The effect of chronotype on emotional memory, sustained attention and stress response. Polymorphisms of the PER3 gene are associated with extreme circadian rhythm sleep disorders: Advanced Sleep Phase Syndrome (ASPS) and Delayed Sleep Phase Syndrome. PER3 polymorphisms result in either shortened circadian rhythms (as exhibited in ASPS) or extended circadian rhythms (as associated with DSPS) and affect an individual’s preferred time of day to function, or chronotype. It has been demonstrated that psychological functioning and stress response vary among these different circadian rhythms.
ABSTRACT

Polymorphisms of the PER3 gene are associated with extreme circadian rhythm sleep disorders: Advanced Sleep Phase Syndrome (ASPS) and Delayed Sleep Phase Syndrome. PER3 polymorphisms result in either shortened circadian rhythms (as exhibited in ASPS) or extended circadian rhythms (as associated with DSPS) and affect an individual’s preferred time of day to function, or chronotype. It has been demonstrated that psychological functioning and stress response vary among these different circadian rhythm sleep disorders. The present study examined the extent to which chronotypes and polymorphisms associated with extreme circadian rhythm sleep disorders affect the interaction between stress and performance on an emotional memory and sustained attention task during the morning versus evening. Our results suggest that sleep quality has the greatest effect on psychosocial variables (i.e. depressive symptoms, total mood disturbances, confusion, fatigue, and vigor), correct as well as false recognition of emotional stimuli, and response inhibition. Sleep duration affects false recognition of negative stimuli and attention. Furthermore, time of day effects were only present for a component of an attention task, suggesting that time of day seems to specifically have an effect on a non-emotional task versus an emotional one. Combined, these data suggest that sleep quality’s effects of psychological functioning may affect sensitivity to negative stimuli. In addition, inadequate sleep results in decreased attention performance demonstrated through a deficit in response inhibition and cognitive slowing.
THE EFFECT OF CHRONOTYPE ON EMOTIONAL MEMORY, SUSTAINED ATTENTION, AND STRESS RESPONSE

The Effect of Chronotype on Emotional Memory, Sustained Attention, and Stress Response

Master of Science in Experimental Psychology

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABSTRACT .........................................................1</td>
</tr>
<tr>
<td>2</td>
<td>INTRODUCTION ....................................................2</td>
</tr>
<tr>
<td>3</td>
<td>METHODS ..........................................................7</td>
</tr>
<tr>
<td></td>
<td>Participants .....................................................7</td>
</tr>
<tr>
<td></td>
<td>Materials .......................................................7</td>
</tr>
<tr>
<td></td>
<td>Procedure .......................................................12</td>
</tr>
<tr>
<td>4</td>
<td>RESULTS ..........................................................14</td>
</tr>
<tr>
<td>5</td>
<td>DISCUSSION .......................................................23</td>
</tr>
<tr>
<td>6</td>
<td>CONCLUSION ......................................................28</td>
</tr>
<tr>
<td>7</td>
<td>REFERENCES .......................................................32</td>
</tr>
<tr>
<td>8</td>
<td>TABLES ...........................................................36</td>
</tr>
<tr>
<td>9</td>
<td>FIGURES ..........................................................38</td>
</tr>
</tbody>
</table>
Abstract

Polymorphisms of the PER3 gene are associated with extreme circadian rhythm sleep disorders: Advanced Sleep Phase Syndrome (ASPS) and Delayed Sleep Phase Syndrome. PER3 polymorphisms result in either shortened circadian rhythms (as exhibited in ASPS) or extended circadian rhythms (as associated with DSPS) and affect an individual’s preferred time of day to function, or chronotype. It has been demonstrated that psychological functioning and stress response vary among these different circadian rhythm sleep disorders. The present study examined the extent to which chronotypes and polymorphisms associated with extreme circadian rhythm sleep disorders affect the interaction between stress and performance on an emotional memory and sustained attention task during the morning versus evening. Our results suggest that sleep quality has the greatest effect on psychosocial variables (i.e. depressive symptoms, total mood disturbances, confusion, fatigue, and vigor), correct as well as false recognition of emotional stimuli, and response inhibition. Sleep duration affects false recognition of negative stimuli and attention. Furthermore, time of day effects were only present for a component of an attention task, suggesting that time of day seems to specifically have an effect on a non-emotional task versus an emotional one. Combined, these data suggest that sleep quality’s effects of psychological functioning may affect sensitivity to negative stimuli. In addition, inadequate sleep results in decreased attention performance demonstrated through a deficit in response inhibition and cognitive slowing.
**Introduction**

Sleep is regulated by the circadian rhythm, an endogenous day-night biological cycle that operates on an approximately 24 hour rotation for humans. Alterations in the circadian rhythm can be attributed to environmental or internal factors and result in circadian rhythm disorders. Environmental circadian rhythm disorders are triggered by changes in the environment such as jet lag or shift work. Internal circadian rhythm sleep disorders are attributed to gene expression in the suprachiasmatic nucleus (SCN) of the hypothalamus. Alterations in an individual’s circadian rhythm affect his/her preference for times of day to function, or chronotype. A very early or late preference for sleep and wakeful periods in relation to conventional sleep/wake time preferences are classified as extreme sleep chronotypes or circadian rhythm sleep disorders. For example, Delayed Sleep Phase Syndrome (DSPS) is characterized as an extreme evening chronotype. Individuals diagnosed with this circadian rhythm sleep disorder prefer very late sleep times and very late wake times. In contrast, Advanced Sleep Phase Syndrome (ASPS) is referred to as an extreme morning chronotype and is characterized by a preference for very early sleep and wake times. Diagnoses of these circadian rhythm sleep disorders are obtained via genetic analysis and self-report measures. Genetic analysis is used to indicate the polymorphisms in specific sleep genes associated with these circadian rhythm sleep disorders while self-report measures are used to analyze sleep quality and disturbances.

**Delayed Sleep Phase Syndrome (DSPS)**

Delayed Sleep Phase Syndrome is the most common circadian rhythm sleep disorder (CRSD) and is associated with an extended circadian rhythm wherein sleep-
wake cycles are delayed (Okawa & Uchiyama, 2007). Individuals diagnosed with this CRSD are referred to as “night owls” (Mahowald & Schenck, 2005). Preferred sleep hours for “night owls” are usually after midnight while the preferred wake times are in the late morning or early afternoon. Preference for a delayed time of day to function conflicts with conventional social and occupational schedules. Thus, individuals with DSPS have difficulties functioning and adjusting to the daily demands of typical school and work schedules. They also usually exhibit physical and psychological symptoms such as loss of appetite, headaches, loss of concentration and depressed mood (Okawa & Uchiyama, 2007).

**Advanced Sleep Phase Syndrome (ASPS)**

Advanced Sleep Phase Syndrome is characterized by a shortened circadian rhythm in which sleep-wake cycles are usually two or more hours earlier than conventional societal times. Individuals with this CRSD are known as “larks.” Larks, unlike owls, typically do not have difficulties functioning and adjusting to early school and work schedules since these conventional schedules are within their preferred time window. However, since they prefer to go to bed at early evening hours, they usually miss opportunities for nocturnal social interaction. If they do choose to resist sleeping at their preferred time in order to partake in night activities, they exhibit symptoms associated with sleep deprivation (Mahowald & Schenck, 2005).

**Polymorphisms Associated with CRSD**

The circadian rhythm is partly regulated by genes such as \( Per1/2/3, \) **CLOCK**, **Cry** \( \frac{1}{2}, \) **CK1** and **BMALL** (Dunlap, 1999). DNA sequence variations, or polymorphisms, in
these particular genes can alter the circadian rhythm and result in extreme sleep chronotypes (Robilliard, et al., 2002).

Polymorphisms on the PER3 gene are linked with both Advanced Sleep Phase Syndrome and Delayed Sleep Phase Syndrome. The longer allele on the PER3 polymorphism as well as homozygosity for the five repeat allele \((PER3^{5/5})\) of a variable number tandem repeat polymorphism in PER3 is associated with ASPS. The shorter allele and a homozygosity for the four repeat allele \((PER3^{4/4})\) is linked to DSPS (Dijk & Archer 2010).

**Extreme Chronotypes and Psychological Factors**

Differences exist between chronotypes in emotion processing and mood disturbances. For example, Dagan and Einstein (1999) found a high prevalence of learning (19.3%) and personality disorders (22.4%) in individuals with DSPS. Shirayama and colleagues (2003) also found that these individuals commonly exhibit negative psychological problems such as nervousness and depression. In addition, studies have found that DSPS individuals display less emotional control, coping, volition, and caution, and more affective instability and externalization (Ottoni, Antoniolli & Lara, 2012). For individuals with ASPS, the prevalence of depressive disorders is greater (Xu, et al., 2005). NSS individuals, in contrast, exhibit a more optimal psychological well-being. Personality tests indicate that they are extroverted, aggressive, ambitious and less anxious (Monk, et al., 2001). Thus, for the purposes of this study, psychological well-being, as assessed by self-report measures, is a variable of interest to determine the relationship between psychological well-being, extreme sleep chronotypes and genes. Differences in psychological well-being and emotion processing may also influence emotional memory
consolidation. Thus, also for the purposes of this study, memory retention for emotionally arousing stimuli versus neutral stimuli is of interest. Furthermore, this study will examine the differences in the perception of emotional valence among chronotypes. In addition, the psychological problems exhibited in individuals with DSPS might be a result of the societal demands of early work and school hours which are inconsistent with these individuals’ chronotypes. Thus, this study will additionally explore if negative emotion processing is still exhibited among these individuals if they perform an emotional memory task during a time of day consistent with their chronotypes.

**Extreme Chronotypes and Stress Response**

Stress response is also a variable to be considered among chronotypes in this study. It is clear that changes to the stress system, or hypothalamic pituitary adrenal (HPA) axis can greatly affect sleep. There is a strong, bi-directional communication between sleep and HPA axis functioning wherein changes in glucocorticoid levels can affect sleep and changes in sleep can affect glucocorticoid levels. For example, slow-wave sleep (SWS) has an inhibitory influence on cortisol secretion in humans (Weitzman, Zimmerman, Czeisler, & Ronda, 1983), and conversely, intracerebroventricular administration of the major glucocorticoid secretagogue, corticotrophin-releasing hormone (CRH) (Opp, 1989) (Opp, Obal, & Krueger, 1995). DSPS has been associated with lower sleep quality and higher stress response than ASPS individuals. In addition, these individuals report higher self-perceived stress after a stress task than ASPS individuals (Roeser, Muele, Schwerdtle, Kubler & Schlarb, 2012).

The hormones of the HPA axis influence memory in situations of acute and chronic stress. Glucocorticoids (GC) are released during acute and chronic stress. During
chronic stress, GCs impair memory. In contrast, GCs facilitate memory consolidation during acute stressors. However, if the acute stressor is unrelated to the cognitive task, memory performance has been shown to be impaired (Wolf, 2003). For example, the acute stress one feels before one takes an exam is a stressor related to the task/exam. An example of an acute stressor unrelated to the task/exam would be the cold pressor test. Thus, this study will examine the effects of an unrelated acute stress condition (cold pressor test) on self-perceived stress response and emotional memory task performance among different chronotypes.

**Time of Day**

An individual forced to function at a time of the day incompatible with his/her preferred hours of functioning is likely to report sleepiness. Sleepiness has been shown to result in impairments in cognitive performance and reductions in alertness. Consequently, this study will also examine the relationship between preferred time of day and performance for an emotional memory and sustained attention task either in the morning or the evening.

**Current Study**

The current study had two main aims. The first aim was to assess the effect of chronotype versus genotype on psychosocial and sleep functioning. The second aim was to determine the effect of chronotype on an emotional and non-emotional task at congruous and incongruous times of day. It is hypothesized that greater psychosocial and sleep dysfunction would be expected for the participants who self-reported as evening chronotypes as well as those who have genetic marker for eveningness. It is also hypothesized that individuals who perform both the emotional memory task and the
sustained attention task at a time of day consistent with his/her chronotype will perform better than those who are completing the tasks at a time of day inconsistent with his/her chronotype. Groups in the stress condition administered an acute stressor (cold pressor test) prior to performing the cognitive tasks are expected to have impaired performance compared to the non-stress condition administered the control pressor test. Differences are predicted to exist in how each chronotype is affected by the acute stressor. Individuals are expected to be more impaired in cognitive task performance if the stressor and the tasks are both administered at a time of day inconsistent with their chronotypes. Differences in emotional valence processing, emotional memory task performance and reactions to the stress condition are expected to be exhibited among the different chronotypes.

Methods

Participants

A sample of 127 undergraduate students from Nova Southeastern University were recruited and given either two hours of research credit for an introductory psychology course or a $20 Target gift card as compensation for completing this study.

Materials

Cold-Pressor Test

The cold-pressor test (CPT) is a widely used and well-validated approach of inducing general stress through submerging one’s non-dominant hand in a bucket of ice-cold water (at most 5°C) for at least one minute (Lovallo, 1975) (Hines & Brown, 1932) (Loyke, 1995). This test is commonly used to determine the effect of stress on memory
(Buchanan, Tranel & Adolphs, 2006) (Duncko, Johnson, Merikangas & Grillon, 2009) (Cahill, Gorski & Le, 2003).

**Alpha Amylase**

Saliva samples were collected three times, throughout various points in the experiment and later analyzed with an ELISA to determine alpha amylase levels. Alpha-Amylase is a digestive enzyme that is produced in the salivary glands and increases in response to psychological and physical stress through interactions with the autonomic nervous system (Salimetrics, 2014). The first saliva sample was collected at least five minutes after the participant arrived for the study. The delay in collecting the first sample was to ensure an accurate baseline saliva sample. The second saliva sample was collected about 10 minutes after the stressor, This second sample was used to determine the stress response by later analyzing the alpha amylase activity from the baseline sample at time one to the second sample after the stressor. The third saliva sample was collected about 35 minutes after the stressor and 25 minutes after the second saliva sample. Due to a collection error, the saliva samples were not analyzed.

**Morningness-Eveningness Questionnaire**

The Morningness-Eveningness questionnaire (MEQ) is a widely administered self-report measure composed of 19 items used to determine if one’s peak sleepiness and alertness is in the morning or in the evening (Horne & Ostberg, 1976). This questionnaire has high internal consistency. The score is calculated using the summation of the items, and then determining chronotype using a range of scores from 16 to 86. Evening types are indicated with a score of 41 or lower. Intermediate types are classified with a score between 42 and 58. Morning types are signified with a score of 59 or higher.
Visual Analog Scale

After completing either the cold or control pressor test, the participants completed a four-item Visual Analog Scale (VAS) to determine if the participants perceived the task as a threat or a challenge.

International Affective Picture System

The International Affective Picture System (IAPS) is a collection of positive, negative, and neutral pictures with standardized valences and ratings for each image (Lang, Bradley & Cuthbert, 2008). For this study, a total of 120 negative stimuli were selected from the IAPS for use in the exposure and test sessions. Only negative pictures with a valence of about 3 or less were chosen as the negative stimuli for this study. The average valence rating for all 120 negative stimuli used in this study was 2.36. The 120 neutral pictures chosen for this study to represent neutral stimuli were selected in accordance to a valence rating of between 4 and 6. The average valence for all 120 neutral pictures chosen was 5.20.

Sustained Attention to Response Task

The Sustained Attention to Response Task (SART) was used to examine sustained attention. The SART measure provides several outcome variables: target accuracy, non-target accuracy, target reaction time, and non-target reaction time (Robertson, Manly, Andrade, Baddeley & Yiend, 1997). During the task, participants were instructed to press the spacebar whenever they saw a digit on the screen. They were also informed to refrain from pressing the response key whenever they saw the number, “3.” The target is this task is the number, “3.” The non-target refers to any of the other numbers, “1-9.” Target accuracy refers to the total number of times that the participant
refrained from hitting the spacebar upon seeing the number, “3” on the screen. Non-target accuracy refers to the number of times that the participant pressed the spacebar in response to any number between 1 and 9, excluding the number, “3.” Target reaction time refers to the time in milliseconds in which the participant incorrectly responded to the target when he/she should not have. Non-target reaction time refers to the time in milliseconds in which the participant did not respond to the non-target when he/she should have.

**The Pittsburgh Sleep Quality Index**

The Pittsburgh Sleep Quality Index (PSQI) is a reliable self-report measure used to assess sleep quality and disturbances over a one month time interval. There are 19 items which indicate seven component scores used to relay subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, the use of sleep medications, and daytime dysfunction. The summation of these seven components yield a global score ranging from 0 to 21 in which a global score of above five is used to differentiate between those with poor sleep versus those with good sleep (Buysse, Reynolds, Monk, Berman & Kupfer, 1989). This questionnaire was administered after all of the cognitive tasks.

**Profile of Mood States**

The Profile of Mood States (POMS) questionnaire is a reliable self-report measure, with internal consistency, used to determine transient mood states. The full length version used in this study consists of 65 adjectives rated by participants on a 5-point Likert scale. The 65 items are used to yield six subscales: Anger-Hostility, Confusion-Bewilderment, Depression-Dejection, Fatigue-Inertia, Tension-Anxiety and
Vigor-Activity. A seventh sub scale referred to as the friendliness scale is scored separately (McNair, Lorr, & Droppelman, 2013). This scale was not used for this study since it has poor psychometric properties.

Center for Epidemiologic Studies Depression Scale

The Center for Epidemiologic Studies Depression Scale (CES-D) is a short self-report measure, with internal consistency, used to determine depressive symptoms. Across a variety of demographic characteristics in the general population, this scale has shown to be reliable and valid. (Radloff, 1977). This measure consists of 20 items asking questions about the frequency of symptoms associated with depression in the past week. A score of less than 15 points is categorized as no depressive symptoms. Scores of 15-21 represent mild to moderate depressive symptoms, and scores of over 21 indicate a possibility of major depression.

State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) is a self-report measure, with internal consistency, used to assess state and trait anxiety. State anxiety refers to current anxiety about an event. Trait anxiety indicates an overall anxiety level as a personal characteristic. This inventory is composed of 40 questions associated with symptoms of anxiety which are rated by participants on a four point Likert scale. The scores are positively correlated with anxiety levels (Tilton, S., 2008).

Genetic Analysis

Two buccal swabs were collected from each participant and later assessed using DNA extraction, Polymerase chain reaction (PCR) and Electrophoresis to determine if the participants have the 4/4 homozygous repeat allele (indicative of DSPS), the 4/5
heterozygous allele (signifying an intermediate type with no strong preference for extreme morning or extreme evening), or the 5/5 homozygous allele (related to ASPS). DNA extraction is a molecular biology technique used to purify DNA from cells through the disruption of the cells and removal of membrane lipids, proteins, and RNA. PCR then amplifies a single copy of a DNA piece. Prior to PCR, DNA is mixed with a solution of primers and DNA polymerase. This solution enables specific and repeated amplification of the DNA. This technique utilizes thermal cycling which repeatedly heats and cools the samples. Electrophoresis is the final technique used to classify the DNA fragments. This method uses a constant electric field which causes the DNA fragments to migrate across a buffer solution. The final length of the fragments is compared to a standard ladder (Genetic Science Learning Center, 2014).

Procedure

Participants were instructed to correlate the Morningness-Eveningness Questionnaire (MEQ) online prior to coming in for the study. After, each participant’s MEQ was scored to determine his/her chronotype. Each participant was then pseudo randomly assigned to a morning/evening time slot and a stress/non stress condition. If the participants were completing the study for class credit, they were given a password to sign up for their designated time slots on SONA. If the participants were completing the study for the gift card, communication took place via email in which they were given a time condition to come in, and they confirmed which day they could come in at that time.

Stress Condition

Participants were randomly assigned to this condition to complete this experiment at either 9am or 9pm. Upon arriving to the study, all participants provided written
informed consent. The first saliva sample was then collected to determine baseline alpha amylase levels. Next, the participants completed the cold pressor test. In this test, the participant submerged his/her non-dominant hand in a bucket of ice cold water (5°C) for one minute. They then completed the Visual Analog Scale (VAS) questionnaire.

Next, participants were shown a series of 120 picture stimuli (60 emotionally negative and 60 neutral) from the International Affective Picture System (IAPS) in randomized order without knowing that they will later be tested on their memory for those pictures. By rating the pictures in the IAPS, we were able to determine if there were differences among chronotype, stress, and time of day in how the pictures were perceived. During the picture viewing, they were instructed to rate on a scale of 1-10 how positive, negative or neutral they perceived the pictures to be. A rating of 1 was “very negative,” 5 was “neutral,” and 10 was “very positive.” The task of rating the pictures generally lasted for about five to seven minutes per participant. Rating the pictures helped to ensure that the participants were actually paying attention to the pictures on the screen. Another saliva sample was collected after this task.

Participants then completed the Sustained Attention to Response Task (SART) in order for us to assess sustained attention. This task lasted approximately 22 minutes. The duration of the task served as a delay period from the time participants were exposed to the picture stimuli to the time they were tested on their memory for the pictures in the test session. Following this task, a final saliva sample was collected. Participants were then presented the original 120 picture stimuli in addition to 120 new picture stimuli (60 new negative and 60 new neutral stimuli) in randomized order. A response screen instructed the participants to indicate (by pressing the “1” key corresponding to “yes” and the “2”
key corresponding to “no” on the keyboard) whether each picture shown was either old (one from the previous exposure session) or novel. Finally, participants provided responses to the demographic questionnaire, Profile of Mood States (POMS) questionnaire, the Center for Epidemiologic Studies Depression Scale (CES-D), State-Trait Anxiety Inventory (STAI), and the Pittsburgh Sleep Quality Inventory (PSQI). Cheek cells were collected and the participants were debriefed.

**Non stress condition**

Participants were randomly assigned to this condition to complete this experiment at either 9am or 9pm. The procedure for the non-stress condition was the same as described for the stress condition. However, participants completed the control pressor test for one minute with lukewarm water instead of ice cold water. The temperature of the water was about 25°C. The aforementioned procedure was implemented thereafter.

**Results**

**MEQ and PER3**

A Chi Square was conducted to correlate MEQ type (morning, intermediate, evening) with PER3 polymorphism type (5/5, 4/5, 4/4). Significance was not reached $\chi^2(4, N = 123) = 4.06, p > .05$. However, the distribution of the observed counts was as expected for a college aged population. There were more heterozygous intermediates (4/5) self-reporting as morning types (52.9%) and as intermediate types (55.7%). There were more homozygous evening types (4/4) self-reporting as evening types (52.8% as seen in Fig.1). To explain these results, it is important to note that chronotype varies with age. Specifically, college-aged individuals tend to over report being evening chronotypes
(Crowley et al., 2007; Roenneberg et al., 2007). Thus, a college-aged sample is not representative of all ages.

Stress Measure

A stress measure manipulation check was done by looking at the effect of the stress condition on the VAS. A one way ANOVA was conducted to determine the effect of stress on the total VAS. A significant effect of stress was found on the total score for this measure $F(1, 125) = 40.5, p < .000, \eta^2 = .25$. The stress condition reported feeling more stressed compared to the non-stress condition as seen in Table 1. A one way ANOVA was conducted to determine the effect of stress on the first VAS question used to assess if the participants perceived the stressor as stressful. The stress condition rated the stressor as significantly more stressful than the non-stress condition, $F(1, 125) = 136.4, p < .000, \eta^2 = .52$; as seen in Table 1). Finally, a one way ANOVA was conducted to determine the effect of stress on the second VAS question used to assess if the participants perceived the stressor as a challenge. A significant effect of stress was found on this measure ($F(1, 124) = 201.8, p < .000, \eta^2 = .62$). Those in the stress condition perceived the stressor to be a challenge relative to the non-stress condition as seen in Table 1.

AIM 1: MEQ and Psychosocial Functioning

Four separate ANOVA’s were conducted to determine the effect of MEQ on the CES-D, POMS, STAY and TAY. No effect of MEQ was found on these psychosocial questionnaires, all $p$’s > .05.
MEQ and Sleep Quality

A Chi square was conducted to compare sleep quality (good versus poor sleep quality) and MEQ type (morning, intermediate or evening chronotype). To assess this, the sleep quality subscale on the PSQI was first made categorical. This subscale assesses sleep quality using four different options: very good, good, bad, and very bad. The choices, “very good” and “good” were collapsed into an overall categorization of good sleep quality. The remaining selections, “bad” and “very bad” were grouped together to create an overall classification of poor sleep quality. A significant Chi square was found, \( \chi^2 (2, N = 127) = 6.76, p < .05 \). More morning types (83.3%) reported good sleep quality than intermediate types (75.7%) and evening types (55.3%) respectively as seen in Fig. 2.

PSQI and Psychosocial Functioning

From the Chi square findings comparing MEQ and sleep quality, a follow up analysis was conducted to determine if sleep quality was a predictor of psychosocial functioning. Linear regressions were conducted to determine if the PSQI total score was a predictor of different psychosocial variables. The PSQI total score is a combination of seven subscales; sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, the use of sleep medication, and daytime dysfunction. A total score of five or higher indicates poor sleep quality. Linear regressions found the PSQI total score to be a significant predictor of greater depressive symptoms as measured by the CES-D and POMS. The total PSQI score was also a significant predictor of greater trait anxiety as measured by the TAY and fatigue as measured by the POMS, as seen in Table 2.
AIM 2: MEQ, Time of Day and Stress on Emotional Memory Recognition

A 3 X 2 X 2 factorial ANOVA was conducted to determine the effect of MEQ (Moderate morning, intermediate, or moderate evening), stress condition (stressor or control), and time tested (9am or 9pm) on the percentage of negative hits for the IAPS. No main effects or interactions were found, all p’s > .05.

MEQ, Time of Day, and Stress on Emotional Rating

Two separate 3 X 2 X 2 factorial ANOVAs were conducted to determine the effect of MEQ (morning, intermediate, or evening chronotypes), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the average rating for neutral stimuli as well as negative stimuli. No significant main effects or interactions were found for the average rating of neutral or negative stimuli, all p’s > .05.

MEQ and Stress Response

Three 3 X 2 X 2 factorial ANOVAs were conducted to determine the effect of MEQ (morning, intermediate, or evening chronotype), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the total VAS score in addition to the two subscales measuring the perception of the stressor as a threat or a challenge. No interactions were found for MEQ, stress, and time of day. A main effect of stress was found on the total VAS score \( (F(1, 115) = 32.68, p < .000, \eta^2 = .22) \), the VAS stress question \( (F(1, 115) = 91.21, p < .000, \eta^2 = .44) \), and the VAS challenge question \( (F(1, 114) = 142.58, p < .000, \eta^2 = .56) \). A main effect of MEQ was also found on the VAS stress question. However, this effect appears to be noise since there would have to be an interaction between MEQ and the stress condition to show that chronotypes responded differently to the stressor.
MEQ, Time of Day, and Stress on the SART

Four separate 3 X 2 X 2 factorial ANOVAs were conducted to determine the effect of MEQ (morning, intermediate, or evening chronotype), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the SART measures: non-target accuracy, non-target reaction time, target accuracy, and target reaction time. No significant main effects or interactions were found, all \( p \)'s > .05.

Time Tested on the SART

Four separate one-way ANOVAs were conducted to assess the effect of time of day (9am or 9pm) on each of the four SART measures: non-target accuracy, non-target reaction time, target accuracy and target reaction time. Significance was reached for non-target accuracy \( F(1, 123) = 4.09, p < .05, \eta^2 = .03 \) in which participants were overall more accurate when pressing the spacebar in response to numbers (1 through 9, except 3) in the evening than in the morning, as seen in Fig. 4. No effect of time of day was found on the other three SART measures, all \( p \)'s > .05.

PER3, Time of Day, and Stress on Emotional Memory Recognition

Since only nine participants had the 5/5 homozygous polymorphism on the PER3 gene, the participants with the 5/5 allele and the 4/5 allele were collapsed into one group in order to categorize those participants as individuals with a non-evening genotype. A 2 X 2 X 2 factorial ANOVA was conducted to determine the effect of PER3 (non-evening genotypes vs. evening genotypes), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the percentage of total hits and total false alarms, percentage of negative test hits and negative false alarms, and the percentage of neutral hits and neutral false alarms. No significant main effects or interactions were found, all \( p \)'s > .05.
**PER3, Time of Day, and Stress on Emotional Rating**

Two separate 2 X 2 X 2 factorial ANOVAs were conducted to determine the effect of PER3 (non-evening genotypes vs. evening genotypes), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the average rating for neutral stimuli as well as negative stimuli. No interactions were found for the average rating of neutral or negative stimuli. A main effect of time tested was found on the average rating of the neutral stimuli, $F(1, 115) = 4.45, p < .05, \eta^2 = .04$. Participants in the 9pm condition rated the neutral stimuli as more positive ($M = 6.29, SE = .13$) than participants in the 9am condition ($M = 5.91, SE = .13$).

**PER3 and Stress Response**

Three 2 X 2 X 2 factorial ANOVAs were conducted to determine the effect of PER3 (non-evening genotypes vs. evening genotypes), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the total VAS score in addition to the two subscales measuring the perception of the stressor as a threat or a challenge. No significant interactions were found. A main effect of stress was found on the total VAS score ($F(1, 119) = 39.76, p < .000, \eta^2 = .25$), the VAS stress question ($F(1, 119) = 129.16, p < .000, \eta^2 = .52$), and the VAS challenge question ($F(1, 118) = 202.29, p < .000, \eta^2 = .63$).

**PER3, Time of Day, and Stress on the SART**

Four 2 X 2 X 2 factorial ANOVAs were conducted to determine the effect of PER3 (non-evening genotypes vs. evening genotypes), stress condition (stress vs. no stress), and time tested (9am vs. 9pm) on the SART measures: non-target accuracy, non-target reaction time, target accuracy, and target reaction time. A significant effect of time
tested was found on non-target accuracy \((F(1, 113) = 4.05, p < .05, \eta^2 = .04)\) in which participants were more accurate when pressing the spacebar in response to numbers (1 through 9, except 3) in the evening than in the morning, as seen in Fig. 4. No significant interactions or other main effects were found, all \(p\)’s > .05.

*Follow up Analyses: Sleep Quality and Emotional Memory*

The hypotheses pertaining to the second aim of the study were generally unsupported. However, since it was found that sleep quality was an important predictor of psychosocial functioning, follow up analyses were conducted to further explore the effects of sleep quality on the cognitive measures. An ANOVA was conducted to determine the effect of the categorical sleep quality (PSQI) subscale (good vs. poor) on the percentage of correct recognition for the negative pictures. A significant effect was found, \(F(1,125) = 5.87, p < .05, \eta^2 = .05\). Those with poor sleep quality had a higher percentage of correct recognition for the negative pictures relative to those with good sleep quality, as seen in Table 3. This suggests that the individuals with poor sleep quality are more sensitive to the negative stimuli.

An ANOVA was also conducted to determine the effect of sleep quality on total false alarms on the IAPS. A significant main effect \((F(1, 125) = 4.43, p < .05, \eta^2 = .034)\) was found in which those with good sleep quality had less false alarms for the total picture set (negative and neutral pictures) than those with poor sleep quality, as seen in Table 3. Another ANOVA found a significant effect of sleep quality on false alarm rates for the negative pictures \((F(1, 125) = 6.44, p < .05, \eta^2 = .05)\) in which those with poor sleep quality had a higher percentage of false alarms compared to those with good sleep quality, as seen in Table 3. ANOVAs conducted to determine the effect of sleep quality on
the average ratings for the negative stimuli and the average ratings for the neutral stimuli revealed no significant differences \((p’s > .05)\).

**Sleep Quality and the SART**

Four separate ANOVAs were conducted to determine the effect of sleep quality on each of the SART measures. Only the ANOVA assessing sleep quality on target accuracy was significant, \(F(1, 123) = 7.13, p < .01, \eta^2 = .06\). Participants who reported good sleep quality were better able to withhold their response to the target stimuli than those who reported poor sleep quality, as seen in Table 3.

**Sleep Duration and Sleep Quality**

Considering the effects of sleep quality, it was hypothesized that sleep duration might be related to individuals’ subjective sleep quality. To assess this, sleep duration was first split into two categories: those who slept six hours or less and those who slept seven hours or more. A Chi square was then conducted to compare sleep duration \((\leq 6 \text{ hours or } \geq 7 \text{ hours})\) with sleep quality \((\text{good versus poor})\). Significance was reached, \(\chi^2(1, N = 127) = 16.32, p < .000\). Compared to individuals who reported at least seven hours of sleep, a greater percentage of individuals reporting at most six hours of sleep experienced poor sleep quality \((67.6\%)\). A greater percentage of individuals who reported seven hours of sleep or more experienced good sleep quality \((71.1\% \text{ as seen in Fig. 3})\) compared to those who reported sleeping six hours of sleep or less.

**Sleep Duration and Psychosocial Functioning**

Since sleep quality and sleep duration were significantly overlapped in the aforementioned Chi Square, ANCOVA’S were conducted to determine the effect of sleep quality on psychosocial functioning while controlling for the variance of sleep quality.
There was no effect of sleep duration while controlling for sleep quality on the STAI, CES-D and the POMS, all p’s > .05.

**Sleep Duration on Emotional Memory Recognition**

An ANCOVA was conducted to determine the effect of sleep duration on the percentage of false alarms for the negative stimuli while controlling for sleep quality. A significant effect of sleep duration, while controlling for sleep quality, was found \((F(2, 124) = 4.27, p < .05, \eta^2 = .03)\) in which those who reported sleeping six hours or less had a greater percentage of false alarms for the negative stimuli compared to those who reported sleeping seven hours or more, as seen in Table 4. There were no effects of sleep duration on the percentage of total hits and total false alarms, the percentage of negative hits, and the percentage of neutral hits and neutral false alarms while controlling for sleep quality, all p’s > .05.

**Sleep Duration and Emotional Rating**

An ANCOVA was conducted to determine the effect of sleep duration on the emotional ratings of the stimuli while controlling for sleep quality. There was no effect of sleep duration while controlling for sleep quality on the average ratings for negative or neutral stimuli, all p’s > .05.

**Sleep Duration on the SART**

An ANCOVA was conducted to determine the effect of sleep duration on non-target reaction time while controlling for sleep quality. A significant difference was found \((F(2, 122) = 4.11, p < .05, \eta^2 = .03)\) in which those who slept six hours or less had a greater reaction time than those who slept seven hours or more when responding to the non-target numbers (1 through 9 except 3), as seen in Table 4. This means that when
participants who had six hours of sleep or less took longer to press the spacebar in response to the non-target numbers. There was no effect of sleep duration while controlling for sleep quality on target reaction time, target accuracy, or non-target accuracy, all p’s > .05.

**Discussion**

The hypothesis for the first aim of the study was generally not supported. The chronotypes did not differ on the psychosocial variables assessed by the CES-D, POMS, STAY, and TAY. However, chronotypes did show a significant difference on sleep quality. More morning chronotypes reported good sleep quality while more evening chronotypes reported poor sleep quality. A follow-up analysis from this finding showed that sleep quality had an effect on depressive symptoms; participants who experienced poor sleep reported more depressive symptoms (Prather, Bogdan & Hariri, 2013). Also, poor sleep quality was shown to significantly result in more negative mood states: a greater total mood disturbance on the POMS, greater fatigue, and less vigor. Significant linear regressions showed that the total PSQI score of sleep quality predicted trait anxiety as well as depression and fatigue on the POMS subscales.

It was also found that the hypotheses of the second aim of this study were unsupported. There were no effects of chronotype or genotype on emotional memory recognition, emotional ratings of the stimuli, stress response, and sustained attention performance. The manipulation of time of day and stress also did not significantly interact with chronotype or genotype on these measures. However, time of day was found to affect ratings of neutral stimuli. Participants in the 9pm evening condition relative to the 9am morning condition rated the neutral stimuli as more positive. Time of day was
also found to have a main effect on the SART measure of non-target accuracy.

Participants in the evening had a better accuracy when pressing the spacebar in response to certain numbers presented on the screen (1 through 9, except 3). There were no effects of time tested on emotional memory recognition. Thus, these results underscore the specificity of time of day effects on a non-emotional task rather than on an emotional task.

Considering our results showing the effect of sleep quality on various psychosocial variables, follow-up analyses were conducted to explore the effects of sleep quality on the cognitive measures. New to our study, sleep quality was found to affect memory recognition on the IAPS and target accuracy on the SART. Participants with poor sleep quality had a greater percentage of hits and false alarms for negative stimuli, suggesting a response bias to the negative stimuli. Participants who slept six hours or less also had a higher percentage of false alarms for negative stimuli relative to those who slept for seven hours or more.

It has been demonstrated that younger adults generally have a better memory for negative stimuli versus neutral stimuli since negative stimuli are more salient (Kensinger, 2007). The amygdala is integrally involved with remembering negative stimuli (Yoo, Gujar, Hu, Jolesz & Walker, 2007). Specifically, individuals exhibit greater amygdala activation when viewing negative stimuli relative to neutral stimuli, and they show an even greater increase in amygdala activity following sleep deprivation. Yoo and researchers (2007) have found that there is greater connectivity between the amygdala and the autonomic-activating centers of the brainstem (e.g. midbrain and locus coeruleus), in the brains of sleep-deprived individuals, suggesting a hyper-limbic
response generated by the amygdala to negative stimuli for these individuals. Moreover, they have shown that the amplified limbic activity for these sleep deprived individuals was correlated with a decrease of functional connectivity with the medial prefrontal cortex (Mpfc), a brain region involved in appropriate emotional responses through the inhibitory control of amygdala function. This suggests a down-regulation of top-down prefrontal control which can ultimately affect emotional regulation. Their findings greatly overlap with the present study’s findings. For example, in the current study, sleep quality positively correlated with sleep duration in which those who reported poor sleep quality were significantly more likely to report less sleep (six hours or less). This suggests that these individuals with poor subjective sleep quality may not be getting enough sleep. The neurological findings in Yoo and researchers’ study bridges the gap between the self-report data and behavioral data in the present study. Extrapolating from their findings, it is possible that the inadequate sleep experienced by participants in the current study led to diminished functional connectivity between the amygdala and the mPFC. If this were the case, it would follow that these participants would exhibit less emotional regulation. The study at hand did in fact find this result as demonstrated through the greater total mood disturbances, greater trait anxiety, and more depressive symptoms associated with poor sleep quality. In addition, Yoo and researchers’ conclusion that a hyper-limbic amygdalar response is also associated with inadequate sleep suggests another neurological link between the current study’s self-report data and behavioral data. For example, the individuals with poor sleep in this study might be exhibiting a better memory for the negative stimuli as a result of a hyper-limbic response by the amygdala. It is possible that a decreased emotion regulation, combined with an increased sensitivity
to negative stimuli, can account for the significantly greater percentage of negative false alarms for individuals with poor sleep quality. Though individuals with poor sleep quality also had a greater percentage of false alarms for the total stimuli in general, this effect seems to be driven by the negative stimuli rather than the neutral stimuli since there was a significant main effect on false alarms for negative stimuli ($F (1, 125) = 6.44, p < .05, \eta^2 = .05$) and not on false alarms for neutral stimuli.

In the current study, participants with poor sleep quality also had decreased target accuracy in the SART task; they had less accuracy when refraining from pressing the spacebar upon seeing the number “3” on the screen. Researchers, Anderson and Platten (2011) found that sleep deprived individuals demonstrate quicker incorrect responses as well as a failure to inhibit a response to negative stimuli. However, our study’s findings suggest that individuals with inadequate sleep may also have a compromised response inhibition for non-emotional stimuli. Moreover, Chuah and colleagues (2006) found that sleep deprived individuals demonstrate decreased activation in ventral and anterior prefrontal regions, areas implicated in sustained attention tasks. They also found that there are substantial individual differences following a short period of 24 hours of sleep deprivation in which individuals less vulnerable to sleep loss showed less difficulty in recruiting the ventrolateral PFC than those individuals more vulnerable to sleep loss. The influence of individual differences may be involved in the results of the present study that show sleep quality’s effects on sustained attention. For example, though sleep duration significantly positively related to sleep quality, there was a main effect of sleep quality but not sleep duration on response inhibition. This could perhaps suggest that some individuals with a shorter duration of sleep are not as vulnerable to the effects of sleep
loss, and thus, the assessment of sleep quality might be an indicator of individual differences underlying vulnerability following sleep loss. It is thus possible that these individuals with poor sleep quality have a decreased activation of the ventrolateral PFC like the individuals vulnerable to sleep loss in Chuah and colleagues’ study. Since the ventrolateral PFC has been linked with inhibitory control, it would thus make sense why individuals with poor sleep quality showed this cognitive deficit. The mood states, fatigue and vigor, were also found to be significant predictors of response inhibition in which fatigue negatively correlated and vigor positively correlated with accuracy in inhibiting responses to a target stimulus. Since fatigue was significantly negatively correlated and vigor was significantly positively correlated with sleep quality, sleep quality might account for some of the variance relating these mood states to response inhibition. Vigor was also found to be a significant predictor of response accuracy to stimuli in the SART.

It was also found that inadequate sleep duration resulted in a greater reaction time in milliseconds when pressing the spacebar in response to numbers presented on the screen (any number 1 through 9 except 3). It appears that those who slept less experience cognitive slowing. It has been demonstrated that when individuals are fatigued, the time it takes to perform cognitive actions is increased (Bratzke et al., 2007; Dinges & Kribbs, 1991). Since poor sleep quality is related to shorter sleep duration, it is possible that the greater fatigue and less vigor associated with poor sleep lends some overlap with the cognitive slowing effects of insufficient sleep.
Limitations

The present study did not reach the target sample size of 150 which serves as a limitation to the study’s power. Furthermore, the study sought to determine the effect of extreme chronotypes on sustained attention, emotional memory and stress response. However, the sample only consisted of moderate evening, intermediate, and moderate morning types. The study also attempted to correlate genetic markers for extreme chronotypes with psychological and behavioral measures. However, there were not enough individuals with the 5/5 homozygous allele to make a meaningful comparison. The current study also did not collect data on female participants’ menstrual cycles to control for any hormonal influences on any of the measures. Controlling for this measure, however, should not influence the results since previous studies show that this information accounts for little variance on stress response. In addition, due to a labeling error with saliva tubes, a number of participants’ data was not included when comparing alpha amylase levels for stress and non-stress groups. Nevertheless, this action should not have made a difference in demonstrating that our stress manipulation worked since only the participant data collected from the researcher suspected of making an error was excluded. There was still enough data from the other researchers to show a comparison between the stress and non-stress group. In addition, the VAS self-report measure of perceived stress serves as an additional method of determining if the stress manipulation worked.

Conclusion

The present study showed the importance of adequate sleep on psychological functioning, emotional memory, and sustained attention. However, inconsistent with our
hypothesis, time of day (compatibility or incompatibility with one’s chronotype) did not affect stress response or vary the effects of a stressor on an emotional memory or sustained attention task. These findings could be the result of an inability to acquire extreme morning and evening chronotypes. For example, none of the participants were diagnosed with an extreme circadian rhythm disorder, and only two individuals scored as a definite evening chronotype on the MEQ. The rest of the participants reported as moderate morning, intermediate or moderate evening chronotypes. Perhaps individuals without a preference for extreme morning or extreme evening times are not as affected by time of day influences as an extreme chronotype population might be. Future research should thus explore the effects of time of day on stress response, emotional sensitivity and sustained attention on a population of individuals diagnosed with extreme circadian rhythm disorders. In addition, the study did not find any effects of stress on the cognitive measures. This could be due to the type of stressor that we used (cold-pressor test). It is possible that any effects of stress may have been too transient with the cold pressor test. Perhaps, a psychological stressor may have lingering effects on the participants which could subsequently affect their performance. Furthermore, the possible lingering effects of psychological stress may differentially affect performance on an emotional versus non-emotional task. Future research could explore this avenue of the effects of a physical versus psychological stressor on an emotional and non-emotional task.

Our study also did not find any effects of the polymorphisms of the PER3 gene with psychosocial functioning, emotional memory and sustained attention. There were no influences of time of day (compatibility or incompatibility with these markers for extreme morning or evening preference) on the effects of a stressor on emotional memory
and sustained attention. These results could be due to the loss in power attributed to a very small number of participants with the 5/5 homozygous polymorphism indicating morning preference. In addition, the sample was drawn from a college population which is not representative of the general population since younger adults tend to self-report as being more of an evening type. The influences of these genetic markers seem to appear as one ages. Thus, even if younger adults have a genetic marker for a non-evening type, they typically still prefer an evening type during this younger time period.

While both chronotype and genetic markers for extreme chronotypes did not affect time of day influences on stress response, emotional memory, and sustained attention for this normal college population, the effect of sleep quality and sleep duration demonstrated to be very important. Also, time of day only seems to matter for this population for a non-emotional attention task. Individuals who reported sleeping a shorter duration were significantly likely to report poor sleep quality. These individuals with poor sleep quality were more likely to experience depressive symptoms, confusion, fatigue, trait anxiety, and less vigor. Poor sleep quality also resulted in a greater sensitivity to negative stimuli (interpreted as such from the greater percentage of negative hits and false alarms) and a deficit in response inhibition performance. Although there was significant overlap with sleep duration and sleep quality, all of these effects, aside from false alarms for negative stimuli, were not seen for sleep duration. This could be explained by individual differences. For example, even though less sleep is correlated with poor sleep quality, not everyone is vulnerable to these effects of sleep loss. Thus, one’s subjective assessment of sleep quality might be the better indicator of these psychological effects than the sleep loss itself. Sleep duration did, however, have an
effect on reaction time (ms) when responding to numbers on the screen. Individuals with less sleep showed a form of cognitive slowing by taking longer to make errors when responding to a target stimulus. Sleep quality did not have a main effect on this measure. However, sleep duration only showed this effect when controlling for sleep quality, suggesting that the effect of sleep duration on this variable is demonstrated when accounting for the variance of sleep quality.

The data combined suggest the importance of sleep quality on psychological functioning, emotion sensitivity and sustained attention for a normal college population. Future research should explore these measures for individuals diagnosed with extreme circadian rhythm disorders and sampled from the general population. Furthermore, neuroimaging should be incorporated to assess if these effects of sleep quality are attributed to decreased activation of different areas in the brain implicated in emotional regulation, response inhibition, and sustained attention.
References


Tilton, S. (2008). Review of the State-Trait Anxiety Inventory (STAI), News Notes, archived from the original on 2008-12-03.


### Table 1

*The Effects of Stress*

<table>
<thead>
<tr>
<th>Dependent Var.</th>
<th>Stress</th>
<th>Nonstress</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Means</td>
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<tr>
<td>VAS Total</td>
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<td>VAS Challenge</td>
<td>3.74</td>
<td>1.18</td>
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Note. SD = Standard Deviation. n = Number of Participants. VAS = Visual Analog Scale
Table 2  
*Linear Regression of PSOI Total Score*

<table>
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<tr>
<th>Variable</th>
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<th>SE</th>
<th>$\beta$</th>
<th>t</th>
<th>Sig.</th>
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<td>CES-D</td>
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<td>1.26</td>
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<td>POMS_Dep.</td>
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<td>.20</td>
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<td>.024</td>
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<tr>
<td>Constant</td>
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<td>POMS_Fatigue</td>
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<td>.27</td>
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Table 3

**The Effects of Sleep Quality**

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<th>Dependent Var.</th>
<th>Good</th>
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<th>Poor</th>
<th></th>
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<td></td>
<td>Means</td>
<td>$SD$</td>
<td>$n$</td>
<td>Means</td>
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<tr>
<td>Hits for Neg Stimuli</td>
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<td>14.16</td>
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<td>89.73</td>
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<td>FA for Total Stimuli</td>
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<td>4.91</td>
<td>90</td>
<td>10.59</td>
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<tr>
<td>FA for Neg Stimuli</td>
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<td>3.31</td>
<td>90</td>
<td>15.32</td>
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<tr>
<td>Target Accuracy</td>
<td>67.77</td>
<td>25.28</td>
<td>89</td>
<td>54.50</td>
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</table>

Note. SD = Standard Deviation. n = Number of Participants. FA = False Alarms
Table 4

*The Effects of Sleep Duration*

<table>
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<th></th>
<th>≥ 7 hours</th>
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<tr>
<td></td>
<td>Means</td>
<td>SD</td>
<td>n</td>
<td>Means</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>FA for Neg. Stimuli</td>
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<td>51</td>
<td>9.58</td>
<td>6.42</td>
<td>76</td>
</tr>
<tr>
<td>NTAC RT</td>
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<td>94.67</td>
<td>51</td>
<td>185.39</td>
<td>69.51</td>
<td>76</td>
</tr>
</tbody>
</table>

Note. SD = Standard Deviation. n = Number of Participants. FA = False Alarms.

NTAC RT = Non-Target Reaction Time
Figures

Figure 1

Relationship between MEQ & PER3

![Bar graph showing the relationship between MEQ and PER3 across different times of the day.]

- Morning
- Intermediate
- Evening

Figure 2
Figure 3

Relationship between MEQ & Sleep Quality

Percentage of Counts

Sleep Quality

Morning
Intermediate
Evening

Figure 3

Relationship between Sleep Quality & Sleep Duration

Percentage of Counts

Sleep Duration

≤6 hrs
≥7 hrs
≤6 hrs
≥7 hrs

Good
Poor
Figure 4

Non-Target Accuracy

Mean # of Hits

0 850 900 950 1000

9am 9pm

Time Tested

*