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14. ABSTRACT
PTSD is a growing concern for both active duty personnel and Veterans. Fear conditioning is implicated in the development of PTSD, while successful acquisition, consolidation, and recall of extinction memory are implicated in both the natural reduction of initial PTSD symptoms and as the mechanism underlying the most successful treatment for PTSD, Prolonged Exposure. In animal models, sleep deprivation has been shown to impair extinction memory, although this has never been directly tested in humans. This project is the first to examine the role of sleep and sleep loss in acquisition, consolidation, and generalization of extinction memory in humans.

15. SUBJECT TERMS
PTSD, sleep deprivation, fear conditioning, extinction memory, humans

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Introduction

PTSD is a growing concern for both active duty personnel and Veterans. Fear conditioning is implicated in the development of PTSD, while successful acquisition, consolidation, and recall of extinction memory are implicated in both the natural reduction of initial PTSD symptoms and as the mechanism underlying the most successful treatment for PTSD, Prolonged Exposure. In animal models, sleep deprivation has been shown to impair extinction memory. Indirect evidence in humans also supports that notion, but it has never been tested directly in humans. Some of the most ubiquitous and distressing symptoms of PTSD are insomnia and nightmares. The resultant sleep deprivation may actually serve to perpetuate the disorder by interfering with treatments designed to promote extinction memories. Before this hypothesis can be tested in clinical populations, however, well-controlled experimental studies need to establish the exact role of sleep deprivation in extinction acquisition, consolidation, and recall in humans. This study will do just that. This is a mixed-effects study designed to examine the impact of 36 hours TSD on fear conditioning and consolidation (Aim 1), as well as extinction memory acquisition, recall, and generalization (Aim 2). A total of 60 subjects will participate across 3 years. Following recruitment and screening, subjects will spend 4 nights and days in the laboratory: a) adaptation to the lab (Night/Day0); b) normal sleep followed by fear memory acquisition (Night/Day1); c) sleep or TSD followed by fear recall and extinction memory acquisition (Night/Day2); and d) sleep or TSD followed by a test of extinction recall and generalization (Night/Day3). Group1 will receive sleep prior to each testing day, Group2 will be sleep deprived prior to Day2, and Group3 will be sleep deprived prior to Day3.

Body

This report covers the first No Cost Extension year of the project. This year focused on data processing and analysis, as well as dissemination and publication of our findings. Specific activities include:

a. Data Processing. As describe in a series of quarterly reports, we spent a significant amount of effort developing the best ways to account for the significant inter-individual variability in our main physiological outcome data during the sleep deprivation nights. After several iterations and consultation with colleagues in the field, we settled on the most appropriate method and are now reprocessing all relevant data.

b. Data Analysis. We have analyzed data for our first two manuscripts, and are working an analysis for the third.

c. Dissemination. In addition to the manuscripts described below, Dr. Drummond has made several national and international presentations of our study findings. These include: a) an invited presentation at the Canadian Sleep Society meeting in Halifax, Nova Scotia; b) a departmental colloquium at Monash University in Melbourne, Australia; and c) an invited presentation at the Australian Centre for Posttraumatic Mental Health. Dr. Drummond also conducted a number of media interviews related to the Journal of Neuroscience paper described below.

d. Manuscripts. We have published two manuscripts, thus far, from this study (see Reportable Outcomes, below).

e. We are currently working on a third paper and anticipate a total of four papers based on the data from this study.

Key Research Accomplishments

Data collection completed

Two published manuscripts:

1. van Enkhuizen, J., Acheson, D., Risbrough, V., Drummond, S., Geyer, M., Young, J. Sleep deprivation impairs performance in the 5-choice continuous performance test; Similarities between humans and mice. Behavioural Brain Research. 2014, 261:40-48.

2 Marshall, AJ, Acheson, DT, Risbrough, VB, Straus, LD, Drummond, SPA. Fear Conditioning, Safety Learning, and Sleep in Humans. Journal of Neuroscience. 2014 34(35):11754 –11760.

Several national and international conference presentations

Reportable Outcomes

1. van Enkhuizen, J., Acheson, D., Risbrough, V., Drummond, S., Geyer, M., Young, J. Sleep deprivation impairs performance in the 5-choice continuous performance test; Similarities between humans and mice. Behavioural Brain Research. 2014, 261:40-48.

In this paper, we leveraged the work being done in the current project with the larger collaboration between Dr. Drummond's group and Dr. Risbrough's group to extend the translational nature of the current project. In particular, we combined secondary data from the current project examining the effects of our sleep deprivation manipulation on attention with animal model data from Dr. Risbrough's lab also examining the effects of sleep loss on attention. A summary of the paper follows.

Several groups undergo extended periods without sleep due to working conditions or mental illness. Such sleep deprivation (SD) can deleteriously affect attentional processes and disrupt work and family functioning. Understanding the biological underpinnings of SD effects may assist in developing sleep therapies and cognitive enhancers. Utilizing cross-species tests of attentional processing in humans and rodents would aid in mechanistic studies examining SD-induced inattention. We assessed the effects of 36 hours of: 1) Total SD (TSD) in healthy male and female humans (n=50); and 2) REM SD (RSD) in male C57BL/6 mice (n=26) on performance in the cross-species 5-Choice Continuous Performance Test (5C-CPT). The 5C-CPT includes target trials on which subjects were required to respond and non-target trials on which subjects were required to inhibit from responding. TSD-induced effects on human Psychomotor Vigilance Test (PVT) were also examined. Effects of SD were also examined on mice split into good and poor performance groups based on pre-deprivation scores. In the human 5C-CPT, TSD decreased hit rate and vigilance with trend-level effects on accuracy. In the PVT, TSD slowed response times and increased lapses. In the mouse 5C-CPT, RSD reduced accuracy and hit rate with trend-level effects on vigilance, primarily in good performers. In conclusion, SD induced impaired 5C-CPT performance in both humans and mice and validates the 5C-CPT as a cross-species translational task. The 5C-CPT can be used to examine mechanisms underlying SD induced deficits in vigilance and assist in testing putative cognitive enhancers.

2 Marshall, AJ, Acheson, DT, Risbrough, VB, Straus, LD, Drummond, SPA. Fear Conditioning, Safety Learning, and Sleep in Humans. Journal of Neuroscience. 2014 34(35):11754 –11760.

This paper focuses on the main translational questions in the project. Specifically, this is the first paper to directly translate animal models of fear condition-sleep relationships into humans. Other human studies have examined extinction learning, but not the initial fear acquisition. This is critical to understanding PTSD, as without the initial fear acquisition, there would be no PTSD. We were also able to examine, for the first time in any species, the relationship between sleep and safety signals. A summary of the paper follows.

Fear conditioning is considered an animal model of Posttraumatic Stress Disorder (PTSD). Such models have shown fear conditioning disrupts subsequent Rapid Eye Movement Sleep (REM). Here, we provide a translation of these models into humans. Using the Fear Potentiated Startle (FPS) procedure, we examined the effects of fear conditioning and safety signal learning on subsequent REM sleep in healthy adults. We also examined the effects of changes in REM sleep on retention of fear and safety learning. Participants (n=42 normal controls) spent 3 consecutive nights in the lab. The first was an adaptation night. Following the second night, we administered a FPS procedure that included pairing a wrist shock with a threat signal and a safety signal never paired with a shock. The next day, we administered the FPS procedure again, with no wrist shocks to any stimulus, in order to measure retention of fear and safety. Canonical correlations assessed the relationship between FPS response and REM sleep. Results demonstrated increased safety signal learning during the initial acquisition phase was associated with increased REM sleep consolidation that night, with 28.4% of the variance in increased REM sleep consolidation from baseline accounted for by safety signal learning. Overnight REM sleep was, in turn, related to overnight retention of fear and safety learning, with 22.5% of the variance in startle retention accounted for by REM sleep. These data suggest sleep difficulties, specifically REM sleep fragmentation, may play a mechanistic role in PTSD via an influence on safety signal learning and/or threat-safety discrimination.

Conclusion

This past year, we published two peer-reviewed manuscripts, including one in the highest rated neuroscience journal in the world that also received a fair amount of media coverage. We also successfully solved our data processing issues, which has lead to our ability to reprocess the sleep deprivation data and published two additional manuscripts in the requested second NCE period.

There are two major implications of our findings. First, both papers show we can effectively and validly translate basic science conducted with animal models into humans, thereby capitalizing on basic mechanism work done in animals to better understand cognition and fear processes underlying PTSD in humans. This will contribute to development of preventative and treatment strategies. Second, our Journal of Neuroscience paper points two a completely novel mechanism in understanding the critical role sleep plays in the maintenance treatment of PTSD. Impaired safety learning is a hallmark of PTSD, as well as other anxiety disorders. Our data suggest REM sleep is critical for consolidating safety learning and the ability to discriminate safe from threatening stimuli. This, in turn, argues treating sleep problems prior to administering evidence based PTSD treatments may improve clinical outcomes dramatically.

References

See above

Appendices

- Updated Quad Chart (showing figures from Outcome reported above)
- Two published manuscripts

Supporting Data

N/A

Role of Sleep Deprivation in Fear Conditioning and Extinction: Implications for Treatment of PTSD
Proposal ID: DM102425, funding Source: DMRDP

PI: Sean P.A. Drummond, PhD **Org:** Veterans Medical Research Foundation Award Amount: $1,091,578.00

Study Aim

- Overall: Provide first translation study of impact of sleep deprivation on fear conditioning and extinction memory in humans
- Specific Aim 1: Determine if total sleep deprivation (SD) alters consolidation of fear conditioning
- Specific Aim 2: Determine if total SD impairs extinction memory acquisition, recall, or generalization

Approach: Between subjects study comparing normal night of sleep to 26 hours total SD wrt impact on fear conditioning, and extinction acquisition, recall, and generalization. Subjects are healthy human controls.

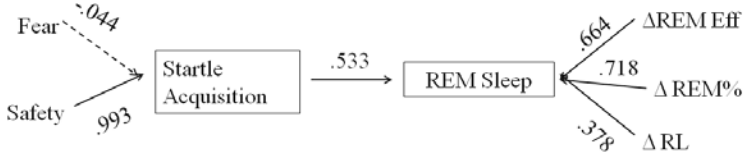

Fig 1: Model showing impact of startle paradigm on subsequent REM sleep

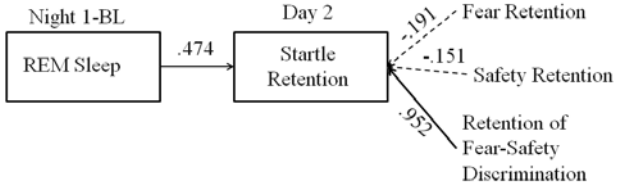

Fig 2: Model showing effect of REM sleep on fear/safety memory consolidation

Timeline and Cost

Activities	FY 11	FY12	FY13
***Task 1**: Regulatory Approval*			
***Task 2**: Hire and train*			
***Task 3**: Enrollment*			
***3a**: Enroll 14 subjects*			
***3b**: Cumulative enrollment of 53*			
***3c**: Cumulative enrollment of 72*			
***Task 4**: Analyze data & submit publication*			

Updated: 22 Oct 2014

Goals/Milestones

FY 11 Goals

☑ Regulatory approval
☑ Hire and train staff
☑ Enroll 14 subjects

FY 12 Goals

☑ Cumulative Enrollment of 53 subjects

FY13 Goals

☑ Cumulative enrollment of 72 subjects
☐ Analyze data and submit manuscripts

NCE Year 1 Goals

☑ Determine Proper Processing Pathway
☑ Published 1-2 Manuscripts

Behavioral/Cognitive

Fear Conditioning, Safety Learning, and Sleep in Humans

Anisa J. Marshall,[1] Dean T. Acheson,[1,3,4] Victoria B. Risbrough,[1,3,4] Laura D. Straus,[1,5] and Sean P.A. Drummond[2,3,4,5]

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Fear conditioning is considered an animal model of post-traumatic stress disorder. Such models have shown fear conditioning disrupts subsequent rapid eye movement sleep (REM). Here, we provide a translation of these models into humans. Using the fear potentiated startle (FPS) procedure, we examined the effects of fear conditioning and safety signal learning on subsequent REM sleep in healthy adults. We also examined the effects of changes in REM sleep on retention of fear and safety learning. Participants ($n = 42$ normal controls) spent 3 consecutive nights in the laboratory. The first was an adaptation night. Following the second night, we administered a FPS procedure that included pairing a wrist shock with a threat signal and a safety signal never paired with a shock. The next day, we administered the FPS procedure again, with no wrist shocks to any stimulus, to measure retention of fear and safety. Canonical correlations assessed the relationship between FPS response and REM sleep. Results demonstrated that increased safety signal learning during the initial acquisition phase was associated with increased REM sleep consolidation that night, with 28.4% of the variance in increased REM sleep consolidation from baseline accounted for by safety signal learning. Overnight REM sleep was, in turn, related to overnight retention of fear and safety learning, with 22.5% of the variance in startle retention accounted for by REM sleep. These data suggest that sleep difficulties, specifically REM sleep fragmentation, may play a mechanistic role in post-traumatic stress disorder via an influence on safety signal learning and/or threat-safety discrimination.

Key words: **fear conditioning; REM sleep; safety learning; translational**

Introduction

Sleep difficulties are among the most common symptoms in post-traumatic stress disorder (PTSD) (Germain et al., 2008; Spoormaker and Montgomery, 2008), with 70% of civilians (Ohayon and Shapiro, 2000), and up to 90% of combat veterans with PTSD (Neylan et al., 1998) reporting sleep difficulties. More than just a symptom, sleep appears to play a role in development and maintenance of PTSD (Germain et al., 2008). Although sleep disruption has been linked with the development, exacerbation, and possibly poor recovery from PTSD (Babson and Feldner, 2010; Nadorff et al., 2011; van Liempt et al., 2013), the exact mechanism underlying the role sleep plays in PTSD is not well known.

PTSD is a disorder of abnormal fear processes, wherein learned fear responses are greater and more difficult to inhibit (Johnson et al., 2012). One translational model for addressing the role of sleep in fear processes, and thus PTSD, is fear conditioning. Fear conditioning is a Pavlovian response whereby a neutral stimulus is paired with an aversive stimulus until the previously neutral stimulus elicits a conditioned fear response and is considered a good animal model of PTSD (Grillon, 2002). Animal models have shown consistent relationships between fear conditioning and sleep, especially rapid eye movement (REM) sleep. For example, fear conditioning disrupts and fragments REM sleep (Sanford et al., 2001; Wellman et al., 2008), even after a single trial of conditioning (Sanford et al., 2003). REM sleep fragmentation or deprivation, in turn, impairs extinction memory, the process by which an animal learns the previously feared stimulus no longer signals threat (Silvestri, 2005; Fu et al., 2007). Furthermore, greater REM sleep fragmentation before fear conditioning predicts greater acoustic startle responses 1 month after fear conditioning (Polta et al., 2013). These findings suggest that REM sleep disruption may play a role in PTSD via interactions with fear and/or extinction processes. Few studies, though, have translated the animal model findings into humans. While studies have shown sleep in general (Pace-Schott et al., 2009; Sturm et al., 2013), and REM sleep in particular (Spoormaker et al., 2010, 2012), play a role in extinction memory in humans, no published studies have examined whether initial fear conditioning disrupts overnight REM sleep in humans.

In contrast to a conditioned threat signal, fear conditioning procedures can also include a safety signal. This is a stimulus presented during fear learning but never paired with the aversive unconditioned stimulus (US). Impairments in safety signal learning are seen when someone shows a strong fear response to

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Author contributions: D.T.A., V.B.R., and S.P.A.D. designed research; A.J.M., D.T.A., L.D.S., and S.P.A.D. performed research; A.J.M., D.T.A., V.B.R., L.D.S., and S.P.A.D. analyzed data; A.J.M., D.T.A., V.B.R., L.D.S., and S.P.A.D. wrote the paper.

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The authors declare no competing financial interests.

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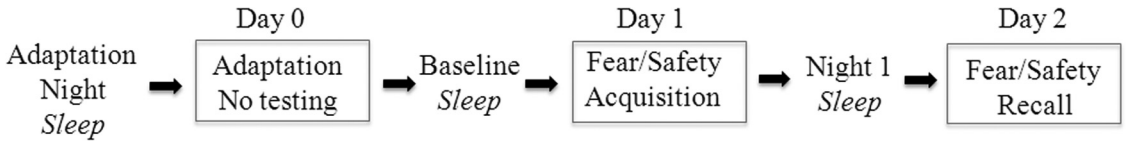

Figure 1. Study time line. Participants entered the laboratory on the evening before the Adaptation Night and remained there until the study was completed. Startle procedure was scheduled for 10–12 h after awakening.

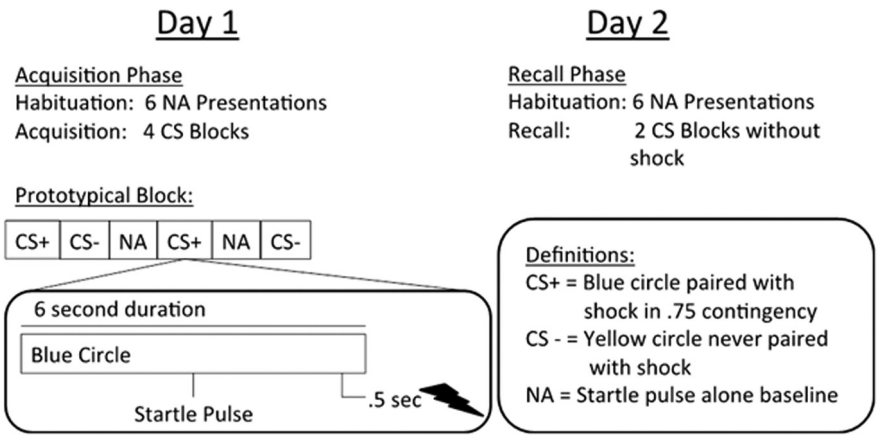

Figure 2. Fear potentiated startle procedure. Schematic of the fear potentiated startle procedure detailing the fear acquisition and recall phases, as well as illustrating a prototypical block and trial.

a safety signal (e.g., as strong as that shown to the threat signal), despite repeated presentations of the stimulus without ever pairing it with the aversive stimulus. There are no published studies examining sleep in relation to safety signals. However, given PTSD is the only clinical population to show impairments in safety signal learning (Jovanovic et al., 2010), it is of interest to know if safety learning interacts with REM sleep, as well.

Our aim was to examine effects of fear conditioning and safety learning on subsequent sleep in humans. Our main hypothesis, based on the animal literature, was greater levels of fear conditioning during the day would lead to more fragmented REM sleep at night. Although there is no literature upon which to develop hypotheses related to safety learning, we anticipated the opposite relationship: greater levels of safety learning would be associated with consolidated REM sleep. We also examined whether changes in REM sleep following fear/safety learning was associated with next day retention of conditioned responses.

Materials and Methods

Participants. Forty-two healthy normal controls were recruited from the San Diego area, completed the study, and were included in these analyses (age = 24.1 ± 5.0 years, 42% female, 37% racial/ethnic minority). In addition, one subject was excluded because of invalid sleep data, and 13 were excluded for not showing a startle response to 12 108-dB acoustic pulses administered at screening (startle reactivity was defined as exhibiting responses to >50% of trials). After written informed consent, participants were screened for drug use, sleep disorders, and psychiatric and medical conditions via structured interview and laboratory tests. Inclusion criteria included the following: (1) age 18–39 years old; (2) ≥12 years of education; and (3) consistent sleep–wake schedule, including 7–9 h of overnight sleep/night. Female participants were studied in the early follicular phase of the menstrual cycle, as estrogen can affect fear learning processes (Milad et al., 2006). Participants maintained a regular sleep–wake schedule, corresponding to their habitual schedule, for 1 week before entering the laboratory portion of the study. Sleep diaries and actigraphy monitored adherence with this schedule, and anyone deviating >15 min on ≥2 nights was not studied. Participants refrained from alcohol and caffeine for 48 h before entering the laboratory.

Sleep procedures. Participants entered the laboratory ~2 h before bed time on the adaptation night and lived in the laboratory until the end of the study, including three overnight sleep studies (Fig. 1). Each night, a polysomnogram, including EEG, EOG, and chin EMG, monitored sleep. To screen for unreported sleep apnea and periodic leg movements, monitors were added on adaptation night. Three REM sleep variables served as our main measures: REM% (proportion of sleep spent in REM); REM sleep efficiency (RE: proportion of epochs within REM episodes scored as REM sleep, as opposed to wake or non-REM sleep; this is parallel to the concept of sleep efficiency); and REM sleep latency (RL: time from sleep onset to the first REM epoch). We took lower REM%, lower RE, and shorter RL as indices of REM sleep fragmentation. To examine change in REM sleep after the startle procedure, a difference score was calculated as follows: (Night 1 − baseline).

Fear potentiated startle (FPS). We used FPS model of cued fear conditioning. Participants learned to associate a visual conditioned stimulus (CS) with an US of electrical shock to the wrist. Conditioned fear responses to the CS were measured using eyeblink magnitude in response to acoustic stimuli presented in the presence/absence of CS. The degree of relative startle potentiation in the presence of the CS, compared with absence of CS, was used as an operational measure of fear response.

On Day 1, participants completed the fear-conditioning procedure to acquire conditioned FPS, as described in detail previously (Acheson et al., 2013). Participants were seated in a lounge chair in a sound-attenuated testing chamber. The visual CSs (colored circles) were presented using an LCD computer monitor connected to a Dell desktop computer. Startle pulses were 108 dB, 40 ms bursts of broadband noise. EMG responses were recorded from electrodes placed at the orbicularis oculi muscle (San Diego Instruments; for details on apparatus, startle measurement and processing see Acheson et al., 2013). The electrical shock stimuli were delivered via a Contact Precision Instruments SHK1 aversive shock stimulator coupled with an IBM ThinkPad notebook computer as previously described by Acheson et al. (2013). On the first day of testing, shock intensity levels were set manually for each individual by delivering gradually more intense shocks (0–5 mA range) until the subject reported that the shock level was "highly annoying yet not painful." Figure 2 contains a schematic of the FPS procedure. Acquisition began with a 1 min acclimation period consisting of 70 db of white noise followed by six startle pulses presented in the absence of any other stimuli in order for the participants to acclimate/habituate startle responses to baseline level. After acclimation, visual cues were as follows: (1) conditioned via pairing with wrist shock in 0.75 contingency (CS^+); or (2) never paired with the shock (CS^-), thus serving as safety signals. The acquisition session included 8 CS^+ trials (6 paired with shock), 8 CS^- trials, and 8 noise alone (NA) trials where no visual stimuli were presented, providing baseline startle reactivity. The auditory stimulus during the NA trials, a brief (40 ms) pulse of 108 dB is used to induce a startle response, the magnitude of which is the operational measure of threat response or "fear." The auditory stimulus is not a unconditioned

stimulus, as it is presented during all conditions (NA, CS^-, and CS^+ trials). Thus, because this probe happens in every trial type, it is not predicted by the presence or absence of any specific stimulus. To the extent the startle pulse has arousing/aversive qualities, then such an effect is controlled for by using a difference score of potentiation above background for the stimuli of interest (i.e., the differential between NA trials and the CS^+ or CS^- trials). Stimulus presentation was block randomized with the constraint of two trials of each type (CS^+, CS^-, and NA) per block. This approach prevents confounds of uneven habituation effects on any one stimulus type and assures accurate temporal match of NA baseline responses to CS^+ and CS^- trials. Participants returned on Day 2 for the recall phase. FPS recall was assessed via four presentations of each stimulus type (CS^+, CS^-, and NA) in block randomized order as in acquisition. No shocks were presented during this phase.

Data were analyzed by averaging peak responses to each stimulus type within a block. Responses were examined trial by trial to remove voluntary eyeblink and movement artifact, with responses only being scored if they were within 100 ms of the onset of the startle pulse. The NA average was subtracted from the CS^+ and CS^- responses to create a score representing startle above baseline for each CS type in each block (e.g., $(CS^+) - (NA)$) (Norrholm et al., 2006, 2011; Acheson et al., 2013). Three variables served as outcomes for the acquisition phase: (1) acquisition to the threat signal (i.e., fear potentiation): mean CS^+ response during the last two blocks of the session; (2) safety signal learning: difference in CS^- response from the first block to the last block; and (3) differential conditioning: difference between mean CS^+ versus CS^- responses during last two blocks (i.e., differentiation of threat from safety signals). Safety signal learning is measured slightly differently than acquisition to the threat signal and differential conditioning because it is conceptualized as a within-subject change across the session (Norrholm et al., 2011; Acheson et al., 2013). The extent to which a subject learns over time that the CS^- is, indeed, a safety signal, is best captured by examining the reduction in potentiation to the CS^- stimulus from the beginning of the task (when they do not know it represents safety) to the end of the task (when they should have learned it is a safety signal). Variables of interest on Day 2 related to retention of learning from Day 1: (1) fear retention: change in response to CS^+ from the last block of Day 1 to the first block of Day 2; (2) safety signal retention: change in response to CS^- from the last block of Day 1 to the first block of Day 2; and (3) retention of differential learning: difference in response to CS^+ and CS^- during first two blocks of Day 2.

Statistical analyses. To document the anticipated differential effects between fear potentiation to the CS^+ and safety signal learning on the CS^-, we compared the FPS response to each stimulus type during the relevant blocks, as defined above, with a paired-samples *t* test.

For the main analyses, because we anticipated two startle variables from Day 1 (fear acquisition and safety signal learning) being associated with three REM sleep variables (RE, REM% RL), we used a multivariate approach: canonical correlation analysis. This analysis forms canonical variates (i.e., latent variables) from each set of input variables, assessing the correlation between those canonical variates. If that correlation is significant, one examines the correlation (i.e., structural coefficients) between individual measures and their respective canonical variate to determine which measures make significant contributions to the overall relationship (Thompson, 2000; Tabachnick and Fidell, 2001). Based on recommendations of Tabachnick and Fidell (2001), we considered any structural coefficient $>|0.30|$ to indicate a significant contribution to the respective canonical variate and thus the overall relationship between FPS and REM sleep measures.

After determining the relationship between fear/safety acquisition and subsequent REM sleep, we examined whether those changes in REM sleep on Night 1 were associated with overnight retention of fear/safety, as measured on Day 2. Here, we used a similar multivariate approach using the REM canonical variate developed in the analysis above to predict response to FPS measures on Day 2. Outcome variables were as follows: fear retention, safety retention, and differential conditioning retention. For this analysis, those participants who did not show a potentiation response to the threat signal on Day 1 (potentiation response defined as $(CS^+) - (NA) > 0$) were dropped because, in those individuals, our operational measure of fear responding did not exist and thus there was no fear conditioning to "retain" on Day 2. The reason for this is that some people require very loud stimuli (>108 dB) to show a reliable startle response, or they habituate their startle response very quickly. Subjects who do not startle to the 108 db stimulus provide no data with which we could assess fear conditioning and safety signal learning. Additionally, it is unclear whether a small startle response to the safety signal in those participants was due to learning safety or simply an overall nonresponse to the task. Similar exclusions for low baseline response rates also occur in studies using other physiological measures, such as galvanic skin response. Given this exclusion, the sample size for this analysis was 38. As with the first analysis, significance testing with $\alpha = 0.05$ was used to determine whether REM sleep predicted the FPS measures and structural coefficients $>|0.30|$ defined which measures made significant contributions to the startle retention canonical variate.

After determining the findings from the main analyses above, two *post hoc* analyses were conducted to examine alternative explanations for the relationships between FPS and REM sleep reported here. First, we conducted a canonical correlation to determine whether initial REM sleep adaptation to the sleep laboratory predicted fear and/or safety acquisition. Specifically, we calculated change in REM sleep variables across the initial nights as (baseline − adaptation). We then entered those variables into the canonical correlation as one set and the same FPS variables used in the main analysis (i.e., fear potentiation and safety learning) as the other set. Second, to test whether REM sleep contributes variance to startle retention above and beyond that accounted for by initial startle acquisition, we conducted another canonical correlation, including both the startle and REM sleep canonical variates as predictors of startle retention. Here, we entered both startle canonical variate and REM sleep canonical variate as predictors of our three startle retention variables into a canonical correlation. For both *post hoc* analyses, we evaluated the outcome in the same manner described above.

Examination of age and sex showed neither one was related to any sleep measure, FPS variable, or canonical variate. Thus, they were not included in the analyses.

Results

Table 1 shows sleep measures from adaptation, baseline, and Night 1. We observed the expected improvements in sleep from adaptation to baseline, and no sleep variable showed further significant changes between baseline and Night 1 within the entire sample. Figure 3 demonstrates the expected differential response to CS^+ and CS^- at the end of the acquisition phase. There was no difference in the response to CS^+ and CS^- at Block 1 ($t_{(41)} = -0.502$, $p = 0.618$), whereas there was a significant difference at the end of acquisition ($t_{(41)} = -2.44$, $p = 0.019$).

Figure 4*A* shows results of the canonical correlation testing whether FPS measures on Day 1 were associated with changes in REM sleep on Night 1. The two sets of measures shared one significant factor (Hotelling's Trace $F_{(6,72)} = 2.44$, $p = 0.033$, $r^2 = 0.284$). Only safety signal learning contributed in a meaningful way to the startle acquisition canonical variate. Standardized canonical coefficients (equivalent to standardized β weights in a regression analysis) were as follows: safety signal learning = 1.00, fear acquisition = −0.116, confirming the findings from the structural coefficients. All three REM sleep variables made meaningful contributions to the REM sleep canonical variate. Standardized canonical coefficients were as follows: RE = 0.466; REM% = 0.652; RL = 0.589. The direction of the relationships between the REM sleep measures and the REM canonical variate were all in the direction of more consolidated REM sleep.

Figure 4*B* shows results of the analysis assessing the relationship between REM sleep and retention of fear/safety on Day 2. REM sleep significantly predicted values on the retention canonical variate (Hotelling's Trace $F_{(3,34)} = 3.29$, $p = 0.032$, $r^2 = 0.225$). Only differential conditioning retention made a meaningful contribution

Table 1. Sleep measures[a]

Measure	Adaptation (mean ± SD)	Baseline (mean ± SD)	Night 1 (mean ± SD)	Adaptation − baseline		Baseline − Night 1	
				p	Effect size	*p*	Effect size
Total sleep Time (min)	438.6 ± 45.9	463.0 ± 43.7	467.6 ± 35.2	0.00	−0.57	0.32	−0.16
				0.00	0.58		
Wake after sleep onset (min)	48.6 ± 34.0	30.1 ± 21.6	28.1 ± 17.0			0.49	0.11
Sleep latency (min)	12.8 ± 11.1	13.5 ± 14.7	10.8 ± 8.4	0.78	−0.04	0.21	0.22
				0.01	−0.44		
Sleep efficiency (TST/TIB)	87.8 ± 7.4	91.4 ± 6.0	92.3 ± 3.9			0.28	−0.18
N1%	8.6 ± 4.7	7.5 ± 3.3	7.9 ± 2.9	0.16	0.23	0.37	−0.14
N2%	53.6 ± 6.6	49.8 ± 6.8	48.4 ± 6.5	0.00	0.67	0.08	0.28
N3%	17.9 ± 8.1	18.9 ± 7.3	19.0 ± 6.9	0.15	−0.23	0.83	−0.03
				0.00	−0.74		
REM %	20.0 ± 5.4	23.9 ± 5.0	24.6 ± 4.3			0.26	−0.18
				0.00	0.60		
REM latency (min)	106.9 ± 56.9	77.4 ± 22.6	76.8 ± 26.9			0.88	0.02
REM efficiency (%)	89.4 ± 7.5	87.9 ± 8.1	87.1 ± 9.2	0.32	0.16	0.57	0.09

[a]Sleep measures on adaptation night, baseline, and Night 1. *p* values are for paired sample *t* test comparing each night. Effect size is Cohen's *d*.

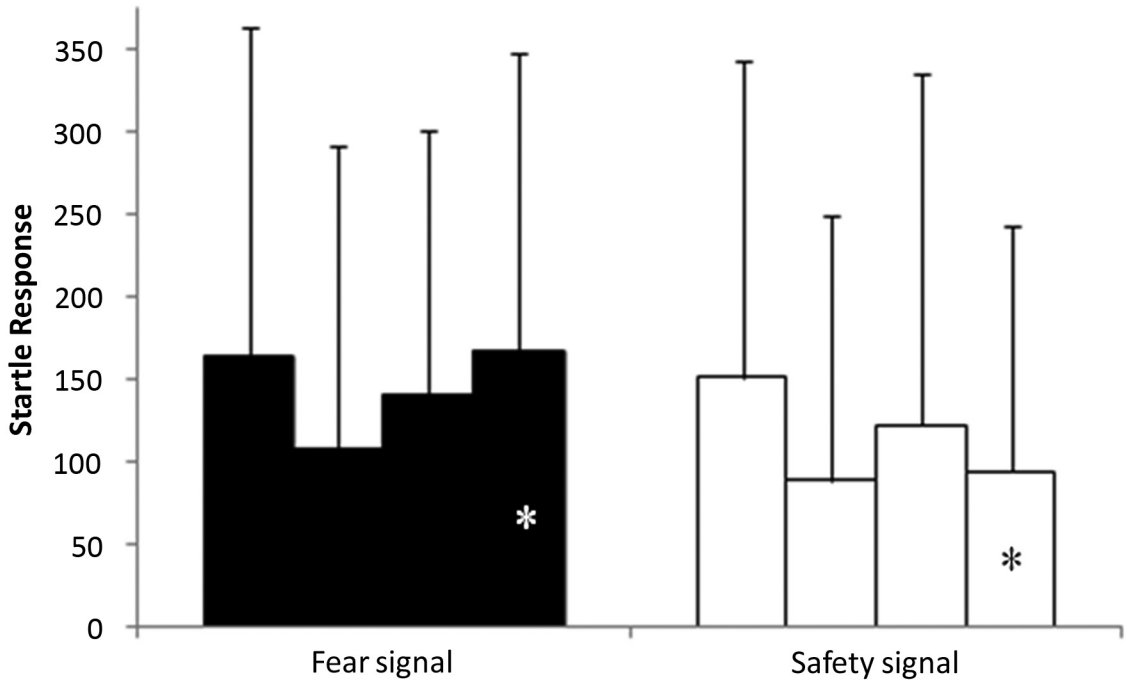

Figure 3. Startle acquisition. Startle response to fear/threat signal (left) and safety signal (right) across 4 blocks of initial acquisition phase. *Significant difference at the end of acquisition between startle response to threat signal and safety signal ($p < 0.05$). Data are mean ± SD.

to the canonical variate (which we labeled startle retention). Standardized canonical coefficients were as follows: fear retention = 0.322, safety retention = 0.303, differential conditioning retention = 1.07, confirming that differential conditioning retention was the startle retention measure most strongly influenced by REM sleep.

The first *post hoc* analysis showed there is no association between the extent of habituation in REM sleep to the sleep lab and initial startle acquisition (Hotelling's Trace $F_{(6,72)} = 0.372$, $p = 0.985$, $r^2 = 0.057$). The second *post hoc* analysis, assessing the combined contribution of startle acquisition and REM sleep to startle retention, was significant (Hotelling's Trace $F_{(6,64)} = 2.61$, $p = 0.025$, $r^2 = 0.286$). Both the startle and REM sleep canonical variates made meaningful contributions to the relationship, with very similar correlations to the new predictor canonical variate ($r = 0.912$ and 0.837, respectively). This was confirmed by the structural coefficients (startle latent = 0.649; REM latent = 0.487). With respect to the startle retention variables, the basic results did not change, and the structural coefficients confirmed differential conditioning was the measure most strongly affected by the combination of the initial startle response and REM sleep (standardized canonical coefficients: fear retention = −0.011; safety retention = 0.036, differential conditioning retention = 0.996).

Discussion

This study examined the relationship between strength of FPS (fear conditioning and safety signal learning) and REM sleep in healthy humans. Our findings did not directly replicate findings in animals, where fear conditioning leads to fragmentation and disruption of REM sleep. Rather, we found that subsequent sleep was more robustly influenced by the extent of safety signal learning. Specifically, stronger safety signal learning was associated with increased REM sleep consolidation that night, with 28.4% of

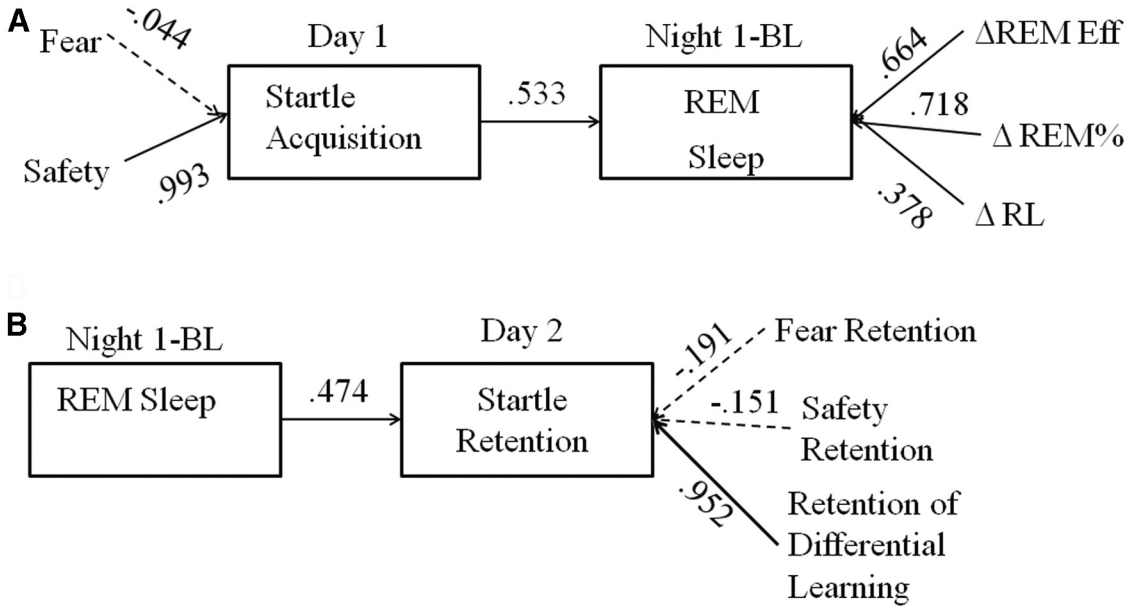

Figure 4. Canonical correlation results. ***A***, Canonical correlation between Startle Acquisition on Day 1 and change in REM sleep on Night 1. ***B***, Canonical correlation between change in REM sleep on Night 1 and Startle Retention on Day 2. For both, canonical variates (i.e., latent variables) are in the boxes. Arrows connecting canonical variates indicate the relationship between the two canonical variates; value is the canonical correlation between the variates. Measures associated with each variate are connected by arrows, and values along the arrows represent the correlations between the measures and the associated canonical variate. Any value $>|0.30|$ is considered to represent a meaningful contribution to the canonical variate structure (solid arrow).

the variance in REM sleep changes from baseline to Night 1 accounted for by safety signal learning. Interestingly, increased REM sleep was, in turn, related to overnight consolidation of the ability to discriminate between the threat and safety stimuli (i.e., differential conditioning). Overall, the REM sleep canonical variate accounted for 22.5% of the variance in startle retention the next day.

Based on the animal literature, we anticipated that the strength of fear potentiation would predict REM sleep fragmentation, and we did not find that. This may relate to the extent of fear generated by the FPS procedure. The shock we delivered, although aversive, was not as strong or putatively aversive as that delivered in animal studies and certainly not as strong as that experienced by humans during trauma exposure. Alternatively, it may be that sleep in animals is equally sensitive to fear and safety signal learning, and future studies should examine this question across species. Although no other studies report the relationship between safety learning and sleep, animal and human studies show extinction recall and/or generalization are related to REM sleep (Silvestri, 2005; Fu et al., 2007; Spoormaker et al., 2010, 2012). Extinction and safety learning are both forms of fear inhibition (Jovanovic and Norrholm, 2011), so the REM sleep–extinction connection reported previously supports the REM sleep-safety signal link observed in the present study.

The overlap in neural substrates of REM sleep and safety learning provides further support for their connection. REM sleep is characterized by high activity in the limbic system, including hippocampus, and medial prefrontal cortex (mPFC) (Braun et al., 1998; Maquet, 2000). The neural circuits for safety learning are not fully determined, but both hippocampus (Pollak et al., 2008) and mPFC (Jovanovic and Norrholm, 2011) are implicated. Within the hippocampus, safety signal learning results in increased neurogenesis and survival of newborn cells in the dentate gyrus (Pollak et al., 2008). Preventing this neurogenesis slows safety learning significantly, including preventing safety learning in the first day of training. REM sleep promotes neurogenesis, particularly cell proliferation and survival, and REM sleep deprivation impairs those processes (Guzman-Marin et al., 2008; Meerlo et al., 2009). Neurogenesis during REM sleep is important for memory consolidation (Meerlo et al., 2009; Pan et al., 2013), and it may be specifically related to extinction memory (Pan et al., 2013). Although causal relationships were not tested in the present study, this literature suggests that an increase in REM sleep subsequent to safety learning may allow increased neurogenesis, which in turn may contribute to enhanced fear/safety retention and/or discrimination the next day.

The notion that increased REM sleep may help play a role in overnight startle retention receives further support from studies showing that REM sleep plays an active role in consolidation of emotional memories. A growing number of human studies demonstrate that REM sleep plays a facilitatory role in consolidation of emotional memory (Nishida et al., 2009; Baran et al., 2012; Payne et al., 2012; Groch et al., 2013), including positive emotions (Gujar et al., 2011), which are arguably closer to safety signals than the negative emotions typically studied. Several of these studies report that REM sleep increases emotional reactivity, as well as enhancing memory (Gujar et al., 2011; Baran et al., 2012; Groch et al., 2013), helping explain why the ability to discriminate threat from safety showed the most robust association with prior REM sleep. Nishida et al. (2009) further showed that prefrontal theta activity during REM sleep was related to emotional memory consolidation, providing a link to the vmPFC role in safety learning proposed by Jovanovic and Norrholm (2011).

This study implies a causal chain whereby increased safety signal learning during the day influences increased REM sleep consolidation that night, which in turn influences the increased ability to discriminate threat from safety the next day. Two alternative explanations to this chain were considered. First, the FPS–REM sleep relationship may be more general and trait-like rather than specific and state-like. That is, perhaps those who more easily adapt to fear and/or more easily learn safety are also those who more easily adapt to a new sleeping environment. This might

make sense evolutionarily because a new sleeping environment appears to be a stressor in some animals. If the FPS–REM sleep relationship is trait-like, one would expect that the initial change in REM sleep from adaptation night to baseline night would predict initial fear and/or safety acquisition. However, this was not the case, thus arguing that the FPS–REM sleep relationship is state-like and directional. Second, it is possible that initial startle acquisition accounts for both REM sleep and startle recall, so REM sleep, itself, does not account for unique variance in startle recall. An analysis, including both the startle and REM sleep canonical variates as predictors of startle recall, showed that REM sleep does have an independent and significant effect on startle retention beyond that accounted for by initial startle acquisition.

The findings in this study have potential implications for understanding the physiological mechanism underlying the prominent role of sleep difficulties in PTSD. Sleep disruption, especially nightmares, subsequent to trauma exposure predicts the onset of PTSD (Babson and Feldner, 2010; Germain, 2013; van Liempt et al., 2013). One behavioral feature salient in the development and maintenance of PTSD is avoidance, in part because of an inability to accurately discriminate threatening environments from safe environments (Foa and Kozak, 1986). If increased REM sleep consolidation facilitates discriminating threat from safety at the physiological level (via overnight retention of conditioning), the REM sleep disruption characteristic of acute trauma responses and PTSD may impair the ability to retain learning associated with safety and/or threat-safety discrimination. Indeed, impaired safety signal learning appears to be unique to PTSD among clinical populations (Jovanovic et al., 2010), and the ability to modulate fear responses to safety signals is impaired in these patients (Jovanovic et al., 2012). Perhaps a stronger clinical parallel is during the treatment of PTSD. Prolonged exposure is one of two gold standard treatments for PTSD. It involves, among other things, exposure to avoided environments in an effort to extinguish the fear response and allow the patient to relearn the environment is safe. If the REM sleep deprivation produced by nightmares, insomnia, and/or early morning awakenings in PTSD (Spoormaker and Montgomery, 2008; Germain, 2013) impairs the ability to retain learning of threat-safety discrimination and/or the ability to retain newly formed safety memories, such sleep disruptions may reduce the ability to benefit maximally from a treatment, such as prolonged exposure. Although there are studies showing that PTSD treatments do not correct sleep problems (Nappi et al., 2012), none has examined whether nightmares or insomnia during treatment predicts worse outcomes. The clinical connection between sleep and PTSD before, during, and after treatment remains a critical area of study (Germain, 2013).

Although the clinical implications of these findings are important, we must note they were not the focus of study. Rather, we enrolled healthy controls in an effort to take necessary initial steps in advancing translation of relevant animal models into humans. Future research is needed to extend these findings and to test our proposed hypotheses of REM sleep playing a role in the development and/or maintenance of PTSD. One such study would be to actively manipulate REM sleep to test the causal role of REM sleep in fear and safety learning. Spoormaker et al. (2012) reported experimental REM sleep deprivation impaired measures of extinction recall, another process clearly important in PTSD. Those findings further argue for studies systematically testing the effect of manipulating REM sleep on acquisition and/or recall of fear and safety signals. Another avenue for future studies would be to more formally determine whether REM sleep partially or fully mediates the relationship between initial FPS responses on the acquisition day and startle retention. The pattern in our data (startle acquisition response affects subsequent REM sleep, which affects subsequent startle retention) and our *post hoc* analyses suggests such a meditational role, although we could not formally test that here because it would require ~3 times as many subjects as we had in this study (Fritz and Mackinnon, 2007). Finally, studies need to test these relationships directly in clinical samples, as well as examine the effects of improving sleep, especially REM sleep, on clinical symptoms and/or treatment response in PTSD.

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Research report

Sleep deprivation impairs performance in the 5-choice continuous performance test: Similarities between humans and mice

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HIGHLIGHTS

- The 5-choice continuous performance test assesses attention across species.
- 36 h total sleep deprivation impairs attentional performance in humans.
- 36 h REM sleep deprivation impairs performance in mice similarly.
- The 5C-CPT may be used to test putative cognitive enhancers after sleep deprivation.

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ABSTRACT

Several groups undergo extended periods without sleep due to working conditions or mental illness. Such sleep deprivation (SD) can deleteriously affect attentional processes and disrupt work and family functioning. Understanding the biological underpinnings of SD effects may assist in developing sleep therapies and cognitive enhancers. Utilizing cross-species tests of attentional processing in humans and rodents would aid in mechanistic studies examining SD-induced inattention. We assessed the effects of 36 h of: (1) Total SD (TSD) in healthy male and female humans ($n = 50$); and (2) REM SD (RSD) in male C57BL/6 mice ($n = 26$) on performance in the cross-species 5-choice continuous performance test (5C-CPT). The 5C-CPT includes target trials on which subjects were required to respond and non-target trials on which subjects were required to inhibit from responding. TSD-induced effects on human psychomotor vigilance test (PVT) were also examined. Effects of SD were also examined on mice split into good and poor performance groups based on pre-deprivation scores. In the human 5C-CPT, TSD decreased hit rate and vigilance with trend-level effects on accuracy. In the PVT, TSD slowed response times and increased lapses. In the mouse 5C-CPT, RSD reduced accuracy and hit rate with trend-level effects on vigilance, primarily in good performers. In conclusion, SD induced impaired 5C-CPT performance in both humans and mice and validates the 5C-CPT as a cross-species translational task. The 5C-CPT can be used to examine mechanisms underlying SD-induced deficits in vigilance and assist in testing putative cognitive enhancers.

1. Introduction

All species, including humans, require some state of sleep [1]. Despite the ubiquity of this phenomenon, much of the underlying mechanisms, long-term effects, and the actual function that sleep provides are still poorly understood. Nevertheless, it is well known that deprivation from sleep negatively affects general health and cognition in humans [2–4]. The extent to which sustained wakefulness impairs cognitive performance in particular seems to depend on the task at hand. For example, sleep deprivation (SD) has a more profound effect in tasks requiring the maintenance of attention than in tasks assessing working memory and executive functions [5].

The increasingly fast-paced nature of society requires people to work longer hours resulting in sleeping fewer hours per day with irregular patterns of sleep [6]. For example, several professions

Abbreviations: 5C-CPT, 5-Choice Continuous Performance Test; 5CSRTT, 5-Choice Serial Reaction Time Task; CR, Correct rejection; FA, False alarm; FAR, FA rate; HR, Hit rate; ITI, Inter-trial interval; PVT, Psychomotor Vigilance Test; REM, Rapid eye movement; RI, Responsivity index; RSD, REM sleep deprivation; RT, Reaction time; SD, Sleep deprivation; TSD, Total sleep deprivation; vRT, variable RT.

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including piloting or the military require vigilance (attending to relevant stimuli over time), yet involve extended periods without sleep, which impairs vigilance [7,8]. Moreover, certain psychiatric populations exhibit abnormal sleeping patterns, which may further impact their already deficient cognitive performance and possibly impair efficacy of some treatments (*e.g.*, cognitive behavioral therapy). Patients with bipolar disorder for instance are well known for experiencing disrupted sleep patterns, SD, and concomitantly suffer from cognitive symptoms [9]. Furthermore, SD can precipitate manic and hypomanic episodes [10], yet benefit patients in depressive episodes [11,12]. Investigating the mechanisms of SD-induced effects on behaviors including vigilance would aid in developing cognition-enhancing pharmaceuticals or behavioral countermeasures to cognitive deficits for certain professions and psychiatric disorders. While humans can be experimentally sleep deprived, animal models are more suitable for investigating underlying mechanisms of SD-induced deficits in vigilance. Additionally, SD may serve as an environmental challenge in animal models of psychiatric disorders [13,14]. The limited cross-species tests of attention/vigilance in humans and animals hampers such investigations however.

Attentional performance during SD in humans has commonly been assessed using the psychomotor vigilance test (PVT) [15]. This reaction time (RT) task requires responding to a visual cue (target stimulus) presented at pseudo-random intervals. Generally, RTs are slowed and more variable, while omissions are increased in humans subjected to SD [7]. SD-induced impaired performance has been observed in rats in a PVT analog [16] and the 5-choice serial reaction time task (5CSRTT), the latter of which requires responding in varied locations [17]. These tasks require only responses to target stimuli however, despite the important and distinct role that inhibiting from responding to irrelevant (non-target) stimuli has in attentional processes [18]. Specifically, with only target stimuli, separating attentional lapses from response fatigue is difficult. By including non-target stimuli, one can determine whether response rates are globally or specifically diminished due to inattention to relevant stimuli. Likewise, treatments that increase global responsiveness may not be useful when one's environment is littered with irrelevant (non-target) stimuli. Hence, cross-species studies are required on the effects of SD on attentional performance that is specific to responding to relevant (target) stimuli.

The combination of both target and non-target stimuli is the hallmark of tests labeled as continuous performance tests (CPT; [18]). With the inclusion of non-target stimuli, CPTs measure vigilance and are the gold-standard tests of attention in psychiatric populations [19]. In the limited studies conducted on the effects of SD on CPT performance, several days of sleep restriction increased misses to target stimuli and reduced responses to non-target stimuli, thereby overall impairing vigilance and reducing responsiveness [20,21]. Other studies using total SD (TSD) report modest but non-significantly increased misses to targets but no change in non-target responses after TSD in healthy subjects; however stronger attentional disruption is reported in methadone-maintained subjects [22,23]. TSD primarily increased non-target responses compared to target responses in a go/no-go task however, despite this task not being a true CPT [24]. Determining the effects of SD on a cross-species vigilance task is required however, for examining putative underlying mechanisms.

The 5-choice (5C-CPT), based on the 5CSRTT, was developed to assess vigilance in mice [25–27] and rats [28,29], and is now available in humans [30], including in an fMRI setting [31]. Consistent with other CPTs, the 5C-CPT presents target stimuli to which the subject is required to respond as well as non-target stimuli, to which the subject is required to inhibit from responding. To date, no studies have assessed whether SD affects mouse or human performance in this cross-species CPT. Thus, the present studies investigated whether SD would affect 5C-CPT performance similarly in both mice and humans. We hypothesized that: (a) 36 h of TSD in humans; and (b) 36 h of rapid eye movement (REM) SD (RSD) in mice would similarly impair 5C-CPT performance. Since inter-individual differences were expected on mice 5C-CPT performance [27], and treatments can affect rodent performance differentially dependent upon baseline performance [32,33], we split the animals in good and poor performers. Finally, to ensure the validity of our TSD protocol, we also assessed TSD-induced effects in the human PVT.

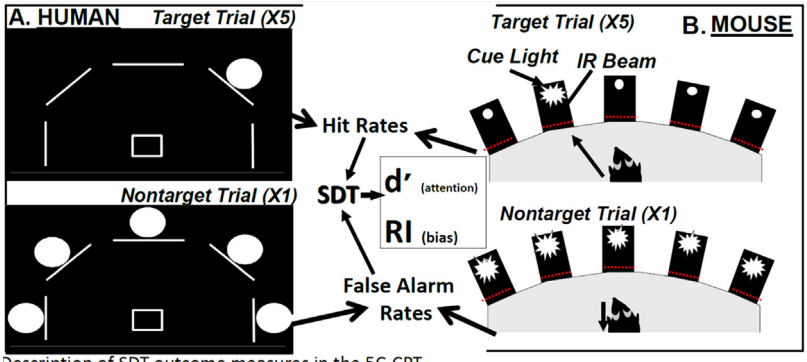
2. Methods

2.1. Humans

Fifty human subjects (23 female) aged between 18 and 39 years were recruited through flyers, newspaper, and radio from the general San Diego community to participate in this study. Subjects were initially screened via telephone for eligibility. Informed consent was signed at an in-person screen, which included a complete medical history and a Structured Clinical Interview for DSM-IV. Inclusion criteria were at least 12 years of education, a consistent sleep-wake schedule (7–9 h sleep each night), and for women to be tested in the early follicular phase of their menstrual cycle. Exclusion criteria were history of any sleep disorder, Axis I psychopathology or immediate family history of mood or psychotic disorders; head injury followed by unconsciousness, migraine headaches requiring treatment, seizures, neurological symptoms of the hand, wrist, or arm; current use of nicotine or in the past 2 years; current use of psychotropic medications, hormone-based birth control; high caffeine (>400 mg/day) or alcohol (>2 ounces/day) use; positive urine toxicology screen for illegal substances; hearing threshold above 45 dB(A) at 500–6000 Hz; non-responsiveness to startling stimuli or any other medical condition which might pose a health risk for the subject. Subjects were instructed to maintain a regular sleep-wake schedule at home for at least one week prior to the study, which was monitored with sleep diaries and actigraphy. Sleep monitoring on the first night of the study screened for unreported sleep disorders. This study was conducted at the VA San Diego with the approval of the IRBs of UCSD and VA and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.1.1. Total sleep deprivation

Subjects spent four nights and days in the laboratory: (a) adaptation to the lab (night/day 0); (b) normal sleep followed by a battery of testing including the PVT and then the 5C-CPT (night/day 1); (c) sleep or TSD followed by a similar battery of testing (night/day 2); and (d) sleep or TSD followed by a similar battery of testing as night/day 2 (night/day 3). Subjects were randomly assigned to one of three groups. Group 1 received normal sleep throughout the study; group 2 was sleep deprived for 36 h prior to day 2; and group 3 was sleep deprived for 36 h prior to day 3. Subjects assigned to group 1 were included in the 'normal sleep' group ($n = 18$). Post-deprivation night data for subjects in groups 2 and 3 were collapsed into the TSD group ($n = 32$). The data from group 1 used for analysis was taken from day 2 or 3 in order to match with subjects from groups 2 and 3 therefore minimizing practice effects as a putative confound. Sleep schedules were made as similar to those maintained at home as possible with sleep being monitored with a standard overnight polysomnogram, including EEG, EOG, and EMG. At each point, subjects were free to engage in activities such as reading, watching television, or socializing. No exercise more strenuous than walking was allowed, nor any form of stimulant. Light snacks and meals were provided. Lights were kept at a constant low



Description of SDT outcome measures in the 5C-CPT

d′	Responsivity Index (RI)	Hit Rate	False Alarm Rate	Explanation of performance
+5	0	1.0	0.0	Perfect attention: Responding to every target and inhibiting from responding to all non-target stimuli
0	+1	1.0	1.0	Chance: Responding to every stimulus irrespective of whether it is a target or non-target
	-1	0.0	0.0	Chance: Not responding to any stimulus irrespective of whether it is a target or non-target
-5	0	0.0	1.0	Rule reversed: Inhibition of responding to all targets while responding to all non-target stimuli

Fig. 1. Schematic of the human and mouse 5C-CPT.
In both the human and mouse 5C-CPTs, there are 5 stimuli locations. For humans, stimuli are presented in 1 of 5 locations arrayed in an arc on a computer screen, and subjects respond using a 5-way joystick (A). For mice, stimuli are presented in 1 of 5 holes located in an arc at the rear of a 5-hole operant chamber and responses are recorded by infrared beams in each hole (B). The task design is the same in both cases, whereby: (1) a single stimulus represents a target trial to which subjects must respond; and (2) all 5 stimuli being presented simultaneously represents a non-target trial to which the subject must inhibit from responding. Target trials generate measures of hits and misses (target responses and omissions), which are used to calculate a subjects' hit rate, while non-target trials generate measures of correct rejections and false alarms, which are used to calculate a subjects' false alarm rate. Using signal detection theory (SDT), the non-parametric measure of vigilance (d') and bias (responsivity index (RI)) are generated. The table provides examples of what permutations of hit and false alarm rates result in various d' and RI levels and its interpretation.

level, with no sunlight introduced. Wakefulness was documented through (1) a staff-completed monitoring log every 15 min with subjects' activities and mental status and (2) actigraphy.

2.1.2. Psychomotor vigilance test

During the PVT, subjects were presented with a blank box in the middle of a screen. At pseudo-random intervals ranging from 2 to 10 s, a bright red light millisecond (ms) counter started to scroll, and subjects had to press the space bar to stop the counter as quickly as possible. After pressing the button, the counter displayed the achieved RT for 1 s, providing the subject with feedback on performance. The PVT task lasted 10 min and was programmed in E-prime (Psychology Software Tools (Sharpsburg, PA, USA)). Median RT, fastest and slowest 10% of RTs, and number of lapses (RTs > 500 ms) were measured.

2.1.3. Human 5C-CPT apparatus

The task appeared on a 56 cm CRT computer screen (60 cm from subject). Subjects used an arcade joystick to make responses. The joystick was spring-mounted so that it would return to the center after each response. A Dell PC with E-Prime2 software (Psychology Software Tools) was used for stimulus presentation and data acquisition.

2.1.4. Human 5C-CPT

A schematic of the paradigm is presented in Fig. 1 and described elsewhere [30]. In brief, participants were briefly introduced to the task and were told that they would see 5 white lines (3 cm) in an arc on a black background. Subjects were instructed that if a white circle (≈2 cm) appeared behind a line (target stimuli), the joystick should be moved in that direction, but if circles appeared behind every line (non-target stimuli) they should inhibit from responding. Stimuli appeared for 100 ms with a response window of 1 s after the stimuli disappeared. A variable inter-trial interval (ITI; 0.5, 1, or 1.5 s) occurring 1 s after the stimulus of the previous trial was presented in a pseudo-random order between trials. Before the actual task, subjects were given a practice session, which consisted of 12 trials (10 target and 2 non-target stimuli randomly presented). The full task consisted of 270 trials (225 target and 45 non-target stimuli pseudo-randomly presented). Several measures were determined from this task (Table 1) and calculations based on hit rates (HR), false alarms (FA), FA rates (FAR), and correct rejections (CR) were made accordingly:

$$\text{Accuracy} = \frac{\text{Hit}}{\text{Hit} + \text{Incorrect}}$$

$$\%\ \text{Omissions} = \left(\frac{\text{Miss}}{\text{Total trials}}\right) \times 100$$

$$\text{Reaction Time} = \frac{\text{Cumulative correct latency}}{\text{Corrects}}$$

$$\text{HR} = \frac{\text{Hit}}{\text{Hit+Miss}} \quad \text{FAR} = \frac{\text{FA}}{\text{FA+CR}}$$

Signal detection indices were calculated based upon these basic parameters to assess both sensitivity and responsivity indices:

$$d' = z(\text{HR}) - z(\text{FAR}) \quad \text{RI} = \frac{\text{HR} + \text{FAR} - 1}{1 - [\text{FAR} - \text{HR}]^2}$$

d' provides a parametric assessment of sensitivity to appropriate responding. The non-parametric response bias measure RI provides a measure of the 'tendency to respond'. Low numbers indicate a conservative response strategy, while high numbers indicate liberal responding [34,35].

2.2. Animals

Male C57BL/6 mice (n = 26) were 12–14 months old at the time of testing and weighed between 23–30 g. All animals were group housed (maximum 4/cage) and maintained in a temperature-controlled vivarium (21 ± 1 °C) on a reversed day-night cycle (lights on at 7.00 pm, off at 7.00 am) and tested during the dark phase of the day-night cycle between 8.00 am and 11.00 am. All mice had *ad libitum* access to water and were food-restricted at 85%

Table 1
Description of the behavioral measures used in the human and rodent 5C-CPTs.

Measure	Description
Hit	Response to target stimulus in correct location
Miss	Non-response to target stimulus
Incorrect	Response to target stimulus but in wrong location
Correct rejection (CR)	Correct non-response to non-target stimulus
False alarm (FA)	Incorrect response to non-target stimulus
Premature response	Response to no stimuli during the inter-trial interval
Mean reaction time (RT)	Mean latencies to correct responses
Variable RT	Standard deviation of the RT
Hit rate (HR)	Proportion of correct responses to target stimuli
False alarm rate (FAR)	Proportion of incorrect responses to non-target stimuli
Vigilance (d')	Parametric measure examining the difference between hit and false alarm rates to determine performance
Responsivity index (RI)	Non-parametric measure examining the combination of hit and false alarm rates to determine responsivity to stimuli
Accuracy	Proportion of correct compared to incorrect responses
% Omissions	Percentage of misses/lapses

of their free-feeding weight during periods of testing. All procedures were approved by the UCSD Institutional Animal Care and Use Committee. The UCSD animal facility meets all federal and state requirements for animal care and was approved by the American Association for Accreditation of Laboratory Animal Care.

2.2.1. REM sleep deprivation

Mice receiving normal sleep ($n = 13$) and mice on RSD ($n = 13$) were baseline matched on training performance as measured by their average d' 3 days prior to testing. The conventional 'inverted flower pot' technique was used, originally designed by Jouvet et al. in 1964 [36] and still used in RSD studies in animals [13]. In brief, group-housed mice were sleep deprived by placing the same number of small inverted cups (4 cm diameter) as there were mice in the cage in a pool of water (37 °C; 2 cm height) for 36 h prior to testing. Control animals had bigger inverted cups (7 cm diameter), which because of its size allowed for sleep, in a pool of water for the same period.

2.2.2. Mouse 5C-CPT

A schematic of the paradigm is presented in Fig. 1 and is described elsewhere [25,27]. Consistent with the human task, mice were required to make a hole poke if 1 of the 5 holes lit up (target trials) in order to obtain a food reward, but inhibit from responding when all 5 holes lit up (non-target trials) in order to obtain a reward (see Supplemental Material and Methods). In brief, mice were progressively trained to conduct this task using simple choice progressing to use the entire 5-hole array and until performance was stable on d', % omissions, and RTs when tested for baseline performance over 3 days before SD (~70 5C-CPT training sessions). Measures were calculated as described for the human 5C-CPT (see Table 1 for measures).

2.3. Statistics

Human 5C-CPT performance was analyzed using the general linear model (GLM) with TSD and gender as between-subject factors and trial period as a within-subjects factor. Mouse 5C-CPT performance was analyzed using a repeated measure ANOVA with stimulus duration as a within-subject factor and RSD as a between-subject factor. Where appropriate, planned comparison Tukey *post hoc* analyses were conducted between groups and Cohen's d effect sizes were calculated. Two animals from the RSD group were removed from statistical analyses because of a lack of responding (>95% omissions). In order to explore the effects of SD on individual differences in performance, a median split was conducted on vigilance performance (d') measured during 3 days of baseline testing to group subjects into good and poor performers. Performance group was entered into the model as a between subject factor. The level of probability for statistical significance was set at 0.05. All statistics were performed using SPSS (19.0, Chicago, IL, USA).

3. Results

3.1. Humans

3.1.1. Effects of TSD on PVT performance

The effects of TSD on PVT performance in humans are detailed in Table 2. In brief, TSD slowed overall RTs, including the fastest and slowest 10% of responses during the task. TSD also increased the number of attentional lapses (RTs > 500 ms).

3.1.2. Effects of TSD on human 5C-CPT performance

Because there were no interactions of TSD with trial period, gender, or baseline performance ($F < 1.8$ ns), data were pooled and analyzed. As hypothesized, TSD impaired vigilance as measured by reduced d' ($F_{(1,42)} = 5.7$, $p < 0.05$; Cohen's $d = 0.6$; Fig. 2a). TSD also reduced hit ($F_{(1,42)} = 4.8$, $p < 0.05$; Cohen's $d < 0.5$; Fig. 2b) and false alarm rates ($F_{(1,42)} = 4.2$, $p < 0.05$; Cohen's $d = 0.15$; Fig. 2c). TSD tended to reduce responsivity as measured by reduced RI ($F_{(1,42)} = 3.5$, $p < 0.1$; Cohen's $d = 0.2$; Fig. 2d), and tended to decrease accuracy ($F_{(1,42)} = 3.4$, $p < 0.1$; Cohen's $d = 0.1$; Fig. 2e). There was no effect of TSD on omissions ($F_{(1,42)} = 2.5$ ns; Fig. 2f). Interestingly, TSD did not affect RT ($F_{(1,42)} < 2.2$ ns; Fig. 2g), but tended to increase variability of RT ($F_{(1,42)} = 4.0$, $p < 0.1$; Fig. 2h).

3.2. Mice

3.2.1. Effects of RSD on mouse 5C-CPT performance

Interestingly, while longer stimulus durations improved hit rate in control mice (stimulus duration; $F_{(2,22)} = 12.3$, $p < 0.0001$), this effect was not present in the RSD mice (stimulus duration; $F < 1$ ns). *Post hoc* analyses revealed that RSD mice exhibited a reduced hit rate at the 2 s stimulus duration compared with control mice ($p < 0.05$; Fig. 3b). Similar benefits to lengthening the stimulus duration were observed in fewer omissions in control mice (stimulus duration; $F_{(2,22)} = 12.0$, $p < 0.0001$) and again this effect was not present in the RSD mice (stimulus duration; $F < 1$ ns). *Post hoc* analyses revealed that RSD mice exhibited increased omissions at the 2 s stimulus duration compared with control mice ($p < 0.05$; Fig. 3f). RSD tended to reduce accuracy ($F_{(1,21)} = 3.5$, $p < 0.1$; Fig. 3e), without interacting with stimulus duration. Overall, RSD did not affect d', false alarms, RI, RTs, or vRTs (Fig. 3). RSD also did not affect premature responses, but reduced the amount of trials completed in mice ($F_{(1,21)} = 4.6$, $p < 0.05$), without interacting with stimulus duration (see Supplemental Table 1).

Table 2
Means, standard errors of the mean, and statistical comparison of human PVT performance after normal sleep *vs.* TSD.

Variable	Normal sleep mean (SEM)	TSD mean (SEM)	d.f.	*F*	*p*-value
Lapses	0.4 (1.2)	5.2 (0.9)	1.48	10.5	<0.005
Median RT	278.2 (8.2)	312.2 (6.2)	1.48	11.0	<0.005
Fastest 10% RT	230.2 (5.6)	245.6 (4.2)	1.48	4.8	<0.05
Slowest 10%RT	389.9 (154.8)	816.0 (116.1)	1.48	4.8	<0.05

RT, reaction time (in milliseconds).
TSD, total sleep deprivation.

3.3. Good and poor performing mice

Consistent with previous reports [27], inter-individual differences in performance were observed in mice, with several subjects performing at a low baseline level. Treatments can differentially affect rodent performances in operant tasks dependent upon baseline level of performance [32,33]. Therefore, we investigated the effects of RSD in good *vs.* poor performing mice. Good and poor performers ($n = 12/12$) were identified as described above (see Section 2.3).

3.3.1. The effects of RSD on good performing mice in the 5C-CPT

In good performing mice, RSD deleteriously affected accuracy ($F_{(1,9)} = 5.7, p < 0.05$), with its effect tending to interact with stimulus duration ($F_{(2,18)} = 4.6$, $p = 0.051$). RSD specifically reduced accuracy at the 0.75 s stimulus duration ($p < 0.05$; Cohen's $d = 1.34$; Fig. 4e). For percentage omissions there was a trend effect of RSD ($F_{(1,9)} = 3.5$, $p < 0.1$). Again, longer stimulus durations resulted in fewer omissions in control mice (stimulus duration; $F_{(2,10)} = 12.3$, $p < 0.005$), but this effect was not present in the RSD mice (stimulus duration; $F < 1$ ns), who exhibited more omissions at the 2 s stimulus duration compared with control mice ($p < 0.05$; Cohen's $d = 1.79$; Fig. 4f). No main effect of RSD or interaction with stimulus duration was observed for d'. Longer stimulus durations tended to result in increased d' in control mice however (stimulus duration; $F_{(2,10)} = 3.3$, $p < 0.1$), with this effect not being present in RSD mice (stimulus duration; $F < 1$ ns), who tended to exhibit reduced d' at the 1.25 s stimulus duration compared with control mice ($p < 0.1$; Cohen's $d = 1.18$; Fig. 4a). There was a trend effect of RSD impairing hit rate ($F_{(1,9)} = 4.7$, $p < 0.1$). Although longer stimulus durations improved hit rate in control mice (stimulus duration; $F_{(2,10)} = 17.3$, $p < 0.005$), this effect was not present in RSD mice (stimulus duration; $F_{(2,6)} = 2.0$ ns), whom exhibited a reduced hit rate at both the 0.75 ($p < 0.05$; Cohen's $d = 1.28$; Fig. 4b) and the 2 s stimulus durations ($p < 0.05$; Cohen's $d = 1.89$) compared with control mice. For the RI, a trend stimulus duration by RSD interaction was observed ($F_{(2,18)} = 3.6$, $p < 0.1$; Fig. 4d), but no *post hoc* effect of RSD was observed. RSD did not affect RTs, vRTs, false alarms (Fig. 4), trials completed, or percentage premature responses (See Supplemental Table 2).

3.3.2. The effects of RSD on mice performing at low baseline levels in the 5C-CPT

The effects of RSD on poor performing mice in the 5C-CPT are detailed in Supplemental Table 3. In brief, RSD did not affect trials completed, percentage premature responses, RTs, vRTs, accuracy, false alarms, d', or RI of these mice, overall, nor at any specific stimulus duration.

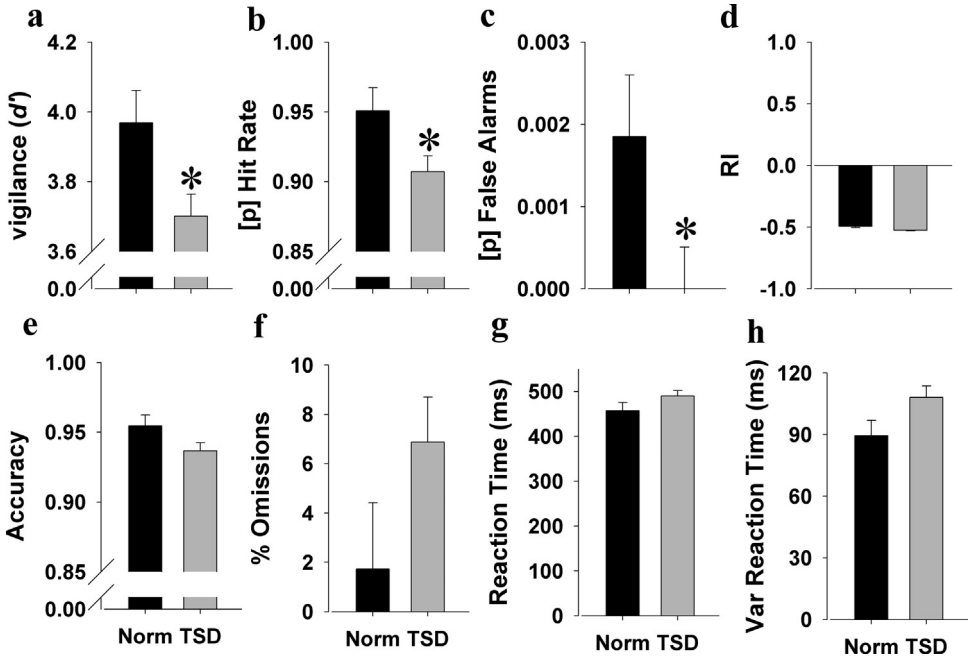


Fig. 2. Effects of TSD on 5C-CPT performance in humans.TSD impaired vigilance as measured by reduced d' (a) with a large effect size (Cohen's $d = 0.6$). This TSD-impaired vigilance was partially driven by reduced overall hit rate (b; Cohen's d effect size = 0.5) and lower non-target responses (c; Cohen's d effect size = 0.15). Humans during TSD were slightly less responsive (d) and also made slightly less target responses (e) compared to humans after normal sleep. No significant difference between normal sleep and TSD was observed on the number of omitted trials (f). Mean RTs did not differ (g), but humans during TSD exhibited slight increased variable RTs compared to humans after normal sleep (h). Data are presented as the mean ±SEM, *denotes $p < 0.05$ when compared with humans after normal sleep.

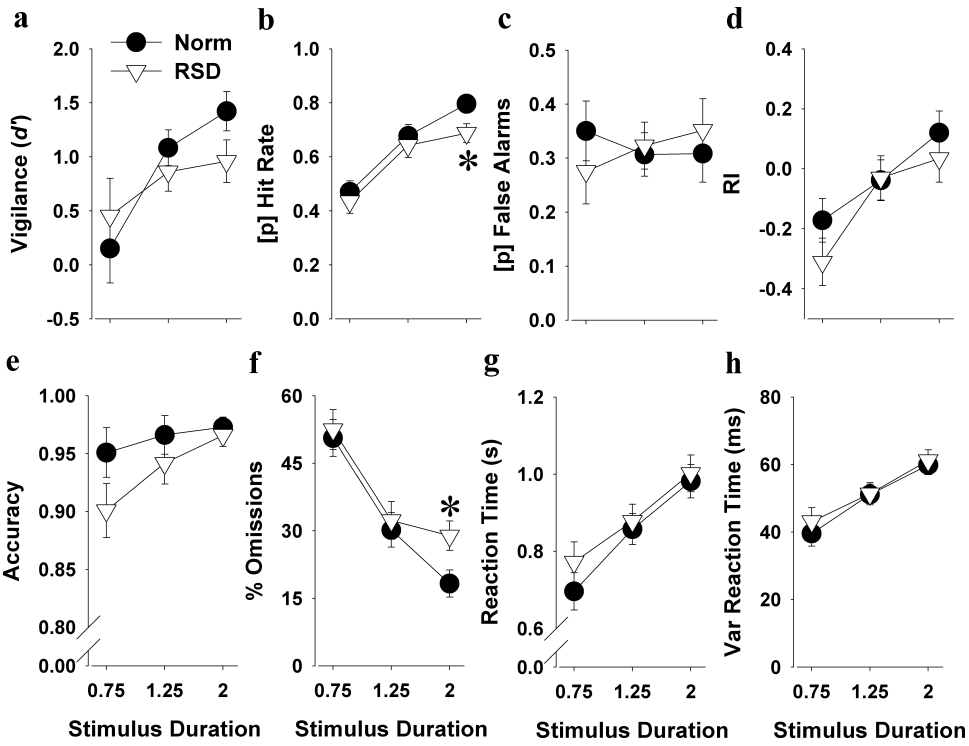


Fig. 3. Effects of RSD on 5C-CPT performance in all C57BL/6 mice.RSD had only subtle effects when looked at the overall group performance of mice in the 5C-CPT. Overall, mice seemed to perform better with longer stimulus duration (a–h). However, this effect was less pronounced in mice during RSD, where RSD decreased hit rate (b) and increased the amount of omissions (f) at the longest stimulus duration of 2 s. Data are presented as the mean ±SEM, *denotes $p < 0.05$ when compared with mice after normal sleep.

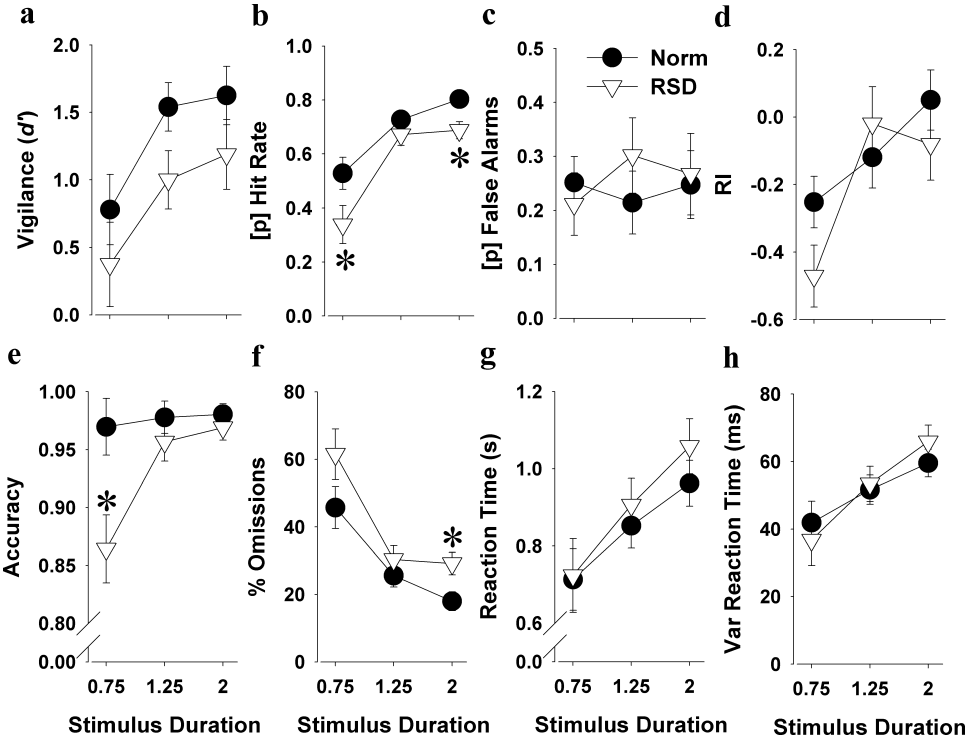


Fig. 4. Effects of RSD on 5C-CPT performance in good performing mice.In the good performing subgroup of mice, RSD more severely impaired 5C-CPT performance. RSD negatively impacted vigilance as measured by slight reduced d' at the 1.25 s stimulus duration (a). RSD decreased hit rate, specifically at the 0.75 s and 2 s stimulus durations (b), while leaving non-target responses unaffected (c). No effect of RSD was observed on responsiveness (d), but after RSD, mice made fewer target responses compared to mice after normal sleep, specifically at the 0.75 s stimulus duration (e). Although longer stimulus durations reduced the number of omitted trials in control mice, this effect was less pronounced in the mice after RSD, where RSD increased omissions at the highest stimulus duration (f). No effect of RSD was observed on both mean and variable RTs (g,h). Data are presented as the mean ±SEM, *denotes $p < 0.05$ when compared with mice after normal sleep.

4. Discussion

We report that 36 h of TSD and RSD impaired 5C-CPT performance in humans and mice respectively. This SD-impaired 5C-CPT performance was driven by more misses of target stimuli, consistent with previous CPT studies in humans [20,21]. TSD-induced attentional lapses in the PVT confirm the efficacy of the TSD procedure in humans. Importantly, the present data reveal that despite SD-induced reduced responsiveness of humans and mice overall, inattention specific to relevant stimuli was still observed, particularly in good performing subjects.

Over the last decade, the PVT has been used as the 'gold standard' to assess the effects of SD on alertness [4]. Broadly, PVT studies reliably find that SD slows RTs and increases lapses (omissions) of attention [7]. Similarly here, 36 h of TSD slowed RTs and increased PVT lapses (RTs > 500 ms). This behavior has been associated with increased activation of the prefrontal region part of the 'default mode network', which is generally activated when subjects are at rest and not engaged in goal-directed behaviors [37]. Our PVT data confirm that our TSD protocol reliably affected attentional performance. Nevertheless, the TSD-induced reduction in PVT responsiveness could reflect generalized reduced responding rather than vigilance *per se*, since this distinction cannot be made using the PVT because it contains only target stimuli.

Specifically examining vigilance requires assessing both accurate responding to target stimuli as well as the inhibition of responding to non-target (irrelevant) stimuli. Using the 5C-CPT, we observed that TSD overall reduced target responding, as in the PVT, and also reduced non-target responding. Importantly, the greater decrease in target responding (as indicated by greater effect sizes) resulted in a lower d' score of vigilance. Hence, these 5C-CPT findings indicate that TSD impairs attention beyond simply reducing responding as seen in the PVT, supporting the use of both stimulus types. Furthermore, mood questionnaires (PANAS) [38] completed by the subjects indicated that feelings of alertness correlated significantly with d' in the 5C-CPT ($r = 0.42$, $p < 0.005$), but less with attentional lapses in the PVT ($r = -0.31$, $p < 0.05$), whereas pleasantness correlated with PVT ($p < 0.05$), but not 5C-CPT performance ($p > 0.1$). These data support TSD-induced deficits in 5C-CPT as reflecting attentional dysfunction.

Consistent with the present results, mild cumulative sleep restriction impaired CPT performance of healthy controls and children with ADHD [21]. Joo et al. reported that impaired attention in subjects performing a complex CPT after 24 h of TSD was also driven by reduced target responding, which was accompanied by increased non-target responses [20]. The discrepancy of SD effects on non-target responding between that report and the present study could have resulted from their small study population of only 6 young male adults and/or the complexity of the CPT used. In healthy and methadone-treated humans, a non-significant reduction in target responding was observed following 36 h of TSD [22], which may have been limited by low sample sizes, practice effects, and/or poor performing subjects. In another study, SD did not significantly affect CPT performance in Korean medical residents and interns [23]. In the present studies using healthy subjects from the general population and matching post TSD-testing days to account for possible practice effects, we observed that 36 h of TSD clearly impaired 5C-CPT performance.

Similar to our human study, 36 h of RSD impaired performance in mice in the rodent 5C-CPT, an effect that was not observed in poor performing mice. Interestingly, the TSD-induced reduction in d' and target responding in humans was primarily driven by affecting good performing humans, without significantly affecting poor performers (data not shown). RSD in good performing mice decreased their target (correct) responses, resulting in more omitted trials and a trend-level vigilance deficit as measured by reduced d'. Comparable results have been observed in the 5CSRTT where 10 h TSD rats made fewer correct responses and omitted more trials compared to rats with normal sleep [17]. Additionally, 24 h of TSD slowed responses and increased lapses in a rat PVT [16]. These tasks support our findings in the 5C-CPT. Because these tasks include only target trials however, and no measure of false alarm rates, a simple reduction in responding could not be discounted. When developing treatments by using these tasks, it would be unclear therefore if developed treatments simply increased responding to any presented (even irrelevant) stimuli. In contrast, the 5C-CPT measures both correct responses to target trials and failures to inhibit responding to non-target trials [25]. Hence, responsiveness can be dissociated from target responding and our data support that RSD affects attentive responding beyond simply reducing responding. To date, the rodent 5C-CPT has been successfully used to assess genetic and pharmacological manipulations on attentional measures in both rats [28,29] and mice [25–27,39]. Thus, the current study validates the 5C-CPT as a test suited for translational studies because SD manipulations induce similar 5C-CPT effects in both humans and mice. Consequently, the 5C-CPT will be useful to examine the mechanism(s) underlying SD-induced impairment of attentional performance.

A cross-species comparison between 5C-CPT performance of mice and humans revealed that 36 h of SD decreased hit rate and vigilance and tended to decrease accuracy in humans, whereas it decreased accuracy and hit rate while tending to decrease vigilance in mice, primarily in those with a good baseline performance. The lack of SD-induced deleterious effect in poorly performing mice could be due to a floor effect wherein performance could not be made worse in these mice (see Supplemental Table 3). The stronger overall vigilance deficits observed in humans may have also resulted from the different SD technique used compared to mice. TSD in humans slowed RTs in the PVT and increased variable RTs in the 5C-CPT, but did not affect 5C-CPT RTs in mice. The 'inverted flower pot' technique used here in mice affects various forms of sleep including deep slow wave sleep [40], but primarily deprives animals from REM sleep [41,42]. TSD also affects non-REM sleep and reduces the overall amount of sleep to a greater extent than RSD. Thus, the TSD we administered in humans may have exerted a stronger effect on attention than the RSD we administered in mice. The extended training used in mice, which may have resulted in greater use of procedural memory and hence different circuitry activation patterns, may have also resulted in some of the differences between species.

With cross-species similarity of SD effects on 5C-CPT performance, the mechanism(s) underlying these effects can be investigated. Some putative mechanisms have been tested, *e.g.*, that microdialysis perfusion-induced elevation of basal forebrain adenosine, a key mediator of sleep homeostasis, impaired rat PVT performance [43]. Similarly, SD increased basal levels of adenosine in rats [44]. Furthermore, the adenosine antagonist caffeine is commonly consumed by humans to increase wakefulness. Serotonergic mechanisms could also be examined given that RSD for 24 h increased serotonergic activity in the hypothalamus in rats [45]. Therefore, various mechanisms that may underlie SD-impaired 5C-CPT performance could be targeted in the future to improve attention following sleep loss.

The consistency of SD-induced impaired human and mouse 5C-CPT performance could also prove useful when investigating aspects of psychiatric disorders. As described above, SD can switch people with bipolar disorder into a mania episode. In fact, SD has been used to model mania in rodents [13,46,47]. Such studies are limited however because healthy humans do not become manic

after SD [14]. Thus, people with bipolar disorder have an underlying sensitivity to SD-induction of mania [10]. Therefore, using the 5C-CPT and SD technique described here may enable the examination of susceptibility genotypes that result in impaired attention in bipolar disorder patients [48].

SD impaired 5C-CPT performance in both humans and mice, primarily by reducing target responding. The SD-induced deficits in mice were only significant in good performers and at longer stimulus durations. Mouse 5C-CPT performance consistently improved with longer stimulus durations. It is clear that SD disrupted the benefit of longer stimulus durations leading to pronounced effects at these durations. With larger sample sizes however, SD would likely impair performance at all stimulus durations. Besides smaller sample sizes after the median split, differences in training and TSD *vs*. RSD techniques discussed above could have also contributed to the limited effects observed in mice. In addition to not affecting all forms of sleep, the 'flower pot' technique can be stressful for animals [49], even more so in combination with food restriction [50]. Other techniques such as the gentle handling method [17] may therefore be useful in future studies. Future studies with larger sample sizes will be conducted in order to account for inter-individual differences and SD effects.

5. Conclusion

In conclusion, 36 h SD deleteriously affected 5C-CPT performance of both humans and mice. Importantly, SD primarily reduced target responding in both species, with a smaller effect on reducing non-target responding, indicating that SD is primarily deleterious to vigilance and not overall responding. These data validate using the 5C-CPT as a cross-species test of vigilance. Therefore, the rodent and human 5C-CPTs can be used in the future across species to examine mechanisms underlying SD effects, susceptibility of psychiatric disorders to such effects, and test pro-vigilance medication for affected groups.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.12.003.

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