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Sleep is critical for physical and mental health and peak cognitive performance (Watson et al., 2015). While main effects of genetic and environmental factors, including stress, on sleep quality are well-established, the influence of gene-environment interactions on sleep merits further study. The present research evaluated the relationship between serotonergic genetic variation, exposure to recent perceived life stress, and sleep quality outcomes among a sample of emerging adults. Given the polygenic influences underlying sleep processes, the cumulative effect of single nucleotide polymorphisms (SNPs) in and near several serotonin system genes was examined. A genetic profile score was constructed using a sum of alleles hypothesized to confer risk for sleep disruption. Undergraduate participants provided DNA samples, wore wrist-actigraphs to record sleep-wake patterns over three nights, and completed daily diary entries for two weeks. Diaries included assessments of sleep quality and exposure to daily hassles, a form of life stress. Using hierarchical linear modeling, we evaluated the hypotheses that 1) daily hassles would be associated with poorer quality of sleep in main effect and 2) genetic profile scores would significantly moderate the relationship between hassles and sleep, such that higher genetic risk scores would predict poorer quality of sleep in the context of increased daily hassles. Findings indicated that interpersonal and non-interpersonal daily hassles did not predict self-reported or actigraph-measured sleep outcomes in main effect, and the interaction of hassles and serotonergic MLP score did not predict sleep outcomes. Results suggested that the study sample exhibited relatively healthy sleep patterns and

reported relatively few hassles. The findings suggest avenues for future research, including exploration of the roles of additional forms of stress in sleep disruption.

PREDICTORS OF SLEEP QUALITY: STRESS EXPOSURE AND THE ADDITIVE INFLUENCE OF SIX SEROTONERGIC POLYMORPHISMS

by

Gail M. Corneau

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Approved by

Dr. Suzanne Vrshek-Schallhorn
Committee Chair

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APPROVAL PAGE

This thesis written by GAIL M. CORNEAU has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair_____
Dr. Suzanne Vrshek-Schallhorn

Committee Members_____
Dr. Kari M. Eddington

_____
Dr. Laurie Wideman

June 7, 2018
Date of Acceptance by Committee

June 7, 2018
Date of Final Oral Examination

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CHAPTER I

INTRODUCTION

Despite debate regarding the functions of sleep at the cellular, tissue, and organismal levels, a large body of research clearly demonstrates its importance for both physical and mental health (Perry, Patil, & Presley-Cantrell, 2013). Inadequate sleep, however, is common in the United States (Liu et al., 2016) and is associated with a range of psychopathologies (American Psychiatric Association, 2013). In particular, the majority of individuals with depression—including an estimate of up to 90%— report sleep disturbances, especially related to insomnia (Franzen & Buysse, 2008). Therefore, empirical work that elucidates factors influencing sleep quality is likely to benefit public health.

Exposure to life stress has been associated with sleep disruption (Åkerstedt, 2006), and stress exposure also represents a well-characterized risk factor for depression (Paykel, 2003). Further, individual differences in the serotonin system have been associated with variation in stress reactivity, sleep functioning, and depression risk, all of which involve at least a moderate genetic contribution. One approach for probing individual differences in the serotonin system involves examination of relevant genetic variants, and recent advances suggest the use of multilocus profile scores, which permit examination of the collective effect of multiple genetic variants. The present study tested whether a serotonergic genetic multilocus profile score moderated the relationship

between perceived daily hassles, a form of life stress, and sleep quality variables measured both subjectively through diary report and objectively using sleep actigraphy.

Life Stress and Sleep

Forms of stress. Past research suggests associations between exposure to stress and sleep variables (e.g., Van Reeth, 2000), including reduced duration and quality of sleep (e.g., Åkerstedt, 2006), differences in sleep physiology (e.g., Kim & Dimsdale, 2007), increased daily variability in sleep (Mezick et al., 2009), and clinically significant sleep disturbance (Healey et al., 1981). These associations have been found in the context of the three main types of threatening experiences emphasized in the objective life stress and depression literature (Harkness & Monroe, 2016), including episodic stressors, chronic stressors, and daily hassles. Briefly, episodic stressors are time-limited events, such as a serious argument with a close friend, romantic break-up, or failed academic course; among these, major severity but not minor severity events significantly predict depressive episode onset (Vrshek-Schallhorn, Stroud, Mineka, Hammen, et al., 2015). Chronic stress captures dimensions of ongoing quality of life (and lack thereof), such as long-term strained relations with a close family member, a prolonged period of financial instability, or frequent exposure to community violence. Daily hassles are episodic events of relatively lower severity, such as obtaining an unsatisfactory grade on an exam or having a mild argument with a friend; because more severe episodic events are relatively rare, hassles are often studied as a less severe but more common model of episodic events. Finally, the stress and depression literature emphasizes a distinction between interpersonal and non-interpersonal aspects of each of these three forms of stress, as

interpersonal stress has been shown to have significantly greater unique variance for predicting prospective depression than non-interpersonal stress (Vrshek-Schallhorn, Stroud, Mineka, Hammen, et al., 2015), and interpersonal but not non-interpersonal chronic stress predicted recurrence of major depression (Sheets & Craighead, 2014).

Evidence of associations between stress and sleep. Regarding episodic stressors, early work demonstrated that people with insomnia reported a significantly greater number of undesirable life events in the year their insomnia began compared to a control group during the same period (Healey et al., 1981). Similarly, a more recent study showed that sleep quality and duration were reduced among high school students during examinations, a time-limited period of increased stress (Astill, Verhoeven, Vijzelaar, & Van Someren, 2013).

Chronic stress has also been implicated in sleep disruption. Among college students, for example, chronic interpersonal conflict interacted with negative affectivity to predict sleep quality, such that those who endorsed more frequent conflicts *and* higher negative affectivity reported poorer quality of sleep (Fortunato & Harsh, 2006). Similarly, a significant effect of interpersonal chronic stress on sleep quality was also found among middle aged adults, such that increased interpersonal stress predicted greater sleep disturbance (Aanes, Hetland, Pallesen, & Mittelmark, 2011). Finally, a meta-analysis examining associations between work-related chronic stress and physical symptoms supported that higher levels of various chronic stressors in the work environment—including lack of control over tasks, organizational constraints preventing fulfillment of responsibilities, receipt of conflicting messages about responsibilities,

interpersonal conflicts, larger workloads, and longer work hours—predicted increased sleep disturbance (Nixon, Mazzola, Bauer, Krueger, & Spector, 2011).

Objective, repeated measures approaches to the stress-sleep relationship. Complimenting cross-sectional self-report studies, research designs incorporating daily diaries and actigraphy allow for a more fine-grained analysis of the daily associations between stressors and sleep variables. Daily diary methodologies use self-report measures completed outside of the laboratory setting across multiple days, which facilitate more accurate recall of daily events than assessment over longer retrospective periods of time (Lischetzke, 2014). Actigraphy involves the tracking of small movements with a device worn on the body (in an area such as the wrist or hip) that contains an accelerometer and measures activity in short epochs. This portable technology allows for objective measurement of daily activity level and various sleep characteristics in naturalistic settings. Sleep actigraphy provides the ability to examine objective characteristics of sleep quality, including sleep onset latency (SOL) and wake after sleep onset (WASO). SOL represents the total amount of time taken to initiate sleep following bedtime, and WASO represents the total amount of time spent awake during the night (Ancoli-Israel et al., 2003). Studies comparing actigraphy to polysomnography largely indicate adequate validity for the use of actigraphy as an objective measure of sleep variables in non-clinical populations, although polysomnography remains the gold standard measurement of objective sleep variables (for a review, see Sadeh, 2011).

Studies incorporating actigraphy and daily diary methods provide further evidence for the relationship between stress and sleep. In one study, people who reported,

on average, more daily hassles slept for significantly shorter amounts of time and exhibited reduced sleep efficiency (i.e., the ratio of sleep time to total time in bed; e.g., Doane & Thurston, 2014). In a separate study, daily hassles were not associated with sleep duration in main effect; however, number of hassles interacted significantly with childhood family environment, such that people who grew up in riskier environments (e.g., characterized by more family conflict) slept less on nights following exposure to more hassles (Hanson & Chen, 2010). To build on these studies, the present study used daily diary and actigraphy methodologies in conjunction with a novel genetic approach to examine the relationship between perceived daily hassles, serotonergic genetic variation, and both subjective and objective measures of sleep quality.

Genetics and Neurobiology of Sleep

Beyond the influence of stressful experiences, genetics play a substantial role in sleep functioning. Heritability estimates from twin studies suggest a substantial genetic influence on sleep duration (31% to 55%; Watson et al., 2015) and quality (43%; Barclay, Eley, Buysse, Archer, & Gregory, 2010). Basic animal research has advanced knowledge of the physiological processes of sleep and the genes involved (e.g., for reviews, see Andretic, Franken, & Mehdi, 2008; Sehgal & Mignot, 2011). Notably, while many genes contribute to sleep processes, none are thought to contribute exclusively to sleep (Sehgal & Mignot, 2011).

Neurobiology of sleep. An understanding of the neurobiology governing sleep and the sleep-wake cycle can inform which neurobiological systems to target in candidate genetic research on sleep. Although a full review is beyond the scope of this project,

briefly, wakefulness is controlled by the ascending reticular activating system, which sends excitatory signals to cortical regions via two main neural pathways (Swick, 2005). First, the dorsal branch is composed of cholinergic neurons and ascends from nuclei in the brainstem and basal forebrain to the thalamus, which sends signals to the cortex. Second, the ventral branch is composed of monoaminergic neurons (e.g., serotonergic and noradrenergic neurons) and ascends from several nuclei in the brainstem to the hypothalamus and basal forebrain, which transmit excitatory signals to the cortex. In order to induce sleep, the ascending excitatory signals to the cortex must be inhibited. The ventrolateral preoptic nucleus (VLPO, located in the hypothalamus) is responsible for the secretion of GABA and galanin, neurotransmitters that inhibit the activity of the monoaminergic neurons in the reticular activating system. Conversely, in the waking state, the neurotransmitters released by the monoaminergic neurons (including serotonin and noradrenaline) inhibit activation of the VLPO, maintaining a state of arousal. This bi-directional feedback loop acts as a "sleep switch" (Schwartz & Roth, 2008, p. 370) to shift the body between sleeping and waking states.

The homeostatic sleep drive is in part responsible for "flipping" the switch towards sleep (Swick, 2005). One mechanism of the sleep drive involves the accumulation of adenosine due to cellular metabolism over the waking period (Schwartz & Roth, 2008). Adenosine inhibits excitatory cholinergic neurons in the basal forebrain and dis-inhibits the GABA- and galaninergic neurons in the VLPO that promote sleep (Bjorness & Greene, 2009).

In addition to the sleep drive, arousal state is also influenced by the circadian system, known as the body's "biological clock" (Lu & Zee, 2010). The suprachiasmatic nucleus (SCN) in the hypothalamus acts as a "master clock" that synchronizes various physiological processes, including the sleep-wake cycle, with the 24-hour clock. Neuronal activity in the SCN is entrained to the 24-hour clock through exposure to periods of light and dark. The SCN sends projections to the VLPO and reticulating activating system: overall, these neural influences act to promote a state of wakefulness. Taken together, the neurobiological mechanisms involved in sleep implicate a number of neurochemical systems as potential targets for genetic studies, including GABAergic, galaninergic, acetylcholinergic, noradrenergic, and serotonergic systems.

The serotonin system and sleep. While multiple neurochemical systems are involved in the regulation of sleep processes, the present research focused specifically on the serotonin (5-HT) system as a potential moderator of the stress-sleep relationship for several reasons. First, the serotonin system is involved in stress responding (Chaouloff, Berton, & Mormède, 1999) as well as arousal and sleep-wake processes (Swick, 2005). Second, people differ in their amount of sleep disruption when exposed to a recent stressor or anticipating an upcoming stressor, a characteristic known as sleep reactivity (Drake, Richardson, Roehrs, Scofield, Roth, 2004; Drake & Roth, 2006), and genetic factors contribute to this variation (heritability estimates range from 39% to 43% in males and 26% to 29% in females; Drake, Friedman, Wright, & Roth, 2011). While specific genetic variants influencing sleep reactivity are not yet well-characterized, prior research has implicated the short allele of the serotonin transporter polymorphism, a commonly

studied genetic variant that may impede sleep during periods of heightened stress by contributing to a state of hyperarousal (Harvey, Gehrman, & Espie, 2014). Third, the three serotonin receptor subtypes (5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}) and the enzyme tryptophan hydroxylase-2 (the rate limiting enzyme in the synthesis of central serotonin) that were also examined in this study have been implicated in sleep (Chen & Miller, 2012; Landolt et al., 1999).

As noted above, serotonergic neurons (originating in the raphe nuclei) represent a component of the reticular activating system. Neuronal recordings demonstrate that serotonergic activity declines substantially across transitions from waking to NREM sleep, and finally to REM sleep, lending support to the role of serotonin in the arousal processes of the waking state (Ursin, 2002). However, it should also be noted that serotonin serves multiple, complex roles in sleep and wakefulness (for a review, see Ursin, 2002).

The role of the serotonin system was also of particular interest in this study given the longer-term goal of informing future investigations into a potential pathway to depression involving genetic factors, stress exposure, and sleep disturbance. Although research continues to evaluate the precise role of the serotonin system in the causal pathway to depression (Andrews, Bharwani, Lee, Fox, & Thomson, 2015; Cowen, 2008), empirical evidence—including findings from serotonin depletion studies, psychopharmacological studies on selective serotonin reuptake inhibitors, and gene-environment interaction studies—suggest that serotonergic dysregulation plays a role in depression (Cowen & Browning, 2015; Karg, Burmeister, & Shedden, 2011).

Gene x environment interactions and sleep. Only a small number of studies have characterized the interactive effects of molecular genetic risk and environmental factors, including stress exposure, on sleep variables (e.g., Barclay, Eley, Rijsdijk, & Gregory, 2011). In large part, this research has focused on genetic variants in the circadian system (i.e., *CLOCK* and *PER3* genes) and serotonin system (i.e., the *SLC6A4* gene encoding the serotonin transporter). In a study examining circadian system genes, the genotype of one particular *CLOCK* genetic variant significantly interacted with stress exposure, such that increased stress exposure and the presence of two copies of the variant predicted altered sleep patterns (Antypa et al., 2012).

Studies examining interactions between serotonergic genes and stress exposure in the prediction of sleep have largely focused on the serotonin transporter-linked polymorphic region, known as 5-HTTLPR, a commonly studied functional 44-base pair insertion/deletion genetic variant in the promoter region of the *SLC6A4* gene. Compared with the long (L) allele of this variant, the short (S) allele is associated with reduced transcriptional efficiency of the serotonin transporter gene (Heils et al., 1996) and heightened stress sensitivity (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010). One study focusing on the main effects of 5-HTTLPR reported a higher prevalence of the S allele among people with primary insomnia compared to controls (Deuschle et al., 2010). Further, in one study, 5-HTTLPR genotype significantly interacted with chronic stress—indicated by being a caregiver for a family member with dementia—to predict sleep quality over the past month (assessed using the Pittsburgh Quality Sleep Index, PSQI; Brummett et al., 2007). Caregivers who were homozygous for the S allele (S/S) self-

reported worse global sleep quality compared to non-caregivers with S/S, S/L, or L/L genotypes, as well as caregivers with the S/L or L/L genotypes. However, separate findings did not indicate the presence of an interaction between 5-HTTLPR genotype and episodic stress predicting sleep; rather, the L/L genotype predicted poorer PSQI global sleep quality in main effect (Barclay et al., 2011). Overall, the mixed findings in this area suggest that additional studies of gene-environment interactions are necessary to characterize the specific serotonergic genetic variants that affect sleep quality.

Additive Influence of Serotonergic Genes

To date, the majority of studies examining the main effects and interactions of genetic variables in psychological processes have focused on the role of single genetic variants. Theoretical conceptualizations, however, of behavioral genetics indicate that complex diseases and behaviors, including sleep processes, have polygenic additive bases: many genetic variants each contribute a small amount of variance to the outcome in a cumulative fashion (Fisher, 1918). One novel and theory-consistent approach developed to address the polygenic contributions of multiple genetic variants involves the use of multilocus genetic profiles (MLPs; Nikolova et al., 2011). MLPs are composed of variants from several genes that are hypothesized to act in concert to contribute to individual differences, and are created by summing values assigned to each allele based on its hypothesized relationship with the outcome. In the present study, a person's MLP score represents the total number of alleles hypothesized to confer risk for disrupted sleep in the context of heightened stress.

A recent study provided evidence for the additive influence of serotonergic genes in interaction with stress in predicting: 1) major depressive episodes among emerging adults and 2) depressive symptoms in early adolescent girls (Vrshek-Schallhorn, Stroud, Mineka, et al., 2015). To create this MLP, based on a literature review, the authors identified five single nucleotide polymorphisms (SNPs) in the serotonin system—including functional SNPs present on three serotonin (5-HT) receptor genes (*HTR1A*, *HTR2A*, and *HTR2C*) and two SNPs in the promotor region of the tryptophan hydroxylase-2 gene (*TPH2*)—as well as the specific allelic variants hypothesized to confer risk for depression.

A brief overview of the five genetic variants included in this MLP is merited. First, the *HTR1A* SNP (rs6295) is a C/G polymorphism found on the gene coding for the 5-HT_{1A} receptor. This G protein-coupled receptor is found throughout the brain, including in the raphe nuclei. Heterozygotes (C/G) and homozygotes for the G allele (G/G) have previously been found to have an increased risk for depression (Anttila et al., 2007; Lemonde et al., 2003). The *HTR2A* SNP (rs6314) is a C/T polymorphism found on the gene coding for the 5-HT_{2A} G protein-coupled receptor. Some meta-analytic results suggest an association between the C allele and suicidal behavior (Li, Duan, & He, 2006). The *HTR2C* SNP (rs6318) is a G/C polymorphism located on the gene coding for the 5-HT_{2C} G protein-coupled receptor. *HTR2C* is a sex-linked gene; because it is located on the X chromosome, females have two alleles for *HTR2C* (only one of which is expressed in any given cell) and males have one allele. The C-allele is associated with increased stress reactivity (Brummett, Babyak, Kuhn, Siegler, & Williams, 2014). The two *TPH2*

promotor SNPs (rs4570625 G/T and rs11178997 T/A) are found on the gene coding for the critical rate-limiting enzyme (tryptophan hydroxylase-2) catalyzing serotonin synthesis in the brain. A random-effects meta-analysis indicated a significant association between the rs4570625 G allele and depression risk (Gao et al., 2012). In the same study, a significant association was found between the rs11178997 T allele and risk for depression using a fixed effects model, but not a random effects model.

In the study examining serotonergic MLP and depression, higher serotonergic MLP score predicted a greater likelihood of depression onset in the context of exposure to major interpersonal episodic stressors among emerging adults, and greater depression symptoms in the context of elevated interpersonal episodic stressors in early adolescent girls. However, in both samples examined, in contexts of lower interpersonal stress, the MLP had a protective effect, such that higher MLP values corresponded to reduced risk for depression onset in emerging adults and lower symptom levels in the adolescent girls. Taken together, these findings suggest that the serotonergic MLP score might confer risk for or protection against maladaptive outcomes based on features of the environment, a concept known as differential susceptibility (Belsky & Pluess, 2009). This concept posits that, rather than solely conferring risk for negative outcomes, certain genetic variants may heighten a person's sensitivity to the environment. This heightened sensitivity may lead to *either* maladaptive or adaptive outcomes, depending on the quality of the environment.

To the writer's knowledge, no existing research has investigated a multilocus genetic profile score from any neurotransmitter system in the context of sleep variables. However, both theoretical perspectives regarding polygenic influences on behavior and

empirical findings on the role of the serotonin system in sleep suggest that evaluating a serotonergic multilocus profile score might provide a fruitful avenue for sleep-related gene-environment studies. The current research tested the serotonergic multilocus profile score previously characterized (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). Additionally, given that some empirical work has established significant associations between 5-HTTLPR variation and sleep quality, the profile also incorporated variants (i.e., the S and L alleles) in this region of the *SLC6A4* gene promoter. The *SLC6A4* gene encodes the serotonin transporter, a monoamine transporter found on presynaptic cells that facilitates the reuptake of serotonin from the synapse.

The Present Research

The present research evaluated whether a previously characterized serotonergic MLP (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015) plus 5-HTTLPR variation interacts with daily perceived hassles in a daily diary study to predict sleep quality outcomes (i.e., self-reported quality and sleep onset latency, SOL; actigraphy-based sleep onset latency and wake after sleep onset, WASO). Hassles and sleep measures were recorded daily, allowing for the temporal ordering of exposure to hassles and sleep disturbance. Multilevel regression modeling examined the hypotheses that 1) daily hassles would be significantly associated with reduced quality of sleep (including longer SOL and greater WASO) and 2) MLP score would significantly moderate the relationship between exposure to hassles and sleep quality, such that higher MLP score would predict poorer quality of sleep in the context of more hassles. Because of evidence for a differential susceptibility model in which individuals benefit from higher MLPs

under better conditions and suffer from higher MLPs under poorer environmental conditions, no main effect of the MLP on sleep variables was hypothesized.

CHAPTER II

METHOD

Participants

As part of a larger daily diary study on patterns of mood, goal-directed behaviors, HPA axis functioning, physical activity, and sleep, participants were undergraduate students recruited through the human subject research pool at the University of North Carolina at Greensboro. Students were awarded research credit for their participation. Data collection took place from February 2015 through November 2016, excluding summer and winter recesses. The minimum age for participation was 18 years, and a total of 161 participants meeting this criterion provided informed consent. Demographic data is reported for the two subsamples analyzed—one for self-reported sleep outcomes and one for actigraph-recorded outcomes—after accounting for missing day-level data and exclusions, which are described subsequently. Among participants in the subsample capturing self-reported sleep (N = 147; demographic information available for 146 participants), the average age was 18.9 years (SD = 1.65, range: 18-32) and gender composition was 77.4% female, 21.9% male, and 0.7% preferred not to say. Self-reported ethnic and racial composition was 41.1% White/Caucasian, 30.1% Black/African American, 11.6% Asian, 4.1% Hispanic/Latino, 2.7% Middle Eastern/Arab, and 10.3% multiple races/ethnicities. Among the subsample capturing actigraph-measured sleep (N =

121; demographic information available for 119 participants), the average age was 18.9 (*SD* = 1.7, range: 18-32) and gender composition was 78.2% female, 21.0% male, and 0.8% preferred not to say. Ethnic and racial composition was 42.0% White/Caucasian, 28.6% Black/African American, 11.8% Asian, 3.4% Hispanic/Latino, 2.5% Middle Eastern/Arab, and 11.8% multiple races/ethnicities.

Measures

Electronic daily diaries. Participants completed an online questionnaire (daily diary) between the hours of 5 pm and 12 am each day for 14 consecutive days. The diary assessed participants' activities that day, including school and work-related activities, and their exposure to daily hassles. The diary also assessed self-reported sleep quality on the previous night. Because actigraph measures of sleep were collected over three nights, diary data from only the three days corresponding to these time points were used for analyses of objective sleep outcomes. By contrast, 14 days of diary data were available for analysis of subjective sleep outcomes.

Daily hassles (Appendix C). Daily hassles experienced by the participant were captured using a measure targeting objective exposure to stressful daily experiences rather than emotional responses to these hassles (Sheets & Armey, 2014). Further, due to evidence of the importance of a distinction between interpersonal and non-interpersonal forms of stress in emotional functioning, questions probed both types of hassles. Interpersonal hassles assessed included being ignored or snubbed by someone, being disappointed or let down by someone, and experiencing an interpersonal conflict. Non-interpersonal hassles included whether the participant had given a presentation, taken an

exam, received a poor grade, experienced a negative event related to his/her job, or was ill or injured that day. An additional question asked participants about experiencing any other "type of stressful event" and provided space for a description. Two research assistants independently coded event descriptions as interpersonal or non-interpersonal, described subsequently.

Data preparation – Write-in hassles coding (Appendix D). A total of 248 diary entries included responses to the question, "What was the other type of stressful event you experienced today?" Research assistants coded each description as interpersonal or non-interpersonal in nature using provided instructions (Appendix D). Interpersonal hassles were defined as those primarily impacting the quality or quantity of the participant's relationships with other individuals; all other reported experiences were considered non-interpersonal hassles. Interrater reliability was calculated for a subsample of 231 entries, excluding entries that indicated no additional hassles (e.g., "N/A"; $N = 3$) and for which raters' codes could not be readily compared due to missing codes ($N = 3$), differences in the number of codes given on entries describing multiple hassles ($N = 10$), or coding in a different manner than specified in the instructions ($N = 1$). Reliability was substantial ($\kappa = 0.75$, 95% CI [0.66-0.84], $p < 0.0001$). The author then reviewed all descriptions to screen for and exclude duplicates (i.e., if a participant responded "yes" to the question about having an argument and also provided a write-in response relating to an argument or conflict on the same day, the written response was not counted in the summed interpersonal hassles score; $N = 71$). Discrepancies between raters' coding decisions were resolved through a consensus discussion. The author also excluded

descriptions that were generally agreed by the raters and author to be too vague to interpret (e.g., “drama,” “personal,” “everything is stressful”; $N = 14$). A total of 5 additional descriptions were excluded, 2 for content that appeared to indicate either a minor chore (e.g., “laundry”) or suggested a facetious response about the nature of activities in the current study, and 3 for responses that—as described previously—indicated no additional hassle. Following exclusions, including through creation of the lagged hassles variables as described subsequently, 40 interpersonal and 90 non-interpersonal written-in hassles were included in the respective summed variables.

Numbers of daily interpersonal and non-interpersonal hassles were summed and examined separately. Descriptive data was used to determine whether sufficient variation existed in the number of reported hassles to allow for construction of a dimensional score (i.e., a sum of daily events) or whether hassles should be treated categorically (i.e., whether the participant experienced at least one stressful event or no events).

Subjective sleep quality (Appendix E). Participants provided reports in the daily diary about the time taken to fall asleep, and self-reported time to sleep onset was used as a subjective measure of sleep onset latency. Subjective sleep quality was assessed through two questions on the daily diary, one of which was worded such that higher scores indicated higher sleep quality, and the other of which was worded such that higher scores indicated poorer sleep quality. Responses to this latter question were reverse scored, and the responses to the two questions were then averaged to generate a subjective sleep quality composite score, with higher scores corresponding to better sleep quality.

Actigraph and objective sleep quality. Participants wore actigraphs (wActiSleep+, ActiGraph, Pensacola, Florida) on their non-dominant wrists during the first three days of the 14-day diary study. Using ActiLife software (ActiGraph), data collection was programmed to begin at 5 pm on the day of the initial laboratory visit (day 0) and to continue until 5 pm on the third day of the study. During the initial lab visit, participants provided information on height, weight, gender, and dominant hand to the research assistant, who inputted these variables into ActiLife for calibration. A sampling rate of 30 Hz was selected. Participants were instructed to wear the actigraph on their non-dominant wrists and to remove the device only when bathing or swimming. Objective sleep quality was assessed by examining sleep onset latency (SOL) and wake after sleep onset (WASO). Because SOL and WASO measure distinct phenomena (early insomnia, or difficulty initiating sleep, versus middle insomnia, or difficulty maintaining sleep), they were treated separately in analyses. SOL and WASO were calculated for each of the three nights using ActiLife software and the automatic scoring algorithm developed by Cole, Kripke, Gruen, Mullaney, and Gillin (1992). Sleep variables are reported in minutes.

DNA collection and genotyping. Saliva samples were collected from participants by passive drool during the initial laboratory session, and samples were stored at -20 °C. DNA genotyping for SNPs rs6295, rs6314, rs6318, rs11178997, and rs4570625 was performed by the Molecular Core Lab at the University of North Carolina at Greensboro. Briefly, DNA was extracted from thawed saliva samples using a Maxwell 16 Cell DNA Purification Kit and Maxwell 16 MDx Instrument (Promega, Madison, Wisconsin).

Amplification and allelic discrimination at each SNP were performed using TaqMan SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, Massachusetts) on an ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, California). Genotyping for the 5-HTTLPR variant was performed at the University of Wisconsin-Madison Biotechnology Center. Briefly, 5-HTTLPR was amplified with PCR using a Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany) and submitted to a restriction enzyme digest (MspI (HpaII), Thermo Fisher Scientific). Sizes of resulting fragments were then determined (ABI 3730XL Genetic Analyzer, Life Technologies, Carlsbad, California; and GeneMark HID v2.6.0 STR Human Identity Software, SoftGenetics, State College, Pennsylvania).

Serotonergic multilocus profile score (Table 1). The multilocus profile score was constructed using six polymorphisms (*HTR1A* rs6295, *HTR2A* rs6314, *HTR2C* rs6318, *TPH2* rs11178997, *TPH2* rs4570625, and *SLC6A4* 5-HTTLPR) located in and near serotonin system genes. SNP alleles, with the exception of *HTR2C* rs6318, were assigned values of 0 or 1 corresponding to hypothesized risk for greater stress-sensitivity in sleep disruption, with higher scores indicating greater hypothesized risk in keeping with a prior report on stress-sensitivity and depression (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015; Table 1). As *HTR2C* rs6318 is sex-linked—meaning that males have one copy of the allele and females have two copies, with only one allele expressed in any given cell—this SNP was coded as 1 or 0 for C- or G-carrier status, respectively. SLC6A4 5-HTTLPR, not included in the prior report, was coded such that the short ("S") allele was assigned a value of 1, and the long ("L") allele was assigned a

value of 0. Risk scores were then summed to generate an overall profile score. Higher profile scores indicate a higher risk for sleep disruption based on a greater number of "risk" alleles. Participants missing one genotype in the MLP were included by prorating the score based on the participant's remaining number of alleles (i.e., the number of risk alleles was divided by the total number of alleles and then multiplied by 11, the number of alleles in the full profile). For example, if 9 out of 11 possible alleles were present and the number of risk alleles was 6, the prorated score is 7.33 (i.e., 0.67 x 11 possible alleles). Individuals missing more than one genotype ($N = 1$) were excluded from analyses.

Data Preparation

Lagging hassles variables. To examine the relationship between the previous day's hassles and the same night's sleep quality, diary entries completed more than 36 hours or less than 12 hours apart were excluded from analysis. Additionally, single diary entries that were completed over a period of 10 or more hours were excluded from analysis. Creation of the lagged hassles variable also eliminated entries on days that were not preceded by an entry, including the first diary entry completed. If a diary was completed on the following day, the hassles reported on the previous day's entry were shifted to the following day. A hypothetical example is provided to illustrate this data preparation: A participant completed six diaries, including on February 1st at 5 pm, February 2nd at 7 pm, February 3rd at 9 pm, February 4th at 7 am, February 5th at 11 pm, and February 6th at 10 pm. The entry on February 4th would be eliminated because it was completed less than 12 hours following the previous entry. The hassles reported on

February 1st, 2nd, and 5th would be lagged, such that they would appear in the following day's entry. The entries on February 2nd, 3rd, and 6th would be included in analysis because both previous day's hassles and previous night's sleep quality variables would be available. Prior to exclusions due to missing diary entries, 1591 diary entries from 156 participants were available (6 participants with intake measures were missing all diary entries, and 1 participant with diary entries was missing intake measures). In total, 310 entries were removed because they were completed more than 36 or less than 12 hours apart from the preceding or subsequent entry, and 3 of these entries were also started and completed over a period greater than 10 hours. Following lagging of hassles, 1234 entries from 148 participants were available for analyses.

Actigraph compliance and sleep scoring. Participants' compliance with actigraph use was evaluated through answers to the daily diary question, "Did you wear an actigraph watch for the study today?" Prior to removal of non-consecutive diary entries for lagged analysis of previous day's hassles, 151 participants (93.8% of the total sample) responded that they had worn the actigraph during the study (M = 2.86 days, SD = 0.97, range: 1-5 days). In all, 435 entries indicated that the actigraph was worn, 1133 entries reported that it was no longer scheduled to be worn, and 12 entries indicated that the actigraph was scheduled to have been worn but was not.

Responses to actigraph compliance questions—"Since your last diary entry, did you take off your actigraph watch off for any reason except to bathe?", "Why did you take it off?", and "For what period(s) of time did you take it off"?—and to the items, "I took a nap today" and "Please list what time your nap began and ended" were used to

screen actigraphy files for anomalous sleep periods. Files for participants who indicated their actigraphs were removed or they took a nap were examined to determine whether substantial discrepancies existed between actigraph- and self-reported sleep and wake times. Given that discrepancies between participants' self-reports and actigraph measurements of sleep variables have been established in the literature (Lauderdale, Knutson, Yan, Liu, & Rathouz, 2008), bed and wake time adjustments were conservative: If self-report and actigraph times differed by 3 hours or more, self-reported bed or wake time was inputted into the corresponding actigraph sleep period. An exception to this guideline was used in cases in which a participant's self-reported nap time or non-wear period overlapped with a bed or wake time. Adjustments to wake/bed times that were less discrepant than 3 hours were made if warranted by examination of participant's diary responses and corresponding sleep periods.

Additionally, all files were checked for the number of sleep periods reported, and files with more or less than 3 sleep periods—corresponding to the number of nights participants were asked to wear an actigraph—were compared to diary reports. Sleep periods during the day were removed. Sleep periods were added via visual inspection of actigrams and use of self-reported bed and wake times when available. Actigraph sleep periods were also examined for length: For periods longer than 12 hours, bed and wake times were compared to diary reports. When times were discrepant by at least three hours, self-reported times were inputted into actigraphy files. Actigraphy entries that recorded zero activity counts during sleep, indicating a nonwear period, were removed. Table 2 details the reasons for adjustments to sleep periods and provides the respective

number of participants whose files were adjusted. In total, actigraphy files from 48 participants were adjusted based on the data preparation protocol described. Additionally, actigraph data was missing from 18 participants, the reasons for which are also detailed in Table 2.

Procedure

Undergraduate research assistants who were trained in study protocols conducted the initial laboratory session, which occurred on either a Monday or Tuesday to ensure that all actigraphy collection occurred during weekdays. During the session, following informed consent procedures and overview of the study, participants provided saliva samples for DNA extraction and completed a battery of baseline questionnaires, including demographic information. Research assistants then presented a brief slideshow to participants to provide guidelines for use of the actigraph. The procedure for completion of nightly diaries was also reviewed, including completion between 5 pm and midnight each day. It was explained to participants that study credit was contingent upon completion of at least 12 of 14 diaries and an exit survey. A Qualtrics link containing the daily diary questions was sent to participants at 5 pm each day. Completion of surveys was checked by research assistants, and follow-up emails or phone calls were initiated to remind participants about survey completion. Participants returned equipment, including actigraphs, to the lab on the fourth day (Thursday or Friday) of data collection.

Analytic Plan

Preliminary analyses. Descriptive statistics (means, standard deviations, skew, and kurtosis) were examined for hassles variables, MLP score, and sleep outcomes.

Genetic data was checked for deviation from Hardy-Weinberg Equilibrium using chi-squared tests and evidence for gene-environment correlations was examined using zero-order correlations between MLP score and hassles variables.

Power considerations. Past studies have not examined interactions between a multilocus genetic profile score and exposure to daily hassles in predicting sleep quality, and therefore effect size estimates are not yet well-established. However, among a sample of emerging adults ($N = 387$), findings of a significant interaction between a serotonergic profile score and life stress in predicting depression risk replicated in a second and smaller sample ($N = 105$; Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). Given the sample size of the current study ($N = 161$) as well as the use of repeated measures, adequate power was expected.

Multilevel regression modeling (MRM; Appendix F). Due to the nested data structure (days within people) and collection of repeated measurements for each participant, multilevel modeling was used to address the two hypotheses. Intraclass correlation coefficients were examined for unconditional models of all sleep outcomes to verify the presence of between-person clustering of data, which would support an MRM approach (Appendix F, Table 5). Modeling of actigraph-measured SOL produced a very low ICC (0.01), suggesting that a non-nested approach such as multiple linear regression was a more appropriate choice for modeling this outcome. The remaining three sleep outcomes (self-reported quality and SOL, and actigraph-measured WASO) produced higher ICC values, providing support for the use of MRM in the remaining models.

Within-person or day-level variables (Level 1) for MRM analyses include the interpersonal and non-interpersonal daily hassles and sleep outcome. Because the distribution of hassles variables deviated significantly from normality, hassles were collapsed into dichotomous variables and coded as 0 for the absence of daily hassles and 1 for the presence of at least one hassle, by type (i.e., interpersonal or non-interpersonal). Serotonergic MLP score represents the between-person variable (Level 2), and this data was grand-mean centered, following accepted best practices (Enders & Tofighi, 2007).

Two sets of MRM analyses were conducted, including within-person tests of the effects of hassles on sleep, and cross-level within- and between-person tests examining gene-environment (GxE) interaction effects. First, within-person analyses tested the hypothesis that exposure to daily hassles during the day would predict worse sleep quality that evening (i.e., longer self-reported SOL, increased actigraph-measured WASO, and poorer self-reported sleep quality). For this hypothesis, six models were tested in total, including either interpersonal or non-interpersonal hassles as a covariate and actigraph-measured WASO, self-reported SOL or self-reported sleep quality as the outcome (Appendix F, Tables 6 and 8). Second, the hypothesis regarding a GxE effect was tested using an analysis of a cross-level interaction between the level-2 and level-1 predictors (MLP and hassles, respectively). For this hypothesis, models were tested in total and incorporated interpersonal or non-interpersonal hassles at level-1 and serotonergic MLP score at level-2 (Appendix F, Tables 7 and 9). Across all MRM analyses, multivariate likelihood-ratio tests were used to determine the most parsimonious model (random-intercept or random-intercept and -slope) by comparison of

deviance statistics across models. The magnitude of difference in the deviance statistic between models with and without a random slope must be greater than or equal to a critical chi-square value with degrees of freedom equaling the difference in number of parameters between the two models.

Additionally, multiple linear regression with aggregated variables was used to test predictions for average actigraph-measured SOL over the study period. In these analyses, the hassles variables represented the proportion of the number of study days in which at least one interpersonal or non-interpersonal hassle, respectively, was endorsed compared to the total number of completed diaries. Hassles variables and MLP scores were centered prior to construction of interaction terms (i.e., MLP x IP Hassles and MLP x Non-IP Hassles). Two sets of analyses were used, including testing of the main effects of each of the two hassles types on actigraph-measured SOL using simple linear regression (Appendix F, Table 8), and examination of the simple main and interactive effects of hassles and MLP score using multiple linear regression (Appendix F, Table 9).

To account for multiple testing, we planned False Discovery Rate (FDR) adjustment (Benjamini & Hochberg, 1995) separately for interpersonal hassles and non-interpersonal hassles (i.e., correcting across four tests) in the event of significant genetic findings. Adjusted q-values of $q \leq .05$ are considered significant.

Post-hoc tests. To probe significant interactions between serotonergic MLP and the dichotomous daily hassles variables, simple slope analyses were planned. These tests would allow for examination of the influence of the serotonergic MLP score on sleep

with (hassle = 1) and without a daily hassle (hassle = 0) (Curran, Bauer, & Willoughby, 2006; Preacher, Curran, & Bauer, 2006).

Further, to probe the influence of significant GxE effects, two sets of post-hoc sensitivity analyses were planned, following prior work (Vrshek-Schallhorn, Stroud, Mineka, Zinbarg, et al., 2015). An "*N*-1" analysis involves subtraction of one genetic variant from the MLP to create six unique multilocus profiles, each composed of five SNPs. Each of these profiles would then be run in the previously described analyses to determine whether the profile score would be robust to the deletion of any one variant. Similarly, GxE tests would also be conducted separately with each variant to evaluate whether each SNP contributed to the overall significant MLP GxE test in the predicted direction. Further, these tests would provide estimates of effect sizes and allow evaluation of the similarity of effect sizes across the variants.

CHAPTER III

RESULTS

Descriptive Statistics

Daily hassles. Following lagging, perceived interpersonal hassles ($N = 453$; over study period: $M = 2.01$, $SD = 1.90$, range: 0-9) were reported in a total of 293 diary entries and perceived non-interpersonal hassles ($N = 516$; over study period: $M = 2.77$, $SD = 2.08$, range: 0-10) were reported in 410 entries. Figure 1 (Appendix B) illustrates the total number of endorsements for each individual hassle (e.g., argument, exam, bad grade). Given the different numbers of diary entries available across participants, ratios were calculated to reflect the total number of hassles (interpersonal and non-interpersonal, respectively) occurring over the number of study days for each person. The ratio scores indicated a mean of 0.27 interpersonal hassles per day ($N = 147$, $SD = 0.26$) and 0.34 non-interpersonal hassles per day ($N = 148$, $SD = 0.24$). Both the summed interpersonal and non-interpersonal hassles variables and their respective ratio scores were non-normally distributed (Interpersonal hassles ratio score: $W(147) = 0.87$, $p < .001$; Non-interpersonal hassles ratio score: $W(147) = 0.93$, $p < .001$). Therefore, hassles were treated categorically, such that one or more reported hassles were coded as *present* and no reported hassles were coded as *absent*.

DNA. Genotyping was completed for 159 participants, and a serotonergic multilocus profile score was calculated for 158 participants. Up to 1 missing genotype

was permitted per participant, resulting in exclusion of $N = 1$ participant missing 2 genotypes. Profile scores for those missing 1 genotype were prorated as described above. The six variants were checked for Hardy-Weinberg equilibrium (HWE) using genotype frequencies in chi-square tests (Table 3). Because the *HTR2C* gene is sex-linked, the chi-square test for rs6318 included female participants only (i.e., males have a single copy of the gene and thus violate the assumptions of the HWE test). The variants rs6295, rs6314, rs 6318, rs11178997, and rs4570625 were in HWE. The 5-HTTLPR deviated significantly from HWE ($\chi^2 = 7.81$; $p = .005$). Therefore, analyses were conducted both including and excluding the 5-HTTLPR score in the serotonergic MLP. Scores were normally distributed, and descriptive information is provided in Table 4.

Self-reported sleep quality. Self-reported previous night's sleep variables, including sleep onset latency (SOL) and sleep quality, were available in 1222 and 1217 diary entries, respectively. Average sleep quality, but not SOL, was normally distributed (quality: $W(148) = 1.00$, $p = .94$; SOL: $W(148) = 0.70$, $p < .001$). Overall, participants tended to report fair to high quality sleep on average ($M = 4.57$ min, $SD = 0.97$, range: 2.25-7) and short average latency periods ($M = 24.94$ min, $SD = 24.38$, range: 1.90-180 min).

Actigraph-recorded sleep quality. Actigraph-recorded SOL and time awake after sleep onset (WASO) were available for 276 nights. Overall, average SOL was short ($M = 1.88$ minutes, $SD = 3.89$, range 0-78 min), while WASO was longer ($M = 50.62$ minutes, $SD = 26.16$, range 1 – 322 min). Distributions of average SOL and WASO for

participants deviated from normality (SOL: $W(122) = 0.39$, $p < .001$; WASO: $W(122) = 0.94$, $p < .001$).

Zero-order correlations (Table 4). Gene-environment (G-E) correlations were evaluated to examine whether serotonergic MLP score is associated with potential self-selection into environments with more hassles. To account for the unequal number of diary entries completed across participants, proportions were calculated to represent the total number of days in which one or more hassle (interpersonal or non-interpersonal, respectively) was endorsed compared to the total number of diaries completed. The respective G-E correlations between MLP score with 5-HTTLPR and proportions scores for interpersonal and non-interpersonal hassles were both non-significant. This indicates that participants did not report more days affected by hassles as a function of the MLP, reducing the likelihood that MLP influenced either self-selection into more stressful environments or biased recall of hassles. Additionally, the correlation between MLP score without 5-HTTLPR and interpersonal hassles proportion score was non-significant, while the association between this MLP and the non-interpersonal hassles proportion score was positive and significant ($p = .05$).

Zero-order correlations were also calculated for the remaining predictor and outcome variables. The two MLP scores (with and without the 5-HTTLPR) were significantly associated ($r = .88$, $p < .001$). Interpersonal and non-interpersonal hassles proportion scores were also positively correlated ($r = .22$, $p < .01$), consistent with previous findings of correlations between different forms of stress (Vrshek-Schallhorn, Stroud, Mineka, Hammen, et al., 2015). Average self-reported sleep quality and SOL

were negatively correlated (r = -.32, p < .001), such that longer SOL was associated with worse sleep quality. Average actigraph-recorded SOL and WASO were positively correlated (r = .23, p < .05), such that longer time to sleep onset was associated with more time awake throughout the sleep period. Unexpectedly, interpersonal hassles proportion score was negatively associated with WASO (r = -.24, p = .01); however, this should be interpreted cautiously as the hassles proportion score was calculated over the entire study period, whereas actigraph-measured WASO was collected for a subset of that period.

Multilevel Regression and Multiple Linear Regression Modeling

The results from multilevel regression and multiple linear regression modeling are presented separately for self-reported and actigraph-measured sleep quality variables. Results are summarized in the text with t- and p-values, and models with full tables are presented in Appendix F. For MRM, multivariate likelihood-ratio tests indicated that random-intercept models were parsimonious for all models with self-reported outcomes and for models predicting WASO with interpersonal hassles as a covariate. Random-intercept and -slope models provided an improved fit for predicting WASO with non-interpersonal hassles as a covariate.

Self-reported sleep outcomes.

Main effects of hassles. Taken together, results suggest that neither subjective global sleep quality or latency depended on the endorsement of interpersonal or non-interpersonal hassles that day. Interpersonal hassles did not significantly predict SOL (t =

1.14; p = .26) or quality (t = -0.19; p = .85). Non-interpersonal hassles also did not significantly predict SOL (t = 0.62; p = .53) or quality (t = 0.79; p = 0.45).

Interaction effects of hassles x 5-HT MLP. Results from models examining the interactive effects of daily hassles and MLP score indicate that the relationships between hassles and self-reported sleep outcomes did not significantly depend on participants' serotonergic genetic variation. Simple main effects of MLP scores (with or without 5-HTTLPR) and interpersonal hassles did not predict SOL (MLP with 5-HTTLPR: t = 1.19; p = .24; Interpersonal hassles: t = 1.14, p = .26). Similarly, no significant simple main effects of either MLP (with or without 5-HTTLPR) or interpersonal hassles were observed for self-reported quality (MLP with 5-HTTLPR: t = -1.10; p = .27; Interpersonal hassles: t = -0.19, p = .85). Finally, the interaction between MLP score (with or without 5-HTTLPR) and interpersonal hassles did not significantly predict SOL (t = -0.06; p = .95) or quality (t = 0.60; p = .55).

Similar to models with interpersonal hassles, those incorporating non-interpersonal hassles did not significantly predict self-reported sleep outcomes. Specifically, simple main effects of MLP score (with or without 5-HTTLPR) and non-interpersonal hassles did not predict SOL (MLP with 5-HTTLPR: t = 0.91; p = .37; Non-interpersonal hassles: t = 0.57, p = .57) or quality (MLP with 5-HTTLPR: t = -1.32; p = .19; Non-interpersonal hassles: t = 0.76, p = .45). The interaction between MLP score (with or without 5-HTTLPR) and non-interpersonal hassles was also not significant in predicting SOL (t = 0.63; p = .53) or quality (t = 1.12; p = .26).

Actigraph-measured sleep outcomes.

Main effects of hassles. Overall, results indicated that neither actigraph-recorded SOL or WASO depended on the hassles that occurred the previous day. Specifically, interpersonal (t = -0.33; p = .74) and non-interpersonal hassles (t = -0.95; p = .34) were not significantly associated with SOL. Hassles also did not significantly predict WASO in main effect (Interpersonal hassles: t = -1.47; p = .15; non-interpersonal hassles: t = 0.36; p = .72).

Interaction effects of hassles x 5-HT MLP. Taken together, results from modeling of interactions suggested that serotonergic genetic variation did not significantly influence the relationship between hassles and actigraph-measured sleep outcomes. Simple main effects of hassles were not significant in the models for SOL (Interpersonal hassles: t = -0.41; p = .68; non-interpersonal hassles: t = -1.01; p = .31). In models for WASO, simple main effects of both types of hassles were also non-significant (Interpersonal: t = -1.53; p = .13; non-interpersonal: t = 0.28; p = .78).

Serotonergic MLP score (with and without 5-HTTLPR) did not predict either sleep outcome as a simple main effect in models with interpersonal hassles (MLP with 5-HTTLPR; SOL: t = 1.02; p = 0.31; WASO: t = 1.83; p = 0.07) or non-interpersonal hassles (MLP with 5-HTTLPR; SOL: t = 1.26; p = 0.21; WASO: t = 1.03; p = 0.31). Although the simple main effect of the MLP with 5-HTTLPR approached significance in the model with interpersonal hassles predicting WASO, this result was not observed for the MLP without 5-HTTLPR. Cross-level interactions between MLP score and interpersonal or non-interpersonal hassles were not significantly associated with

actigraph-measured SOL (MLP with 5-HTTLPR and interpersonal hassles: $t = 1.32$; $p = 0.19$; MLP with non-interpersonal hassles: $t = -0.98$; $p = 0.33$) or WASO (MLP with 5-HTTLPR and interpersonal hassles: $t = -0.87$; $p = 0.39$; MLP with non-interpersonal hassles: $t = 0.16$; $p = 0.87$). Analyses incorporating the MLP without 5HTTLPR also yielded non-significant findings.

CHAPTER IV

DISCUSSION

The results from this study suggest, contrary to predictions, that the sleep quality indicators examined through both subjective and actigraphic measures did not significantly depend on interpersonal or non-interpersonal hassles reported on the previous day. Further, also contrary to predictions, participants' serotonergic genetic variation—examined in a polygenic manner through a multilocus profile score—did not moderate the relationship between either type of hassle and the sleep quality variables. This study used a repeated-measures, diary approach to capture temporal patterns of perceived daily life stress and sleep quality, measured through both subjective and objective assessment. The design also incorporated DNA analysis of multiple functional variants in the serotonergic system, allowing for testing of a gene-environment interaction between a serotonergic multilocus profile score and daily hassles in the prediction of sleep quality outcomes. Results from this study have several implications for future research.

Main Effects of Hassles

Contrary to hypotheses that hassles would be associated with poorer sleep, interpersonal and non-interpersonal hassles—treated dichotomously as present or absent on the previous day—did not significantly predict any of the four sleep quality outcomes

in main effect models. Although past research has established a relationship between various forms of stress and sleep quality (e.g., Åkerstedt, 2006), findings from previous main effects models of self-reported daily stressors predicting sleep quality are not uniformly significant (e.g., Hanson & Chen, 2010; Van Laethem, Beckers, van Hooff, Dijksterhuis, & Geurts, 2016).

Methodological considerations. One methodological consideration concerns the endorsements of hassles during the study period. A substantial majority of diary entries did not indicate exposure to any interpersonal or non-interpersonal hassles on the prior day, resulting in non-normal distributions and treatment of hassles as present or absent. Further, missing diary entries over the study period resulted in loss of day-level data during lagging of hassles due to the necessity of consecutive diaries for analysis of the relationship between previous day's hassles and previous night's sleep. Relatedly, through examination of responses to the write-in hassle item, it appeared that participants experienced additional hassles that were not captured on the measure but that were perceived as bothersome. Taking these considerations into account, power to detect significant effects was likely lower than anticipated.

A second methodological consideration relates to the sleep characteristics of the population in this study. Contrary to previous work demonstrating disrupted sleep among college students (Lund, Reider, Whiting, & Prichard, 2010), the present sample largely reported satisfactory sleep quality on average. Actigraph measures indicated shorter average SOL and slightly longer WASO periods compared to a typical sleep profile (Spriggs, 2015). Overall, this study captured sleep patterns of a healthy sample who also

endorsed few daily hassles, which likely reduced explainable variance in regression analyses.

Theoretical considerations. In addition to the range constraints among sample variables, a related theoretical consideration arises from the characteristics of the sample. Sleep reactivity, a trait characterizing sleep disruption in the context of stress, differs across people and is heightened in those with chronic insomnia (Drake & Roth, 2006). The present study modeled sleep reactivity in a sample in which sleep was in the relatively healthy range, therefore stress-related changes in sleep may have been smaller than would have been observed in at-risk populations or those with sleep disorders.

Additionally, the proportion variable capturing the number of days with at least one interpersonal hassle was significantly correlated with actigraph-measured WASO, such that a greater proportion of study days with at least one interpersonal hassle was associated with *less* time spent awake throughout the night. This unexpected finding should be interpreted with caution due to the difference in time periods captured by each variable (i.e., hassles proportion score was calculated for the entire two-week study period while WASO was measured over the first three nights only). However, this association hints at the possibility of associations between interpersonal hassles and sleep quality in an unanticipated direction, such that interpersonal hassles may be associated with relatively *better* sleep under certain circumstances.

Some previous evidence supports this relationship: In a repeated-measures study with a healthy sample of emerging adult women, more daily stress was associated with greater somatic arousal, which in turn predicted increased sleep efficiency (Winzeler et

al., 2014). Among other possible explanations, the authors briefly note that this relationship might represent a healthy physiological response to challenge. This explanation is consistent with theories that sleep serves an important role in emotion regulation processes, including through consolidation of memories (Deliens, Gilson, & Peigneux, 2014). In a healthy population, it is possible that the negative aspects of sleep reactivity unfold through exposure to stressors of greater severity, a process that was likely not captured in the present study.

GxE Effects of Hassles and MLP Score

Similar to main effect models, all simple main and interactive effects between interpersonal and non-interpersonal daily hassles and serotonergic MLP were non-significant in models for the four sleep outcomes. These results deviated from the prediction of a significant moderating role for MLP score on the relationship between hassles and sleep quality variables. The simple main effect for MLP score predicting actigraph-measured WASO with interpersonal hassles as a covariate approached significance; however, this finding was not observed when the MLP without 5-HTTLPR was tested in the model. The literature examining interactions between molecular genetic risk and stress exposure in predicting sleep variables is limited and does not yet contain robust findings regarding the particular genetic variants that contribute to sleep reactivity.

Methodological considerations. Preliminary examination of genotype frequencies revealed that the 5-HTTLPR variant deviated from Hardy-Weinberg equilibrium. This suggests possible errors during genotyping, which may introduce bias into estimates of effect size (Trikalinos, Salanti, Khoury, & Ioannidis, 2006), or that 5-

HTTLPR genotype frequencies may vary across racial subgroups. Post-hoc chi-squared tests were conducted separately in White and Black/African American subgroups to determine whether the deviation reflects racial stratification of genotype frequencies. These tests indicated that the 5-HTTLPR did not deviate from Hardy-Weinberg equilibrium within the two subgroups (Black/African American group: $\chi^2 = 0.002$, $p = .96$; White group: $\chi^2 = 0.26$, $p = .61$), providing support for the validity of tests using the six-variant MLP (with the 5-HTTLPR). Further, the MLP with and without the 5-HTTLPR produced similar results across analyses, with the exception of the model with interpersonal hassles, MLP, and their interaction predicting actigraph-measured WASO. Based on the similar pattern of findings, we conclude that the results for the full six-variant MLP are sound. The results suggest that the effect size of the relationship between MLP and sleep outcomes is likely small when sleep quality of the sample is predominantly adequate. Additionally, tests of gene-environment interactions in this study were limited by the weak effects of hassles on sleep outcomes.

Theoretical considerations. Given the methodological considerations discussed previously that appear to have contributed to reduced power, it would not be appropriate to draw a conclusion based on the null findings in this study that forms of stress and serotonergic MLP do not influence sleep quality. It is possible that undetected small to medium effect sizes characterize the relationships between hassles, serotonergic genetic variation, and sleep outcomes; however, range restrictions reduced power to detect these effects. Another consideration regards the severity of stress required to disrupt sleep quality among non-clinical populations. Further, although daily hassles such as those

measured here have predicted changes in self-reported negative and positive affect in prior work, it may be that more robust stressors are necessary to perturb sleep functioning in a relatively healthy emerging adult population.

A second important consideration raised by the results of this study regards the mechanism by which genes and environment interact to disrupt sleep. Additional intermediary factors along the pathway to sleep disruption merit identification and further exploration, particularly in the context of GxE studies. Rather than exerting a direct effect on sleep outcomes, serotonergic genetic variation and exposure to daily stress might interact to influence more proximal variables, such as perseverative cognitions and pre-sleep arousal, which in turn may affect distal outcomes.

Limitations

The current study's strengths include use of a repeated measures approach, multimethod assessment of sleep variables including both self-report and objective indicators, and testing of effects of a multilocus genetic profile, which examined the polygenic influence of genes in the serotonergic system on sleep outcomes; however, there were several limitations as well. First, apparent restriction of range in the hassles variable, along with the loss of participants through removal of non-consecutive diary entries, likely resulted in lower than anticipated statistical power. This precludes firm determination that there is not a predictive role of daily hassles on sleep outcomes, although it seems reasonable to conclude that there are not likely *large* effects of these types of hassles on sleep quality in this population. Relatedly, examination of responses to the write-in hassle item revealed that participants experienced additional bothersome

events that were not included in the hassles measure, which future investigations might assess in all participants.

Future Directions

Past investigations of the interactive effects between stress and person-level variables, such as cognitive response style, in predicting sleep outcomes inform potential mediating factors and intermediary outcomes along the pathway from genes and stress to sleep disturbance. Specifically, patterns of perseverative thought, including rumination, represent aspects of stress *responding* that might serve as proximal mediators, and pre-sleep arousal—a critical variable related to sleep disturbance in models of insomnia (Drake & Roth, 2006)—represents a potential intermediary outcome.

Research supports roles for these intermediate variables in the pathway from genes and stress exposure to sleep disturbance. For example, in one study, the average perceived severity and impact of daily stress was higher among people with insomnia compared to the same amount of stress in healthy controls (Morin, Rodrigue, & Ivers, 2003), suggesting variation in the appraisal of daily stress—rather than magnitude of stress exposure—between the groups. Differences in cognitive responses to stress between people with and without insomnia can inform research on sleep disturbance in healthy populations. For example, among a sample of doctoral students, perseverative cognitions at bedtime or during the sleep period significantly mediated the relationship between perceived stress and objective sleep efficiency and subjective sleep quality (Van Laethem et al., 2016). In a separate study, an interaction between trait rumination and state rumination in response to a stressor predicted actigraph-measured SOL, such that

higher trait rumination and state rumination resulted in longer SOL (Zoccola, Dickerson, & Lam, 2009).

Additionally, pre-sleep arousal represents a potential intermediary outcome variable to be explored in gene-environment interaction studies. In comparison to a healthy control group, participants with insomnia reported elevated daily cognitive and somatic pre-sleep arousal, and across all participants, bedtime arousal level mediated the relationships between exposure to and perceived impact of daily stress and subjective sleep efficiency and quality (Morin, Rodrigue, & Ivers, 2003). A separate study also established mediating roles for cognitive and somatic arousal in the stress-sleep relationship: When examined across people, heightened daily stress and heightened somatic arousal resulted in worse subjective sleep quality, and when examined at the within-person level, higher daily stress and increased cognitive arousal were associated with worse sleep (Winzeler et al., 2014). Taken together, evidence points to roles for rumination and pre-sleep arousal along the pathway to sleep disturbance that would likely enhance the explanatory power of the preliminary gene-environment model examined in this study.

Conclusion

The present study tested relationships between genetic variation, stress, and sleep functioning in a repeated-measures, daily diary study. Specifically, polygenic variation in the serotonergic system and exposure to daily hassles were examined as predictors of self-reported and actigraph-measured sleep variables over multiple study days. Modeling of main effects for daily hassles as well as for simple main and interactive effects of

hassles and serotonergic MLP on both self-reported and objective sleep outcomes produced non-significant results across outcomes. Methodological and theoretical considerations have implications for future research, including exploration of additional forms of life stress contributing to sleep quality, as well as inclusion of intermediary variables, such as patterns of perseverative thought and pre-sleep arousal, in theoretical accounts of the action of genes and stress on sleep quality.

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APPENDIX A

TABLES

Table 1

Genetic Variants in the Serotonergic Multilocus Profile and Genotype Scores

Polymorphism	**Genotypes**	**Coding**[a]
HTR1A rs6295	G/G	2
	G/C	1
	C/C	0
HTR2A rs6314	C/C	2
	C/T	1
	T/T	0
HTR2C rs6318	C-carrier; C/C	1
	G-carrier; G/G	0
TPH2 rs11178997	T/T	2
	T/A	1
	A/A	0
TPH2 rs4570625	G/G	2
	G/T	1
	T/T	0
SLC6A4 5-HTTLPR	S/S	2
	S/L	1
	L/L	0

[a] Higher scores for genotypes indicate greater hypothesized risk for sleep disruption in the context of increased daily hassles.

Table 2

Adjustments to Actigraphy Files and Missing Actigraphy Data

N (participants)	Adjustments
33	Waketime and/or bedtime adjusted
20	Sleep period removed (due to > 3 sleep periods or total actigraph counts = 0)
6	Sleep period added
N (participants)	Missing Actigraphy Data
6	Actigraphs not initialized at intake appointment
3	Raw actigraphy data file could not be located
4	No daily diary, DNA, or actigraphy data available
3	No diary or actigraphy data available (DNA data available)
2	Scoring algorithm detected no sleep periods over study period

Table 3

Hardy-Weinberg Results

SNP (# alleles/genotype)	*N*	0 (%)	1 (%)	2 (%)	χ^2	*p*-value
HTR1A rs6295 (G alleles)	159	42 (26.4)	87 (54.7)	30 (18.9)	1.61	.20
HTR2A rs6314 (C alleles)	158	1 (0.6)	22 (13.9)	135 (85.4)	0.01	.92
HTR2C rs6318 (C alleles)[a]	118	71 (60.2)	39 (33.1)	8 (6.78)	0.67	.41
TPH2 rs11178997 (T alleles)	158	5 (3.16)	41 (25.9)	112 (70.9)	0.27	.60
TPH2 rs4570625 (G alleles)	154	21 (13.6)	59 (38.3)	74 (48.1)	2.64	.10
SLC6A4 5-HTTLPR (S alleles)[b]	155	63 (40.6)	58 (37.4)	34 (21.9)	7.81	.005*

[a] Sex-linked gene; Chi-square calculated for women only

[b] 5-HTTLPR treated as biallelic variant (long and short alleles)

*$p < 0.05$

Table 4

Zero-Order Correlations

Measure	*M* (*SD*)	1	2	3	4	5	6	7	8
1. IP Hassles[a]	.27 (.26)	-	-	-	-	-	-	-	-
2. Non-IP Hassles[a]	.34 (.24)	.22*	-	-	-	-	-	-	-
3. Six-SNP MLP	6.99 (1.65)	.02	.13	-	-	-	-	-	-
4. Five-SNP MLP	6.16 (1.40)	.003	.16*	.88**	-	-	-	-	-
5. SOL (self-report)	24.94 (24.38)	.09	.03	.09	.06	-	-	-	-
6. Quality (self-report)	4.57 (0.97)	-.13	-.03	-.06	-.03	-.32**	-	-	-
7. SOL (actigraph)	1.88 (3.89)	-.0004	.15	.09	.03	-.01	-.05	-	-
8. WASO (actigraph)	50.62 (26.16)	-.24**	.12	.15	.11	.13	-.08	.23*	-

[a] IP and non-IP Hassles variables represent proportions (# days with at least one hassle / # study days). All other variables represent participant averages.

*$p \leq 0.05$

**$p \leq 0.01$

APPENDIX B

FIGURES

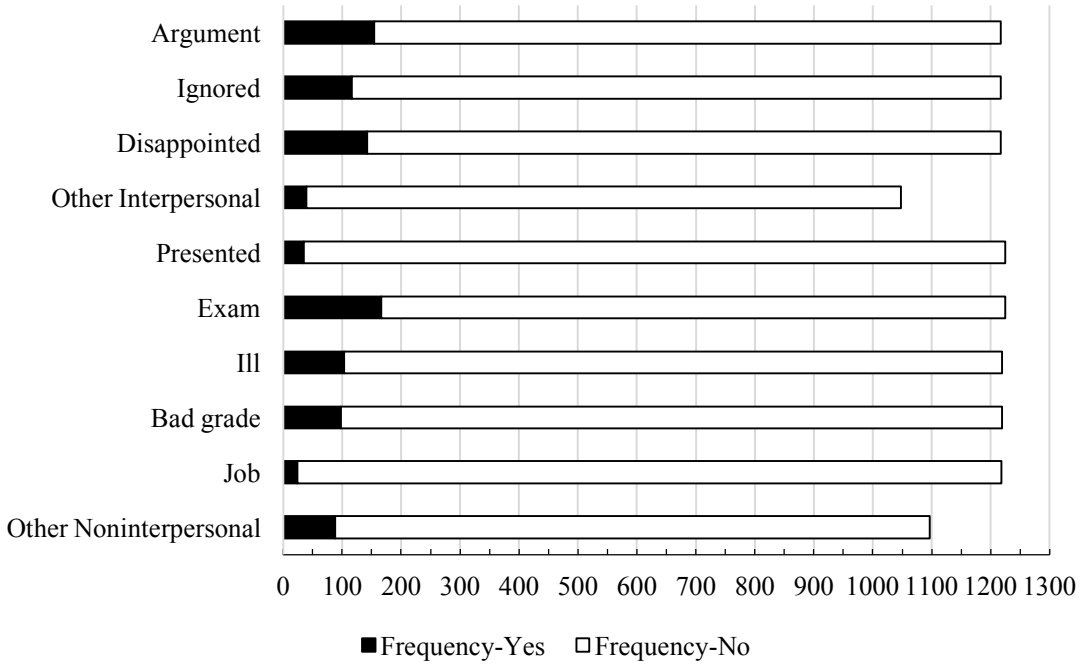

Figure 1. Types of Interpersonal and Non-Interpersonal Hassles Endorsed in Daily Diaries.

APPENDIX C

DAILY DIARY – HASSLES

Interpersonal Hassles

(Yes or No)

I had an argument, conflict, or disagreement with someone else today.

I was ignored or snubbed by someone today.

Someone let me down or disappointed me today.

Non-interpersonal Hassles

(Yes or No)

Today I gave a presentation.

Today I took an exam.

I was physically ill or injured today.

I got a bad grade on an assignment, paper, quiz, or exam today.

I experienced a negative event related to my job today.

I experienced some other type of stressful event today.

(If answered yes to previous question; participants provide a fill-in response)

What was the other type of stressful event you experienced today?

APPENDIX D

INSTRUCTIONS FOR CODING DESCRIPTIONS OF WRITE-IN HASSLES

1. Read the description of the hassle and use the definitions below to evaluate whether it is an **interpersonal** or **non-interpersonal** hassle.

    a. "Interpersonal [hassles] are those that impact the quality and quantity of relationships with others such as intimate relationships, friendships, social life, and family life" (Vrshek-Schallhorn et al., 2015).

    b. Non-interpersonal hassles are those that fail to meet the definition of interpersonal hassles.

2. Under Ip_hassle_FL (F = first initial, L = last initial of your name):

    a. Code **0 if hassle is non-interpersonal** or **1 if hassle is interpersonal.**

3. Under Nonip_hassle_FL (F = first initial, L = last initial of your name):

    a. Code **0 if hassle is interpersonal** (i.e., NOT non-interpersonal) or **1 if hassle is non-interpersonal.**

4. If there are **two or more** hassles that the participant described in one entry, make a note about it in variable "FL_notes" and write-in whether each is interpersonal or non-interpersonal

5. Determine if the hassle(s) written in are a duplicate of any of the following descriptions. If so, write "repeat" in the "FL_notes" column. Still code the hassle using the instructions in #1-3 above.

APPENDIX E

DAILY DIARY – SUBJECTIVE SLEEP QUALITY

Sleep Onset Latency

(Fill-in response)

Last night it took me about ___ minutes to fall asleep.

Quality

(Rated from 1 – Strongly Disagree to 7 – Strongly Agree)

Last night my sleep was high quality.

Last night my sleep was poor quality.

APPENDIX F

REGRESSION MODELING

Table 5

Unconditional Models

Variable	Definition
IP_Hassles	Interpersonal hassles
nonIP_Hassles	Non-interpersonal hassles
actSOL	Sleep onset latency measured with actigraphy
actWASO	Wake after sleep onset measured with actigraphy
srSOL	Self-reported sleep onset latency
srQuality	Self-reported sleep quality
MLP	Serotonergic multilocus profile score

Outcome	β_{00}	*SE*	τ	σ^2	ICC
srSOL	24.81	1.95	420.28	978.74	0.30
srQuality	4.58	0.08	0.65	1.93	0.25
actSOL	1.87	0.32	0.35	28.68	0.01
actWASO	51.79	2.54	235.19	1196.52	0.16

Table 6

Main Effects of Hassles on Self-Reported Sleep Outcomes

Interpersonal hassles predicting self-reported sleep onset latency

Level 1: $srSOL_{ij} = \pi_{0j} + \pi_{1j}(\text{IP\_}Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + r_{0j}$

$\pi_{1j} = \beta_{10}$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (β_{00})	24.19	1.97	12.30	145	<.001
IP Hassles (β_{10})	2.54	2.23	1.14	1052	0.26

Non-interpersonal hassles predicting self-reported sleep onset latency

Level 1: $srSOL_{ij} = \pi_{0j} + \pi_{1j}(nonIP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + r_{0j}$

$\pi_{1j} = \beta_{10}$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (β_{00})	24.47	2.02	12.10	146	<.001
Non-IP Hassles (β_{10})	1.11	1.77	0.62	1058	0.53

Interpersonal hassles predicting self-reported sleep quality

Level 1: $srQuality_{ij} = \pi_{0j} + \pi_{1j}(IP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + r_{0j}$

$\pi_{1j} = \beta_{10}$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (β_{00})	4.58	0.08	54.14	145	<.001
IP Hassles (β_{10})	-0.02	0.10	-0.19	1050	0.85

Non-interpersonal hassles predicting self-reported sleep quality

Level 1: $srQuality_{ij} = \pi_{0j} + \pi_{1j}(nonIP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + r_{0j}$

$\pi_{1j} = \beta_{10}$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (β_{00})	4.55	0.08	53.89	146	<.001
Non-IP Hassles (β_{10})	0.07	0.10	0.76	1055	0.45

Table 7

MLP x Hassles Cross-Level Interaction on Self-Reported Sleep Outcomes

Interpersonal hassles x MLP predicting self-reported sleep onset latency

Level 1: $srSOL_{ij} = \pi_{0j} + \pi_{1j}(IP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + \beta_{01}(MLP_i) + r_{0j}$

$\pi_{1j} = \beta_{10} + \beta_{11}(MLP_i)$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (π_0)					
Intercept, β_{00}	24.18	1.96	12.35	144	<.001
MLP, β_{01}	1.45	1.22	1.19	144	0.24
For IP Hassles Slope (π_1)					
Intercept, β_{10}	2.53	2.22	1.14	1051	0.26
MLP, β_{11}	-0.07	1.26	-0.06	1051	0.95

Non-Interpersonal hassles x MLP predicting self-reported sleep onset latency

Level 1: $srSOL_{ij} = \pi_{0j} + \pi_{1j}(\text{non}IP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + \beta_{01}(MLP_i) + r_{0j}$

$\pi_{1j} = \beta_{10} + \beta_{11}(MLP_i)$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (π_0)					
Intercept, β_{00}	24.47	2.00	12.21	145	<.001
MLP, β_{01}	1.15	1.27	0.91	145	0.37
For Non-IP Hassles Slope (π_1)					
Intercept, β_{10}	1.00	1.75	0.57	1057	0.57
MLP, β_{11}	0.68	1.07	0.63	1057	0.53

Interpersonal hassles x MLP predicting self-reported sleep quality

Level 1: $srQuality_{ij} = \pi_{0j} + \pi_{1j}(IP\_Hassles_{ij}) + e_{ij}$
Level 2: $\pi_{0j} = \beta_{00} + \beta_{01}(MLP_i) + r_{0j}$
$\pi_{1j} = \beta_{10} + \beta_{11}(MLP_i)$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (π_0)					
Intercept, β_{00}	4.58	0.08	54.30	144	<.001
MLP, β_{01}	-0.05	0.05	-1.10	144	0.27
For IP Hassles Slope (π_1)					
Intercept, β_{10}	-0.02	0.10	-0.19	1049	0.85
MLP, β_{11}	0.04	0.07	0.60	1049	0.55

Non-Interpersonal hassles x MLP predicting self-reported sleep quality
Level 1: $srQuality_{ij} = \pi_{0j} + \pi_{1j}(nonIP\_Hassles_{ij}) + e_{ij}$
Level 2: $\pi_{0j} = \beta_{00} + \beta_{01}(MLP_i) + r_{0j}$
$\pi_{1j} = \beta_{10} + \beta_{11}(MLP_i)$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (π_0)					
Intercept, β_{00}	4.55	0.08	54.19	145	<.001
MLP, β_{01}	-0.07	0.05	-1.32	145	0.19
For Non-IP Hassles Slope (π_1)					
Intercept, β_{10}	0.07	0.10	0.76	1054	0.45
MLP, β_{11}	0.06	0.06	1.12	1054	0.26

Table 8

Main Effects of Hassles on Actigraph-Recorded Sleep Outcomes

Interpersonal hassles predicting actigraph sleep onset latency
$actSOL_i = \beta_0 + \beta_1(IP\_Hassles_i) + \varepsilon_i$

	Coefficient (B)	Standard Error	t-ratio	p-value
Intercept, β_0	2.01	0.51	3.96	<.001
IP Hassles, β_1	-0.32	0.96	-0.33	.74

Non-interpersonal hassles predicting actigraph sleep onset latency
$actSOL_i = \beta_0 + \beta_1(nonIP\_Hassles_i) + \varepsilon_i$

	Coefficient (B)	Standard Error	t-ratio	p-value
Intercept, β_0	2.27	0.55	4.15	<.001
nonIP Hassles, β_1	-0.89	0.94	-0.95	.34

Interpersonal hassles predicting actigraph WASO
Level 1: $actWASO_{ij} = \pi_{0j} + \pi_{1j}(IP\_Hassles_{ij}) + e_{ij}$
Level 2: $\pi_{0j} = \beta_{00} + r_{0j}$
$\pi_{1j} = \beta_{10}$

Fixed Effect	Coefficient	Standard Error	t-ratio	Approx. df	p-value
For Intercept (β_{00})	54.01	3.30	16.39	119	<.001
IP Hassles (β_{10})	-6.31	4.30	-1.47	152	0.15

Non-interpersonal hassles predicting actigraph WASO
Level 1: $actWASO_{ij} = \pi_{0j} + \pi_{1j}(nonIP\_Hassles_{ij}) + e_{ij}$
Level 2: $\pi_{0j} = \beta_{00} + r_{0j}$
$\pi_{1j} = \beta_{10} + r_{1j}$

Fixed Effect	Coefficient	Standard Error	t-ratio	Approx. df	p-value
For Intercept (β_{00})	51.76	3.64	14.23	120	<.001
Non-IP Hassles (β_{10})	1.90	5.24	0.36	120	0.72

Table 9

MLP x Hassles Interaction on Actigraph-Recorded Sleep Outcomes

Interpersonal hassles x MLP score predicting sleep onset latency measured with actigraphy

$actSOL_i = \beta_0 + \beta_1(IP\_Hassles_i) + \beta_2(MLP_i) + \beta_3(IP\_Hassles\ x\ MLP_i) + \varepsilon_i$

	Coefficient (*B*)	Standard Error	*t*-ratio	*p*-value
Intercept, β_0	1.89	0.36	5.27	<.001
IP Hassles, β_1	-0.39	0.96	-0.41	.68
MLP, β_2	0.23	0.22	1.02	.31
IP Hassles x MLP, β_3	.83	0.63	1.32	.19

Non-interpersonal hassles x MLP predicting sleep onset latency measured with actigraphy

$actSOL_i = \beta_0 + \beta_1(nonIP\_Hassles_i) + \beta_2(MLP_i) + \beta_3(nonIP\_Hassles\ x\ MLP_i) + \varepsilon_i$

	Coefficient (*B*)	Standard Error	*t*-ratio	*p*-value
Intercept, β_0	1.90	0.36	5.35	<.001
Non-IP Hassles, β_1	-0.96	0.94	-1.01	.31
MLP, β_2	0.28	0.22	1.26	.21
Non-IP Hassles x MLP, β_3	-0.58	0.60	-0.98	.33

Interpersonal hassles x MLP score predicting WASO measured with actigraphy

Level 1: $actWASO_{ij} = \pi_{0j} + \pi_{1j}(IP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + \beta_{01}(MLP_i) + r_{0j}$

$\pi_{1j} = \beta_{10} + \beta_{11}(MLP_i)$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (π_0)					
Intercept, β_{00}	54.11	3.24	16.72	118	<.001
MLP, β_{01}	3.67	2.01	1.83	118	0.07
For IP Hassles Slope (π_1)					
Intercept, β_{10}	-6.47	4.24	-1.53	151	0.13
MLP, β_{11}	-2.45	2.83	-0.87	151	0.39

Non-interpersonal hassles x MLP predicting WASO measured with actigraphy

Level 1: $actWASO_{ij} = \pi_{0j} + \pi_{1j}(nonIP\_Hassles_{ij}) + e_{ij}$

Level 2: $\pi_{0j} = \beta_{00} + \beta_{01}(MLP_i) + r_{0j}$

$\pi_{1j} = \beta_{10} + \beta_{11}(MLP_i) + r_{1j}$

Fixed Effect	Coefficient	Standard Error	*t*-ratio	Approx. *df*	*p*-value
For Intercept (π_0)					
Intercept, β_{00}	52.00	3.71	14.02	119	<.001
MLP, β_{01}	2.61	2.54	1.03	119	0.31
For Non-IP Hassles Slope (π_1)					
Intercept, β_{10}	1.46	5.27	0.28	119	0.78
MLP, β_{11}	0.56	3.42	0.16	119	0.87