self-reported state anxiety (STAI-S), salivary cortisol, and heart rate over time, see descriptive statistics presented in Suppl. Table 1. On average, participants rated their state anxiety (STAI-S) as significantly higher at post1 compared to pre-TSST ($t(131) = 10.40$, $p < .001$, 95% CI[9.10, 13.38], $d = 0.91$), see Fig. 3A. Log-transformed salivary cortisol increased significantly from pre-TSST to post2 ($t(104) = 4.09$, $p < .001$, 95% CI[0.06, 0.18], $d = 0.40$), see Fig. 3B. A one-way repeated measures ANOVA showed a significant main effect of time on heart rate ($F(1.53, 194.65) = 386.92$, $p < .001$, η^2

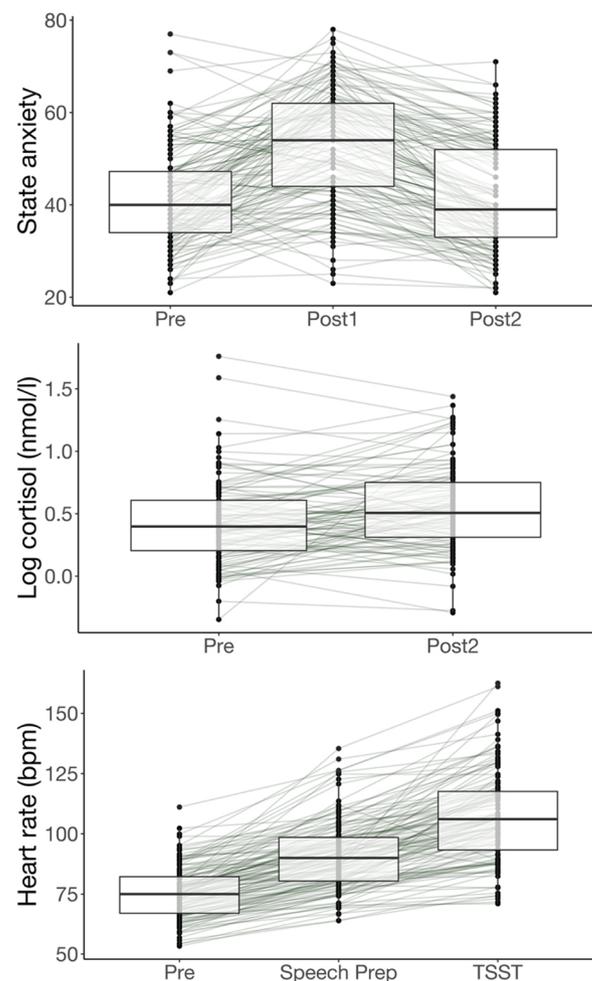


Fig. 3. Boxplots and individual trajectories of stress responses to the Trier Social Stress Test (TSST). *Note:* A State anxiety (STAI-S) at baseline (pre), directly after (post1) and 20 min after exiting the panel room (post2), $n = 132$. B Log-transformed salivary cortisol pre-TSST and 20 min after exiting the panel room (post2), $n = 105$. C Heart rate during baseline video (pre), speech preparation, and during the active part of the TSST, $n = 128$. Linear slopes are presented for each individual participant.

= 0.75), see Fig. 3C. Pairwise comparisons showed that mean heart rate significantly increased from baseline (pre-TSST) to speech preparation ($t(127) = 18.40, p < .001, 95\% \text{ CI}[13.60, 16.88], d = 1.63$) and from speech preparation to undergoing the active component of the TSST ($t(127) = 14.53, p < .001, 95\% \text{ CI}[14.22, 18.70], d = 1.28$). Yet, it bears mentioning that the increase in heart rate during the active component of the TSST (i.e., arithmetic task and speech) may also be explained by the participants engaging in more movement (walking to the panel room and standing) compared to the seated speech preparation (Gibbs et al., 2017; Watanabe et al., 2007). Nonetheless, a significant increase in heart rate was already apparent from baseline to speech instructions and preparation, during which there was no change in movement. Based on these results, we considered the stress-induction as successful.

Ratings of cue familiarity (a measure of the declarative component of the memory) are presented in Table 1. Exploratorily, we merged the categories into either *unfamiliar (not at all and vaguely familiar)* and *familiar (pretty well and very vivid reminder)* to compare frequencies. McNemar’s tests showed that most participants correctly identified the TSST cues as more familiar and the control cues as less familiar per modality (auditory: $\chi^2 = 19.15, p < .001$, olfactory: $\chi^2 = 45.46, p < .001$, visual: $\chi^2 = 109.01, p < .001$). As evident from Table 1, the visual TSST-related cue was most distinguishable from the control cue, which aligns with the visual cues resulting in the strongest differentiation in valence, arousal, and feelings of lack of control. The familiarity ratings also suggest that most participants recognized the voice of their panel member (i.e., auditory TSST cue). However, participants seemed uncertain about the auditory control cue, presumably due to its strong link to the TSST. The opposite pattern can be seen in the ratings of the olfactory cues: participants seemed certain that the control cue was unfamiliar, but seemed unsure regarding the familiarity of the TSST-related odour cue. This finding is in line with the TSST odour being a peripheral cue, suggesting that only a few people may have been aware of the smell.

3.2. Memory induction

With respect to the first aim of this study, we expected the TSST-associated retrieval cues to elicit stronger self-reported and physiological responses compared to control cues. Mean responses per cue and outcome measures are illustrated in Suppl. Table 2. The 2 (cue condition: TSST vs control) x 3 (cue modality: auditory, olfactory, visual) repeated measures ANOVAs on the self-reported outcome measures (negative valence, arousal, and lack of control) showed that participants had an elevated emotional response to TSST-associated compared to control cues, see Fig. 4. There was a main effect of condition for ratings of negative valence ($F(1, 128) = 91.96, p < .001, \eta^2 = 0.42$), arousal ($F(1, 128) = 59.60, p < .001, \eta^2 = 0.32$), and feelings of lack of control ($F(1, 128) = 66.57, p < .001, \eta^2 = 0.34$). Post-hoc pairwise comparison *t* tests with Bonferroni correction confirmed that the TSST cues led to a significantly stronger emotional reactivity compared to control cues across most sensory modalities. On negative valence the induced memory was rated significantly more negative with the auditory TSST cues ($t(128) = 2.94, p < .01, 95\% \text{ CI}[1.65, 7.96], d = 0.26$), the

Table 1
Frequency table of the rating of each cue on its familiarity to the Trier Social Stress Test (TSST).

Cue modality	Condition	Not at all familiar	Pretty well	Vaguely familiar	Very vivid reminder
Auditory	Control	12	28	31	58
	TSST	2	7	27	93
Olfactory	Control	89	32	5	3
	TSST	28	42	22	37
Visual	Control	72	40	11	6
	TSST	1	0	12	116

Note: n = 129. TSST = Trier Social Stress Test.

olfactory TSST cues ($t(128) = 4.63, p < .001, 95\% \text{ CI}[4.44, 11.06], d = 0.41$), and the visual TSST cues ($t(128) = 8.53, p < .001, 95\% \text{ CI}[13.45, 21.57], d = 0.75$) compared to their controls. The memory was rated as more arousing with the use of the auditory ($t(128) = 2.10, p < .05, 95\% \text{ CI}[0.30, 9.73], d = 0.18$) and visual TSST cues ($t(128) = 10.34, p < .001, 95\% \text{ CI}[16.83, 24.80], d = 0.90$), compared to the respective controls. A marginal significant difference was found in the arousal ratings between conditions for the olfactory cues ($t(128) = 1.89, p = .06, 95\% \text{ CI}[-0.21, 8.83], d = 0.17$). Compared to the control cues, the TSST-related cues triggered significantly stronger feelings of lack of control with respect to olfactory ($t(128) = 2.64, p < .05, 95\% \text{ CI}[1.31, 9.19], d = 0.23$) and visual cues ($t(128) = 9.66, p < .001, 95\% \text{ CI}[15.20, 23.02], d = 0.85$) compared to the controls, while there was no difference between the auditory cues ($t(128) = 1.91, p = .58, 95\% \text{ CI}[-0.11, 7.03]$).

No main effect of condition was apparent from the 2 (cue condition: TSST vs control) x 3 (cue modality: auditory, olfactory, visual) ANOVA on the difference in heart rate from the preceding inter-stimulus interval to memory retrieval per cue ($F(1, 127) = 0.00, p = .989$). As exploratory analysis, we compared the 5 min resting heart rate on D7 with D14, which showed that the mean resting heart rate on the day of memory reactivation (D14) was significantly higher by a mean difference of 5.63 bpm ($t(123) = 5.75, p = .001, 95\% \text{ CI}[3.69, 7.57], d = 0.52$). This difference in heart rate may be an indication of increased physiological arousal upon returning to the lab.

Together, these results confirm that we were able to induce an emotional memory with the TSST that was reactivated seven-days later with the aid of retrieval cues. However, the self-reported readouts did not directly correspond to our physiological measure of emotional memory. As hypothesised, the olfactory TSST cues were able to elicit a significantly higher rating on feelings of lack of control and negative valence compared to the control cues. No significant effect of condition was found among the olfactory cues on arousal and in heart rate.

3.3. Individual differences

In addition to the two primary study aims, we ran exploratory correlation analyses to investigate whether individual traits correlate with the emotional response upon memory reactivation by TSST-associated cues compared to control cues.

We explored whether we could limit the probability of type I error in our exploratory analyses by reducing our number of self-reported outcomes and thereby the number of tests. Bartlett’s test of sphericity showed a significant redundancy between the 18 variables (2 conditions x 3 sensory modalities x 3 self-reported measures), $\chi^2(153) = 1353.75, p < .001$. The KMO test was indicative of great factorability (KMO = .83; Hutcheson and Sofroniou, 1999). After removing the effect of sensory modalities, a principal component analysis was done to determine whether the self-reported measures of emotional reactivity could be captured in fewer variables. As the majority of variance in the data was explained by one factor (component 1: 71%, component 2: 18 %, component 3: 11%), we opted for a one-factor solution, namely a mean score across modalities (auditory, olfactory, visual) and self-reported measures (negative valence, arousal, lack of control) per condition, see Suppl. Table 3. To interpret whether specific traits may lead to a larger emotional response to the memory evoked by TSST cues vs to control cues, the difference score between condition was used as additional outcome variable.

Correlations are presented in Table 2. The explored individual traits are significantly correlated with the overall response to the TSST cues. All traits, besides HRV, are positively associated with the emotional response to the TSST cues. While the direction of correlation remains similar when considering the difference in mean self-reported response between conditions, stress sensitivity (STAI-S increase during the TSST) is the only trait showing a significant correlation. Some of the individual traits also show a small correlation with the physiological measure of

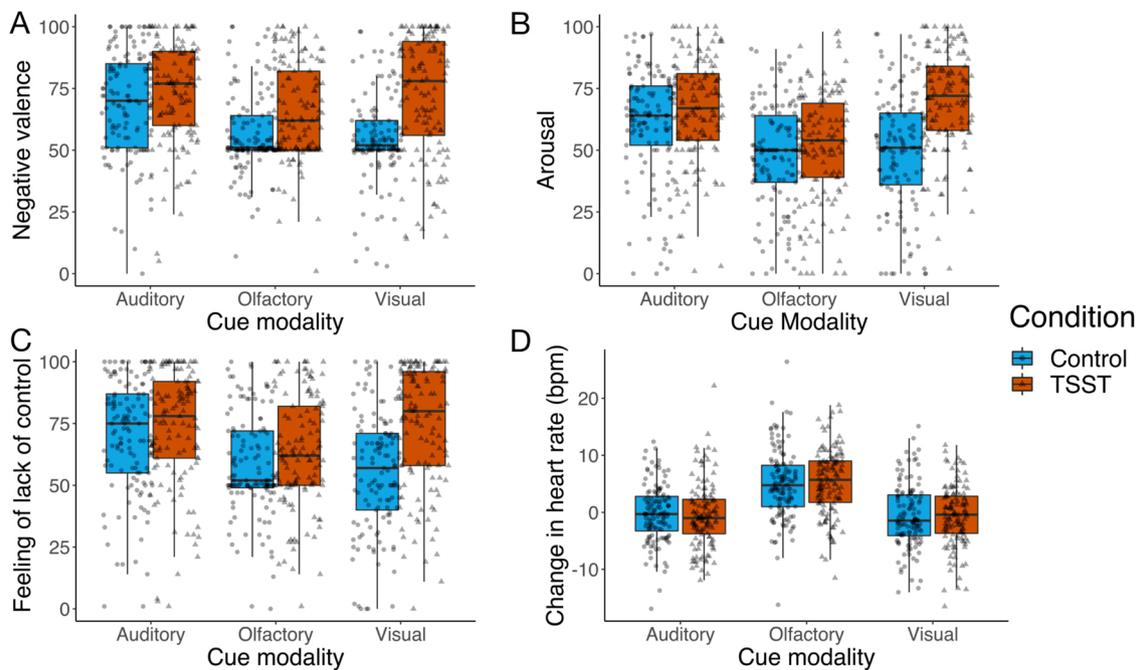


Fig. 4. Boxplots of negative valence, arousal, lack of control, and change in heart rate during memory retrieval per cue modality across condition. *Note:* A Negative valence, n = 129. B Arousal, n = 129. C Feeling of lack of control, n = 129, D Change in heart rate is measured from the mean preceding inter-stimulus interval to memory retrieval, n = 128. TSST = Trier Social Stress Test; bpm = beats per minute.

Table 2
Correlations of outcome variables of the memory reactivation task with individual traits.

	M (± SD)	Range	Mean self-report			Change in heart rate (bpm)		
			TSST	Control	Condition difference	TSST	Control	Condition difference
STAI-T	45.64 (9.15)	24–70	0.29***	0.24**	0.12	0.14	0.12	0.03
LSAS	46.94 (20.87)	6–106	0.35***	0.29**	0.13	0.09	0.19*	-0.07
DASS: Depression	10.97 (8.19)	0–42	0.18*	0.13	0.10	0.05	0.00	0.04
DASS: Anxiety	9.95 (6.88)	0–34	0.20*	0.22*	0.00	0.01	0.06	-0.04
DASS: Stress	16.66 (7.28)	2–38	0.20*	0.15	0.10	0.10	0.10	0.01
HRV (ms)	53.91 (22.77)	20.32–132.71	-0.17#	-0.16	-0.05	-0.03	0.00	-0.02
Stress sensitivity (STAI-S increase)	11.41 (12.35)	-19–39	0.38***	0.26**	0.23 *	0.06	0.06	0.01
Resilience (STAI-S recovery)	11.18 (8.95)	-9–40	0.29***	0.27**	0.08	-0.07	0.05	-0.10

Note: Mean self-reported emotional response per condition (TSST and control) and the difference between condition (TSST-control), averaged across modality and self-reported measures (negative valence, arousal, and lack of control). Heart rate is the change in heart rate (bpm) from the preceding inter-stimulus interval to memory retrieval per condition (TSST and control) and the difference between conditions (TSST-control) averaged across modality. # p = .05-.06; * p < .05 ** p < .01; *** p < .001. SD = standard deviation; TSST = Trier Social Stress Test; bpm = beats per minute; STAI-S/T = Spielberger State-Trait Anxiety Inventory - State/Trait; LSAS = Liebowitz Social Anxiety Scale; DASS = Depression, Anxiety and Stress Scales; HRV = heart rate variability. n = 124.

emotional memory. The change in heart rate with control cues has a significant positive association with social anxiety (LSAS).

3.4. Post-hoc exploratory analyses: Effects of age, sex, and oral contraception

We explored the potential effects of age, sex given at birth, and oral contraception use on stress induction. Firstly, age did not correlate with the stress induction (state anxiety (r(130) = [0.00 p = .982; salivary cortisol (r(103) = -.13, p = .202 and heart rate (baseline to speech preparation: r(126) = -.09, p = .328 speech preparation to TSST: r (126) = .08, p = .346). Secondly, no effects of sex were found on TSST-induced increases in state anxiety (STAI-S; main effect: F(1, 130) = 0.19, p = .665, interaction: F(1.75, 227.96) = 1.71, p = .187) or heart rate (main effect: F(1, 126) = 3.33, p = .070, interaction: F(1.53, 192.9) = 0.84, p = .406). However, for salivary cortisol, an interaction effect was found between sex and time (F(1, 103) = 19.10, p < .001, η²

= 0.16). While both men (t(22) = 4.94, p < .001, 95% CI[0.21, 0.50], d = 1.03), and women (t(81) = 2.00, p < .05, 95% CI[0.01, 0.12], d = 0.22) showed a significant increase in cortisol from pre- to post-TSST, the increase was stronger among men (t(29.92) = 30.80, p < .001, d = 1.03). To assess any possible effects of sex on the ratings of the memory, the ANOVAs were re-run with sex as a between-subjects variable. Descriptive statistics split by sex and oral contraception use are displayed in [Suppl. Table 2](#). A significant interaction between sex and condition (F(2, 254) = 6.61, p < .05, η² = 0.05) was apparent for negative valence. While there was no sex difference in negative valence for the auditory-evoked memory, women had a significantly larger difference in valence ratings of odour-evoked memories (t(127) = -2.54, p < .05, 95% CI[- 18.47, - 2.30], d = -0.56) and a larger difference (marginally significant) in valence ratings of visually-evoked memories (t(127) = -1.89, p = .06, 95% CI[- 19.58, 0.47]. d = -0.41) than men. Thirdly, we post-hoc explored the possible influence of oral contraception on stress induction by re-running the analyses on stress but

excluding participants who used oral contraception ($n = 14$; [Suppl. Table 1](#)). In this subsample the effect sizes of the change in state anxiety (STAI-S; $d = 0.90$) and heart rate (pre-TSST to speech preparation: $d = 1.57$; speech preparation to during TSST: $d = 1.27$) were comparable to the effect sizes seen in the whole sample (reported in [Section 3.1](#)). Yet, the increase in log-transformed salivary cortisol from pre- to post-TSST was stronger in the subsample excluding participants using oral contraception ($d = 0.46$) compared to the whole sample ($d = 0.16$). This may suggest that oral contraception may have diminished the cortisol response to the TSST, although the small sample size did not allow for formal comparisons.

4. Discussion

To test assumptions about the role of emotional memory in (the treatment of) symptoms of psychopathology, we need laboratory paradigms with high ecological validity. The first aim of this study was to examine the utility of the TSST as a model for an aversive real-life event to create an emotional memory that is sensory-rich and personally meaningful. Our slightly adapted version of the TSST produced significant distress, as reflected in self-reported anxiety, salivary cortisol, and heart rate, of comparable effect sizes reported in recent meta-analyses ([Goodman et al., 2017](#); [Helminen et al., 2021](#); [Miller and Kirschbaum, 2019](#)). In accordance with our hypothesis, the distressing experience left an emotional memory that we were able to reactivate with retrieval aids of different sensory modalities seven days later. That is, when the memory was retrieved with cues that referred to the TSST this elicited more negative affect, arousal, and feelings of lack of control compared to when the same memory was retrieved with control cues. We found that besides auditory and visual cues, neutral unfamiliar odours that were paired with the TSST experience were effective retrieval aids as well. Furthermore, the self-reported emotional response to the reactivated memory correlated with indices of anxiety, stress, and depression. Thus, aside from the well-known use of the TSST to induce stress, our findings show its translational utility for research on emotional memory.

While there was a significant increase in heart rate during encoding, specifically during speech preparation (the increase during the TSST itself could be partially attributed to the fact that participants were standing), we observed no change in heart rate in response to memory retrieval when contrasting TSST-associated cues with control cues. Hence, the elevated emotional response to the memory elicited by the directly related retrieval cues in self-report measures was not captured in the physiological readout. However, we noted that the participants' resting heart rate on the day of memory reactivation (D14) was significantly higher compared to the resting heart rate measured before the TSST manipulation (D7). The elevated baseline heart rate on D14 could be representative of a conditioning effect of the TSST experience. Perhaps due to the heightened physiological arousal at baseline on D14, there was no further increase in heart rate during memory reactivation with the use of, relatively brief, retrieval cues. Another potential limitation of heart rate is that it more closely captures action tendency or physiological mobilisation for threat ([Aue et al., 2007](#); [Scherer & Moors, 2019](#)) instead of the affective value of an experience. To address these shortcomings, future research could include more time-sensitive measures of physiological arousal such as skin conductance, both before and during the TSST and at memory retrieval, as well as measures that specifically capture the affective nature of the experience, such as facial electromyography ([Boxtel, 2010](#); [Dimberg et al., 2002](#); [Duken et al., 2021](#); [Golland et al., 2018](#)).

The memory reactivation task that we used in the present study aimed to mimic memory retrieval in a clinical setting: participants were instructed to think about the distressing event on every trial, aided by retrieval cues that differed in the degree to which they referred to the stressful event. Thus, participants rated the emotions related to the retrieved memory, rather than the cues itself. As the control cues presented in the memory reactivation task indirectly referred to the TSST,

participants presumably showed an elevated emotional response to the memory evoked by the TSST-related as well as the control cues. In consequence, the emotionality of the memory (now inferred from the difference in memory ratings aided by the TSST-related and control cues) is likely to be underestimated. A control group that would undergo a friendly TSST (see for example [Wiemers, Schoofs et al., 2013](#)) would allow a between-subjects comparison of emotionality of autobiographical memories of similar events that only differ in how distressing they are, likely resulting in larger differences in valence, arousal, and feelings of lack of control than observed in the present study.

Many therapies rely on exposing patients to (imagined or real) visual or auditory retrieval cues associated with past events. While odours are generally neglected in this area, they have shown to trigger (spontaneous) recollection of vivid episodic memories as well as emotions associated with such memories. Therefore, our second aim was to test whether neutral unfamiliar odours can aid retrieval of the lab-induced episodic memory. We found that with the help of the olfactory TSST-related cue compared to its control cue, the memory evoked more negative valence, feelings of lack of control, and arousal (marginally significant). These findings indicate that neutral odours that have no relevance to the aversive experience can become part of the memory representation, which is in agreement with prior studies that used neutral unfamiliar odours as successful retrieval aids in context conditioning ([Herten, Pomrehn et al., 2017](#); [Kastner et al., 2016](#); [Toffolo et al., 2012](#); [Wiemers et al., 2014](#)). However, there are limitations that warrant further discussion. We used unfamiliar ambient odours that we disseminated with a scented tissue box and a sham microphone during the TSST. During memory reactivation, participants were exposed to the odours by smelling from two glass bottles. While experimenters checked multiple times a day that the odours were still perceivable, we did not measure this using a special device, nor did we ask participants whether they were aware of the odour, to not draw attention to this. If ecological validity is less of a priority, a more automatized odour presentation, such as with an olfactometer, would better control the presentation, intensity, and duration of exposure during the TSST as well as during memory reactivation ([Herten, Pomrehn et al., 2017](#); [Kastner et al., 2016](#)). In addition, while most participants rated the memory reactivated by the control odour as more neutral (i.e., closer to 50) and more unfamiliar than the TSST cue, direct ratings of the odour e.g., before the TSST, could confirm whether they are indeed neutral as intended.

Although the direction of the responses to the reactivated memory was the same across modalities (with TSST cues eliciting more negative emotions than control cues), effects seemed generally smaller for auditory and olfactory cues compared to visual cues. However, the current paradigm does not allow for a direct comparison of the effectiveness of retrieval aids across modality because the extent to which the TSST-associated cues were explicitly distinguishable from the control cues varied across modality. For example, to control for prior associations with the odour, the olfactory control cue was an unfamiliar odour. Meanwhile, the auditory cue was a sentence from the TSST presented either in an unfamiliar voice or in the voice of the panel member, making it more difficult to differentiate between control and TSST-associated cues. Furthermore, the cues differed in their centrality to the TSST experience. The panel members presented in the visual cues and the sentence repeated in the auditory cues were central aspects of the TSST. In contrast, the olfactory cue was an irrelevant, peripheral cue and thereby less related to the gist of the experience because we prioritised ecological validity of the paradigm and avoided deviating from the original TSST too much. Studies that are specifically interested in comparing cue modalities could aim to equalize the retrieval cues across modalities in terms of how related each cue is to the experience and how central the cue was during encoding.

Regarding our third aim of establishing the translational significance of the paradigm at hand, we found that the emotional memory response between TSST and control cues was positively related to stress sensitivity (the degree to which participants showed an increase in anxiety

during the TSST). This suggests that participants who experienced the TSST as more distressing may have formed a stronger emotional memory of the event. Furthermore, a stronger self-reported response to the emotional memory elicited by the TSST-related cues was associated with elevated trait and social anxiety, depression, stress sensitivity, and resilience. In response to the control cues, the self-reported memory readouts also showed positive correlations with trait anxiety, social anxiety, stress sensitivity, and resilience, although to a lesser degree. Moreover, HRV had a marginally significant negative association with the memory evoked by TSST cues. Being a marker of autonomic arousal and emotion regulation (Appelhans and Luecken, 2006; Balzarotti et al., 2017), the negative correlation with HRV may reflect that inferior emotion regulation is associated with an elevated emotional response upon memory reactivation. Based on these exploratory findings, we conclude that our paradigm is related to risk factors for psychopathology, reflecting its potential as a model for studying the role of memory in mental health. Nonetheless, using this paradigm in (sub)clinical populations may raise additional ethical concerns, and participants with increased vulnerability to psychopathology may be more likely to prematurely drop out of the study. Thus, the current paradigm may have translational value, but might require some adjustments for a more vulnerable sample.

Aside from the limitations and suggestions for future research mentioned above, a number of other limitations are worth mentioning. Firstly, saliva samples were collected suboptimally by relatively inexperienced experimenters causing many samples to not have enough saliva in the tube. Secondly, the gender distribution of the sample was uneven. Given that affective disorders are more common in women than in men (Boyd et al., 2015; Wittchen & Jacobi, 2005), the sample may still be considered representative when developing models for investigating how emotional memory underlies symptoms of psychopathology. Nevertheless, participant sex and related factors, such as menstrual cycle and use of oral contraception, can influence stress reactions, including cortisol responses (Goodman et al., 2017). Indeed, post-hoc exploratory analyses showed that while sex had no effect on self-report and heart rate, men had a stronger increase in salivary cortisol during the TSST than women. Interestingly, women perceived the memory retrieved by visual and olfactory TSST-related cues as somewhat more negatively valenced than control cues compared to men. Another factor that should be considered is the age of a TSST panel relative to the age of the participants: the larger the age gap, the more convincing a panel may be, potentially resulting in a more distressing experience. The age range of our sample ended up being quite narrow, and the average age of the participants (first year bachelor students) was lower than the average age of the panel members (more senior bachelor- and graduate students), which may explain why we did not find a correlation between age and the stress manipulation. However, future studies that aim for a more representative (i.e., non-student) sample with a larger age range may want to work with older panel members.

5. Conclusion

We show that the TSST can be harnessed to induce an emotional episodic memory that is sensory-rich and personally meaningful. Although physiological responses (i.e., heart rate) were only observed during learning, and not at retrieval, participants rated the memory reactivated by cues that directly referred to the aversive experience as more emotional compared to control cues. Aside from visual and auditory cues, effective retrieval included neutral olfactory cues, that were ambiently presented during the TSST, and of which participants were not necessarily aware. This tentatively suggests that peripheral odours may be useful aids in exposure treatment to create a more sensory-rich environment, thereby increasing the overlap between the retrieval and the encoding context of the initial experience. Lastly, we show that the self-reported affective experience of the memories induced with this paradigm are associated with individual differences in anxiety, stress,

and depression. With its relation to individual differences, the TSST opens new avenues to test whether emotional memory is indeed a critical construct in the onset and treatment of symptoms. Existing emotional memory paradigms, such as fear conditioning and the trauma-film paradigm, have proven to be very useful for modeling certain aspects of anxiety- and PTSD symptomatology, such as heightened responses to feared objects and intrusive memories following a (life-)threatening event. The TSST models a different type of disturbing experience, i.e., a negative social (evaluative) event, which in real life could give rise to symptoms such as intrusions, feelings of inferiority, shame, and avoidance as seen in depression and anxiety disorders. Thus, the TSST has the potential to extend our arsenal of tools for studying the role of emotional memory in symptoms of psychopathology across a range of disorders. Overall, this study contributes to the development of ecologically valid methods for inducing and assessing episodic memory in the lab, which is a necessary first step towards critically testing assumptions regarding the role of emotional memory in psychopathology.

CRediT authorship contribution statement

Inga Marie Freund: Conceptualization, Methodology, Investigation, Analysis, Visualization, Writing – Original Draft, Writing – Review & Editing. **Jacqueline Peters:** Methodology, Investigation, Writing – Review & Editing. **Merel Kindt:** Conceptualization, Methodology, Writing – Review & Editing, Supervision. **Renée M. Visser:** Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psychneuen.2022.105971](https://doi.org/10.1016/j.psychneuen.2022.105971).

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