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PHYSIOLOGICAL DIFFERENCES IN STRESS REACTIVITY BETWEEN MORNING AND EVENING CHRONOTYPES

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Departmental Honors

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This is to certify that the thesis prepared by Megan E. Jacobs entitled: "Physiological Differences in Stress Reactivity between Morning and Evening Chronotypes" has been approved by her committee as satisfactory completion of an honors thesis as partial fulfillment for the degree of Bachelor of Science.

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Author Notes

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Physiological Differences in Stress Reactivity Between Morning and Evening Chronotypes

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Abstract

Morning and evening chronotypes (circadian preference) differ on several factors, such as stress response and sleep quality. Previous cardiovascular findings support the assumption that evening types exhibit a greater response to stress. Previous cortisol literature, in contrast, suggests that morning types have a greater response to stress. The two measures have not yet been investigated together in relation to chronotypes. The study explores differences in cardiac measures (heart rate (HR), heart rate variability (HRV)) as well as salivary cortisol in morning and evening types at baseline and under stress at different times of the day (7-11 AM or 4-7 PM). Students (n = 53) were pre-screened for chronotype preference. Participants provided salivary samples and completed a computerized mental arithmetic task while HR was recorded. Heart rate significantly increased from baseline during the task, and HRV significantly decreased. Evening types had significantly higher cortisol concentrations in the morning session, and significantly higher performance in the evening session. The interaction of chronotype and testing time did not reach significance for any of the dependent variables. General patterns partially support the idea that evening types may exhibit higher stress markers that could impair task performance.

Physiological Differences in Stress Reactivity Between Morning and Evening Chronotypes

Every individual varies in when they prefer to start and end their day. Diurnal preference is whether an individual is considered to be a morning or evening individual, which is based on the individual's preferred times of sleep and when tasks are best completed. Diurnal preference is primarily determined by an endogenous clock in the brain that independently generates near 24h rhythms that control the timing of rhythmic behavioral, physiological, and metabolic functions. These endogenous rhythms synchronize with environmental cues called zeitgebers, such as the 24h light-dark cycle, to determine circadian rhythm (Lockley & Foster, 2012).

Morning and evening types differ physiologically with regards to “alerting signals”. Endogenous melatonin is secreted by the pineal gland and is a hormone that induces heat loss and reduces neural arousal. Melatonin secretion is associated with an increase in sleep propensity and closely follows sleep-wake cycle timing. During the day, melatonin release is inhibited; during the night, the inhibition is released, which leads to more melatonin being released, thus facilitating sleepiness. Morning types begin to release melatonin earlier in the day, characterizing increased sleepiness earlier in the day, and suppress melatonin earlier in the morning, characterizing increased alertness in the morning (Maierova et al., 2016). Glucocorticoids, such as cortisol, are released from the adrenal glands into the bloodstream. Basal concentrations of cortisol follow a circadian rhythm, with the highest levels occurring after waking and gradually decreasing throughout the course of the day (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). Under normal conditions, cortisol levels peak in the early morning, are at half of morning levels by afternoon, and are insignificant by midnight (Schmidt, 1997). Cortisol regulates its own release via anegative feedback loop and binds to receptors throughout limbic system. Higher concentrations of cortisol are indicative of an increase in sleepiness (Maierova et al., 2016). Morning types have been found to have higher concentrations of cortisol earlier in the day, which declines earlier in the evening (Maierova et al., 2016; Kudielka, Bellingrath, & Hellhammer, 2007; Marvel-Coen, Nickels, & Maestripieri, 2018).

These differences in physiological alerting signals leads to different characteristics of morning and evening chronotypes. Morning types, or “larks,” are characterized by being alert in the mornings and going to bed early at night. Larks wake at an earlier clock time, but actually sleep and wake at a relatively later circadian phase, or later in their ‘day.’ This is because their circadian phase is shifted forward, or earlier than the average. High alerting signals results in higher alertness and performance in the morning. Morning types prefer day activity, as their performance and alertness rapidly declines in the evening and they find it difficult to stay awake past their typical bedtime. On the other hand, evening types, or “owls,” prefer to go to sleep at later hours and find difficulty getting up in the morning. Owls’ circadian phase is shifted backward, or later than the average. Thus, when they wake, evening types wake earlier in their ‘day’. Low alerting signals results in sleepiness and poorer performance in the morning. Owls prefer nighttime activity, as their alertness and performance increases throughout the evening and into the night (Cavallera & Giampietro, 2007; Giannotti, Cortesi, Sebastiani, & Ottaviano, 2002; Lockley & Foster, 2012).

Morning and evening types differ on various factors, such as punctuality, personality, and performance. Morning types have been shown to have greater punctuality and highly regular school attendance than evening types (Werner, Geisler, & Randler, 2014; Giannotti et al., 2002). Additionally, evening types have been found to be more extraverted and impulsive, whereas morning types lean more towards introversion and conscientiousness (Muro, Gomà-i-Freixanet,

& Adan, 2009; Tsaousis, 2010). Poor academic performance has been associated more with evening types (Giannotti et al., 2002; Preckel, Lipnevich, Schneider, & Roberts, 2011; Tavernier & Willoughby, 2014). Generally, individuals who are characterized as evening types often have more problems adjusting to having classes that are not synchronized with their preference, which occurs in most academic institution settings, where there are few classes offered after 5pm generally (Tavernier & Willoughby, 2014; Preckel et al., 2011).

While morning and evening types differ on many factors, one big difference seems to be in their reactivity to stress, although how they differ remains unclear. There are a variety of ways to measure stress, such as by self-report, heart rate variability (HRV), and cortisol release. Evening types have been shown to report higher self-reported stress after the Trier Social Stress Task (TSST) arithmetic stress task (Roeser, Obergfell, Meule, Vögele, Schlarb, & Kübler, 2012b). Additionally, research has demonstrated that evening types have more problems coping with environmental and social demands (Meccacci & Rocchetti, 1998; Roeser et al., 2012b). When measuring cardiac and cortisol differences in stress response between morning and evening types, the differences are inconsistent in what they suggest about which chronotype is more reactive to stress.

Cardiac Measures and Chronotype

One physiological measure of stress is through cardiac variables, such as heart rate (HR) and heart rate variability HRV). Heart rate is the number of times the heart beats per minute (BPM) and is defined as the average BPM over baseline and task periods. At rest, the sympathetic and parasympathetic nervous system are in a dynamic balance, and at any given time, HR represents the relative activity of both of these systems (Shaffer, McCraty, & Zerr, 2014). Heart rate variability is the variability of the time that elapses between two consecutive heart beats, representing the body’s regulatory abilities for a number of processes (Roeser et al., 2012b; Shaffer et al., 2014), and is measured by the root mean square of successive differences (RMSSD). RMSSD is acquired by calculating the time difference between two consecutive heartbeats (ms) and squaring the value. The total value is averaged before the root square is obtained. RMSSD reflects beat-to-beat variance in heart rate and is the preferred measure for heart rate variability (Roeser et al., 2012b; Task Force, 1996). RMSSD was log-transformed, as is it typically done with non-normal HRV data (Roeser et al, 2012b).

HR and HRV are correlates of the physiological response to stress. When a stressor occurs, the sympathetic nervous system becomes activated, causing physiological responses such as pupil dilation and increased HR. As HR increases in response to sympathetic nervous system arousal, HRV decreases, since a higher heart rate allows less room for variability between heartbeats (Roeser et al., 2012b; Shaffer et al., 2014).

Evening types have been found to have a significantly higher HR and systolic blood pressure (SBP) and lower HRV than morning types at rest (Roeser et al., 2012b; Willis, O’Connor, & Smith, 2005). Additionally, morning and evening types differ in their HR and HRV in response to stress. Roeser et al, (2012b) explored the relationship between chronotype and the cardiovascular response to a mental arithmetic stress task at different times of the day. Researchers collected baseline HR and continued to collect data while participants were engaging in the stress task. Evening types had exhibited significantly higher HR, and thus lower HRV, than morning types during the stress task, suggesting that evening types have a higher reactivity to stress. Additionally, Nebel et al, (1996) and Willis et al, (2005) both found that the time of testing mattered, such that HR was found to significantly increase when stress was induced in the evening, and that evening types showed higher HR in the afternoon than in the morning in response to stress. Roeser et al, (2012b) failed to find that time of day mattered.

Cortisol Measures and Chronotype

Another physiological measure of the stress response is cortisol. Cortisol is released in response to hypothalamic-pituitary-adrenal (HPA) axis activation (Tsigos & Chrousos, 2004). The HPA axis is formed by the hypothalamus and structures in the brain stem, areas that work together to initiate and maintain the stress response. Cortisol works to increase glucose production for energy and metabolic processes, playing a major role in maintaining homeostasis in the response to stress (Dedovic et al., 2009; Dickmeis, 2009). Cortisol is positively linked with stress, such that higher levels of cortisol are indicative of higher reactivity to stress (Pruessner, Hellhammer, & Kirshbaum, 1999; Schulz et al., 1998). With chronic stress, the continuous stimulation of the HPA axis works to eliminate cortisol's cyclic nature (increasing as HPA axis is activated, decreasing as HPA axis is returning to rest), thus leading to increased levels of cortisol throughout the course of the day (Schmidt, 1997).

Morning and evening types differ in endogenous cortisol release. Morning types exhibit significantly higher cortisol levels, especially after awakening, compared to evening types (Axelsson, Akerstedt, Kecklund, Lindqvist, & Attefors, 2003; Bailey & Heitkemper, 1991; Kudielka et al., 2007; Schulz, Kirschbaum, Prüßner, & Hellhammer, 1998). Additionally, morning and evening types differ in cortisol release as part of the stress response. Morning types have been found to show increased concentrations of cortisol compared to evening types (Kudielka et al., 2007; Marvel-Coen et al., 2018). In addition, Marvel-Coen et al, (2018) assessed cortisol concentrations in both baseline and stress conditions, in relation to chronotype. Participants were randomly assigned to either a stress or control condition; they either took part in the TSST or they sat in a room doing nothing for the same amount of time. Marvel-Coen et al, (2018) found a significant interaction between chronotype and the induction of stress on cortisol changes, such that morning types had a significantly larger increase in cortisol than evening types in response to stress, suggesting that morning types have a higher reactivity to stress. There were no differences in cortisol concentrations between chronotypes in the control condition.

Sleep Quality

An additional factor that can affect cortisol concentrations is sleep quality. Disturbed sleep may lead to an increase in cortisol concentrations, thus poor sleep quality and sleepiness thus may be a factor that leads to a higher reactivity to stress (Dahlgreen, Kecklund, & Akerstedt, 2005; Lac & Chamoux, 2003, Roeser, Meule, Schwerdtle, Kübler, & Schlarb, 2012a). Roeser et al, (2012a) found that evening types were more likely to experience social jetlag, which is an inconsistency between social and biological times, leading to greater reports of poor sleep quality and feelings of tiredness. Sleep quality is linked to daytime functioning, such that chronically disturbed sleep results in daytime fatigue and poorer task performance. These results are consistent with previous studies suggesting that evening types report lower academic performance than morning types (Giannotti et al., 2002; Preckel et al., 2011; Tavernier & Willoughby, 2014). Additionally, Roeser et al, (2012a) found that subjective sleep quality mediates the relationship between chronotype and self-reported stress.

Present Study

Currently, the literature on the cardiac and cortisol responses to stress are independent, and both are inconsistent in what both suggest about reactivity to stress in morning and evening chronotypes. The cardiac literature suggests that evening types are more reactive to stress, but the cortisol literature suggests that morning types have a higher reactivity to stress. To resolve this difference, it would help to collect both cardiac and cortisol measures within the same study. There is evidence to suggest that cardiac and cortisol measures of stress reactivity are related (Johnsen, Hansen, Murison, Eid, & Thayer, 2012). Johnsen et al, (2012) found a negative association between HRV and cortisol secretion, which could be because both low HRV and high cortisol secretion are related to the central autonomic network that controls the sympathetic response. However, no one thus far has measured both HRV and cortisol concentrations in reaction to stress.

The present study adapted the stress induction task from Roeser et al, (2012b), in which participants were asked to complete a mental arithmetic task while cardiac input was measured. In addition to measuring HRV, salivary cortisol samples were collected from both morning and evening types in the present study. We manipulated testing time, measuring both in the morning and evening to account for the natural circadian changes in cortisol (e.g. highest right after waking and decreases gradually throughout the day). Generally, it was predicted that individuals with higher cortisol concentrations would exhibit a higher HR, and therefore lower HRV, in their reactivity to stress. Specifically, if participants defined as ‘morning’ types had higher cortisol levels and lower HRV, this would support the cortisol literature suggesting that morning types have a higher reactivity to stress. However, if ‘evening’ types had lower HRV and higher cortisol levels, it would suggest evening types have higher reactivity.

Method

Participants

The participants in this study were students who met the criteria of being either morning or evening types as determined by their answers on a pre-screening survey consisting of the

Morningness-Eveningness Questionnaire (MEQ; Horne & Ostberg, 1976) that was administrated using the participant pool software SONA. A total of 201 students responded to the prescreening questionnaire. Results of the prescreening showed that 0 participants scored as definite evening types, 54 scored as moderate evening types, 128 scored as intermediate, 12 scored as moderate morning types, and one student scored as definite morning type. Initially, SONA was used to invite the students who scored as ‘definite’ and ‘moderate’ evening types (scores of 16-41) and morning types (scores of 59-86) on the MEQ to participate in the study.

The ‘definite’ and ‘moderate’ evening and morning type groups had relatively low numbers, participants who scored intermediate, but as close as possible to each end of the spectrum, were also invited.

Participants (*N* = 53) were undergraduates at the University of Mary Washington who completed the study in exchange for credit towards a course requirement. The participants’ age ranged from 18-26 years of age. Eighty-one percent of the participants (*n* = 43) identified as female, and 19% (*n* = 10) identified as male. Participants described themselves as Caucasian (67.9%), African American (5.6%), Hispanic/Latino (15.1%) and other (9.6%). A total of 10 participants were dropped for either unreadable cardiac (*n* = 3) and cortisol (*n* = 7) data.

Participants who signed up to participate were asked to do the following in preparation for the study (Roeser et al., 2012b; Salimetrics, 2019): 1) refrain from physical strain for at least 1 hour prior to the experiment, 2) not eat, drink or smoke for at least 1 hour prior to the study 3) avoid foods with high sugar/acidity, high caffeine, or alcohol immediately before collection to avoid compromising the assay by lowering pH and increasing bacteria growth; and 4) refrain from brushing their teeth for at least 1 hour prior to the experiment to minimize the risk of blood contamination.

Survey Measures

Morning-Eveningness Questionnaire (MEQ). The MEQ is a self-rated questionnaire used to determine whether a person's circadian rhythm reaches its climax in the morning, evening, or in-between. The scale consists of 19 multiple choice questions, each question having either four or five response options. Responses are totaled, forming a composite score and indicating the degree to which the individual has a morning versus evening preference. Scores can range from 16-86, with scores of 41 and below indicating 'evening' preference, scores of 59 and above indicating as 'morning' preference, and scores between 42-58 indicating 'intermediate' types (Horne & Ostberg, 1976). The present study had scores ranging from 32-46 for 'evening' types and 47-64 for 'morning' types.

Pittsburgh Sleep Quality Index (PSQI). The PSQI is a self-assessment questionnaire assessing sleep quality over a period of one month. The scale has 19 items that generate seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, sleeping medication use, daytime dysfunction) and one global, composite score. Each item has response options from 0 to 3. The global score is calculated by totaling the component scores, which provides an overall score that ranges from 0 to 21. Higher scores indicate poorer sleep quality (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The PSQI has also been found to mediate the relationship between morning-eveningness and stress response in a previous study (Roeser et al., 2012a).

Chronic Sleep Reduction Questionnaire (CSRQ). The CSRQ is a 20-item self-rated questionnaire that consists of four subscales: 'sleepiness' (four items), 'shortage of sleep' (six items), 'loss of energy' (five items), and 'irritation' (five items) and it assesses chronic sleep reduction. Each question has three response options, ranging from 1 to 3. Higher scores indicate greater chronic sleep reduction (Dewald, Short, Gradisar, Oort, & Meijer, 2012). The CSRQ has also been shown to be a good predictor of insufficient sleep (Dewald et al., 2012).

Global Vigor and Affect Scale (GVA). The GVA is a computerized, visual-analogue scale that consists of eight subscales. Four of the subscales (alertness, sleepiness, effort, and weariness) indicate subjective activation of vigor, and the other four (happiness, sadness, calmness, and tension) indicate affective state. Each group of four subscales is summed to give a global value of vigor (GV) and affect (GA). Each subscale ranges from 0 to 100; higher scores indicate stronger expression of the state (Monk, 1989).

Stress Coping Style Inventory (SCSI). The SCSI is a 28-item questionnaire that identifies possible individual differences in coping with stress. Each item describes a possible response to a stressor, and participants are asked to indicate on a 5-point Likert scale to what extent they use the proposed strategy (1 = completely disagree, 5 = completely agree). The questionnaire is divided into four factors: active emotional, passive emotional, active problem, and passive problem. The higher the score in each factor, the higher the rate an individual uses the coping style (Lin & Chen, 2010).

Subjective Stress Rating Scale (SSRS). The SSRS is a self-assessment of the degree of perceived stress participants currently feel. The scale contains one-question, and responses to this question range from 0 to 10. Higher scores indicate a higher level of subjective stress (Roeser et al., 2012b).

Physiological Measures

Heart Rate. An AdInstruments finger pulse transducer recorded heart rate throughout the study during baseline and during the stress task. Heart rate is measured by beats per minute

(BPM), and HRV is measured by the root mean square of successive differences (RMSSD). RMSSD reflects beat-to-beat variance and is the preferred measure of variability between beats (Roeser et al., 2012b; Shaffer et al., 2014).

Cortisol. Salimetrics SalivaBio Passive Drool collection kits were used to collect salivary cortisol samples from participants at baseline and after the stress task. The Salimetrics Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit was used to analyze the cortisol data (Salimetrics, 2019).

Procedure

Each participant was run individually in the University of Mary Washington Physiology Lab. Invitations to participate were sent to the top 76 scorers (characterized as ‘morning’ types) and the bottom 82 scorers (characterized as ‘evening’ types. Participants were randomly assigned to be invited to either a morning or evening session. There were 15 individuals in the morning type, morning (M/M) session (average MEQ score = 55), 13 in the morning type, evening (M/E) session (average MEQ score = 56.8), 11 in the evening type, morning (E/M) session (average MEQ score = 38.7), and 14 in the evening type, evening (E/E) session (average MEQ score = 39.7). Morning sessions ran between 7-11AM and evening sessions ran between 4-7 PM to replicate the Roeser et al, (2012b) study.

Salivary Cortisol Preparation. Each participant was instructed to rinse their mouth thoroughly with water at least 10 minutes before the sample was collected, because bovine hormones (normally present in dairy products) can cross-react and cause false results, and acidic/high sugar foods can compromise assay performance and lower pH of the sample (Salimetrics, 2019).

Questionnaires. Participants completed a number of questionnaires on Qualtrics that included the MEQ, CSRQ, PSQI, and GVA, which took at least 10 minutes, allowing for proper preparation for the salivary cortisol collection.

Baseline Cortisol Measurement. After completing the survey measures, participants were asked to provide a baseline saliva sample to measure cortisol concentrations using a Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit. Salivary cortisol has been found to be directly proportional to serum concentrations of cortisol. In accordance with the unstimulated passive drool protocol, participants were instructed to tilt their head forward, allowing saliva to pool on the floor of their mouth. They then passed the saliva through a short straw into a polypropylene vial, collecting at most 0.5mL (Salimetrics, 2019). The researcher then placed the vial in the freezer.

Baseline Heart Rate (HR) Measurement. After the baseline cortisol sample, participants were hooked up to an AdInstruments heart rate pulse transducer, placed via velcro on the left index finger. Participants rested for 5 minutes to collect baseline HR data. Following Roeser et al, (2012), participants wore headphones for sound insulation and a sleep mask to avoid visual distractions. After 5 minutes, participants were instructed to take off the mask and headphones, but to leave the HR monitor on for the next part of the study. Participants were then asked to rate their current level of perceived stress by answering the SSRS.

Stress Task: TSST Mental Arithmetic Task. After the baseline HR measurement, participants completed an adapted version of the TSST mental arithmetic stress task (Kirschbaum, Pirke, & Hellhammer, 1993). The mental arithmetic task involves counting backwards by subtracting the number 13 from a 4-digit number, 2022, as quickly as possible for a period of five minutes. Research has shown that vocalization of answers during the task potentially interferes with the analysis of HRV (Roeser et al., 2012b; Sloan, Korten, & Myers, 1991); therefore, the task responses were entered on a computer instead of being verbalized. This e-task was presented using E-Prime, a stimulus presentation software. Participants used the number keypad to type their answers. After each wrong answer, the participant heard an aversive negative feedback sound (a buzzing sound) and had to start again at the very beginning. Consistent with previous studies, the experimenter was in the room to further induce stress while the participant was completing this task (Kirschbaum et al., 1993; Roeser et al., 2012b).

After 5 minutes has passed, the program automatically stopped the task. Heart rate was measured throughout the task in the manner described above. To incentivize the participants and to create even more stress (i.e., a reason to do better), they were told that they would be given a prize (i.e., a piece of candy) at the end of the study if their performance met a certain standard. Everybody got to choose a piece of candy, regardless of their performance, during debriefing. After completing the task, participants were instructed to remove the HR monitor, then participants were immediately asked to again rate their current level of stress via the SRSS.

Post-Task Cortisol Measurements. Directly after the task, participants were asked to provide another saliva sample. They followed the same protocol as described above. Participants then completed the SCSI. Cortisol that is released in the bloodstream reaches saliva in as little as five minutes, thus is representative of unbound cortisol plasma levels (Schmidt, 1997).

Results

Sleep and Coping Characteristics

PSQI. An ANOVA was conducted to assess the differences in sleep quality between morning and evening types. Evening types (*M* = 9.00, *SD* = 3.59) had a significantly higher mean PSQI scores, indicating lower sleep quality compared to morning types (*M* = 6.46, *SD* =

3.31), $F(1, 51) = 7.16$, $p = .010$, $\eta p^2 = .123$. The PSQI has a cut-off score of 5, such that any score greater than 5 is indicative of poor sleep (Grandner, Kripke, YOON, & Youngstedt, 2006). Eighty percent evening people scored over a 5 on the PSQI, while only 53% of morning people did.

CSRQ. An ANOVA was conducted to assess the differences in chronic sleep reduction between chronotypes. Evening types ($M = 39.60$, $SD = 5.95$) had a significantly higher mean CSRQ score compared to morning types ($M = 34.46$, $SD = 3.41$), indicating a greater level of chronic sleep reduction, $F(1, 51) = 15.25$, $p < .001$, $\eta p^2 = .230$.

GVA. A 2 x 2 between-subjects ANOVA was conducted to assess the differences in expression of global vigor and affect between chronotypes, morning type versus evening type, and testing time, morning session versus evening session. Mean global vigor was significantly higher for morning types ($M = 62.71$; $SD = 16.23$) compared to evening types ($M = 52.36$, $SD = 21.69$), $F(1, 49) = 4.77$, $p = .034$, $\eta p^2 = .089$. Also, there was a significant interaction between testing time and chronotype, $F(1, 49) = 7.13$, $p = .01$, $\eta p^2 = .127$. The main effect of testing time was not significant, $F(1, 49) = .259$, $p = .613$, $\eta p^2 = .005$. A *t* test for independent means was conducted to determine if global vigor was higher in the morning or evening sessions, on averages. During the morning session, morning types ($M = 67.73$, $SD = 9.59$) had a significantly higher mean global vigor scores than evening types ($M = 43.45$, $SD = 20.35$), $t(24) = 4.07$, $p = .000$, $d = 1.08$. There was no significant difference between mean global vigor scores of morning ($M = 56.92$, $SD = 20.43$) and evening types ($M = 59.36$, $SD = 20.74$) in the evening sessions, $t(25) = -.307$, $p = .762$, $d = 0.08$.

SCSI. An independent *t* test was conducted to assess the differences in the likelihood that morning and evening types would use both positive and negative coping strategies. There was no significant difference in the mean likelihood of the use of positive strategies by morning and evening types, *t*(51) = .681, *p* = .499, *d* = .133, and negative strategies by morning versus evening types, *t*(51) = -1.47, *p* = .147, *d* = .285.

Task Performance

TSST. A 2x2 between-subjects ANOVA was conducted to assess the difference in mental arithmetic task performance between chronotypes, morning type versus evening type, and testing time, morning session versus evening session. Evening types (*M* = 18.90, *SD* = 14.59) had a significantly larger number of correct trials in a row compared to morning types (*M* = 10.07, *SD* = 7.63), *F*(1, 49) = 7.58, *p* = .008, ηp^2 = .134. There was not an effect of testing time, *F*(1, 49) = .529, *p* = .471 ηp^2 = .011, but the interaction of chronotype and testing time was close to significant, *F*(1, 49) = 3.90, *p* = .054, ηp^2 = .074. The pattern of the means suggests that the difference between chronotypes especially true in the evening sessions.

Physiological and Subjective Stress Measures

A 2 (trial; pre versus post stress task) x 2 (chronotype; morning versus evening type) x 2 (testing time; morning versus evening session) mixed ANOVA was conducted individually on each of the physiological and stress measures collected before, during, and after the TSST mental arithmetic stress task, including HR, HRV, cortisol, and subjective stress. The significant effects are presented below for each measure.

Heart Rate (BPM). There was a significant main effect of trial, *F*(1, 49) = 68.83, p < .001, ηp^2 = .584. Heart rate increased significantly from the baseline period (*M* = 76.61, *SD* = 12.90) to the task period (*M* = 85.99, *SD* = 13.83). The main effect of testing time was not significant, *F*(1, 49) = 1.50, *p* = .227, ηp^2 = .030. The main effect of chronotype was also marginally insignificant, *F*(1, 49) = 3.57, *p* = .065, ηp^2 = .068. There is a pattern that suggests that evening types (*M* = 84.56, *SD* = 14.59) exhibited higher HR than morning types (*M* = 78.38, SD = 11.66). The three-way interaction of HR x chronotype x testing time was also not significant, *F*(1, 49) = 0.095, *p* = .759, ηp^2 = .002.

Heart Rate Variability (HRV). There was a significant main effect of trial, *F*(1, 49) = 11.49, *p* = .001, ηp^2 = .190. RMSSD decreased significantly from the baseline period (*M* = 1.70, *SD* = .26) to the task period (*M* = 1.59, *SD* = .27; see Figure 1). The main effect of chronotype was not significant, *F*(1, 49) = 1.665, *p* = .203, ηp^2 = .033, as well as the main effect of testing time, *F*(1, 49) = 3.147, *p* = .082, ηp^2 = .060. The three-way interaction of HRV x chronotype x testing time was also not significant, *F*(1, 49) = .150, *p* = .700, ηp^2 = .003.

SSRS. There was a significant main effect of subjective stress, *F*(1, 49) = 78.32, *p* = .000, ηp^2 = .615. Subjective stress increased significantly from baseline (*M* = 3.34, *SD* = 2.24) to after stress induction (*M* = 5.91, *SD* = 2.39). The main effect of chronotype was not significant, *F*(1, 49) = .115, *p* = .736, ηp^2 = .002, nor was the main effect of testing time, *F*(1, 49) = 1.147, *p* = .289, ηp^2 = .023. The three-way interaction of subjective stress rating x chronotype x testing time was also nonsignificant, *F*(1, 49) = .718, *p* = 401, ηp^2 = .014.

Cortisol Concentration. There was a significant main effect of trial, *F*(1,42) = 8.80, *p* = .005, ηp^2 = .173. Cortisol concentration (in ug/dL units) increased significantly from baseline (*M*=.27, *SD*=.27) to after stress induction (*M*=.35, *SD*=.28). There was also a main effect of chronotype on cortisol, *F*(1,42) = 5.89, p = .020, ηp^2 = .123. Evening types (*M*=.37, *SD* = .36) had significantly higher cortisol concentrations than morning types (M=.26, *SD* =.15). There was also a main effect of testing time on cortisol, *F*(1,42) = 16.73, p = .000, ηp^2 = .285. Cortisol levels were significantly higher in the morning sessions (M=.43, *SD* =.34) than in the evening sessions (M=.19, *SD* =.10). See Figure 2. The interaction of chronotype x testing time was marginally insignificant, $F(1,42) = 3.997$, $p = .052$, $\eta p^2 = .087$.

Discussion

Previous to this study, the literature on both cardiac and cortisol responses to stress were independent and inconsistent in what they suggested about reactivity to stress in morning and evening types. To resolve these differences, the present study collected both cardiac (HR and HRV) and cortisol measures. Overall, regardless of chronotype, HR and cortisol significantly increased from baseline to during the stress task and HRV (RMSSD) significantly decreased from baseline to during the stress task, suggesting that the mental arithmetic part of the TSST used was successful in inducing stress. These findings are consistent with the cardiac literature, such that in response to stress, HR increases and HRV decreases (Roeser et al, 2012b; Shaffer et al, 2014). While cortisol did differ in a way that seems to affect testing performance, there was no evidence of differences in stress reactivity between chronotypes.

The computerized adaptation of the TSST mental arithmetic stress task was successful in inducing stress. A computerized stress task was used because research has shown that vocalization of answers potentially interferes with the sensitiveness of HRV (Roeser et al., 2012b; Sloan et al., 1991). Self-reported stress scores increased from baseline to after the stress task. Following the predicted pattern, HR and cortisol concentrations significantly increased from baseline to after the stress task. Additionally, HRV followed the predicted pattern and significantly decreased from baseline during the stress task. This is an important finding, since most research using the TSST use the verbalized mental arithmetic stress task. This study provides validation that the computerized version is also a viable measure to induce stress.

While there wasn't a significant main effect of chronotype on HR, evening types showed a pattern of higher heart rate compared to morning types. Heart rate variability (RMSSD) decreased from baseline to during the stress task, independent of chronotype and testing time, yet followed in the predicted pattern. This observation is consistent with Roeser et al, (2012b) and others, who found that evening types exhibit significantly higher HR than morning types (Nebel et al., 1996; Roeser et al., 2012b; Willis et al., 2005).

In addition, Nebel et al, (1996) found significant interactions between chronotype and testing time, such that morning individuals exhibited higher HR in the morning, and evening individuals exhibited higher HR in the evening. Willis et al, (2005) also found a significant interaction of chronotype and testing time, in which evening individuals exhibited significantly higher cardiac activity in the evening compared to the morning. The present study shows a pattern that suggests that evening types may have higher HR in the evening, but overall did not find any significant differences in stress reactivity that were based on chronotype or testing time. It's possible that the lack of extreme chronotypes ('definite' morning and evening) in the present study led to an underestimation of these effects.

Additionally, evening types were found to have significantly higher cortisol concentrations in morning sessions compared to morning types. These findings are in contrast to previous literature, which it was consistently reported that morning types showed an increased concentration of cortisol, both at baseline and after induction of stress (Axelsson et al., 2003; Bailey & Heitkemper, 1991; Kudielka et al., 2007; Schulz et al., 1998; Marvel-Coen et al., 2018). Specifically, Marvel-Coen et al, (2018) found that morning types had a significantly larger increase in cortisol than evening types in response to stress. In Marvel-Coen et al.'s study, saliva samples were all collected between 12:30 PM and 4:30 PM, while in the present study, saliva samples were collected between 7:00 AM - 11:00 AM and 4:00 PM - 7:00 PM. Cortisol is not just a stress marker, but a sleep marker as well. Cortisol is highest right after waking and decreases gradually throughout the day, and it’s possible that in the present study, the morning types in the morning sessions had already been awake for a while and thus their cortisol levels had already started to decrease. However, evening types had woken up earlier in their ‘day’, or circadian phase, just to get to the study, and thus their cortisol levels were at its peak.

These cortisol findings make sense if task performance results are taken into consideration. Evening types performed better on the mental arithmetic task, and the pattern suggests that this was especially true in the evening. Generally, evening types experience an increase in performance throughout the course of their day and into the evening, whereas morning types find performance to be at its peak in the morning. When looking at the results of the cortisol data, evening types were exhibiting high levels of cortisol in the morning, which is most likely due to the natural rhythm of cortisol rather than a response to stress. Furthermore, evening types showed a pattern of decreased performance in the morning, which is when their cortisol levels were highest. Therefore, it’s possible that the relationship between cortisol and task performance is not due to stress, but something that has to do with the natural circadian rhythm of cortisol. In which case, evening individuals were performing worse in the morning, because they were up at a much earlier time in their circadian phase. Waking up earlier in the circadian phase results in low alerting signals, which leads to decreased performance in the morning. Additionally, the present study found evidence to suggest that morning types had significantly higher levels of vigor in the morning, compared to evening types. This further supports that evening individuals may be performing worse in the morning because they are being tested at a time where alerting signals are low and cortisol concentrations are high, leading to decreased vigor and performance.

Furthermore, evening types are typically found to have overall poorer performance compared to morning types. Nevertheless, Preckel et al, (2011) found that eveningness was positively associated with cognitive ability. Evening types generally have more problems adjusting to having classes/duties that are not synchronized with their preference, which generally occurs at most academic institutions, so evening types have a more frequent and recurring need to overcome this social jetlag. This might lead to evening types developing greater problem-solving skills, thus they are able to perform better on tasks that require them to solve problems, such as the mental arithmetic required of the stress task in the present study. So, in the present study, evening types might have shown increased performance and cognitive ability due to these training effects in their everyday life.

One of the biggest limitations for the present study was the relatively low sample size, and thus low power. There were a few interactions that were close to being significant. It is possible that having equal groups and a larger sample size would have increased power and, in turn, the study would have been able to produce significant effects.

Another big limitation is the issue of self-selection. Participants were randomly assigned to either a morning or evening session. Within the respective session, participants were able to choose a time to participate based on what worked best for them; they could reject the invitation and not sign up if they were invited to a session they didn't prefer. This likely led to the small group sizes (e.g. evening individuals in the morning session, $n = 11$). Chronotypes generally tend to complete tasks that are in line with when they are the most alert, and if they aren't forced to do so, they likely won't complete the task outside of that preferred time.

Lastly, there were a relatively large number of intermediate types who participated in the study. Originally, there were very few ‘definite’ and ‘moderate’ morning and evening types. I accepted the risk that by pooling from the intermediate group, even if those pooled from this group were as close to their respective end of the spectrum as possible, the effects could be underestimated. Previous studies have been able to get a larger number of these more extreme chronotypes, and future research should open participation to a community rather than relying solely on a general psychology subject pool, of whom are mostly freshmen in college.

The present study did find that evening types had reported a significantly poorer sleep quality compared to morning types. Since poorer sleep quality can affect various factors such as impulsivity, mood, and cognition, it would be interesting if future studies investigated these factors of sleep quality between chronotypes. Additionally, given the present study’s findings on task performance and cortisol concentrations, future studies should investigate the relationship between the two, independent of stress.

Overall, there is little evidence from the present study to support any differences in stress reactivity between chronotypes; however, the performance and cortisol data are suggestive of the problems with asking evening types to perform in the morning (e.g. university settings). Generally, university institutions offer many of their classes starting in the early morning, and relatively few after 5pm. Essentially forcing evening types to conform to an early-rising society leads to social jetlag, or a discrepancy between sleep and social schedules, which can have negative effects not only on academic performance and stress response, but also psychological and psychosomatic distress. Universities should offer more classes in the evening to accommodate to these students, and further research should be completed to assess the effects of testing time and performance between chronotypes.

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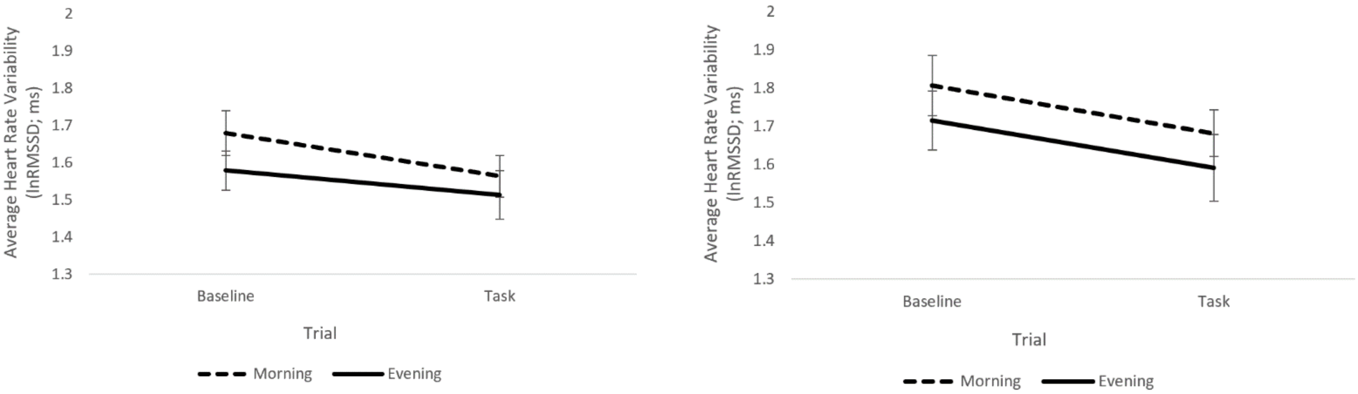
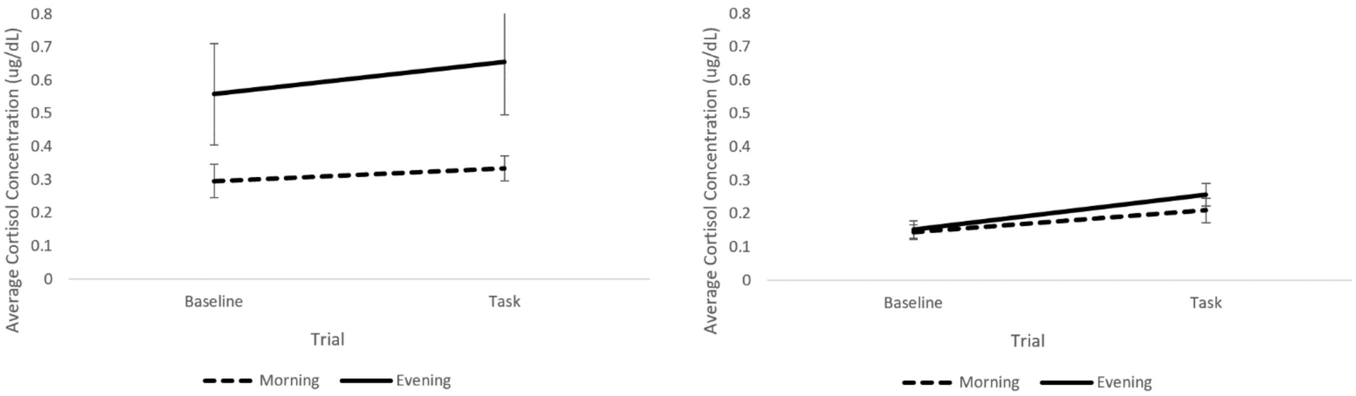


(a) (b)

Figure 1. Heart rate variability expressed as average root mean square of successive difference (lnRMMSD; ms) in morning and evening chronotypes by morning (a) and evening (b) sessions.



(a) (b)

Figure 2. Average salivary cortisol concentrations (ug/dL) in morning and evening chronotypes during morning (a) and evening (b) sessions.