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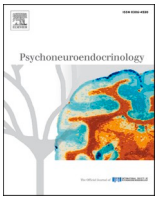

Acute stress reactivity and intrusive memory development: a randomized trial using an adjusted trauma film paradigm

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ABSTRACT

Understanding the neurobiological and cognitive processes underlying the development of posttraumatic stress disorder (PTSD) and its specific symptoms may facilitate preventive intervention development. Severe traumatic stress and resulting biological stress system activations can alter contextual memory processes. This may provide a neurobiological explanation for the occurrence of intrusive memories following trauma. Investigating the associations between temporal aspects and individual variation in peri- and post-traumatic hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system (SNS) stress reactivity and memory processing may increase our understanding of intrusive symptom development. The experimental trauma film paradigm is commonly used for this purpose but lacks robust SNS and HPA axis activation. Here, we performed an RCT to investigate the effect of an adjusted trauma film paradigm containing an added brief psychosocial stressor on HPA and SNS stress reactivity throughout the experiment and intrusive memory frequency in the following week in healthy males ($N = 63$, mean age = 22.3). Secondary, we investigated effects on film-related declarative memory accuracy and intrusion-related characteristics, and associations between acute HPA and SNS stress reactivity, film-related memory, glucocorticoid receptor functioning and intrusion frequency and characteristics. Participants were randomized to the socially-evaluated cold pressor test (seCPT $n = 29$) or control condition (warm water $n = 34$) immediately prior to a trauma film. Linear Mixed Models revealed increased acute SNS and cortisol reactivity, lower recognition memory accuracy and more intrusions that were more vivid and distressing during the following week in the seCPT compared to control condition. Linear regression models revealed initial associations between cortisol and alpha amylase reactivity during the experimental assessment and subsequent intrusions, but these effects did not survive multiple comparison corrections. Thus, with this adjustment, we increased the translational value of the trauma film paradigm as it appears to elicit a stronger stress response that is likely more comparable to real-life trauma. The adapted paradigm may be useful to investigate individual variation in biological and cognitive processes underlying early post-trauma PTSD symptoms and could advance potential preventive interventions.

1. Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric disorder occurring in approximately 10% of trauma-exposed individuals (De Vries and Olff, 2009). As PTSD is by definition preceded by traumatic events, this theoretically provides the opportunity for interventions early post-trauma to prevent PTSD development. To facilitate establishment of effective preventive interventions, further elucidation of the neurobiological and cognitive processes underlying the development of PTSD and its specific symptoms is warranted.

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PTSD symptoms include intrusive re-experiencing of the traumatic event, in the form of recurrent distressing involuntary memories, nightmares or dissociative flashbacks. Trauma-related involuntary memory phenomena have been conceptualized to lie along a continuum, with overlapping and distinctive quantitative and qualitative characteristics between memory types. In this continuum, PTSD's intrusive re-experiencing symptoms are placed at its most severe end (Meyer et al., 2014). Yet, trauma-related involuntary memories, including intrusive re-experiencing, are not specific to (prodromal) PTSD and are common after trauma, especially in the first weeks (e.g. Michael et al., 2005). In a prospective study, the presence and frequency of trauma-related involuntary memories in the first weeks post-trauma had limited predictive value for PTSD symptom severity 6 months after assault. However, the extent of distress, feelings of 'nowness', and lack of context associated with these intrusive memories explained almost half of the variance in symptom severity (Michael et al., 2005). This latter observation fits with several cognitive PTSD models posing that intrusive re-experiencing results from poor contextualization during memory encoding and consolidation in the first hours post-trauma, which leads to fragmented ('disjointed') memories that are prone to spontaneous or triggered automatic retrieval (Brewin, 2015; Ehlers et al., 2004). Recent neurobiological PTSD models also have addressed the accumulating evidence for the importance of altered contextual processing in the pathophysiology of PTSD (Liberzon and Abelson, 2016). In line with these models, lower general ability to contextualize emotional memories predicted subsequent intrusive memories development following experimental trauma (Meyer et al., 2017). However, it remains unknown whether peri- and acute post-traumatic contextual memory processing is indeed associated with subsequent intrusive re-experiencing (van Rooij et al., 2021).

There is increasing evidence that severe stress and resulting sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis activation together impact hippocampal-dependent contextual memory encoding (Schwabe et al., 2012; Lesuis et al., 2021). Specifically, non-genomic effects of cortisol on glucocorticoid receptors (GRs) within one hour post-stress decrease memory contextualization, while contrastingly later occurring genomic effects of cortisol increase memory contextualization (Sep et al., 2020). Thus, if contextual memory processing is indeed involved in intrusion development, these previous observations may provide a neurobiological explanation for the common occurrence of intrusive re-experiencing following traumatic stress. Yet, this provides no explanation for interindividual differences in the severity of intrusive memories following trauma and why only a minority of trauma-exposed individuals experiences long-lasting intrusive re-experiencing symptoms and develop PTSD.

A growing number of prospective studies link individual differences in SNS and HPA axis reactivity around the time of traumatic stress to subsequent PTSD development. Higher GR signalling as measured before, two days and within 1,5 week post-trauma predicted subsequent high long-term PTSD symptom levels in predominantly male samples (Engel et al., 2020; McFarlane et al., 2011; Steudte-Schmiedgen et al., 2015; van Zuiden et al., 2013). Additionally, low cortisol in the first hours post-trauma was repeatedly observed to predict PTSD development, potentially as a result of enhanced negative feedback on cortisol release following initial cortisol release due to high GR signaling (e.g. Mouthaan et al., 2014; Schultebraucks et al., 2021). Regarding SNS reactivity, the most consistent associations with PTSD development have been found for higher heart rate within 72hrs post-trauma (Morris et al., 2016). Additionally, PTSD development was found to be associated with blood pressure (Schultebraucks et al., 2021) and skin conductance reactivity to trauma reminders in the immediate post-trauma period (Hinrichs et al., 2019).

Importantly, these prospective studies typically only used PTSD diagnostic status or total PTSD symptom severity as outcome, and it has rarely been investigated whether identified predictors were associated with development of specific PTSD symptoms in the early post-trauma period. It seems worthwhile to investigate whether HPA and SNS reactivity around the time of trauma is associated with subsequent intrusive re-experiencing in the early post-trauma period, and whether this is mediated via trauma-related contextual memory encoding. For this purpose, the associations between temporal aspects and individual variation in peri- and post-traumatic stress reactivity, various types of trauma-related declarative memory, and subsequent trauma-related intrusive memories should be studied in further detail.

As repeated in-depth biological and cognitive assessment is neither feasible nor ethical during real-life trauma and subjective characteristics of intrusions cannot be reliably assessed in animals, currently this can only be investigated in healthy participants using experimental trauma paradigms. The trauma film paradigm is a commonly used experimental trauma paradigm that consistently induces short-term mild intrusive memories that share characteristics with trauma-related intrusive symptoms (James et al., 2016). There commonly is considerable variation in induced intrusive memory frequency and characteristics (Clark et al., 2015), as is the case for intrusive memories and PTSD development upon real-life trauma-exposure. However, the previous studies using the trauma film paradigm have not shown robust and consistent SNS and HPA axis activation (Chou et al., 2014; Rombold et al., 2016a; Rombold et al., 2016b; Weidmann et al., 2009). This lack of a reliably induced naturalistic stress response diminishes the paradigms' translational value as the magnitude, timing and duration of stress responses influences memory consolidation (Joëls et al., 2011; Schwabe et al., 2012) and intrusion development (Bryant et al., 2013).

In this randomized-controlled study in healthy male adults, our primary aim was to investigate the effect of an adjusted version of the trauma film paradigm containing a brief psychosocial stressor immediately prior the trauma film on HPA and SNS stress reactivity throughout the experimental paradigm as well as intrusive memory frequency in the following week. The socially-evaluated cold pressor test (seCPT) was used as psychosocial stressor, as it was previously shown to reliably induce HPA and autonomic nervous system (ANS) activation (Sänger et al., 2014; Schwabe et al., 2008). One previous study also adjusted the trauma film paradigm by adding a longer psychosocial stressor immediately prior to the trauma film in a female sample (Schultebraucks et al., 2019). This adjustment increased SNS reactivity prior to the trauma film and cortisol levels after the trauma film, but did not influence intrusion frequency. In contrast to this previous study, we additionally investigated the effects of the adjusted paradigm on declarative memory accuracy related to the trauma film and intrusion characteristics as a secondary objective. As a secondary aim, to further investigate the biological and cognitive processes underlying interindividual variability in trauma-related intrusive memories, we investigated whether acute SNS and HPA axis stress reactivity to the paradigm, acute film-related declarative memory accuracy, as well as salivary cortisol suppression upon oral dexamethasone ingestion as a measure of GR functioning were predictive of trauma film-related intrusion frequency and characteristics in the following week.

2. Methods

2.1. Participants

In this single-blind randomized-controlled trial (NL6550/NTR6739, Appendix A11), $N = 68$ healthy males (aged 18–40, all university educated) were randomized to the experimental seCPT (n = 34) or control condition (warm water condition; n = 34; details regarding sample size calculations, blinding and randomization in Appendix A1). Ultimately, $N = 63$ participants completed all procedures and were included in analyses (seCPT $n = 29$, warm water $n = 34$; Appendix A2 for flowchart). Inclusion criteria were Caucasian ethnic background (to prevent confounding of forthcoming genetic analyses), fluency in Dutch, Body Mass Index (BMI) of 18.5–30, and smartphone possession (required to report intrusions). Exclusion criteria were current (sub)

Table 1
Participant characteristics, memory accuracy at the experimental (T2) and follow-up (T3) assessment, and intrusion characteristics of the most prominent intrusion and film-related PTSD symptoms assessed at follow-up (T3).

	SeCPT (n = 29)	**Warm-water (n = 34)**		**Statistics**
Age (years)	22.52 (4.83)	22. 90 (3.89)		$U = 490.00$, $p = .97$
BMI (kg/m^2)	22.53 (2.51)	22.75 (2.25)		$T_{61} = -0.37$, $p = .72$
Smoking behaviour (n (%))				
Occasional smoker	7 (24.1%)	9 (26.5%)		$p = 1.00$
Screening prior T2				
DASS-21[1]				
Depression	2.03 (2.28)	1.32 (1.80)		$U = 379.500$, $p = .11$
Anxiety	1.41 (1.24)	1.29 (1.29)		$T_{61} = 0.37$, $p = .71$
Stress	3.86 (3.29)	2.47 (2.25)		$U = 372.00$, $p = .09$
Total score	7.31 (5.75)	5.09 (3.62)		$U = 393.00$, $p = .17$
PCL5[2]				
Total score	3.45 (3.45)	3.21 (3.89)		$U = 451.00$, $p = .56$
Cluster B - Intrusions	0.59 (0.98)	0.79 (1.43)		$U = 523.50$, $p = .63$
Cluster C - Avoidance	0.21 (0.49)	0.35 (0.60)		$U = 553.00$, $p = .27$
Cluster D - Negative Cognitions and Mood	1.24 (2.08)	0.74 (1.46)		$U = 423.50$, $p = .27$
Cluster E - Arousal and Reactivity	1.41 (1.78)	1.32 (1.59)		$U = 486.00$, $p = .92$
LEC5[3]	4.90 (3.28)	6.47 (4.07)		$T_{61} = -1.67$, $p = .10$
GR functioning prior T2				
CAR[4]				
AUCg	1083.46 (61.59)	1051.83 (139.36)		$T_{38.85} = 0.65$, $p = .52$
AUCi	303.28 (68.90)	309.73 (70.11)		$T_{52} = -0.07$, $p = .95$
DST[5]				
AUCg	105.20 (11.18)	144.97 (21.96)		$T_{40.81} = -1.62$, $p = .11$
AUCi	8.95 (4.85)	26.82 (15.58)		$T_{32.06} = -0.84$, $p = .41$
	SeCPT (n = 29)	**Warm-water (n = 34)**	**Between-subject Statistics**	**Within-subject Statistics**
Declarative memory accuracy			***Condition effects***	***Time effects***
At T2				
Cued Recall	6.40 (0.23)	6.82 (0.21)	F(1)= 1.00, $p = .32$,	F(1)= 5.28, $p = .03$
Recognition	9.46 (0.21)	10.24 (0.19)	F(1)= 4.30, $p = .04$	F(1)= 7.03, $p = .01$
Sequential Recall[6]	0.96 (0.01)	0.96 (0.01)	F(1)= 0.09, $p = .77$	F(1)= 7.97, $p < .01$
At T3				
Cued Recall	6.11 (0.21)	6.09 (0.20)		
Recognition	9.18 (0.26)	9.38 (0.24)		
Sequential Recall[6]	0.95 (0.01)	0.94 (0.01)		
	SeCPT (n = 28)	**Warm-water (n = 24)**		**Statistics**
Characteristics of most prominent intrusion at T3				

(*continued on next page*)

Table 1 (*continued*)

	SeCPT ($n = 29$)	Warm-water ($n = 34$)		Statistics
Vividness		0.41 (0.27)	0.47 (0.25)	$T_{50} = -0.90$, $p = .37$
Anxiousness	0.28 (0.23)	0.35 (0.27)		$T_{50} = -0.95$, $p = .35$
Unpleasantness	0.40 (0.30)	0.39 (0.28)		$T_{49} = 0.15$, $p = .88$
Distress	0.26 (0.23)	0.26 (0.24)		$T_{50} = 0.14$, $p = .89$
Disjointedness/fragmentation	0.55 (0.35)	0.44 (0.35)		$T_{50} = 1.20$, $p = .23$
Film-related PCL5 at T3[2]				
Total score	4.93 (3.99)	3.38 (4.47)		$T_{61} = 2.33$, $p = .02$
Cluster B-Intrusions	2.03 (1.80)	1.15 (1.37)		$T_{61} = 2.22$, $p = .03$
Cluster C-Avoidance	0.38 (0.62)	0.35 (0.65)		$U = 475.00$, $p = .75$
Cluster D-Negative Cognition and Mood	1.45 (2.38)	0.85 (1.21)		$T_{61} = 1.04$, $p = .30$
Cluster E-Arousal and Reactivity	1.07 (1.19)	0.71 (1.22)		$U = 390.50$, $p = .12$

Scores are displayed as raw, non-transformed mean(SD) and for memory tasks mean(SE) or n(%).[1]DASS-21: Depression, Anxiety and Stress Scale; [2]PCL5: PTSD Checklist for DSM5; [3]LEC5: Life Events Checklist, number of experienced traumatic event types when experienced personally, witnessed it, learned about it happening to close family members or friends, or if it happened at work; [4]CAR: cortisol awakening response; [5]DST: cortisol suppression using the dexamethasone suppression test; [6]Sequantial recall task accuracy was calculated by Spearman's Rank correlations (range 0–1); AUCg: area under the curve with respect to the ground, AUCi: area under the curve with respect to the increase; $p < 0.05$

clinical depressive, anxiety or PTSD symptoms; current major medical disorder; habitual smoking; use of medication known to impact HPA/ANS functioning; and lifetime diagnosis of any psychiatric disorder. Additionally, participants were excluded upon previous exposure to an event resembling trauma film-related events. Conditions did not differ regarding demographic characteristics, psychological screening or baseline GR functioning (Table 1). The Institutional Review Board of the Academic Medical Center approved the study, performed in accordance with the Medical Research Involving Human Subjects Act (WMO) and Declaration of Helsinki. All participants provided verbal and written informed consent and received a monetary reward (€40,-) or Student Course Credits.

2.2. Assessment procedures

2.2.1. Recruitment and screening

Participants were recruited through flyers and online advertisements targeted at university students. After indicating interest, a 10-minute screening took place by telephone. When eligible and upon continued interest, a face-to-face assessment (T1) was scheduled wherein current depressive, anxiety-related or PTSD symptoms and previous trauma exposure were screened using self-report questionnaires (details in Appendix A3). Weight was measured to determine BMI. Instructions for saliva collection and behavioural restrictions were provided and procedures practiced (Stalder et al., 2016). Behavioural restrictions for the experimental assessment (T2) included: no caffeine/nicotine/medication/drug use < 24hrs; no alcohol use during the prior evening; no physical exercise on the T2 day; no brushing teeth < 1hr. Participants were requested to eat a light lunch (low protein amount) before T2 began (details in Appendix A9).

2.2.2. Experimental assessment (T2)

The 95-minute T2 was scheduled in the afternoon to account for cortisol's diurnal rhythm (Fig. 1 visualizes procedure). Firstly, participants ate a candy bar for glucose level standardization and collected saliva was handed in. Thereafter, two experimental manipulations (seCPT and trauma film, see below), two resting measurements (*Baseline* and *Recovery*) and film-related declarative memory assessment were performed. Furthermore, participants were instructed how to report film-related intrusions experienced in the following 7 days (day 1 = T2 day). These procedures were interspersed with six 2.5-minute stress reactivity and recovery measurements.

2.2.3. Follow-up assessment (T3)

The 30-minute follow-up assessment (T3) took place exactly 7 days after T2. Film-related PTSD symptoms over the previous week were assessed using an adjusted PTSD Checklist for DSM-5 (PCL5), followed by re-assessment of film-related declarative memory and debriefing (Appendix A3 for details). When intrusion validity was unclear, additional details of reported intrusions were inquired upon and video recorded for reliability assessment purposes.

2.3. Experimental manipulations

2.3.1. Socially-evaluated cold pressor test (seCPT)

The seCPT is a well-validated brief, mild experimental stressor that induces acute subjective and HPA responses up to 60 min and ANS responses up to 20 min (Schwabe et al., 2008; Sänger et al., 2014; Appendix A4.1 for details). In the seCPT condition a female experimenter instructed participants to immerse their dominant hand up to their wrist into a plastic container filled with 0–3°C ice water (mean(SD)= 3.31°C (1.26)) and to persist as long as possible or until they could no longer tolerate the cold, without knowing the exact test duration (maximum 3 min). The experimenter took an impatient and non-appeasing demeanor and recorded facial expressions during the seCPT with the stated purpose of later evaluation (although not truly analysed). In the warm water condition, participants were instructed in a calm, friendly manner to immerse their dominant hand in water at body temperature (35–37°C; mean(SD)= 37.50°C(1.08)), not inducing any stress responses. In both conditions systolic blood pressure (SBP) was measured 1-min after the seCPT started, unless participants withdrew their hand earlier ($n = 3$ seCPT).

2.3.2. Trauma film paradigm

The well-validated trauma film paradigm was administered to induce intrusions (see Appendix A4.3 for ethical considerations; Holmes and Bourne, 2008). Participants watched a 15-minute aversive graphic scene from the movie *Irréversible* by Gaspar Noé (2002; Appendix A4.2

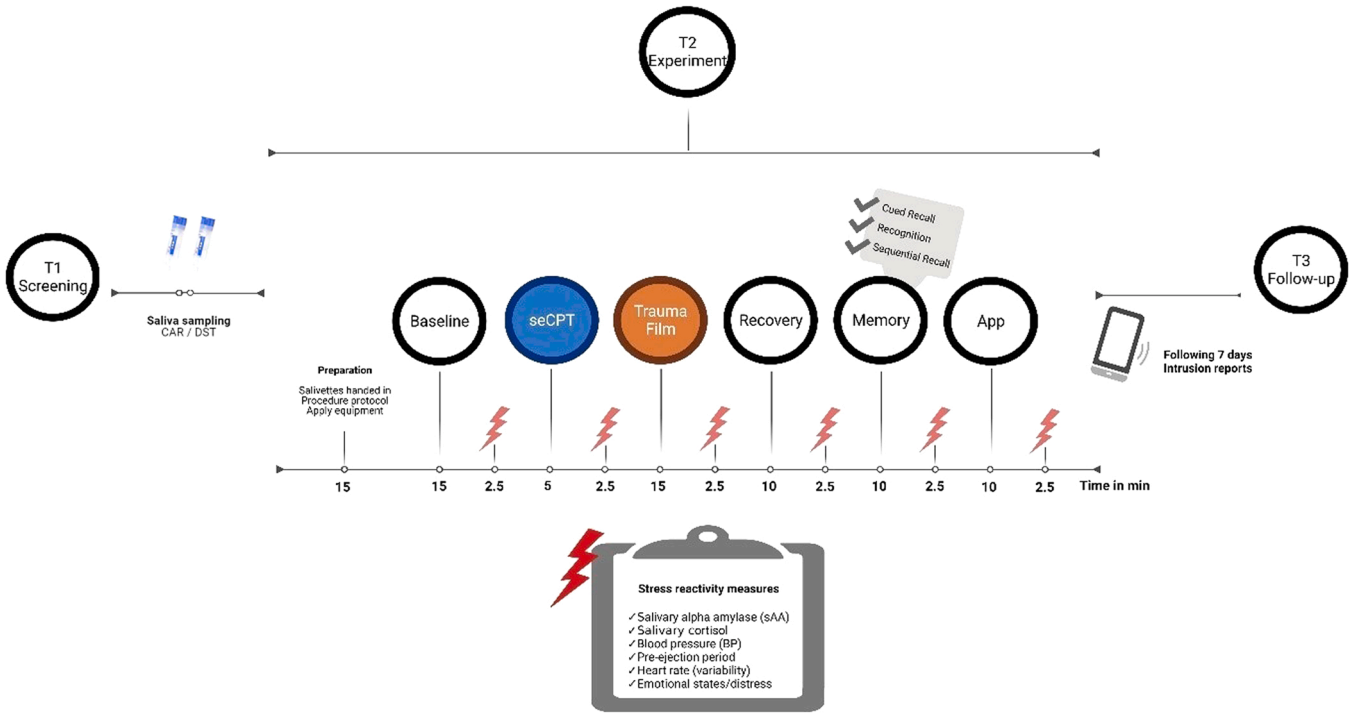

Fig. 1. Overview of all assessments, including a detailed procedure of the experimental assessment (T2). CAR: cortisol awakening response, DST: dexamethasone suppression test, seCPT: socially-evaluated cold pressor test, sAA: salivary alpha amylase, BP: blood pressure, PEP: pre-ejection period, HR(V): heart rate (variability).

for details). The fragment displays a woman suffering severe sexual and physical violence, and was previously found to induce short-term mildly distressing intrusions, immediate distress and negative emotions equally in women and men (Weidmann et al., 2009; James et al., 2016).

2.4. Measures

2.4.1. Acute stress reactivity

During the six stress measurements at T2, we collected; 1) salivary cortisol and alpha amylase (sAA) reactivity, 2) cardiac reactivity, i.e. pre-ejection period (PEP), heart rate (HR) and heart rate variability (HRV), 3) SBP, and 4) subjective emotional states and distress (Appendix A10 for details).

Salivary cortisol levels were assessed as a marker of HPA reactivity and recovery and sAA levels as a marker of ANS reactivity and recovery. To enhance reliability of sAA analyses, the unstimulated spitting method was applied using a standardized timing (2 min; Bosch et al., 2011). Cortisol levels were determined using ELISA (IBL International GmbH, Hamburg, Germany; Intra-assay variations <2.25%). For sAA a quantitative kinetic determination kit was used (Lyophilized, IBL International GmbH, Hamburg, Germany; Intra-assay variations <4.18%). Each sample was assayed in duplicate and their means were calculated. Additionally, 'Area under the Curve with respect to Ground' (AUCg) and 'with respect to Increase from baseline' (AUCi) were calculated for cortisol and sAA during stress reactivity (samples 2–3; after seCPT and trauma film) and recovery (samples 4–5–6; from *Recovery* measurement onwards (Pruessner et al., 2003)).

PEP, HR, and HRV were measured using the VU-Ambulatory Monitoring System. SBP was measured during each stress measurement using a separate monitor. PEP, HR and SBP were assessed to reflect SNS functioning. Root Mean Square of the Successive Differences (RMSSD) was assessed to measure vagal-parasympathetic modulation (Kleiger et al., 2005). Ultimately, mean RMSSD, PEP, HR (beats/min) for each separate stress measurement were averaged over three raters.

2.4.2. Intrusion frequency and characteristics

Participants were instructed to report all experienced film-related intrusions (i.e. involuntary, spontaneous memories) for 7 days in a smartphone app designed for this study (Appendix A5). The app was a digital version of the commonly used paper diaries in trauma film studies. For each intrusion, participants reported 1) date and time of occurrence, 2) short description of content, 3) *vividness* and *distress* (range 0–10), and 4) type (image-related, thought, mixture of both). To keep participants engaged, daily reminder notifications were sent (10 am and 10 pm, 13 total), upon which participants indicated if and how many intrusions they experienced since their last report. If participants did not respond > 24hrs, they were contacted by the researchers.

In line with previous studies, reported intrusions were considered valid when their nature was intrusive; their content film-related; and both *vividness* and *distress* > 0 (e.g. Ehlers et al., 2004; Schultebraucks et al., 2019). Additionally, participants needed to have rated their compliance at T3 ≥ 7. A second rater (blind to condition) scored reported intrusions of 20% randomly selected participants using app reports and videos of clarifying questions asked during T3. Interrater reliability was excellent (two-way mixed effects model, consistency, single measure interclass correlation=1.00, $p < .01$, Koo and Li, 2016).

For intrusion frequency, we counted the number of valid intrusions on every day separately and additionally calculated the total sum over 7 days. For intrusion characteristics *vividness* and *distress,* we calculated mean scores by dividing total *vividness/distress* scores by the number of reported intrusions (valid and invalid) for each day separately. We included both valid and invalid intrusions to avoid overestimation of *vividness* and *distress* scores across all experienced intrusive memories. Furthermore, we calculated total *vividness* and *distress* sum scores over 7 days. Additionally, Visual Analogue Scales (VAS; all ranges 0–1) were administered at T3 to rate intrusion reporting compliance and characteristics of the most prominent intrusion (i.e. the intrusion indicated by the participants to be most significant, unpleasant and distressing in the past 7 days, Davies and Clark, 1998).

2.4.3. Declarative memory accuracy

Participants completed three commonly used film-related memory tasks in standardized order at T2 and T3 (James et al., 2016). To prevent learning effects, two versions consisting of different questions were administered in randomized and counterbalanced order between sessions and conditions. The *Cued Recall* task consisted of 9 open questions on details of the victim and surroundings portrayed in the film (e.g. '*What colour was the victim's purse?*'). For each item there was only one unambiguously correct answer. If the whole or part of the answer was wrong, the whole item was scored as incorrect. Total scores were calculated by counting the number of correct answers (range 0–9). The *Recognition* task consisted of 12 true/false statements regarding either film-related gist or peripheral/central details. Total scores were again calculated by counting the number of correct answers (range 0–12). The *Sequential Recall task* consisted of 10 film-related events that had to be placed in order of occurrence, measuring contextual memory. Accuracy was calculated per participant as Spearman's correlation between ranks of correct and recalled orders (range 0–1; Wegner et al., 1996).

2.4.4. Subjective experience of the experimental assessment

Digital VAS were used during stress measurements at T2 (range 0–1; PsychoPy (v1.81)) to assess emotional states (*Anxious, Angry, Happy, Sad, Disgust, Distress*). These specific states were selected based on previous studies using the trauma film paradigm observing an impact on these particular states (Clark et al., 2015; James et al., 2016; Schultebraucks et al., 2019; Weidmann et al., 2009), and for how *Painful, Unpleasant, Difficult* and *Stressful* the seCPT was. Also, using VAS, participants indicated how well they maintained their focus while viewing the film and how much they felt that they empathized with and were immersed in the film fragment ('To what extent were you able to focus on the film?', 'To what extent were you able to empathize with the film?', 'To what extent were you immersed in the film') as this may influence the feeling of realism (i.e. feeling of being physically present as if they were witnessing the events happening in the film) that is associated with eliciting emotional responses such as subjective distress and changed emotional state to a film (Visch et al., 2010).

2.4.5. GR functioning

Participants collected saliva at home for CAR assessment on two mornings between T1 and T2 on prescheduled time points: immediately upon awakening, 30 min and 45 min after awakening (Stalder et al., 2016) using synthetic salivettes (Sarstedt, Rommelsdorf, Germany). Saliva from day 2 was used for GR sensitivity assessment using the dexamethasone suppression test (DST; Yehuda et al., 1991). Participants were asked to administer 0.5 mg dexamethasone (exogenous glucocorticoid) at 11 pm on the evening before sample collection. To check compliance with prescribed time points, participants were asked to take time-stamped photos of themselves at time of assessment. Cortisol levels were determined as outlined above.

AUCg and AUCi were calculated for CAR (2 ×3 samples). Since potential delays between saliva collection time points might cause false-low estimates of CAR (Stalder et al., 2016), we included individual sampling times between time points in our calculations and excluded samples when awakening times were missing ($n = 2$) or ≥ 5 min delayed (CAR $n = 7$, DST $n = 8$).

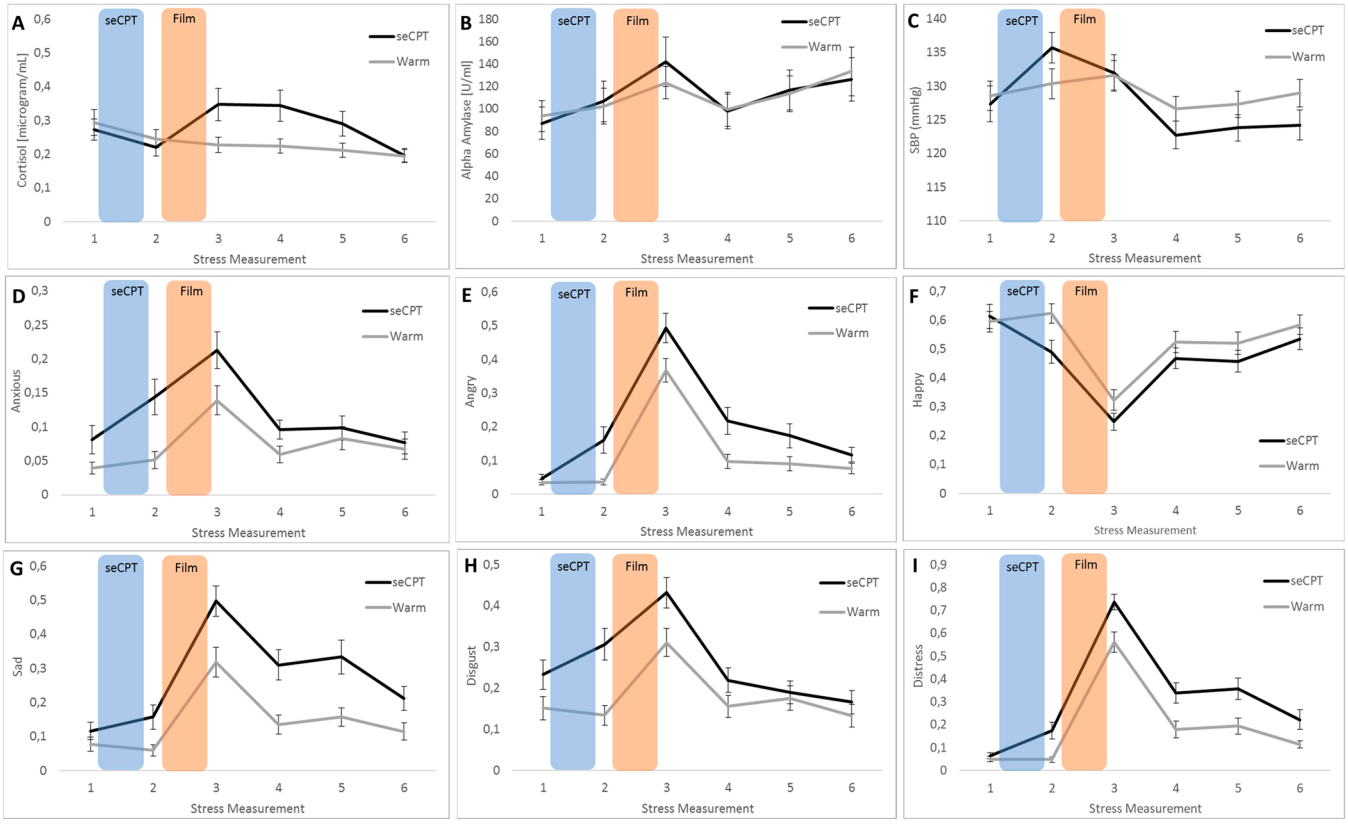

Fig. 2. Biological reactivity and emotional states and distress across the experimental assessment (T2). A. Cortisol reactivity. SeCPT 1: $n = 29$, 2: $n = 27$, 3: $n = 29$, 4: $n = 29$, 5: $n = 29$, 6: $n = 29$, warm water all measurement times 1–6: $n = 34$. **B.** Alpha amylase reactivity. SeCPT 1: $n = 29$, 2: $n = 28$, 3: $n = 29$, 4: $n = 29$, 5: $n = 29$, 6: $n = 29$, warm water 1: $n = 33$, 2: $n = 34$, 3: $n = 34$, 4: $n = 34$, 5: $n = 34$, 6: $n = 34$. **C.** Systolic BP reactivity. SeCPT $n = 29$, warm water $n = 34$. **D.** Anxious feelings. **E.** Angry feelings. **F.** Happy feeling. **G.** Sad feeling. **H.** Feeling distressed **I.** Feelings of disgust. For all emotional states: seCPT 1: $n = 29$, 2: $n = 29$, 3: $n = 29$, 4: $n = 29$, 5: $n = 29$, 6: $n = 29$, warm water 1: $n = 34$, 2: $n = 33$, 3: $n = 34$, 4: $n = 34$, 5: $n = 34$, 6: $n = 34$; Data are displayed as raw, non-transformed means (SE); sAA: salivary alpha amylase, SBP: systolic blood pressure.

2.5. Statistical analyses

All data were analysed using SPSS 26 (IBM SPSS Statistics Software). When assumptions for normality of distributions were not met, data was log-transformed before presence of outliers was checked ($Z \geq 3.29$). Outliers were winsorized to obtain a normal distribution (Reifman and Garrett, 2016) and excluded if winsorizing was not successful.

To assess differences in cortisol and sAA reactivity and recovery, RMSSD, PEP, HR, SBP, and subjective emotional states and distress across the six assessments, Linear Mixed Models (LMMs) with Restricted Maximum Likelihood (REML) were performed. Standard error covariance first-order autoregressive covariance structure (AR1) with random intercept and fixed slope were used as this has been recommended for randomized-controlled trials (model details and formula are provided in Appendix A6).

An overdispersed Poisson Generalized Linear Mixed Model (GLMM) with canonical link function was performed to assess differences between conditions in intrusion frequency per day, due to multicollinearity and zero-inflation dependent overdispersion ≈1.5 within Poisson-distributed count data (right-skewed approximating bi-nominal for days 1–7 with variance approximately equal to the mean; Crawley-Boevey, 2011; Payne et al., 2018). Negative Binomial Regression GLMMs were used to assess differences in mean *vividness* and *distress* scores per day, due to zero-inflation dependent overdispersion <1.2 within Poisson-distributed count data. In all GLMMs, condition, time and the interaction effect between time and condition were included as fixed predictors, and intercept was included as the only random effect. Details on sensitivity analyses for intrusion frequency of all reported intrusions, including invalid intrusions with vividness and/or distress scores = 0, are provided in Appendix A7.

Additionally, repeated measures Analyses of Covariance (rmANCOVAs) were performed to assess differences in memory task accuracy including condition as between-subject factor, time as within-subject factor, an interaction effect between time and condition, and task version as covariate.

Differences in all other variables were assessed using Independent samples *T*-tests and Mann-Whitney U tests for continuous variables and Pearson Chi-square tests, Fisher's or Fisher-Freeman-Halton exact tests (if>2 categories) for categorical variables.

Multiple Linear Regressions were performed to identify predictors of intrusion development (7-day frequency; *vividness* and *distress*; T3 PCL total score and Cluster B score) using the following separate models; baseline GR functioning: (1) DST AUCg, (2) DST AUCi; declarative memory accuracy at T2: (3) *Cued Recall*, (4) *Recognition*, (5) *Sequential Recall*; cortisol/sAA stress reactivity: (6) AUCg, (7) AUCi, and recovery (8): AUCg, (9) AUCi. Predictors were centered to prevent multicollinearity. For all models, main effects for condition and model predictors and interaction terms between condition and model predictors were included. Models 1 and 2 were corrected for CAR AUCg/AUCi and models 3, 4 and 5 for task version. To correct for multiple comparisons in the regression models, a False Discovery Rate threshold (5%) was applied (Benjamini and Hochberg, 1995).

3. Results

3.1. Primary outcomes

3.1.1. Acute stress reactivity

Biological stress reactivity in cortisol, sAA and all cardiac measures significantly changed across T2, with changes in cortisol and SBP differing between conditions.

For cortisol reactivity we found a significant interaction between condition and time ($F(5,292.04) = 7.66, p < .01$) and main effect of time ($F(5,292.04) = 13.89$, $p < .01$), but not condition ($F(2,69.64) = 1.51$, $p = .22$; Fig. 2 A). The seCPT participants showed a stronger increase in cortisol levels compared to *Baseline* after the film ($B = 0.14$, 95% CI 0.05–0.24, SE = 0.05, $t = 3.11$, $p < .01$) and during *Recovery* ($B = 0.14$, 95% CI 0.03–0.25, SE = 0.05, $t = 2.59$, $p = .01$) than warm water participants, but not for the other stress measurements (all p values >.05).

A significant time effect was found for sAA reactivity ($F(5,172.32) = 12.34$, $p < .01$; Fig. 2B). *s*AA significantly increased from *Baseline* after the seCPT ($B = 0.06$, 95% CI 0.01–0.12, SE = 0.03, $t = 2.29$, $p = .02$), after the film ($B = 0.19$, 95% CI 0.13–0.25, SE = 0.03, $t = 6.16$, $p < .01$), after the memory tasks ($B = 0.12$, 95% CI 0.05–0.18, SE = 0.03, $t = 3.73$, $p < .01$) and at the final assessment after explanation on how to report intrusions ($B = 0.16$, 95% CI 0.10–0.23, SE = 0.03, $t = 5.27$, $p < .01$), and were only not significantly higher during *Recovery* ($B = 0.04$, 95% CI −0.02 to 0.10, SE = 0.03, $t = 1.35$, $p = .18$). No significant interaction of condition and time ($F(5,172.32) = 0.44$, $p = .82$) or main effect of condition ($F(1,61.07) = 0.03$, $p = .86$) was found.

For SBP, a significant interaction effect between condition and time ($F(5,185.13) = 4.36$, $p < .01$) and main time effect ($F(5,185.13) = 13.86$, $p < .01$) was found, while the effect of condition was non-significant ($F(1,60.98) = 0.26$, $p = .61$). The seCPT participants showed a stronger increase in SBP levels compared to *Baseline* after the seCPT ($B = 0.03$, 95% CI 0.02–0.05, SE = 0.01, $t = 4.05$, $p < .01$) and the film ($B = 0.02$, 95% CI <0.01–0.03, SE = 0.01, $t = 2.02$, $p = .045$) than warm water participants, but not for the other stress measurements (all p values >.05; Fig. 2 C). Moreover, the additional SBP measured during the seCPT was significantly higher in the seCPT (mean(SD)= 141.32(18.17)) than warm water condition (123.85(11.34), $U = 166.50$, $p < .01$).

For RMSSD, PEP and HR, significant time effects were found (RMSSD $F(5,160.18) = 5.11$, $p < .01$; PEP $F(5,137.22) = 9.58$, $p < .01$; HR $F(5,162.91) = 9.86$, $p < .01$), without significant interactions between condition and time or main condition effects (RMSSD condition*time $F(5,160.18) = 1.80$, $p = .12$, condition $F(1,54.00) = 0.03$, $p = .87$; PEP condition*time $F(5,137.22) = 0.57$, $p = .72$, condition $F(1,54.25) = 0.002$, $p = .97$; HR condition*time $F(5,162.91) = 1.65$, $p = .15$, condition $F(1,54.16) = 0.17$, $p = .68$). RMSSD significantly decreased from *Baseline* after the film ($B = -0.04$, 95% CI −0.06 to −0.01, SE = 0.01, $t = -2.69$, $p < .01$). PEP significantly increased from *Baseline* after the film ($B = 3.12$, 95% CI 0.90–5.35, SE = 1.13, $t = 2.77$, $p < .01$), during *Recovery* ($B = 7.12$, 95% CI 4.67–9.56, SE = 1.23, $t = 5.76$, $p < .01$), during the memory tasks ($B = 7.23$, 95% CI 4.67–9.79, SE = 1.29, $t = 5.61$, $p < .01$), and final assessment ($B = 6.67$, 95% CI 4.04–9.30, SE = 1.32, $t = 5.06$, $p < .01$). HR significantly increased from *Baseline* after the film ($B = 1.17$, 95% CI −0.13 to 2.21, SE = 0.53, $t = 2.22$, $p = .03$), but significantly decreased from *Baseline* during the last assessment ($B = -2.22$, 95% CI −3.38 to −1.07, SE = 0.58, $t = -3.81$, $p < .01$). During the other stress measurements RMSSD, PEP or HR did not significantly differ from *Baseline* (all p values >.05).

3.1.2. Intrusion frequency

Of all $n = 341$ reported intrusions in the 7 days after T2, $n = 111$ (32.6%) were excluded from our analyses based on validity according to our definition. Ten participants (15.9%) did not report any intrusions, either valid or invalid (see appendix A7 for results on sensitivity analyses with intrusion frequency including invalid reports). Seventeen (27.0%) participants reported no valid intrusions, which did not differ between conditions (Pearson's Chi-square (1)= 1.08, $p = .30$). We found significant main effects of time (Wald Chi-square(6)= 90.96, $p < .01$) and condition (Wald Chi-square(1)= 5.11, $p = .02$), but no significant interaction between condition and time (Wald Chi-square (6)= 3.38, $p = .76$) on intrusion frequency per day. The seCPT participants reported significantly more intrusions per day across the 7 days than warm water participants ($B = 0.44$, 95% CI 0.10–0.78, SE = 0.17, Wald Chi-square = 6.31, $p = .01$; Fig. 3A), and both conditions showed a steady decline in the number of reported intrusions per day across the week ($B = -0.46$, 95% CI −0.57 to −0.36, SE = 0.05, Wald Chi-square = 72.32, $p < .01$). However, a summation of all reported valid intrusions

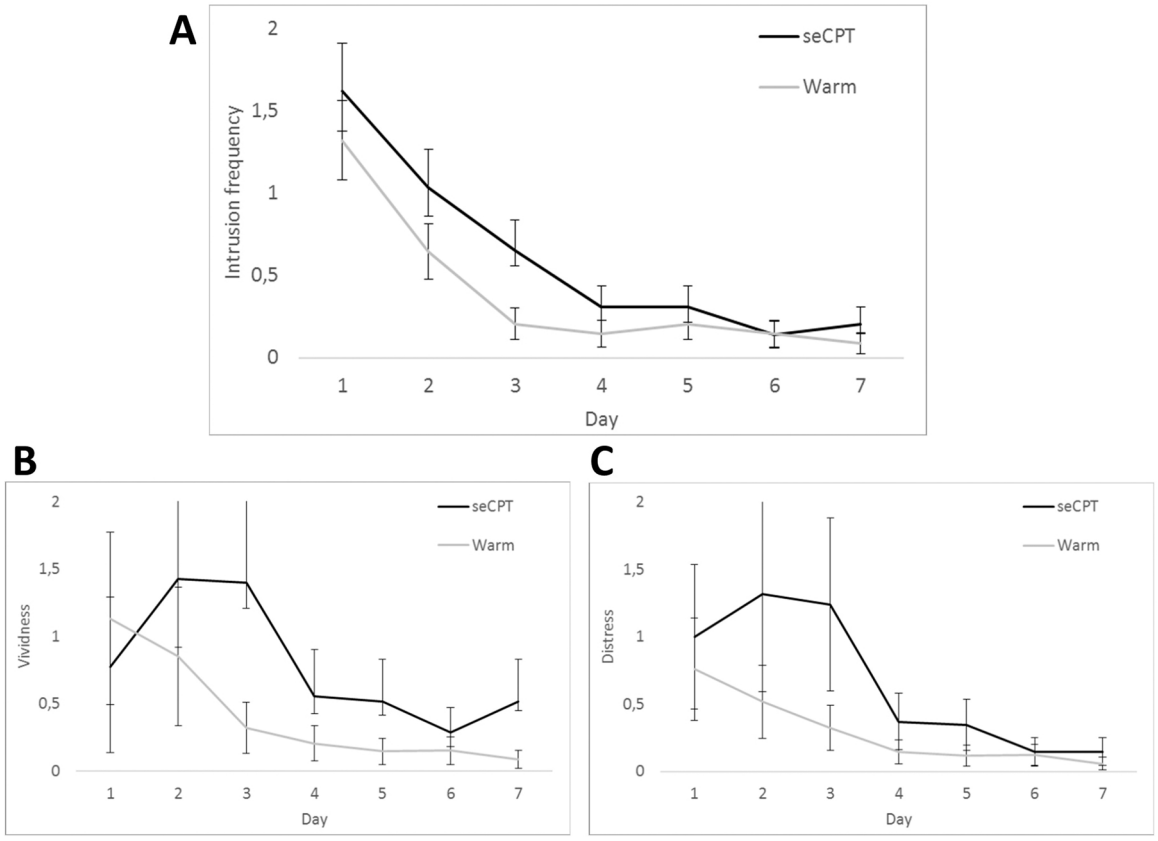

Fig. 3. Intrusion development during the 7-days after T2. A. Intrusion frequency during 7-days after T2, **B.** *Vividness* of intrusions reported during 7-days after T2, **C.** *Distress* of intrusions reported during 7-days after T2; Data is displayed as estimated marginal means (SE); seCPT $n = 29$, warm water $n = 34$.

across the week did not differ significantly between conditions (seCPT 4.48(5.42), warm water 2.94(3.30); $T_{61}= 1.62$, $p = .11$, $d = .41$). Notably, conditions differed significantly with regard to types of reported intrusions (Pearson's Chi-square(2)= 15.13, $p = .01$). In the seCPT condition, most intrusions were image-based (48.5%) rather than thoughts (35.8%) or a mixture of both (15.7%), while in the warm water condition types were almost equally distributed with slightly more intrusions being a mixture of images and thoughts (mix 37.5%; image-based 31.3%; thought 31.3%).

3.2. Secondary outcomes

3.2.1. Intrusion characteristics

For intrusion characteristics *vividness* and *distress* per day across the 7 days after T2, we found significant main effects of time (*vividness* Wald Chi-square(6)= 14.29, $p = .03$, *distress* Wald Chi-square(6)= 26.60, $p < .01$) and condition (*vividness* Wald Chi-square(1)= 6.82, $p = .01$, *distress* Wald Chi-square(1)= 6.32, $p = .01$), but no significant interactions (*vividness* Wald Chi-square(6)= 3.81, $p = .70$, *distress* (Wald Chi-square(6)= 1.63, $p = .95$). The seCPT participants reported more vivid ($B = 0.93$, 95% CI 0.34–1.53, SE = 0.30, Wald Chi-square = 9.45, $p < .01$) and distressing intrusions ($B = 0.31$, 95% CI 0.08–0.54, SE = 0.12, Wald Chi-square = 6.72, $p = .01$) than warm water participants. For both conditions the vividness and distress associated with the intrusions declined over time (*vividness* $B = -0.33$, 95% CI −0.47 to −0.18, SE = 0.07, Wald Chi-square = 19.08, $p < .01$; *distress* $B = -0.12$, 95% CI −0.18 to −0.06, SE=0.03, Wald Chi-square = 14.97, $p < .01$; Fig. 3B-C). Additionally, no differences between conditions were found in the summation of *vividness* (T_{61}=1.20, $p = .24$, $d = .39$) and *distress* (T_{61}=1.54, $p = .13$, $d = .30$) across the 7 days. Likewise, there were no significant differences between conditions in most prominent intrusion characteristics as reported during T3. seCPT participants had significantly higher PCL5 total scores during T3, indicating more film-related PTSD symptoms than warm water participants, mainly due to significantly higher scores on Cluster B-*Intrusions* (details are reported in Table 1).

3.2.2. Memory accuracy

seCPT participants had significantly lower scores on the *Recognition* task than warm water participants across both assessments (main condition effect: F(1)= 4.30, $p = .04$, $\eta_2 = 0.07$). No significant differences between conditions were found for *Cued Recall* (F(1)= 1.00, $p = .32$, $\eta_2 = 0.02$) and *Sequential Recall* (F(1)= 0.09, $p = .77$, $\eta_2 < 0.01$). On all tasks, memory accuracy significantly declined from T2 to T3 (main time effect: *Cued Recall* F(1)= 5.28, $p = .03$, $\eta_2 = 0.08$; *Recognition* F(1)= 7.03, $p = .01$, $\eta_2 = 0.11$; *Sequential Recall* F(1)= 7.97, $p < .01$, $\eta_2 = 0.12$), without significant differences in this decline between conditions (condition*time: *Cued Recall* F(1)= 1.00, $p = .32$, $\eta_2 = 0.02$; *Recognition* F(1)= 1.81, $p = .18$, $\eta_2 = 0.03$; *Sequential Recall* F(1)= 0.10, $p = .76$, $\eta_2 < 0.01$; Table 1).

3.2.3. Subjective experiences of the experimental manipulations

All assessed emotional states and subjective distress significantly changed across T2, with most changes differing between conditions (Fig. 2D-I; Anxious: condition*time F(5,189.52) = 2.29, $p < .05$, condition F(1,61.68)= 6.19, $p = .02$, time F(5,189.52)= 21.55, $p < .01$; Angry: condition*time F(5,188.04)= 2.27, $p < .05$, condition F(1,61.31)= 11.21, $p = .01$, time F(5,188.04)= 88.15, $p < .01$; Happy: condition*time F(5,177.92)= 2.63, $p = .03$, condition F(1,61.23)= 1.64, $p = .21$, time F(5,177.92)= 58.14, $p < .01$; Sad: condition*time F(5,188.12)= 2.28, $p < .05$, condition F(1,61.17)= 13.00, $p = .01$, time F(5,188.12)= 47.87, $p < .01$; Distress: condition*time F(5,189.52)= 2.29, $p < .05$, condition F(1,61.68)= 6.19, $p = .02$, time F(5,189.52)= 21.55, $p < .01$; Disgust: condition*time F(5,194.43)= 2.01, $p = .08$,

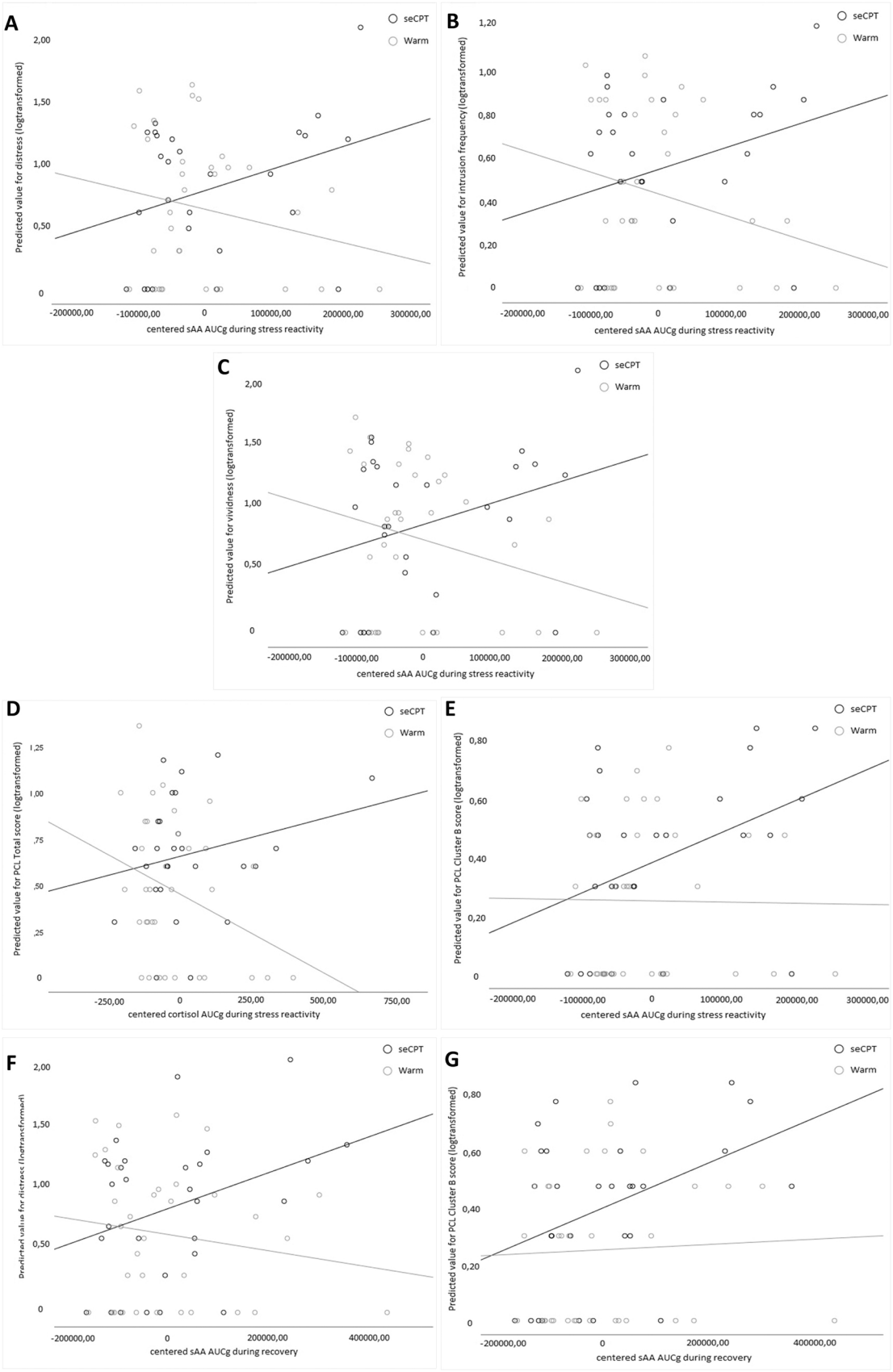

Fig. 4. Biological stress reactivity of the HPA axis and ANS predicting intrusive memory development. Graphs display the significant (uncorrected) interaction effects between condition and sAA AUCg **(A-C)**; between condition and cortisol AUCg during stress reactivity **(D)**; between condition and sAA AUCg during recovery **(E)**, and main effect of sAA AUCg during stress reactivity **(F)** and recovery **(G)**. Scores are displayed as log transformed estimated marginal means, predictors were centered; seCPT $n = 29$, warm water $n = 34$; sAA: salivary alpha amylase; $p < 0.05$.

Table 2
Results of linear regression analyses of GR functioning, biological stress reactivity and memory accuracy on intrusion frequency and intrusion characteristics reported during the 7 days after T2 and film-related PTSD symptoms administered during 7 day follow-up (T3).

Predictor variable	Intrusions									Film-related PTSD symptom scores					
	Frequency			*Vividness*			*Distress*			*Total*			*Cluster B-Intrusion*		
Model 1: GR functioning – AUCg *corrected for CAR*															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		8.096	< .001		7.938	< .001		7.652	< .001		9.454	< .001		7.317	< .001
Condition	-.211	-1.629	.109	-.153	-1.165	.249	-.190	-1.440	.155	-.304	-2.324	.024	-.275	-2.080	.042
DST	.090	0.324	.747	.065	0.233	.817	.102	0.362	.719	.011	0.040	.968	.145	0.512	.611
CAR	-.531	-1.570	.122	-.462	-1.350	.183	-.382	-1.112	.271	-.090	-0.263	.793	.019	0.057	.955
DST*Condition	-.278	-0.999	.322	-.244	-0.865	.391	-.292	-1.032	.306	-.014	-0.051	.959	-.215	-0.758	.452
CAR*Condition	.421	1.235	.222	.315	0.913	.365	.308	0.889	.378	-.100	-0.292	.771	-.122	-0.353	.726
Model 2: GR functioning – AUCi *corrected for CAR*															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		7.761	< .001		7.527	< .001		7.503	< .001		9.307	< .001		7.097	< .001
Condition	-.182	-1.400	.167	-.117	-0.886	.380	-.167	-1.287	.203	-.272	-2.094	.041	-.236	-1.820	.074
DST	-.033	-0.074	.942	-.204	-0.450	.654	-.007	-0.015	.988	-.326	-0.734	.466	-.370	-0.830	.410
CAR	.033	0.167	.868	.029	0.148	.883	.078	0.399	.691	-.048	-0.249	.804	.079	0.407	.686
DST*Condition	-.182	-0.411	.683	-.010	-0.023	.982	-.221	-0.498	.621	.270	0.610	.544	.222	0.501	.619
CAR*Condition	.156	0.805	.424	.107	0.544	.588	.113	0.582	.563	.060	0.309	.759	-.015	-0.080	.937
Model 3: Memory - Cued Recall *corrected for task version*															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		3.617	.001		3.422	.001		3.059	.003		2.335	.023		2.360	.022
Condition	-.224	-1.730	.089	-.170	-1.301	.198	-.206	-1.577	.120	-.269	-2.148	.036	-.269	-2.125	.038
Cued Recall	.201	1.015	.314	.183	0.911	.366	.104	0.521	.604	-.145	-0.759	.451	.084	0.434	.666
Task version	-.078	-0.506	.615	-.051	-0.325	.746	-.008	-0.051	.959	.241	1.617	.111	.110	0.727	.470
Cued*Condition	-.081	-0.457	.649	-.087	-0.482	.632	-.041	-0.230	.819	-.039	-0.225	.823	-.211	-1.215	.229
Model 4: Memory - Recognition *corrected for task version*															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		3.916	< .001		3.764	< .001		3.643	.001		3.678	.001		3.314	.002
Condition	-.145	-1.081	.284	-.098	-0.722	.473	-.124	-0.929	.357	-.247	-1.885	.064	-.244	-1.864	.067
Recognition	-.101	-0.539	.592	-.098	-0.517	.607	-.133	-0.714	.478	-.115	-0.628	.532	.072	0.394	.695
Task version	-.039	-0.299	.766	-.022	-0.165	.870	-.016	-0.122	.904	.113	0.891	.377	.047	0.373	.710
Recognition*Condition	-.109	-0.605	.548	-.098	-0.538	.593	-.121	-0.681	.499	-.038	-0.216	.829	-.248	-1.415	.162
Model 5: Memory - Sequential Recall *corrected for task version*															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		3.555	.001		3.451	.001		3.269	.002		3.333	.001		2.851	.006
Condition	-.204	-1.606	.114	-.153	-1.194	.237	-.194	-1.529	.132	-.297	-2.406	.019	-.280	-2.234	.029
Sequential Recall	-.157	-0.663	.510	-.194	-0.813	.420	-.214	-0.900	.372	.061	0.265	.792	.115	0.492	.624
Task version	.020	0.153	.879	.032	0.246	.807	.044	0.339	.736	.173	1.377	.174	.097	0.756	.453
Sequential *Condition	.282	1.197	.236	.310	1.304	.197	.301	1.274	.208	.089	0.389	.699	.011	0.049	.961
Model 6: Stress Reactivity AUCg															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		7.528	< .001		7.426	< .001		7.190	< .001		9.471	< .001		7.212	< .001
Condition	-.151	-1.167	.248	-.100	-0.773	.443	-.135	-1.037	.304	-.261	-2.122	.038	-.232	-1.869	.067
sAA	.279	1.593	.117	.292	1.663	.102	.311	1.772	.082	.261	1.568	.123	.399	2.379	.021
sAA*Condition	-.362	-2.074	.043	-.366	-2.097	.041	-.355	-2.033	.047	-.149	-0.901	.371	-.260	-1.556	.125
Cortisol	.060	0.339	.736	.082	0.462	.646	.074	0.418	.678	.220	1.310	.196	.184	1.087	.282
Cortisol*Condition	-.076	-0.433	.666	-.148	-0.843	.403	-.093	-0.530	.598	-.399	-2.395	.020	-.266	-1.586	.118
Model 7: Stress Reactivity AUCi															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		7.931	< .001		7.938	< .001		6.874	< .001		8.690	< .001		6.788	< .001
Condition	-.204	-1.518	.135	-.204	7.077	< .001	-.111	-0.800	.427	-.212	-1.569	.123	-.214	-1.585	.119
sAA	.180	1.039	.303	.180	-0.462	.646	.281	1.516	.135	.213	1.175	.245	.287	1.584	.119
sAA*Condition	-.206	-1.185	.241	-.206	1.512	.136	-.187	-1.013	.316	-.150	-0.830	.410	-.174	-0.964	.339
Cortisol	-.070	-0.367	.715	-.070	-1.052	.297	-.057	-0.277	.783	.156	0.776	.441	-.018	-0.088	.930
Cortisol*Condition	-.017	-0.091	.928	-.017	-0.274	.785	.043	0.210	.835	-.101	-0.508	.614	.015	0.076	.939
Model 8: Recovery AUCg															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		8.099	< .001		8.074	< .001		7.881	< .001		9.944	< .001		7.759	< .001
Condition	-.185	-1.425	.160	-.131	-1.003	.320	-.162	-1.260	.213	-.271	-2.175	.034	-.253	-2.005	.050
sAA	.314	1.660	.102	.317	1.671	.100	.360	1.926	.059	.350	1.929	.059	.408	2.219	.030
sAA*Condition	-.342	-1.820	.074	-.365	-1.930	.059	-.378	-2.029	.047	-.114	-0.633	.529	-.266	-1.457	.151
Cortisol	.042	0.269	.789	.043	0.273	.786	.080	0.513	.610	.166	1.100	.276	.084	0.552	.583
Cortisol*Condition	.033	0.215	.831	.047	0.302	.764	.054	0.356	.723	-.145	-0.980	.331	-.019	-0.124	.902
Model 9: Recovery AUCi															
	β	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***	**β**	***T***	***p***
(Constant)		7.954	< .001		8.011	< .001		7.675	< .001		9.956	< .001		7.727	< .001
Condition	-.185	-1.378	.174	-.123	-0.916	.364	-.169	-1.256	.214	-.234	-1.838	.071	-.257	-1.969	.054

(*continued on next page*)

Table 2 (*continued*)

Predictor variable	Intrusions									Film-related PTSD symptom scores					
	Frequency			*Vividness*			*Distress*			*Total*			*Cluster B-Intrusion*		
sAA	-.013	-0.073	.942	.032	0.187	.852	-.002	-0.014	.989	.110	0.664	.509	.063	0.370	.713
sAA*Condition	.063	0.363	.718	.090	0.517	.607	.073	0.419	.677	.120	0.722	.473	.028	0.163	.871
Cortisol	-.045	-0.171	.865	-.081	-0.309	.758	-.008	-0.031	.975	.046	0.185	.854	-.154	-0.604	.548
Cortisol*Condition	.077	0.296	.768	.188	0.725	.471	.069	0.266	.791	.146	0.596	.554	.240	0.952	.345

Significant results uncorrected for multiple testing. After False Discovery Rate correction ($p = .05$) for multiple comparisons according to Benjamini and Hochberg (1995) no results remained significant. sAA: salivary alpha amylase, AUCg: area under the curve with respect to the ground, AUCi: area under the curve with respect to the increase, DST: cortisol suppression using the dexamethasone suppression test, β: Standardized coefficients; seCPT $n = 29$, warm water interaction with cortisol $n = 34$, interaction with sAA $n = 33$.

condition F(1,63.31)= 15.38, $p < .01$, time F(5,194.43)= 137.69, $p < .01$). The seCPT participants showed a stronger increase in negative emotions and distress from *Baseline* after the seCPT (*Anxious* $B = 0.02$, 95% CI 0.01–0.04, SE = 0.01, $t = 2.07$, $p < .04$; *Angry* $B = 0.04$, 95% CI 0.01–0.06, SE = 0.01, $t = 2.95$, $p < .01$; *Distress* $B = 0.03$, 95% CI 0.01–0.06, SE = 0.01, $t = 2.29$, $p = .02$) and film (*Angry* $B = 0.03$, 95% CI 0.01–0.06, SE = 0.02, $t = 2.16$, $p = .03$; *Sad* $B = 0.04$, 95% CI 0.01–0.08, SE = 0.02, $t = 2.69$, $p = .01$), and during *Recovery* (*Angry* $B = 0.04$, 95% CI 0.01–0.07, SE = 0.02, $t = 2.35$, $p = .02$; *Sad* $B = 0.04$, 95% CI 0.01–0.08, SE = 0.02, $t = 2.74$, $p = .01$) and the memory tasks (*Sad* $B = 0.04$, 95% CI 0.01–0.07, SE = 0.02, $t = 2.56$, $p = .01$) than warm water participants, but not during the last assessment (all p values >.05). Positive emotions followed the opposite pattern, with seCPT participants showing stronger decreased happiness from *Baseline* after the seCPT ($B = -0.04$, 95% CI −0.07 to −0.02, SE = 0.01, $t = -3.61$, $p < .01$) and film ($B = -0.03$, 95% CI −0.05 to <−0.01, SE = 0.01, $t = -2.04$, $p = .04$) than warm water participants. Only for disgust no interaction effect was found, but main effects indicated that feelings of disgust increased from *Baseline* after the seCPT ($B = 0.02$, 95% CI 0.01–0.03, SE = 0.01, $t = 2.41$, $p = .02$), after the film ($B = 0.19$, 95% CI 0.17–0.20, SE = 0.01, $t = 20.87$, $p < .01$), during *Recovery* ($B = 0.07$, 95% CI 0.05–0.09, SE = 0.01, $t = 7.10$, $p < .01$), memory tasks ($B = 0.07$, 95% CI 0.05–0.09, SE = 0.01, $t = 7.52$, $p < .01$), and the final assessment ($B = 0.04$, 95% CI 0.02–0.06, SE = 0.01, $t = 3.83$, $p < .01$). Overall, the seCPT participants experienced more disgust across the experimental assessment than warm water participants ($B = 0.04$, 95% CI 0.02–0.06, SE = 0.01, $t = 3.58$, $p < .01$).

The seCPT participants experienced the seCPT as significantly more painful, unpleasant, difficult and stressful than warm water participants (all p values <.01; Appendix A8). There were no significant differences between conditions in film-directed focus ($T_{61} = 0.41$, $p = .68$, $d = .10$), empathizing ($T_{61} = 1.13$, $p = .27$, $d = .25$) and immersion ($T_{61} = -0.32$, $p = .75$, $d = -.08$).

3.2.4. Predictors of intrusion development

We found initially significant interaction effects between condition and sAA AUCg during stress reactivity on intrusion frequency and associated *vividness* and *distress* (frequency: $\beta = -0.362$, $p = .04$, $f^2 = 0.11$; *vividness:* $\beta = -0.366$, $p = .04$, $f^2 = 0.11$; *distress* $\beta = -0.355$, $p = .05$, $f^2 = 0.11$), and between condition and sAA AUCg during recovery on *distress* ($\beta = -0.378$, $p = .05$, $f^2 = 0.13$), indicating differential associations within both conditions (Fig. 4; Table 2). However, these effects were no longer significant upon corrections for multiple comparisons. Significant main effects of sAA AUCg during reactivity and recovery on film-related PCL5 Cluster B-*Intrusions* scores were initially found (stress reactivity: $\beta = 0.399$, $p = .02$, $f^2 = 0.21$; recovery: $\beta = 0.408$, $p = .03$, $f^2 = 0.18$), yet were also no longer significant upon multiple comparison corrections. Similarly, the interaction effect between condition and cortisol AUCg during stress reactivity on film-related PCL5 total scores was also no longer significant upon multiple comparison corrections ($\beta = -0.399$, $p = .02$, $f^2 = 0.23$).

4. Discussion

As our primary aim, we investigated whether adding a brief psychosocial stressor immediately prior to a trauma film increased acute HPA and SNS axis reactivity and subsequent film-related intrusion frequency across the following week in healthy men. Secondary, we investigated the effects of adding the psychosocial stressor on intrusion characteristics and film-related declarative memory. Lastly, we investigated associations between sAA and cortisol stress reactivity to the experimental paradigm, film-related declarative memory accuracy, GR sensitivity and film-related intrusive memory development. We found stronger HPA and SNS reactivity and more intrusions in the psychosocial stressor condition compared to the warm water condition. Men undergoing the psychosocial stressor also had lower film-related recognition memory accuracy and their intrusive memories were associated with higher levels of vividness and distress than men in the warm water condition. Secondary, we found indications for associations between cortisol and sAA levels throughout the experimental session and subsequent intrusion development, but these predictive effects did not survive corrections for multiple comparisons.

We found a stronger increase in cortisol and SBP reactivity to the trauma film in the experimental condition compared to the control condition. The original trauma film paradigm lacked reliably induced biological stress responses (Chou et al., 2014; Rombold et al., 2016a, Rombold et al., 2016b) and therefore has limited suitability for investigation of neurobiological and related cognitive processes underlying intrusive symptom development following traumatic stress. Our findings indicate that adjustment of the paradigm by adding the seCPT as a psychosocial stressor resulted in a more robust naturalistic biological stress response that is more similar to what is expected within a real-life trauma condition. This holds in particular for activation of the HPA axis, as we observed a less pronounced effect on SNS activation: we only identified a stronger increase in SBP, and not sAA, PEP or HR within the experimental condition.

Similar to Schultebraucks et al. (2019), who previously added a longer lasting psychosocial stressor (Trier social stress test) immediately prior to the trauma film in university educated healthy females, we demonstrated a stronger increase in cortisol after the film in men receiving the psychosocial stressor. Our findings regarding the SNS parameters differed from Schultebraucks et al., as we observed a stronger increase in SBP both after the psychosocial stressor and the film in the seCPT condition, while Schultebraucks et al. observed differences in SBP and sAA immediately after the stressor but not after the film. Such seemingly inconsistent effects on different measures of the SNS within and between studies are not uncommon and may be explained by their rapid changes upon and in the aftermath of stress exposure, making it difficult to capture SNS activation with all these measures at exactly the right moment (Bosch et al., 2011; Nagy et al., 2015).

Additionally, in this previous study, addition of the psychosocial stressor did not influence intrusion frequency, whereas we did find a higher frequency of intrusions upon psychosocial stress exposure. Also, addition of the psychosocial stressor in our study resulted in more pronounced subjective distress and negative emotions during the experimental session, while this was not observed by Schultebraucks et al. (2019). The different applied psychosocial stressors (seCPT versus Trier social stress test) may obviously have impacted these differential findings. Moreover, differences may also be due to samples consisting of females versus males respectively. Women are at increased risk for PTSD development following most types of traumatic events (Olff, 2017) and the increased risk for PTSD one year after traumatic injury within women was previously found to be mediated by higher initial PTSD symptom severity (Shalev et al., 2019). Although these findings concern PTSD diagnosis and not intrusive symptoms in particular, it seems counterintuitive at first sight that adjustment of the paradigm had a larger impact on our male sample than on the female sample of Schultebraucks et al., especially as our used film fragment portrays a victimized woman. Yet, previously no sex differences were observed in intrusion frequency after several commonly used trauma films including the fragment from *'Irreversible'* (Weidmann et al., 2009), used by both Schultebraucks et al. and ourselves. Moreover, there is increasing evidence that neurobiological processes underlying development of early post-trauma PTSD symptoms differ between males and females, and within females are also dependent on menstrual cycle phase, hormonal contraception and related estrogen and progesterone levels (Engel et al., 2020). Previous studies indicating predictive value of GR function and early post-trauma cortisol levels for early and long-term PTSD symptoms have been performed in predominantly male populations (Steudte-Schmiedgen et al., 2015; van Zuiden et al., 2013; McFarlane et al., 2011; Engel et al., 2020) and sex differences herein remain largely uninvestigated. Future studies using the (adjusted) trauma film paradigm to investigate intrusion development and its underlying processes should directly contrast male and female participants, preferably with various hormonal (estrogen and progesterone) statuses as well.

Contrary to our expectations, we did not observe differences between conditions in contextual film-related memory accuracy. We did however observe decreased accuracy on the *Recognition* task across T2 and T3 in the seCPT condition. In the absence of an effect on the other two memory tasks, at first sight this suggests poorer ability to recall facts about trauma film-related details and gist specifically. The used tasks were all based on previous trauma film studies (James et al., 2016), but it is well possible that more complex tasks should be used to capture potential effects on contextual memory and recall, especially as the participants performed quite well and there was very limited variability across participants.

Our secondary aim was to further investigate the biological and cognitive processes underlying interindividual variability in development of intrusive memories following traumatic stress, by means of analyzing whether acute cortisol and sAA reactivity and recovery as well as declarative memory accuracy during the experimental assessment predicted trauma film-related intrusion frequency and characteristics in the week following the experimental assessment, including interaction effects with allocated condition. We found some initial associations between cortisol and sAA levels throughout the experimental assessment and subsequent intrusion development, but none of these predictive effects remained significant after our stringent multiple comparison corrections. Although this clearly urges caution in interpreting these findings, we believe that the observed predictive effects with moderate effect sizes are worth a brief mentioning in light of future research into these processes.

In an exploratory data-driven analysis across both conditions to predict intrusion frequency following the trauma film from several biological and psychological features, Schultebraucks et al. (2019) observed that higher cortisol increases during the experimental paradigm were associated with higher subsequent intrusion frequency. Here, we found tentative evidence that cortisol AUCg levels during specifically the acute stress reactivity phase predicted PTSD-related total symptom scores the following week, with the directionality of the associations differing between the seCPT and warm water conditions. These differential effects are noteworthy given the fact that the seCPT condition showed a stronger increase in cortisol levels in response to the experimental paradigm and warrant further investigation. Similar to Schultebraucks et al., we observed tentative associations between higher overall sAA AUCg levels during both reactivity and recovery phases and more self-reported film-related intrusive symptoms using the PTSD symptom questionnaire at follow-up across conditions, which is interesting in light of previous meta-analytic findings across observational cohort studies that high SNS activity within the first 72hrs post-trauma predicted subsequent PTSD symptom severity (Morris et al., 2016). Although both Schultebraucks et al. and we focused on intrusive memories within one week post-trauma and not on sustained intrusive memories nor long-term PTSD outcome, the combined findings tentatively indicate that this previously observed predictive effect with the cohort studies may not be merely associated with sustained high SNS activity during early recovery following trauma, but also with high peri-traumatic SNS reactivity.

We did not observe any associations between film-related declarative memory accuracy, including the sequential recall task thought to reflect contextual memory, and subsequent intrusion development. Thus, our findings do not support the hypothesized mechanism of decreased contextual encoding of traumatic memories mediating associations between HPA and ANS functioning and intrusive symptom development, but as stated above, it may be worthwhile to investigate contextual memory encoding using another more complex task and not only focus on sequential recall.

A methodological strength of our study is that we used a digital application to increase accurate real-time intrusion reports immediately upon occurrence and to miss fewer reports, thought to lead to less recall bias and more accurate measures of intrusion frequencies and their related characteristics (Moskowitz and Young, 2006). At first sight, the observation that 27% of our participants reported no valid intrusions may indicate that our adapted paradigm and used experimental procedures did not result in reliably induced intrusions. However, Laposa and Alden, 2008 previously found a comparable percentage of 28% of their sample not reporting any distress-inducing intrusions after a trauma film. Also, if we take both valid and invalid intrusions into account, our observation that 15.9% of participants did not report any intrusions is highly similar to meta-analytic results of 15.5% of healthy individuals not reporting any intrusions (irrespective of vividness and distress) following trauma film viewing (Clark et al., 2015). Still, our strict in- and exclusion criteria, precluding all potential low-threshold psychological problems due to ethical considerations (James et al., 2016), may have influenced the generalizability of our findings as pre-trauma psychopathology has been identified as a risk factor for not only trauma film-related intrusive memories (Clark et al., 2015) but also PTSD development (Sayed et al., 2015). While it has been found that the majority of trauma-exposed individuals reports intrusive memories in the first weeks following trauma, these memories commonly decrease within the first months after trauma. Only a minority experiences long-term or sustained intrusive memories, of which an even lower percentage will fulfil diagnostic criteria for PTSD (Iyadurai et al., 2019). Thus, including a more heterogeneous population in terms of pre-existing psychological problems could give important additional insights into the development of these longer-lasting intrusive memories, although this also brings along additional ethical considerations and should be very carefully considered. In addition, the generalizability of our findings may be limited by the fact that we included only university educated Caucasian men, while lower educational level, minority ethnic status and being women have been found to be risk factors for PTSD (Brewin et al., 2000; Olff, 2017). Furthermore, as only a minority of trauma-exposed individuals experiences sustained intrusive

memories and eventually fulfil diagnostic PTSD criteria, it is important to be cautious in interpreting how our findings on the development of early post-(experimental) trauma intrusive memories may relate to sustained trauma-related intrusive re-experiencing, both in the absence or presence of PTSD diagnosis.

Several experimental procedure-related aspects may also have impacted subsequent intrusion development. These include the potential influence of the presence of the experimenter in the room during viewing of the trauma film, which has not been investigated currently. Studies using the trauma film paradigm have used a varying approach; in some studies the experimenter left the room (e.g. Holmes et al., 2009; Holmes et al., 2010; James et al., 2015; Lau-Zhu et al., 2019), while in others studies the experimenter was present to monitor whether participants looked away from the screen (Chou et al., 2014; Rombold et al., 2016a; Meyer et al., 2017; Rombold et al., 2016b). For this reason, as well as for safeguarding purposes in case of potential elicited distress, we also opted to have the experimenter in the room during film viewing (although fully out of sight). Furthermore, although common practice in studies using the trauma film paradigm, we cannot exclude that memory tasks and explanation on how to report intrusions during recovery at T2 – when it was not yet possible to report intrusions in the application – could have influenced intrusion development, particularly as we found increased sAA during recovery specifically during the memory tasks and intrusion reporting instructions. Finally, because of participant dropouts as well as technical issues with the VU-AMS that resulted in >10% of cardiac reactivity missing measures, our power was limited here.

5. Conclusions

We found that adding a brief psychosocial stressor prior to viewing a trauma film resulted in stronger increases in HPA and SNS axis activation during the experimental session, as well as increased intrusion frequency and associated vividness and distress during the following week in healthy men. The elicitation of a more robust stress response in this adapted version, likely more comparable to real-life trauma exposure, increases the translational value of the trauma film paradigm. The adapted paradigm may be useful to investigate effects of individual variation in and potentially pharmacological manipulation of biological stress reactivity, as well as underlying cognitive processes, on development of intrusive symptoms, as more insights into the biological and cognitive processes underlying development of early post-trauma PTSD symptoms could advance future effective prevention.

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Declaration of Competing Interest

None.

Data Availability

The data that support the findings of this study are available on request from the corresponding author, MVZ.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105686.

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