Circadian Phase in Chronic Insomnia

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ABSTRACT

Introduction:

Melatonin and melatonin agonists like ramelteon have been shown to be successful at treating insomnia without the significant side effects and withdrawal effects of the sedatives and hypnotics that are traditionally prescribed for this condition. Furthermore, traditional pharmacological treatments for insomnia do not address the underlying physiological, psychological, and behavioral causes of insomnia. When combined with behavioral therapy, treatments for insomnia are more effective and longer lasting. In addition, some types of insomnia are connected with circadian misalignment with the external environment. Melatonin and melatonin agonists may treat these types of insomnia by adjusting circadian phase. The aim of this thesis was to compare the change in circadian phase, as determined by salivary melatonin levels, from pre- and post-treatment with 8mg of ramelteon combined with multi-component behavioral therapy (MCBT) or with ramelteon alone compared to placebo in chronic insomniacs. The hypothesis of this thesis is that there will be a greater change in circadian phase with ramelteon and MCBT than in the placebo group. We expect that a change in circadian phase will be correlated with improvement in subjective sleep quality.

Methods:

Thirty one adults aged 18-64 that met the criteria for chronic insomnia (sleep onset latency of more than 30 minutes on at least 3 nights a week and average total sleep time less than 6.5 hours) were studied at the University of Arizona under the supervision of Dr. Richard Bootzin. Subjects received either 8mg ramelteon or placebo daily in combination with MCBT. Subjects in the MCBT/ramelteon condition received weekly therapy sessions in addition to the drug. Sleep diaries and subjective sleep measures were recorded throughout the treatment period. Saliva samples were taken under dim light conditions (<25 lux) every hour during pre and post-treatment visits to assess circadian phase. Frozen saliva samples were shipped to the University of Colorado where they were assayed using a high sensitivity ALPCO ELISA kit. DLMO was determined using a 3pg/mL threshold and the change in circadian phase was calculated by comparing the difference between pre- and post- treatment bedtimes and DLMO.

Results:

A significant ($p<.05$) change in phase angle was found between the ramelteon-only group and placebo. Significant improvements in subjective measures were seen in all groups. There was no association between change in phase angle and treatment efficacy.

Conclusion:

Our findings further support existing evidence that a standard 8mg dose of ramelteon can effectively shift the circadian clock so that individuals sleep at a more appropriate biological time. Although we did not find that a change in phase angle was associated with treatment efficacy, ramelteon alone or in combination with MCBT continues to show promise in the treatment of insomnia. However, further research needs to be done to verify and test the scope of these results.

INTRODUCTION

Although most humans spend about one third of their lives sleeping, scientists in the field of sleep medicine are still just beginning to grasp the scope and complexity of how sleep and circadian rhythms interact with other biological processes such as hormone secretion, metabolism, and learning and development. Through this research, adequate sleep and proper circadian alignment has proven to play a critical role in health and well-being in various areas of medicine. For example, recent studies implicating inadequate sleep in the etiology of obesity, diabetes, and cardiovascular disease underscore the importance of the field of sleep in modern medicine as the incidence of these diseases rises (Scheer, Hilton et al. 2009; Cardinali, Cano et al. 2011). Sleep disorders can have far-reaching consequences, from hormonal and metabolic imbalances to fatigue that results in workplace or motor vehicle accidents. A 2004 study in Australia calculated the approximate costs of sleep disorders to be 7.5 billion USD across a population of 20.1 million, out of whom less than 10% were estimated to suffer from a chronic primary sleep disorder (Hillman, Murphy et al. 2006). Although insomnia was only estimated to affect 5% of the Australian population, this cost analysis included an estimated $8.8 million spent on pharmaceuticals to help induce and maintain sleep. Furthermore, 76% of the drugs prescribed were hypnotics, which have been shown to increase the risk of motor vehicle accidents (Gustavsen, Bramness et al. 2008). The injuries and property damage resulting from the drugs used to treat sleep disorders further add to the indirect costs of these disorders. It is clear that the physiological effects and economic costs of sleep disorders are far-reaching, and more needs to be done to ensure that treatments are easily available, low-risk, and effective.

Insomnia

Insomnia, broadly defined as difficulty initiating or maintaining sleep that results in sleep of poor duration and quality[1], is reported to affect anywhere between 4-50% of the population (Wade 2010). Because of the broad use of the term "insomnia" among the general public, more stringent clinical criteria (such as the presence of daytime impairment) are typically used to obtain closer estimates, which fall around 10% (Roth, Krystal et al. 2007). To further complicate this matter, insomnia often appears in conjunction with other disorders like depression and anxiety, potentially spurning a vicious cycle of anxiousness, frustration, and poor sleep. Figure 1 shows the results from a 1998 study in France that described some of the complexities in the interactions between depressive and anxiety disorders with insomnia (Ohayon, Caulet et al. 1998). The data in Figure 1 is from the 17.7% of the studied population who fit the criteria listed in the DSM-IV[2] to further classify insomnia into these six categories. Over half of those interviewed who met the criteria for insomnia also experienced depression or anxiety symptoms. In cases like these, it can be difficult to discern the causal relationship – if any – between insomnia and these disorders, which makes it difficult both to study and to treat (Simon and VonKorff 1997).

Figure 1. Distribution of insomnia by diagnostic category

Primary Insomnia	7.3%
Insomnia related to a depressive disorder	8.1%
Insomnia related to an anxiety disorder	8.4%
Depressive disorder with insomnia symptoms	10%
Anxiety disorder with insomnia symptoms	37.7%
Isolated insomnia symptoms	28.4%

(Ohayon, Caulet et al. 1998)

[1] Poor sleep can be measured subjectively (ie. not feeling refreshed, feeling like one spent the night tossing and turning) or objectively, with measures like sleep onset latency (SOL), total sleep time (TST), EEG parameters, etc.
[2] The DSM-IV (Diagnostic and Statistical Manual) is published by the American Psychiatric Association and includes descriptions and classifications of mental disorders.

Treatments for insomnia

Because there are different types of insomnia with different causes and comorbidities[3], there are a variety of psychological, pharmacological, and behavioral treatments that can be implemented to treat insomnia and its symptoms. However, not all of these treatments are equally sought or prescribed. One study revealed that nearly one third of insomniacs use psychotropic medications but only 13% sought mental health services. Of those who utilized mental health services, 36% had comorbid depression and only 5% were seeking treatment for insomnia alone (Simon and VonKorff 1997). This indicates that insomnia is often treated as a symptom, not as an independent disorder which often has its own underlying cause. More surprising is the overwhelming use of psychotropic drugs, given that they must be taken continually, and sleep problems tend to re-emerge upon discontinuance. In one two-year study, only 22% of those with severe insomnia who were taking hypnotics reported significant improvements in their sleep (Hohagen, Rink et al. 1993).

The most commonly prescribed sleep drugs are hypnotics such as the non-benzodiazepines Ambien and Lunesta, which are $GABA_A$ receptor agonists. GABA, the brain's primary inhibitory neurotransmitter, promotes sleep by causing wide-spread inhibition across the wakefulness-promoting regions in the cerebral cortex, thalamus, and hypothalamus (Gottesmann 2002; Szabadi 2006). Both benzodiazepines and non-benzodiazepines cause this general central nervous system depression, and may have serious side-effects including daytime impairment, rebound effects after discontinuing use, and potential for abuse (Szabadi 2006). These drugs have also been shown to alter sleep architecture[4], usually causing an increase in stage 2 sleep and a decrease in slow wave activity (Parrino and Terzano 1996).

[3] Comorbidity is the medical term for when one or more diseases or disorders occur together.
[4] Sleep architecture refers to the EEG measure of how sleep is distributed across sleep stages throughout the night.

To avoid the next-day "hangover effect" resulting from the use of drugs that act on the GABAergic system, scientists have recently invested in exploring the use of melatonin and melatonin agonists to improve sleep in insomniacs and healthy sleepers. Melatonin is a naturally occurring hormone that is secreted by the pineal gland and plays an important role in the circadian regulation of the sleep/wake cycle. The synthesis and release of melatonin from the pineal gland is almost entirely inhibited during the day by the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. The SCN receives information about environmental light conditions from the retina, and passes this information on through its projections to other brain areas, which are summarized later in this paper in Figure 2. When the SCN's inhibition of the pineal gland is removed, circulating melatonin levels rise and feed back to the SCN (Moore 1997). The sleep-promoting functions of melatonin are facilitated through its actions on the MT_1 and MT_2 receptors that are located throughout the body but primarily in the SCN (Pandi-Perumal, Srinivasan et al. 2007; Pandi-Perumal, Srinivasan et al. 2009). Because melatonin works independently of the GABA system, it does not result in the general central nervous system depression that causes morning impairment and other unwanted side effects (Roth, Stubbs et al. 2005; van den Heuvel, Ferguson et al. 2005).

Melatonin has been successfully used in the treatment of insomnia in older individuals and shows promise as a safe and effective alternative to sedative or hypnotic drugs (Haimov, Lavie et al. 1995; Zhdanova, Wurtman et al. 2001; Leger, Laudon et al. 2004). Not all studies, however, demonstrate a significant effect of melatonin on improving sleep. While most studies find that melatonin significantly decreases sleep onset latency (SOL)[5], others conclude that the effects of melatonin are not strong enough to promote and maintain sleep in all populations (van den Heuvel, Ferguson et al. 2005). For example, since melatonin does not cause an involuntary

[5] Sleep latency is a measure of how long it takes someone to fall asleep while lying in bed.

loss of consciousness even at very high doses, its effects can be modified by an individual's desire to stay alert (ie. it is easy to make the decision to stay up past one's bedtime) (van den Heuvel, Ferguson et al. 2005). Melatonin's short half-life of approximately 20 minutes in blood and the variability of effect depending on time of administration have also been implicated as limits to its use as a sleep aid (van den Heuvel, Ferguson et al. 2005; Pandi-Perumal, Srinivasan et al. 2007) .

Because melatonin has not been proven as an effective sleep aid in all cases, pharmaceutical companies are now exploring synthetic drugs that act similarly to melatonin. One melatonin agonist in particular, ramelteon, has been shown to reduce SOL and improve total sleep time (TST) (Roth, Stubbs et al. 2005). Ramelteon binds specifically to melatonin receptors with a higher affinity than melatonin and has a longer half-life, which could help enhance and prolong its sleep-promoting effects (Pandi-Perumal, Srinivasan et al. 2007).

In addition to pharmacological interventions, behavioral therapies have been shown to help improve sleep in insomniacs. The type of therapy used in this study, Multi-Component Behavioral Therapy (MCBT), consists of different modules (stimulus control instructions, sleep restriction therapy, sleep education, and cognitive therapy) which address different factors that may contribute to poor sleep. Variations of these therapeutic tools have been applied in numerous studies, and overall results conclude that cognitive behavioral therapies achieve positive results in both subjective and objective measures of sleep quality including improving TST, reducing wakefulness after sleep onset (WASO), and reducing SOL (Morin, Bootzin et al. 2006). These treatments can also help reduce the dosages of sleep medications being used by insomniacs and the effects tend to persist for months after discontinuing treatment (Morin, Bootzin et al. 2006). Among the types of therapy that were found to be particularly effective

treatments for insomnia include stimulus control therapy, sleep restriction, and cognitive behavioral therapy, which are three of the four components of MCBT (Morin, Bootzin et al. 2006). More information on the components of MCBT can be found in the Appendix.

BACKGROUND

In healthy individuals sleep and circadian rhythms are timed so that brain functions, physiological processes, and behaviors occur at optimal environmental times corresponding with the light-dark cycle and its changes across the year. These rhythms can even be observed in organisms as simple as cyanobacteria and molds, playing an important role in both biochemical and behavioral aspects of life across all species from seasonal reproductive cues to memory formation (Gillette and Tischkau 1999).

Homeostatic sleep drive and circadian rhythm

In humans and many other species, daily patterns of sleep and wakefulness are the result of interactions between the homeostatic drive for sleep and the circadian rhythm in what is known as the two-process model of sleep regulation (Borbely and Achermann 1999). Homeostatic sleep drive, known as Process S, increases across the day and dissipates during sleep. With prolonged wakefulness, brain glycogen[6] levels decrease as the brain uses its energy stores, but are restored after sufficient recovery sleep (Kong, Shepel et al. 2002). In addition to decreased brain glycogen levels with prolonged wakefulness, adenosine[7] accumulates in the brain, which may act as a signal to activate the brain's sleep-promoting centers (Saper, Scammell et al. 2005). These biochemical changes in the brain are proposed to be mechanisms by which

[6] Glycogen is the storage form of glucose, the brain's primary source of energy.
[7] Adenosine is a metabolic by-product resulting from the breakdown of ATP for energy.

the homeostatic sleep drive promotes sleep to occur. These homeostatic signals continuously interface with the circadian rhythm, or Process C, which is a near 24-hour endogenously driven rhythm controlled by the SCN. Process C promotes wakefulness over the course of the day to counteract the increasing homeostatic sleep drive, thereby maintaining wakefulness. During the biological night, and under control of the circadian system, the pineal hormone melatonin is synthesized and released into the blood as described previously. Melatonin levels typically rise around 2 hours prior to habitual bedtime, and decline shortly before waking (Wright, Gronfier et al. 2005). Sleep is initiated after the circadian peak, where the homeostatic and circadian systems are both promoting sleep. This zone of high propensity for sleep corresponds with a decrease in cognitive performance because of the strong drive for sleep being enforced by both the homeostatic and circadian processes at this time (Wright, Hull et al. 2002; Rogers, Dorrian et al. 2003).

Chronotypes – owls and larks

The period (τ) of the circadian rhythm varies between individuals and typically falls within 1 hour of the 24 hour earth day, with an average of ~24.2 hours (Duffy and Wright 2005). In one 1999 study, the intrinsic circadian period of 11 young adult men and 13 older adults was assessed over a month-long forced desynchrony protocol[8]. The researchers found a standard distribution of periods ranging from 23.9 to 24.5 hours with the peak at 24.2 hours (Czeisler, Duffy et al. 1999). While this group of researchers found no difference in circadian period between older and younger subjects, they did report that the older subjects studied did have earlier entrained circadian rhythms and early morning awakening that is typical in the elderly (Duffy, Dijk et al. 1998; Czeisler, Duffy et al. 1999).

[8] In a forced desynchrony protocol, subjects are kept in dim-light conditions with a scheduled sleep/wake cycle that is several hours shorter or longer than the 24 hour day (ie. 28 hours), which desynchronizes the sleep wake cycle from the endogenous circadian rhythm.

At the extremes of the circadian spectrum are morning and evening-types, known colloquially as "larks" and "owls". These chronotypes express significant differences in alertness and cognitive performance across the day with morning-types being more alert and having better performance on cognitive tasks earlier in the day than their evening-type counterparts (Horne, Brass et al. 1980). However, alertness and cognitive performance are also closely tied to the sleep/wake cycle. A 2003 study suggests that the contributions of homeostatic and circadian processes to sleep pressure differ between morning and evening types (Taillard, Philip et al. 2003). The differences in circadian and homeostatic interactions between chronotypes has implications for the effects of people sleeping outside their optimal circadian time, as occurs in some types of insomnia.

Entrainment

Despite differences in τ, most individuals are able to synchronize their circadian rhythm to periodic cues from the external environment and maintain a very tight distribution around the 24-hour earth day. Entrainment is said to occur when the internal clock is synchronized with environmental time cues, known as zeitgebers[9] (Lack and Wright 2007). For humans, light is the primary zeitgeber, which coordinates sleep and wake activity with the alternating periods of environmental darkness and light (Wehr, Aeschbach et al. 2001). As most individuals have innate circadian periods slightly longer or shorter than the 24 hour earth day, they must entrain themselves daily to maintain synchrony between their internal clock and environmental time. A 2005 study revealed that for healthy individuals, an adjustment of only 10-15 minutes is needed to re-entrain the circadian clock to the external environment each day depending on the individual's circadian period (Czeisler, Duffy et al. 1999). Individuals with a period shorter

[9] Zeitgeber – German for "time giver" (Lack and Wright 2007).

than 24 hours require a daily phase delay, while those with a longer period must undergo a daily phase advance in order to re-synchronize their biological clock to the 24-hour day.

The relationship between biological time and environmental time is known as the phase angle of entrainment (Duffy and Wright 2005). The phase angle of entrainment can be described by any circadian marker (ie. dim light melatonin onset or core body temperature minimum), relative to environmental or physiological events (ie. habitual sleep time).

The SCN

The SCN, located in the anterior hypothalamus, is known as the "master clock". The SCN acts as a synchronizer for other "clocks" located peripherally throughout the entire body (Dibner, Schibler et al. 2010). The position of the SCN in the hypothalamus is biologically convenient for the transmittance of photic cues from the environment as it is positioned just above the optic chiasm and receives light cues from the retina through the retinohypothalamic tract (Dibner, Schibler et al. 2010). These input signals are critical to the entrainment of the circadian clock, and are found to be most effective at shifting the clock when they occur during specific phases of the circadian cycle. For example, in a normally entrained individual, light received at night is perceived as an error to which the biological clock must adjust (Gillette and Tischkau 1999). These periods of sensitivity to the phase resetting effects of light have implications for the treatment of circadian disorders with light therapy, as described later in this paper.

The SCN projects to other hypothalamic areas to synchronize processes throughout the body, as shown in Figure 2 below (Moore 1997). Synchronization of these processes can occur indirectly via body temperature rhythms or feeding and activity rhythms controlled by the hypothalamus, or directly via humoral and neural signals from the SCN (Dibner, Schibler et al.

2010). For example, neuronal firing rate and vasopressin secretion are two primary SCN output signals that oscillate across the circadian cycle and provide information about the passage of time and the phase of the clock (Gillette and Tischkau 1999).

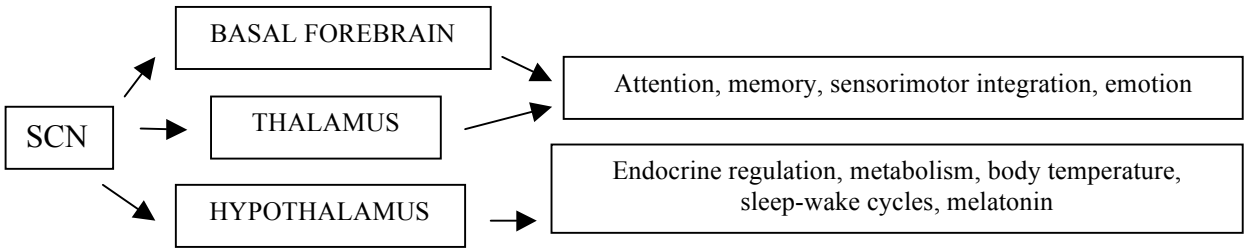

Figure 2. SCN projections within the brain and some the functions they regulate. The circadian rhythm is primarily regulated by secondary projections from the hypothalamus to the anterior pituitary, reticular formation, and pineal gland.

Constant Routine

In humans, SCN output rhythms such as core body temperature (CBT) and melatonin are used to infer the status of the biological clock. However, these rhythms are sensitive to environmental and behavioral factors such as external temperature, food intake, light exposure, physical activity, and even posture, and therefore must be studied under very controlled conditions. For example, when a person stands up, gravity immediately causes blood to pool in the lower extremities, which in turn causes plasma fluid to leak into the surrounding tissues. This has been shown to raise the concentration of plasma components, particularly proteins (which melatonin is usually bound to), by 10-20% (Deacon and Arendt 1994). Because salivary melatonin levels closely reflect levels circulating in the blood, it is important to control for posture regardless of sampling plasma or saliva to measure the melatonin rhythm (Nagtegaal, Peeters et al. 1998).

Light is another factor that must be controlled for when assessing circadian phase, as it is the most potent zeitgeber for the mammalian circadian clock (Roenneberg and Foster 1997).

The timing, intensity, and wavelength of light are all important factors in entrainment, as the sleep-promoting hormone melatonin is acutely suppressed by light, and the degree of melatonin suppression is correlated with the intensity, timing, and duration of light exposure. Wavelengths in the blue-green area of the visible spectrum (470-525nm) have been shown to be most effective at suppressing melatonin (Lack and Wright 2007). This has important implications for circadian entrainment to the external environment. For example, the spectral composition of twilight (dawn or dusk) differs from that of daylight, having enriched shorter wavelength light (<500nm). The detection of such a difference of wavelength at the beginning and end of the day could be an important cue for the entrainment of the circadian system (Roenneberg and Foster 1997).

Similarly, the circadian resetting response to light varies depending on the timing of light exposure. A phase response curve (PRC) can show how induced phase changes can vary across the day (Roenneberg and Foster 1997). The degree and direction of phase shifting varies depending on where the stimulus is given in relationship to circadian phase. A study in 1991 found that bright light (~5000-9000 lux) given before CBT minimum produces a phase delay, while light exposure after CBT minimum produces a phase advance. The greatest phase shifts were found for light given within two hours of the CBT minimum, suggesting that the window surrounding body temperature minimum is the period most susceptible to phase shifting by light (Minors, Waterhouse et al. 1991). However, it has been found that light exposure throughout the day contributes to circadian entrainment (Duffy and Wright 2005).

The intensity of light exposure can also affect the degree of phase shifting. Light intensity is measured in lux, which is based on the sensitivity of the human visual system to light (Duffy and Wright 2005). While it is well established that bright light (for example, 10,000 lux) shifts the circadian clock, it has been found that dim light ~100 lux exposure could produce

nearly half the effect seen by 10,000 lux exposure (Zeitzer, Dijk et al. 2000). It has also been demonstrated that light as low as ~1.5 lux (about equivalent to very dim candle light) was sufficient to maintain entrainment to a 24 hour day but not sufficient to entrain to longer or shorter days (Wright, Hughes et al. 2001).

Delayed and advanced sleep phase syndrome

Two types of insomnia, early morning awakening and sleep onset insomnia, have been shown to be associated with changes in melatonin and CBT rhythms. Early morning awakening insomniacs express advanced rhythms and sleep onset insomnias express delayed rhythms (Morris, Lack et al. 1990; Lack, Mercer et al. 1996; Shibui, Uchiyama et al. 1999). Furthermore, these types of insomnia have been successfully treated with light therapy and melatonin (Lack and Wright 2007). In two studies of evening bright light administration (2500 lux for 4 hours) among early morning awakening insomniacs, CBT and dim light melatonin were significantly phase delayed by around 2 hours (Lack and Wright 1993; Lack, Wright et al. 2005). In both of these studies, TST increased and subjective ratings of sleep improved. Melatonin has also been used successfully as a treatment for elderly insomniacs (Haimov, Lavie et al. 1995; Zhdanova, Wurtman et al. 2001). This evidence strongly suggests an underlying circadian component to these types of insomnia, such as advanced or delayed sleep phase syndrome. In advanced and delayed sleep phase syndromes there is an inappropriate phase relationship between biological time and environmental time, which results in sleep occurring at a socially acceptable but biologically inappropriate time (Duffy and Wright 2005). Optimally, sleep is initiated ~5 hours before the CBT minimum and waking occurs at ~2-3 hours after the CBT minimum. In normally entrained sleepers, this occurs across the period of environmental darkness, for example between 23:00 and 7:00. Surrounding the window for optimal sleep are two zones

during which either the circadian or homeostatic component of sleep drive is promoting wakefulness, thus making it an inopportune time to sleep. Individuals who attempt sleep during these windows report difficulty sleeping and poor sleep quality (Lack and Wright 2007). For example, if an individual's circadian rhythm is delayed, the individual would have difficulty initiating sleep at an appropriate clock time, as their CBT does not begin to decline until much later.

The associations between CBT, melatonin, and sleep/wake activity are well-known, but how these factors interact is still being explored. It is known that sleep is facilitated by the loss of heat through distal body sites such as the hands and feet and it is suspected that melatonin plays a key role in CBT regulation through peripheral heat loss (Krauchi, Cajochen et al. 1999; Markwald, Lee-Chiong et al. 2010). Sleepiness is correlated with increasing melatonin levels and decreasing body temperature. It is hypothesized that decreasing CBT signals the ventrilateral pre-optic area (VLPO) of the hypothalamus (a primary sleep-promoting area of the brain) to initiate sleep (Krauchi, Cajochen et al. 2006). Skin on the palms of the hands has been shown to have an increase in blood flow with a dose of exogenous melatonin, which further strengthens the evidence that melatonin may contribute to heat loss through peripheral vasodilation (Aoki, Zhao et al. 2008).

Similar to melatonin, it is proposed that ramelteon works partly through modulation of core body temperature via distal vasodilation to promote heat loss at the hands and feet (Markwald, Lee-Chiong et al. 2010). Ramelteon reaches peak serum levels of 5,700 pg/mL within 30-90 minutes of administration, but has a half-life 1-2 hours longer than that of melatonin (Karim, Tolbert et al. 2006). This effect is compounded by the presence of ramelteon's M-II metabolite, which remains in circulation an additional 2-5 hours longer than

ramelteon. Although M-II has a lower potency than ramelteon, it reaches much higher concentrations in the blood and therefore results in 20-100% greater systemic exposure (Karim, Tolbert et al. 2006; Pandi-Perumal, Srinivasan et al. 2007). Ramelteon has no rebound or withdrawal effects and does not alter sleep architecture like hypotic drugs do, which results in a more natural night's sleep according to EEG parameters (Roth, Stubbs et al. 2005; Erman, Seiden et al. 2006). Ramelteon has also been shown to produce phase advances in humans when administered in the evening, which makes it an optimal candidate for treating some of the chronobiological underpinnings of insomnia (Richardson, Zee et al. 2008).

CURRENT STUDY

Aims, hypotheses

This thesis was conducted as part of a larger collaborative study between Richard Bootzin, PhD at the University of Arizona and Kenneth P. Wright, PhD, University of Colorado at Boulder to assess sleep, daytime functioning, and circadian phase effects of a selective melatonin receptor agonist (ramelteon) combined and separately with multi-component behavioral therapy in patients with chronic insomnia. The secondary aim of this study, from which this thesis project was derived, was to determine if a change in sleep/wake circadian phase was associated with treatment efficacy. It was hypothesized that both ramelteon and MCBT will be more effective than placebo at post-treatment and that the combined ramelteon/MCBT treatment will be more effective than either treatment alone. We also expected that SOL would be related to phase angle and that the change in phase angle pre to post treatment would correlate with improvement in treatment efficacy as measured by decreased SOL and increased TST.

Methods

Participants had to meet the standard criteria of primary insomnia, reporting a SOL of more than 30 minutes on at least 3 nights a week and average TST less than 6.5 hours. Participants underwent physical and mental health screening including a physical exam, interviews about medical and sleep/wake histories, and alcohol, drug, and caffeine use. Participants with a diagnosis or treatment of other sleep disorders including obstructive sleep apnea, narcolepsy, and period limb movement disorders were excluded from the study, as were subjects who had received treatment for insomnia in the past 6 months. Other exclusion criteria were having a history of drug or alcohol abuse or dependency, psychiatric disorders, shift work or travel across more than three time zones within 6 months of the study.

For two weeks prior to the study, subjects' sleep-wake activity was monitored using actigraphy and with sleep diaries in which subjects reported bed and wake times in addition to SOL, WASO, number of awakenings, SQ, TIB, TST, and subjective ratings on the quality of sleep. Subjects were scored for sleep quality using the Pittsburgh Sleep Quality Index (PSQI)[10]. Subjects also completed a baseline DLMO assessment in the laboratory. Throughout the 6 week study subjects lived at home. Subjects in the MCBT treatment group received four weekly 90-minute group sessions and either 8mg of ramelteon daily or placebo. The remaining subjects received 8mg ramelteon or placebo with no therapy component. Ramelteon or placebo was taken in the evening, approximately 30-60 minutes prior to bedtime. Sleep and wake activity were measured with sleep diaries for two weeks pre-, during, and post-treatment.

During the pre- and post-treatment saliva collection, wakefulness light exposure remained <25 lux maximum anywhere in the room. Saliva samples were collected with salisaver

[10] The PSQI is a questionnaire that calculates subjective sleep quality and self-reported sleep disturbances into an overall score of sleep quality. A score of ≤5 is associated with good sleep quality, while a score >5 indicates poor sleep quality.

ALPCO Inc. saliva collection devices every 60 minutes during pre- and post-treatment visits. Frozen samples were shipped to the University of Colorado at Boulder, where they were assayed for melatonin content using a high sensitivity ALPCO ELISA with a detection range of .3pg/mL-25pg/mL. DLMO was assessed using a threshold of 3pg/mL. Change in the phase angle of entrainment between melatonin onset and sleep time for pre- and post-treatment were examined. Our treatment outcome measures were sleep quality on the PSQI, and SOL and TST based on sleep diaries. Data were analyzed using repeated measures ANOVA with group as a between subject factor (placebo, ramelteon and combined MCBT/ramelteon) by pre post treatment as a within subject factor.

Results

Figure 3 shows the relationship between SOL and phase angle. Consistent with our expectations, the degree of sleep onset disturbance was associated with phase angle such that subjects who went to be too close to their melatonin onset showed a longer time to fall asleep.

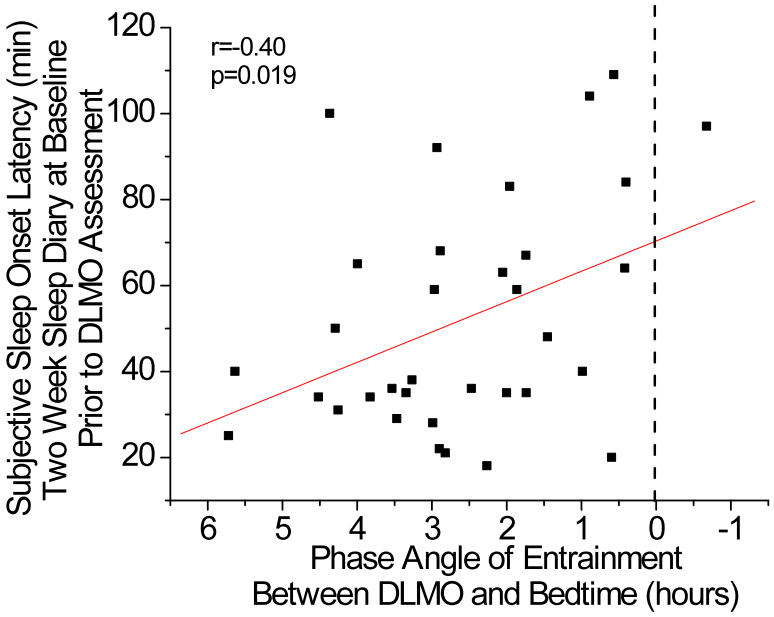

Figure 3. The relationship between phase angle and SOL before treatment. DLMO is marked with a dashed line at 0. The longer after DLMO subjects attempted sleep, the shorter perceived SOL.

We found a statistically significant ($p<.05$) difference in phase angle between pre- and post-treatment nights in the ramelteon condition but not in the combined treatment or placebo conditions (Table 1). No association was found between the change in phase angle and treatment efficacy for SOL ($r=0.09$, p=ns) or TST ($r=0.04$, p=ns).

Table 1. Phase angle changes between baseline and week 6		
Placebo	Baseline	2.18 ± 0.47
	Week 6	2.17 ± 0.73
Ramelteon	Baseline	2.94 ± 0.62
	Week 6*	1.44 ± 0.96
MCBT+ramelteon	Baseline	2.82 ± 0.39
	Week 6	2.59 ± 0.61
*significant change from baseline ($p<.05$)		

A main effect of pre post treatment was observed for SOL, WASO, number of awakenings, SQ, TST, and sleep efficiency.

For most of the sleep outcome measures we did not find a significant difference between groups. The number of awakenings decreased significantly in the placebo and combined treatment groups, but not in the ramelteon-only condition. SQ was significantly increased in the combined group condition, and TST was significantly increased in the placebo condition. These results are summarized in Table 2.

Table 2.	Placebo (n=7)			Ramelteon (n=7)			MCBT+ramelteon (n=12)		
Parameter	Baseline	Post Treatment	P	Baseline	Post Treatment	P	Baseline	Post Treatment	P
SOL (min)	50.4 ± 10.6	32.9 ± 8.3	< .05	60.1 ± 10.6	38.3 ± 8.3	< .05	51.3 ± 8.1	26 ± 6.4	< .001
Number of Awakenings	1.9 ± .4	1.1 ± .4	< .05	2.1 ± .4	1.8 ± .4	0.329	2.2 ± .3	1.3 ± .3	< .001
WASO (min)	31.7 ± 8.6	16.7 ± 4.2	0.066	18.1 ± 8.6	12.6 ± 4.2	0.481	47.6 ± 6.5	16.9 ± 3.2	< .001
Sleep Quality	2.6 ± .1	2.9 ± .2	0.180	2.9 ± .1	3.0 ± .2	0.709	2.7 ± .1	3.1 ± .1	< .05
TIB (min)	474.1 ± 27.3	513.7 ± 28.7	0.272	464.7 ± 27.3	479.4 ± 28.7	0.679	493.7 ± 20.8	444.2 ± 21.9	0.078
TST (min)	349.9 ± 27.8	432.9 ± 25.3	< .05	344.6 ± 27.8	391.9 ± 25.3	0.116	351.3 ± 21.2	391.7 ± 19.3	0.081
SE (%)	74 ± 4	85 ± 3	< .05	74 ± 4	82 ± 3	< .05	71 ± 3	88 ± 2	< .001

We found a significant improvement in PSQI scores for all groups in post-treatment and follow-up compared to baseline. These data are shown in Figure 4. Furthermore, 50% of the subjects in the MCBT-ramelteon condition improved their PSQI scores to ≤5 for the post-treatment and follow-up evaluations; whereas zero subjects in the placebo and ramelteon only conditions improved their PSQI scores to ≤5 for the post-treatment evaluation and zero in the placebo condition and only one out of five subjects in the ramelteon condition showed a PSQI ≤5 for the follow-up evaluation. A PSQI score of ≤5 is considered to be good sleep quality, so these subjects could be considered to be effectively treated for insomnia.

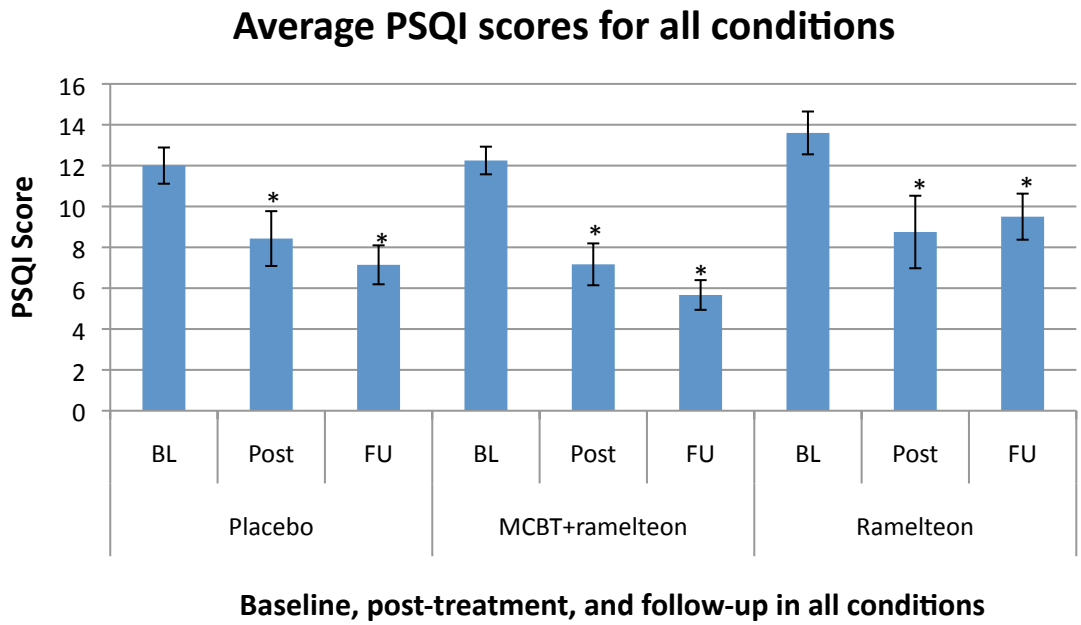

Figure 4. This figure shows the change in PSQI scores from baseline (BL) to the post-treatment (post) assessment at week 6, and a follow-up (FU) assessment after the study. Significant improvements were found in all groups between the baseline assessment and the post-treatement and follow-up assessments.
* different from baseline ($p<.05$)

Discussion

Findings from the current study show for the first time a significant correlation between phase angle and SOL in patients with insomnia. The larger the phase angle, the greater the distance between DLMO and bedtime, with negative numbers indicating bedtime occurring before DLMO. In this population, all but one subject went to bed after melatonin onset, and those who attempted sleep closer to or before melatonin onset had longer sleep latencies. This indicates that some of these insomniacs could be attempting to fall asleep at biological time when the circadian clock is promoting wakefulness; that is, subjects may be going to bed too early.

Phase angle, as described previously, was calculated by measuring the difference in average bedtime from the time of DLMO. Insomniacs can have incredibly variable bedtimes from day to day, as was evidenced in the sleep diaries – some showing subjects going to bed as much as 6 hours later on one night than the previous night. Although most subjects kept fairly consistent bedtimes (±2hrs) throughout the treatment period, the subjects who deviated further could have skewed the post-treatment phase angle calculation.

We found that ramelteon significantly changed phase angle. The sample size was too small to determine whether the degree of change in phase angle was associated with the degree of sleep improvement.

All treatments and placebo groups significantly reduced sleep onset latency, reduced the number of awakenings and increased sleep efficiency. Placebo also significantly increased TST. Such findings are typical in other insomnia studies, with subjects in the placebo group showing improvements when participating in research studies. The combination of MCBT and ramelteon also significantly reduced WASO and improved sleep quality.

All groups showed improved sleep quality on the PSQI, but only the combined treatment group showed subjects who achieved a level of improvement to ≤5 which an in indication of remission of insomnia.

Limitations of the current study:

The primary limitation in this study is the small sample size within groups. Due to drop-outs and missing data for some subjects, there was limited post treatment data for some groups. Due to incomplete post treatment data, we were only able to calculate the change in phase angle for 21 subjects – 4 in the ramelteon only group, 10 in the combined therapy group, and 7 in the placebo condition. Thus a study with a larger completed sample size may be necessary to examine whether a change in phase angle in the combined MCBT and ramelteon groups would be associated with improved sleep. It is also possible that not all of these subjects had an underlying circadian component to their insomnia.

Another factor that influences phase angle is changes in light exposure between the pre- and post-treatment visits. For example, a subject who has to get up early for work each day will be more likely to be exposed to the short-wavelength "twilight" exposure that appears to be a stronger signal for entrainment than normal daylight (Roenneberg and Foster 1997). An individual who rises in the late morning or early afternoon may miss this important environmental cue for appropriate synchronization of internal and external time. Also, as subjects were not restricted to laboratory-controlled light conditions, they may have inadvertently phase-delayed themselves through exposure to light during the 2 hour window before CBT minimum where the clock is more sensitive to phase delay as was described previously.

Conclusions and directions for future research

Although we did not see the hypothesized effect that change in phase angle would be correlated with treatment efficacy, we did find results that are promising for future research. The correlation between phase angle and sleep onset latency should be validated in a larger population of insomniacs, and more work should be done to explore how phase angle relates to subjective sleep quality in patients with insomnia. A follow-up study with a similar design but more subjects may be able to show a significant relationship between phase angle and treatment efficacy and might be able to show a more robust relationship between phase angle and SOL; especially using objective outcome measures The implications of this finding are important for the treatment of insomnia, as it highlights the importance of attempting to sleep at an appropriate biological time. As more evidence arises that implicates circadian misalignment as an underlying cause of insomnia, better treatment strategies can be developed like the combinations of light, melatonin agonists and cognitive behavioral therapy that are more likely to address underlying mechanisms of insomnia and result in a more effective and more sustained improvement in sleep quality.

APPENDIX:

Multi-component behavioral therapy

MCBT integrates stimulus control instructions, sleep restriction therapy, sleep education, and cognitive therapy to help address many of the complex issues contributing to insomnia and poor sleep quality.

Stimulus Control

Stimulus control is a form of conditioning that helps strengthen cues for sleep and weaken cues for activities and behaviors that are incompatible with sleep. Stimulus control attempts to curb behaviors like watching television and reading in the bedroom, and attempts to retrain individuals to the bedroom as a place for sleep instead of a place for worrying, planning, or eating (Bootzin and Nicassio 1978).

Sleep Restriction Therapy

Sleep restriction therapy helps insomniacs improve sleep efficiency by limiting the amount of time they spend laying in bed.

Sleep Education

Sleep education gives people a basic understanding of sleep and factors that promote, interrupt, or otherwise affect it. For example, this education might involve information about the effects of alcohol, caffeine, daytime naps, or exercise on sleep.

Cognitive Therapy

Cognitive therapy addresses irrational beliefs about sleep held by insomniacs and helps promote more constructive attitudes and beliefs through providing accurate information and realistic expectations.

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