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PERSPECTIVES

Molecular Clock Genes in Man and Lower Animals: Possible Implications for Circadian Abnormalities in Depression

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This paper reviews the recent discovery of clock genes that provide the mechanism for the regulation of circadian and seasonal rhythms in lower organisms and in humans and relates these clock genes to the circadian abnormalities in depression. (1) A subgroup of depressed patients have documented circadian abnormalities in mood, sleep, temperature and neuroendocrine secretion; (2) It is also suggested that seasonal affective disorder (SAD) patients may show an abnormality in their ability to shift their daily circadian rhythms in response to seasonal light changes; (3) The dramatic improvements in some depressions in response to three treatment modalities which manipulate circadian rhythms suggest that circadian abnormalities reported in patients may constitute a core component of the pathophysiology in depression; (4) Mutations in clock genes have been discovered that accelerate or delay circadian cycles; (5) It is hypothesized that 24-hour rhythm abnormalities in major depression and SAD may be due to altered clock genes. ***[Neuropsychopharmacology 22: 335–345, 2000]*** *© 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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Each year the journal *Science* evaluates all fields of science and lists the ten most important breakthroughs of the year. For two years in a row (1997,1998), clock genes made the Runners Up list. The following is a quote from *Science* (December, 1998): "Nineteenth-century philosophers proposed that God was a clockmaker who created the world and let it run. Modern biologists might in part agree, for it's clear that evolution has carefully crafted clocks that allow almost all organisms to follow the rhythm of the sun. In 1998, a volley of rapid-fire discoveries revealed the stunning universality of the clock workings. Across the tree of life, from bacteria to humans, clocks use oscillating levels of proteins in feedback loops to keep time. Perhaps more amazing, fruit flies and mice–separated by nearly 700 million years of evolution–share the very same timekeeping proteins. Now that they better understand the cellular clock, scientists can begin to manipulate it, with applications from curing jet lag to brightening winter depression."

BACKGROUND

One of the most pervasive epigenetic influences in the evolutionary process from single cell organisms to man is the 24-hour light/dark cycle. Exposure to light stimulates a cascade of molecular events in the circadian clock involving clock gene expression, which facilitates the daily synchronization to changes in photoperiod. Clock genes must cycle and respond rapidly to environmental signals (e.g. light) that affect the phase of the

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clock to regulate the daily patterning of physiology and behavior. By serving as transcriptional activators, clock genes regulate their own expression by building up over a 24-hour period until they turn their genes off and start their cycles again. Virtually all body processes systematically fluctuate during the 24-hour period and shift daily in response to the changes in the length of the period of light and darkness. The actual control of circadian rhythms is a complex process involving many environmental, genetic and physiological factors as reviewed by Wehr (1996).

Most clock genes have a Per-Arnt-Sim (PAS) domain that mediates protein-protein interaction, regulates circadian rhythms and is related to transcription factors that act as heterodimers. PAS domains confer target-gene specificity. The PAS domain is found in clock genes which span over 700,000 years of evolution and have been observed in plants (e.g., photoactive yellow protein), in Neurospora (e.g., mesphytochromes and algae cytochromes), in Drosophila and in mammals. Each of these clock-associated genes is almost identical with respect to their PAS domain, suggesting a common evolutionary origin and conservation of structure (Nambu et al. 1991).

The suggested criteria for clock genes include: (1) that they maintain circadian rhythmicity in constant darkness and (2) that they can be entrained to a new light/dark cycle (advance or delay in response to changes in light) (Albrecht et al. 1997). Over a decade ago, one clock gene was identified in the fruit fly, Drosophila (Bargiello et al. 1984). Little progress was made despite intensive effort until recently. During the last few years, a large number of clock genes have been cloned. These include in the bread mold, Neurospora, *white-collar 1* and *white collar 2* (*wc-1; wc-2*) and *frequency* (*frq*) (Crosthwaite et al. 1997) in Drosophila, *period* (*per*), *timeless* (*tim*), *cycle* (*cyc*), d*Clock* and *doubletime* (*dbt*), and in mammals, period genes (*per1*; *per2*; *per3*), *timeless* (*tim*), *clock* and *bmal1.* In humans, recently cloned circadian clock genes include *period* (*per*) (Sangoram et al. 1998), *timeless* (*tim*) (Sangoram et al. 1998; Koike et al. 1998), and candidate clock genes, *cry1* and *cry2* (van der Horst et al. 1999). In 1999, the human clock gene named *Clock* was cloned (Steeves et al. 1999; Abe et al. 1999). In situ hybridization in human brain tissue revealed the localization of *clock* mRNA in SCN (Steeves et al. 1999; Abe et al. 1999), hippocampus (Abe et al. 1999), cerebellum (Steeves et al. 1999) and piriform cortex (Abe et al. 1999).

AUTOREGULATORY LOOP OF THE CIRCADIAN CLOCK

The generation of internal circadian rhythms in many physiological and behavioral processes are under the control of clock genes which do not oscillate with a precise 24-hour rhythm. Thus, the entrainment of the mo-

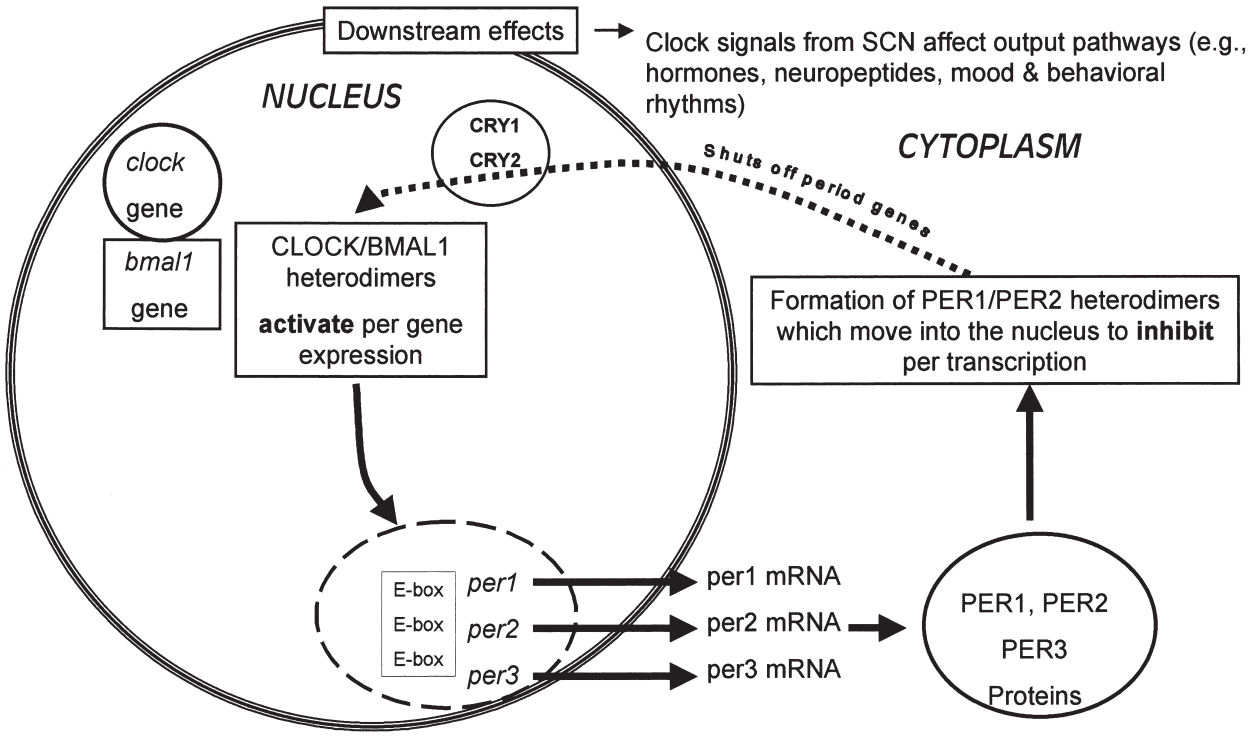

Figure 1. Mammalian Clock Gene Cycle. A theoretical model of the clock gene feedback loop in the SCN of mammals. (Note that clock genes are italicized in lower case while clock gene proteins are in upper case)

lecular clocks to light/dark cycles is a critical component for the adaptation of organisms to diurnal and/or seasonal changes in the length of the photoperiod. In mammals, the major circadian pacemaker is in the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus above the optic chiasm. Single cells isolated from the SCN demonstrate circadian rhythms of electrical activity suggesting that each cell has the capacity to regulate its own rhythms (Welsh et al. 1995). The synchronization of these neurons is thought to provide temporal organization for the SCN-regulated effector systems (Moore, 1999). Lesions to the SCN disrupt circadian oscillations producing arrhythmicity. Implantation of fetal SCN cells restores the rhythm in lesioned animals, although the new circadian oscillations mimic those from the donor, rather than those from the host SCN (Sollars and Pickard, 1998, for review).

The "generic" circadian clock which is found in virtually every organism containing a nucleus (eukaryote), as well as in some bacteria, serves three functions. First, the clock pacemaker can generate endogenous rhythms. Second, the clock contains an input pathway that links the internal cycle to external light-dark stimuli (entrainment). Finally, an output pathway regulates downstream physiological and behavioral rhythms.

A model of the circadian clock in the SCN of mammals, based in part, on data from lower organisms (Dunlap 1999), is illustrated in Figure 1. A circadian autoregulatory loop incorporates activators and suppressors of *period* gene expression. As detailed below, two pairs of protein heterodimers, CLOCK/BMAL1 and PER1/PER2, respectively activate or suppress *period* gene transcription.

MAMMALIAN CLOCK GENE CYCLE

Figure 1 illustrates the known components of the mammalian clock gene mechanism. In the nucleus, CLOCK/BMAL1 heterodimers activate *period* genes, *per1*, *per2*, and *per3* through E-Box, a transcription factor binding site, located near the promoter region of the *period* genes (Ikeda and Nomura 1997; Darlington et al. 1998; Gekakis et al. 1998; Honma et al. 1998; Jin et al. 1999). *Per1, per2* and *per*3 mRNAs are transcribed and moved from the nucleus to the cytoplasm where their encoded proteins are translated into PER1, PER2, and PER3 proteins. As the levels of PER1 and PER2 proteins increase and begin to form PER1/PER2 heterodimers, the PER1/PER2 heterodimers enter the nucleus. Cryptochrome proteins (CRY1; CRY2) act in the negative feedback limb of the clock feedback loop. They are nuclear proteins that interact with the PER proteins, translocating mPER proteins from the cytoplasm into the nucleus (Kume et al. 1999). CRY1 and CRY2 act as light-independent inhibitors of CLOCK/BMAL1, the activator driving *per1* transcription as well as interactions with *per1* and tim (Griffin et al. 1999). Thus far, studies do not support a role for mammalian *cry* genes in photoentrainment (Griffin

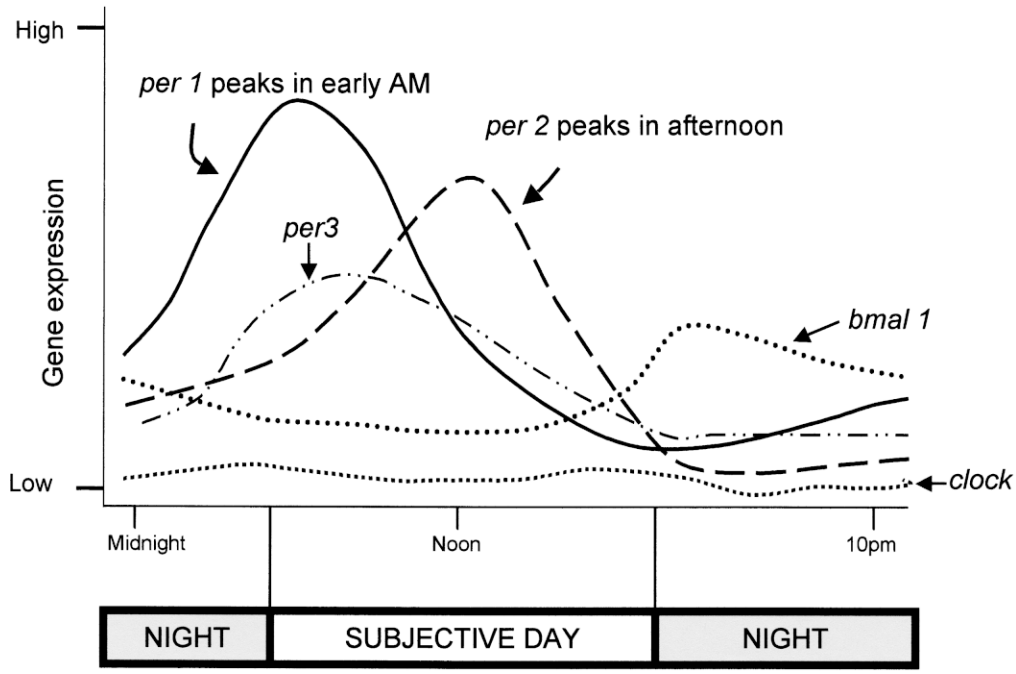

Figure 2. Theoretical model of mammalian circadian gene expression in 12 hour light/12 hour dark cycle. Note that *per1* peaks in the morning hours while *per2* activity occurs later in the afternoon with a temporal separation that approximates 8 hours. (Adapted from Dunlap, 1999)

et al. 1999). Following the blockade of CLOCK/BMAL1 activity, *period* gene transcription is turned off (Jin et al. 1999; Zylka et al. 1998a,b).

ENTRAINMENT OF CLOCK GENES TO THE LIGHT/DARK CYCLE

Entrainment is accomplished through photic phase shifts that are initiated by light activation of retinal photoreceptors in the eye and conveyed directly via the retinohypothalamic tract (or indirectly via the geniculohypothalamic tract) to the SCN. Figure 2 illustrates the model of mammalian clock gene expression in a 12 hour light/12 hour dark cycle. As can be seen, *period* is differentially expressed throughout the 24-hour cycle. *Per 1* peaks in the early subjective morning hours while *per 2* activity peaks in the early subjective afternoon. *Per 3* is somewhat increased in the subjective morning. *Bmal1* reaches its peak level at the time of dark-light transition, maintaining its highest levels in the subjective night while *Clock* shows minimal variation during the 24 hour cycle (Takumi et al. 1998; Oishi et al. 1998; Honma et al. 1998; Albrecht et al. 1997). There is a rapid induction of *per1* in response to a light pulse during a dark period (subjective night) (Albrecht et al. 1997).

The study of mammalian clock genes is a constantly evolving field and new data has recently been published on the role of *timeless (mtim)* and the *period 3* (*mPer3*) genes in the mammalian clock gene cycle. Tischkau et al. (1999) utilized novel probes and demonstrated that the mammalian gene, *timeless* (*mtim*), has a three-fold diurnal variation in expression. Light pulses known to induce phase delays cause significant elevations in *mtim* RNA. The mTim expression profile and the response to nocturnal light are similar to *mPer2* (Tischkau et al. 1999).

Recent work by Takumi et al. (1999) on studies of the *mPer3* demonstrate circadian rhythmicity in the SCN. However, in contrast to *mPer1* and *mPer2* genes, *mPer3* RNAs do not appear to be acutely altered by light (Takumi et al. 1999; Zylka et al. 1998b).

OUTPUT PATHWAY

The interactions of the SCN with the pineal gland produce changes in physiology and behavior that affect sleep, nocturnal temperature, cortisol, and the synthesis of melatonin and neuropeptides. One pathway involved in these interactions is the cyclic-AMP-responsive element modulator (CREM) which is expressed cyclically in the pineal gland in response to signals from the SCN. CREM exhibits circadian rhythmicity so that levels peak at night and are absent during the day. As reviewed by Sassone-Corsi (1998), an inducible cAMP early repressor (ICER) interacting with CREM could provide potential mechanisms for shaping oscillatory synthesis of hormones (e.g., melatonin) by periodically repressing gene transcription. In addition, changes in photoperiodicity may help explain downstream changes affecting pineal activity.

The precise organization of the body clock supports a critical role in clock gene function for the adaptation and internal regulation of physiological mechanisms and behavior in a large spectrum of organisms, including humans. What happens if a clock gene is defective? Data reviewing the action of mutant clock genes and clinical evidence for circadian disturbances in depression and seasonal affective disorder are presented below.

MUTANT CLOCK GENES

There is a high degree of homology between mammalian clock genes and those in lower organisms (for review see Dunlap 1999). Thus, it may follow that mutant clock genes identified in lower organisms (e.g., Young 1998) are likely to have mammalian counterparts and may be candidate genes for human diseases, particularly those associated with circadian rhythm abnormalities. It is hypothesized that mutations or allelic variations in clock genes may be associated with clinical symptoms in SAD or in specific populations of depressed patients with circadian abnormalities. Various mutant forms delay, accelerate, shorten, or lengthen circadian rhythms and/or produce arrhythmicity. In Drosophila, mutations of period and tim as well as short- and long alleles have been identified. There are a number of classical mutations such as perS, *per1* and *per0*, the *tim0* and the long period of *tim.* A few recently identified mutations include: *doubletime* (*dbt*) which significantly speeds up period length (Price et al. 1998; Kloss et al. 1998), *cycle* (*cyc*) which alters circadian rhythm by interfering with the transcription of *per* and *tim* genes (Rutila et al. 1998a), *perSLIH* which produces a delayed evening peak of locomotion (Hamblen et al. 1998), *cryb* mutations which are associated with a poor synchronization to light-dark cycles (Stanewsky et al. 1998) and *timSL* mutants which alter period length (Rutila et al. 1998b). In the hamster, the mutant gene, *tau*, produces robust reductions in the period of circadian activity, melatonin and cortisol rhythms (Lucas et al. 1999). In mice, a mutant gene, *CLOCK-Δ19*, after forming a heterodimer with *Bmal1*, fails to perform its normal action of activating transcription of the *period* genes (Gekakis et al. 1998). Mutant mice lacking the cryptochrome2 blue light photoreceptor gene (*mcry2*) had a lower sensitivity to acute light induction of *mper1* in the SCN and had an intrinsic circadian period one hour longer than normal (Thresher et al. 1998). This offers an additional mechanism that could be defective in diseases such as SAD.

Thus, it is hypothesized that mutations or allelic variations in clock genes might contribute to the symptoms of depression in seasonal affective disorder (SAD) and subgroups of major depression (Bunney and Akil, 1998). A clock gene hypothesis adds a further dimension in the theoretical conceptualization of the molecular biological understanding of depression and could provide potential alternative therapies. Evidence for circadian abnormalities associated with SAD and depressive disorders is reviewed below.

SEASONAL AFFECTIVE DISORDER (SAD)

Seasonal affective disorder (winter depression), is a syndrome characterized by recurrent depressions that occur at the same time every year (Rosenthal et al. 1984). Depressive phases are associated with hypersomnia, overeating, and carbohydrate craving, symptoms that have been compared to "hibernation". The prevalence of SAD in the United States is estimated at 6%, affecting approximately 11 million people. Epidemiological studies reveal a high incidence of SAD in northern latitudes, theoretically due to the decreased amount of daylight and increased hours of darkness during fall/winter periods. The incidence of SAD ranges from 1.4% in Southern Florida and South Texas (latitude 25–30°) to over 10% in Washington and northern Maine (latitude 45–50°). SAD affects females more often than males, occurs most frequently between the ages of 20-40 years and may run in families (Rosenthal 1993). Abnormalities in circadian rhythms in SAD include sleep disturbances (Rosenthal et al. 1984), increases in the minima of nocturnal core body temperature (Schwartz et al. 1997a) and disturbances in cortisol (Avery et al. 1997) and melatonin (Dahl et al. 1993; Lewy et al. 1987, 1998) secretion. In addition, a subgroup of winter depressives have phase-delays reported in temperature, cortisol and melatonin secretion (Dahl et al. 1993; Avery et al. 1997). It is hypothesized that environmental and social zeitgebers (e.g., sleep, meals, light) mask the expression of the circadian pacemaker. To control for these factors, Wirz-Justice (1998) used a 40-hour sleep deprivation constant routine protocol. (In the constant routine method, patients are studied in a laboratory controlled for temperature, humidity and light and are fed small isocaloric meals. Wirz-Justice suggests that this method minimizes exogenous effects and unmasks circadian amplitude and phase. Results from studies of SAD patients and controls in the constant protocol routine revealed that mood in the patients was generally worse in the early morning hours (5-6 A.M.), improved slightly in the afternoon and declined during the night, particularly during the first day. Following sleep deprivation, patients and controls were significantly differentiated. The SAD patients showed improvement in behavioral ratings while the controls tended to become worse.

MAJOR DEPRESSION

Numerous studies conducted over a period of more than 25 years provide data to suggest that a subgroup of depressed patients may have a circadian rhythm disorder. A meta-analysis of data reveals that circadian rhythm disorders including temperature and cortisol secretion abnormalities which are often manifested during the sleep period are most likely to distinguish subgroups of depressed patients from controls (Benca et al. 1992). A subgroup of these patients has disturbances in circadian rhythms that are manifested by daily mood swings. Some patients have marked diurnal mood swings in which they are severely psychotically depressed in the early morning and become almost euthymic by evening. This pattern of 24-hour striking alterations in mood can persist for many months in untreated individuals. Elevated nocturnal body temperature is one of the more consistently observed circadian abnormalities in depression (Duncan 1996; Souétre et al. 1988; 1989; Avery et al. 1982; Szuba et al. 1997). However, this is clearly not the case in all patients. The effect may be due to blunted amplitude of the central circadian pacemaker. Masking effects of night-time arousal and sleep could complicate the interpretation of some studies (Duncan 1996). A phase-advance in overall 24 hour pattern of body temperature is reported in many depressed patients (Dietzel et al. 1986; Goetze and Tölle 1987; Kripke et al. 1978; Wehr et al. 1980). A non-significant trend has been reported in other studies by Atkinson et al. (1975); Parry et al. (1989); Pflug et al. (1976); Souétre et al. (1988;1989). However, von Zerssen et al. (1985) reported a lack of phase-advance in patients compared to themselves after clinical recovery and with controls. They reported a reduction in the amplitude of body temperature but suggested that it might be due to a negative masking of the temperature rhythm by the patients' sleep disturbances. Also, Buysse et al. (1995) studied circadian patterns of unintended sleep episodes in remitted depressed patients. They reported no phase or amplitude changes in sleep propensity. However, they note that these patients had only minor sleep changes and normal temperature profiles even while depressed which they suggest could have biased against finding significant differences. In addition, a subgroup of depressed patients have increases in diurnal and nocturnal cortisol secretion (Carpenter and Bunney 1971; Branchey et al. 1982; Souétre et al. 1988; Jarrett et al. 1983; von Zerssen et al. 1985;1987; Linkowski et al. 1985). Phase advances in nocturnal cortisol secretion relative to sleep onset provide clinical evidence compatible with a disturbance in circadian rhythms (Car-

penter and Bunney 1971; Halbreich et al. 1985; Jarrett et al. 1983; Linkowski et al. 1985; Lisansky et al. 1987; Steiger et al. 1989). Finally, abnormal sleep patterns including shortened REM latency and early morning awakening are associated with depression (Gillin et al. 1979). It is suggested that, perhaps, depression may involve a weaker coupling process between internal pacemakers and involve abnormal sensitivity to environmental cues such as light (Souétre et al. 1989). This could be a result of mutant clock genes or allelic variations, leading to abnormal clock cycles or altered photosensitivity. Historically, prior to the discovery of clock genes, concepts of "susceptible circadian phases" during which sudden shifts in circadian rhythm lead to depression in predisposed individuals, was initially proposed by Papousek (1975) and refined by Wehr and Justice, 1982 and others (see review, Wirz-Justice 1998).

MANIPULATIONS OF THE CIRCADIAN CYCLE AS TREATMENT

We have asked the question: since circadian abnormalities have been documented in SAD and major depression, are they central to the illness or epiphenomena? If one manipulates circadian rhythms (e.g., sleep/rest/activity), are there profound improvements in depressive symptoms? If so, it would argue that circadian rhythms under the regulation of clock genes might be etiologically linked to depression. Three treatment approaches, each involving circadian manipulations, have been used to treat depressive disorders. Light therapy, sleep deprivation and phase-advance treatment have been used either separately or in combination to treat SAD and depressive illness. Results from these studies as reviewed below document a striking and sometimes rapid treatment response in a subgroup of patients.

BRIGHT LIGHT THERAPY

Bright light therapy is one of the most effective treatments for SAD depressions (Rosenthal 1993). Typically, patients are exposed to 2500 lux or greater for approximately two hours per day for one week or longer. Studies in SAD patients suggest that circadian rhythms are often phase-delayed relative to the sleep/wake or light-dark cycle. Morning bright light (producing phase advance of the light period) may be more effective than evening bright light (associated with phase delay) in elevating mood and phase advancing melatonin offset in a subgroup of patients (Lewy et al. 1998). Thus, two hours of additional bright light can lengthen a short winter-day light photoperiod into a summer-like photoperiod. Eastman et al. (1998) published a study addressing the issue of whether light treatment in winter seasonal affective disorder (SAD) is demonstrated to have an effect beyond its placebo effect. In a study of SAD patients treated either with bright light (6000 lux) or placebo (sham negative-ion generators), patients that were treated with the bright light for at least three weeks had an antidepressant effect beyond its placebo effect in producing full remissions. Jacobsen et al. (1987) reported data that the antidepressant effects of phototherapy in SAD may not depend entirely on its ability to lengthen the daylight photoperiod. They suggest that its effects may not be due to shifting the timing of the circadian rhythm. Based on animal data (Goldman and Elliot, 1988), Wehr suggests that bright light therapy could be expected to reduce the magnitude of the circadian pacemaker's phase resetting response to light (Wehr 1996). This change could decrease the pacemaker's capacity to synchronize the sleep-wake cycle with the day-night cycle. Additionally, bright light lowers the increased nocturnal core body temperature in SAD patients (Schwartz et al. 1997a). A study of 17 SAD patients and controls reported that light-associated temperaure decreases are in direct proportion to antidepressant responses (Schwartz et al. 1997b).

A review of the literature in major depression suggests that bright light therapy is not as effective for treatment of nonseasonal depression as compared with seasonal depression (Gordijn et al. 1998; Thalaen et al. 1997). However, some nonseasonal patients may show improvement (Dietzel et al. 1986; Kripke, 1998; Yamada et al. 1995). For example, Dietzel et al. (1986) demonstrated improvements in mood, attention, concentration and psychomotor activity after treatment with 3000 lux of bright light in a subgroup of non-seasonal depressed patients. These changes were accompanied by normalization of sleep, cortisol rhythms, and melatonin suppression.

PHASE ADVANCE OF THE SLEEP/WAKE CYCLE AS AN ANTIDEPRESSANT

Wehr et al. (1979) in a pioneer study, treated depression in a bipolar patient by advancing the time of sleep and awakening in an attempt to synchronize the circadian rhythms. Advancing the sleep and awake times on two occasions by 6 hours earlier than normal for a period of two weeks produced a striking but temporary improvement in symptoms. The theory suggests that moving the sleep/wake activity cycle earlier to coincide with a hypothesized already advanced circadian rhythms such as cortisol and temperature will synchronize the cycles and result in an improvement in depression. Sack et al. (1985) conducted a phase-advance study on four depressed patients who were unresponsive to antidepressants and reported successful antidepressant effects.

For example, one patient presented with constant and severe depression that persisted for 30 days. Within two days after a five-hour phase advance in sleep/wake cycle, there was a striking and long-lasting response in terms of a decrease in depressive symptoms.

SLEEP DEPRIVATION

The circadian manipulation of the sleep/activity rest/wake cycle with sleep deprivation in depression is a rapidly-acting although transient treatment for depression. A review by Wu and Bunney (1990) documents that in more than 61 studies with 2000 patients during the last three decades, one night of total sleep deprivation completely reversed depressive symptoms in 50% of severely depressed patients. Relapse often occurs with recovery sleep and even sleep for very short periods of time can induce switches back into depression (Wu and Bunney, 1990).

COMBINATION TREATMENT STRATEGIES WHICH MANIPULATE CIRCADIAN RHYTHMS

A strategy to block relapse in successful sleep deprivation responders includes therapeutic interventions combining the three modalities of sleep deprivation, phase advance and light therapy. One such combination includes total sleep deprivation plus phase advance (Vollmann and Berger 1993; Riemann et al. 1995; 1996; Berger et al. 1997a;b; Albert et al. 1998). One possible theory is that following successful sleep deprivation, avoiding recovery sleep during a hypothesized "critical" early morning time period prevents sleep deprivation-associated relapses. Following a night of total sleep deprivation, Berger et al. (1997a) advanced sleep-deprivation responders five hours by putting them on a sleep schedule from 5:00 P.M. to 12:00 midnight. A significant decrease in relapses occurred following recovery sleep. By advancing the sleep, patients remained awake during the hypothesized critical period in the early morning. In contrast, sleep deprivation combined with a 5-hour phase delay in sleep each night was not as effective in blocking post sleep-deprivation relapses (Berger et al. 1997a). Riemann has enlarged his patient sample and has continued to observe therapeutic responses (in press). Forty depressed patients off medication for 7 days who responded to total sleep deprivation were either phase-advanced or phase-delayed. Seventy-five percent were stabilized by the phase-advance condition while only 40% of the patients in the phase-delayed condition did not relapse.

An alternative treatment involves the combination of sleep deprivation with bright light therapy. Data suggests that exposure to bright light on the night of (Wehr et al. 1985) or on the night following sleep deprivation (Neumeister et al. 1996) decreases the incidence of relapses associated with recovery sleep in successful sleep deprivation responders. As a control, dim light was shown to be less efficacious (Neumeister et al. 1996).

DISCUSSION

A growing body of data, collected over a period of more than two decades, provides evidence that SAD, as well as subtypes of major depression may be, in part, circadian-related disorders. Converging lines of evidence suggest that through evolution, clock genes have regulated circadian processes controlling behavioral and physiological parameters. Abnormalities in clock gene function are speculated to be associated with diseases characterized as having circadian-related abnormalities including disturbances in sleep, temperature, hormones, and behavior. SAD patients, as well as patients with subtypes of major depression may be prime candidates for clock gene research.

It is speculated that SAD patients may have altered sensitivity to light. In these patients it is possible that the morning component of the clock gene mechanism that is responsive to decreased photoperiods (as occurs in fall and winter) is not adequately functional with respect to photoperiod phase shifts. Thus, it is hypothesized that the circadian clock genes that are reset in response to shorter winter light periods could be altered in depression in SAD. Furthermore, mood is frequently worse in the early subjective morning hours in a subgroup of patients. One of the challenges in clock gene investigations is the relationship of clinical symptoms to underlying clock gene function. For example, the mammalian *period* genes show a diurnal variability in expression with higher levels of expression of *mper1* in subjective morning in contrast to *mper2* levels which are more highly expressed in subjective evening (Takumi et al. 1998) (see Figure 2). It is suggested that a mutant form of the *per1* gene could play a role in the pathophysiology of SAD. The process, however, is complex in that the *Clock* gene plays a modulatory role in the photic induction of *per1* in the SCN. Homozygous *Clock* mutant mice show a significantly reduced light response (Shearman and Weaver 1999). Furthermore, the morning induction of *per1* also involves CLOCK/BMAL1 interactions with the PER1/PER2 heterodimers and possibly with yet to be identified loop modifiers.

Steeves et al. (1999) suggest that depression is a candidate disease as the symptoms are associated with abnormalities of circadian period and entrainment and are, in turn, responsive to manipulations that change the circadian parameters. Steeves et al. (1999) speculate that the *clock* gene is a possible locus for analyses of hu-

man circadian disorders. Two recent studies provide some of the first evidence compatible with a role for clock genes in altered circadian rhythms in man. Evidence is beginning to emerge that individuals carrying different *clock* gene alleles may have altered circadian activity. In one of the first studies of its kind, Katzenberg et al. (1998) demonstrated an increase in the sequence variation of the *clock* gene (3111C/3111T) in a population of 410 normal adults. These were characterized as "late to bed, late to rise" or "early to bed, early to rise". Those individuals carrying the 3111C allele appeared to have a diurnal preference for evening activity. In a second human study, Jones et al. (1999) presented evidence for a profound phase advance of the sleep-wake, melatonin and temperature rhythms in a set of three kindreds. The trait, associated with a very short *tau*, segregates as an autosomal dominant with high penetrance. The investigators suggest that these kindreds represent a well-characterized familial circadian rhythm variant in humans which could provide an opportunity for genetic analysis of human circadian physiology.

Finally, a large body of data, including the therapeutic efficacy of the selective serotonin reuptake inhibitors (SSRIs), suggests that the serotonin system is involved in depressive illness. Morin (1999) reviews the evidence supporting a modulatory role of serotonin in the regulation of mammalian circadian rhythmicity. The SCN contains one of the densest serotonergic plexes in the brain and receives much of its serotonergic input from the median raphe nucleus. Loss of serotonergic neurons in the median raphe results in a later offset of the nocturnal activity phase, a longer duration of the activity phase and increased sensitivity of the circadian rhythm response to light. The suggested mechanism involves the presynaptic 5HT1A receptor and the 5HT1B and the 5HT7 postsynaptic receptors in the retinal hypothalamic tract. The activation of these 5HT receptors decreases the photic input to the SCN thus reducing the phase response to light (Pickard and Rea, 1998). Morin (1999) states that the most convincing role is the apparent ability of serotonin to modulate sensitivity of the circadian rhythm to light. Thus, photic information essential for the daily phase resetting of the SCN circadian clock is sent directly to the SCN through the retinohypothalamic tract. This daily phase resetting process is hypothesized to be abnormal in SAD. Additional work potentially linking serotonin to biological rhythms involves the role of the serotonin transporter promoter repeat length polymorphism in SAD (Rosenthal et al. 1998; Sher et al. 1999).

Future research could involve cDNA microarray profiling for abnormal clock genes in the postmortem tissue of depressed patients contrasted with matched normal controls. Although speculative, it is intriguing to propose that the analyses of clock genes in depression will add a new dimension to the understanding and potential treatment of this serious illness.

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