Author/s:
Schenker, MT;Ince, S;Ney, LJ;Hsu, CMK;Zuj, DV;Jordan, AS;Nicholas, CL;Felmingham, KL

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Sex differences in the effect of subjective sleep on fear conditioning, extinction learning, and extinction recall in individuals with a range of PTSD symptom severity

Authors

Maya T. Schenker [a*] (* corresponding author: Level 9, Redmond Barry Building, Parkville Campus, Melbourne VIC 3010, Tel: +61 (0) 3834 44911, m.schenker@unimelb.edu.au, https://orcid.org/0000-0003-3811-2940)

Sevil Ince [a] (sevil.ince@unimelb.edu.au, https://orcid.org/0000-0001-9461-2398)

Luke J. Ney [b,c] (luke.ney@qut.edu.au, https://orcid.org/0000-0003-0209-8366)

Chia-Ming K. Hsu [b] (cmkhsu@utas.edu.au)

Daniel V. Zuj [b] (daniel.zuj@utas.edu.au)

Amy S. Jordan [a] (ajordan@unimelb.edu.au, https://orcid.org/0000-0001-8561-9766)

Christian L. Nicholas [a] (cln@unimelb.edu.au, https://orcid.org/0000-0002-3837-3609)

Kim L. Felmingham [a,b] (kfelmingham@unimelb.edu.au, https://orcid.org/0000-0002-0749-538X)

[a] Melbourne School of Psychological Sciences, The University of Melbourne, Parkville, VIC, Australia

[b] School of Psychological Sciences, University of Tasmania, Hobart, TAS, Australia

[c] School of Psychology and Counselling, Queensland University of Technology, Brisbane, QLD, Australia

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Abstract

Sleep has been found to play a key role in fear conditioning, extinction learning and extinction recall, and sleep disturbances are linked to many mental disorders including post-traumatic stress disorder (PTSD). Previous studies examining associations between sleep and fear or extinction processes primarily focused on objectively measured sleep architecture. Little research has so far focused on subjective sleep measures and particularly in clinical populations, which often experience subjectively poor sleep, including PTSD. Here we investigated whether subjective sleep disturbance, sleep onset latency, wake after sleep onset or sleep efficiency were related to fear conditioning, extinction learning or extinction recall in a large sample of individuals with a range of PTSD symptom severity (n=248). Overall, we did not find that subjective sleep was associated with fear conditioning or extinction processes. However, exploratory analyses examining the moderating effect of sex found that shorter sleep onset latency and greater sleep efficiency were associated with improved extinction recall in women with higher PTSD symptom severity. This suggests that less time falling asleep and longer time asleep while in bed may be protective in highly symptomatic women against the commonly observed impaired extinction recall in PTSD. More studies are needed to explore sex-specific effects further.

Keywords

Fear Conditioning, Extinction Learning, Extinction Recall, Sleep, Sex, PTSD

Background

Post-traumatic stress disorder (PTSD) is a debilitating mental disorder with a lifetime prevalence of approximately 8% (de Vries & Olff, 2009; Kilpatrick et al., 2013). Key mechanisms underlying the development and maintenance of PTSD are thought to be impairments in the capacity to regulate fear to a previously - but no longer - threatening stimulus (i.e., fear extinction, Jovanovic et al., 2013; Pitman et al., 2012; Zuj, Palmer, Lommen, et al., 2016) as well as impairments in the ability to maintain extinction learning (i.e. extinction recall, e.g. Milad et al., 2008; Milad et al., 2009; Pitman et al., 2012). Recent research has highlighted the powerful influence of sleep on extinction learning processes through alterations in emotion regulation and emotional memory consolidation (Pace-Schott et al., 2015a). However, this relationship has largely been established in healthy individuals, and empirical evidence in clinical populations with PTSD, who commonly suffer from sleep disturbances, is largely lacking.

Fear conditioning paradigms model the development, maintenance, and treatment of anxiety disorders as well as PTSD in a controlled laboratory setting and allow the underlying mechanisms of these conditions to be explored (Zuj & Norrholm, 2019). Fear conditioning involves an associative learning process during which an aversive, unconditioned stimulus (US, e.g. electric shock) is repeatedly paired with an inherently neutral, nonthreatening stimulus (e.g. coloured circle) to elicit a conditioned fear response (threat signal or CS+, Graham & Milad, 2011; VanElzakker et al., 2014). The conditioned response leads to sympathetic arousal and the associated psychophysiological reactivity is commonly measured using skin conductance response (SCR; Milad & Quirk, 2012). Along with the CS+, a second stimulus (e.g. different coloured circle) is introduced but never associated with the US to indicate safety (safety signal or CS-; Laing & Harrison, 2021). Following fear acquisition, fear extinction learning occurs when the individual is repeatedly exposed to the CS+ in the absence of the US, resulting in a gradual reduction of the conditioned response. Rather than

erasing the original fear memory, extinction forms a new association between the CS+ and the absence of the US, competing with the original conditioned CS+-US association through inhibition and reducing arousal (Bouton, 2004; Milad & Quirk, 2012). Extinction memory is commonly tested after 24-48 hours during the extinction recall task. Central mechanisms in PTSD that prevent recovery from trauma and contribute to its maintenance are thought to be impairments in extinction learning (e.g. Guthrie & Bryant, 2006; Jovanovic et al., 2013; Norrholm et al., 2011; Suarez-Jimenez et al., 2020; Wicking et al., 2016; Zuj, Palmer, Lommen, et al., 2016) and extinction recall (Garfinkel et al., 2014; Milad et al., 2008; Milad et al., 2009). Alterations in extinction processes are indicated by greater psychophysiological reactivity towards the CS+ throughout the extinction learning and recall phases, which have been commonly observed in individuals with PTSD compared to those without PTSD (e.g. Garfinkel et al., 2014; Wicking et al., 2016). Further, evidence emerged that individuals with PTSD have difficulties in differentiating between threatening (CS+) and safe (CS-) cues (impaired safety learning and safety recall; Jovanovic et al., 2013; Jovanovic et al., 2012; van Rooij & Jovanovic, 2019). Extinction learning underpins exposure-based therapy approaches, the gold standard in treatment for PTSD and anxiety disorders (Graham et al., 2014; Myers & Davis, 2007; Raeder et al., 2020). Limited treatment gains in individuals with PTSD may be attributed to reduced speed of extinction learning as well as impairments in the recall of extinction (Bradley et al., 2005; Garfinkel et al., 2014; Milad et al., 2008; Milad et al., 2009).

In recent years, the role of sleep in the processing of trauma memories has sparked increasing interest. With up to 90% of individuals with PTSD experiencing insomnia and/or nightmares (Cox & Olatunji, 2020; Ohayon & Shapiro, 2000), sleep disturbances have been acknowledged to be not only a symptom, but also a key etiological factor in the disorder (Germain, 2013; Germain et al., 2008; Koffel et al., 2016). This, in turn, has led to the study of sleep measures in experimental fear conditioning paradigms. In healthy individuals, sleep

compared to wakefulness has been found to facilitate recall of both fear and extinction (Davidson et al., 2018; Lerner et al., 2021; Menz et al., 2013; Pace-Schott et al., 2014; Seo et al., 2021; Straus et al., 2017; Zenses et al., 2020). Studies of natural variations in sleep in healthy controls found that individuals with shorter total sleep time, as well as longer sleep onset latency (time it takes to fall asleep) and longer time awake after initial sleep onset, showed impaired extinction recall (Spoormaker et al., 2010). The level of fear and extinction learning during the experimental session may on the other hand have an impact on the following sleep, indicating a bidirectional relationship between sleep and fear and extinction learning processes. For example, greater intensity of the US (Sturm et al., 2013) as well as higher fear expression during fear acquisition and extinction learning (Seo et al., 2021; Spoormaker et al., 2014) has been associated with longer sleep onset latency (Seo et al., 2021) as well as longer time spent awake after initial sleep onset (Spoormaker et al., 2014; Sturm et al., 2013).

A large body of literature has focused on rapid eye movement (REM) sleep and its role in the context of trauma and fear conditioning/extinction learning (for review see Colvonen et al., 2019; Davidson & Pace-Schott, 2020; Pace-Schott et al., 2015a, 2015b; Schenker et al., 2021; Tempesta et al., 2018). REM sleep is a unique sleep stage commonly associated with vivid dreaming and the processing of emotional memories (Goldstein & Walker, 2014; Rasch & Born, 2013; Rasch & Born, 2015; Walker & Stickgold, 2006). Alongside comorbid insomnia and reduced sleep efficiency (proportion of time asleep while in bed; Cox & Olatunji, 2020; Zhang et al., 2019), PTSD has also been associated with REM sleep disturbances including lower REM sleep percentage compared to healthy- and trauma-control populations (Kobayashi et al., 2007; Zhang et al., 2019). However, in relation to fear learning and extinction processes, consensus of the exact role of REM sleep is lacking between studies. A recent meta-analysis found no meaningful general effect of REM sleep on fear acquisition, extinction learning or extinction recall when looking at both healthy controls

and clinical samples (Schenker et al., 2021). But, Schenker et al. (2021) found preliminary evidence that in clinical populations, REM sleep is associated with impaired extinction learning and extinction recall. They highlighted, however, that the number of clinical studies is very small and further research looking at sleep including individuals with PTSD is warranted.

While polysomnography provides the gold standard in objective sleep recording (Berry et al., 2012), subjective measures such as sleep diaries and questionnaires are highly valuable to gain insight into the perception of sleep. PTSD has been associated with paradoxical insomnia, which means that there is often a discrepancy between subjectively reported sleep disturbances and a lack of objectively measured alterations in sleep (e.g. Ghadami et al., 2015; Hurwitz et al., 1998; Klein et al., 2003; Werner et al., 2016). Subjective measures may provide a better understanding of an individual's sleep quality, which has clinical implications on daytime functioning (Pilcher et al., 1997; Rezaie et al., 2018). For example, Zuj et al. (2018), one of the few studies looking at subjective sleep measures in a clinical sample, found a moderating effect of sleep disturbances and sleep onset latency in the relationship between the level of fear reinstatement and PTSD symptom severity. Therefore, more research into the role of subjective sleep in fear acquisition, extinction learning and extinction recall within PTSD populations is urgently needed.

Importantly, emerging evidence has highlighted a critical role of sex in PTSD risk following trauma as well as in fear conditioning paradigms. Women are about twice as likely to develop PTSD than men (Felmingham et al., 2010; Kessler et al., 2017) regardless of trauma type (Blanco et al., 2018). Sex differences (including varying levels of sex hormones) have been found to modulate fear conditioning and extinction learning processes (Garcia et al., 2018; Hsu et al., 2021; Li & Graham, 2017; Ney et al., 2019; Peyrot et al., 2020; Wen et al., 2022). In addition, differences in sleep between sexes are also well recognised with women being more likely to self-report insomnia symptoms and reduced sleep quality, despite

generally lacking evidence for disrupted objective sleep (Baker et al., 2020; Mong & Cusmano, 2016; Suh et al., 2018). Taken together, sex, sleep and PTSD seem to be interconnected, but more research is needed (Kobayashi et al., 2012; Richards et al., 2022; Schenker et al., 2021). In the abovementioned meta-analysis by Schenker et al. (2021), sex-specific findings were observed in the association between REM sleep and fear conditioning and extinction processes which partially accounted for the overall observed null-finding. In predominantly female samples, more REM sleep was associated with increased arousal during fear acquisition as well as impaired extinction recall, while the opposite was found in male samples. Following this discovery, Richards et al. (2022) also reported a moderating effect of sex in the sleep-fear processing relationship. Hence, those findings highlight the critical need to study both male and female sexes as well as their differences and to consider sex as a moderating variable in the relationship between sleep and fear acquisition, extinction learning and extinction recall.

We therefore aimed to further investigate the relationship between sleep, and fear acquisition, extinction learning, and extinction recall in relation to severity of PTSD symptomatology. We hypothesize that firstly, baseline subjective sleep disturbances will be associated with heightened fear acquisition and impaired fear extinction learning (greater psychophysiological reactivity towards the CS+ and CS-). Secondly, subjective sleep including longer sleep onset latency and wake after sleep onset as well as lower sleep efficiency will be associated with worse extinction and safety recall (increased reactivity to both the CS+ and CS- during early recall compared to late extinction, respectively). Thirdly, those associations will be stronger with increasing PTSD symptom severity. Lastly, exploratory analyses will examine the role of sex in the associations between fear and extinction learning processes and sleep.

Methods

Participants

Data was collected across four years at two sites (the University of Tasmania and the University of Melbourne) using an identical fear conditioning and extinction learning paradigm. 307 participants aged 17-69 were recruited through the respective universities, psychology clinics and the public. Exclusion criteria included use of psychoactive medication including illicit drugs, as well as history of a psychiatric disorder apart from PTSD (n=48). In addition, participants were excluded for the present analysis if they were classified as 'non-responders' to the paradigm (n=11, see *Skin conductance data processing*). At the baseline assessment, participants reported whether they had previously experienced a traumatic event using the trauma events questionnaire (TEQ, Vrana & Lauterbach, 1994) and completed the PTSD Checklist (PCL, Weathers et al., 1994,Weathers et al., 2013) to asses PTSD symptom severity. To visualise the data and descriptive analyses, participants were divided into three groups based on their trauma history and PTSD symptoms using the TEQ and PCL. A clinician (KLF) determined whether participants were likely to meet the diagnostic criteria for post-traumatic stress disorder (i.e. scoring ≥2 on the PCL items that map onto diagnostic criteria, see see *PTSD Checklist (PCL)*) and grouped those into the probable PTSD group (n=47). The remaining individuals with previous trauma exposure but unlikely to have PTSD, and trauma-naïve individuals were grouped into trauma-exposed (TE, n=104) and non-trauma exposed (NTE, n=97) controls, respectively. Due to the project not testing extinction recall during earlier stages of recruitment, only a proportion of participants (n=133) completed the recall session. Of those, 17 had probable PTSD, 54 were TE and 62 were NTE. The main analyses testing the hypotheses included PTSD symptom severity dimensionally using PCL scores rather than the diagnostic status. Subsamples of this database have been analysed in previous publications exploring sleep and fear reinstatement (Zuj, Palmer, Hsu, et al., 2016; Zuj et al., 2018) as well as cannabinoids and fear extinction learning (Ney et al., 2021). The

data presented here has not previously been published, especially the recall data from a new subset of participants, who have not been included in previous publications and only have been analysed in this study.

Measures

Trauma Exposure Questionnaire (TEQ)

The TEQ (Vrana & Lauterbach, 1994) is a brief self-report checklist in which participants report whether ('yes') or not ('no'), they have ever experienced a trauma from a list of common criteria-A events as defined by the Diagnostic and Statistical Manual (DSM, American Psychiatric Association, 2013).

PTSD Checklist (PCL)

The PCL-5 assesses the presence and severity of 20 PTSD symptoms over the past month, which map onto DSM-5 criteria. Participants indicate whether they were bothered by a given symptom on a scale between 0 ('not at all') and 4 ('extremely'; Weathers et al., 2013). While higher scores indicate more severe PTSD symptom severity, PTSD diagnosis is probable if at least one intrusive, one avoidance, two negative cognition and mood as well as two hyper arousal symptoms are scored as ≥ 2 (Weathers et al., 2013). Here, a dimensional approach was chosen due to the small number of male individuals with probable PTSD tested at recall (n=6), including the PCL score as an indicator for PTSD symptom severity. A subsample completed the PCL for DSM-4 (Weathers et al., 1994) prior to the release of the DSM-5. To account for different scoring criteria, total PCL scores were scaled using standardized Z scores in all analyses including both versions (acquisition and extinction session).

Pittsburgh Sleep Quality Index (PSQI)

This self-report questionnaire assesses sleep habits and sleep related issues (Buysse et al., 1989). Seven component scores can be derived including sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleep medication and daytime

dysfunction. The component score indicates whether the individual has difficulties in any aspect of sleep over the past month ranging from 0 (‘no difficulty’) to 3 (‘severe difficulty’). Here, only the sleep disturbances component was included in the analysis to test the first hypothesis.

Depression Anxiety and Stress Scale (DASS-21)

DASS-21 (Lovibond & Lovibond, 1995) a 21-item self-report questionnaire measuring states of depression, anxiety and stress in the past week on a scale from 0 (‘not at all’) to 3 (‘very much or most of the time’). The summary score within each domain gives an indication on the current symptom severity ranging between normal and extremely severe (Henry & Crawford, 2005).

Alcohol Use Disorder Identification Test (AUDIT)

AUDIT is a screening tool to identify harmful drinking and potential alcohol use disorder. Each item is scored between 0 and 4 with higher scores indicating greater level of drinking and/or associated issues. Scores above 8 suggest hazardous drinking and above 15 indicates alcohol dependence or alcohol use disorder (Saunders et al., 1993)

Sleep diary

The diary recorded the participants’ sleep during the previous night including bedtime, sleep time, the number of awakenings and when the participant woke up in the morning. Total time in bed, total time asleep, sleep onset latency (SOL), wake after sleep onset (WASO) and sleep efficiency (SE; calculated as total time asleep /total time in bed *100%) can be calculated from the diary. Generally, SE ≥ 85% is considered as good sleep (Baglioni et al., 2014; Kryger et al., 2022; Natale et al., 2015)

Procedures

Participants were invited to attend the laboratory on two occasions in the afternoon to account for circadian effects on fear extinction (Pace-Schott et al., 2014). The first session included a baseline questionnaire battery including PCL, TEQ, PSQI, DASS-21, AUDIT and

general demographics. This was followed by a differential fear conditioning and extinction learning paradigm adapted from Orr et al. (2000), where a mild electric shock (US) was paired with a previously neutral stimulus to evoke a conditioned fear response. The US was an 500ms shock to the first interosseous muscle of the dominant hand which was individually calibrated to be "highly annoying, but not painful" prior to the beginning of the experiment. The CS were blue and red circles, each presented for 12s with the inter-stimulus interval ranging from 12-21s. One of the coloured circles (counterbalanced between participants) was reinforced with the shock during the acquisition phase using a 100% reinforcement schedule (threat signal or CS+) while the other one was never paired with the shock (safety signal or CS-). The conditioned response was measured by SCR reflecting sympathetic arousal recorded continuously throughout the task. The first session of the paradigm was divided into four phases, starting with the habituation phase where participants were exposed to four randomized trials of both CS+ and CS- without any shocks. The participants were then instructed that from now on, they may or may not receive the shock. The acquisition phase consisted of five presentations of each stimulus with the CS+ being immediately followed by the shock. The CS- was never associated with the shock. This session concluded with the extinction phase which was divided into two blocks labelled early and late extinction. Each extinction phase consisted of five trials of both CS+ and CS- presented in the absence of the shock. Acquisition, early and late extinction were separated by a short break to allow participants to rest before they initiated the start of the next phase (after habituation, researchers did not interact with participants anymore). No new instruction was given to participants after acquisition to allow extinction learning to occur (measured as the speed with which the conditioned response to the CS+ reduced across trials). Two days later, a subsample of participants (n= 133) returned to the lab for the extinction recall phase. To begin the recall session, participants completed a sleep diary reporting their sleep during the previous night before they were set up for the experiment. The recall session involved a repetition of the

extinction phase including the attachment of the shock electrode without any instructions on what to expect. The amount of conditioned response at the beginning of recall compared to the end at extinction learning reflected the return of fear or rather the extent that extinction (CS+) and safety (CS-) were recalled.

Skin conductance data processing

To test acquisition, extinction and recall of conditioned fear, skin conductance was measured in microSiemens (μS) through bipolar electrodes attached to two fingers on the non-dominant hand. An amplifier with low constant-voltage AC excitation (22 mV_{rms} at 75Hz) and automatic zeroing was used sampling at 512Hz and storing at 64Hz (ADInstruments). SCR was calculated for each trial as the difference between the mean response during the 2s before the onset of the CS (baseline skin conductance level), and the highest peak response during the CS presentation (Milad et al., 2013; Milad et al., 2008; Milad et al., 2005; Zuj et al., 2018). This created a baseline-corrected SCR value for each trial (Lonsdorf et al., 2017; Pineles et al., 2009). Non-responders were defined as those having a SCR less than 0.01 μS to CS+ three or more trials (i.e. more than 50%, of trials 2-5) during fear acquisition (Ney et al., 2021) and their data was excluded from the analysis.

Statistical analysis

Demographic differences were calculated using Kruskall-Wallis rank sum test (group differences) due to assumption violations as well as linear regression (differences in PTSD symptom severity, see supplementary methods). Fear acquisition, extinction learning, and extinction recall were analysed using separate linear mixed models within each phase including PTSD symptom severity dimensionally (Ney et al., 2021; Richards et al., 2022; Tabachnick & Fidell, 2007). *PCL* (scaled PCL scores for the acquisition and extinction analyses, raw PCL scores for the recall analysis), *stimuli* (CS+, CS-) and *trial* were included as fixed effects and *participant* as random effect with a random intercept using restricted maximum likelihood estimation (REML). Trial was considered as a random effect with

random intercept and slope. Model fit did not differ with or without trial as a random intercept (χ^2=0, p=1). In addition, trial as a random slope highly correlated with the random intercept for participant even after assuming uncorrelated effects (r>.90). Thus, trial was included as fixed effect only. Further, age was considered as a covariate due to the significant differences between groups (see table 1), but the results did not differ. For the fear acquisition analysis, the first CS+ and CS- trial during fear acquisition was removed since no learning has occurred at this stage. The trials at extinction were divided into early (first 5 trials) and late (trials 6-10) phase, due to a short intermission after the first five trials where saliva samples were collected (data not presented here). To account for potential changes in contingencies because of the short break, separate analyses were run within each phase (an approach used previously, e.g., Milad et al., 2005; Ney et al., 2021; Wen et al., 2022; Zuj et al., 2018). For the extinction recall analyses, averages were taken of the last two trials at extinction (trials 9 and 10) and the first two trials at recall (trials 1 and 2) respectively and included as the *time* variable to test the change from late extinction to early recall. Due to lack of consensus in operationalising extinction recall (Lonsdorf et al., 2022; Lonsdorf et al., 2019), a fear recovery index (e.g. Li & Graham, 2016; White & Graham, 2016), or averaged conditioned response during recall trials only (first 2 or 3) were examined and model results can be found in Tables A.28-A.39 in the appendix. Prior to the analyses, model assumptions were checked, and no concerns were identified. Raw SCR data were skewed, thus square-root data transformation using absolute values (Lonsdorf et al., 2022; Lonsdorf et al., 2017) were compared to the raw data to test whether model assumptions improved. Overall, the distribution of the residuals versus fitted values was similar and the models using raw and transformed data yielded similar effects (see Tables A.2-A.27 in the appendix). Therefore untransformed values were used for the present analyses to facilitate interpretation (Gelman & Hill, 2006; Tabachnick & Fidell, 2007).

To test the moderating influence of the sleep variables and sex, their interactions with the other fixed effects were explored after stepwise inclusion into the models described above.

Since the temporal change within session was not of interest for the moderation analyses, trial was not included for the fear acquisition and extinction phases. At recall, the temporal change from late extinction to early recall was of interest. Therefore, the time variable remained in the model after the sleep variable and sex were added, but separate models were run for each stimulus (CS+ and CS-).

Post-hoc analyses included comparison of estimated marginal means and/or simple slopes where appropriate using ratio *t*-test with Tukey HSD or Bonferroni-correction. To estimate the slope of continuous variables, the mean, as well as one standard deviation above and below was included for average, high and low values (Aiken et al., 1991). If low or high values were beyond the range, then the minimum or maximum were used instead. All analyses were conducted in R (R Core Team, 2021), using lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017) and emmeans (Lenth, 2021) packages. Alpha level was set at .05 and confidence level at .95. Additional methods and results are reported in the appendix.

Results

The final sample included 248 participants aged 17-69 years (M=25.94, SD=10.02), 57.66% of whom were female. Sample demographics can be found in Table 1 and group-wise trial-by-trial mean SCR values for the CS+ and CS- are displayed in Fig. 1. Overall, individuals with probable PTSD scored highest on the PCL reflecting greater PTSD symptom severity. Additionally, the probable PTSD group also scored highest on the PSQI (sleep disturbances component), DASS sub-scores (depression, anxiety and stress) as well as AUDIT, and were generally older than TE or NTE controls.

Table 1: Baseline sample characteristics

		PTSD	TE	NTE	Test statistic[#]	*p*
Sex		27F 20M	60F 44M	56F 41M	χ^2 (2) = .001	.999
Age		30.28 (12.35)	27.27 (10.44)	22.41 (6.68)	χ^2 (2) = 18.64	<.001
PCL[*]	4:	44.71 (14.26)	14.35 (12.52)	7.48 (8.79)	χ^2 (2) = 50.04	<.001
	5:	51.00 (11.69)	26.84 (6.18)	21.00 (5.69)	χ^2 (2) = 74.48	<.001
PSQI sleep disturbances		1.41 (1.17)	1.09 (0.86)	0.83 (0.64)	χ^2 (2) = 9.38	.009
DASS-21 depression		7.82 (4.27)	2.87 (3.47)	1.90 (2.42)	χ^2 (2) = 67.02	<.001
DASS-21 anxiety		10.70 (5.73)	4.45 (3.60)	2.63 (2.89)	χ^2 (2) = 68.68	<.001
DASS-21 stress		4.98 (4.92)	2.93 (3.14)	2.60 (2.75)	χ^2 (2) = 6.86	.032
AUDIT risk		5.83 (5.92)	3.57 (4.03)	2.96 (3.78)	χ^2 (2) = 11.15	.004

Note: mean and standard deviation per group for each questionnaire. PTSD: post-traumatic stress disorder, TE: trauma exposed, NTE: non-trauma exposed. PCL: PTSD Checklist DSM, PSQI: Pittsburgh Sleep Quality Index, DASS: Depression Anxiety and Stress scale, AUDIT: Alcohol Use Disorder Identification Test.

*133 individuals completed the PCL-5, 115 completed the PCL-4

Kruskal-Wallis chi-square and p-value

Acquisition of conditioned fear

During the acquisition phase, linear mixed model including PTSD symptom severity (scaled PCL score), stimuli (CS+, CS-) and trial (trial: 2-5) as fixed effects revealed a significant main effect for stimuli, $F(1, 1772)=91.168$, p <.001. Across the trials at acquisition, SCR estimates were significantly higher for the CS+ than the CS-, $t(1722)=9.548$, 95% CI [0.125, 0.189], p<.001. Additionally, a significant trial*stimuli*PCL interaction was found, $F(3, 1722)=3.821$, p=.010. In trial 3, increasing PCL scores were associated with increased SCR amplitudes towards the CS+ (CS: β=0.059, 95%CI [0.001, .0118], p=.047) while a trend for the opposite was found in trial 5 (β=-0.055, 95%CI [-.0114, 0.003], p=.064). No association with the PCL score was found in any trials showing the CS-.

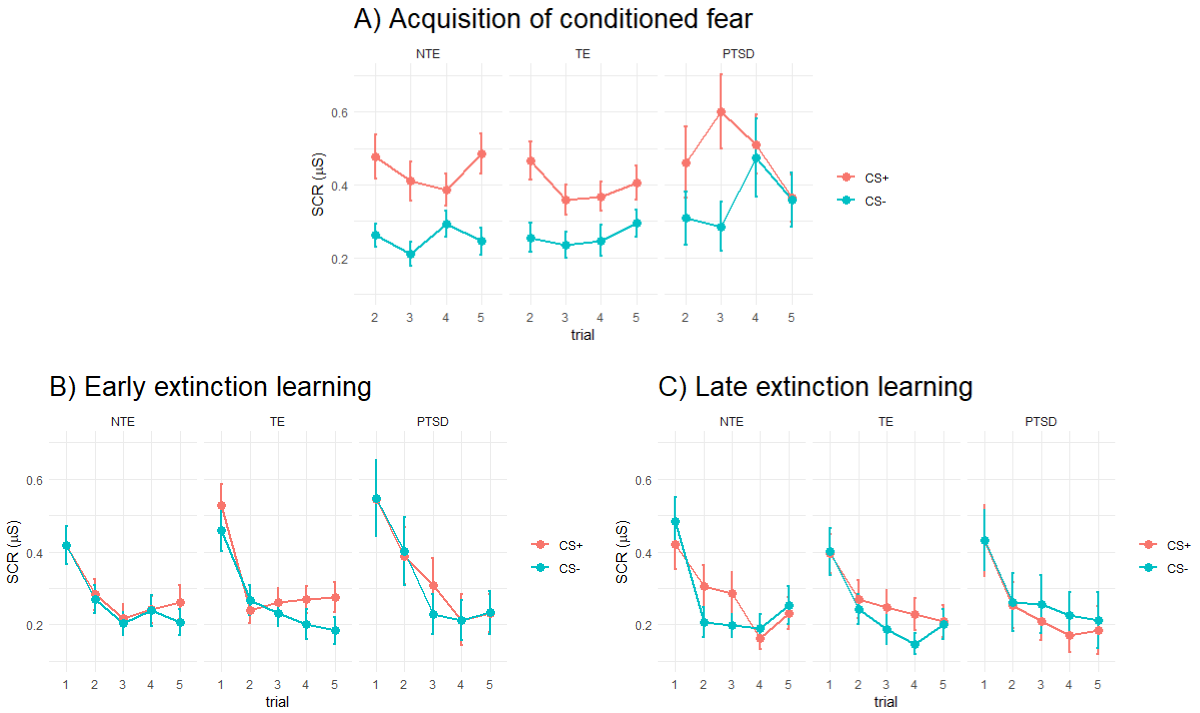

Figure 1: Group-wise mean and standard error of raw SCR values in microSiemens (µS). NTE: non-trauma exposed, TE: trauma-exposed, PTSD: posttraumatic stress disorder. Separate plots for males and females can be found in Fig. A.2.

Extinction learning

Early Extinction

During the early extinction learning phase (trials 1-5 of the extinction session), the linear mixed model revealed a significant main effect for trial, $F(4, 2214)=40.834$, $p<.001$, as well as a significant main effect for stimuli, $F(1, 2214)=3.959$, $p=.047$. Similar to the acquisition phase, SCR estimates were higher for the CS+ compared to the CS- across trials, $t(2214)=1.990$, 95% CI [0.001, 0.057], $p=.047$. The trial main effect showed a reduction in SCR amplitudes across the early extinction phase and across stimuli. Post-hoc contrasts revealed that there was a significant decrease between the first and the second trial, but this difference subsided after trial 2 (e.g. contrast between trial 1-2: $t(2214)=8.058$, 95% CI [0.1223, 0.248], $p<.001$; contrast between trial 2-3: $t(2214)=2.209$, 95% CI [-0.012, 0.113], $p=.177$). None of the interactions reached significance.

Late Extinction

In the late extinction phase (trials 6-10), only the trial main effect remained significant, $F(4, 2214)=50.149$, $p<.001$, with a similar reduction in SCR amplitude across stimuli compared to the early extinction phase. Again, post-hoc contrasts showed a significant difference between trial 6 (the first trial at late extinction) and the following (e.g. trial 6-7: $t(2214)=6.864$, 95% CI [0.1018, 0.236], $p<.001$) as well as between trial 7-9, $t(2214)=2.800$, 95% CI [0.002, 0.136], $p=.041$. The remaining comparisons were not significant.

Extinction Recall

One hundred and thirty-three participants completed the extinction recall session the following day. Fig. 2 illustrates the group-wise SCR amplitudes, separately averaged across the last two trials at late extinction (E) and the first two trials at recall (R), respectively, to create the time variable.

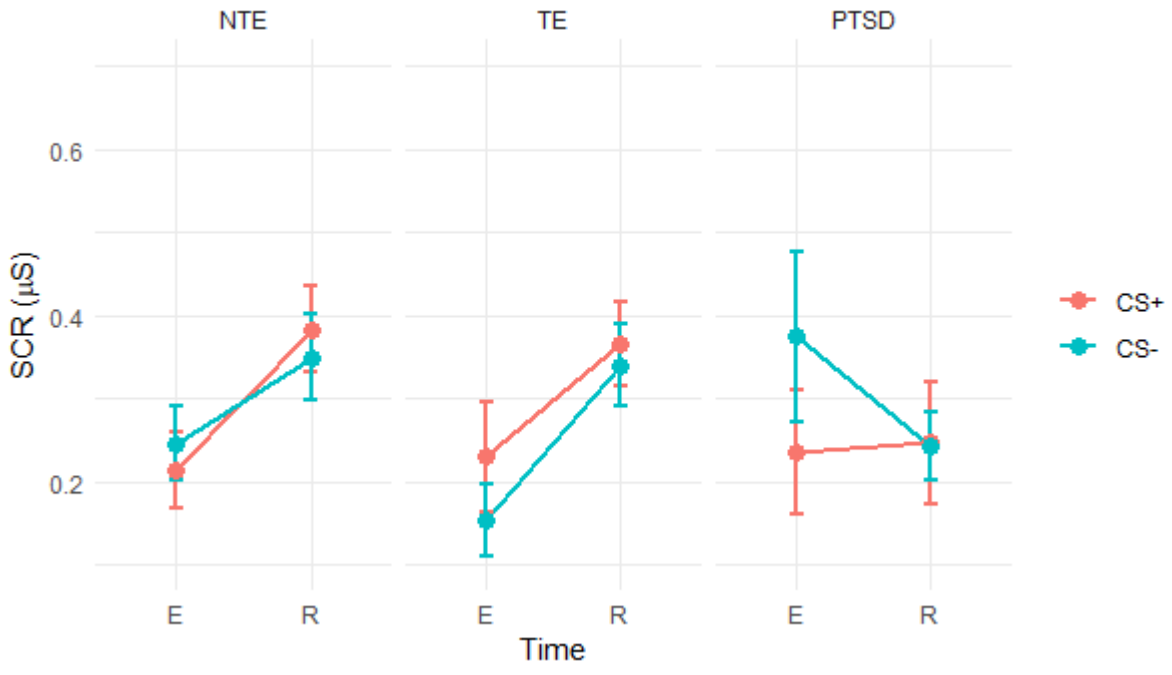

Figure 2: Group-wise mean raw SCR values microSiemens (µS) and standard error of the last two trials at late extinction (E) and first two trials at recall (R). NTE: non-trauma exposed, TE: trauma-exposed, PTSD: posttraumatic stress disorder. Separate plots for males and females can be found in Fig. A.3.

The linear mixed model revealed a significant main effect for time, $F(1, 386.10)=15.196$, $p<.001$. SCR amplitudes across both stimuli were significantly lower at late extinction compared to early recall, $t(385)=-4.353$, 95% CI [-0.174, -0.066], $p<.001$. None of the other main effects (stimuli or PCL) nor any interaction reached significance. The results of the models using alternative extinction recall operationalisations can be found in Tables A.28-A.39 in the appendix. As per their operationalisation of the outcome measure, none of the other models included a repeated measure, thus both stimuli were included in the same model. Similar to the findings reported here, none of the main effects for stimuli or PCL score, nor their interaction reached significance in the alternative analyses (see appendix).

Moderation analyses

Overall, individuals in the high PTSD symptom group had greater sleep disruptions including higher PSQI (sleep disturbances component), shorter total sleep time, shorter time in bed, longer sleep onset latency, longer time awake after initial sleep onset as well as poorer sleep efficiency than trauma-exposed and non-trauma exposed controls (see Tables 1 and 2). Additional linear regression investigated the associations between sleep variables and sex on PTSD symptom severity using PCL scores (see Tables A.1 and Fig. A.1 in the appendix).

Table 2: Sleep diary

	PTSD	**TE**	**NTE**	**Test statistic**	***p***
Sex	11F 6M	33F 21M	34F 28M	χ^2 (2) = 0.76	.683
Total sleep time	5.51 (2.64)	7.24 (1.49)	7.46 (2.08)	χ^2 (2) = 5.99	.050
Time in bed	7.23 (3.00)	8.18 (1.49)	8.13 (2.10)	χ^2 (2) = 1.33	.514
Sleep onset latency	54.07 (73.76)	25.61 (27.14)	16.93 (16.10)	χ^2 (2) = 6.34	.042
Wake after sleep onset	23.27 (42.98)	5.56 (10.42)	2.03 (4.68)	χ^2 (2) = 8.82	.012
Sleep efficiency	77.40 (20.76)	88.46 (7.98)	91.43 (5.44)	χ^2 (2) = 10.56	.005

Note: Mean and standard deviations. Total sleep time and time in bed in hours. Sleep onset latency and wake after sleep onset in minutes. Sleep efficiency in percentage 100*(total sleep time/time in bed).

Sleep disturbances

In the moderation analysis at fear acquisition (removing trial and adding the PSQI sleep disturbances component score as a fixed effect in the mixed model), the main effect for stimuli remained significant, $F(1, 1627)=42.571$, $p<.001$. There was no significant main effect or interaction with the PSQI sleep disturbances component score. This remained unchanged after including sex (stimuli main effect: $F(1, 1623)=39.315$, $p<.001$). During the early and the late extinction phases after excluding the fixed effect for trial, the stimuli main effect disappeared in both moderation analyses including either sleep alone or sleep and sex. SCR amplitudes did not differ depending on stimuli, PCL score, sleep, sex nor was there a significant interaction.

Sleep onset latency

At recall, looking at sleep onset latency only, the main effect for time remained significant in the model for the CS-, $F(1, 122.51)=4.341$, $p=.039$, but not CS+ ($p=.152$). This indicated that for the CS- only, SCR amplitudes were higher at early recall compared to the end of the extinction session. After including sex, the sleep onset latency*sex*PCL interaction was significant within the CS+ $F(1, 117.59)=6.769$, $p=.010$. In females and those with short sleep onset latency, higher PCL scores were associated with lower SCR amplitude towards the CS+ across the levels of time (β=-0.008, 95%CI [-0.015, -0.002], $p=.012$). This interaction is displayed in Fig. 3. The equivalent three-way interaction within the CS- reached trend level CS+ $F(1, 117.00)=3.135$, $p=.079$. In addition, the sex*sleep onset latency interaction was significant for both the CS+, $F(1, 117.70)=5.297$, $p=.023$, and CS-, $F(1, 117.11)=4.295$, $p=.040$. Regardless of time or PCL score, increasing sleep onset latency was associated with increasing SCR amplitudes in males and decreasing amplitudes in females in both stimuli. However, post-hoc simple slope analyses were not significant.

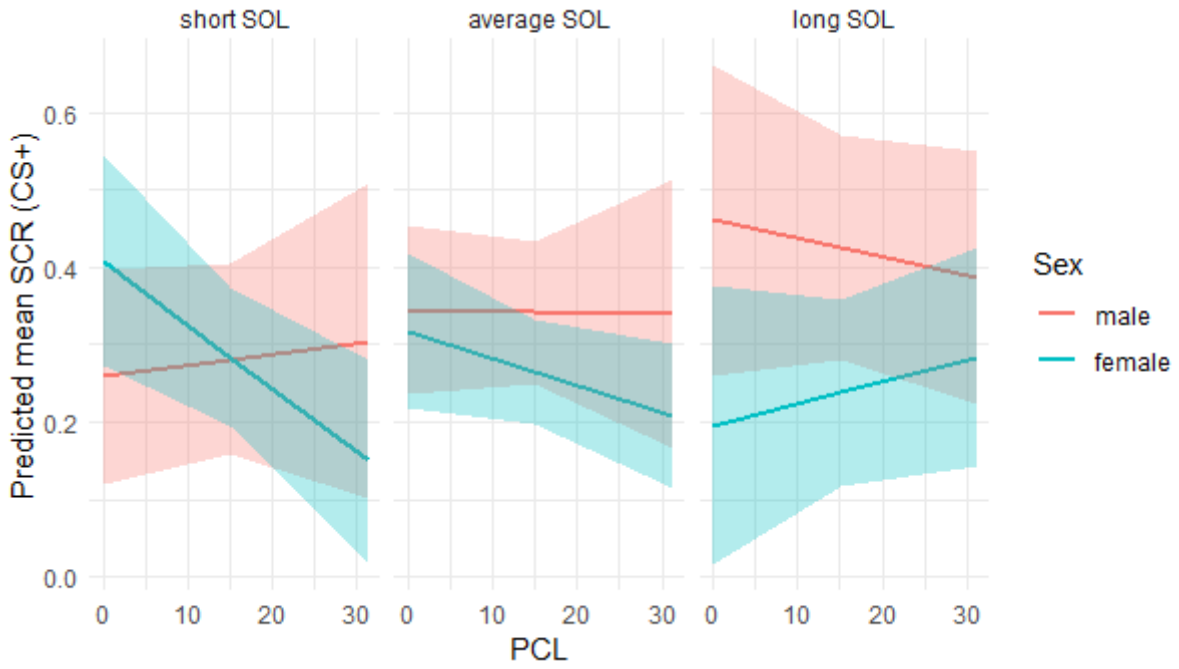

Figure 3: Simple slope analyses illustrating the PCL*sleep onset latency (SOL)*sex interaction using one standard deviation above the mean and minimum for long and short latencies, respectively. Shaded areas represent confidence bands.

Wake after sleep onset

In the models including wake after sleep onset, time remained a significant main effect with greater arousal towards both the CS+ and CS- at recall compared to late extinction trials (CS+: $F(1, 125.20)=6.803$, $p=.010$; CS-: $F(1, 123.94)=8.581$, $p=.004$). No main effect or interaction including wake after sleep onset reached significance for either CS+ or CS-. This remained unchanged after including sex.

Sleep efficiency

At recall, none of the effects looking at sleep efficiency alone were significant for either the CS+ or CS-. However, after including sex, there was a significant interaction for the CS+ between sex*PCL, $F(1,118.45)=4.547$, $p=.035$, such as in females, PCL was significantly associated with SCR amplitudes (β=-0.005, 95%CI [-0.010, -0.0002], $p=.043$), but not in males ($p=.909$). Additionally, there was a significant interaction between sleep

efficiency*sex*PCL, $F(1, 118.84)=5.327$, $p=.023$. In females with average or high sleep efficiency, greater PCL scores were associated with lower SCR amplitude across time points (average efficiency: $\beta=-0.006$, 95%CI [-0.010, -0.0002], $p=.042$, high efficiency: $\beta=-0.011$, 95%CI [-0.018, -0.003], $p=.004$). Within males, there was no significant association. The interaction is illustrated in Fig. 4. The equivalent effects within the CS- were in the same direction, however, did not reach significance.

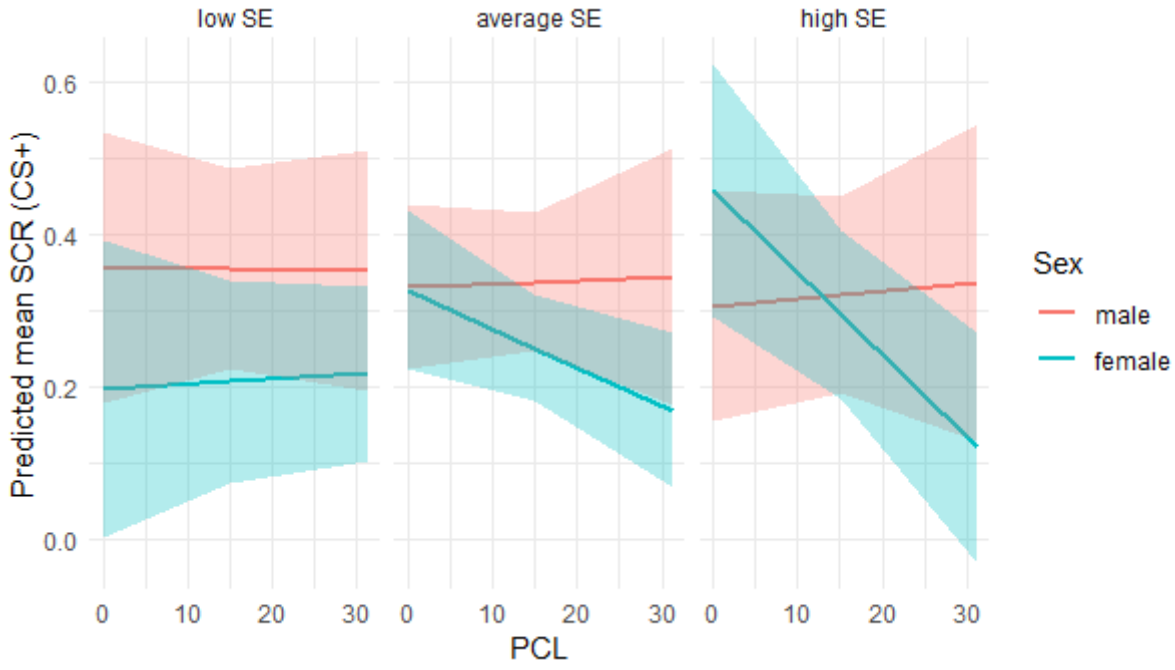

Figure 4: Simple slope analyses illustrating the PCL*sleep efficiency (SE)*sex interaction using one standard deviation above and below the mean for high and low percentages, respectively. Shaded areas represent confidence bands.

Discussion

The present study is the first to our knowledge to investigate the role of various facets of subjectively measured sleep on the acquisition and extinction of conditioned fear as well as extinction recall in a large sample of individuals with varying levels of PTSD symptom severity and non-trauma exposed controls. Contrary to our prediction, we did not find

evidence for disrupted sleep to be related to conditioned response (SCR amplitudes) during the fear conditioning and extinction learning paradigm. Baseline sleep disturbances were not associated with fear acquisition or extinction learning. Likewise, sleep onset latency, wake after sleep onset and sleep efficiency were not related to extinction recall. These associations were further not related to the level of PTSD symptom severity. However, the present study notably deviated from previous studies, which used objective (i.e. polysomnography) rather than subjective measures to assess sleep (e.g. Spoormaker et al., 2010), mostly included healthy controls only (e.g. Spoormaker et al., 2010) or investigated different outcome variables (i.e. fear reinstatement, Zuj et al., 2018). Nonetheless, we did find preliminary evidence for sex-specific effects on the sleep-extinction recall relationship, which is in line with our recent meta-analysis (Schenker et al., 2021). Our results suggest that there is evidence for less sleep disruptions to promote extinction recall for women with higher PTSD symptoms. Specifically, shorter sleep onset latency and greater sleep efficiency in women with greater PTSD symptom severity were both associated with reduced conditioned fear response at recall. Therefore, being able to fall asleep faster and staying asleep for longer while in bed may be protective in women with greater PTSD symptom load. In men however, sleep disruptions were not associated with extinction recall, irrespective of their PTSD severity. These findings add to the growing body of literature describing the role of sleep in fear acquisition, extinction learning and extinction recall (e.g. Peyrot et al., 2020; Richards et al., 2022; Schenker et al., 2021).

Regardless of sleep and sex, the paradigm achieved successful fear acquisition and extinction learning with greater conditioned SCR response observed to the threat signal (CS+) during the acquisition phase and reduction in conditioned response across the extinction phases. During the acquisition phase, individuals with greater PTSD symptom severity, indicated by higher PCL scores, showed greater conditioned response to the threat signal. Some studies have shown that individuals with PTSD show greater differential conditioning

(difference between conditioned response to the threat and safety signals; Orr et al., 2000), but it is important to acknowledge that many other studies did not find enhanced conditionability in PTSD (Duits et al., 2015). More consistently, PTSD has been associated with reduced extinction learning (Duits et al., 2015; Suarez-Jimenez et al., 2020; Zuj, Palmer, Lommen, et al., 2016) and extinction recall (Milad et al., 2008; Milad et al., 2009; Pitman et al., 2012). In contrast to these findings, the present analysis did not find any significant differences in conditioned response during the extinction phase regardless of PTSD symptom severity. A key methodological difference from earlier studies was that we examined the impact of PTSD symptom severity dimensionally rather than in specific groups of patients with PTSD relative to controls (e.g., Acheson et al., 2015; Blechert et al., 2007; Wicking et al., 2016). Additionally, we employed a 100% reinforcement schedule which may have led to more rapid extinction learning, reducing the sensitivity of our extinction measures (Lonsdorf et al., 2017). This may have reduced the strength of effects and explain the discrepancies. During the first trials at early and late extinction, there was a rise in SCR followed by a rapid fall during the following trials. While this reduction indicated successful extinction learning, the spike at the beginning of the late extinction phase was presumably due to the short break resulting in an orienting response or rather a slight rise in contingency uncertainty. At recall, there was a significant return of fear across the sample and stimuli. In fact, the conditioned response was higher during early recall trials compared to late extinction towards the threat signal in 94 individuals (76.42%) and higher towards the safety signal in 86 individuals (69.92%). It needs to be acknowledged that this generalised return of fear across both stimuli may not necessarily represent impaired extinction and safety recall but be due to the orienting response during early recall trials. To gain confidence in the generalisation effect, future studies could include an un-extinguished control stimulus to clarify whether fear generalisation occurred as well as measure the level of fear extinction retained as previously used (e.g. Menz et al., 2013). Again, in contrast to previous research (e.g. Milad et al., 2009), we did not find any

association between PTSD symptom severity and the amount of extinction recalled. Earlier studies commonly used an extinction retention or fear recovery index, respectively, as the proportion of extinction or fear retained at recall compared to maximum conditioned response shown during fear acquisition (e.g. Helpman et al., 2016; Li & Graham, 2016; Milad et al., 2009; Shvil et al., 2014; White & Graham, 2016). Despite these operationalisations being subject to critical discussion (Lonsdorf et al., 2019), we considered such indices and investigated whether this may explain the divergent finding (see Table A.36 in the appendix). Similar to the model used here, applying a fear and safety recovery index (Li & Graham, 2016; White & Graham, 2016) did not reveal increasing impairments in extinction recall in individuals with greater PTSD symptom severity.

Clinical implications

The current study adds to the growing literature around the bidirectional relationship between sleep and fear conditioning processes, which is important for clinical practice as sleep is a modifiable etiological factor (Germain, 2013; Germain et al., 2008; Spoormaker & Montgomery, 2008). Focusing on the treatment of sleep disturbances may mitigate PTSD symptom severity (Ho et al., 2016; Schoenfeld, 2012) and may increase gains from exposure therapy (Kleim et al., 2014; López et al., 2017; Pace-Schott et al., 2012; Sexton et al., 2017). This study supports the need to not only investigate objective sleep measures when studying underlying mechanisms involving sleep in exposure-based therapies, but to also examine subjective sleep. Self-reported sleep measures and associated perception of sleep is particularly important in the context of PTSD, where discrepancies between objective and subjective sleep is often observed (Ghadami et al., 2015; Hurwitz et al., 1998; Klein et al., 2003; Werner et al., 2016). The present study highlights that there is a potential sex-specific effect of subjective sleep on fear extinction processes. Women in particular may benefit from sleep-targeted interventions to maximise benefits from PTSD treatment. However, there are only limited laboratory-controlled intervention studies assessing the role of sleep in exposure-

based therapies to support these claims (Pace-Schott et al., 2012). Substantially more research is required in both male and female participants and assessing subjective measures of sleep.

Limitations and future research

There are several limitations to the present study which could be addressed in future research. First, the present study used PSQI to measure baseline sleep disturbances across the previous month. A sleep diary measuring sleep onset latency, wake after sleep onset and sleep efficiency during the preceding night may provide insight into the immediate impact of sleep (and concurring sleep perception) on the acquisition and extinction of conditioned fear. In addition, no sleep diary was completed during the first night between the acquisition/ extinction session and recall session. As mentioned above, the level of conditioning has been found to immediately affect objectively measured sleep during the following night (Seo et al., 2021; Spoormaker et al., 2014; Sturm et al., 2013). Rather than testing the effect of sleep on fear conditioning, the effect of fear acquisition and extinction learning on subsequent subjective sleep could be explored in future research. Next, while this study included a comparably large sample size, only a subsample of the participants (n=133, 53.63%) completed the recall session. Out of those, only 15 participants had probable PTSD with higher symptom severity including six males. Due to small variability between the male PTSD participants, a categorical analysis including the clinical and control groups separately to test the influence of sex was not possible at recall and warranted the dimensional approach. The small number of male participants with probable PTSD and the inclusion of the PTSD symptom severity dimensionally rather than categorically may have contributed to the failure in replicating earlier patterns of enhanced fear recall found in PTSD groups (e.g. Acheson et al., 2015; Blechert et al., 2007; Wicking et al., 2016). In addition, the smaller number of male participants, particularly on the higher end of the PTSD spectrum, means that the non-significant results in men may not indicate the absence of an effect and the findings have to be interpreted with caution. Therefore, studies with greater sample sizes testing extinction recall

in both men and women with a range of PTSD symptom severity are needed to replicate the findings and clarify the effect. Lastly, while a preliminary effect in women was found here, the current analysis did not include hormonal differences, nor take hormonal contraception or menstrual cycle into account. As previously mentioned, fear and extinction learning capabilities are modulated by the fluctuations of sex hormones within the menstrual cycle of female participants (Garcia et al., 2018; Li & Graham, 2017; Peyrot et al., 2020) and may be the main driver behind the sex effect. To understand the role of sex better, sex hormones and/or menstrual cycle should be accounted for in future research.

Conclusion

In summary, the present study adds to the growing body of literature assessing the role of sleep in fear conditioning/ extinction learning paradigms with relevance to PTSD as well as exploring sex-specific differences in these relationships . While no evidence was found for subjectively measured sleep disruptions to be related to acquisition and extinction of conditioned fear, there might be different effects for men and women with regards to extinction recall. Exploratory analyses suggest shorter sleep latency and greater sleep efficiency may be protective of poor extinction recall in women with higher PTSD symptom load. However, no association was found in men in this study, highlighting the need to study sex-differences in sleep-related fear conditioning processes further. If these effects are replicated, this finding has clinical implications which indicate that in women with greater PTSD symptom severity, sleep-focused PTSD interventions may be particularly beneficial.

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