CLOCK GENES, CIRCADIAN RHYTHMS, AND MOOD DISORDERS: THE ROLE OF POSITIVE AFFECT

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CLOCK GENES, CIRCADIAN RHYTHMS, AND MOOD DISORDERS:

THE ROLE OF POSITIVE AFFECT

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Background: The master circadian clock maintains a ~24 hour rhythm via genetic feedback loops. Polymorphisms in master clock genes have been associated with markers of delayed rhythms and depression, suggesting delayed rhythms originating from variants in the master clock may create vulnerability to depression. Further, evening chronotype, a marker of delayed rhythms, is associated with depression. Recent research has found positive affect (PA) to be an important mediator in the relationship between evening chronotype and depression severity. PA exhibits a diurnal rhythm that is delayed and blunted in those with evening chronotype, suggesting the same master clock polymorphisms that predict chronotype may also predict PA rhythms. Therefore, it is hypothesized that polymorphisms in master clock genes will predict variations in PA rhythms and further, chronotype will mediate this relationship. **Methods:** Affect was measured every 60 minutes during waking hours for at least two workdays and one non-workday in 381 healthy Caucasian adults. Participants completed questionnaires on affect and chronotype. Genetic information was extracted from blood samples and genotyped for four polymorphisms (*CLOCK* 3111 C/T, *PER3* G647V, *BMAL* 1420 A/G, *CRY1* rs8192440) that were used to create a gene risk score. **Results:** The PA rhythm on workdays differed significantly based on chronotype. PA phase timing, but not amplitude, significantly differed across chronotype. The proposed clock gene risk score did not predict either PA rhythm measures or chronotype. **Discussion**: Study results support the association between chronotype and PA rhythm, specifically in phase timing. Although the

gene risk score was not predictive, other polymorphisms that may affect the circadian clock may be important for future studies. Results suggest that chronotype is more predictive of phase timing than amplitude, and may be an important factor in continuing research in vulnerabilities to depression.

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1.0 INTRODUCTION

Mood disorders, along with other poor outcomes including obesity and mortality, have been linked to abnormalities in daily or circadian rhythms of endogenous factors, such as body temperature and hormone release. Genetic studies have found genes within the circadian clock to be significantly associated with both mood disorders and abnormal rhythms in sleep, activity preference, and hormone release (see Fig 1.a; Lamont, Legault-Coutu, Cermakian, & Boivin, 2007; McClung, 2007; Etain, Milhiet, Bellivier, & Leboyer, 2011). However, it remains unclear how individual differences in clock genes, associated with abnormal sleep or circadian rhythms could lead to mood disorders. Positive affect (PA) may help to explain the association between circadian rhythms and mood disorders. PA distinguishes mood disorders from anxiety disorders (Watson, Clark, & Carey, 1988), as it is decreased in mood disorders but not anxiety disorders (Watson, Clark, & Carey, 1988). Further, PA exhibits a clear circadian pattern (e.g. Clark, Watson, & Leeka, 1989) with daily peaks reported during the afternoon and evening hours (e.g. Stone et al., 2006), which are blunted in those with delayed rhythms and mood disorders (see Figure 1: b, d; Murray, 2007). Thus, it is possible that clock gene variations lead to depression by altering daily rhythms in PA (see Figure 1: c). Although plausible, little research has explored the potential links between clock genes, abnormal rhythms, and patterns of PA. Therefore, this study aimed to test (1) associations between the clock gene risk score and PA rhythm estimates including amplitude and acrophase, (2) associations between the clock gene risk score and chronotype, a proximal measure

of circadian rhythms and, lastly, (3) chronotype as a potential mediator in the relationship between the clock gene risk score and PA rhythm estimates. The current project aimed to fill a gap in knowledge about how clock genes influence mood, and to further our knowledge of the circadian etiology of depression.

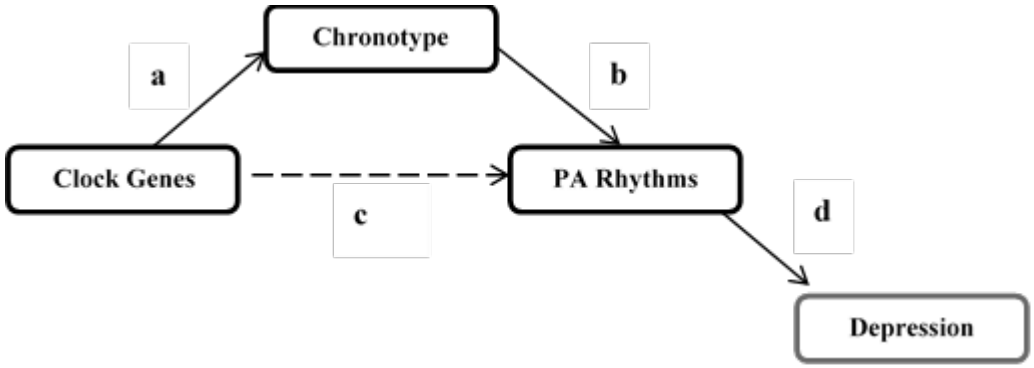

Figure 1. Schematic of proposed role of PA in the link between clock genes, chronotype and depression. Solid lines represent established relationships. Dotted line represents proposed relationship.

1.1 CIRCADIAN RHYTHMS AND THE MOLECULAR CLOCK

Daily variations in bodily processes such as core body temperature, cortisol, and melatonin release are driven by the molecular clock. These variations are rhythmic in nature and can be described by both amplitude, and amount of daily variation, (see Figure 2: a) and acrophase, the timing of the

peak of the rhythm (see Figure 2: b). The genes involved in the molecular clock contain sequence variations that lead to variations in individual circadian rhythms, and therefore make them targets of the present study. Located in the suprachiasmatic nucleus (SCN), the circadian clock maintains a ~24 hour period via a transcription-feedback loop. This loop is transcriptionally activated by a dimer between the circadian locomotor output cycles kaput (CLOCK) protein and the brain and muscle ARNT-like protein 1 (BMAL1). The CLOCK/BMAL heterodimer then bind and increase transcription and translation of both PER and CRY. Completing the loop, PER and CRY form their own heterodimer and inhibit the actions of CLOCK/BMAL, therefore inhibiting their own transcription and translation (Lee, Etchegaray, Cagampang, Loudon, & Reppert, 2001). At night, the PER/CRY heterodimer is degraded, allowing the *CLOCK* and *BMAL1* genes to be translated once again (see Figure 3). This molecular loop is the driving force of mammalian circadian rhythms (Hardin, 2000). Importantly for the present project, sequence variations in these clock genes may alter this feedback process leading to downstream effects on circadian rhythms such as delayed acrophase or dampened amplitude.

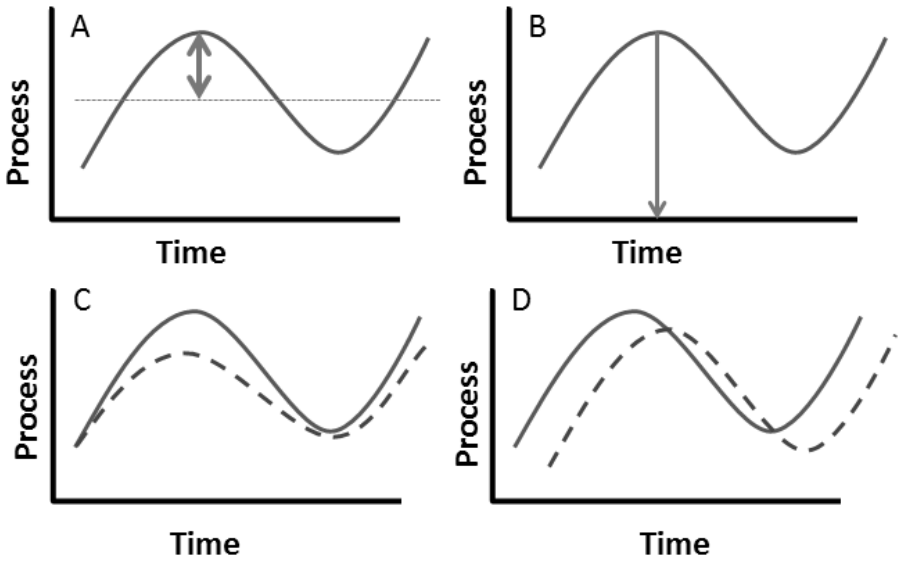

Figure 2. Schematic of proposed role of PA in the link between clock genes, chronotype and depression. Solid lines represent established relationships. Dotted line represents proposed relationship.

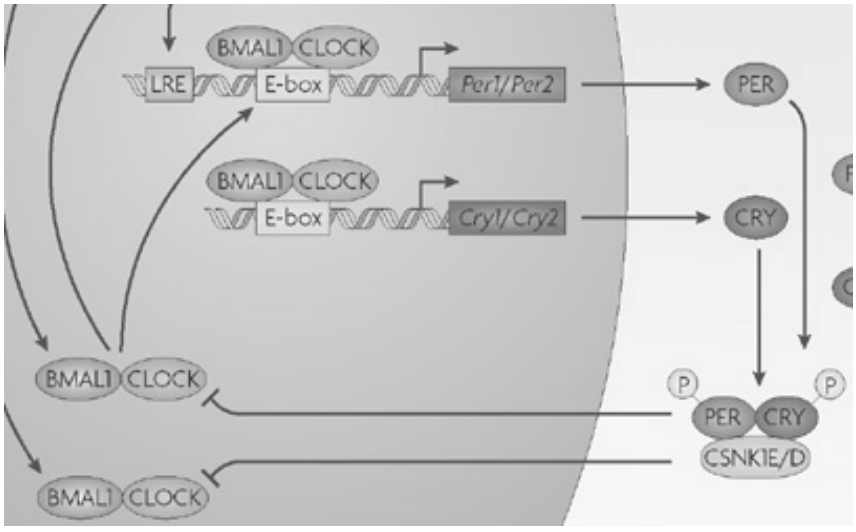

Figure 3. Representation of genetic transcriptional-translational feedback loop. BMAL/CLOCK complex increases translation and transcription of PER/CRY, which in turns inhibits the actions of the BMAL/CLOCK complex. Adapted from "The genetics of mammalian circadian order and disorder: implications for physiology and disease." Takahashi, J. S., Hong, H. K., Ko, C. H., & McDearmon, E. L., 2008, *Nature Reviews Genetics*,9..

1.2 CLOCK GENES AND ABNORMAL RHYTHMS

Mutations and deletions of the molecular clock's genes have been shown to impact circadian rhythms in both humans and animals (see Lowrey & Takahashi, 2004). Impacts include blunting a rhythm's amplitude and delaying a rhythm's acrophase (see Figure 2: c, d). Utilizing genetic knockout techniques, animal studies have illuminated the role of individual clock genes in circadian rhythms. For example, rats with chemically deleted *Per1* genes have shown delayed circadian rhythms compared to their wildtype counterparts (Cermakian, Monaco, Pando, Dierich, & Sassone-Corsi, 2001), and mice lacking the *Cry* gene display severely disrupted rhythms (van der Horst et al., 1999). Therefore, animal studies have provided direct evidence of the pervasive impact of clock gene mutations on circadian rhythms in mammals, and similar studies suggest the same may be true in humans.

In humans, the self-report measurement of chronotype is used as a proxy for circadian rhythms. Chronotype is defined as a preference in timing of activity and sleep and is a noninvasive way of investigating the association between mutations of the circadian clock genes and circadian rhythms. Recent human research has focused on naturally occurring polymorphisms in human clock genes and measures of chronotype. Chronotype categorization is moderately correlated with direct biological markers of circadian rhythms (Duffy, Rimmer, & Czeisler, 2001), making chronotype a useful proxy measurement for internal circadian rhythms. For example, those who report an evening chronotype will most likely exhibit delayed biological rhythms when measured objectively. Additionally, delayed sleep phase disorder (DSPS), a disorder characterized by persistent delayed sleep/wake times, provides a naturally occurring model for delayed rhythms in humans. Individuals with DSPS exhibit delayed biomarkers of circadian phase (Shibui, Uchiyama, & Okawa, 1999) and are more likely to carry certain mutations of the master clock genes (Hamet

& Tremblay, 2006). Both chronotype measures and the study of disorders like DSPS support an association between clock gene variations and abnormal circadian rhythms in humans.

CLOCK, the most extensively investigated clock gene in humans, has been implicated in circadian rhythm control. As a part of the internal clock's transcriptional feedback look, *CLOCK* variants have been shown to directly impact circadian rhythm in animals (Vitaterna et al., 1994) and in humans (Katzenberg et al., 1998). A single-nucleotide polymorphism, *CLOCK* 3111 C/T, has been associated with evening chronotype and delayed sleep in several human studies (Garaulet et al., 2011; Katzenberg et al., 1998; Mishima, Tozawa, Satoh, Saitoh, & Mishima, 2005; see Table 1). Specifically, in healthy populations, individuals carrying two C alleles at 3111C/T have significantly lower MEQ scores, indicating a tendency towards eveningness (Katzenberg et al., 1998). Additionally, a 40-50 minute delay in sleep time has been found in those carrying the *CLOCK* 3111 C allele (Katzenberg et al., 1998; Mishima, Tozawa, Satoh, Saitoh, & Mishima, 2005). The C allele has also been associated with evening preference in specific populations including obese individuals (Garaulet et al., 2012), individuals with bipolar disorder (Lee et al., 2010), and those with initial or onset insomnia in depression (Serretti et al., 2003). Other studies have failed to replicate the C allele association with evening preference, perhaps as a result of limited sample size (Choub et al., 2011; Johansson et al., 2003; Robilliard et al., 2002) and low effect size, an issue that may be addressed by our composite risk score, as described below. Inconsistent results may also be due to multi-ethnic samples (Chang, Buch, Bradstreet, Klements, & Duffy, 2011; Pedrazzoli et al., 2007), as such studies are likely affected by population stratification. Among studies not vulnerable to population stratification and with sufficient sample sizes, the C allele appears to increase risk for evening chronotype.

Table 1. Summary of clock gene polymorphism studies and findings

Gene	Polymorphism	Study	Sample		Findings
CLOCK	3111 C/T	Katzenberg et al., 1998	410 healthy Caucasian individuals	+	C allele associated with eveningness
		Mishima et al., 2005	421 healthy Japanese individuals	+	CC associated with eveningness and 40-50 minute delay in sleep
		Garaulet et al., 2011	1495 Spanish overweight/obese individuals	+	C allele carriers associated with eveningness
		Lee et al., 2010	108 Korean individuals with bipolar depression	+	C allele carriers associated with eveningness
		Serretti et al., 2003	234 individuals with depression (ethnicity not reported)	+	C allele associated with initial insomnia
		Choub et al., 2011	152 healthy Italian Caucasians	*	3111 C/T not associated with chronotype
		Johansson et al., 2003	92 Caucasian individuals including those with SAD and healthy controls	*	3111 C/T not associated with chronotype
		Robilliard et al., 2003	105 healthy individuals (ethnicity not reported)	*	3111 C/T not associated with chronotype
		Chang et al., 2011	147 multi-ethnic population	*	3111 C/T not associated with chronotype, DSPS
		Pedrazzoli et al., 2007	multi-ethnic population	*	3111 C/T not associated with chronotype
PER3	G647V	Ebisawa et al., 2001	36 Japanese sleep patients including DSPS	-	G allele associated with DSPS
		Johansson et al., 2003	92 Caucasian individuals including those with SAD and healthy controls	+	G allele associated with morningness
BMAL	A1420G	Pedrazzoli et al., 2010	98 multi-ethnic population	+	G allele most often in genotype combinations associated with

					eveningness
CRY	rs8192440	Reszka et al., 2012	709 female shift workers (ethnicity not reported)	+	T allele associated with morningness

Note. Regarding symbols used in findings, '+' indicates that a given study reported the predicted association, '-' indicates an opposite or contradiction association, and '*' indicates no significant association.

The *PER*3 gene may also significantly impact individual circadian rhythms (See Table 1 for summary of studies). Several versions of *PER* (*PER1, 2, & 3*) are important for regulation of the circadian clock. Analyses exploring different combinations of polymorphisms across the *PER3* gene found a specific combination of polymorphisms to be associated with DSPS (Ebisawa et al., 2001). The T → G allele change in the sixteenth exon, associated with a functional amino acid change to glycine of the G647V *PER3* SNP was hypothesized to be the driving force of this association. Given the small sample size and indirect association, this association remains tentative. In contrast, the G allele was *directly* and significantly associated with morningness in a much larger sample of individuals with seasonal affective disorder (SAD) and controls (Johansson et al., 2003). Overall, results suggest an important role for the *PER3* G647V SNP in morningness.

Although less research has been published testing associations between *BMAL, CRY* and chronotype compared to *CLOCK* and *PER3*, there is evidence to suggest these next two genes may impact circadian rhythms in humans as well. Variation in the BMAL protein could theoretically impact circadian timing as the gene creates a dimer with CLOCK to increase transcription of *PER* and *CRY*, as described above. A recent study exploring combinations of clock genes predicting chronotype hinted at an association with the A1420G polymorphism in the *BMAL* gene and

chronotype (Pedrazzoli et al., 2010). Although results would need to be replicated in a larger sample, preliminary results found the G allele was most strongly associated with evening chronotype. Further, variation in the *CRY* gene may impact the molecular clock because it inhibits transcription of *BMAL* and *CLOCK*; although only one study has explored the impact of polymorphisms in the *CRY* gene on human circadian rhythms (Reszka et al., 2012). Specifically, the T allele of SNP rs8192440 has been associated with morningness in a sample of day and night shift workers (Reszka et al., 2012). While further investigation of the influence of the *BMAL* and *CRY* genes on human circadian rhythms is required, preliminary findings indicate possible associations. In addition, the integral roles of *BMAL* and *CRY* in the clock suggest that these two genes should be included in a clock gene risk score.

1.3 CLOCK GENE RISK SCORE PREDICTING CHRONOTYPE

Recent studies have begun to recognize that the quantitative nature of many phenotypes, such as chronotype, may be better accounted for by a risk score compiling multiple polymorphisms rather than with only one SNP. Many disorders are quantitative traits representing extreme ends of a normally distributed phenotype (Plomin, Haworth, & Davis, 2009), pointing to a polygenic phenotype that may be more accurately reflected by the additive effects of several risk genotypes instead of single polymorphisms. Recent studies with other phenotypes have used a multilocus strategy and successfully accounted for variance in the phenotype above and beyond what would be found in single locus studies. For example, Nikolova, Ferrell, Manuck, & Hariri (2011) constructed a gene risk score comprised of genes linked to increased dopamine signaling. This risk score significantly explained 10.9% of variability in striatum reactivity—a large finding compared

to single locus studies that accounted for a relatively small variance in the phenotype (e.g. 0.2-5.2%; Nikolova et al., 2011). Therefore, a cumulative risk gene profile approach may account for more variance in quantitative phenotypes. However, this unique methodological approach has yet to be applied to chronotype.

Chronotype may be best predicted by a gene risk score given the quantitative nature and normal distribution of chronotype (Chelminski, Ferraro, Petros, & Plaud, 1997). Recent literature has recommended a multilocus approach to predict abnormal rhythms (Takahashi, Hong, Ko, & McDearmon, 2008), although only one previous clock gene study utilized a multilocus approach (Pedrazzoli et al., 2010). Specifically, Pedrazzoli et al. (2010) examined the frequency of 31 different combinations of four clock gene genotypes in individuals with extreme morningness and eveningness. Findings supported the utility of a multilocus approach, though the study was most likely greatly under powered given there were 31 categories of polymorphic combinations tested in only 98 participants. In contrast to an approach like that of Pedrazzoli et al. (2010), the currently proposed risk score compiles a cumulative risk score within one continuous independent variable, allowing us to increase the Bonferroni corrected alpha level from 0.0025 (corrected alpha with four described genotypes and five dependent variables as in the Pedrazzoli type of study) to 0.01 (corrected alpha with risk score and five dependent variables) in our study. To date, no study exploring chronotype has utilized a gene risk score; therefore, it remains unclear whether a summed risk score of previously studied risk genotypes may better predict chronotype. This study aims to extend the current literature by creating a clock gene risk score for evening chronotype constructed from previously established clock gene and chronotype associations. The proposed clock gene score may account for more of the association between clock genes and abnormal

rhythms, potentially improving our understanding of the etiology of associated poor outcomes such as mood disorders.

1.4 BLUNTED & DELAYED CIRCAIDAN RHYTHMS IN DEPRESSION

Variations in clock genes may contribute to the abnormal rhythms often found in mood disorders (McClung, 2007; Mitterauer, 2000). Although limited, evidence suggests a link— perhaps causal—between abnormal circadian rhythms and mood disorders (e.g. Germain & Kupfer, 2008; McClung, 2013). Abnormalities in circadian rhythms include blunted, as well as delayed rhythms. Specifically, depressed individuals exhibit blunted amplitudes in several biological rhythms including cortisol levels, core body temperature, melatonin, and thyroid secreting hormone as compared to non-depressed controls (Claustrat, Chazot, Brun, Jordan, & Sassolas, 1984; Daimon, Yamada, Tsujimoto, & Takahashi, 1992; Posener et al., 2000). Further, decreased amplitude of cortisol, temperature, and melatonin rhythms predicts higher depression severity scores (Souętre et al., 1989), suggesting that blunted rhythms may exacerbate depression. It has been postulated that the amplitude of circadian rhythms may be a result of the relative strength of the underlying master clock (Souętre et al., 1989). Given the impact of clock gene variations on the timing of the master clock, genetic factors shown to impact phase and rhythm may create a chronobiological vulnerability for rhythms in depression.

Along with blunted rhythms, evidence also supports a role for delayed rhythms in mood disorders. In seasonal depression, some patients exhibit a phase delays relative to controls in biological rhythms such as body temperature and melatonin release (Daimon et al., 1992; Lewy, Lefler, Emens, & Bauer, 2006). Further, rhythmically expressed genes have been shown to be

delayed in those who are depressed as compared to non-depressed controls (Li et al., 2013). Behavioral data also suggest an association between evening chronotype and mood disorders. When comparing mean chronotype scores between depressed individuals and matched controls, a significantly lower mean (i.e. more evening type) was found in those with depression (Drennan, Klauber, Kripke, & Goyette, 1991). This association was replicated in studies of two student samples (Chelminski, Ferraro, Petros, & Plaud, 1999; Hirata et al., 2007), which found that individuals who were categorized as depressed were more likely to be evening types. Hasler et al. (2010) replicated this association, finding Beck Depression Inventory (BDI; Beck, Steer, & Brown, 1996) scores were higher in those who were more evening types in a sample with a wide range of depression severity. Consistent with predictions, those in the sample who had never experienced depression or dysthymia had greater morningness (Hasler, Allen, Sbarra, Bootzin, & Bernert, 2010). Further, individuals reporting extreme morningness in a Japanese sample had a decreased incidence of depressive symptoms when controlling for measured sleep parameters (Kitamura et al., 2010). While evening chronotype may increase risk of depression, the Kitamura et al. (2010) findings suggest morningness may be a protective factor. Although further investigation is needed, research supports an important role of chronotype, specifically evening chronotype, in depression.

1.5 THE ROLE OF POSITIVE AFFECT IN DEPRESSION

Although it is not yet known how these delayed or blunted rhythms may lead to depression, one hypothesis invokes positive affect (PA). Preliminary research has suggested a role for PA in the relationship between evening chronotype and decreased mood. Affect is often represented as a circumplex with interim mood descriptions organized in a circular manner along two distinct affect

dimensions (see Figure 4; e.g. Russell, 1980; Watson & Tellegen, 1985). Russell (1980) defined the affect circumplex around an arousal dimension, describing perceived arousal associated with the emotional experience, and a valence dimension, descriptive of the hedonic quality of emotion (see Figure 4:a). In contrast, others have defined the affect circumplex as two mutually independent factors of positive affect (PA) and negative affect (NA: Watson, Clarck, & Tellegen, 1988, Watson & Tellegen, 1985). In research combining the two models, Russell and Feldman Barrett (1999) found that rotating the PA and NA model by 45 degrees led to a match with the arousal/valence model (see Figure 4: b). Therefore, PA and NA can be viewed as having both valence and arousal components. For example, high levels of PA and high arousal are associated with enthusiasm, interest while increased NA levels with high arousal are representative of nervousness and fear. Because most literature exploring rhythms and mood disorders utilize the Watson & Tellegen (1985) NA/PA circumplex, it is the focus of the following discussion.

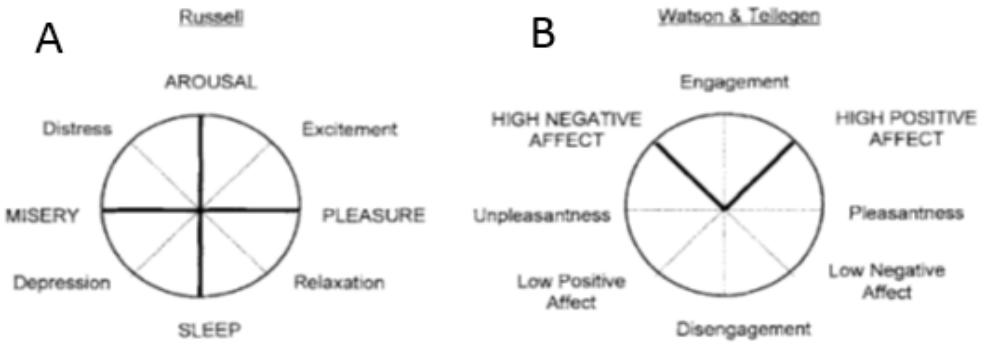

Figure 4. The circumplex model of affect proposed by Russell (A:1980) and Watson and Tellegen (B: 1985). Bold lines represent the rotation required in the Watson and Tellegen model to replicate the Russell model. Adapted from "Core affect, prototypical emotional episodes, and other things called emotion: dissecting the elephant." Russell, J. A., & Barrett, L. F, 1999, *Journal of personality and social psychology*,76.

The dimensions of PA and NA have been used clinically to differentiate between depression and anxiety. Unlike NA, which is associated with both depression and anxiety, low PA is uniquely related to depression (Watson, Clark, & Carey, 1988). Specifically, decreased levels of PA are associated with depression diagnosis (Watson et al., 1988), and with high scores on the BDI (Hasler et al., 2010). In a study that collected PA 8 times in one day in those with high and low levels of depression, Murray (2007) found that those in the high depression group had lower amplitude PA and less robust PA rhythms than those in the lower depression group. Further, a significant difference in PA patterns was found between depressed and control individuals (Peeters, Berkhof, Delespaul, Rottenberg, & Nicolson, 2006). Decreased levels of PA have also been observed in children at high risk to develop depression as compared to control children (Olino et al., 2011), which suggests that differences in PA increase risk for the development of mood disorders. Given that decreased levels of PA play an important role in depression and circadian rhythms may create vulnerability to decreased mood, it is important to understand how PA may be influenced by circadian rhythms.

PA may be an important mediator in the established relationship between abnormal rhythms and depression. A recent study of groups of individuals with current, past, or no diagnosis of depression tested whether PA, as measured once, mediated the relationship between evening chronotype and depression (Hasler et al., 2010). A mediation analysis of the sample found PA to be a statistically full mediator of the relationship between chronotype and depression severity score. This implies the correlation between eveningness and depression may be largely accounted for by PA levels across the day. Although preliminary, these results give credence to the importance of PA in depression and, perhaps, an explanation of the association between chronotype and depressive symptoms. However, this study utilized a single PA score, and a better

understanding of the rhythm of PA across the day may provide more information about the circadian-related etiology of depression.

1.6 CIRCADIAN ASPECTS OF POSITIVE AFFECT

Given PA's potential role in the relationship between evening chronotype and depression, it is not surprising that PA levels also exhibit a circadian rhythm. Murray et al., (2009) investigated PA and NA rhythms in multiple study designs to explore a potential endogenous rhythm of mood. One study design assessed PA 8 times during the "natural day" to acquire a baseline measurement, finding 13% of PA variation explained by a sinusoidal curve, consistent with previous studies (Clark et al., 1989; Golder & Macy, 2011; Murray, 2007; Murray, Allen, & Trinder, 2002; Stone et al., 2006; Vittengl et al., 1998; Wood & Magnello, 1992). To remove the effect of environmental cues like social interaction, light, sleep, and food intake, Murray et al. (2009) employed a forced desynchrony (FD) study design in the second study. In an FD protocol participants in a constant environment entrain to a 28 hour schedule allowing circadian rhythms to be "unmasked" from sleep/wake schedules. Results from the FD study demonstrated that PA correlated with the established circadian rhythm in core body temperature. As predicted, NA did not fit a sinusoidal curve (Murray, 2007; Murray et al., 2002; Wood & Magnello, 1992; Clark et al., 1989), though some have found significant diurnal variation in NA (Stone et al., 2006; Vittengl et al., 1998, Golder & Macy, 2011). Results from this study support an endogenous, circadian rhythm of PA.

1.6.1 Chronotype and Positive Affect Rhythms

Although evidence points to a circadian rhythm in PA levels, reported peak times vary across studies with reported peaks in the late morning (Wood & Magnello, 1992), two separate peaks in

the afternoon and/or evening (Stone et al., 2006; Vittengl & Holt, 1998), or an extended peak from noon to evening (Clark et al., 1989). A study measuring audible behavior associated with PA, like laughing, found a single peak of PA eight to 10 hours after waking (Hasler, Mehl, Bootzin, & Vazire, 2008). The range in observed peak times may be explained by the relative distribution of chronotype groups across samples if PA peaks vary by chronotype.

Individual differences in PA rhythm may be driven by chronotype. Indeed, a correlation between chronotype and affect has been supported in the literature (Figure 1: b). Morningness is associated with higher levels of PA (Biss & Hasher, 2012; Clark et al., 1989) as well as higher levels of alertness and energy (Fröberg, 1977). Similarly, lower levels of daily PA are correlated with eveningness in a population with a wide range of depression severity (Hasler et al., 2010). In addition, those with lower chronotype scores, representing evening chronotype, exhibit significantly later peaks of PA (acrophase) compared to those with higher morningness (Porto, Duarte, & Menna-Barreto, 2006). Specifically, the individual with the lowest chronotype score had a peak PA time of 20:43 as compared to the individual with the highest chronotype score who had a peak PA time of 12:33—more than an eight hour difference (Porto et al., 2006). In further support, a study of individuals with insomnia found evening types had decreased amplitude *and* delayed phase as compared to morning types (Hasler et al. 2012). Interestingly, no association has been found between individual chronotype and NA levels, further supporting a unique circadian pattern of PA. These lines of evidence point to an association between reported chronotype and PA patterns across the day. Therefore, clock gene variations influencing chronotype may also impact daily PA timing. Though further research is needed, delayed PA rhythms in evening types may be a promising mechanistic hypothesis for the development of mood disorders.

1.6.2 The Effect of Sleep Homeostasis on Positive Affect Rhythms

Circadian mechanisms are expected to account for some proportion of the daily variation of PA, while other factors such as sleep homeostasis may account for additional variation in PA (Murray et al., 2009; Boivin et al., 1997). In an FD study, the impact of hours since wake on PA varied based on circadian phase. For example, the lowest point of PA was closely tied to the amount of hours since wake. Therefore, without an FD study, it is difficult to separate variation in PA diurnal rhythms due to circadian rather than homeostatic mechanisms.

1.7 HOW MIGHT ABNORMAL POSITIVE AFFECT PATTERNS RESULT IN DEPRESSION

Lewinsohn's behavioral model describes depression as a result of reduced experience of reward in the social environment (Lewinsohn & Atwood, 1969). Lacking the motivation of reward to continue behaviors like social interaction, the model predicts individuals will socially isolate ultimately resulting in depressed mood. Given that reduced PA is correlated with decreases in reward sensitivity (e.g. Hasler et al., 2010), Lewinsohn's behavioral model may point to the importance of abnormal rhythms of PA in the development of depression. An overall decrease in or blunted amplitude of PA across the day may lead to reduced reward from social interaction throughout the day. Additionally, misaligned timing of peak PA may create and/or maintain depressed mood. Individuals with delayed rhythms may experience peak PA levels and time that are not in sync with their environment's peak reward. For example, an individual with a peak PA timing that is delayed compared to that of others around them may miss the maximal reward to be gained from social interactions, daily accomplishments, and other gratifying behavior. Further,

given that PA tends to hit its lowest point during the early morning hours while most individuals are asleep, those who are delayed may wake up during this "trough" and experience low PA that others have slept through. Therefore, individuals with delayed PA rhythms may miss opportunities for reward during the day while still experiencing the lowest PA avoided by their non-delayed counterparts. Depression, then, may be a result of decreased levels of PA or peaks of PA occurring at maladaptive times of day.

1.8 GENES AND POSITIVE AFFECT

Although PA patterns may constitute a vulnerability to developing depression, the presumed genetic etiological factors driving individual differences in PA remain understudied. A study including 158 monozygotic twin and 102 dizygotic twin pairs collected PA data once within 90-minute time blocks between 7:30 and 10:30 pm. Although average levels of PA across five days were best explained by solely environmental factors, the study failed to control for age that may explain the unlikely finding of zero heritability (Menne-Lothmann et al., 2012). An additional study utilizing the same dataset explored daily variation in PA by comparing standard deviations of average PA, and found genetic factors explained 18% of PA variability on the sample (Jacobs et al., 2012). Although PA was found to be mildly heritable when analyzed using standard deviation as a proxy for daily variation, the study points to the influence of genetics in PA, which may be magnified when actual daily PA rhythms are measured. Further investigation is needed to explore the heritability of PA amplitude and acrophase, which are more accurate measures of circadian variation than standard deviation, and also may convey risk for mood disorders. Additionally, no research currently exists exploring the candidate genes or risk gene profiles in PA rhythms

throughout the day. The proposed study would be the first to our knowledge to explore the influence of candidate clock genes on daily PA patterns.

1.9 POSITIVE AFFECT: THE LINK BETWEEN CLOCK GENES, CHORNOTYPE AND MOOD?

To our knowledge, the current study is the first to connect converging lines of research to explore potential clock gene predictors of abnormal patterns of PA. As described above, clock genes are associated with individual differences in chronotype (Figure 1: a); and evening chronotype and blunted rhythms are associated with mood disorders (Figure 1: d). Further, evening chronotype has been associated with decreased levels of PA (b). Given the impact of clock genes variants on chronotype, we hypothesize an association between a clock gene risk profile associated with evening chronotype and blunted or delayed PA rhythms (c). Evidence suggests that the resulting altered rhythms in PA may be a precursor of depressed mood and may increase risk for developing MDD (d). As a potential precursor to depressed mood, diurnal PA rhythms will be investigated in a healthy population to more accurately understand the range of variance in PA as well as control for the potential confound of reciprocal effects of depression on PA patterns. Therefore, this project aims to look at a potential link between a unique clock gene risk score and blunted or delayed PA, and further, to explore chronotype as a potential mediator of this relationship in a healthy population.

2.0 RESEARCH DESIGN AND METHODS

The current study relies on ecological momentary assessment (EMA) data collected via personal electronic devices to assess diurnal variation of PA. Participants used Personal Digital Assistants (PDA) devices to complete multiple assessments, beginning upon awakening, over workdays and one non-workday. Participants were instructed to "sleep" their PDAs when they went to sleep. Individuals were contacted throughout participation to address equipment concerns. Blood samples for DNA assays were collected, and additional questionnaires were administered during a laboratory visit.

2.1 PARTICIPANTS

Participants were adults (N = 498) from the greater Pittsburgh area in the Adult Health and Behavior project registry (AHAB II). Individuals were excluded if they were taking antidepressant medications or any psychoactive drugs that might alter their response to questionnaire measures, or if they reported a history of schizophrenia, major neurological disorders or other psychiatric illness. Further, all night-shift workers were excluded from the study. All individuals included in the current analyses were non-Hispanic Caucasian adults to minimize the risk of population stratification.

2.2 MEASURES AND ASSESSMENTS

2.2.1 DNA Collection and Genotyping

DNA was extracted from blood samples collected during laboratory visits. A single iPLEX assay was used to amplify and genotype all SNPs simultaneously. Preliminary analyses ensured the sample was in Hardy Weinberg Equilibrium for all proposed polymorphisms. Chi-square and analysis of variance tests were used to explore potential genotype group associations with gender and age.

2.2.2 Clock Gene Cumulative Risk Score

The cumulative risk score was calculated using individual genotype risk assessment as summarized in Table 2. Genotypes previously associated with eveningness were assigned a risk score of 1. Genotypes not containing the risk allele were assigned a score of 0. Importantly, because chronotype is a continuous measure, with morningness and eveningness representing extremes of the same scale (Natale & Cicogna, 2002), variants previously associated with morningness were scored 0 and the non-associated genotype was considered a risk for eveningness and accordingly scored as 1. Genotype scores were summed for each individual and used as a continuous independent variable in analyses with evening chronotype and PA diurnal rhythm estimates.

Table 2. Clock gene risk score assignments**.**

Gene	Polymorphism	Expecten MAF	Observed MAF	HWE (p)	Genotype	Assigned Cumulative Risk Score
CLOCK	3111 C/T	C: 0.26	0.29	0.43	CC, CT	1
					TT	0
PER3	G647V	G: 0.18	0.16	0.78	GG, GT	1
					TT	0
BMAL	1420 A/G	A: 0.27	0.28	0.71	GG, AG	1
					AA	0
CRY	rs8192440	T: 0.37	0.34	0.49	TT, CT	1
					CC	0

Note. Expected minor allele frequency data was extracted from NCBI database (Sherry et al., 2001). MAF=Minor Allele Frequency (MAF)

2.2.3 Chronotype

The Composite Scale of Morningness (CSM; Smith, Reilly, & Midkiff, 1989) was used to assess chronotype during an in-lab visit. The CSM scale is a continuous measure based on 13 items assessing activity and sleep preference. For example, the item “How alert do you feel during the first half hour after having awakened in the morning?” would most likely be rated “very alert” by an extreme morning individual and “not at all alert” by an extreme evening individual. Higher scores indicate morningness and lower scores indicate eveningness. Group chronotype analyses were based off of pre-established cutoffs (Natale & Alzani, 2001): 13–26 evening-type; 27–41 intermediate-type; 42–55 morning type. The scale has acceptable internal consistency (Cronbach’s

α = .83; Smith et al., 1989) and good test-retest reliability and predictive validity (Guthrie, Ash, & Bendapudi, 1995; Greenwood, 1994).

2.2.4 Positive and Negative Affect

PA and NA were collected both during a one-time assessment using the Positive Affect Negative Affect Schedule- Expanded From (PANAS-X; Watson & Clark, 1999) *and* via a personal digital assistant (PDA) every hour since time awake during the participation period. EMA Affect was assessed with an adapted version of the Positive Affect Negative Affect Schedule Short Form (PANAS-SF; Thompson, 2007) using a six point Likert scale to measure endorsement of 13 affect items. In an effort to reduce overall participant burden in the AHAB II study, the "ashamed," "active," and "alert" items were deleted from the scale due to rotated principal components analysis, which revealed low factor loading on these items. Additional items, "happy" and "cheerful" from the Profile Of Mood States scale (McNair, Lorr, & Droppleman, 1989) were added to represent the lower activation PA terms. The resulting survey included "inspired", "determined," "attentive," "happy," and "cheerful" items in the PA scale. The NA scale consisted of "upset," "hostile," "afraid," "nervous," "angry," "lonely", and "sad." The PANAS-SF has good internal consistency (Cronbach's α = .73-.78) and acceptable test-retest reliability (Thompson, 2007).

Importantly, the modifications to the PANAS-SF created a scale that was not equivalent to previous research on PA rhythms (see Table 3). Previous studies used the full PANAS (Clark et al., 1989; Hasler, 2012; Murray, Allen, & Trinder, 2002; Porto et al., 2006; Vittengl et al., 1998; Wood & Magnello, 1992), or a modified version that included "excited", "interested", "determined", and "active" items (Hasler, 2009; Murray, 2007; Murray et al., 2009) or created their own scale with

the PA items “warm” “happy” and “enjoy” (Stone et al., 2006). See Table 3 for NA scale comparisons with previous literature.

Table 3. Affect scale comparisons with previous literature.

Items	Home Questionnaire	Current Study	Previous Studies
PA Scale			
Happy	POMS	X	8
Cheerful	POMS	X	
Warm	DRM		8
Enjoy	DRM		8
Interested	PANAS		1,2,3,4,5,6,7,9,10
Alert	PANAS		1,3,4,7,9,10
Attentive	PANAS	X	1,3,4,7,9,10
Active	PANAS		1,2,3,4,5,6,7,9,10
Strong	PANAS		1,3,4,7,9,10
Enthusiastic	PANAS		1,3,4,7,9,10
Inspired	PANAS	X	1,3,4,7,9,10
Excited	PANAS		1,2,3,4,5,6,7,9,10
Proud	PANAS		1,3,4,7,9,10
Determined	PANAS	X	1,2,3,4,5,6,7,9,10
NA Scale			
Angry	PANAS-X	X	8
Lonely	PANAS-X	X	
Sad	PANAS-X	X	
Criticized	DRM		8
Impatient	DRM		8
Frustrated	DRM		8
Worry	DRM		8
Hassled	DRM		8
Depressed/Blue	DRM		8
Distressed	PANAS		1,3,4,7,9,10
Upset	PANAS	X	1,2,3,4,5,6,7,9,10
Guilty	PANAS		1,2,3,4,5,6,7,9,10
Scared	PANAS		1,2,3,4,5,6,7,9,10
Hostile	PANAS	X	1,2,3,4,5,6,7,9,10
Irritable	PANAS		1,3,4,7,9,10
Ashamed	PANAS		1,3,4,7,9,10
Nervous	PANAS	X	1,3,4,7,9,10
Jittery	PANAS		1,2,3,4,5,6,7,9,10
Afraid	PANAS	X	1,3,4,7,9,10

Note. Numbers represent the following publications: 1-Clark et al., 1989; 2- Hasler, 2009; 3 - Hasler, 2012;4- Murray, Allen, & Trinder, 2002;5 - Murray, 2007; 6-Murray et al., 2009;7 - Porto et al., 2006;8-Stone et al., 2006; 9- Vittengl et al., 1998; 10- Wood & Magnello, 1992. POMS = Profile of Mood States, DRM= Daily Reconstruction Method, PANAS= Positive Affect Negative Affect Schedule, PANAS-X = Positive Affect Negative Affect Schedule-Expanded Form.

2.2.5 Depression Severity

The Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) is a 21 item rating scale that assesses depression symptom severity. Each item has a list of four increasingly severe statements about a particular symptom of depression with each response scored between 0 and 3. The measure was used as a means of preliminary investigation of the study model predicting depression (Figure 1; d).

2.2.6 Social Jet Lag

In order to calculate the social jet lag (SJL) variable, actigraphy data was collected for at least 7 nights (including 4 workdays and 1 non-workday) using an Actiwatch-6. Sleep onset was defined as a period lasting at least 10 consecutive minutes with activity counts lower than 40 per epoch. Wake onset was defined as 10 consecutive minutes of greater than or equal to 40 activity counts per epoch. As previously suggested by (Wittmann et al., 2006), the SJL variable was calculated by taking the absolute value of the difference between mid-sleep on non-work days and mid-sleep on work days. For the present study, mid-sleep on one non-workday was subtracted from the average mid-sleep time across 3 workdays.

2.3 ANALYSES OF AFFECT DATA

Given that individuals may have had a forced wake-up time on workdays, all affect analyses were divided into workday and non-workday in order to separate potential effects of work start time on rhythms. Preliminary analyses using a cosinor analysis variant of multilevel modeling (MLM) was used to confirm a rhythm in PA and to replicate previous findings associating chronotype group (i.e. morning, intermediate, and evening) and PA. In this type of MLM, cosinor analysis was used

at level one of the model to fit affect data to a sinusoidal curve with a 24 hour period through the addition of a sine term. A cosine term was added to the model to account for phase shifts in the diurnal rhythms resulting in the following equation:

$$f(t) = \beta_0 + \beta_1 \sin(2\pi t / 24) + \beta_2 \cos(2\pi t / 24) + e$$

Time (t) was kept as clock time, as opposed to time since wake. As shown in Hasler et al. (2012), using time since wake values negated acrophase differences by chronotype originally detected in clock time analyses. This may be due to the high correlation of chronotype and wake-up time. Therefore standardizing wake up time with time since wake values may decrease variation in affect acrophase and limit the study findings.

To complete the MLM, fixed effects for chronotype, age, and gender were included along with the random effects of individual rhythms within chronotype groups. Due to the inability to extract a formal R^2 value from MLM analysis, a pseudo R^2 statistic previously used by Hasler (2009) was calculated by comparing the residual covariance parameters in an "empty" model to the complete model. Estimates of amplitude and acrophase were calculated using cosinor modeling parameters. The delta method, a version of the central limit theorem that allows for the calculation of estimate precision measures based on asymptotic variance, was utilized in the current project to estimate standard errors for the comparison of chronotype groups on acrophase and amplitude (Mikulich, Zerbe, Jones, & Crowley, 2003). All cosinor MLM was completed in SAS, Version 9.3 (SAS Institute Inc, Cary, NC).

2.3.1 Individual Analyses of PA Data

In order to test for individual differences based on the genetic risk score, individual PA amplitude and acrophase were also extracted from the EMA data for each study participant using similar statistics to the above group analyses. Two Ordinary Least Squares (OLS) regression models were

constructed using chronotype score to predict individual PA amplitude and individual PA acrophase to investigate whether the individual rhythmicity data replicated chronotype group comparisons.

2.4 PRIMARY AIMS ANALYSES

Following preliminary analyses, the three main aims of the project were investigated including the covariates described below. To test Aim 1, an OLS model was constructed using the genetic cumulative risk score to predict individual chronotype scores. Additionally, further exploration into individual polymorphism impact on chronotype was explored via a stepwise regression model, which entered each included polymorphism into a model predicting chronotype. To test Aim 2, two similar OLS models were created with the cumulative risk score predicting either PA amplitude or acrophase. Again, further exploration into individual polymorphism contribution in predicting both amplitude and acrophase was conducted using a stepwise regression model entering each polymorphism individually. To explore Aim 3, mediation analyses investigated an indirect effect of chronotype on the association between the cumulative risk score and PA diurnal rhythm estimates. Mediation was tested using the INDIRECT syntax (Preacher & Hayes, 2008), which calculates both the traditional Sobel test and also produces bootstrap confidence intervals (CI). The Sobel test (Sobel, 1982) calculates the indirect effects of the mediator by calculating the IV to mediator pathway (*a*) and the mediator to DV pathway (*b*) and assumes that the product of these (*ab*) would be equal to the difference between the total effect between the independent and dependent variable and the effect between the IV and DV while considering the mediator. Because the Sobel test assumes a normal distribution of *ab*, which is rarely the reality, bootstrapping may

provide a more accurate prediction of the significance of the indirect effect by repeatedly sampling the data and calculating estimates of *ab* for each resampled data set. This repeated process allows for the creation of a normal distribution of *ab* and is used to calculate confidence intervals for the indirect effect (Preacher & Hayes, 2008). All OLS regression models and mediation analysis were completed in SPSS 20 (SPSS Inc, Chicago, Ill).

2.4.1 Covariates

Age and gender were included in analyses as covariates in both mediation models. Age is an important covariate in circadian rhythm research, as older individuals have relatively advanced rhythms (e.g. Sherman, Wysham, & Pfoh, 1985) and are more likely to report morning chronotype (Paine, Gander, & Travier, 2006). Further, gender has been shown to significantly impact chronotype, with females tending to be more morning types compared to males (Chelminski et al., 1997).

2.5 ANCILLARY AIMS

2.5.1 Negative Affect

Multiple studies have found a lack of diurnal rhythmicity in daily NA measurements. In order to potentially replicate this finding, cosinor MLM was used to test for a daily rhythm in NA.

2.5.2 PANAS vs. Rhythm Modeling

An argument could be made that modeling daily affect variation does not add information above and beyond a one-time measurement of affect, especially because past studies have found one-time measurement associations with chronotype (Hasler et al., 2010). In order to investigate whether

modeling daily variation in PA contributes to the literature to warrant multiple day and time point measurement, the current study looked at Pearson correlations between one-time measurement of the PANAS, PA amplitude and PA acrophase. Further, a stepwise regression was used to explore how much variation each of these variables explained in chronotype.

2.5.3 PER 1

A recent study utilizing actigraphy measures of daily activity has found SNP rs7221412 on the PER1 gene to be associated with a 67 minute delay of activity rhythms in those with the homozygous risk genotype (GG; Lim et al., 2012). Although significant, the allele was not associated with chronotype and therefore omitted from our proposed clock gene risk score. However, given the significant activity delay associated with the GG genotype, OLS equations were built using both a dominant and additive model of rs7221412 to predict study measures of circadian rhythm, PA amplitude and acrophase.

2.5.4 Alternative Cumulative Risk Scores

Although the statistical advantages of a clock gene risk score over individual genotype analysis is clear, it is important to note that the cumulative score can only be as accurate as the literature that informed the risk assignments (i.e., scores of 0, 0.5, or 1 for each genotype). However, most of the previous research exploring the relationship between clock genes and chronotype only explores a dominant model (comparing individuals with the risk allele to those without) because many of these studies were likely underpowered to explore additive or non-dominant models of inheritance. As a result, additive models in which heterozygotes inherit an intermediate amount of risk remain largely underexplored in clock gene research. It therefore remains unclear if the proposed genotypes truly follow a dominant model of inherited risk (genotypes scored 0 or 1) or if, instead, additive model may be more appropriate (genotypes scored 0, 0.5, or 1). Therefore, as an

alternative approach to the proposed risk score, an additive model of cumulative risk for each locus was created. This risk score was then replaced in all relevant analyses.

2.5.5 Depression Severity

Given that the study sample was relatively healthy, BDI score variation was extremely limited and therefore BDI was not included in the primary study analyses. However, as part of the ancillary aims, analyses were conducted to replicate and extend previous findings between chronotype, PA, and depression (Hasler et al. 2010). Specifically, OLS regression models were used to explore associations between CSM score, PA amplitude and acrophase, and BDI scores. Further, the Preacher & Hayes (2008) mediation analysis was used to test the measures of PA rhythmicity as an indirect effect on the relationship between CSM and BDI scores.

2.5.6 Social Jet Lag

The social jet lag (SJL) theory of depression posits that depressed mood is a result of the misalignment between internal biological time and the socially created external schedule (Wittmann, Dinich, Merrow, & Roenneberg, 2006). According to the SJL theory, evening type individuals are more likely to have delayed sleep onset, despite needing to wake in time for regular work hours, creating a sleep debt during weekdays associated with decreased psychological wellbeing. In the context of the current study, SJL may explain an association between chronotype and PA amplitude. Individuals who are delayed may have decreased PA due to the large differences in their socially imposed rhythms and their natural rhythms. To test this hypothesis, mid-sleep time on workday and non-workdays, and the SJL variable, were compared across chronotype groups. Subsequently, the SJL variable was entered into two mediation models: the first exploring the indirect effects of SJL on the relationship between chronotype and individual PA

amplitude and the second investigating the indirect effects of SJL on the association between chronotype and individual PA acrophase.

2.5.7 Mood Diagnosis

Individuals with mood disorders were included in the original analyses to provide more variation in the variables of interest, as it was hypothesized that delayed or evening chronotype individuals would be more likely to also be depressed. To see how these individuals may influence the results, individuals with current or past mood disorders were removed from analyses to compare results with the original study analyses.

3.0 RESULTS

3.1 PARTICIPANTS

Of the 498 individuals who had completed the EMA portion of the AHAB II study, 89 were not Caucasian, 24 were unable to be genotyped for the SNPs included in the cumulative risk score, and 4 were not included due to missing chronotype, leaving 381 individuals for analysis. The average age of the sample was 42.58 years old (*SD* = 7.30) and was 49% female. Participants had an average of 17.21 years of education (*SD* = 2.80) and 68% (*n* = 259) of participants were married. Additionally, 92% (*n* = 349) of participants reported full-time employment and median family income was between $65,000 and $79,999.

3.2 GENETIC DATA

Genotype frequencies and Hardy Weinberg Equilibrium data are in Table 2. No significant associations with *CLOCK* 3111 C/T, *PER3* G647V, *BMAL* 1420 A/G, *CRY1* rs8192440 and gender (χ^2 = 0.50, p = 0.78; χ^2 = 3.84, p = 0.15; χ^2 = 0.98, p = 0.61, and χ^2 = 2.35, p = 0.31 respectively) or age (F_{380} = 0.90, p = 0.60; F_{380} = 0.96, p = 0.52; F_{380} = 0.77, p = 0.78, and F_{380} = 1.42, p = 0.09) were found. The cumulative risk score ranged from 0 to 4 (*M* = 2.3; *SD* = 0.89).

3.3 CHRONOTYPE DATA

Individuals were grouped by CSM scores into evening, intermediate, or morning based on previously established ranges (Natale & Alzani, 2001; see Table 4). Average CSM scores were in the intermediate range (M = 39.5; SD = 7.08). As predicted, the evening chronotype group was younger than other groups (F_{380} = 8.64, p < 0.001). PA scores measured by one time administration of the PANAS were higher in the morning group compared to evening (F_{380} = 8.63, p<0.001). Also as predicted, NA did not significantly differ by chronotype (F_{380} = 2.40, p = 0.09).

Table 4. Demographic variables by chronotype group

	Chronotype Group			
Variable	Morning (n=166)	Intermediate (n=196)	Evening (n=19)	*p*
Female n(%)	82(49%)	97(49%)	9(47%)	0.98
Age	43.76 (6.87)	42.13 (7.51)	36.89 (5.62)	<0.001
Years of Education	17.42 (2.69)	17.02 (2.89)	17.32 (2.77)	0.39
BDI	3.61 (3.91)	4.99 (4.92)	4.74 (4.21)	0.01
CSM	45.81 (3.15)	35.76 (3.87)	23.32 (2.79)	<0.001
PA	35.17 (5.62)	33.13 (5.75)	30.84 (5.83)	<0.001
NA	15.04 (4.98)	16.18 (5.13)	15.32 (3.90)	0.09

Note. Standard deviations listed in parenthesis. Chi-square test used for group comparisons of gender. All other group comparisons performed with ANOVA. BDI= Beck Depression Inventory, CSM=Composite Scale of Morningness, PA= Positive Affect, and NA=Negative Affect.

3.4 ECOLOGICAL MOMENTARY ASSESSMENT DATA

All individuals had at least two days of workday data and one of non-workday data, except two participants missing non-workday data. Most (90%) had measurements for at least 3 workdays. Daily times assessed ranged from midnight to 23:59 with an average 14 (*SD* = 2.5) times sampled per day. PA scores averaged 19.21 (*SD* = 4.37) on workdays and 19.04 (*SD* = 4.48) on non-workdays. NA scores averaged 13.22 (*SD* = 5.93) on workdays and 12.81 (*SD* = 5.78) on non-workdays.

3.4.1 Workday PA Diurnal Rhythms

Workday PA showed a diurnal rhythmicity, with a significant fit to both sine (B = -0.19, t_{15968} = -3.44, $p < 0.001$) and cosine (B = -1.08, $t_{1596\ 7}$= -17.87, $p < 0.0001$) terms (Table 5). The intermediate chronotype term was significant (B = -0.93, t_{390} = -2.70, $p < 0.01$) as well as the sine interaction term (B = -0.27, t_{15968} = -3.44, $p < 0.001$), indicating a difference in diurnal PA rhythm between intermediate and morning chronotype groups. Although the evening chronotype term was not significant, the cosine interaction term was (B = 0.61 t_{15968} = 3.85, p = 0.0001), indicating a significant difference in diurnal PA rhythm in evening types compared to morning types. See Figure 5 for a depiction estimated rhythms by chronotype group. The pseudo R^2 statistic revealed the cosinor model explained 4% ((8.89-8.55)/8.55) of the variance in PA.

Table 5. Chronotype group cosinor MLM results for both PA on workday and non-workdays.

	Workdays			Non-Workdays		
Variable	B	SE	*p*	B	SE	*p*
Sine	-0.19	0.06	0.0006	-0.32	0.11	0.0049
Cosine	-1.08	0.06	<0.0001	-0.91	0.10	<0.0001
Intermediate	-0.93	0.34	0.0073	-0.88	0.37	0.0194
Sine*Intermediate	-0.27	0.08	0.0006	-0.26	0.16	0.1049
Cosine*Intermediate	0.08	0.08	0.3080	0.19	0.14	0.1654
Evening	-0.73	0.80	0.3637	-0.10	0.87	0.2495
Sine*Evening	-0.37	0.19	0.0515	0.69	0.37	0.0603
Cosine* Evening	0.61	0.16	0.0001	0.03	0.28	0.9059
Age	0.02	0.02	0.3812	0.02	0.02	0.5315
Gender	-0.59	0.33	0.0759	-0.62	0.36	0.0808

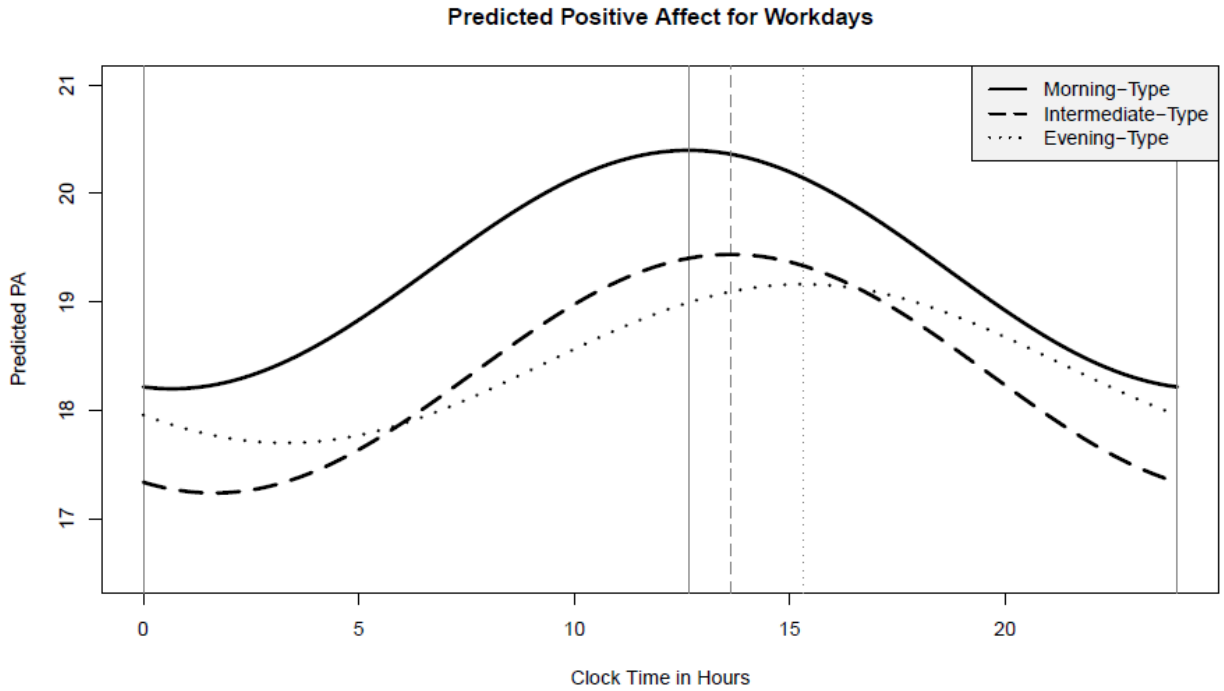

Figure 5. Workday PA rhythm modeled by chronotype group. Dotted lines mark acrophase for each group.

Estimates for amplitude and acrophase by chronotype group were extracted from regression results to allow for statistical group comparisons utilizing the delta method (Mikulich et al. 2003). Amplitude estimates were similar for both the morning (1.10, *SE* = 0.06) and the intermediate groups (1.10, *SE* = 0.06). Although amplitude differences between groups were not significant (M vs. E: *amp diff z-value* = -1.87, *p* = 0.06; I vs. E: *amp diff z-value* = -1.89, *p* = 0.06), they were in the hypothesized direction, with the evening chronotype group exhibiting a lower amplitude (0.73, *SE* = 0.19) than both the morning and the intermediate groups. Acrophase differed significantly between chronotype groups (Table 6). The acrophase of morning types occurred at 12:40 hours (*SE* = 0:11), intermediate acrophase occurred at 13:38 hours (*SE* = 0:10), and evening acrophase occurred at 15:18 hours (*SE* = 0:43).

Table 6. Chronotype group estimates and group comparisons for PA estimates on workdays and non-workdays

Workdays

Group Estimations	PA Amplitude			Acrophase in Clock Time		
	Estimate	SE	*p*	Estimate	SE	*p*
Morning	1.10	0.06	10^{-7}	12:40	00:11	10^{-7}
Intermediate	1.10	0.06	10^{-7}	13:38	00:10	10^{-7}
Evening	0.73	0.19	10^{-4}	15:18	00:43	10^{-7}
Estimate Differences	PA Amplitude Differences			Acrophase Differences in Hours		
	Estimate	SE	*p*	Estimate	SE	*p*
Evening vs. Morning	-0.37	0.20	0.06	02:38	00:44	10^{-3}
Evening vs. Intermediate	-0.37	0.20	0.06	01:40	00:44	0.02
Intermediate vs. Morning	0.00	0.09	0.99	00:58	00:14	10^{-4}

Non-Workdays

Group Estimations	PA Amplitude			Acrophase in Clock Time		
	Estimate	SE	*p*	Estimate	SE	*p*
Morning	0.96	0.12	10^{-7}	13:17	00:20	10^{-7}
Intermediate	0.92	0.12	10^{-7}	14:35	01:26	10^{-7}
Evening	0.95	0.25	10^{-3}	10:28	00:23	10^{-7}
Estimate Differences	PA Amplitude Differences			Acrophase Differences in Hours		
	Estimate	SE	*p*	Estimate	SE	*p*
Evening vs. Morning	-0.01	0.28	0.96	-02:49	01:29	0.06
Evening vs. Intermediate	0.03	0.28	0.92	-04:07	01:29	0.01
Intermediate vs. Morning	-0.04	0.17	0.80	01:18	00:31	0.01

3.4.2 Non-Workday PA Diurnal Rhythms

MLM analysis of PA on non-workdays revealed a diurnal PA rhythm with significant sine (B = -0.32, t_{4857} = -2.82, $p < 0.01$) and cosine (B = -0.91, t_{4856} = -8.68, $p < 0.0001$) terms (Table 5). The intermediate group term was significant (B = -0.88, t_{420} = -2.82, p = 0.02), though both sine and cosine intermediate interaction terms were not, suggesting PA rhythms exhibited by those in the intermediate chronotype group did not differ significantly from morning types. Neither the evening group nor evening interaction terms were significant. See Figure 6 for a depiction of estimated rhythms by chronotype group. Because the residual parameter estimate was larger in the "empty" model than in the current model, the pseudo R^2 statistic was not calculated.

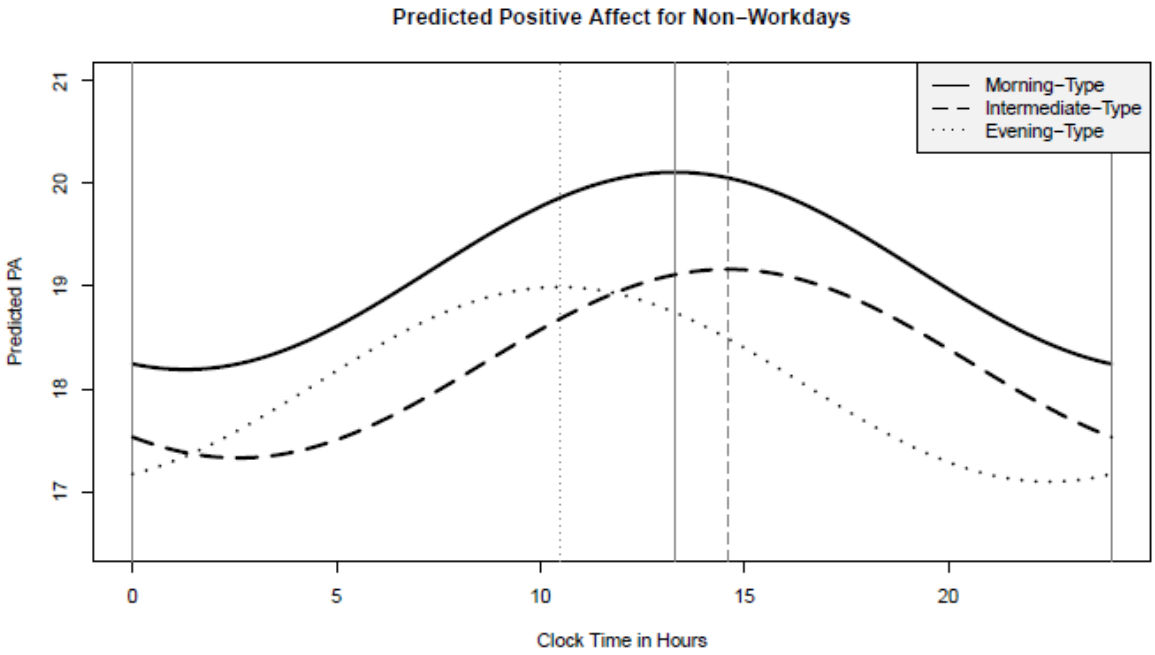

Figure 6. Non-Workday PA rhythm modeled by chronotype group. Dotted lines mark acrophase for each group.

Estimates for chronotype group amplitude and acrophase on non-workdays did not follow predictions. PA amplitude did not differ by chronotype group. In addition, evening chronotype had the earliest acrophase time at 10:28 hours (*SE* = 0:23), morning chronotype at 13:17 hours (*SE* = 0:20) and intermediate at 14:35 hours (SE = 1:26) on non-workdays. Group comparisons of estimates showed a significant phase difference between morning and intermediate (*amp diff z-value* = 2.53, $p = 0.01$) as well as intermediate and evening groups (*amp diff z-value* = -2.78, $p < 0.01$; Table 6).

3.4.3 Individual Analyses of PA Data

Using similar methods as those for the chronotype group analyses, individual PA amplitude and acrophase estimates were calculated for both workdays and non-workdays. Average workday amplitude was lower (M = 1.69, SD = 1.27) than average non-workday amplitude (M = 2.45, SD = 1.67). Average work acrophase (M = 12:56, SD = 4:40) was similar to non-workday average (M = 12:25, SD = 5:35). Individual amplitude scores are smaller than self-reported peak values because amplitude is calculated as a measurement of variance around the intercept.

In order to replicate group comparisons with individuals, an OLS regression model was used including age, gender, and chronotype to predict workday PA amplitude and acrophase. The model predicting amplitude was not significant, while the model predicting acrophase was significant (2.8% of variance explained; p = 0.01). Increasingly evening chronotype was associated with relatively delayed PA acrophase on workdays (β = -0.17, p = 0.001). Similar models predicting non-workday PA amplitude and acrophase were not significant (Table 7). Given these significant circadian rhythms of PA in both the chronotype group and individual analyses, we proceeded with our primary aims testing the gene risk score.

Table 7. Models of chronotype predicting individual PA rhythm estimates on work and non-workdays.

	Predicting PA Amplitude					
	Workday			Non-Workday		
Variable	β	SE	*p*	β	SE	*p*
Gender	0.08	0.13	0.14	0.10	0.17	0.06
Age	-0.02	0.01	0.70	-0.05	0.01	0.31
Chronotype	0.03	0.01	0.53	0.04	0.01	0.47
R^2		0.01	0.47		0.01	0.21

	Predicting PA Acrophase					
	Workday			Non-Workday		
Variable	β	SE	*p*	β	SE	*p*
Gender	0.01	0.48	0.90	-0.04	0.58	0.49
Age	0.02	0.03	0.75	0.09	0.04	0.08
Chronotype	-0.17	0.03	< 0.01	0.01	0.04	0.10
R^2		0.03	0.01		0.01	0.33

3.5 PRIMARY AIMS

3.5.1 Aim 1

Aim 1 hypothesized that the gene risk score and individual polymorphisms would be associated with chronotype group. In an OLS regression model using genotype risk score to predict chronotype, the gene risk score term was not significant (Table 8), and no polymorphism contributed to the stepwise model (Table 9).

Table 8. Model using genetic risk score and alternative risk score to predict chronotype

	Original Risk Score			Alternative Risk Score		
Variable	β	SE	*p*	B	SE	*p*
Gender	-0.01	0.72	0.90	-0.01	0.72	0.91
Age	0.22	0.05	< 0.01	0.23	0.05	0.00
Risk Score	-0.01	0.40	0.79	0.02	0.58	0.69
R^2		0.05	<0.001		0.05	<0.001

Table 9. Individual genotypes predicting chronotype with both original and alternative scoring

	Original Risk Score				Alternative Risk Score			
Variable	ΔR^2	β	SE	*p*	ΔR^2	β	SE	*p*
CLOCK 3111C/T	0.004	0.07	0.73	0.21	0.005	0.07	1.05	0.18
PER3 G647V	<0.001	0.01	0.79	0.90	<0.001	0.02	1.38	0.67
BMAL 1420A/G	0.001	-0.03	1.35	0.56	<0.001	0.01	1.15	0.80
CRY rs8192440	0.007	-0.09	0.74	0.10	0.004	-0.06	1.10	0.24

3.5.2 Aim 2

The OLS models predicting individual amplitude from cumulative genetic risk score were not significant for workdays (R^2= 0.01; p = 0.42) or non-workdays (R^2 = 0.01; p = 0.21). Acrophase models for workdays (R^2 = 0.01 p = 0.90) and non-workdays (R^2= 0.01; p = 0.14) were also not significantly associated with risk score (Table 10). Further, no individual polymorphism contributed significantly to the stepwise model (Table 11).

Table 10. Gene risk score predicting individual PA amplitude and acrophase on workdays and non-workdays

	Predicting PA Amplitude					
	Workday			Non-Workday		
Variable	β	SE	*p*	B	SE	*p*
Gender	0.08	0.13	0.15	0.10	0.17	0.05
Age	-0.02	0.01	0.77	-0.04	0.01	0.41
Gene Risk Score	-0.04	0.07	0.42	0.03	0.10	0.51
R^2		0.01	0.42		0.01	0.21

	Predicting PA Acrophase					
	Workday			Non-Workday		
Variable	β	SE	*p*	B	SE	*p*
Gender	0.01	0.48	0.90	-0.03	0.58	0.52
Age	-0.02	0.03	0.66	0.10	0.04	0.06
Gene Risk Score	-0.04	0.27	0.50	0.07	0.32	0.15
R^2		0.01	0.90		0.01	0.14

Table 11. Individual loci included in the original risk score predicting either amplitude or acrophase

	Amplitude				Acrophase			
Variable	ΔR^2	β	SE	*p*	ΔR^2	B	SE	*p*
CLOCK 3111C/T	0.002	-0.05	0.13	0.37	<0.001	-0.01	0.48	0.81
PER3 G647V	0.001	-0.60	0.14	0.55	0.001	-0.02	0.53	0.69
BMAL 1420A/G	<0.001	0.00	0.24	0.99	<0.001	-0.02	0.90	0.73
CRY rs8192440	<0.001	-0.01	0.13	0.92	<0.001	-0.02	0.49	0.75

3.5.3 Aim 3

Due to the lack of significant associations between the genetic risk score and PA rhythm estimates, conditions for mediation analyses were not met (Barron & Kenny 1986). Therefore, analyses exploring the mediation of chronotype were not performed.

3.6 ANCILLARY AIMS

3.6.1 Negative Affect

A diurnal rhythm in workday NA was evidenced by significant sine (B = 0.19, t_{15968} = 2.86, p < 0.01) and cosine (B = -0.26, t_{15967} = -3.67, p < 0.001) terms. Chronotype terms and interaction terms were not significant (Table 12), indicating NA rhythms did not differ significantly by chronotype group. Amplitude and acrophase group estimates were not significant, similar to other NA findings. Non-workday analyses were not significant.

Table 12. Chronotype group cosinor MLM results for both NA on workday and non-workdays

	Workdays			Non-Workdays		
Variable	B	SE	*p*	B	SE	*p*
Sine	0.19	0.07	0.0043	0.22	0.12	0.0611
Cosine	0.26	0.07	0.0002	-0.08	0.11	0.4929
Intermediate	0.54	0.52	0.3036	0.47	0.53	0.3809
Sine* Intermediate	-0.11	0.09	0.2223	-0.16	0.17	0.3413
Cosine* Intermediate	-0.13	0.09	0.1715	-0.08	0.15	0.6127
Evening	0.81	1.22	0.5069	0.81	1.24	0.5148
Sine*Evening	-0.16	0.22	0.4767	-0.68	0.40	0.0867
Cosine* Evening	-0.37	0.19	0.0458	0.39	0.31	0.2041
Age	0.02	0.04	0.4769	0.03	0.04	0.4286
Gender	-0.31	0.51	0.5370	-0.34	0.51	0.5033

3.6.2 PANAS vs. Rhythm Modeling

Pearson correlations with PANAS one-time PA measurements revealed significant correlations with workday PA amplitude (r = 0.17, p < 0.01), but not workday PA acrophase (Table 13). In stepwise regression predicting chronotype scores, acrophase contributed significantly to the variation in chronotype scores above and beyond the one time PANAS measurement (ΔR^2 = 0.03, p < 0.01; Table 14). Including age and gender as covariates did not significantly change the results.

Table 13. Correlations between PA variables

	PANAS	Amplitude	Acrophase
PANAS	1	0.17*	-0.01
Amplitude	0.17*	1	0.02
Acrophase	-0.01	0.02	1

Note. Workday amplitude and acrophase were used for correlations. * p<0.01

Table 14. Stepwise regression model measuring contribution of PA variables in predicting chronotype

Variable	ΔR^2	β	SE	*p*
PANAS	0.05	0.22	0.06	<0.001
Amplitude	0.00	-0.01	0.28	0.91
Acrophase	0.03	-0.16	0.08	<0.01

Note. Workday amplitude and acrophase were used in model

3.6.3 PER 1 SNP

The additive model of rs7221412 did not significantly predict workday PA amplitude (R^2 = 0.01, *p = 0.52*), non-workday amplitude (R^2 = 0.01, *p = 0.25*), workday acrophase (R^2= 0.00, *p = 0.90*) or non-workday acrophase (R^2 = 0.01, *p = 0.25*). Similarly, the dominant model did not significantly predict workday PA amplitude (R^2 = 0.01, *p* = 0.54), non-workday amplitude (R^2= 0.01, *p = 0.25*), workday acrophase (R^2 = 0.00, *p* = 0.65) or non-workday acrophase (R^2 = 0.01, *p = 0.28*).

3.6.4 Alternative Genetic Risk Score.

While the additive model risk score did significantly predict chronotype (R^2 = 0.05, *p* < 0.01), the risk score term was not significant (Table 8). The OLS models predicting individual amplitude

from the alternative genetic risk score were not significant using either workday (R^2 = 0.01; p = 0.52) or non-workday data (R^2 = 0.01; p = 0.24). Acrophase models for workday (R^2 = 0.00; p = 0.95) and non-workdays (R^2 = 0.01; p = 0.18) were also not significant (Table 10).

3.6.5 Beck Depression Inventory

Consistent with the literature, chronotype significantly predicted variance in BDI (R^2 = 0.03, p < 0.01). Eveningness was associated with increased BDI (β = -0.17, p = 0.001; Table 15). BDI was not associated with PA amplitude and acrophase, however (Table 16). Mediation analyses did not support either amplitude or acrophase effects on the relationship between chronotype and BDI.

Table 15. Model of chronotype predicting BDI score

	β	SE	*p*
Gender	0.07	0.46	0.18
Age	0.02	0.03	0.69
Chronotype	-0.17	0.03	0.001
R^2		0.03	<0.01

Table 16. Models of PA amplitude and acrophase to predict BDI score

	Amplitude					
	Workday			Non-Workday		
Variable	β	SE	*p*	β	SE	*p*
Gender	0.07	0.47	0.18	0.08	0.47	0.15
Age	-0.02	0.03	0.74	-0.02	0.03	0.74
Amplitude	0.01	0.18	0.83	-0.02	0.14	0.68
R^2		0.01	0.59		0.01	0.54
	Acrophase					
	Workday			Non-Workday		
Variable	β	SE	*p*	β	SE	*p*
Gender	0.07	0.47	0.17	0.07	0.47	0.16
Age	-0.02	0.03	0.72	-0.02	0.03	0.76
Acrophase	-0.05	0.05	0.30	<-0.01	0.04	0.95
R^2		0.01	0.40		0.01	0.58

3.6.6 Social Jet Lag

The Social Jet Lag (SJL) variable could be calculated in 362 individuals who had no missing data for workday and non-workday mid-sleep times. Both workday and non-workday mid-sleep were significantly different by chronotype group, though the SJL variable did not differ by chronotype group (Table 17). SJL was found to be a significant mediator *(*$\Delta\beta$ = 0.0016, *95% CI* = 0.0001-0.0051) in the relationship between chronotype and PA amplitude (see Figure 7). Mediation analyses investigating mediation of SJL on the relationship between chronotype and PA acrophase, however, were not significant *(*$\Delta\beta$ = -0.0038, *95% CI* = -0.019-0.004).

Table 17. Social Jet Lag variables by chronotype group M (SD)

	Chronotype Group			
Variable	Morning (n = 155)	Intermediate (n = 190)	Evening (n = 17)	p
Workday Mid-sleep Time	2:49 (00:31)	3:08 (00:29)	3:44 (00:39)	0.00
Non-Workday Mid-sleep Time	3:26 (00:39)	3:49 (00:43)	4:07 (00:44)	0.00
Social Jet Lag	0.66 (0.56)	0.76 (0.64)	0.76 (0.58)	0.27

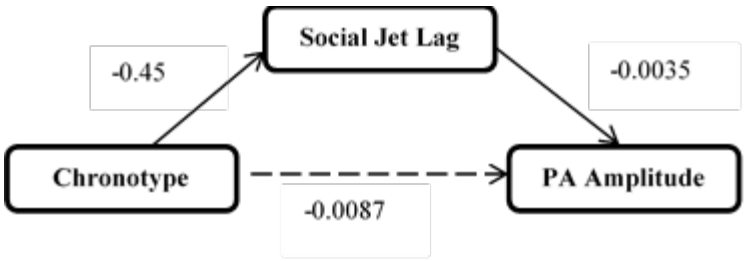

Figure 7. Social jet lag as a mediator in the relationship between chronotype and PA amplitude. Coefficients extracted from Sobel test and were not significant. Model became significant after bootstrapping.

3.6.7 Mood Diagnosis

Excluding 40 individuals with past or current mood diagnosis did not greatly change our results. However, the cosinor MLM for non-workdays PA acrophase changed from 10:28 to 12:04 when those with mood diagnosis were excluded, indicating that individuals with mood disorders pulled PA acrophase towards earlier morning hours on non-workdays. The implication and possible explanations for this apparent move towards morningness in those with mood diagnosis are discussed below.

4.0 DISCUSSION

The current study aimed to explore associations between clock genes, chronotype, and diurnal variations in PA, as measured by PA amplitude and acrophase. Preliminary MLM cosinor analyses revealed a significant rhythm of PA on both workdays and non-workdays, which significantly differed between chronotype groups. Although non-workday findings did not follow study hypotheses, workday values for acrophase demonstrated the predicted pattern, such that the morning chronotype group exhibited the earliest time of peak PA, the intermediate group peaked in the middle, and the evening group peaked last. In the current sample, the evening group peaked more than four hours after the morning type, supporting previous literature that has shown that individuals with evening chronotype experience delays in several rhythms including PA (Porto et al., 2006; Hasler et al. 2012). Further supporting our hypotheses, individual PA rhythm analyses revealed that eveningness was significantly associated with later acrophase. Contrary to study predictions, chronotype group and individual analyses predicting amplitude were not significant, though they were in the predicted direction. Taken together, the study results indicated a strong association between an individual's chronotype and the acrophase of PA rhythms.

In contrast to workday PA acrophase, workday PA amplitude did not differ significantly by chronotype group, nor was there a significant association between chronotype as a continuous measure and individual PA amplitude estimates. However, although not statistically significant, amplitude analyses revealed the predicted pattern of results such that individuals with evening

chronotype exhibited the most blunted rhythms while morning types exhibited the highest peaks in PA (Figure 5). The lack of amplitude findings may be a result of the restricted variation in workday PA amplitude estimates (*SD* = 1.27) as compared to PA acrophase estimates (*SD* = 4:40). Because the current study utilizes statistical methods, which rely on association of variation, restricted variation in amplitude may have impaired our ability to detect existing associations between amplitude and chronotype. Although the lack of amplitude findings may be a result of restricted variance, it is also important to remember that only one study reported a significant association between PA amplitude and chronotype in an insomnia population (Hasler et al., 2012), indicating further research is needed to confirm this finding. In the current healthy population, the lack of amplitude findings may suggest that individual differences in chronotype may more closely associate with PA phase timing than with daily PA amplitude. Alternatively, the small percentage of individuals with evening chronotype in this study may have served to restrict variance in the chronotype measure.

Further supporting the notion that PA acrophase may be a more important measure in predicting chronotype than PA amplitude, ancillary results found that PA acrophase, but not amplitude, significantly predicted variation in chronotype above and beyond the one-time measurement of PA employed in this study. Although the amount of variation in chronotype explained by any of the PA variables was small (0% - 5% variance explained), the findings suggest that estimates of acrophase added a statistically significant amount of information about chronotype beyond a once-daily measurement of affect. These results not only point to the value of PA rhythm estimates, especially acrophase, in circadian research, but also suggest that PA phase time may be more tightly linked with chronotype than PA amplitude.

Study hypotheses for PA acrophase and chronotype were only supported for workday models and not for non-workday models. This inconsistency may be due to the multiple days measured on workdays compared to the single non-workday measurement. Given the single day measurement and resulting limited measurement time points, non-workday estimates may be more influenced by event-related mood changes than workday estimates, creating less accurate estimates. Further, participants tended to have mood measurements during several consecutive workdays, which may have allowed individuals more time to adjust to the workday schedule and provided a more accurate measurement of diurnal mood patterns during workdays. In contrast, the single day measurement of mood on non-workdays may have captured an in-between period when individuals are still adjusting from a workday rhythm to a non-workday rhythm. It may be that multiple, consecutive measurements on workdays, provided a more accurate estimate of diurnal mood variation. To increase confidence in our non-workday measures, future studies could include multiple non-workdays over several weeks, or a vacation period of consecutive non-workdays.

Of specific interest in non-workday estimates is the reverse PA acrophase order, such that the evening group had the earliest acrophase. Although contradictory to study hypotheses, this may be consistent with the literature suggesting that evening chronotypes have the most dramatic changes in rhythm from workday, or socially imposed schedules, to their natural rhythms on non-workdays (Wittmann et al., 2006). As shown in Table 6 and Figures 4 & 5, workday PA acrophase was more than four hours earlier than PA acrophase on non-workdays in the evening chronotype group, a difference not found in the morning and intermediate groups. It may be that evening type individuals were so delayed in their acrophase on non-workdays that their peak was actually most evident the following morning after awakening. Indeed, this is supported by the observation in the present study that individuals with mood disorders, who mostly had extreme evening scores

relative to the rest of the sample, were pulling the average evening group acrophase towards early morning. Evening group acrophase may differ between workday and non-workday due to greater changes between workday and non-workday schedules, which may cause them to become extremely delayed on those days they are not on a socially or work-defined schedule when their schedule is free to delay.

Interestingly, all analyses including clock gene predictors of these variables were not significant. Specifically, the proposed clock gene risk score did not predict chronotype or individual PA rhythm variables. The lack of genetic associations in this sample may be, in part, due to the size of the sample. Post hoc power analysis for the original gene risk score predicting chronotype revealed the current study was underpowered to find a significant association between the gene risk score and chronotype (power = 46%, α = 0.01), and the sample would need to include 765 individuals to reach significance given the effect size obtained. However, it is important to note that post hoc power analyses confirmed the current study was powered properly for the gene risk score predicting both workday amplitude (95%, α=0.01) and acrophase (91%, α=0.01), suggesting the lack of significant findings in PA rhythms and genetic risk score may be a result of other factors discussed below. Further impairing the current study's ability to find significant findings with chronotype is the lack of evening types in the study (n = 19; 5%). Given the genes included in the risk score were selected due to their ability to predict eveningness, the paucity of evening types in the current sample likely limited our ability to find gene-chronotype associations. Therefore, the absence of findings with the risk score may be due to the lack of evening types in the sample, and consequently restricted range of chronotype and acrophase/amplitude PA scores.

It is also important to note that the current study's genetic risk score may have failed to include genes that could significantly impact circadian variation. First, the gene risk score was

created based on previous literature. The strength of the risk score relied on the quality and number of clock genes previously explored. It may be that genes that are more important to predicting chronotype have yet to be reported. Second, regulating factors that directly control the transcription and translation of the core clock genes, but are not part of the core clock mechanism, were not represented in the clock gene risk score. Interestingly, recent research has found the rhythmic expression of clock genes is abolished when calcium is blocked from entering SCN cells (Lundkvist, Kwak, Davis, Tei, & Block, 2005), suggesting calcium levels may indirectly effect the transcription of clock genes. Further, appropriate membrane depolarization (Nahm, Farnell, Griffith, & Earnest, 2005) and proper daily cAMP signaling (O'Neill, Maywood, Chesham, Takahashi, & Hastings, 2008) has been shown to be essential in maintaining rhythmic expression of the clock genes. Given that calcium, membrane depolarization, and cAMP seem to modulate the transcriptional/translational loop of the molecular clock, these additional factors may obscure the effect of the genetic risk score included in this study. Future studies should include a larger array of circadian genes, including those that control regulating factors of the clock.

Further, included genes may have predicted chronotype indirectly, weakening the gene risk score's predictive capabilities. Specifically, the *PER3* G647V polymorphism may influence circadian measurements indirectly via mechanisms that control the sleep homeostat or sleep drive (Dijk & Archer, 2010; Mongrain, Carrier, & Dumont, 2006). Mice deficient in the *PER3* gene had decreased wake time and more time in NREM sleep immediately after sleep deprivation compared to wildtypes (Hasan, van der Veen, Winsky-Sommerer, Dijk, & Archer, 2011) suggesting *PER3* has a direct impact on homeostatic sleep drive, affecting circadian rhythms downstream. If indeed *PER3* only directly impacts homeostatic sleep drive, inclusion of the *PER3* G647 polymorphism may introduce a weak, indirect predictor of circadian rhythms. The proposed genetic risk score

may have been a stronger predictor of chronotype if additional clock related genes, described above, were included.

Lastly, the lack of associations with the core clock genes may be due to the relatively small amount of variance explained by PA rhythms. In the current sample, 4% of the variance in PA was explained by the modeled curve, in contrast to previous studies that found up to 13% of PA variance explained by a daily rhythm including the sleep homeostat (Murray et al., 2009). Given the 4% variance explained by the rhythm in our study, and the likelihood that sleep homeostasis will also explain some the variance explained by the modeled curve, there is little variance left to explained by the core clock gene risk score used in the study. Our sample is larger and older than those used in previous studies (e.g. Murray et al., 2009), which may explain the difference in variance present to be predicted by clock gene risk score.

Beyond the sleep homeostat, the modifications to the PANAS PA scale in the current study may have attenuated detectable PA rhythmicity relative to previous reports of PA rhythms. In reviewing Table 3, only the "determined" item in the PA scale overlaps with previous research reporting variance in PA explained by a sinusoidal rhythm (Hasler, 2009; Murray et al., 2009), suggesting item differences may explain why only 4% variance was explained in the current study relative to 22% and 13% in previous reports. Further, "active," was deleted from the measure due to low factor loading with PA, and in an effort to reduce participant burden given that EMA was conducted hourly. Importantly, this item may tap into the arousal dimension of the mood circumplex mentioned above (see Figure 4; Russell, 1980), which has been shown to have a circadian rhythm in FD studies (Van Dongen & Dinges, 2005). To the extent that arousal is a component of PA, the circadian rhythm in arousal may be driving previous robust PA rhythm measurements (Hasler, 2009; Murray et al., 2009) that included the "active" item. Therefore, it

may be the measure of arousal that is ultimately driving the previously detected robust PA rhythm, and that explains the smaller amount of variance explained in our study. Future research should explore the potential impact of arousal via the inclusion of more arousal items in MLM cosinor analysis.

As reported in the ancillary results, NA exhibited a significant rhythm in the current study sample. This is in contrast to several studies (Murray et al., 2009; Murray, 2007; Murray et al., 2002; Wood & Magnello, 1992; Clark et al., 1989) that did not find NA to have a significant diurnal variation. However, it is consistent with three studies (Golder & Macy, 2011; Stone et al., 2006; Vittengl et al., 1998) that did find a rhythm. The inconsistency with the bulk of the literature may be due to the current sample's population size, which is greater than most of the samples represented in the literature (except for Stone et al. 2006 and Golder & Macy, 2011), as well as the multiple mood assessments throughout the day in the present study. Interestingly, the current results found no significant impact of chronotype on NA rhythms, indicating that individual differences in circadian rhythms were not associated with the daily variation in NA. Further investigation is needed to confirm a diurnal pattern in NA and whether individual differences in circadian rhythms co-vary with NA rhythms. It appears that, even if a rhythm in NA is evident, it is not as clearly associated with chronotype as PA rhythm.

4.1 LIMITATIONS

Although the current study provided the first healthy population of this magnitude with multiple workday measurements, several study limitations may impact results and interpretation. The use of self-report measures, like the CSM, is fraught with reporting bias in that individuals may not

accurately report their activity timing preference (Podsakoff, MacKenzie, Lee, & Podsakoff, 2003). Further, chronotype may be influenced by both circadian and sleep homeostasis effects, such that an evening type individual may be classified as such due to delayed circadian rhythms, abnormal homeostatic sleep drive, or both (Mongrain, Lavoie, Selmaoui, Paquet, & Dumont, 2004). Adding a measure of homeostatic sleep drive may explain the null finding with the clock gene risk score, as we may have been utilizing circadian elements to predict homeostatic sleep effects. In order to circumvent the limitations of chronotype measurement, utilizing a more direct measure of circadian rhythms, such as dim light melatonin onset (DLMO; Pandi-Perumal et al., 2007), could provide a more accurate measure of an individual's circadian rhythm in future studies.

Additional limitations include the number of individuals with evening chronotype. In the current sample, there were 19 (5%) individuals with evening chronotype. With fewer participants in the evening type group, the range for chronotype was restricted and estimates may be less accurate. However, the fact that using chronotype as a continuous variable revealed similar results as the group analyses increases confidence that our sample included enough variance in chronotype to predict PA acrophase. Future studies could benefit from recruiting an equal representation of individuals for each of the three chronotype groups, or samples including those with depression or otherwise more likely to be evening types.

The present study aimed to investigate how variation in the genetic clock, chronotype, and PA rhythms may be related, and to explore the potential of predicting depression. Although the sample was mostly healthy, an association with the study model (Figure 1) and BDI scores was explored. Due, perhaps, to the limited variation and skew in BDI scores in the current sample (M = 4.38; SD = 4.51), we did not find an association between PA rhythms and BDI. Although eveningness was significantly associated with higher BDI scores, the effect size was small (β = -

0.17). Repeating analyses in a depressed sample should introduce a wider range of BDI scores and more eveningness and may identify associations between PA rhythm and chronotype.

4.2 SUMMARY

The lack of findings in genetic risk score analyses points to the need for future research exploring samples with greater evening type representation, and polymorphisms that may have a more robust impact on circadian variation. Given the supported association between chronotype and PA rhythms, extending the current research into a depressed population may identify a mediating factor of PA rhythms in explaining the existing association between evening chronotype and depression. To date, the current study is the largest reported sample investigating daily affect rhythms using EMA techniques exploring chronotype differences on a group and individual basis, and therefore, further supports the existence of diurnal PA rhythms, and that they vary by individual and group chronotype. Specifically, individuals with evening chronotype had later PA acrophase times. Further, the current study provides evidence for the importance of PA acrophase information above and beyond a single affect measurement, suggesting EMA collection of PA may be a significant contribution to studies that are concerned with the circadian variations in affect such as those testing the Social Jet Lag hypothesis, or the role of PA and chronotype in depression.

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