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Review article

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

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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.

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

Contents

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✠ 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.

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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 time-keeping 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 sychronizers (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 (Monk, 2005). The administration of the pineal product melatonin has been found to stabilize sleep/wake timing even in the blind whose circadian systems are not entrained to the 24 h day (Lockley et al., 2000; Sack et al., 1991, 2000). Melatonin administration induces differences in phase of circadian rhythms, either by accelerating (phase advance) or slowing down (phase delay) human circadian rhythms with the direction of shift being dependent upon the time of administration (Lewy et al., 1998).

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 Voultsios 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).

Leibenluft 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