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Estradiol during (analogue-)trauma: Risk- or protective factor for intrusive re-experiencing?

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ABSTRACT

Intrusions, a key symptom of posttraumatic stress disorder (PTSD), can occur in the form of images but also as pain sensations. Similar to audiovisual intrusions, the frequency and persistence of pain intrusions varies greatly between individuals. In the current study, we examined whether peritraumatic circulating 17β-estradiol (E2) levels are a biologic factor associated with subsequent audiovisual (i.e., film) and pain intrusion development, and whether peritraumatic stress levels modulate this relationship. Forty-one free-cycling women participated in an ecologically informed trauma-pain-conditioning (TPC) paradigm, using trauma-films and pain as unconditioned stimuli. Independent variables were salivary peritraumatic E2 levels and stress indexed by salivary cortisol and self-reported state-anxiety during TPC. Outcomes were film- and pain-intrusions occurring during daily-life in the week following TPC and a Memory-Triggering-Task in response to conditioned stimuli 24 h after TPC. In the week after analogue-trauma, higher peritraumatic E2 levels were associated with a greater probability of experiencing film-intrusions in the beginning of the week, which switched to a lower probability toward the end of the week. This time-dependent relationship between E2 and film-intrusions only held for higher state-anxious women. In contrast, results indicated a consistent inverse relationship between peritraumatic E2 levels and pain-intrusions during daily-life and Memory-Triggering-Task. Together, these data suggest that higher peritraumatic E2 levels could be associated with lower long-term visual trauma intrusions, as well as lower pain-intrusions, and thereby possibly constitute a protective biologic factor for PTSD and potentially also for chronic pain.

1. Introduction

Intrusive memories, i.e., the distressing, involuntary, and recurrent retrieval of the traumatic event, are a core symptom of PTSD. Empirical studies support that intrusions emerge as conditioned responses (CRs) to previously neutral cues (conditioned stimuli, CSs) that have been associated with aversive stimuli (USs) (Franke et al., 2021; Wegerer et al., 2013). Recent evidence suggests that cue-driven intrusions can arise as mental images (e.g., the perpetrator's face) but interestingly also as pain sensations (Franke et al., 2022; Macdonald et al., 2018). Re-experiencing may thus not only perpetuate distressing images of the traumatic event, but also pain beyond tissue healing. Yet so far, factors associated with intrusion development after trauma within the somatosensory domain remain poorly understood.

Not all individuals automatically re-experience the traumatic event when exposed to trauma reminders. Evidence from the traditional audiovisual field suggests that whether individuals experience involuntary intrusions upon exposure to trauma reminders may depend on (1) how strongly the memory is consolidated and on (2) how well the memory is integrated in time and place (i.e., contextualized). Strongly

Abbreviations: E2, 17β-estradiol; P4, progesterone; US, unconditioned stimulus; CS, conditioned stimulus; UR, unconditioned response; PTSD, posttraumatic stress disorder; TPC, trauma-pain-conditioning paradigm; MTT, memory-triggering task; AP, aversive-film/painful stimulation; AnP, aversive-film/no-painful stimulation; NP, neutral-film/painful stimulation; NnP, neutral-film/no-painful stimulation; CORT, cortisol; STAI-S, State-Trait Anxiety Inventory-State.

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consolidated, but poorly contextualized memories are thought to be particularly prone to be re-experienced as intrusions (Pitman and Delahanty, 2005). More specifically, manipulations enhancing memory consolidation have been associated both with enhanced (Rombold et al., 2016) and reduced (Kleim et al., 2016) intrusions after trauma. Based on theoretical models of PTSD, the direction of this relationship depends on the extent to which individuals encode and stabilize *sensory cues* that later function as triggers for intrusions vs. the extent to which individuals concomitantly encode and stabilize *contextual details* which aid integrating the event in context, and thereby can inhibit involuntary retrieval (Brewin et al., 2010; Ehlers and Clark, 2000).

Research supports that *stress* enhances memory consolidation but reduces the ability to successfully encode contextual information due to hippocampal-function loss (de Quervain et al., 2016). For example, one study showed that exogeneous hydrocortisone administration immediately before fear acquisition reduced contextualization of fear. Interestingly, this finding of stress reducing fear memory contextualization was only observed in women, with the opposite pattern showing in men (van Ast et al., 2012). Thus, stress may reduce fear contextualization and thereby underly intrusion development especially in women.

A factor that may significantly influence fear contextualization in women is the sex hormone 17β-estradiol (E2). Research has strongly supported that E2 boosts the development of contextual memories and hippocampal-mediated memory consolidation (Taxier et al., 2020). Importantly, some studies have suggested that E2 also protects from stress-induced associative and declarative memory impairments (Antov and Stockhorst, 2018, 2014). Thus, the presence of high E2 levels may be especially important in preventing decontextualized, easily-triggered memories during highly stressful situations. In other words, women with high peritraumatic E2 levels (i.e., during high-stress) may remain more able to consolidate not only the sensory, but also the contextual details of the traumatic event. This enhanced memory consolidation but also contextualization could foster women's ability to integrate the event in time and place and thus prevent automatic cue-driven retrieval of the event.

Fear conditioning studies have offered a straightforward way of investigating cue-driven fear memories. They allow examining the ease with which CS ("trauma-reminders") trigger US ("trauma")-representations after individuals learned the CS-US association. Yet so far, while a host of studies has investigated the role of E2 levels on fear-conditioning processes, they have not yet tapped well into the process of CSs triggering a US memory that has already been consolidated into long-term memory (McGaugh, 2000). Concretely, from results suggesting that E2 facilitates fear acquisition we can infer that women with higher E2 possibly more easily develop associations between stimuli present at the time of the trauma and the event itself. However, from these stronger associations we may not automatically infer that trauma-related stimuli more easily trigger US("trauma") representations following the event. As discussed earlier, we expect this could, amongst other factors, depend on the degree to which women contextualized their fear memory into time and place. Similarly, studies suggesting a facilitating effect of E2 on fear extinction learning and recall (Garcia et al., 2018; Li and Graham, 2017; Merz et al., 2018; Ney et al., 2019) inform us on the association between E2 and processes involving the learning of an inhibitory CS-US memory or the updating of the original CS-US memory (Gershman and Niv, 2012), but not exactly on the association between peritraumatic E2 and spontaneous, involuntary recall of a consolidated memory.

In this vein, this study investigated whether natural peritraumatic E2 levels were associated with intrusion development following analogue-trauma. To examine both film- and pain-intrusions, we used a fear-conditioning procedure with trauma-films and pain stimulation as USs (Trauma-Pain-Conditioning (TPC) paradigm) (Franke et al., 2022). Main outcomes were film- and pain-intrusions occurring during daily-life in the week after TPC, and during Memory-Triggering-Task (MTT) in response to the CSs from TPC (24 h later). Predictors were salivary E2 levels, as well as stress indexed by salivary cortisol (CORT) levels and state anxiety during TPC. We expected an inverse relationship between E2 and film- and pain-intrusions during daily-life and MTT, and that this relationship would show especially in highly-stressed women.

2. Methods

2.1. Participants

As part of a larger study (Franke et al., 2022)we tested 74 healthy women between 18 and 35 years. The aim of the larger study ($N = 65$) was to investigate whether pain can occur as a conditioned response in the absence of nociceptive stimulation using functional magnetic resonance imaging (fMRI). Thus, while the larger study mainly investigated the classical conditioning nature of pain intrusions using, amongst others, multivariate fMRI-based pain markers, the current manuscript investigated the relationship between estradiol and stress in the development of pain- (and film-) intrusions in a final subsample of 41 naturally-cycling women. Common exclusion criteria were smoking, medication use, cardiovascular, neuroendocrinological, and pain-related disorders, plaster allergies, reports of current psychological/psychiatric disorders, blood-injury-injection phobia, absence of self-reported physical and psychological resilience, pregnancy, current breast-feeding, as well as high (>2–3x/week) consumption of extremely violent media. We excluded participants due to drop-out ($N = 3$), technical difficulties ($N = 3$), and suspected brain anomaly ($N = 1$). Since the current manuscript investigated the association between endogenous E2 and visual and pain intrusions, we excluded oral-contraceptive (OC)-users ($N = 26$) from main analyses,[1] leaving a final sample of 41 naturally-cycling women.

2.1.1. Hormonal status

Due to practical restrictions and the current study's focus on continuous E2 levels participation days were not precisely scheduled to coincide with a specific cycle phase. Nevertheless, for descriptive purposes we asked participants to report pre- and post-study menses start dates and assessed menstrual cycles based on these two dates. We used two approaches for assigning cycle days to one of the four phases (follicular, periovulatory, luteal, perimenstrual): (1) The forward-count cycle day approach which assigns each cycle day a number based on the last menstrual onset (i.e., day +1) by counting forward, and (2) the backward-count cycle day approach which assigns each cycle day a number based on the subsequent menstrual onset (day 1 = onset menses), by counting backward from that day. The day before the next menstrual onset is day − 1 and so forth. Based on a recent analysis of menstrual cycle characteristics of > 600.000 menstrual cycles (Bull et al., 2019), we estimated ovulation to fall on day − 12 for women with a typical cycle length (25–30 days, $N = 22$), day − 13 for women with longer cycles (31–50 days, $N = 13$), day − 11 for women with normal butshorter cycles (21–24 days, $N = 5$), day − 8 for very short cycles (15–20 days, $N = 1$). Cycle days three days before or after ovulation were coded as periovulatory. Cycle days after the periovulatory phase until day − 3 were coded as luteal. Cycle days before the periovulatory phase until day + 3 were coded as follicular. Cycle days − 3 to + 3 were coded as perimenstrual.

Table 1 details the distribution of menstrual cycle phases and respective E2/P4 levels.

2.1.2. Clinical characteristics

We assessed participants' current depression, anxiety, and stress symptomatology with the German versions of the Depression-Anxiety-Stress-Scale (Nilges and Essau, 2015) and the

[1] For the interested reader we exploratively analyzed the development of visual and pain intrusions in OC-users and naturally-cycling women and report results in Supplement 11.

Table 1
Distribution of menstrual cycle phases and respective estradiol and progesterone levels in the current study.

		Estradiol		Progesterone	
	N	*M*	*SD*	*M*	*SD*
Perimenstrual	11	0.86	0.35	55.96	29.48
Follicular	13	0.80	0.48	32.31	26.58
Periovulatory	4	0.94	0.39	18.64	6.03
Luteal	13	1.21	0.58	164.06	103.40

Note: Based on P4 levels, we re-assigned menstrual cycle phases of six participants. Allowing a human error in reporting menses by 2–3 days, women whose P4 levels strongly deviated from the assigned menstrual cycle phase were re-assigned to the more likely menstrual cycle phase. Specifically, two participants tested on days − 19 and − 18 (coded as follicular phase) showed P4 levels > 100 pg/ml, which suggests that these women were more likely already in the luteal phase (P4 levels in the follicular phase are generally < 43 pg/ml). Similarly, two other participants tested in the periovulatory phase on days − 13 and − 12 also showed P4 levels > 100 pg/ml (typical for the mid-luteal phase) and were thus re-assigned to the luteal phase. Further, a participant tested on day + 4 showed P4 levels > 100 pg/ml was re-assigned to the perimenstrual phase. Since the start of menses was self-reported by participants, it may be the case that this participant mistook smear bleeding (already starting in the luteal phase) by her actual menses. Noting that one day later (Memory-Triggering-Task session) her P4 levels dropped to 45 pg/ml, we assume that the participant may not yet have been in the follicular phase during the Trauma-Pain-Conditioning paradigm but instead still been in the perimenstrual phase of the menstrual cycle. Finally, one participant tested on day − 4 was re-assigned to the perimenstrual phase based on P4 levels < 43 pg/ml (unlikely for luteal phase). Abbreviation: *M* = mean; *SD* = standard deviation; P4 = progesterone.

State-Trait-Anxiety-Inventory (Laux et al., 1981). Further, we assessed somatization symptoms during the seven days before TPC with the Screening for Somatoform Symptoms-(SOMS-7) (Rief and Hiller, 2003). To assess PTSD-symptomatology, we used the German version of the revised Impact-of-Event Scale (Maercker and Schützwohl, 1998) applied to the individually-rated most distressing life event. As displayed in Table S1 in *Supplement1*, participants' scores were within normal ranges.

2.2. Ethics statement

The study was approved by the local Ethics Committee. All participants provided written informed consent prior to participation and were reimbursed with course credit or 80 Euro.

2.3. Screening procedures

Participants completed online-questionnaires screening for inclusion criteria up to one week before the first laboratory session. Participants were asked to refrain from eating, nicotine, and caffeine for 2 h prior to the study, and to avoid alcohol or excessive exercise for 24 h prior to the study.[2]

2.4. Experimental design

We employed a classical conditioning procedure with a 2 (*Valence*: aversive-films/neutral-film) x 2 (*Pain*: painful-stimulation/no-painful stimulation) within-subject design. This resulted in four USs (AP: aversive-film/painful stimulation; AnP: aversive-film/no-painful stimulation; NP: neutral-film/painful stimulation; NnP: neutral-film/no-painful stimulation) and four CSs signaling those USs. As detailed below, we paired the CSs with the USs during Day 1, and re-exposed participants to the CSs 24 h later during MTT. See Fig. 1 for details.

2.4.1. Trauma-pain-conditioning (TPC)

Sessions started between 15:30–19:30 with participants signing informed consent, completing the pre-TPC State-Trait-Anxiety-Inventory-State (STAI-S) (Laux et al., 1981), and providing the first saliva sample.

The TPC consisted of a conditioning procedure containing highly aversive trauma-films and electrocutaneous pain-stimulation as USs. *Supplement2a* provides further details on the used film-clips. During one of the aversive and one of the neutral film-clips, participants received painful electrocutaneous stimulation, individually calibrated to be rated as painful between '6′ and '7′on a scale ranging from 0 (*not painful at all*) and 10 (*maximum pain tolerance*), for a total duration of 12.5 s applied in seven pulse trains with a duration of 988 ms each, and an inter-stimulus interval of 400–1300 ms. The first pulse always coincided with film-clip onset. *Supplement2b* details the calibration procedure. Together, the aversive trauma-films and pain-stimulation (i.e., the US_{AP}, the US_{AnP}, and the US_{NP}, and after successful learning also the related CSs) aimed at provoking a highly stressful experimental situation.

CSs were four images resembling contextual elements of each film-clip, lasted 4 s and immediately preceded the respective USs; see Fig. 1 for details. After TPC, participants were immediately released from the MRI-room, provided the second saliva sample, and completed the post-STAI-S questionnaire.

2.4.2. Memory triggering task

To secure full memory consolidation (McGaugh, 2000), participants underwent the MTT 24 h after TPC. As we wanted to keep US-expectancy high during MTT, we started the session by repeating the pain calibration and showing the US_{AnP}. As displayed in Fig. 1, during MTT participants saw the CSs from the TPC without corresponding USs intermittently in a block design.

2.4.2.1. Fear-conditioning ratings

2.4.2.1.1. Unconditioned responses (URs). Participants rated each of the four US conditions on pain (0 =*not painful*, 10 =*maximally tolerable*) and valence (0 =*very pleasant*, 10 =*very unpleasant*) at the end of acquisition.

2.4.2.1.2. Conditioned responses (CRs). Participants rated each CS on pain (0 =*not painful*, 10 =*maximally tolerable*) and valence (0 =*very pleasant*, 10 =*very unpleasant*) while seeing a screenshot of the respective CS at the end of acquisition.

2.4.3. Intrusion assessments

2.4.3.1. Daily-life intrusions. We assessed daily-life intrusions over seven consecutive days through an e-diary application "PsyDiary" for smartphones. After TPC, we instructed participants to register intrusive memories, i.e., spontaneously occurring memories in form of pictures, sounds, feelings, or thoughts regarding the film-clips or pain stimulation, as well as sudden recurring thoughts, feelings, or physical sensations experienced while watching the film-clips or experiencing the painful stimulation. We informed participants of the possibility that film- and pain-intrusions could mingle, and instructed them to decide whether their memory concerned primarily a film-clip or the painful sensation from the experiment. Further, we instructed participants to register every intrusion, together with associated distress (rated on a visual analog scale from 0 *(not at all distressing)* to 100 *(extremely distressing))* in the e-diary-app upon occurrence, i.e., in an event-based manner. In order to monitor participants' compliance, we sent text-message reminders for a questionnaire at 10 pm each day, where we explicitly assessed whether participants reported all intrusions throughout the day. If participants indicated non-compliance, they were asked to retrospectively estimate the true number and distress

[2] As this study was part of a larger investigation including neural measures, participants underwent both the TPC (laboratory-session 1) and the MTT (laboratory-session 2) in the magnetic-resonance-imaging (MRI)-scanner. Data from this investigation are reported elsewhere (Franke et al., 2022).

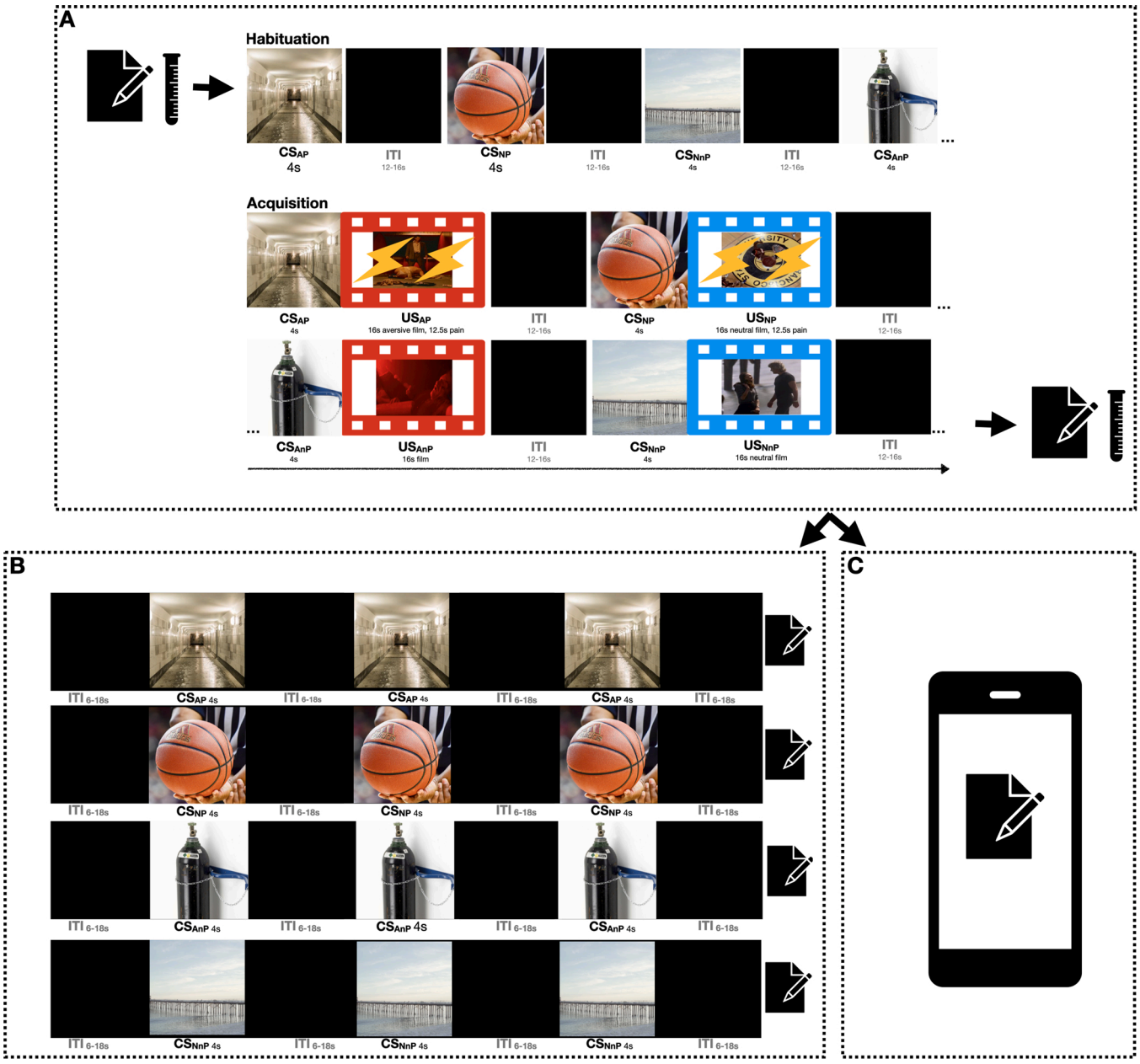

Fig. 1. Schematic overview the study's experimental procedure. **Panel A** depicts the analogue-trauma, i.e., the Trauma-Pain-Conditioning (TPC) paradigm. TPC started with a habituation phase, where each CS was presented four times for 4 s without being followed by an US. The acquisition phase consisted of each CS being presented eight times for 4 s, with a reinforcement rate of 50%. In reinforced trials, each CS was followed by one of the four corresponding USs: aversive film+pain, aversive film+no-pain, neutral film+pain, neutral film+no-pain, lasting 16 s coinciding with or without 12.5 s intermittent pain stimulation. In unreinforced trials, no film-clips or pain stimulation followed the CSs. Stimuli were presented in a pseudorandom order (not more than two consecutive stimuli of the same type), with ITIs ranging between 12 and 16 s. For stress and hormonal assessments, participants filled out the STAI-S and provided a saliva sample immediately before and after TPC. **Panel B** and C depict our intrusion sampling: Panel B depicts the Memory-Triggering-Task (MTT), where CSs appeared three times for 4 s within 52 s-long blocks; the interval between CS presentations ranged between 6 and 18 s and consisted of a black screen. Following each block, participants reported the % of time they experienced memories of the film-clips from the first session and how distressing they perceived these film-memories to be, as well as the % of time experiencing painful bodily sensations. **Panel C** depicts ecological ambulatory assessments in daily-life, acquired event-based for 7 days via a smartphone application. Abbreviations: CS=conditioned stimulus; N = neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P = painful stimulation; ITI= intertrial interval. ITI= intertrial interval; STAI-S: State-Trait Anxiety Inventory-State.

separately for film- and pain-intrusions, and we substituted the event-based intrusion score by the retrospectively-estimated intrusion scores (2% of data).

Since persistent PTSD is primarily linked to intrusions perceived as very distressing (Marks et al., 2018), we aimed to obtain a more clinically-relevant variable by weighting intrusions by their distress; i.e., operationalizing both visual and pain-intrusions as "intrusion load" (i.e., daily intrusion number × average distress; equivalent to the sum of daily intrusive distress).

2.4.3.2. Memory-triggering-task (MTT)-intrusions. In addition to daily-life-intrusions, we also aimed to measure intrusions in the laboratory in response to CSs from TPC. After each CS-block during MTT, participants indicated the percentage of time (0 =*0% of the time*, 10 =*100% of the time*) they experienced memories of the film-clips from session 1 (i.e., "film-intrusions"), as well as painful bodily sensations (i.e., "pain-

intrusions") during each CS-block. In order to weight film-intrusions for associated distress, participants also indicated how distressing they experienced the film-memories (0 =*not distressing at all*, 10 =*extremely distressing*). In this way, akin to daily-life intrusions, we quantified film-intrusions as intrusion load (i.e., frequency × distress of film-intrusions).[3]

2.5. Hormonal assessments

We determined CORT, E2, and P4 levels from saliva samples collected via an unstimulated passive drool method in 15 ml Greiner tubes. Samples were immediately frozen and stored at − 20° until analysis. To remove particulate matter prior to analysis, samples were centrifuged twice for 15 and 10 min respectively at 3000 rpm in an Eppendorf 3750 centrifuge. Whereas we analyzed CORT separately for pre- and post-TPC, we pooled pre- and post-TPC saliva samples prior to E2 and P4 analyses to account for fluctuation in hormone release and saliva production. Note that in line with evidence suggesting that stress induces increases in P4 levels (Herrera et al., 2016), pooling pre- and post-TPC P4 levels may likely also reflect individual differences in the P4 stress response. E2 and P4 were assessed using the high sensitivity ELISA by Salimetrics with a sensitivity of 1 pg/ml for E2, and 10 pg/ml for P4. Cortisol was assessed using ELISA by DeMediTec with a sensitivity of 0.019 ng/ml. All hormone levels were quantified using two duplicate measures for each sample to increase reliability, and samples with intra-assay coefficients of variability above 25% were repeated (undergoing an additional freeze/thaw cycle).

2.6. Stress indices

We used salivary CORT levels and self-reported STAI-S scores to index stress. Table 2 displays intercorrelations between stress indices. Possibly due to the aversive nature of the experiment, we already registered relatively high CORT levels at pre-TPC (CORT-pre: M=5.77, SD=3.73; CORT-post: M=3.68, SD=1.61). To also capture this potential anticipatory stress-response, we averaged pre- and post-TPC stress (CORT/STAI-S) scores to indicate overall stress levels during memory encoding.

2.7. Statistical analyses

All statistical analyses were performed in R-Studio (RStudio Team, 2020). For manipulation checks and main analyses, we fitted Bayesian multilevel regression models (BMLMs) via the *brms* package using Stan in R-Studio (Bürkner, 2017). All models contained repeated measurements over participants (over *days* in daily-life intrusions; over *US/CS-conditions* in MTT data and manipulation checks). Thus, to account for the dependency between observations over participants, responses by the same person were modelled with varying intercepts. Rating data were fitted with ordinal (cumulative) BMLMs (Bürkner and Vuorre, 2019). Daily-life intrusions were fitted with a hurdle lognormal distribution to account for the inflation of zero intrusions in the data. With this approach, we fitted data in two parts where (A) estimated the probability of *intrusion absence*, more specifically the probability of *not experiencing* (i.e., zero) vs. *experiencing* (i.e., non-zero) intrusions (hurdle part, modeled as a Bernoulli distribution); and (B) estimated the intrusion load (i.e., *severity of*) intrusions > 0 (lognormal part, modeled with a lognormal distribution) (Li et al., 2011; Tooze et al., 2002). Finally, we modelled film-intrusions during MTT with a skewed normal distribution to account for the left-skewed distribution of the response variable (Bürkner, 2017). For an overview of fitted models, see *Supplement3*.

Table 2
Intercorrelations between stress indices.

		CORT POST-TPC	CORT PRE-TPC	STAI-S PRE-TPC
CORT PRE-TPC	Pearson's *r*	**0.457**	–	–
	p-value	0.003	–	–
STAI-S PRE-TPC	Pearson's *r*	**0.345**	0.209	–
	p-value	0.027	0.189	–
STAI-S POST-TPC	Pearson's *r*	0.164	0.111	**0.654**
	p-value	0.304	0.488	< 0.001

Note. Significant correlations are highlighted in bold. Abbreviations: TPC: Trauma-Pain-Conditioning Paradigm; CORT = cortisol; STAI-S = State-Trait Anxiety Inventory-State

2.7.1. Manipulation checks

2.7.1.1. URs. To check whether Pain and Valence manipulations were successful, we analyzed URs by means of two ordinal BMLMs. Condition (US_{AP}, US_{AnP}, US_{NP}, US_{NnP}) was entered as a factor, and centered on US_{NnP} in BMLMs. In this way, we contrasted each pain condition (US_{AP}, U_{NP}) and aversive film-clip condition (US_{AP}, US_{AnP}) against a neutral film-clip, no-pain condition.

2.7.1.2. CRs. To test to what extent participants displayed CRs during TPC, we repeated the above-described UR-analyses with participants' Pain and Valence ratings to CSs, and added an interaction between Condition × Phase (habituation; acquisition) to check whether CRs increased from habituation to acquisition.

2.7.2. Main analyses: the relationship between peritraumatic E2 levels, stress, and intrusions

2.7.2.1. Daily-life intrusions. To examine the relationship between peritraumatic E2 levels, stress, and daily-life intrusions we fitted separate hurdle-lognormal BMLMs for film- and pain-intrusions. Independent variables were E2 concentrations (pg/ml) and Stress (indexed by (I) CORT (ng/ml), and (II) STAI-S) levels from TPC, Day (i.e., experimental day on which intrusive memory was registered), as well as the interactions E2 × Stress (CORT/STAI-S)×Day. Further, we controlled for P4 levels and TPC testing time in all models.[4] Factors were centered and standardized before being entered in BMLMs: E2, P4, and Stress indices were centered to their respective means, and Day was centered on the first 24 h day after TPC, i.e., on the second experimental day.[5] As we expected that the effect of Day on intrusions (i.e., the decay of intrusions) could vary between participants, we added a varying slope for the effect of day.

2.7.2.2. MTT-intrusions. To examine the relationship between peritraumatic E2 levels, stress, and MTT-intrusions we fitted separate ordinal BMLMs for film- and pain-intrusions. Independent variables were equivalent to those in daily-life-intrusion models, except for 'Day', which was substituted by 'Condition': during MTT, participants reported intrusions four times, once for each CS-conditions. Again, E2, P4, and Stress indices were centered to their means, and Condition was centered

[3] To reduce subjects' burden in the MRI-scanner, we refrained from asking for distress ratings for pain-intrusions during MTT, because pain is inherently a distressing experience.

[4] Given that these covariates were of no-interest and yielded no significant effects, we refrained from reporting them in results tables to improve readability.

[5] To allow full consolidation of the CS-US memory and assure an equal assessment period for all participants, we excluded the first intrusion assessment day (starting directly after the TPC-session), and started counting assessment days on the day after TPC.

on CS_{NnP}. We excluded one outlier in MTT-intrusions (+/−3SDs from the mean) from analyses due to implausible/inconsistent rating, likely owed to a missed response.

2.7.3. BMLMs model summaries

For a summary of model parameters, we reported regression coefficients and 95% credible intervals (CIs; i.e., Bayesian confidence intervals). Based upon CIs, we can state that there is a 95% probability that the respective parameter falls within this interval, given the evidence provided by the data, priors, and model assumptions. Results were considered significantly different from zero if the estimate's 95% CIs did not include zero (this would indicate two-sided statistical significance on a 5% level). As priors we used the weakly- or non-informative default priors of *brms*, which have only negligible influence on the obtained results. We report Bayesian R^2 as our measure for effect sizes. All Bayesian models converged according to common algorithm-agnostic and algorithm-specific diagnostics. There were no divergent transitions, *Rhat*< 1.01 and *ESS*> 400 for all relevant parameters.

3. Results

3.1. Manipulation-checks

In relation to the CS_{NnP}, after conditioning participants reported stronger pain sensations to CSs signaling the USs with pain-stimulation ($CS_{AP,}$ CS_{NP}), as well as more unpleasantness regarding CSs signaling the aversive film-clip ($CS_{AP,}$ CS_{AnP}). In addition, participants also regarded the CS_{NP} as more unpleasant than the CS_{NnP}, and reported stronger pain sensations to the CS_{AnP} than to the CS_{NnP}. See Fig.S4 in *Supplement4* for details.

3.2. Daily-life intrusions

3.2.1. Film-intrusions

Analyses estimating the probability of *film-intrusion absence* in daily-life suggested no significant main effects of peritraumatic E2 levels (Fig. 2AI-II).

In models with Stress indexed by CORT (i.e., CORT-models), results suggested no significant interactions between E2 × CORT, E2 × Day and E2 × CORT×Day (Fig. 2B-DII). In models with Stress indexed by STAI-S (i.e., STAI-S-models), an interaction between E2 × Day suggested that higher E2 levels were associated with a lower initial probability of film-intrusion absence (see Fig. 2CII). This interaction was further modulated by STAI-S levels: as displayed in Fig. 2DII, a significant E2 × STAI-S×Day interaction suggested that higher E2 was associated with an initial lower probability of film-intrusion absence during the first assessment days which, however, switched to a slightly higher probability of film-intrusion absence in moderately-to-highly state-anxious toward the end of the week. In neither CORT- nor STAI-S-models P4 showed significant main effects on film-intrusion absence. Table 3 $AI\text{-}II_{hurdle}$ provides corresponding regression coefficients and 95%-CIs. -

Results regarding *film-intrusion severity* in daily-life revealed no main effects of E2, P4 or significant interactions between E2, Stress, and Day. See Table 3 $AI\text{-}II_{lognormal}$ and Supplemental Fig. S5 in *Supplement5* for details.

3.2.2. Pain-intrusions

Analyses estimating the probability of *somatosensory-intrusion absence* in daily-life indicated that higher peritraumatic E2 levels were significantly associated with a higher probability of pain-intrusion absence (Fig. 3AI-II). Non-significant E2 × Stress and E2 × Day interactions suggested that the relationship between E2 and pain-intrusions held regardless of individuals' peritraumatic stress responses (Fig. 3BI-II), and was stable over days (Fig.3CI-II). Interactions between E2 × Stress×Day were also non-significant (Fig. 3DI-II).

Results suggested no main effects of P4 levels on pain-intrusion absence. Table $3BI\text{-}II_{hurdle}$ provides corresponding regression coefficients and 95%-CIs.

Analyses estimating daily-life *pain-intrusion severity* suggested no significant main-effects of E2, nor interactions between E2, Stress, and Day (Fig. S6, *Supplement6*). Results suggested no main effects of P4 on pain-intrusion severity. See Table $3BI\text{-}II_{lognormal}$ for respective regression coefficients and 95%-CIs.

3.3. MTT-intrusions

Across E2 analyses, in relation to the CS_{NnP}, participants reported more film-intrusions and pain-intrusions to the $CS_{AP,}$ CS_{NP}, and the CS_{AnP} (Table 4ABI-II). See Fig.S7 in *Supplement7* for details.

3.3.1. Film-intrusions

Analyses revealed no significant main effects of E2 nor interactions between E2 × Stress, or E2 × Stress×CS-conditions (Fig. S8 in *Supplement8*). However, both CORT- and STAI-S-models suggested interactions between Stress×CS-conditions. Results indicated that peritraumatic CORT-levels correlated positively with MTT film-intrusions to the CS_{AnP} (Fig. S9BI, *Supplement9*). Further, STAI-S scores correlated positively with film-intrusions to the CS_{NP} (Fig. S9BII, *Supplement9*). Across models, results suggested no significant main effects of P4 on film-intrusions during MTT. See Table 4A for statistical parameters.

3.3.2. Pain-intrusions

In CORT-models, results suggested no significant main effect of E2 (Fig. 4AI) or interactions between E2 × CORT (Fig. 4BI). Critically though, results revealed significant interactions between E2 × CS_{AP} and E2 × CS_{NP}, suggesting that higher E2 levels were negatively associated with pain-intrusions to the CSs that did not signal the pain-stimulation (CS_{AnP},CS_{NnP}). In contrast, E2 levels were not significantly associated with pain-intrusions to the CSs that signaled the pain-stimulation ($CS_{AP,}$ CS_{NP}), see Fig. 4CI. Non-significant interactions between E2, CS-Conditions and CORT suggested that these relationships were not modulated by CORT (Fig. 4DI). STAI-S models did not reveal a significant main effect of E2 (Fig. 4AII) or interactions between E2 × STAI-S (Fig. 4BII).Interactions between E2 and CS-conditions largely followed the same pattern as in CORT-models, but only reached statistical significance for the E2 × CS_{NP} interaction. Confidence intervals pertaining to the E2 × CS_{AP} interaction contained zero and were thus more uncertain (Fig. 4CII). Analyses further suggested that STAI-S-levels were positively associated with pain-intrusions across CS-conditions (Fig. S10AB-II, *Supplement10*). Finally, P4 yielded no significant main effects on pain-intrusions during MTT either. Table 4B provides regression coefficients and 95%-CIs of the aforementioned results.

4. Discussion

This study scrutinized the relationship between peritraumatic endogenous E2 levels and the development of film- and pain-intrusions following analogue-trauma while considering modulatory effects of stress. Results pertaining pain-intrusions suggest an inverse relationship between E2 and pain-intrusions during daily-life and MTT regardless of participants' stress levels. MTT-results further specified this result by suggesting that higher E2 levels were associated with fewer intrusions specifically to safety cues. Results concerning daily-life film-intrusions suggested time- and stress-dependent results: higher E2 levels were associated with an initial greater probability of experiencing film-intrusions in daily-life, which switched to a slightly lower probability of experiencing film-intrusions later on (i.e., from day 3) in moderately-to-highly state-anxious women.

This study provides novel data on increased peritraumatic E2 levels being associated with a reduced development and severity of pain-intrusions. In line with this, a prospective study also found an inverse

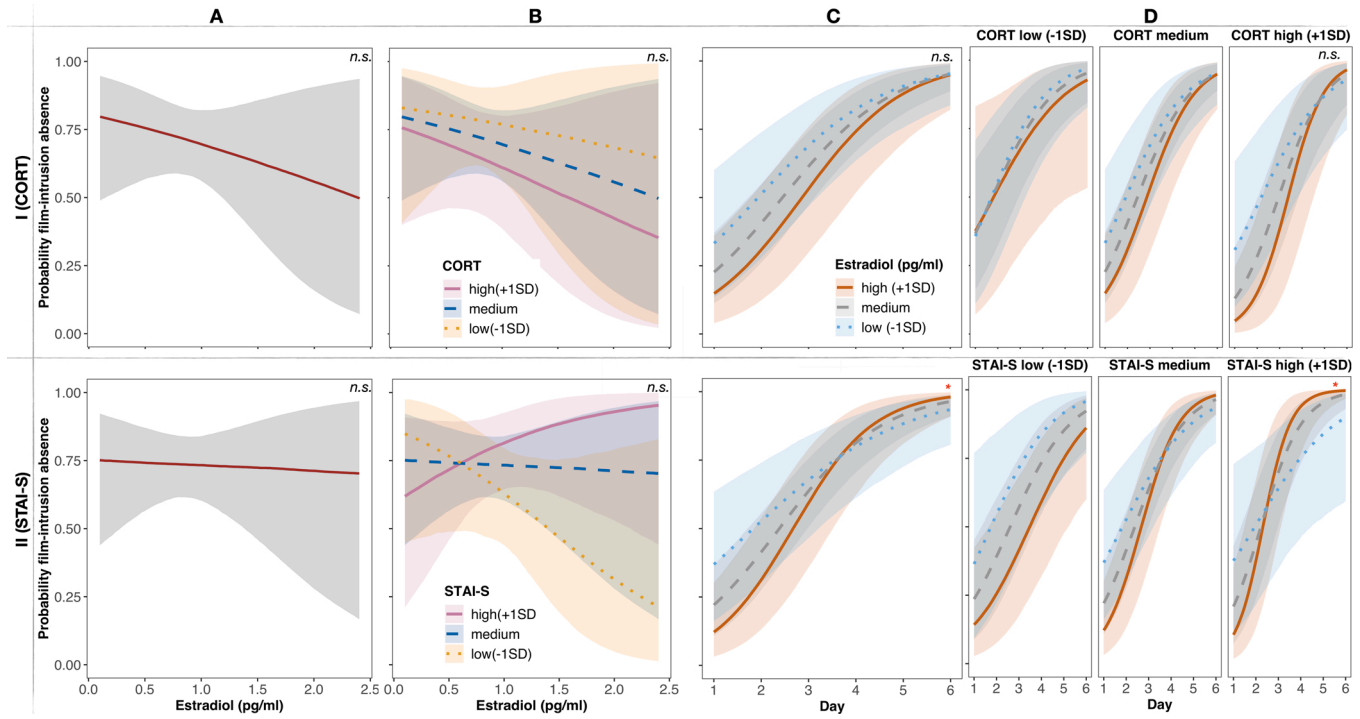

Fig. 2. . Estradiol effects on daily-life film-intrusions over days, considering modulatory effects of stress. Lines depict fitted values of regressions' hurdle part (i.e., estimating the probability of "zero" film-intrusions [film-intrusion absence]) predicting film-intrusions during daily-life by E2 (panel A), E2 × Stress (panel B), E2 × Day (panel C), and E2 × Stress×Day (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying significant effects are marked by an asterisk, plots displaying non-significant results for informative purposes are marked with "n.s" on the right upper corner. Abbreviations: CI=credible interval; E2 = estradiol; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; n.s. = non-significant.

relationship between peritraumatic E2 levels and the development and severity of chronic posttraumatic (but not acute) pain (Linnstaedt et al., 2021). Though pain-intrusions only started to receive empirical attention, they could constitute a promising *pathway* underlying the inverse relationship between peritraumatic E2 and chronic posttraumatic pain (Franke et al., 2022; Macdonald et al., 2018; Morgan and Aldington, 2020) and thereby importantly extend this previous result.

Within the intrusive memory research field, our finding that increased peritraumatic E2 levels during analogue-trauma were associated with a lower probability for developing pain-intrusions falls in line with previous observations where increased E2 was associated with fewer intrusions (Wegerer et al., 2014). These results could point at E2 being related with fewer CRs in the form of pain-intrusions; i.e., trauma-related cues automatically eliciting representations of the consolidated US memory (Franke et al., 2022). Results from MTT further specified this effect by suggesting that higher E2 levels were specifically associated with a lower probability of experiencing pain-intrusions to CSs which did not signal the pain-stimulation and therefore functioned as safety cues.

Interestingly, a growing literature has highlighted a role of E2 in reducing CRs to safety cues. In specific, a series of studies showed that women with high vs. low E2 were not only better at discriminating between safety and danger conditions, but also at inhibiting fear in the presence of safety cues (Day and Stevenson, 2020). Recently, researchers found that higher E2 protected women from generalized heightened physiological responses to CS- after fear-reinstatement[6] (Felmingham et al., 2021). Our results add to this literature by suggesting that higher peritraumatic E2 levels also seem to be related to fewer pain-intrusions to safety cues after consolidation of the original CS-US memory.

One potential explanation for our results could be related to E2 strengthening the formation and consolidation of hippocampal-dependent memories (Taxier et al., 2020), possibly related to its boosting effects on hippocampal functioning (Pletzer et al., 2019). Besides supporting contextualized memories by providing a spatial and temporal framework for relating experiences, the hippocampus has been involved in promoting danger from safety discrimination through pattern separation processes (Besnard and Sahay, 2016) and conditioned inhibition of threat responding (Meyer et al., 2019). Additionally, some studies have suggested that higher E2 inhibited negative emotional responding to threatening stimuli (Goldstein et al., 2010; Miedl et al., 2018). This potential stress-inhibiting effect of E2 is particularly important when considering evidence suggesting that fear spreads to a wider array of harmless stimuli when the threat (US) is more intense (Dunsmoor et al., 2017). Following, if women with higher E2 are more efficient at regulating negative emotional responses and experience the US as less threatening, they may be less likely to respond to cues that only *might* signal the US (such as the no-pain-stimulation signaling CSs during MTT).

Intriguingly, as opposed to the robust inverse relationship with pain-intrusions, we found that higher peritraumatic E2 levels were positively associated with the probability of experiencing film-intrusions in the first days after analogue-trauma. Considering E2′s enhancing effects on memory consolidation (Taxier et al., 2020), our finding agrees with other research observing detrimental effects of sleep-related enhanced memory consolidation specifically on early intrusions (Porcheret et al., 2015). During memory consolidation, individuals consolidate not only contextual details, but also sensory-perceptual elements encountered during trauma. Importantly, these sensory-perceptual cues can trigger intrusions of the traumatic event (Brewin et al., 2010; Ehlers et al., 2002; Franke et al., 2021), particularly in the early days after the event (Marks

[6] Fear-reinstatement is an experimental manipulation which aims to trigger the return of a conditioned fear response after extinction by unexpectedly re-exposing individuals to the US following extinction.

Table 3
Bayesian multilevel model predicting daily-life (A) film-intrusions and (B) pain-intrusions by E2, (I) CORT, (II) STAI-S, and Day.

	(A) Film-intrusions						(B) Somatoform Intrusions					
	Hurdle		Lognormal				Hurdle		Lognormal			
	b	95% CI	*b*	95% CI	R^2	95% CI	*b*	95% CI	*b*	95% CI	R^2	95% CI
I. CORT					**0.50**	**[0.33, 0.64]**					**0.39**	**[0.12, 0.51]**
E2	-0.27	[− 1.26, 1.26]	-0.03	[− 0.39, 0.45]			**1.75**	**[0.27, 3.70]**	0.55	[− 0.70, 1.86]		
P4	0.21	[− 0.61, 1.06]	0.05	[− 0.39, 0.49]			-0.75	[− 2.16, 0.38]	-0.59	[− 1.79, 0.57]		
CORT	-0.82	[− 1.84, 0.08]	-0.04	[− 0.42, 0.33]			0.01	[− 1.27, 1.24]	-0.40	[− 1.36, 0.55]		
Day	**0.85**	**[0.59, 1.17]**	**-0.23**	**[− 0.34, − 0.12]**			**0.51**	**[0.06, 1.02]**	-0.15	[− 0.72, 0.38]		
E2 × CORT	-0.76	[− 1.83, 0.20]	0.09	[− 0.31, 0.49]			0.15	[− 1.18, 1.46]	-0.39	[− 1.43, 0.60]		
E2 × Day	0.08	[− 0.19, 0.39]	-0.04	[− 0.16, 0.09]			-0.24	[− 0.70, 0.22]	0.12	[− 0.53, 0.71]		
Day × CORT	0.15	[− 0.13, 0.45]	0.05	[− 0.07, 0.16]			-0.22	[− 0.60, 0.15]	-0.05	[− 0.50, 0.46]		
Day × E2 × CORT	0.22	[− 0.08, 0.52]	0.04	[− 0.07, 0.16]			-0.05	[− 0.46, 0.35]	-0.04	[− 0.51, 0.50]		
II. STAI-S					**0.49**	**[0.33, 0.63]**					**0.44**	**[0.16, 0.50]**
E2	-0.48	[− 1.46, 0.44]	0.02	[− 0.40, 0.44]			**1.83**	**[0.32, 3.87]**	0.23	[− 1.57, 1.84]		
P4	0.12	[− 0.75, 1.01]	-0.01	[− 0.46, 0.45]			-0.69	[− 2.09, 0.49]	-0.10	[− 1.62, 1.59]		
STAI-S	-0.16	[− 1.01, 0.69]	0.19	[− 0.20, 0.57]			-0.54	[− 1.97, 0.73]	0.62	[− 0.89, 2.18]		
Day	**0.92**	**[0.64, 1.25]**	**-0.21**	**[− 0.34, − 0.08]**			**0.62**	**[0.09, 1.26]**	-0.15	[− 1.66, 1.10]		
E2 × STAI-S	-0.38	[− 1.26, 0.46]	-0.06	[− 0.45, 0.34]			-0.15	[− 1.70, 1.18]	0.23	[− 1.29, 1.92]		
E2 × Day	**0.26**	**[0.00, 0.56]**	0.01	[− 0.11, 0.12]			-0.10	[− 0.62, 0.49]	-0.11	[− 2.01, 1.36]		
Day × STAI-S	0.21	[− 0.06, 0.51]	-0.01	[− 0.13, 0.12]			-0.27	[− 0.80, 0.20]	-0.20	[− 1.46, 1.15]		
Day × E2 × STAI-S	**0.31**	**[0.03, 0.61]**	0.05	[− 0.07, 0.18]			-0.14	[− 0.68, 0.36]	-0.01	[− 1.43, 1.78]		

Note. Coefficients are considered significantly different from zero if the corresponding 95% CI does not contain zero, and are highlighted in bold. For improved readability, we do not display intercepts in the current Table nor parameters concerning the control variable testing time. The models' hurdle part predicted the probability of "zero" intrusions [intrusion absence]; The lognormal part of the models predicted the amount of intrusions [intrusion severity]. Abbreviations: *b*= regression coefficient; CI=credible interval; E2 = estradiol, CORT=cortisol; STAI-S=State-Trait Anxiety Inventory-State

et al., 2018). In line, interfering specifically with the consolidation of these sensory-perceptual elements of the trauma memory through tasks with high visuospatial demands appears to significantly prevent intrusions (Iyadurai et al., 2019). As such, higher E2 may have been linked to an increased probability of intrusion occurrence in our study through fostering consolidation of sensory-perceptual cues associated with the analogue-trauma.

Interestingly, the initial positive association between E2 and film-intrusions flipped to a negative association in moderately-to-highly anxious women toward the end of the week. This result again resonates with sleep research finding a protective effect of sleep on intrusion development only after the passage of three days (Kleim et al., 2016). Conceivably, by strengthening the consolidation of hippocampal-dependent memories, higher peritraumatic E2 may, similarly to sleep, secure greater availability of trauma-related contextual details. This contextual information allows individuals to properly integrate the traumatic event in place, time, and autobiographical memory base, and thereby prevents a fragmented, poorly-contextualized memory as commonly observed in PTSD patients (Brewin et al., 2010; Ehlers and Clark, 2000). Importantly, gist-like memory representations increase over time, as memory precision fades (Wiltgen and Silva, 2007), and fear generalization arises (Dunsmoor et al., 2017). Hence, yielding more precise, contextualized memories may turn particularly relevant after some days have passed after the traumatic event.

Noteworthy, we only observed a negative association between E2 and film-intrusions in moderately-to-highly state-anxious women. Probably, women with higher state-anxiety levels (which in the current sample highly correlated with trait-anxiety levels, $p < .001$) are more vulnerable to show stress-induced impaired hippocampal functioning (Pitman et al., 2012). Echoing previous findings suggesting that E2 protects from stress-induced memory impairments (Antov and Stockhorst, 2018, 2014), and evidence suggesting that overgeneralization of threat is enhanced in anxious individuals (Duits et al., 2015), it is possible that higher E2 may have been especially important to secure a more precise and contextualized memory consolidation in highly anxious individuals.

Curiously, we did not find any significant associations between E2 and CS-elicited film-intrusions during MTT. One noteworthy difference between intrusions assessed in daily-life and MTT relates to the nature of retrieval cues. Specifically, during MTT participants were exposed to embedded visual CSs which, by depicting contextual elements from the film-clips (e.g., a beach pier resembling the pier in the beach-walk scene), probably provided cues that strongly, and perhaps invariantly, lead to retrieval of the film-clips. The fact that participants were young, healthy and perceived CSs signaling aversive vs. neutral film-clips as more unpleasant even before conditioning (see Fig. S4B in *Supplement4*), renders difficulties in retrieving precise film-US representations from overt CSs rather unlikely. In daily-life however retrieval cues are weaker (e.g., a bridge resembling the pier). Consequently, film-intrusion

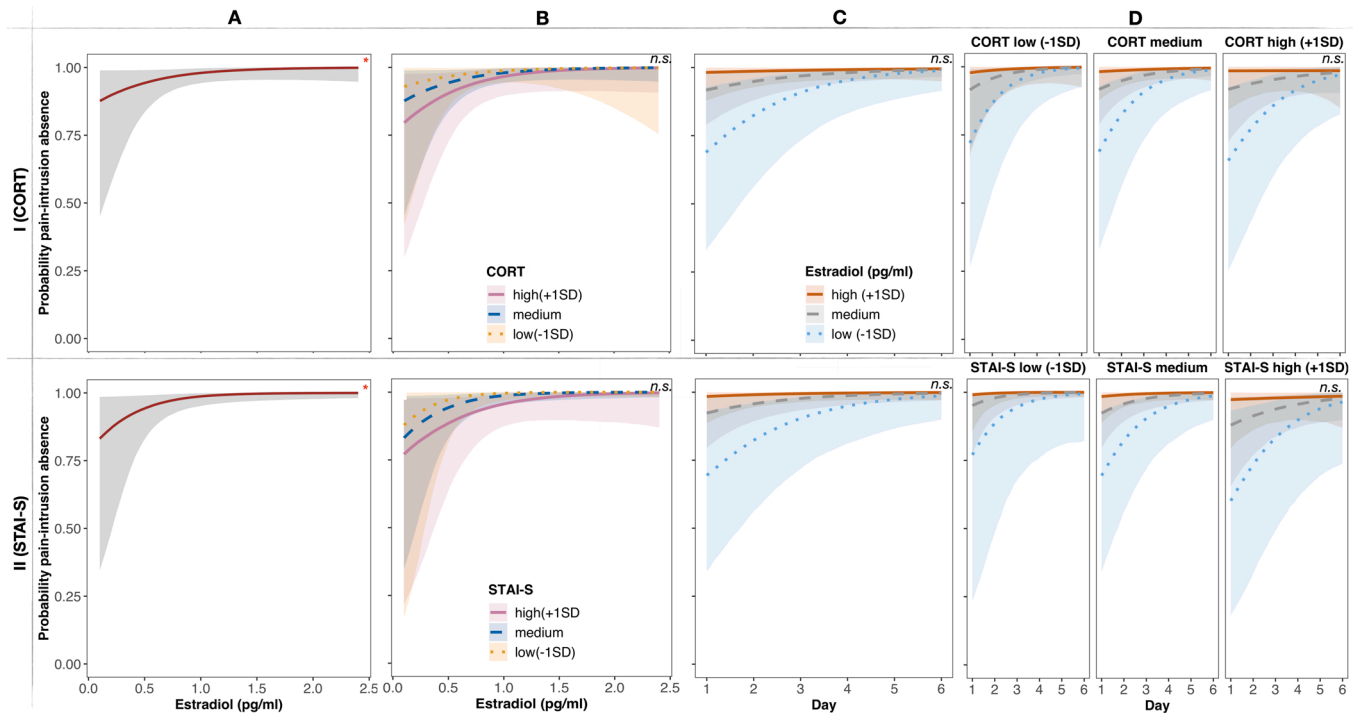

Fig. 3. . Estradiol effects on daily-life pain intrusions over days, considering modulatory effects of stress. Lines depict fitted values of regressions' hurdle part (i.e., estimating the probability of "zero" pain-intrusions [pain-intrusion absence]) predicting pain-intrusions during daily-life by E2 (panel A), E2 × Stress (panel B), E2 × Day (panel C) and E2 × Stress × Day (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying significant effects are marked by an asterisk, plots displaying non-significant results for informative purposes are marked with "n.s" on the right upper corner. Abbreviations: CI=credible interval; E2 = estradiol; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; n.s. = non-significant.

assessment during daily-life may have provided a significantly wider array of trauma-related cues and thereby permitted the emergence of more inter-individual differences than the MTT film-intrusion assessment. Conversely, CSs contained no features that overtly signaled the pain-US and may thus have been more ambiguous, which could explain why we observed E2 effects on pain-intrusions but not on film-intrusions. To overcome the limitation of having too overt CSs, future studies using embedded CSs may opt for using cues of different modality (e.g., odors) than the USs.

We also found different associations between E2 and daily-life pain-intrusions and E2 and film-intrusions. Before, we argued that the positive association between E2 and intrusions could be linked to E2 strengthening the consolidation of sensory-perceptual elements. Cues sensory-perceptually resembling those encoded during analogue-trauma can function as triggers for intrusions (Ehlers et al., 2002; Franke et al., 2021). Although we expect that such cues also trigger pain-intrusions (Franke et al., 2022), since humans are inclined to predominantly focus on visual information, most of the re-encountered trauma-related cues might have rather resembled elements concerning the film-US than the pain-US. Due to greater sensory-perceptual resemblance (Ehlers and Clark, 2000), participants probably more readily recalled the films and reported most cue-elicited intrusions as film-related. Therefore, the initial positive relationship between E2 and intrusions may not have applied to pain-intrusions because most sensory-perceptual cues encountered during daily-life prompted film- over pain-intrusions. Moreover, although we observed that participants reported some sensory reminder cues (e.g., muscular tension), these cues still yielded relatively little resemblance with our very specific pain-US. This may have prevented automatic triggering of pain-intrusions, unless if individuals only retained a very imprecise, poorly contextualized representation of the pain-stimulation and were more prone to over-associate harmless cues to pain, as might be the case in women with lower E2 levels. Hence, women yielding lower peritraumatic E2 levels could be more vulnerable to deficits in inhibiting fear and, as we suggested here, also film- and pain-intrusions to harmless cues. Since such deficits are a core characteristic of PTSD (Duits et al., 2015), it may be interesting to further prospectively investigate whether peritraumatic E2 levels are associated with a greater risk for PTSD and chronic pain symptoms, and whether this risk is conveyed by overgeneralization tendencies.

It is worth discussing that our results seem to stand in contrast with PTSD symptom studies, which have so far mainly suggested that women experience more intrusions when exposed to trauma during the mid-luteal phase of the menstrual cycle, where E2 levels reach a moderate second peak (lower than at ovulation). Critically, most PTSD-symptom studies compared women in the (mid)luteal phase with women in the follicular phase (Garcia et al., 2018). This may be problematic because during the (mid)luteal phase progesterone (P4) levels are high and may oppose E2′s effects. Specifically, while E2 enhances excitatory neurotransmission, P4 enhances inhibitory neurotransmission (Barth et al., 2015). Moreover, studies showed that hippocampal activation was elevated in women during the pre-ovulatory phase where E2 is unopposed by P4 (i.e., high E2, low P4), but dropped during the luteal phase (Pletzer et al., 2019); and that otherwise inhibitory effects of unopposed E2 on the arousal circuitry (Goldstein et al., 2010) disappeared during the luteal phase (Andreano and Cahill, 2010). Possibly then, our results concerning the relationship between peritraumatic E2 and intrusions diverges from what has been suggested by PTSD symptom studies because around ¾ of our participants were in phases where E2 was unopposed by P4. To examine the possibility that P4 could have nevertheless opposed or influenced E2′s actions in part of our sample, we excluded women in the luteal phase ($N = 13$[7]) in supplementary

[7] Note that we excluded 10 participants falling in the luteal phase based on pre- and post-study menses, as well as three participants who were assigned to the luteal phase based on overly high P4 levels (see 2.1.1.).

Table 4
Bayesian multilevel model predicting laboratory CS-elicited (A) film-intrusions and (B) pain-intrusions by E2, (I) CORT, (II) STAI-S, and CS-conditions.

	(A) Visual				(A) Somatosensory			
	b	95% CI	R^2	95% CI	*b*	95% CI	R^2	95% CI
I – CORT			**0.53**	**[0.44, 0.59]**			**0.55**	**[0.46, 0.63]**
E2	-1.87	[− 7.99, 3.87]			-1.45	[− 3.33, 0.24]		
P4	-0.17	[− 3.87, 4.07]			-0.41	[− 1.85, 0.98]		
CORT	0.63	[− 5.98, 7.11]			0.88	[− 1.03, 2.81]		
CS_{NP}	**6.87**	**[0.37, 13.65]**			**2.77**	**[1.53, 4.13]**		
CS_{AnP}	**10.11**	**[3.42, 17.07]**			**2.28**	**[0.99, 3.61]**		
CS_{AP}	**12.11**	**[5.14, 19.40]**			**2.77**	**[1.52, 4.13]**		
E2 × CORT	3.45	[− 3.38, 11.00]			0.76	[− 1.35, 2.94]		
E2 × CS_{NP}	1.69	[− 4.85, 8.49]			**1.65**	**[0.36, 3.10]**		
E2 × CS_{AnP}	-3.58	[− 10.74, 3.70]			0.36	[− 0.98, 1.86]		
E2 × CS_{AP}	-2.20	[− 9.01, 4.78]			**1.46**	**[0.18, 2.96]**		
CORT × CS_{NP}	2.45	[− 5.94, 10.65]			-1.41	[− 3.18, 0.31]		
CORT × CS_{AnP}	**9.28**	**[0.87, 17.77]**			-0.55	[− 2.34, 1.20		
CORT × CS_{AP}	5.79	[− 3.74, 15.03]			-0.71	[− 2.47, 0.98]		
E2 × CS_{NP} × CORT	-1.48	[− 10.31, 7.11]			-1.79	[− 3.81, 0.06]		
E2 × CS_{AnP} × CORT	5.42	[− 4.52, 15.25]			-1.02	[− 3.12, 0.88]		
E2 × CS_{AP} × CORT	-2.41	[− 11.61, 6.71]			-0.87	[− 2.87, 0.96]		
II – STAI-S			**0.51**	**[0.43, 0.57]**			**0.56**	**[0.46, 0.63]**
E2	-0.32	[− 5.81, 4.78]			-1.09	[− 2.70, 0.43]		
P4	-0.80	[− 5.04, 3.66]			-0.69	[− 1.96, 0.50]		
STAI-S	0.57	[− 4.04, 5.18]			**1.52**	**[0.29, 2.79]**		
CS_{NP}	**8.13**	**[1.66, 14.70]**			**2.75**	**[1.53, 4.11]**		
CS_{AnP}	**10.26**	**[3.76, 17.06]**			**1.91**	**[0.57, 3.33]**		
CS_{AP}	**12.45**	**[5.64, 19.47]**			**2.75**	**[1.52, 4.11]**		
E2 × STAI-S	-0.70	[− 5.14, 3.90]			-0.25	[− 1.55, 1.08]		
E2 × CS_{NP}	1.17	[− 4.76, 7.12]			**1.34**	**[0.06, 2.81]**		
E2 × CS_{AnP}	-2.78	[− 8.98, 3.41]			-0.36	[− 1.85, 1.26]		
E2 × CS_{AP}	-1.98	[− 7.99, 3.96]			1.28	[− 0.05, 2.71]		
STAI-S × CS_{NP}	**6.76**	**[0.61, 12.96]**			-0.04	[− 1.13, 1.02]		
STAI-S × CS_{AnP}	4.22	[− 1.32, 9.78]			0.20	[− 0.95, 1.34]		
STAI-S × CS_{AP}	3.77	[− 1.87, 9.22]			-0.17	[− 1.31, 0.92]		
E2 × CS_{NP} × STAI-S	-1.09	[− 6.95, 4.80]			0.15	[− 1.08, 1.30]		
E2 × CS_{AnP} × STAI-S	-1.30	[− 7.17, 4.80]			1.05	[− 0.27, 2.37]		
E2 × CS_{AP} × STAI-S	1.17	[− 4.76, 7.14]			0.37	[− 0.82, 1.52]		

Note: Coefficients are considered significantly different from zero if the corresponding 95% CI does not contain zero, and are highlighted in bold. For improved readability, we do not display intercepts in the current Table. Abbreviations: *b*= regression coefficient; CI=credible interval; E2 = estradiol, CORT=cortisol; STAI-S=State-Trait Anxiety Inventory-State; CS=conditioned stimuli; N = neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P = painful stimulation

analyses (*Supplement12*). The fact that results remained largely identical suggests that the inverse relationship between endogenous peri-traumatic E2 levels and intrusions might hold when P4 levels are enhanced.

Accordingly, rodent studies have challenged a consistent opposing role of P4 on E2′s actions and rather suggest that P4 might, early after administration, even augment E2′s effects. Specifically, one study showed that P4 administration potentiated estradiol's facilitation of fear extinction recall when extinction occurred 6 h after administration, but abolished estradiol's facilitation of extinction recall when extinction occurred 24 h later (Graham and Daher, 2016). Another study showed similar time-dependent effects of P4 administration on estradiol-mediated hippocampal dendritic spine density growth in rats (Woolley et al., 1990). To date, we can only speculate that in our luteal-phase women the timing of rising P4 levels might have been at an optimal point to potentiate, instead of opposing E2′s actions. Follow-up studies may scrutinize whether and when in the human menstrual cycle there might be a "tipping point" between E2-potentiating vs. E2-antagonizing effects of enhanced P4 by, for instance, comparing the relationship between E2 and intrusions in women in the early vs. later stages of the luteal phase.

4.1. Limitations and future directions

First, E2 levels were, in general, relatively low, which could be associated with the fact that our testing sessions were during afternoons/evenings, where E2 concentrations are lower. To secure higher E2 levels, future studies may test in the morning, and recruit women during the pre-ovulatory phase (confirmed by ovulation tests). Second, given that participants arrived relatively stressed/anxious at the laboratory, we averaged pre- and post-analogue-trauma stress levels. Thereby, while we may have captured participants' overall stress levels during analogue-trauma, we did not consider general response tendencies (STAI-S) or potential inter-individual differences in basal CORT-levels. Future studies may overcome this problem by measuring baselines stress levels during a non-threat-inducing situation. Finally, our sample size was relatively low and may have reduced statistical power. Especially higher order interaction effects are thus to be interpreted with caution until replicated in a larger sample.

Finally, our results might raise the question of whether exogenous estradiol administration for instance via OCs could constitute a potential resilience-fostering intervention. Initial evidence already suggests that OC-use lowers retention of negative stimuli (Person and Oinonen, 2020). In line, current exploratory comparisons between naturally-cycling and OC-using women suggested that OC-users had a higher probability of film-intrusion absence than naturally-cycling women (*Supplement11*). Speculatively, it might thus be the case that women benefit from the continuous high levels of the synthetic ethinyl-estradiol contained in OCs. Well-powered studies comparing OC-users (high ethinyl-estradiol but low endogenous E2) to women in the early follicular phase (no supply of ethinyl-estradiol and low endogenous E2) are necessary to further examine this question. Ultimately, OC-use could constitute a safe and cost-efficient way of fostering resilience for intrusions after (potentially) traumatic events in high-risk groups (e.g., emergency workers).

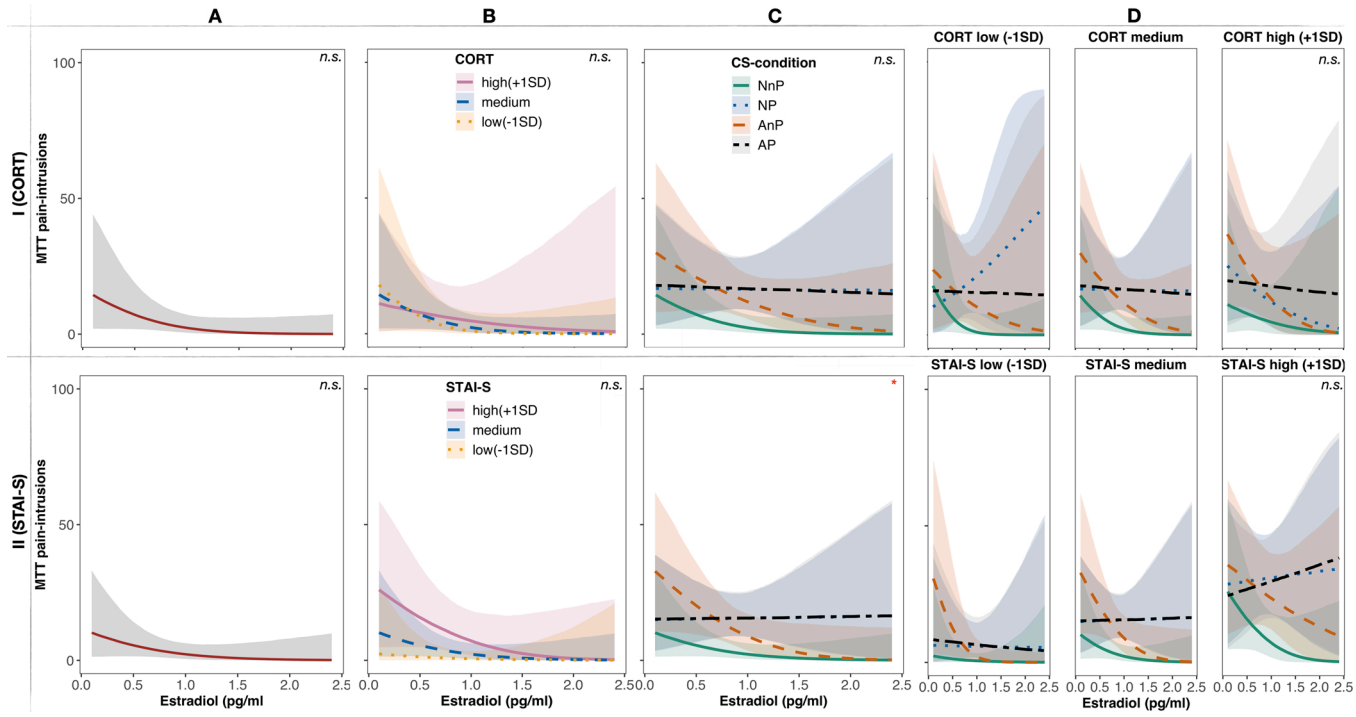

Fig. 4. . Estradiol effects on Memory-Triggering-Task (MTT) pain-intrusions over CS-conditions, considering modulatory effects of stress. Lines depict fitted values of the regressions predicting pain-intrusions during MTT by E2 (panel A), E2 × Stress (panel B), E2 × CS-conditions (panel C), and E2 × CS-conditions × Stress (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying non-significant results for informative purposes are marked with "n.s" on the right upper corner. Abbreviations: CI=credible interval; MTT=Memory-Triggering-Task; CS=conditioned stimuli; E2 = estradiol; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; N = neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P = painful stimulation; n.s. = non-significant.

4.2. Conclusions

Results suggested a positive association between peritraumatic E2 levels and initial daily-life film-intrusions, which however flipped to a negative association toward the end of the week in anxious women. Additionally, results also indicated a consistent inverse relationship between peritraumatic E2 levels and pain-intrusions during daily-life and in response to safety cues during MTT. This could point to a picture where higher E2 during trauma, possibly by enhancing consolidation of sensory-perceptual trauma cues, increases women's initial risk for intrusions. However, higher E2 might concomitantly strengthen the consolidation of more precise and contextualized memories and thereby prevent intrusions in response to cues that only remotely or not at all resemble danger cues, which tends to arise especially in more anxious individuals as memory precision fades over time. Overarchingly, our results could implicate that higher peritraumatic E2 levels may protect women from maintaining long-term involuntary distressing images of the traumatic event, as well as from re-experiencing pain-sensations, both of which could pave the way for PTSD and chronic pain.

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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.psyneuen.2022.105819.

References

Andreano, J.M., Cahill, L., 2010. Menstrual cycle modulation of medial temporal activity evoked by negative emotion. NeuroImage 53, 1286–1293. https://doi.org/10.1016/j.neuroimage.2010.07.011.

Antov, M.I., Stockhorst, U., 2014. Stress exposure prior to fear acquisition interacts with estradiol status to alter recall of fear extinction in humans. Psychoneuroendocrinology 49, 106–118. https://doi.org/10.1016/j.psyneuen.2014.06.022.

Antov, M.I., Stockhorst, U., 2018. Women with high estradiol status are protected against declarative memory impairment by pre-learning stress. Neurobiol. Learn. Mem. 155, 403–411. https://doi.org/10.1016/j.nlm.2018.08.018.

Barth, C., Villringer, A., Sacher, J., 2015. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. Front. Neurosci. 9, 37. https://doi.org/10.3389/fnins.2015.00037.

Besnard, A., Sahay, A., 2016. Adult hippocampal neurogenesis, fear generalization, and stress. Neuropsychopharmacology 41, 24–44. https://doi.org/10.1038/npp.2015.167.

Brewin, C.R., Gregory, J.D., Lipton, M., Burgess, N., 2010. Intrusive images in psychological disorders: characteristics, neural mechanisms, and treatment implications. Psychol. Rev. 117, 210–232. https://doi.org/10.1037/a0018113.

Bull, J.R., Rowland, S.P., Scherwitzl, E.B., Scherwitzl, R., Danielsson, K.G., Harper, J., 2019. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. npj Digit. Med. 2. https://doi.org/10.1038/s41746-019-0152-7.

Bürkner, P.C., 2017. brms: an R package for Bayesian multilevel models using Stan. J. Stat. Softw. 80. https://doi.org/10.18637/jss.v080.i01.

Bürkner, P.-C., Vuorre, M., 2019. Ordinal regression models in psychology: a tutorial. Adv. Methods Pract. Psychol. Sci. 2, 77–101. https://doi.org/10.1177/2515245918823199.

Day, H.L.L., Stevenson, C.W., 2020. The neurobiological basis of sex differences in learned fear and its inhibition. Eur. J. Neurosci. https://doi.org/10.1111/ejn.14602.

Duits, P., Cath, D.C., Lissek, S., Hox, J.J., Hamm, A.O., Engelhard, I.M., van den Hout, M. A., Baas, J.M.P., 2015. Updated meta-analysis of classical fear conditioning in the anxiety disorders. Depress Anxiety 32, 239–253. https://doi.org/10.1002/da.22353.

Dunsmoor, J.E., Kroes, M.C.W., Braren, S.H., Phelps, E.A., 2017. Threat intensity widens fear generalization gradients. Behav. Neurosci. 131, 168–175. https://doi.org/10.1037/bne0000186.

Ehlers, A., Clark, D.M., 2000. A cognitive model of posttraumatic stress disorder. Behav. Res. Ther. 38, 319–345. https://doi.org/10.1016/S0005-7967(99)00123-0.

Ehlers, A., Hackmann, A., Steil, R., Clohessy, S., Wenninger, K., Winter, H., 2002. The nature of intrusive memories after trauma: The warning signal hypothesis. Behav. Res. Ther. 40, 995–1002. https://doi.org/10.1016/S0005-7967(01)00077-8.

Felmingham, K.L., Caruana, J.M., Miller, L.N., Ney, L.J., Zuj, D. v, Hsu, C.M.K., Nicholson, E., To, A., Bryant, R.A., 2021. Lower estradiol predicts increased reinstatement of fear in women. Behav. Res. Ther. 142. https://doi.org/10.1016/j.brat.2021.103875.

Franke, L.K., Rattel, J.A., Miedl, S.F., Danböck, S.K., Bürkner, P.-C., Wilhelm, F.H., 2021. Intrusive memories as conditioned responses to trauma cues: an empirically supported concept? Behav. Res. Ther. 143, 103848 https://doi.org/10.1016/j.brat.2021.103848.

Franke, L.K., Miedl, S.F., Danböck, S.K., Liedlgruber, M., Grill, M., Kronbichler, M., Flor, H., Wilhelm, F.H., 2022. Neuroscientific evidence for pain being a classically conditioned response to trauma- and pain-related cues in humans. Pain. https://doi.org/10.31234/osf.io/84vz2.

Garcia, N.M., Walker, R.S., Zoellner, L.A., 2018. Estrogen, progesterone, and the menstrual cycle: a systematic review of fear learning, intrusive memories, and PTSD. Clin. Psychol. Rev. 66, 80–96. https://doi.org/10.1016/j.cpr.2018.06.005.

Gershman, S.J., Niv, Y., 2012. Exploring a latent cause theory of classical conditioning. Learn. Behav. 40, 255–268. https://doi.org/10.3758/s13420-012-0080-8.

Goldstein, J.M., Jerram, M., Abbs, B., Whitfield-Gabrieli, S., Makris, N., 2010. Sex differences in stress response circuitry activation dependent on female hormonal cycle. J. Neurosci. 30, 431–438. https://doi.org/10.1523/JNEUROSCI.3021-09.2010.

Graham, B.M., Daher, M., 2016. Estradiol and progesterone have opposing roles in the regulation of fear extinction in female rats. Neuropsychopharmacology 41, 774–780. https://doi.org/10.1038/npp.2015.202.

Herrera, A.Y., Nielsen, S.E., Mather, M., 2016. Stress-induced increases in progesterone and cortisol in naturally cycling women. Neurobiol. Stress 3, 96–104. https://doi.org/10.1016/j.ynstr.2016.02.006.

Iyadurai, L., Visser, R.M., Lau-Zhu, A., Porcheret, K., Horsch, A., Holmes, E.A., James, E. L., 2019. Intrusive memories of trauma: a target for research bridging cognitive science and its clinical application. Clin. Psychol. Rev. 69, 67–82. https://doi.org/10.1016/j.cpr.2018.08.005.

Kleim, B., Wysokowsky, J., Schmid, N., Seifritz, E., Rasch, B., 2016. Effects of sleep after experimental trauma on intrusive emotional memories. Sleep 39, 2125–2132. https://doi.org/10.5665/sleep.6310.

Laux, L., Glanzmann, P., Schaffner, P., Spielberger, C., 1981. Das state-trait angstinventar. Weinheim (GER). Beltz. https://doi.org/10.1002/da.21968.

Li, Graham, B.M., 2017. Why are women so vulnerable to anxiety, trauma-related and stress-related disorders? The potential role of sex hormones. Lancet Psychiatry 4, 73–82. https://doi.org/10.1016/S2215-0366(16)30358-3.

Li, N., Elashoff, D.A., Robbins, W.A., Xun, L., 2011. A hierarchical zero-inflated log-normal model for skewed responses. Stat. Methods Med. Res. 20, 175–189. https://doi.org/10.1177/0962280208097372.

Linnstaedt, S.D., Mauck, M.C., Son, E.Y., Tungate, A.S., Pan, Y., Rueckeis, C., Yu, S., Lechner, M., Datner, E., Cairns, B.A., Danza, T., Velilla, M.-A., Pearson, C., Shupp, J. W., Smith, D.J., McLean, S.A., 2021. Peritraumatic 17β-estradiol levels influence chronic posttraumatic pain outcomes. Pain 162, 2909–2916. https://doi.org/10.1097/j.pain.0000000000002282.

Macdonald, B., Salomons, T. v, Meteyard, L., Whalley, M.G., 2018. Prevalence of pain flashbacks in post-traumatic stress disorder arising from exposure to multiple traumas or childhood traumatization. Can. J. Pain. 2, 48–56. https://doi.org/10.1080/24740527.2018.1435994.

Maercker, A., Schützwohl, M., 1998. Erfassung von psychischen Belastungsfolgen: die Impact of Event Skala-revidierte Version (IES-R) [Assessment of post-traumatic stress reactions: The Impact of Event Scale-Revised (IES-R)]. Diagnostica 44, 130–141.

Marks, E.H., Franklin, A.R., Zoellner, L.A., Marks, E.H., Franklin, A.R., Zoellner, L.A., 2018. Can ' t get it out of my mind: a systematic review of predictors of intrusive memories of distressing events can ' t get it out of my mind: a systematic review of predictors of intrusive memories of distressing events. Psychol. Bull. 144, 584–640.

McGaugh, J.L., 2000. Memory - a century of consolidation. Science 287, 248–251. https://doi.org/10.1126/science.287.5451.248.

Merz, C., Kinner, V.L., Wolf, O.T., 2018. Let's talk about sex … differences in human fear conditioning. Curr. Opin. Behav. Sci. 23, 7–12. https://doi.org/10.1016/J.COBEHA.2018.01.021.

Meyer, H.C., Odriozola, P., Cohodes, E.M., Mandell, J.D., Li, A., Yang, R., Hall, B.S., Haberman, J.T., Zacharek, S.J., Liston, C., Lee, F.S., Gee, D.G., Performed, S.J.Z., 2019. Ventral hippocampus interacts with prelimbic cortex during inhibition of threat response via learned safety in both mice and humans 116, 26970–26979. https://doi.org/10.1073/pnas.1910481116/-/DCSupplemental.

Miedl, S.F., Wegerer, M., Kerschbaum, H., Blechert, J., Wilhelm, F.H., 2018. Neural activity during traumatic film viewing is linked to endogenous estradiol and hormonal contraception. Psychoneuroendocrinology 87, 20–26. https://doi.org/10.1016/J.PSYNEUEN.2017.10.006.

Morgan, L., Aldington, D., 2020. Comorbid chronic pain and post-traumatic stress disorder in UK veterans: a lot of theory but not enough evidence. Br. J. Pain. 14, 256–262. https://doi.org/10.1177/2049463719878753.

Ney, L.J., Gogos, A., Ken Hsu, C.M., Felmingham, K.L., 2019. An alternative theory for hormone effects on sex differences in PTSD: The role of heightened sex hormones during trauma. Psychoneuroendocrinology. https://doi.org/10.1016/j.psyneuen.2019.104416.

Nilges, P., Essau, C., 2015. Die Depressions-Angst-Stress-Skalen: Der DASS – ein Screeningverfahren nicht nur für. Schmerz Schmerz 10, 649–657. https://doi.org/10.1007/s00482-015-0019-z.

Person, B., Oinonen, K.A., 2020. Emotional memory in oral contraceptive users: negative stimuli are more forgettable. Psychol. Rep. 123, 2282–2304. https://doi.org/10.1177/0033294119856554.

Pitman, R.K., Delahanty, D.L., 2005. Conceptually driven pharmacologic approaches to acute trauma. CNS Spectr. 10, 99–106. https://doi.org/10.1017/S109285290001943X.

Pitman, R.K., Rasmusson, A.M., Koenen, K.C., Shin, L.M., Orr, S.P., Gilbertson, M.W., Milad, M.R., Liberzon, I., 2012. Biological studies of post-traumatic stress disorder. Nat. Rev. Neurosci. 13, 769–787. https://doi.org/10.1038/nrn3339.

Pletzer, B., Harris, T.A., Scheuringer, A., Hidalgo-Lopez, E., 2019. The cycling brain: menstrual cycle related fluctuations in hippocampal and fronto-striatal activation and connectivity during cognitive tasks. Neuropsychopharmacology 44, 1867–1875. https://doi.org/10.1038/s41386-019-0435-3.

Porcheret, K., Holmes, E.A., Goodwin, G.M., Foster, R.G., Wulff, K., 2015. Psychological effect of an analogue traumatic event reduced by sleep deprivation. Sleep 38, 1017–1025. https://doi.org/10.5665/sleep.4802.

de Quervain, D., Schwabe, L., Roozendaal, B., 2016. Stress, glucocorticoids and memory: implications for treating fear-related disorders. Nat. Rev. Neurosci. 18, 7–19. https://doi.org/10.1038/nrn.2016.155.

Rief, W., Hiller, W., 2003. A new approach to the assessment of the treatment effects of somatoform disorders. Psychosomatics 44, 492–498. https://doi.org/10.1176/appi.psy.44.6.492.

Rombold, F., Wingenfeld, K., Renneberg, B., Hellmann-Regen, J., Otte, C., Roepke, S., 2016. Influence of the noradrenergic system on the formation of intrusive memories in women: an experimental approach with a trauma film paradigm. Psychol. Med. 46, 2523–2534. https://doi.org/10.1017/S0033291716001379.

RStudio Team, 2020. RStudio: Integrated development for R [Computer software]. https://doi.org/http://www.rstudio.com/.

Taxier, L.R., Gross, K.S., Frick, K.M., 2020. Oestradiol as a neuromodulator of learning and memory. Nat. Rev. Neurosci. 21, 535–550. https://doi.org/10.1038/s41583-020-0362-7.

Tooze, J.A., Grunwald, G.K., Jones, R.H., 2002. Analysis of repeated measures data with clumping at zero. Stat. Methods Med. Res. 11, 341–355. https://doi.org/10.1191/0962280202sm291ra.

van Ast, V.A., Vervliet, B., Kindt, M., 2012. Contextual control over expression of fear is affected by cortisol. Front. Behav. Neurosci. 1–14. https://doi.org/10.3389/fnbeh.2012.00067.

Wegerer, M., Blechert, J., Kerschbaum, H., Wilhelm, F.H., 2013. Relationship between fear conditionability and aversive memories: evidence from a novel conditioned-intrusion paradigm. PLoS One 8, e79025. https://doi.org/10.1371/journal.pone.0079025.

Wegerer, M., Kerschbaum, H., Blechert, J., Wilhelm, F.H., 2014. Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life. Neurobiol. Learn. Mem. 116, 145–154. https://doi.org/10.1016/j.nlm.2014.10.001.

Wiltgen, B.J., Silva, A.J., 2007. Memory for context becomes less specific with time. Learn. Mem. 14, 313–317. https://doi.org/10.1101/lm.430907.

Woolley, C.S., Gould, E., Frankfurt, M., Mcewen, B.S., 1990. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J. Neurosci.