



Emotional trait and memory associates of sleep timing and quality

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ABSTRACT

Poor ability to remember the extinction of conditioned fear, elevated trait anxiety, and delayed or disrupted nocturnal sleep are reported in anxiety disorders. The current study examines the inter-relationship of these factors in healthy young-adult males. Skin-conductance response was conditioned to two differently colored lamps. One color but not the other was then extinguished. After varying delays, both colors were presented to determine extinction recall and generalization. Questionnaires measured sleep quality, morningness–eveningness, neuroticism and trait anxiety. A subset produced a mean 7.0 nights of actigraphy and sleep diaries. Median split of mean sleep midpoint defined early- and late-“sleep timers”. Extinction was more rapidly learned in the morning than evening only in early timers who also better generalized extinction recall. Extinction recall was greater with higher sleep efficiency. Sleep efficiency and morningness were negatively associated with neuroticism and anxiety. However, neuroticism and anxiety did not predict extinction learning, recall or generalization. Therefore, neuroticism/anxiety and deficient fear extinction, although both associated with poor quality and late timing of sleep, are not directly associated with each other. Elevated trait anxiety, in addition to predisposing directly to anxiety disorders, may thus also indirectly promote such disorders by impairing sleep and, consequently, extinction memory.

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1. Introduction

This study examines aspects of emotional memory, sleep timing and quality and trait anxiety that, in currently healthy individuals, could potentially interact in such a way as to lead to pathological anxiety. An emotional memory that is of particular importance to regulating normal anxiety is the extinction of conditioned fear, or learning that something that once signaled danger no longer does so (Hermans et al., 2006). For example, exposure therapy for anxiety disorders is based on the formation of therapeutic extinction memories (Craske et al., 2008). Extinction does

not erase conditioned fear; rather, it creates a new, inhibitory emotional memory that coexists and competes with the prior association between the fear signal and fearful event (Milad and Quirk, 2012). Fig. 1 illustrates a 3-component positive feedback mechanism whereby, in initially healthy individuals, abnormal quality and timing of sleep might interact with extinction recall and trait anxiety to, over time, exacerbate abnormalities in each component and lead to pathological anxiety.

1.1. Sleep quality and timing can impact extinction recall

Sleep promotes consolidation in multiple memory systems (Diekelmann and Born, 2010) and thus could also promote consolidation of fear-extinction recall (Pace-Schott et al., 2015). Indeed sleep has been shown to strengthen and help generalize extinction recall (Pace-Schott et al., 2009, 2012, 2015). Therefore, healthy sleep may provide a normal anxiolytic function, viz. overcoming acquired fears by enhancing the consolidation of memories for the extinction of such fears. Rapid eye movement (REM) sleep has, in particular, been reported to exert positive effects on extinction recall in both animals (Fu et al., 2007) and humans (Spoormaker et al., 2012). Both total sleep deprivation and selective REM-sleep

Abbreviations: CS+, reinforced conditioned stimulus; CS−, non-reinforced conditioned stimulus; CS+E, reinforced conditioned stimulus later extinguished; CS+U, reinforced conditioned stimulus left un-extinguished; MEQ, Morningness–Eveningness Questionnaire; NEO-PI-R, NEO Personality Inventory Revised; PSQI, Pittsburgh Sleep Quality Index; PTSD, Posttraumatic Stress Disorder; REM sleep, rapid eye movement sleep; SCR, skin conductance response; SCRd, differential skin conductance response; STAI-T, Spielberger State-Trait Anxiety Index-Trait Version

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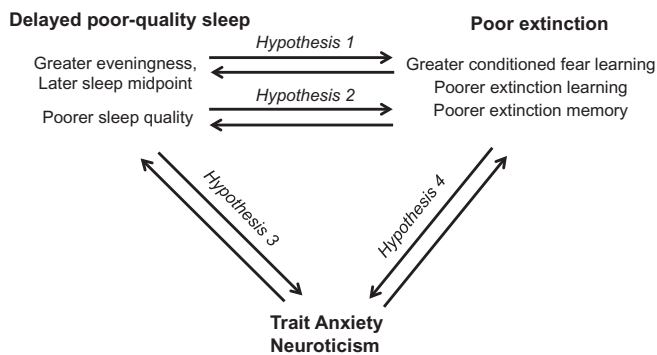


Fig. 1. Hypothetical model of positive relationships between 3 elements – delayed poor-quality sleep, poor extinction and trait anxiety. (See section 1.4 for formal statements of hypotheses.) The bidirectionality of influences (arrows) depicted in this figure indicate that, in the current study, specific causal relationships are not presumed or tested. This does not, however, imply such relationships are equally likely but simply that they are possible. For example, mechanisms by which trait anxiety could have negative effects on sleep can be readily inferred from existing literature whereas the reverse is not as easily envisaged, but is clearly possible (Johnson et al., 2006).

deprivation experiments have shown negative effects on extinction recall (reviewed in Pace-Schott et al., 2015). Therefore, we hypothesized that extinction recall would vary positively with better sleep quality.

Time-of-day also affects extinction learning and recall. For example, among the healthy young-adult males who are also examined in the current study, extinction was better learned in the morning versus evening (Pace-Schott et al., 2013). Similarly, in the morning, extinction recall was better generalized from a conditioned and extinguished stimulus to a similarly conditioned but un-extinguished stimulus (Pace-Schott et al., 2013). One possible explanation for these findings is that circadian rhythms influence extinction either directly or indirectly via hormones such as cortisol and testosterone that show circadian rhythmicity (Pace-Schott et al., 2013).

One important mediator of individual differences in such circadian influences is an individual's subjective and behavioral preference for the morning or evening to engage in wakefulness-related activities, often termed “morningness–eveningness” (reviewed in Adan et al. (2012)). Standardized questionnaires, such as the Morningness–Eveningness Questionnaire (MEQ) (Horne and Ostberg, 1976), have been developed which classify those with the greatest preference for evening and morning as “evening” and “morning” chronotypes respectively with the remaining classified as “neither” or “intermediate” chronotype. Chronotype maps onto physiological rhythms and on average, compared to evening types, morning types go to bed and wake up earlier, have a core temperature nadir about 2 h earlier, melatonin onset about 3 h earlier, and a cortisol acrophase about 1 h earlier (Adan et al., 2012). We therefore hypothesized that morningness–eveningness might influence the degree to which healthy individuals acquire conditioned fear, learn the extinction of this fear or retain this extinction learning.

1.2. Sleep quality and timing can impact anxiety

Anxiety is associated with poor sleep (Kajimura et al., 1998) and evening chronotype (Adan et al., 2012; Lemoine et al., 2013). For example, the neuroticism component of the 5-factor theory of personality, as quantified using the NEO Personality Inventory Revised (NEO-PI-R) (Costa and McCrae, 1992), is a significant predictor of both the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) measure of poor sleep (Calkins et al., 2013) as well as

of poor sleep hygiene (Duggan et al., 2014). Similarly, a large epidemiological study (Taylor et al., 2005) has shown elevated levels of trait anxiety in persons with versus without insomnia using the Spielberger State-Trait Anxiety Index-Trait Version or STAI-T (Spielberger et al., 1990). We therefore predicted that greater self-report measures of NEO-PI neuroticism and STAI-T anxiety would predict both lesser MEQ morningness and poorer PSQI sleep quality. NEO-PI-R neuroticism and STAI-T trait anxiety, although correlated with one another and with poor sleep, do not measure identical constructs. For example, the various facets of neuroticism tap measures of hostility, depression, self-consciousness, impulsivity and vulnerability as well as anxiety per se (Costa and McCrae, 1992). We therefore obtained both measures in order to provide a more complete assessment of traits potentially predisposing otherwise healthy individuals to pathological anxiety, and hypothesized that both measures would be associated with poorer sleep quality.

Sleep deprivation or disturbance is also associated with pathological levels of anxiety. Sleep disturbance is a prominent feature of Posttraumatic Stress Disorder (PTSD), Generalized Anxiety Disorder (GAD) and Panic Disorder (Lepola et al., 1994; Mellman, 2008; Ramsawh et al., 2011; Sheikh et al., 2003) and is a DSM-5 diagnostic criterion for GAD and PTSD (American Psychiatric Association, 2013). Delayed sleep phase may be a circadian abnormality especially common in Obsessive Compulsive Disorder (Paterson et al., 2013). Better sleep quality may predict the success of behavioral therapy for Social Anxiety Disorder (Kushnir et al., 2014; Zalta et al., 2013), and post-exposure sleep may augment exposure therapy for Simple Phobia (Kleim et al., 2013; Pace-Schott et al., 2012).

A large literature also links evening chronotype with sub-clinical poor mood and anxiety (Adan et al., 2012; Hsu et al., 2012). For example, among healthy individuals, morning types show overall greater levels of positive affect (Biss and Hasher, 2012) whereas evening types show more depressive symptoms (Hidalgo et al., 2009). We therefore hypothesized that greater eveningness would be associated with greater trait anxiety and neuroticism.

Evening chronotype is also linked with a greater incidence of mood and anxiety disorders (Adan et al., 2012; Hsu et al., 2012). Greater risk of anxiety disorders in evening types may be mediated, in part, by asynchrony of their activity patterns with normative schedules (“social jetlag”) (Wittmann et al., 2006). Correspondingly, individuals with anxiety disorders are more likely to be evening-types (Lemoine et al., 2013).

1.3. Anxiety can impact extinction recall

There exists little published information on relationships of trait anxiety and neuroticism with fear extinction learning and memory. A single study has shown a lack of relationship of trait anxiety with fear conditioning and generalization (Torrents-Rodas et al., 2013). However, anxiety disorders have been specifically linked with greater fear conditioning (Orr et al., 2000), poorer extinction learning (Bleichert et al., 2007) and poorer extinction recall (Milad et al., 2006, 2008, 2009, 2013). Anxiety disorders have also been linked with greater trait anxiety and neuroticism. For example, both STAI trait anxiety (Hishinuma et al., 2001) and NEO-PI neuroticism (Weinstock and Whisman, 2006) are predictors of anxiety disorders. We therefore hypothesized that greater trait anxiety and neuroticism would be associated with poorer extinction learning and recall.

1.4. Hypotheses

In the current study, we sought to identify, in healthy individuals, interrelationships between sleep timing and quality,

extinction, and trait neuroticism and anxiety that could interact, over time, to produce pathological anxiety. These hypothesized interactions could lead to positive feedback whereby worsening of deficits at one node could exacerbate problems at the others leading ultimately to pathological anxiety in vulnerable individuals. Fig. 1 depicts these putative positive feedback mechanisms. Because the initial level of fear conditioning can influence the degree to which extinction is learned and recalled, the influences of sleep quality, sleep timing and trait anxiety on fear conditioning were also examined.

Formal statements of our hypotheses are as follows: *Hypothesis 1*: greater eveningness, expressed as later sleep midpoint (see Methods) and lower MEQ, and taking into account the time-of-day at testing, are associated with poorer extinction learning, recall and generalization. *Hypothesis 2*: poorer average sleep quality is associated with greater fear conditioning and poorer extinction learning and recall. *Hypothesis 3*: greater levels of NEO-PI-R neuroticism and STAI-T trait anxiety predict later sleep timing, greater eveningness and poorer average sleep quality. *Hypothesis 4*: higher NEO-PI-R neuroticism and STAI-T trait anxiety are associated with poorer extinction learning, recall and generalization.

2. Methods

2.1. Participants

Participants were drawn from 109 healthy young adult males, aged 18–29 y (mean 20.8, SD 2.6), who provided skin conductance response (SCR) data in a prior study (Pace-Schott et al., 2013). The great majority of individuals studied were undergraduate or graduate students at the University of Massachusetts, Amherst with a few from nearby colleges. Participants were restricted to males because extinction recall varies with estradiol level and menstrual cycle phase in females (Graham and Milad, 2013; Milad et al., 2010). Exclusion criteria, based upon self-report, included any history of neurological illness (e.g., seizures, significant head trauma) or diagnosed DSM IV Axis I mental disorder or sleep disorder. Also excluded were those reporting current medical illness potentially interfering with sleep, use of sleep-altering drugs, average sleep per night < 6 or > 10 h, shift work or irregular sleep schedule, cigarette smoking, > 5 caffeinated beverages per day, > 12 alcoholic beverages per week, or problems with alcohol or drug abuse. Sample size varied between analyses as detailed below and in Supplementary methods. Procedures accorded with the Declaration of Helsinki and were approved by the University of Massachusetts, Amherst and the Partners Healthcare Institutional Review Boards. All participants provided written informed consent and were paid or earned academic credit.

2.2. Pre-study week

During the week prior to the experiment, sleep–wake behavior was monitored using wrist actigraphy and a sleep diary. During this week, participants were asked to keep a regular sleep schedule consisting of a minimum of 7 h in bed each night, bedtime no later than 2:00 a.m. and no daytime napping, alcohol, recreational drugs or, on experimental days only, caffeine. Curfew was prescribed to support the primary goal of the parent study, viz. to differentiate effects of time-of-day, inter-session delay and intervening sleep on extinction recall (Pace-Schott et al., 2013). During this week participants also completed the PSQI, MEQ, NEO-PI-R and STAI-T.

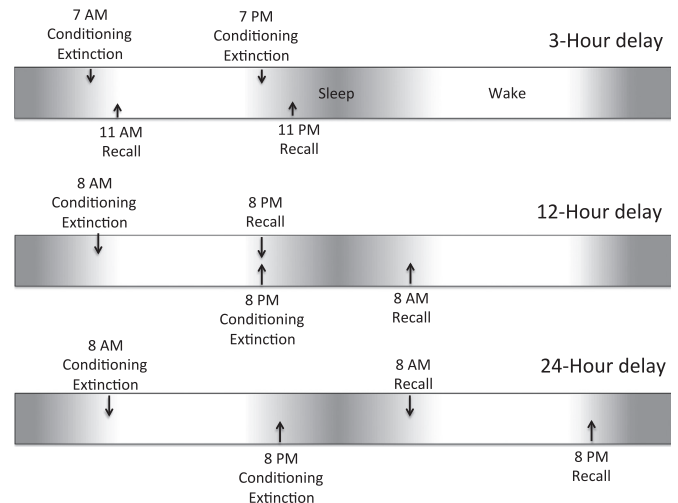


Fig. 2. Experimental fear conditioning and extinction schedule showing the 6 combinations of time-of-day trained and tested with delays of 3, 12 or 24 h between Sessions 1 and 2. Note that those with delays of 3 or 24 h were trained and tested at the same time of day. All individuals with delays of 3 h remained awake between sessions whereas all those with 24-h delays all had a normal night's sleep between sessions.

2.3. Experimental schedule

Four experimental phases took place over two sessions. Habituation, Fear Conditioning and Extinction Learning phases occurred in Session 1; recall of extinction memory (Extinction Recall) occurred in Session 2. Fifty-two subjects underwent Session one in the morning between 7 and 10 a.m. and Session two 3, 12, or 24 h later (Fig. 2). The other 57 subjects underwent Session one in the evening between 7 and 10 p.m. and Session two 3, 12, or 24 h later. Timing was designed to accomplish above-described goal of the parent study – to determine the effects of time of day, intervening sleep and inter-session delay on fear conditioning, extinction learning and extinction recall – and is depicted in Fig. 1 of Pace-Schott et al. (2013) as well as Fig. 2.

2.4. Stimuli and experimental protocol

Stimuli and protocol have been extensively described in previous publications (e.g., Milad and Quirk, 2012; Pace-Schott et al., 2013). Briefly, each subject selected a level of an electric-shock unconditioned stimulus (US) that was “highly annoying but not painful” (Orr et al., 2000). Conditioned stimuli (CS) were digital photographs of 3 differently colored lamps (blue, red or yellow) displayed within the images of 2 different rooms (“contexts”).

During the Habituation phase each combination of CS color and context were presented once across 6 trials. The remaining 3 experimental phases contained 32 trials each. During the Fear Conditioning phase, 2 of the 3 colored lamps (CS+) were presented 8 times each and were immediately followed by the US after 5 of these presentations (63% partial reinforcement). The third colored lamp was never followed by the US (CS–) and was presented 16 times pseudorandomly interspersed among the CS+. During the Extinction Learning phase, 1 of the 2 CS+ colored lamps appeared 16 times (CS+E) in the extinction context along with 16 interspersed CS– trials. The other CS+ was not presented and thus remained conditioned but un-extinguished (CS+U). During the Extinction Recall phase, the CS+E and CS+U were each presented 8 times in the extinction context with 16 interspersed CS– trials and no US.

During Fear Conditioning, the two different CS+ colors were presented as blocks, i.e., all 8 of one color CS+ were presented

with 8 accompanying, unreinforced CS– then all 8 of the other color CS+ were presented with 8 accompanying, unreinforced CS– (see Table S1). During Fear Conditioning, either the first-presented or the second-presented CS+ color could subsequently become the CS+E with the other color then becoming the CS+U. To counteract order effects during the two experimental phases in which both CS+ colors were presented (i.e., Fear Conditioning and Extinction Recall), eight different counterbalanced versions of the protocol were presented to approximately the same number of subjects across the total sample and within each delay (3, 12 and 24-h) group. The colors assigned as CS+E, CS+U and CS– were also counterbalanced across subjects. Within each block of CS+ color (or during presentations of the CS+E alone during the Extinction Learning phase), CS– trials were interspersed and pseudorandomly arranged such that no more than 2 CS+ or 2 CS– occurred in a row.

In each trial, the context picture first appeared alone for 3 s and then the CS appeared in that context for an additional 6 s. The inter-trial interval (CS offset to next context onset) averaged 15 s and varied pseudo-randomly between 12 and 18 s. Stimuli were presented on a 17" PC monitor at approximately eye level using SuperLab 4.0 (Cedrus Corporation, San Pedro, CA).

2.5. Skin conductance response (SCR)

Instrumentation for measuring skin conductance level (SCL) is described in Supplementary methods. SCR was calculated by subtracting the mean SCL in microSiemens (μ S) during the last 2 s of context alone from the maximum skin conductance level achieved during the 6-s CS presentation. SCRs were square-root transformed. If the untransformed SCR was negative, the square root of the SCR's absolute value was calculated and then made negative (Orr et al., 2000).

Differential conditioning procedures provide the most rigorous measure for the degree of association between a CS+ and an unconditioned stimulus (US) by comparing conditioned responses to the CS+ with those to an accompanying CS– that was not previously associated with the US (Lockhart and Grings, 1963; Pineles et al., 2009). In order to measure differential conditioning using SCR, a differential SCR (SCRd) was computed by subtracting from the SCR to each CS+, the SCR to the ordinal corresponding CS– (i.e., first CS+ minus first CS–, second CS+ minus second CS–, etc.). Because no more than 2 CS+ or 2 CS– were presented in succession, each CS+ was always presented in close temporal proximity to its ordinal matched CS–. SCRd was computed in this manner for each CS+ in each experimental phase (detailed in Table S1). For example, at Extinction Recall, 8 of the CS+ were CS+E and 8 were CS+U. The CS– were divided equally between them providing, for each CS+'s SCR, a corresponding SCR to a CS– that could be subtracted to generate that CS+'s SCRd.

2.6. Actigraphy

Participants wore the Actiwatch-2 (Philips Respironics, Bend OR) continuously and were instructed to press an event-marker button when beginning to attempt sleep and when waking for the day. Time stamps inserted by the event button served as demarcation of the subject's time-in-bed within which the default algorithm of Actiware 5.61 software determined total sleep time, sleep onset latency, and sleep efficiency. Within time-in-bed, the default algorithm scores sleep onset at the beginning of the first continuous 10 immobile minutes. Once sleep is initiated, each 1-min epoch is scored as sleep if the sum of the following formula is < 40 : [for epoch 2 s prior to the epoch scored: $(C \times 1/25)] +$ [for epoch 1 s prior to the epoch scored: $(C \times 1/5)] +$ [for the actual 1 s epoch scored: $(C \times 1)] +$ [for epoch 1 s after the epoch scored:

$(C \times 1/5)] +$ [for epoch 2 s after the epoch scored: $(C \times 1/25)]$. In this formula, "C" indicates actiwatch movement counts, and successive summed elements represent the 2 epochs before the scored epoch, the scored epoch itself, and the 2 epochs following the scored epoch. Sleep efficiency was computed as total sleep epochs in minutes divided by total time between event marks $\times 100$.

2.7. Determination of behavioral and subjective morningness–eveningness

Only about 40% of the general population can be classified as morning types or evening types using the standard MEQ criteria (Adan et al., 2012). Among young adults, the distribution is highly skewed toward evening types (Adan et al., 2012), especially in males (Lehnkering and Siegmund, 2007). Therefore, relatively large samples of young adult males can yield very low numbers of MEQ morning types (Kudielka et al., 2006).

Therefore, an indicator of sleep timing was sought that could be derived behaviorally from actigraphy data as well as from self-report sleep diaries. The chosen measure, sleep midpoint, has been shown to be a valid proxy for melatonin onset (Burgess et al., 2003; Martin and Eastman, 2002; Terman et al., 2001) and has been used as sole marker of circadian phase in a number of recent studies (Forbes et al., 2012; Sato-Mito et al., 2011). Sleep midpoint, expressed as minutes past midnight, was determined from actigraph data by computing the time point midway between algorithmically scored sleep onset and the subject's awakening event mark. When the subject failed to make an attempt-sleep or waking event mark, diary entries were used. To support actigraphy-based results, we computed the same measure entirely from diary entries (see Supplementary methods).

To complement behavioral measures with a subjective measure of sleep-timing preference, we modified the MEQ in order to obtain samples segregated into the greatest morningness and eveningness expressed in this population in which only 2 participants met standard criteria for morning type (MEQ=59–86). First, we used standard criteria (Horne and Ostberg, 1976) to define "Evening-Types" (MEQ=16–41). All remaining MEQ scores were then subjected to a median split and only the upper half was retained as "High-Morningness" participants (MEQ=50–70).

2.8. Analyses

2.8.1. Sample sizes

Of the total 109 participants, 107 provided PSQI, and MEQ data, 94 provided STAI-T data and 91 provided NEO-PI-R data. Eighty-five participants (mean age 21.0, SD 2.7) produced 4–9 usable actigraph nights (mean 7.0 nights, SD 1.0, 91% participants > 5 nights, 86% > 6). For Extinction Recall analyses, only the 73 individuals (55 with actigraphy) who learned and recalled extinction at the same time of day, (i.e., 3- or a 24-h delay) were analyzed so as not to confound opposing time-of-day influences for these two phases (Pace-Schott et al., 2013). However, parallel analyses that include the 12-h delay subjects are provided in Supplementary results. The maximum available samples for each comparison were analyzed (excepting diary responses that were limited to those who also provided usable actigraphy). Therefore sample sizes and degrees of freedom could differ among the different comparisons, and the numbers compared in each analysis are provided with each analysis in Results and Supplementary results. Additional sample-size details on subjects with usable actigraph data are provided in Supplementary methods.

2.8.2. Dependent and independent variables

The psychophysiological index of learned fear, SCRd, served as a

repeated dependent measure in mixed ANOVA models (see below) used to test *Hypotheses* 1, 2 and 4. For *Hypothesis* 3, additional dependent measures were sleep quality and timing measured retrospectively by self-report (PSQI, MEQ) and longitudinally by actigraph. During Fear Conditioning, because neither CS+ had yet been extinguished, there were a total of 16 CS+ trials differentiated only by whether they were the first 8 CS+ or the second 8 CS+ presented. Similarly, during Extinction Learning, there were a total of 16 CS+E trials presented. In contrast, during Extinction Recall, there were 8 CS+E (conditioned and extinguished) and 8 CS+U (conditioned only) presented. Therefore, during Fear Conditioning and Extinction Learning phases, mean SCRd to pairs of CS+ trials were analyzed to reduce levels of the within-subject factor “Trial” (see below) from 16 to 8, and to reduce trial-to-trial variability. Table S1 and Supplementary methods detail which pairs of trials were averaged for these two phases. The 8 CS+E and 8 CS+U at Extinction Recall were analyzed as individual trials. Note that computation of the differential conditioning measure, SCRd, incorporates information contained in the SCR to the CS—corresponding to each CS+ (see Table S1 and Section 2.5 above).

Independent variables were dichotomized to serve as between-subjects factors in ANOVAs analyzing SCRd. For *Hypothesis* 1, the primary dichotomized independent variable, Sleep-Timing, was a median split of actigraph-based mean sleep midpoint that defined Late-Timers (larger mean midpoints) and Early-Timers (smaller mean midpoints). A parallel median split of diary-based mean sleep midpoints was also analyzed secondarily (see Supplementary methods). The dichotomized Morningness/Eveningness independent variable was derived from MEQ scores (see Section 2.7 above).

For *Hypothesis* 2, the primary dichotomized sleep-quality independent variable was a median split of actigraph-based sleep-efficiency data (High versus Low Sleep-Efficiency). Parallel median splits of diary-based Sleep-Efficiency values were analyzed (see Supplementary methods). As an additional self-report measure of sleep quality, the standard PSQI cutoff score of > 5 was used to dichotomize participants with and without clinical sleep disturbance.

For *Hypothesis* 3, the 2 dichotomized independent trait variables predicting sleep measures were High and Low Neuroticism and Anxiety measured, respectively, by median splits of the NEO-PI-R neuroticism scale and the STAI-T total score. Continuous values of these 2 scales also served as predictors of sleep measures. For *Hypothesis* 4, High and Low Neuroticism and Anxiety similarly served as dichotomized independent trait variables predicting extinction. (For clarity, experimental phases, dichotomized independent variables and ANOVA factors begin with capital letters).

2.8.3. Statistical tests

The above, dichotomized independent variables served as between-subjects factors in mixed-model ANOVAs analyzing SCRd (*Hypotheses* 1, 2, 4). For Extinction Learning, an additional between-subjects factor was Time-of-Day (Morning, Evening) and a within-subject factor was Trial-Pair (see Section 2.8.2 above, Supplementary methods and Table S1). For Extinction Recall, 2 additional between-subjects variables included Time-of-Day (Morning, Evening) and Delay (3 and 24 h) as well as 2 repeated measures, Trial (trials 1–8) nested in CS+Type (CS+E, CS+U). In each analysis performed, all possible interactions of the between- and within-subject factors noted above were added to mixed ANOVA models (e.g., for Extinction Learning, Time-of-Day; for Extinction Recall, Time-of-Day and Delay). Although reducing degrees of freedom, this provides a conservative analysis of differences between levels of dichotomized independent variables and their interactions.

Because initial presentations of the CS+E and CS+U at Session

2 represented extinction and fear recall least confounded by the subsequent new extinction learning taking place during the Extinction Recall phase itself, all ANOVAs were repeated using SCRd responses to only the first 2 CS+E and first 2 CS+U trials. This approach was favored over measuring the 3-way interaction of dichotomized variables, CS+Type and Trial since, due to such new extinction learning, SCRd to later Extinction Recall CS+ trials (e.g., trials 5–8 and 13–16) was often minimal thereby reducing power to detect such interaction across the full 8 levels of Trial per each CS+Type. In contrast, low SCRd for later CS+ trials added little to overall mean CS+E and CS+U for which the greatest magnitudes are seen in the first 2–4 trials of each CS+Type. Therefore, results of these two ANOVAs should be seen as corroborating one another with similar findings in both providing the strongest evidence for differences or similarities between dichotomized independent variables.

Median splits of data used to form dichotomous predictor factors always used the specific sample on which each analysis would be carried out thereby producing some variability of sample size as well as additional variation in degrees of freedom between analyses. For each analysis, the sample size for each dichotomized variable (or at each Time-of-Day) are provided with results of statistical tests. For *Hypothesis* 3, simple regressions analyzed continuous psychological-trait and sleep variables. Analyses including 12-h delay participants and diary data are provided in Supplementary results.

3. Results

3.1. Characteristics of independent and dependent variables

The total counts, median and ranges of dichotomized independent variables were: MEQ (count 107, median 44, minimum 27, maximum 70); PSQI (count 107, median 4, minimum 0, max 14, of which 27 or 25.2% exceeded the clinical cutoff of 5); sleep midpoint (count 84, median 308 min, minimum 143 min, maximum 483 min); Sleep-Efficiency (count 84, median 82.6%, minimum 66.5%, maximum 98.3%); NEO-PI-R Neuroticism (count 93, median 81, min 32, max 166); STAI-T Anxiety (count 94, median 33.5, minimum 20, maximum 63).

The distributions of all independent (dichotomized) variables did not significantly differ from normality (Fig. S2). The Kolmogorov-Smirnov (K-S) test found no difference from normality for MEQ ($p=0.94$), PSQI ($p=0.24$), sleep midpoint ($p=1.0$), Sleep Efficiency ($p=1.0$), NEO-PI neuroticism ($p=1.0$), and STAI-T ($p=0.55$). Similarly, distributions of the dependent measure (SCRd) as well as SCR to the CS+ from which it was computed did not depart from normality (Figs. S3 and S4) as shown by K-S tests at key points in the protocol including maximum at Fear Conditioning (SCRd $p=0.80$, SCR $p=1.0$), mean of first pair at Extinction Learning ($p=1.0$, $p=0.53$), mean of all 16 at Extinction Learning ($p=0.25$, $p=0.42$), and, for Extinction Recall, mean of first 2 CS+E ($p=1.0$, $p=0.80$), mean of all 8 CS+E ($p=0.95$, $p=0.33$), mean of first 2 CS+U ($p=0.33$, $p=0.42$), mean of all 8 CS+U ($p=0.53$, $p=0.19$).

3.2. Fear conditioning

The degree of fear conditioning was not influenced by sleep timing or quality, psychological trait (e.g., Fig. 3A), or Time-of-Day (Pace-Schott et al., 2013). Fear Conditioning analyses are detailed in Supplementary results.

3.3. Hypothesis 1: Do Late-Timers and Evening-Types show poorer

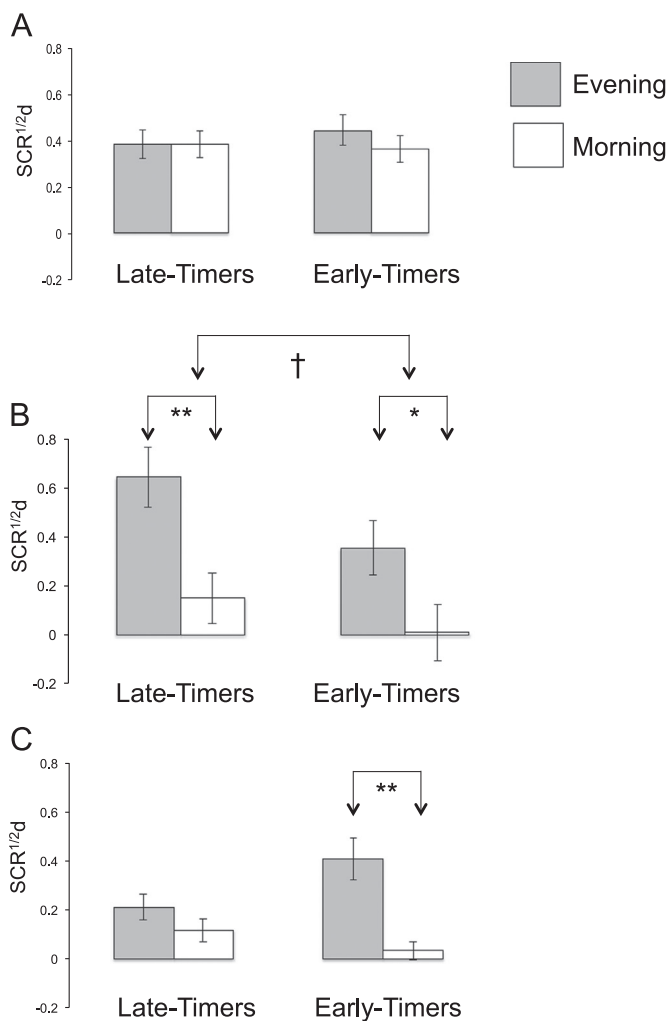


Fig. 3. Effects of actigraph-based Sleep-Timing and Time-of-Day at Session 1 on fear conditioning and extinction learning. (A) Mean SCRd to all 16 CS+ during the Fear Conditioning phase. (B) Mean SCRd to the first CS+E pair during the Extinction Learning phase. (C) Mean SCRd to all 8 CS+E pairs during the Extinction Learning phase among participants in the upper half of a median split of SCRd to the first pair. In this subsample, SCRd to the first pair did not differ between Late-Timers and Early-Timers allowing their comparison during Extinction Learning when beginning from the same level of SCRd to the CS+E. SCR^{1/2}d: differential square-root transformed SCR in microSiemens. † $p < 0.1$, * $p < 0.05$.

extinction learning, recall and generalization?

3.3.1. Extinction learning

Immediately following Fear Conditioning, the average SCRd to the first two CS+E trials at the beginning of Extinction Learning trended lower in the 43 Early-Timers versus the 41 Late-Timers [$F(1,80)=3.46$, $p=0.067$] (Fig. 3B) and in the 33 High-Morningness individuals versus the 40 Evening-Types [$F(1,69)=4.11$, $p=0.047$]. (For details see [Supplementary results](#).)

Given these initial differences, the SCRd starting point for Extinction Learning was equalized across the two levels of Sleep-Timing and Morningness/Eveningness by analyzing only the upper half of a median split of SCRd values for this first trial pair. Within this subset of participants ($N=54$), SCRd for this first trial pair no longer differed by Sleep-Timing ($p=0.17$) or Morningness/Eveningness ($p=0.40$). There was a Sleep-Timing \times Time-of-Day interaction [$F(1,39)=4.63$, $p=0.038$] (Fig. 3C) as well as a Morningness/Eveningness \times Time-of-Day interaction [$F(1,35)=5.49$, $p=0.025$] [Note that additional variation in degrees of freedom result from

varying numbers in this subset of $N=54$ who had actigraphy ($N=44$) or who segregated into categories of the modified MEQ ($N=40$)]. Because Sleep-Timing \times Time-of-Day and Morningness/Eveningness \times Time-of-Day interactions were significant, Time-of-Day effects were examined separately in 21 Early- and 22 Late-Timers as well as in 26 Evening-Types and 13 High-Morningness individuals. A larger evening ($N=12$) versus morning ($N=9$) SCRd was seen in Early-Timers [$F(1,19)=12.68$, $p=0.002$] (Fig. 3C) but not in Late-Timers [$F(1,20)=1.45$, $p=0.24$, $N=14$ tested in evening, 8 in morning]. Similarly, a larger evening ($N=7$) versus morning ($N=6$) SCRd was seen in High-Morningness [$F(1,11)=10.48$, $p=0.008$] but not in Evening-Type participants [$F(1,24)=2.77$, $p=0.11$, $N=15$ tested in evening, 11 in morning]. Although Late-Timers and Evening-Types showed non-significantly larger evening versus morning SCRd, it is striking that this difference was much more pronounced in the Early-Timers and High-Morningness individuals despite low representation in the latter subsample.

Because better learning of extinction results in a more rapid loss of the conditioned SCRd, the decline across the first two Extinction Learning trial pairs (encompassing the first 4 trials when most of the decline in SCRd occurs) was separately compared in Early- and Late-Timers. In Early-Timers there was a Time-of-Day \times Trial-Pair interaction [$F(1,19)=5.89$, $p=0.025$] reflecting more rapid decline from the first to second trial pair in the morning ($N=9$) versus evening ($N=12$). This interaction was absent in Late-Timers ($p=0.49$, 14 tested in evening, 8 in morning).

3.3.2. Extinction recall

Results from only the 73 individuals (55 with actigraphy) who learned and recalled extinction at the same time of day (i.e., the 3- and 24-h delay) are reported below. However analyses are repeated including the 12-h delay subjects in [Supplementary results](#).

3.3.2.1. Sleep-Timing. For SCRd at Extinction Recall, among the 55 individuals with 3 and 24-h delays and good actigraphy data, there were no Sleep-Timing main effects or interactions with Time-of-Day or Delay. However, the Sleep-Timing \times CS+Type interaction was significant across all 8 CS+E and 8 CS+U trials [$F(1,47)=7.12$, $p=0.01$] and across the first 2 trials only [$F(1,47)=5.33$, $p=0.025$]. Decomposing this interaction, within the 27 Late-Timers, SCRd for the CS+U was larger than CS+E across all 8 trials [$F(1,23)=10.25$, $p=0.004$] and across the first 2 trials only [$F(1,23)=7.89$, $p=0.01$], but not in the 28 Early-Timers ($p=0.69$ and 0.45 respectively). In addition, within Late-Timers, across all 8 trials there was a CS+Type \times Time-of-Day interaction [$F(1,23)=5.99$, $p=0.022$] that was a trend across the first 2 trials [$F(1,23)=2.92$, $p=0.10$] but absent in Early-Timers ($p=0.49$ and 0.93 respectively). Within the 27 Late-Timers, this interaction resulted from the SCRd being larger to the CS+U than to the CS+E among the 12 individuals tested in the evening across all 8 trials [$F(1,10)=7.59$, $p=0.02$] (Fig. 4A) and the first 2 trials [$F(1,10)=5.48$, $p=0.041$] (Fig. 4B) but not among the 15 individuals tested in the morning ($p=0.33$ and 0.26 respectively). Additionally, within the Late-Timers, the CS+U SCRd was itself larger in the 12 individuals tested in the evening than in the 15 tested in the morning across all 8 trials [$F(1,23)=12.5$, $p=0.002$] (Fig. 4A) and the first 2 trials [$F(1,23)=5.45$, $p=0.029$] (Fig. 4B), but not in Early-Timers ($p=0.23$ and 0.57 respectively).

3.3.2.2. Morningness/Eveningness. The Morningness/Eveningness factor showed no main effects or interactions with Time-of-Day or Delay. However, there was a trend-level interaction of Morningness/Eveningness with CS+Type (CS+E versus CS+U) [$F(1,41)=3.39$, $p=0.073$] across all 8 trials, becoming significant in the first 2 trials only [$F(1,41)=5.09$, $p=0.03$]. Decomposed, this interaction revealed that the 23 Evening-Types showed a trend-level larger

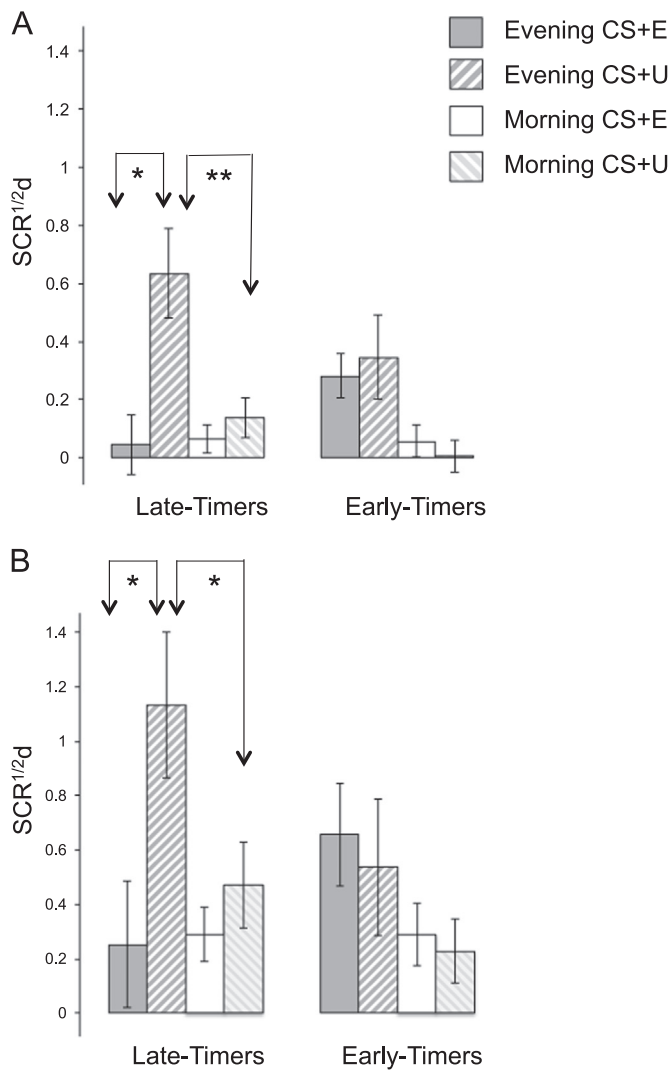


Fig. 4. Effects of actigraph-based Sleep-Timing on recall and generalization of extinction at Session 2. Generalization of extinction memory was operationalized as inversely related to the difference between the SCRd to the extinguished (CS+E) and unextinguished (CS+U) conditioned stimulus. (A) Mean SCRd to all 8 CS+E and all 8 CS+U during the Extinction Recall phase in Late-Timers and Early-Timers in the morning and evening. (B) Mean SCRd to only the first 2 CS+E and first 2 CS+U during the Extinction Recall phase in Late-Timers and Early-Timers in the morning and evening. For clarity, significance levels for Time-of-Day differences in SCRd unrelated to the interaction of Time-of-Day with Sleep-Timing are not shown. See text and Pace-Schott et al. (2013) for these effects. SCR^{1/2d}: differential square-root transformed SCR in microSiemens. * $p < 0.05$, ** $p < 0.01$.

SCRd to the CS+U compared to CS+E across all 8 trials [$F(1,19)=3.38$, $p=0.082$] and the first 2 trials [$F(1,19)=3.48$, $p=0.08$], a difference not seen in the 26 High-Morningness participants ($p=0.67$ and 0.23 respectively). In addition, within Evening-Types there was a CS+Type \times Time-of-Day interaction [$F(1,19)=4.92$, $p=0.039$] across all 8 trials (but not the first 2, $p=0.11$) that was absent in High-Morningness participants (all 8 $p=0.39$, first 2 $p=0.24$). In Evening-Types across all 8 trials, SCRd to CS+U was larger than to CS+E in the 11 subjects tested in the evening [$F(1,9)=5.77$, $p=0.04$], but not in the 15 tested in the morning ($p=0.60$).

3.4. Hypothesis 2: Is poorer sleep quality associated with poorer extinction learning, recall and generalization?

3.4.1. Extinction learning

SCRd to all CS+E during this phase differed neither between

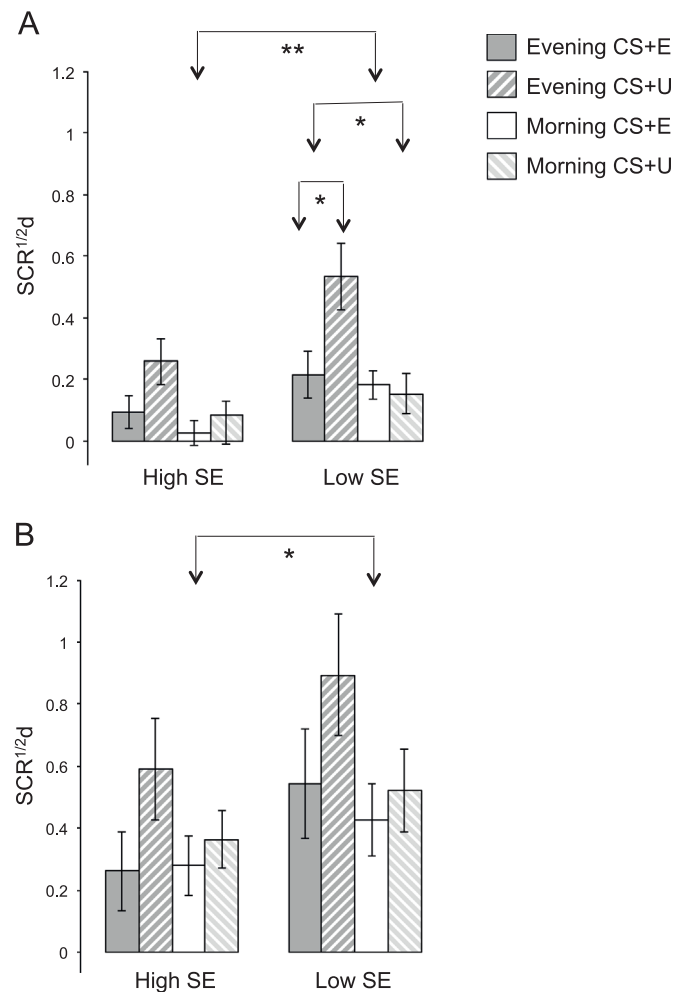


Fig. 5. Effects of High and Low actigraph-based Sleep-Efficiency on extinction recall. (A) Mean SCRd to all 8 CS+E and all 8 CS+U during the Extinction Recall phase in participants with High and Low Sleep-Efficiency in the morning and evening. (B) Mean SCRd to only the first 2 CS+E and first 2 CS+U during the Extinction Recall phase in participants with High and Low Sleep-Efficiency in the morning and evening. For clarity, significance levels for Time-of-Day differences in of SCRd unrelated to the interaction of Time-of-Day with Sleep-Efficiency are not shown. See text and Pace-Schott et al. (2013) for these effects. SE: Sleep Efficiency [(total min sleep/time in bed)] $\times 100$. SCR^{1/2d}: differential square-root transformed SCR in microSiemens. * $p < 0.05$, ** $p < 0.01$.

the 79 participants with PSQI < 5 and the 27 > 5 ($p=0.80$) nor between 41 participants with High Sleep-Efficiency and the 43 with Low Sleep-Efficiency ($p=0.48$). Additionally, none of these dichotomized measures interacted with Time-of-Day (all p 's > 0.57).

3.4.2. Extinction recall

3.4.2.1. PSQI. Across all 8 trials or when only the first 2 trials were analyzed there were neither PSQI main effects ($N=22 > 5$ and $51 < 5$) nor interactions with Time-of-Day, Delay or CS+Type (all p 's > 0.23).

3.4.2.2. Sleep-Efficiency. High versus Low Sleep-Efficiency showed a significant main effect in which, collapsing over CS+Type, the 28 individuals with Low Sleep-Efficiency showed a larger overall SCRd than the 27 with High Sleep-Efficiency when all 8 trials of each CS+ [$F(1,47)=7.77$, $p=0.008$] or when only the first 2 trials of each [$F(1,47)=5.68$, $p=0.021$] were analyzed (Fig. 5). Although Sleep-Efficiency did not interact with Delay or CS+Type across all 8 or just the first 2 trials (all p 's > 0.55), the conditioned and

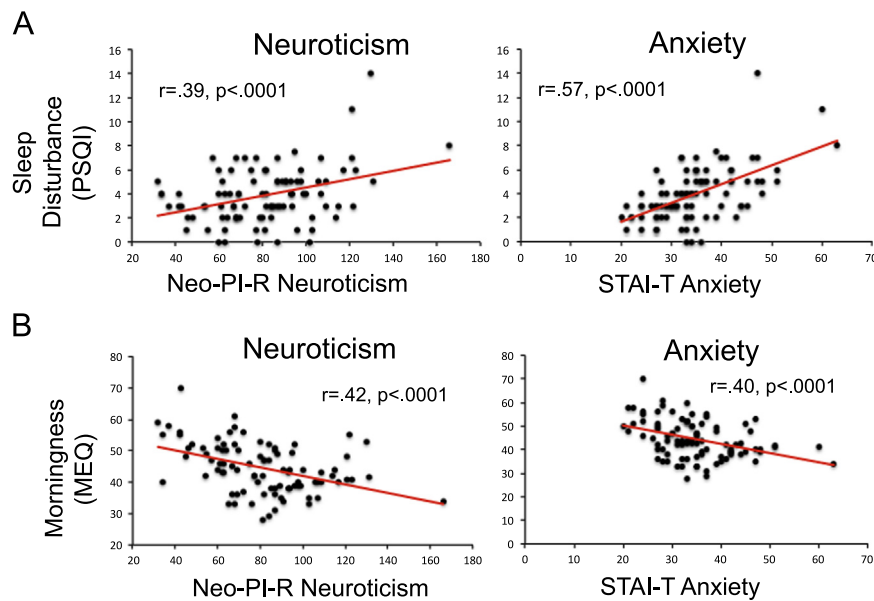


Fig. 6. Relationships between self-report measures of Neuroticism and Anxiety and retrospective subjective measures of sleep quality and Morningness/Eveningness. (A) Relationship of Pittsburgh Sleep Quality index (PSQI) (Buysse et al., 1989) with NEO-PI-R Neuroticism and Spielberger State-Trait Inventory (STAI-T) Anxiety (Spielberger et al., 1990). (B) Relationship of Morningness–Eveningness Questionnaire (MEQ) score (Horne and Ostberg, 1976) with NEO-PI-R Neuroticism scale and STAI-T Anxiety. Higher PSQI indicates greater sleep disturbance. Higher MEQ score indicates greater morningness.

extinguished stimulus (CS+E) alone showed a similar main effect across all 8 trials [$F(1,47)=4.60, p=0.037$] although not across the first 2 trials (CS+E, $p=0.14$). In addition, across all 8 trials (but not the first 2), there was a Sleep-Efficiency \times Time-of-Day interaction [$F(1,47)=4.13, p=0.048$] ($p=0.14$ across the first 2) whereby, collapsing over CS+Type, the 17 participants with Low Sleep-Efficiency who were tested in the evening showed a significantly larger SCRd to CS+ than the 11 tested in the morning [$F(1,24)=8.42, p=0.008$] (Fig. 5A) whereas those with High Sleep-Efficiency (8 tested in the morning, 19 in evening) did not differ by Time-of-Day ($p=0.86$). Among these same Low Sleep-Efficiency individuals, there was also a CS+Type \times Time-of-Day interaction [$F(1,24)=4.83, p=0.038$] whereby SCRd to the CS+U exceeded that to the CS+E in the 17 tested in the evening [$F(1,15)=5.43, p=0.034$] but not among the 9 tested in the morning ($p=0.40$).

3.5. Hypothesis 3: Are neuroticism and trait anxiety associated with greater eveningness and lower sleep quality?

High versus Low Neuroticism ($N=45$ and 46 respectively) was associated with poorer PSQI sleep quality [$F(1,89)=6.65, p=0.012$]. Neuroticism was positively correlated with PSQI ($R=0.39, p<0.0001, N=91$) (Fig. 6A). High versus Low Anxiety ($N=47$ and 47 respectively) was associated with poorer PSQI sleep quality [$F(1,92)=19.38, p<0.0001$] and Anxiety was positively correlated with poorer PSQI ($r=0.57, p<0.0001, N=94$) (Fig. 6A). High versus Low scores of both Neuroticism and Anxiety were associated with greater MEQ eveningness ([$F(1,89)=13.76, p=0.0004$] and [$F(1,92)=10.58, p=0.002$] respectively). Similarly, MEQ morningness was negatively correlated both with Neuroticism ($r=-0.42, p<0.0001, N=91$) and Anxiety ($r=-0.40, p<0.0001, N=94$) (Fig. 6B).

The 39 High-Neuroticism participants with actigraphy showed a significantly later sleep midpoint than the 42 with Low-Neuroticism [$F(1,79)=4.33, p=0.04$]. However the correlation of Neuroticism with sleep midpoint did not reach significance ($R=0.18, p=0.11$). Sleep-Efficiency did not distinguish High versus Low Neuroticism ($p=0.12$) but neuroticism showed a trend-level negative correlation with Sleep-Efficiency ($R=-0.20, p=0.07$). The

41 High-Anxiety participants showed significantly later sleep midpoint than the 42 with Low-Anxiety [$F(1,81)=4.02, p=0.05$], however the correlation of Anxiety with sleep midpoint did not reach significance ($R=0.17, p=0.13, N=83$). Sleep-Efficiency did not distinguish High- versus Low-Anxiety participants ($p=0.15$) nor did Anxiety correlate with Sleep-Efficiency ($R=-0.18, p=0.11$).

3.6. Hypothesis 4: Are higher Neuroticism and Anxiety associated with poorer extinction learning, recall and generalization?

3.6.1. Extinction learning

SCRd to all 8 CS+E trial pairs did not differ between High ($N=46$) versus Low ($N=46$) Neuroticism ($p=0.25$) or High ($N=47$) versus Low ($N=46$) Anxiety ($p=0.42$) individuals nor did these dichotomized variables interact with Time-of-Day ($p=0.09$ and 0.92 respectively).

3.6.2. Extinction recall

During extinction recall, whether all 8 of each CS+ (Fig. 7A) or only the first 2 of each were analyzed, High ($N=28$) versus Low ($N=31$) Neuroticism showed no main effect ($p=0.65$ and 0.84 respectively) or interactions with Time-of-Day ($p=0.54$ and 0.80), Delay ($p=0.81$ and 0.84) or CS+Type ($p=0.18$ and 0.19). Similarly, whether all 8 of each CS+ (Fig. 7B) or only the first 2 were analyzed, High ($N=27$) versus Low ($N=33$) Anxiety showed no main effect ($p=0.63$ and 0.16 respectively) or interactions with Time-of-Day ($p=0.36$ and 0.54), Delay ($p=0.72$ and 0.50) or CS+Type ($p=0.86$ and 0.56).

4. Discussion

Of the three interrelationships proposed in Fig. 1, findings supported two, viz. those between delayed/poor-quality sleep and poor extinction, and between delayed/poor-quality sleep and trait anxiety/neuroticism. However findings did not support the third, between trait anxiety/neuroticism and poor extinction.

Hypothesis 1. was partially confirmed. Extinction was more

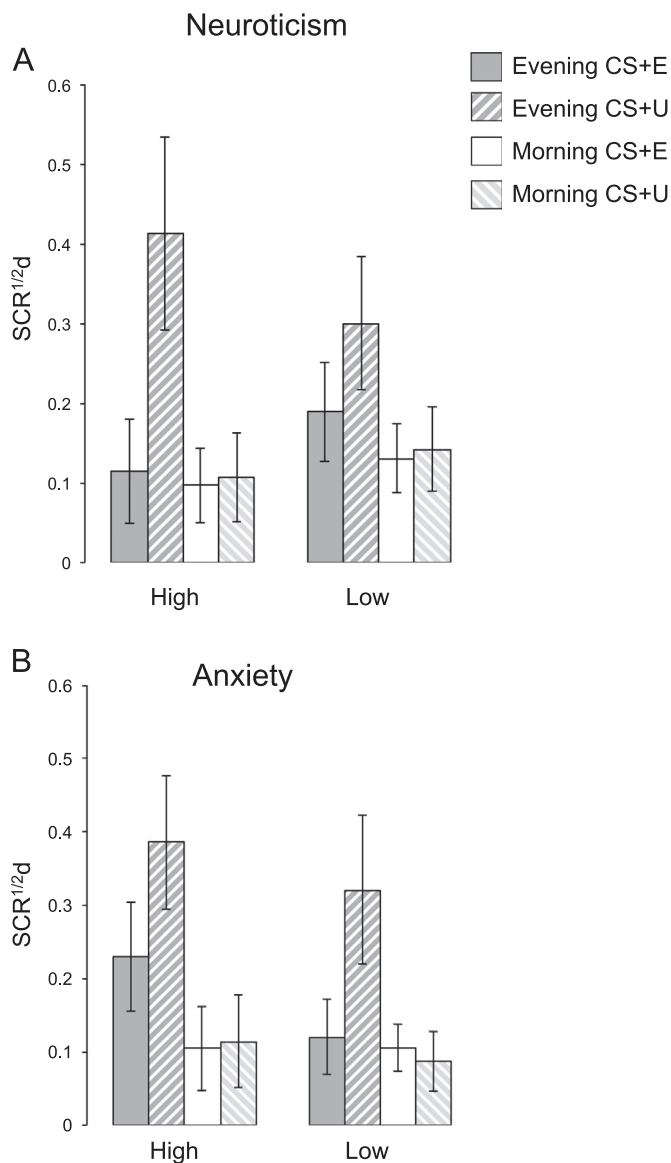


Fig. 7. Effects of High and Low NEO-PI-R Neuroticism and STAI-T Anxiety on extinction recall. (A) Mean of SCRd to all 8 CS+E and all 8 CS+U during the extinction recall phase in participants with High and Low Neuroticism in the morning and evening. (B) Mean of SCRd to all 8 CS+E and all 8 CS+U during the extinction recall phase in participants with High and Low Anxiety in the morning and evening. For clarity, significance levels for Time-of-Day differences of SCRd are not shown. See text and Pace-Schott et al. (2013) for these effects. SE: Sleep-Efficiency (total min sleep/time in bed) \times 100. SCR^{1/2d}: differential square-root transformed SCR in microSiemens. * $p < 0.05$, ** $p < 0.01$.

rapidly acquired in the morning than the evening in Early-Timers and High-Morningness participants but not in Late-Timers or Evening-Types. At Extinction Recall greater SCRd to the CS+U versus CS+E was seen only in Late-Timers and eEvening-Types tested in the evening, whereas the other subgroups (including Late-Timers and Evening-Types tested in the morning) generalized extinction recall for the CS+E to the CS+U. Both superior extinction learning in the morning and poorer extinction-recall generalization in the evening appeared in the sample as a whole (Pace-Schott et al., 2013). The current findings thus specify the sleep-timing characteristics of the subgroups most responsible for these effects.

Specifically, early sleep timing or greater morningness may augment the positive effect of learning extinction in the morning (Pace-Schott et al., 2013) such that this time-of-day advantage

accrues only to such individuals. In contrast, those with later sleep timing or greater eveningness showed the greatest negative effect of evening testing on extinction generalization. Therefore, habitual sleep timing and morningness/eveningness do not enhance extinction learning and recall generalization at their respectively consonant times-of-day as might be expected for psychomotor performance (Adan et al., 2012). Instead, greater morningness and earlier sleep timing may enhance a morning advantage for extinction learning whereas late sleep timing and evening chronotype may exacerbate an evening disadvantage for extinction generalization.

Observed results accord with published findings. Evening chronotype has been widely demonstrated to be associated with negative affect and enhanced risk of mood and anxiety disorders (Adan et al., 2012; Hsu et al., 2012; Lemoine et al., 2013). In healthy individuals, morning types show overall greater levels of positive affect (Biss and Hasher, 2012) whereas evening types show more depressive symptoms (Hidalgo et al., 2009) and nightmares (Nielsen, 2010). Individuals with mood (Adan et al., 2012) and anxiety (Lemoine et al., 2013) disorders are more likely to be evening types, and evening types show more severe symptoms (Hasler et al., 2010). Evening type is also associated with delayed sleep phase syndrome – itself a further risk factor for incident mood and anxiety disorders (Okawa and Uchiyama, 2007).

Hypothesis 2. was also partially confirmed. Poorer sleep efficiency was associated with poorer extinction recall, a finding that was seen collapsing across both CS+ types (CS+E and CS+U) as well as in the conditioned and extinguished CS+ (CS+E) alone.

Hypothesis 3. was that neuroticism and trait anxiety would be associated with greater eveningness, later average sleep timing and poorer average sleep quality. This hypothesis was confirmed as expected from prior reports (Calkins et al., 2013; Duggan et al., 2014; Kajimura et al., 1998; Taylor et al., 2005).

Hypothesis 4. Strikingly, however, although trait anxiety was associated with poorer-quality, delayed sleep, and poorer-quality, delayed sleep was associated with poorer extinction, neuroticism and trait anxiety were not associated with poorer extinction as predicted by Hypothesis 4. Notably, a previous study reported a lack of relationship of trait anxiety with fear conditioning and generalization (Torrents-Rodas et al., 2013). Nonetheless, trait anxiety is a strong correlate of anxiety disorders (Weinstock and Whisman, 2006) and there is clear evidence that pathological levels of anxiety are associated with extinction deficits in experimental paradigms involving extinction of de-novo fear conditioning (Milad et al., 2013; Milad et al., 2009; Milad et al., 2008; Orr et al., 2000). Therefore, the dissociation between poor extinction and trait anxiety reported here may exist only across the normal range of trait anxiety and a negative correlation between anxiety and extinction memory may emerge with the onset of anxiety disorders.

It is possible that poor sleep represents an intermediate step whereby trait anxiety can lead indirectly to poor extinction and thence to positive feedback that further degrades both extinction and sleep (and possibly also exacerbates trait anxiety itself) as depicted in Fig. 1. Therefore, trait anxiety may promote anxiety disorders both directly and by an indirect route via delayed/poor quality sleep and consequent impaired extinction learning, recall and generalization. Across normal levels of trait anxiety, the observed relationship of pathological anxiety with impaired extinction (e.g., Milad et al., 2009) may remain weak.

4.1. Limitations

The following study limitations exist. First, the instruction to be in bed no later than 2:00 a.m. may have advanced bedtime in some Late-Timers. However, sleep-timing preferences would still have influenced choice of bedtimes. Correlation between MEQ and mean sleep midpoint ($R=0.37$, $p=0.0006$) indicates that midpoints observed reflected actual sleep-timing preferences. The 2:00 a.m. limit still provided latitude to delay bedtime. Notably, many participants chose bedtimes throughout their study period that were well before this late limit (e.g., mostly before midnight), suggesting a true early-timing preference. Similarly, others consistently chose times closer to this limit (e.g., mostly after 1:00 a.m.) suggesting a later preference. It is possible also that the imposed 2 a.m. bedtime forced the more extreme Evening-Types/Late-Timers into a sleep schedule that, because unfavorable, worsened their ability to generalize extinction recall. However, the least degree of generalization of extinction recall (i.e., the greatest difference between the CS+U and CS+E) occurred in Evening-Types/Late-Timers tested in the evening. If stress due to imposed sleep timing were to have degraded generalization, this effect would have been expected in Evening-Types/Late-Timers tested in the morning when they would have preferred to be asleep.

Second, a male sample was studied to circumvent the effects of menstrual cycle on extinction recall (Graham and Milad, 2013; Milad et al., 2010). Notably, the prominent time-of-day effect on extinction generalization may not appear in females, possibly due to circadian rhythms of testosterone as well as cortisol in males (Pace-Schott et al., 2013). However, given that females are at heightened risk of anxiety disorders (Lebron-Milad and Milad, 2012) and such risk is modulated by natural cycling, menarche and menopause (Lebron-Milad and Milad, 2012) as is sleep quality (Manber and Armitage, 1999), it will be especially important in the future to examine these phenomena in women. Studies of extinction recall in females as well as the effects of estrogen and menstrual cycle are topic of ongoing research by our colleagues (Lebron-Milad et al., 2012).

Limiting the sample to healthy young adults similarly reduces the generalizability of findings. For example, young adult male college students are especially vulnerable to socially mediated delayed sleep potentially leading to delayed sleep phase syndrome in vulnerable individuals (Adan et al., 2012; Kudiella et al., 2006; Lehnkering and Siegmund, 2007). In addition, sleep physiology, circadian rhythms and their interactions with cognition change markedly with healthy aging (Pace-Schott and Spencer, 2011) and aging is associated with decreased aversive conditioning (Labar et al., 2004) and with decreased extinction in the rat (Kaczorowski et al., 2012). Extending these and other sleep-related findings to extinction learning and recall in the anxiety disorders constitutes the focus of our ongoing and future research.

Third, sleep efficiency and sleep midpoint are inter-correlated and their effects may reflect a shared factor. For example, in actigraphy data, later sleep midpoint predicted poorer Sleep-Efficiency ($r=0.26$, $p=0.015$). Future analyses of larger samples using structural equation modeling and similar techniques may help disambiguate these factors. Ambulatory polysomnography on the night preceding Session 1 as well as between sessions would yield important additional predictors such as sleep-stage percentages (Pace-Schott et al., 2014; Spoormaker et al., 2012) that are potentially more powerful than actigraphy measures.

Fourth, actigraph and diary measures were obtained from both weeknights and free (i.e., weekend) nights. Because the latest bedtime was specified by instruction, sleep on such free nights would have been less indicative of subjects' unrestricted sleep scheduling.

Fifth, the lower SCRd to the first two CS+E presented during

Extinction Learning in the morning versus evening requires further investigation. Processes that differ with time-of-day during the preceding Fear Conditioning phase (e.g., habituation) are one explanation (Pace-Schott et al., 2013). However there was a complete lack of Time-of-Day, Sleep-Timing or Morningness/Eveningness effects across trials during the Fear Conditioning phase (Supplementary results). Therefore, Time-of-Day, Morningness/Eveningness and Sleep-Timing effects on initial Extinction Learning phase trials might have acted immediately after Fear Conditioning occurred and before the Extinction Learning phase began (e.g., differential ultra-short term fear retention during the brief inter-phase interval), or may involve a differential response to the change from the conditioning to the extinction context. Notably, such effects could go on to influence subsequent extinction learning and recall.

Sixth, although data were drawn from an initially large sample ($N=109$), as smaller samples of dichotomized variables (e.g., actigraphy) and interactions of other between- (Time-of-Day) and within-subjects (CS+Type) variables were decomposed, sample sizes sometimes shrunk to levels where Type 1 error became more likely and reduced power similarly increased the likelihood of Type 2 error. This was especially the case for analyses of actigraphy at Extinction Recall when sample size was reduced due to elimination of both participants with poor quality actigraph data and those for whom extinction learning and recall took place at different times of day (12-h delay). However, this latter limitation was partially addressed by including the 12-h delay participants which both increased the sample size and yielded the same results (see Supplementary results).

4.2. Clinical relevance

In exposure-based treatments, it is also important that extinction memories generalize such that they continue to oppose fearful symptoms outside of the therapy context (Craske et al., 2008). A subset of individuals with greater eveningness and/or poor sleep may be particularly vulnerable to poor extinction recall and generalization. If receiving exposure therapy for an anxiety disorder, such individuals may derive less benefit from a given amount of exposure than others. Therefore, titration of the treatment based upon habitual sleep may improve overall outcome. Findings continue to argue for the importance of sleep hygiene and treatment of sleep disorders in the prevention and treatment of anxiety disorders.

Conflict of interest

None of the authors report any conflict of interest.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psychres.2015.05.069](https://doi.org/10.1016/j.psychres.2015.05.069).

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