Dim light melatonin onset (DLMO): A tool for the analysis of circadian phase in human sleep and chronobiological disorders

Seithikurippu R. Pandi-Perumala,⁎, Marcel Smitsb, Warren Spencenc,f, Venkataramanujan Srinivasand, Daniel P. Cardinalie, Alan D. Lowef, Leonid Kayumovf,✠

a Comprehensive Center for Sleep Medicine, Department of Pulmonary, Critical Care, and Sleep Medicine, Mount Sinai School of Medicine, 1176 5th Avenue – 6th Floor, New York, NY 10029, USA
b Gelderse Vallei Hospital, Department of Neurology and Sleep Disorders, Box 9025, 6710 HN Ede, The Netherlands
c Centre for Addiction and Mental Health, 1071-A-1, ARF Division, 33 Russell Street, Toronto, Ontario, Canada M5T-1R8
d Department of Physiology, School of Medical Sciences, University Sains Malaysia, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia
e Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, piso 7, 1121 Buenos Aires, Argentina
f Department of Psychiatry, University of Toronto, and Sleep and Neuropsychiatry Institute, 4325 Sheppard Avenue East, Suite 208, Scarborough ON, Canada M1S 1T7

Received 23 March 2006; received in revised form 23 June 2006; accepted 26 June 2006
Available online 1 August 2006

Abstract

The circadian rhythm of melatonin in saliva or plasma, or of the melatonin metabolite 6-sulphatoxymelatonin (aMT6S) in urine, is a defining feature of suprachiasmatic nucleus (SCN) function, the endogenous oscillatory pacemaker. A substantial number of studies have shown that, within this rhythmic profile, the onset of melatonin secretion under dim light conditions (the dim light melatonin onset or DLMO) is the single most accurate marker for assessing the circadian pacemaker. Additionally, melatonin onset has been used clinically to evaluate problems related to the onset or offset of sleep. DLMO is useful for determining whether an individual is entrained (synchronized) to a 24-h light/dark (LD) cycle or is in a free-running state. DLMO is also useful for assessing phase delays or advances of rhythms in entrained individuals. Additionally, it has become an important tool for psychiatric diagnosis, its use being recommended for phase typing in patients suffering from sleep and mood disorders. More recently, DLMO has also been used to assess the chronobiological features of seasonal affective disorder (SAD). DLMO marker is also useful for identifying optimal application times for therapies such as bright light or exogenous melatonin treatment.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Circadian rhythms; Delayed sleep phase syndrome; Dim light melatonin onset; Light/dark cycle; Melatonin; Mood disorders; Seasonal affective disorder

Contents

1. Assessment of the endogenous circadian pacemaker ........................................... 2
2. Melatonin measurement in body fluids .................................................. 2
3. Melatonin synthesis regulation: role of light ............................................... 3
4. Entrainment of melatonin rhythm by LD cycle. .............................................. 5
5. Phase response curve (PRC) to melatonin vs. PRC to light ....................................... 6
6. DLMO in circadian rhythm sleep disorders ............................................... 8

⁎ Corresponding author. Tel.: +1 212 241 5098; fax: +1 212 241 4828.
E-mail address: pandiperumal@gmail.com (S.R. Pandi-Perumal).

✠ This article is dedicated in honor of the memory of Dr. L. Kayumov, who passed away in unfortunate circumstances. Dr. Kayumov was a dedicated sleep researcher and a great mentor.

0278-5846/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2006.06.020
1. Assessment of the endogenous circadian pacemaker

During the past decade, considerable progress has been made in determining the molecular components of the biological clock (Saper et al., 2005). These molecular mechanisms are universally present in all cells and consist of gene-protein–gene feedback loops in which proteins down regulate their own transcription and stimulate the transcription of other clock proteins. Although anchored genetically, circadian rhythms are synchronized (entrained) by and maintain certain phase relationships to exogenous factors (environmental time cues or Zeitgebers), especially the sleep portion of the light/dark (LD) schedule. The rhythms will persist with a period different from 24 h when external time cues are suppressed or removed, such as when the organism is in complete social isolation or subjected to constant light or darkness (Saper et al., 2005).

Cellular clocks are governed in mammals by a master timekeeping system located in the anterior hypothalamus (SCN). The SCN is the pacemaker that generates a ∼24 h periodic signal which is synchronized to exactly 24 h by external synchronizers (or Zeitgebers) (Saper et al., 2005). Average human endogenous period (tau) is thought to vary between 24.2 h (Czeisler et al., 1999) and 24.9 h (Sack et al., 2000). The circadian component of the sleep/wake cycle is regulated by the circadian pacemaker in the regulation of physiological processes. For instance, the cyclic rise and fall of cortisol and melatonin suppress or removes, such as when the organism is in complete social isolation or subjected to constant light or darkness (Saper et al., 2005).

As research on the circadian system has progressed, several procedures have been developed to document the role of the circadian pacemaker in the regulation of physiological processes. For instance, the cyclic rise and fall of cortisol and melatonin have been used as markers of the circadian phase for measuring the effects of light exposure (Klerman, 2005). Parameters of sleep–wake cycle have also been used as phase markers in humans (Martin and Eastman, 2002). Plasma melatonin has been used for years to assess the circadian phase. Melatonin levels in plasma begin to increase before sleep and peak the first part of the night. Since Lewy’s discovery that bright light can suppress or “mask” nighttime melatonin production (Lewy et al., 1980), it has become recognized that masking is a problem for all marker rhythms. Further, it is not always easy to answer the question of whether there are multiple oscillators for a given cyclic phenomenon or whether one is more reliable than others.

Circulating melatonin levels are often preferred as a circadian marker because they are comparatively robust in the presence of various external influences. For example, while excessive carbohydrate intake can produce significant changes in core body temperature (CBT) and heart rate, melatonin concentration remains essentially uninfluenced by this factor (Krauchi et al., 2002).

One variable which does affect melatonin production however is environmental illumination. It has been recommended that in studies in which melatonin is used as a circadian phase marker, exposure to dim light should be initiated 1–2 h before the earliest melatonin onset (Lewy et al., 1984; Lewy, 1999). This implies that dim light should be started at 17:00 h and blood sampling should be started around 18:00 h. The level of illumination currently recommended for sampling is 10 lx. Absolute darkness appears to have no advantage over dim light for minimizing the suppressant effect of bright light on melatonin production. The plasma levels of the major melatonin metabolite, aMT6S, have also been employed to measure DLMO (Bojkowski et al., 1987).

The fact that melatonin onset is not affected by biochemical and physiological confounding factors accounts for its comparatively greater reliability to measure circadian phase position (Lewy, 1999; Lewy et al., 1999). There may also be individual differences to be accounted for. Human plasma melatonin levels during the day are usually lower than 10 pg/ml, whereas during nighttime values normally exceed 40 pg/ml. In low melatonin producers, plasma levels are considerably lower. Therefore, a 2 pg/ml plasma threshold is often recommended for measuring DLMO (Lewy et al., 1999). Another possibility is to employ the procedure recommended by Voultios et al. (1997) for determining a threshold, i.e., the mean of 3 points plus two times the SD of those 3 points.

2. Melatonin measurement in body fluids

The threshold of melatonin concentration that differentiates nighttime from daytime values is difficult to determine because, as noted above, there exists a subpopulation of low melatonin producers whose peak nighttime values are markedly smaller than those of normal individuals. Lewy et al. (1999) recommended 2 pg/ml as the lowest plasma threshold at which the daytime and nighttime values could be differentiated. The earliest reported assays of plasma melatonin were based on gas chromatography/mass spectrometry (GCMS) but were later replaced by more specific and sensitive radioimmunoassays, suitable to be used in a clinical environment (Lewy et al., 1999).

Leibenuflut et al. (1996) employed salivary samples to measure DLMO and proposed this measurement as the most practical and reliable method for assessing the circadian phase. The correlation coefficient between plasma and salivary assessment of DLMO
was 0.93 (Leibenluft et al., 1996). A salivary melatonin assay described by Voultsios et al. (1997) was found to have the sensitivity and accuracy comparable to that of GCMS assay. This has contributed to the rapid adoption of salivary testing as the preferred method for sampling melatonin, inasmuch as it is relatively non-invasive and more acceptable to patients (Table 1). An additional advantage of the method is that, with proper training, the patients themselves can collect the samples at home and deliver them to the laboratory for assay. In more recent studies salivary sampling has become the primary measurement for assessing physiological levels of melatonin, especially in longitudinal investigations in which repeated measures are needed (Table 2). It must be noted that melatonin levels and consequently DLMO obtained in a sleep laboratory may differ from those obtained in free field, i.e. at home (Roach et al., 2001; Harada, 2004; Kawinska et al., 2005). Comprehensive field studies on normal values of DLMO in healthy adults have not yet been published.

In the Dutch national referral centre for sleep–wake disturbances and chronobiology, headed by one of the authors of this article, salivary DLMO is measured every year in about 1000 patients with a possible circadian rhythm disorder. In adults DLMO is defined as the time at which a salivary concentration of melatonin of 4 pg/ml is reached (Nagtegaal et al., 1998a,b, 2000; Smits and Nagtegaal, 2000; Wieringer et al., 2001; Smits et al., 2002) and is considered to be normal when it occurs between 19:30 and 22:00 h. In children between 6 and 12 year DLMO is considered to be normal if it occurs between 19:00 and 21:00 h (Smits et al., 2001, 2003; Heijden et al., 2005).

3. Melatonin synthesis regulation: role of light

The circadian pattern of melatonin secretion is abolished by lesions of the SCN (Claustrat et al., 2005). The environmental 24 h LD cycle acts as the predominant Zeitgeber that regulates melatonin synthesis (Sheer and Czeisler, 2005). The circadian activity of the SCN is synchronized to the LD cycle by light mainly through a monosynaptic retinohypothalamic tract (RHT) originating from the ganglion cell layer in the retina. Animal studies have now identified the neural pathway which connects the SCN with the pineal gland. This has been shown to include the hypothalamic paraventricular nucleus, and projections of fibers through the medial forebrain bundle and reticular formation to cells of the intermediolateral columns of the cervical spinal cord. Additionally, preganglionic sympathetic fibers project upward to the superior cervical ganglia (SCG). In the final step of this transmission postganglionic sympathetic fibers from the SCG reach the pineal gland and release norepinephrine (NE) (Claustrat et al., 2005).

Activation of pineal β-adrenergic receptors results in an increase of melatonin synthesis; α1-adrenergic receptors are also detectable in the pineal gland and potentiate β-adrenergic receptor activity (Claustrat et al., 2005). During the light phase, the SCN electrical activity is high and under these conditions pineal NE release is inhibited. Conversely, SCN activity is inhibited during darkness and NE release in the pineal gland augments. β-adrenergic blockers have been shown to suppress the nocturnal synthesis and secretion of melatonin in humans, suggesting a similar regulation of pineal melatonin production (De Leersnyder et al., 2003). If the SCN is activated by light at night, NE release in the pineal gland is inhibited. However, there is a lack of correlation between the suppressant effect of light on melatonin synthesis and the pharmacological effect of the β-adrenergic blocker propranolol (Mayeda et al., 1998), thus suggesting that other non-adrenergic mechanisms may be involved.

Melatonin secretion is suppressed by ambient light intensities typically encountered outdoors (i.e., ranging from 3000 to 100,000 lx) but, under certain circumstances, even lower intensities (ranging from 100 to 300 lx) can have a suppressant effect on melatonin (Duffy and Wright, 2005). Light-induced suppression of pineal melatonin synthesis and secretion in humans has peak sensitivity at short wavelength light (446–477 nm) (Brainard et al., 2001; Kayumov et al., 2005).

Originally, it was thought that the LD cycle was relatively less significant when compared to other social cues in resetting the human circadian rhythms. Following the discovery by Lewy et al. (1980) that bright light of 2500 lx or greater suppresses melatonin secretion, it has been repeatedly found that human circadian rhythms are synchronized by the 24 h LD cycle. This opened up a new area not only for studying the synchronization of human circadian systems but also for understanding the

Table 1  Procedures for Dim Light Melatonin Onset (DLMO) measurements in saliva

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. During the saliva collection period consumption of bananas, alcoholic beverages, or coffee are prohibited.</td>
<td>In patients older than 16 years who are suspected of having delayed sleep phase syndrome: saliva is to be collected hourly between 7 p.m. and 11 p.m.</td>
</tr>
<tr>
<td>2. During the time of collection the subject must remain in dim light (10 lx) from 1 h prior to scheduled saliva collection. Curtains in the room must remain closed. Watching TV is permitted.</td>
<td>In patients older than 16 years who are suspected of advanced sleep phase syndrome: saliva is to be collected hourly between 7 p.m. and 11 p.m.</td>
</tr>
<tr>
<td>3. Fifteen minutes before every collection of saliva the subject must rinse his mouth with water.</td>
<td>In children younger than 13 years who are suspected of delayed sleep phase syndrome: saliva is to be collected hourly between 7 p.m. and 11 p.m.</td>
</tr>
<tr>
<td>4. Eating is permitted after saliva collection (except for the food described at “1”).</td>
<td>In children younger than 13 years who are suspected of delayed sleep phase syndrome: saliva is to be collected hourly between 4 p.m. and 9 p.m.</td>
</tr>
<tr>
<td>5. Subjects must not brush their teeth during the saliva collection period.</td>
<td>In children and adults suspected of irregular sleep–wake rhythm / melatonin rhythm: saliva is to be collected hourly between 8 and 12 p.m., once a week for 4 weeks</td>
</tr>
<tr>
<td>6. Nighttime saliva collection must only be done under dim light conditions.</td>
<td>If the approximate time of the DLMO cannot be estimated, all subjects (whether they are adults or children) should start sampling at 1800 and continue until habitual bedtime.</td>
</tr>
<tr>
<td>7. Physical activities during the collection period are to be avoided as much as possible.</td>
<td>24-h melatonin curve:</td>
</tr>
</tbody>
</table>

**Indications:**
- When the partial melatonin curve is inconclusive
- In patients with a suspected free running rhythm (especially in blind people)
Table 2
Studies using DLMO as a tool for assessing circadian phase position, chronobiological sleep disorders and mood disorders

<table>
<thead>
<tr>
<th>Condition or Group</th>
<th>N</th>
<th>Results or conclusion(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD</td>
<td>8 patients, 7 controls</td>
<td>Phase delay of DLMO in winter depressives as compared to controls. Melatonin rhythm noted in winter depressives.</td>
<td>Lewy et al. (1984)</td>
</tr>
<tr>
<td>WD</td>
<td>8 patients, 7 controls</td>
<td>Application of morning bright light phase-advanced DLMO</td>
<td>Lewy et al. (1985)</td>
</tr>
<tr>
<td>WD</td>
<td>6</td>
<td>Phase advancement of DLMO</td>
<td>Lewy et al. (1987)</td>
</tr>
<tr>
<td>WD</td>
<td>8 patients, 5 controls</td>
<td>Phase delay of DLMO in WD patients</td>
<td>Sack et al. (1990)</td>
</tr>
<tr>
<td>Effect of morning BLT</td>
<td>8 patients, 5 controls</td>
<td>Morning bright light phase-advanced melatonin onset in patients but not in controls</td>
<td>Sack et al., (1990)</td>
</tr>
<tr>
<td>Effect of sleep displacement (2 h phase advance)</td>
<td>6</td>
<td>No phase shift of the DLMO</td>
<td>Hoban et al. (1991)</td>
</tr>
<tr>
<td>Melatonin (0.5 mg) administration in normal subjects</td>
<td>9</td>
<td>Morning melatonin phase delays circadian rhythms; afternoon/evening melatonin phase advances circadian rhythms</td>
<td>Lewy et al. (1992)</td>
</tr>
<tr>
<td>SAD</td>
<td>6 patients, 6 controls</td>
<td>Phase delay of circadian rhythms in SAD compared to controls</td>
<td>Dahl et al. (1993)</td>
</tr>
<tr>
<td>BLT (2500 lx) in SAD for 2 h</td>
<td>6 SAD patients</td>
<td>Phase-advanced DLMO in SAD patients</td>
<td>Dahl et al. (1993)</td>
</tr>
<tr>
<td>Bipolar affective disorder</td>
<td>12 bipolar depressives</td>
<td>Salivary DLMO correlates with plasma DLMO (correlation coefficient=0.93)</td>
<td>Leibenluft et al. (1996)</td>
</tr>
<tr>
<td>Non-seasonal depression</td>
<td>8 patients, 8 controls</td>
<td>No phase differences in DLMO between healthy and depressed subjects When given 5 h before DLMO melatonin advanced DLMO by 98 min. Conclusion: to measure DLMO in DSPS patients is important for both diagnosis and therapy.</td>
<td>Gordijn et al. (1998)</td>
</tr>
<tr>
<td>DSPS</td>
<td>13 patients</td>
<td>Melatonin (0.3–3 mg) administered daily 1.5–6.5 h prior to DMLO for 4 weeks advanced the circadian phase of endogenous melatonin and sleep in a phase dependent manner.</td>
<td>Mundey et al. (2005)</td>
</tr>
<tr>
<td>DSPS</td>
<td>20 patients</td>
<td>Melatonin ameliorated some symptoms of subjective and objective measures without any adverse effects in 4-week treatment period</td>
<td>Kayumov et al. (2001)</td>
</tr>
<tr>
<td>Effect of BLT (2500 lx)</td>
<td>12 healthy subjects</td>
<td>Morning light phase-advanced DLMO more than evening light</td>
<td>Gordijn et al. (1999)</td>
</tr>
<tr>
<td>Effect of sleep displacement</td>
<td>12 healthy subjects</td>
<td>3 h phase advance of sleep displacement did not shift DLMO; 3 h phase-delay of sleep caused phase-delay of DLMO</td>
<td>Gordijn et al. (1999)</td>
</tr>
<tr>
<td>Children with idiopathic chronic sleep onset insomnia</td>
<td>40 patients</td>
<td>Melatonin advances mean lights off time by 34 min, sleep onset by 63 min and increases total sleep time by 41 min</td>
<td>Smits et al. (2001)</td>
</tr>
<tr>
<td>Chronic whiplash syndrome</td>
<td>81 patients</td>
<td>DLMO was delayed in chronic whiplash syndrome patients (mean DLMO: 23:20 h), Melatonin administered 5 h before DLMO advanced DLMO and sleep-wake rhythm.</td>
<td>Wieringer et al. (2001)</td>
</tr>
<tr>
<td>CFS</td>
<td>29 patients</td>
<td>In CFS patients with DLMO later than 22:00 h melatonin treatment, administered 5 h before DLMO, decreased fatigue more than in CFS patients with DLMO between 21:30 and 22:00 h.Baseline and final. Various combinations of interventions like wearing sunglasses during the commute home, creating a dark bed room, and adhering to a regular daytime sleep schedule starting soon after the night shift reduced circadian misalignment.</td>
<td>Smits et al. (2002)</td>
</tr>
<tr>
<td>Night-shift workers</td>
<td>67 (median age 22 years)</td>
<td>DLMO was measured before and after nightshifts (baseline and final). Various combinations of interventions like wearing sunglasses during the commute home, creating a dark bed room, and adhering to a regular daytime sleep schedule starting soon after the night shift reduced circadian misalignment.</td>
<td>Crowley et al. (2003)</td>
</tr>
<tr>
<td>Saliva DLMO study in the elderly</td>
<td>85 (over 65 years)</td>
<td>The correlation coefficient for saliva and serum melatonin was r=0.659.</td>
<td>Goonaratne et al. (2003)</td>
</tr>
<tr>
<td>Children with idiopathic chronic sleep onset insomnia</td>
<td>62 patients</td>
<td>Melatonin advanced DLMO by 1:22 h, advanced sleep-wake rhythm and improved health status.</td>
<td>Smits et al. (2003)</td>
</tr>
<tr>
<td>Effect of exercise on circadian melatonin rhythm</td>
<td>18 healthy subjects</td>
<td>Exercise phase-delayed DLMO, thus helping to facilitate circadian adaptation to schedules requiring a phase delay in the sleep-wake cycle</td>
<td>Barger et al. (2004)</td>
</tr>
<tr>
<td>Effect of bedtime on DLMO</td>
<td>10 healthy subjects</td>
<td>No significant correlation between morningness/eveningness and the shift of the DLMO. No significant sex difference in the shift of the DLMO; melatonin rhythm following the late bed night was delayed.</td>
<td>Burgess and Eastman (2004)</td>
</tr>
<tr>
<td>Effect of combination of evening melatonin and BLT</td>
<td>9 healthy young subjects</td>
<td>A single light pulse (5000 lx for 3 h) caused phase delay of DLMO. Melatonin administration caused a phase advance of DLMO. Combination of evening melatonin and light was additive in their phase shifting effects.</td>
<td>Wirz-Justice et al. (2004)</td>
</tr>
<tr>
<td>Effect of different light wavelengths on salivary DLMO</td>
<td>42 healthy subjects</td>
<td>Shorter wavelengths caused greatest melatonin onset (by 40 to 65 min)</td>
<td>Wright et al. (2004)</td>
</tr>
<tr>
<td>Effect of early evening melatonin administration on chronic sleep onset insomnia as predicted by DLMO</td>
<td>110 (aged 6–12 years)</td>
<td>The efficacy of early evening melatonin treatment to advance sleep onset could be predicted by the circadian phase marker DLMO in children with chronic SOI.</td>
<td>van der Heijden et al. (2005)</td>
</tr>
</tbody>
</table>
Lockley et al. (2003) found that exposure of human volunteers to 6 h of monochromatic blue light (460 nm) produced a circadian delay which was two-fold as great as that produced by an equivalent exposure to 555 nm light. Moreover, melatonin secretion was suppressed twice as much by 460 nm light as compared to 555 nm light. This study confirmed that melatonin production and suppression in humans is primarily due to blue wavelength photoreceptors that are distinct from the photopic visual system (which is primarily sensitive to longer wavelengths). The mechanism by which circadian rhythms are regulated by shorter wavelength sensitive photoreceptors was assessed by Cajochen et al. (2005) in phase shifting and melatonin suppression studies. Their findings suggested that photoreceptors, which may contain melanopsin as a photopigment, are involved in several functions, including the regulation of human alerting responses to light, thermoregulation and heart rate.

There are substantial individual variations in sensitivity to the intensity of light required for suppressing melatonin secretion. This is determined not only by environmental factors but also by genetic factors as well. Factors such as the intensity of light applied and duration of exposure seem to determine the level of melatonin suppression. Lewy et al. (1980) showed that light with an intensity of 2500 lx, when administered for 2 h, could suppress the nocturnal melatonin secretion to the low circulating levels seen during daytime. However, partial suppression of melatonin secretion can be achieved with lower intensities of light (200–300 lx) administered for 30 min (Bojkowski et al., 1987). Light intensities of 100 lx have also been shown to suppress melatonin production under certain circumstances (Boivin et al., 1996).

4. Entrainment of melatonin rhythm by LD cycle

Drugs that influence the circadian apparatus are referred to as “chronobiotics” with melatonin being their prototype (Cardinali et al., 2006). Indeed, melatonin secretion is an “arm” of the biologic clock in the sense that it responds to signals from the SCN. In particular the timing of the melatonin rhythm indicates the status of the clock, both in terms of phase (i.e., internal clock time relative to external clock time) and of amplitude. Further, melatonin is also a chemical code for the night: the longer the night, the longer the duration of its secretion. In most species, this pattern of secretion serves as an internal time cue for other rhythms which are governed by seasonal changes (Claustrat et al., 2005).

Melatonin may help the endogenous circadian pacemaker to discriminate the dark phase of the 24-h LD cycle from the sporadic episodes of darkness (Lewy et al., 1996). A study undertaken in Antarctica suggests that a structured social routine in a dim light environment is sufficient to synchronize melatonin to a 24 h cycle (Midwinter and Arendt, 1991). In this context, it is interesting to note that a minority of permanent night shift workers show a shift in peak in melatonin production during the day which eventually mirrors the former nocturnal profile. Zeitzer et al. (2000) demonstrated that humans are highly responsive to phase delaying effects of light during early biological night. In a study of 30 permanent night nurses who worked for 3–9 consecutive nights (midnight to 08:00 h), 5 nurses underwent phase delay adaptation and had a realignment of their melatonin rhythm and sleep, 22 were non-shifters and 3 exhibited an advanced rhythm (Dumont et al., 2001). This study reveals that while the circadian rhythms of some night shift workers are capable of phase shift reversals, most individuals do not exhibit this radical change. A better circadian adaptation can occur when night shift workers receive less morning or afternoon light (Burgess et al., 2002). Exposure to room lighting of about 180 lx for 3 consecutive days in the morning has been shown to significantly phase advance the human circadian phase maker (Boivin et al., 1996).

Zeitzer et al. (2000) found that room light intensity influenced the human circadian pacemaker when applied during the first 6.5 h of biological night. Plasma melatonin concentration became suppressed in a dose-dependent manner during the single 6.5 h light stimulus, administered from 23.00 h to 05.30 h. While low illuminance did not evoke much change, bright room light or higher illuminances completely suppressed plasma melatonin. It was further noted that the acute response of melatonin to increasing illuminance occurred in a stepwise manner with suppression of melatonin at illuminations greater than 200 lx and minimal suppression below 80 lx. Bright room light (above 500 lx) caused an apparent saturating phase shift of the endogenous circadian melatonin rhythm (Zeitzer et al., 2000). The phase resetting response to light and acute suppressive effects of light on plasma melatonin followed a dose response curve. Exposure to a single 6.5 h episode of 100 lx light generated half of the response observed for a stimulus that was nearly 100 fold

### Table 2 (continued)

<table>
<thead>
<tr>
<th>Condition or Group</th>
<th>N</th>
<th>Results or conclusion(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>87 patients</td>
<td>DLMO is delayed in ADHD children with chronic idiopathic sleep onset insomnia. Conclusion: chronic insomnia in ADHD children with chronic sleep onset insomnia is caused by a circadian rhythm disorder.</td>
<td>Van der Heijden et al. (2005)</td>
</tr>
<tr>
<td>CFS</td>
<td>38 patients</td>
<td>In CFS patients with DLMO later than 21:30 h melatonin improves quality of life scores, physical functioning, energy/vitality, bodily pain and general health perception.</td>
<td>van Heukelom et al. (2006)</td>
</tr>
</tbody>
</table>
brighter (9000 lx) (Zeitzer et al., 2000). A photic stimulus in the early subjective night will delay the timing of the clock while light exposure in the late subjective night and early subjective morning will advance the timing of the clock (Duffy and Wright, 2005). Light exposure at either time will induce a suppression of melatonin production. Whether this melatonin suppression is necessary to induce a phase delay is not yet clear since phase shifts occur during daytime when melatonin secretion is almost nil (Khalsa et al., 2003). The DLMO studies undertaken by Wirz-Justice et al. (2004) confirmed the finding that suppression of melatonin does not appear to be a prerequisite for phase shifting the human circadian pacemaker. In that study administration of a single light pulse (5000 lx for 3 h) or a single melatonin pill (5 mg) at 20:40 h caused phase delay or phase advance of DLMO, respectively, when assessed 24 h later. Combinations of both interventions caused additive effects. The Zeitgeber effect of melatonin was shown to be as effective as that of bright light (Wirz-Justice et al., 2004). A number of studies employing DLMO as a tool for assessing circadian phase position, chronobiological sleep disorders and mood disorders are summarized in Table 2.

5. Phase response curve (PRC) to melatonin vs. PRC to light

A PRC is constructed by administering a synchronizing stimulus during different times (or circadian phases) and then evaluating its effect on the phase of the circadian clock. PRC’s generally depict the sensitivity of the circadian clock and the relationship between various stimuli or Zeitgebers and the circadian rhythms (Fig. 1). A schematic PRC to exogenous melatonin or to light illustrates that the timing of melatonin or light administration is crucial for achieving the magnitude and direction of subsequent phase shift (Burgess et al., 2002; Lewy et al., 1998; Minors et al., 1991). However, the relation between melatonin and bright light is not symmetrical (Fig. 1). The measurement of endogenous melatonin in plasma, saliva or urine is helpful in determining the circadian phase (Lewy et al., 1999).

The wave form of human melatonin PRC is about 12-h phase-shifted with respect to the PRC for bright light (Lewy et al., 1998). In the melatonin PRC, the first half of the night reflects the zone of reduced responses whereas in the PRC to light, the phase shifting responses are reduced during the day. Intravenous infusion of a physiological dose of melatonin has been found to produce a melatonin PRC which is similar to pharmacological PRC’s (Zaidan et al., 1994).

Melatonin PRC’s have been experimentally determined in individuals with normal vision as well as in blind people (Arendt et al., 1997; Lewy et al., 1998, 2004). In individuals with normal eyesight who are entrained to the 24 h LD cycle, melatonin administration in the afternoon and evening causes a phase advance. By contrast, melatonin administration in the late night or early morning causes a phase delay. Recently, a PRC was constructed by administration of 0.5 mg of melatonin for 4 consecutive days to six subjects whose sleep schedules had not been altered (Lewy et al., 2004).

The phase advance and phase delay zones of the melatonin PRC are easily delineated in individuals with normal sight by using DLMO as a marker of the circadian phase. By convention, circadian time (CT) 0 is defined as the wake time in visually normal individuals. An increase to 2 pg/ml of melatonin (DLMO2) occurs at CT 13 while a 10 pg/ml plasma concentration (DLMO10) occurs about 1 h later (at CT 14). The phase advance zone for melatonin extends from CT 6 to CT 18 while the phase delay zone extends from CT 18 to CT 6. This implies that the...
The phase shifting properties of melatonin are useful for the entrainment of phase-disordered individuals such as those who are totally blind. Administration of melatonin in the delay zone to blind free running individuals showing circadian rhythms with periods greater than 24 h compromised entrainment (Sack et al., 2000). It is possible that initiation of melatonin treatment in the PRC delay zone of blind free running individuals actually changed melatonin PRC by changing melatonin receptor sensitivity, thus compromising any eventual entrainment (Sack et al., 2000). Lewy et al. (2004) recently showed that initiation of melatonin treatment with a low dose (0.5 mg) in the delay zone of PRC resulted in entrainment. This finding is in contrast to other studies (Hack et al., 2003; Lockley et al., 2000) in which initiation of a 0.5-mg melatonin treatment to blind people in the delay zone failed to produce entrainment. In any event, it does not appear that low-dose melatonin treatment should be initiated exclusively at the advance zone of melatonin PRC to induce eventual entrainment in blind people with free-running rhythms (Lewy et al., 2004). This has a practical consequence: it is not essential that circadian phase be ascertained before starting low-dose melatonin treatment of blind people.

Sharkey and Eastman (2002) used the DLMO as a method for measuring the effect of exogenous melatonin on circadian phase changes in shift work simulation studies. The use of DLMO for phase assessment indicated that the magnitude of the desired phase advance was greater after administering higher doses of melatonin. Subjects who took melatonin adapted to the shifted sleep schedule faster than those who took placebo (56% of subjects with a 0.5-mg dose, 73% of subjects with a 3.0-mg dose) (Sharkey and Eastman, 2002).

Based upon the results of number of studies, a schematic PRC to light has been constructed (Fig. 1). Construction of this PRC included studies in which sleep time was held constant and a high intensity of light was applied before and after sleep to study the phase shifts (Burgess et al., 2002). In other studies, sleep schedule was shifted (up to 12 h) and several pulses of light were applied at various circadian phases to note the course of reentrainment (Burgess et al., 2002). Light pulses were also applied to free running (phase-disordered) individuals. The crossover point of the human light PRC is based on the time of minimum of CBT (CBTmin) that occurs at about the middle of sleep. Application of light before CBTmin produces phase delays while light applied after CBTmax produces phase advances (Jewett et al., 1994). Light exposure close to the CBTmin has been shown to produce the greatest shifts. The magnitude of the phase shift is shown to be dependent on the intensity and duration of light with an increase in magnitude occurring at higher intensities of light (Eastman, 1992).

Several reports on PRCs for light have been published describing the circadian clock responses to light. In a study in which bright light was administered for 5 h on three consecutive days, phase shifts as large as 12 h were observed after light was applied around the CBTmin (type 0 PRC) (Czeisler et al., 1989; Duffy and Wright, 2005). Contrary to other reports that had demonstrated phase delays to single light pulses, Homma and Homma (1988) reported a PRC without a significant phase delay region. A study by Jewett et al. (1994) showed that in human subjects the early portion of the phase delay region is poorly characterized, and that there is a region of peak amplitude as well as a region of phase advance. These findings support the view of the human circadian pacemaker as being not simply a phase-only oscillator and that a full description of human circadian resetting responses to light required an analysis of both the phase and amplitude of phase shift data.

A more comprehensive PRC to single light pulses administered over the entire ranges of circadian phases was undertaken by Khalsa et al. (2003). In that study, 21 healthy, entrained subjects underwent pre- and post-stimulus constant routines in dim light with maintained wakefulness in a semi-recumbent posture. Phase advances (positive values) and delays (negative values) of melatonin rhythm were plotted against the timing of the centre of the light exposure relative to the melatonin midpoint on the pre-stimulus constant routine. Phase delays were generated when light pulses were delivered before the circadian phase 0 h, and phase advances were generated when light pulses were applied after circadian phase 0 h. Delivery of light pulses close to the CBTmin normally produces large phase shifts of 12 h in magnitude (type 0 PRC). However, phase shifts were near zero when the light pulses were delivered close to the circadian phase 0 (the so-called type I PRC) (Khalsa et al., 2003). Thus human beings are capable of type 1 and type 0 resetting depending upon the application of resetting stimulus (Jewett et al., 1994). The peak to trough amplitude of the PRC in the latter study was found to be 5 h and has been shown to be consistent with other reports (Wirz-Justice et al., 2004). It is important to note the relevance of light intensity for accurately predicting the response of the human circadian pacemaker (Khalsa et al., 2003; Zeitger et al., 2000).

Based on studies of the effects of light on plasma melatonin profiles in rodents, Illnerova and Vanecek (1985) hypothesized that two coupled oscillators existed: an evening oscillator associated with melatonin onset, and a morning oscillator associated with melatonin offset. However, studies on the light-induced phase shifts of melatonin onset and offset in humans are controversial. Differences between the DLMO and other phase markers do not necessarily mean that there are separate oscillators inasmuch as relative sensitivity to noise and masking effects could also account for the observed differences.

Results from some studies indicate that phase shifts in melatonin onset are greater than phase shifts in melatonin offset (Cagnacci et al., 1997; Deacon and Arendt, 1994; Hashimoto et al., 1997; Homma et al., 1997; Parry et al., 1997; Van Cauter et al., 1994) whereas other studies have reported opposite effects (Foret et al., 1993; Lewy et al., 1985). Additionally, there have been reports of the comparative effects of bright light or melatonin in their capacity to phase-shift circadian rhythms. Studies by Lewy et al. (1984) demonstrated that the maximal phase shift induced by melatonin was approximately of 1 h whereas the maximum phase shift induced by bright light was 12 h. Sleep phase shifted as much as 12 h with bright light whereas no shift in sleep phase was noted when melatonin was used as a phase resetting agent.
Mitchell et al. (1997), by applying high intensity bright light to human subjects during the night shift (during phase advance portion of light PRC), advanced the CBT\textsubscript{min} by 74% of that which would be required for a full effect. By comparing these effects with a study of phase shifting (Sharkey et al., 2001, Burgess et al. 2002) it was concluded that the phase shifting effect of melatonin appears to be similar to that of high intensity light. In the light exposure study (Eastman et al., 1995) the light stimulation was administered for 8 nights whereas melatonin was only given for 4 nights (Sharkey et al., 2001).

6. DLMO in circadian rhythm sleep disorders

The use of DLMO has been recommended as a method for assessing the phase of the circadian pacemaker in patients suffering from sleep or mood disorders involving a chronobiological component (Table 2). The abnormal timing of the DLMO seen in these patients provides a clue for the optimal timing of treatment. Sleep displacements have been found to cause phase shifts in DLMO. VanCauter et al. (1998) reported an acute 2 h phase advance in the onset, and a 1 h phase advance in the offset of the melatonin rhythm after 8 h advance of the sleep/wake cycle. A 3 h delay in sleep onset applied for 3 days was effective for delaying significantly DLMO when compared to 3 days of sleep at a normal time, or after advancing sleep onset for 3 h (Gordijn et al., 1999; Gooneratne et al., 2003), Buxton and colleagues' finding that total darkness too early in the afternoon (usually accompanied by sleep) causes immediate phase shifts indicates that these extreme conditions could be a potential confound for using the DLMO as a phase marker (Buxton et al., 2000). However, the shifts in DLMO were not accompanied by shifts in body temperature or changes in latency to the first rapid eye movement sleep episode. These findings therefore raise doubts about the reliability of the DLMO as a marker of circadian phase in the presence of sleep disturbances (Gordijn et al., 1999). Indeed, DLMO can be readily estimated in people whose sleep times are minimally affected by work, class and family commitments but it remains to be established whether the DLMO can be accurately estimated in people with greater work and family responsibilities that affect their sleep times (Burgess and Eastman, 2005). It must be noted, however, that DLMO has proven useful for predicting melatonin efficacy in the treatment of children with idiopathic chronic sleep onset insomnia (van der Heijden et al., 2005).

A delayed melatonin onset presumably plays a key role in the pathophysiology of delayed sleep phase syndrome (DSPS), one of the most prevalent circadian rhythm sleep disorders seen in our modern society. A number of studies have examined the efficacy of exogenous melatonin in DSPS patients. Levy et al. (1992) found that melatonin administered 5 h before endogenous melatonin onset advanced the circadian rhythms most. Extending these results, Nagtegaal et al. (1998a) administered melatonin 5 h before melatonin onset and found effective in phase advancing the sleep/wake cycle in DSPS. Hence the administration of melatonin 5 h in advance to DLMO is recommended as the precise time for treating DSPS. In a recent study on 13 subjects with DSPS (Mundey et al., 2005), melatonin (0.3 to 3 mg) administered between 1.5 to 6.5 h prior to DLMO for a 4 week period advanced the circadian phase of endogenous melatonin and sleep in a phase-dependent manner. The magnitude of the phase advance correlated strongly with the time of melatonin administration. Not only in adults but also in children the magnitude of the phase advance in dim light melatonin onset correlates strongly with the time of melatonin administration (Heijden et al., 2005).

Bright light administered during the phase advance part of the PRC also advances the sleep/wake rhythm. Since sleep/wake rhythm (and also the 24 h temperature and cortisol rhythms) is linked to the melatonin rhythm, appropriate administration of melatonin and bright light can be very effective to shift the sleep/wake rhythm.

7. DLMO in mood disorders

The DLMO is not only useful in phase typing circadian phase sleep and mood disorders, but also is helpful in assessing the response to bright light treatment (Cajochen et al., 2005). Circadian rhythm abnormalities are implicated in the pathogenesis of mood disorders (Baumann et al., 2004; Carskadon et al., 2004; Klerman, 2005; Sririvasan et al., in press). Abnormalities of phase positions of cortisol, CBT and melatonin rhythms in depressed patients have all been documented. Salivary melatonin is used as a non-invasive method for studying circadian rhythms in psychiatric patients particularly those on medication. In a study of 12 medicated patients with rapidly cycling bipolar disorder, Leibenuft et al. (1996) found a mean time for DLMO of 22.01±1.86 h in salivary samples and of 22.00±2.06 h in plasma samples.

Various studies have now demonstrated a close correspondence between results obtained with plasma and saliva samples in measurements of melatonin onset. Using DLMO as a marker, Lewy et al. (1987) and Sack et al. (1990) demonstrated that circadian rhythms are phase delayed in patients with seasonal affective disorder (SAD) when compared to normal controls. Phase delay of circadian rhythms in hypersonomolent SAD patients has been confirmed by Dahl et al. (1993) using the DLMO as a phase marker, SAD patients exhibiting a 92-min phase delay in DLMO and a 146-min phase delay in CBT as compared to normal controls. Daily exposure to bright light in the morning and the evening has been found effective in treating patients with SAD (Rosenthal et al., 1985). A number of studies have otherwise shown the advantage of morning light over evening light in treating winter depression (Golden et al., 2005; Mallikarjun and Oyebode, 2005; Sohn and Lam, 2004). Using DLMO, Terman et al. (2001) demonstrated that morning and evening light exposure at 10,000 lx, 30 min per day, caused phase shifts of melatonin rhythms consistent with those predicted from human PRC. A significant finding of those studies was the correlation found between the magnitude of phase advances of DLMO after morning light and the improvement in depression rating. This was in agreement with earlier reports (Lewy et al., 1987; Sack et al., 1990), which showed that improvement after morning light was coincident with the phase advance of DLMO. Based upon DLMO testing, Terman et al. (2001) recommended that for maximum advantage, light therapy of 10,000 lx should be scheduled in a circadian rather than a clocktime manner, i.e., about 8.5 h after baseline DLMO. In SAD patients, the
DLMO/sleep interval may be an important marker for circadian alignment or misalignment (Lewy et al., 2004) as well as the temperature minimum/sleep offset interval (Burgess and Eastman, 2004). This contradicts somewhat with the suggestion that the more a SAD patient is phase-advanced with morning light, the greater the antidepressant response (Terman et al., 2001).

Investigations of melatonin profile provide indirect evidence for the involvement of the SCN in the pathogenesis of SAD and response to bright light treatment. Patients with SAD may generate a biological “signal of change in season” that is absent in healthy volunteers (Golden et al., 2005; Mallikarjun and Oyebode, 2005; Sohn and Lam, 2004). In SAD patients, the duration of the nocturnal period of active melatonin secretion was longer in the winter than in the summer (as compared to no change in normal volunteers). According to Wehr et al. (2001) these abnormalities demonstrate that the pacemaker’s signal of day length is not a passive response to the LD cycle but reflects the processes taking place in SCN cells. Duration of melatonin secretion in constant dim light in SAD patients was modified differently by changes in season as compared to healthy controls. While a 38-min change in the duration of the circadian pacemaker’s day length signal between winter and summer season might be considered small, a change of such a magnitude in photoperiod length was sufficient to elicit behavioral changes in experimental mammals (Wehr et al., 2001). However, it must be noted that these findings were made primarily in men (who represent a small fraction of SAD patients) and that this difference occurred in the summer when compared to normal controls (when SAD patients are euthymic). In any event, DLMO is useful for evaluating patients with winter depression, and when used comparatively during the summer and winter seasons may be useful for elucidating the neural circuits that mediate the pathogenesis of this condition.

8. Conclusions

The DLMO test is one of the most reliable markers of the phase of the circadian pacemaker and is consequently becoming popular as an effective measurement for studies of sleep phase delay. Through the use of the DLMO marker, the phase advance and phase delay zones of the melatonin PRC can be differentiated, thus permitting a diagnosis of whether an individual is entrained to the 24-h LD cycle or is free running. Hence the DLMO is being used to study the phase typing in patients with a chronobiological component of sleep or mood disorder. DLMO can also provide guidance for the optimal timing of bright light treatment or of melatonin administration. Recently, the DLMO has been found useful for assessing effects of the length of day during summer and winter months in patients suffering from SAD. The evaluation of the DLMO and the extent to which it deviates from established normative values has increasingly proven to be a useful test for sleep disorders and for measuring phase disruption in the psychiatric assessment of mood disorders.

A number of potential applications are envisioned for this useful test. The research findings to date suggest that the DLMO marker will make a significant impact on various lines of scientific inquiry and in extending clinical understanding by: (a) improving sensitivity and reliability of melatonin assays; (b) extending its use to patients with rhythm perturbation in a number of clinical settings; and (c) inasmuch as living organisms are predictable resonating dynamic systems that require different amounts of a drug at predictably different times within the circadian cycle, assessment of phase position by DLMO will help to maximize desired and minimize undesired drug effects.

References


Lewy AJ. The dim light melatonin onset, melatonin assays and biological rhythm research in humans. Biol Signals Recept 1999;8:79–83.


