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# Melatonin:
# The Darkness Hormone and its Uses

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CENTER FOR Environmental Therapeutics

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# Melatonin: The Darkness Hormone and its Uses

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CENTER FOR Environmental Therapeutics

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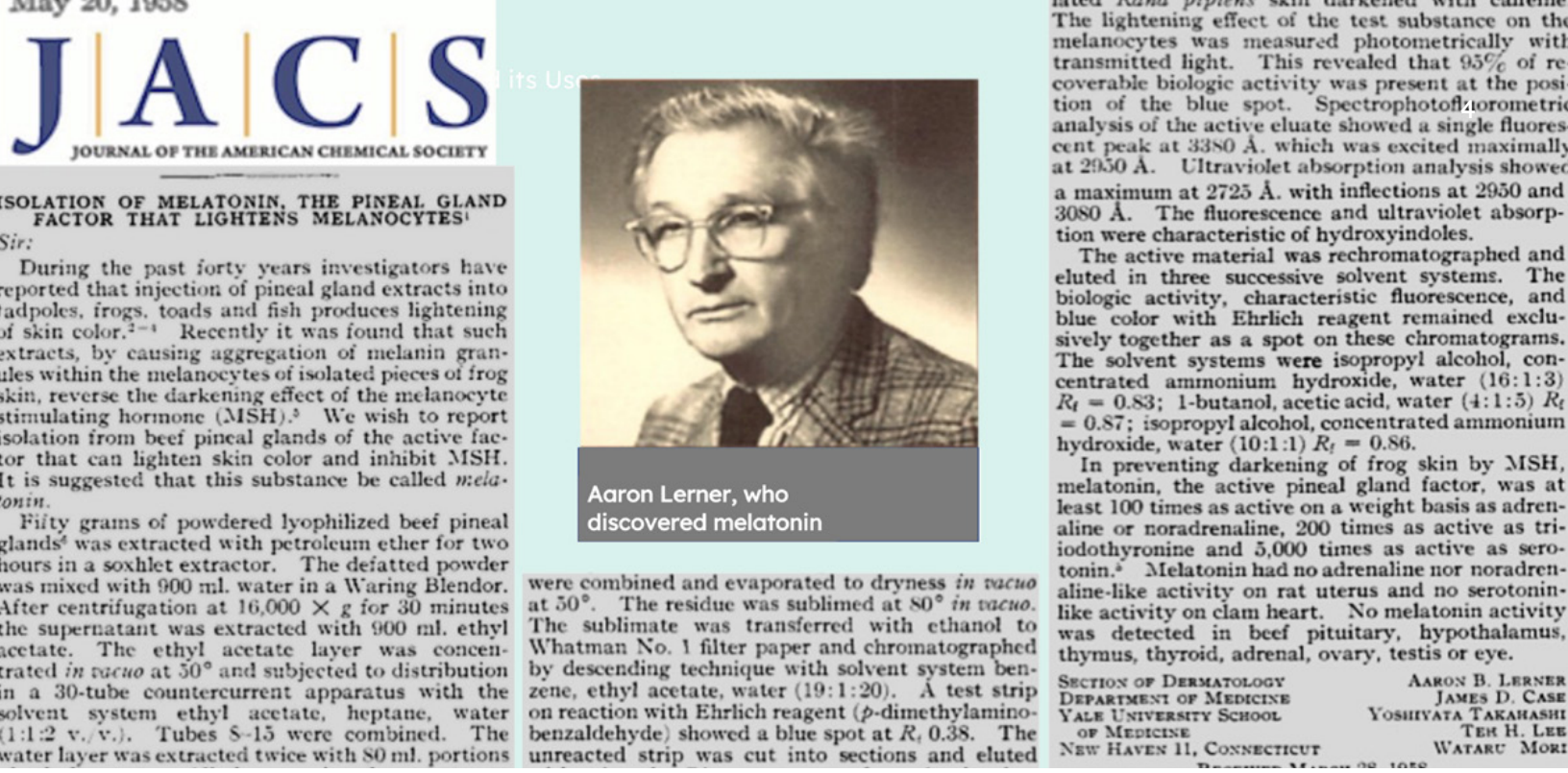

May 20, 1958

JACS

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ISOLATION OF MELATONIN, THE PINEAL GLAND FACTOR THAT LIGHTENS MELANOCYTES[1]

Sir:

During the past forty years investigators have reported that injection of pineal gland extracts into tadpoles, frogs, toads and fish produces lightening of skin color.[2-4] Recently it was found that such extracts, by causing aggregation of melanin granules within the melanocytes of isolated pieces of frog skin, reverse the darkening effect of the melanocyte stimulating hormone (MSH).[5] We wish to report isolation from beef pineal glands of the active factor that can lighten skin color and inhibit MSH. It is suggested that this substance be called *melatonin*.

Fifty grams of powdered lyophilized beef pineal glands[6] was extracted with petroleum ether for two hours in a soxhlet extractor. The defatted powder was mixed with 900 ml. water in a Waring Blendor. After centrifugation at 16,000 × *g* for 30 minutes the supernatant was extracted with 900 ml. ethyl acetate. The ethyl acetate layer was concentrated *in vacuo* at 50° and subjected to distribution in a 30-tube countercurrent apparatus with the solvent system ethyl acetate, heptane, water (1:1:2 v./v.). Tubes 8-15 were combined. The water layer was extracted twice with 80 ml. portions



Aaron Lerner, who discovered melatonin

were combined and evaporated to dryness *in vacuo* at 50°. The residue was sublimed at 80° *in vacuo*. The sublimate was transferred with ethanol to Whatman No. 1 filter paper and chromatographed by descending technique with solvent system benzene, ethyl acetate, water (19:1:20). A test strip on reaction with Ehrlich reagent (*p*-dimethylaminobenzaldehyde) showed a blue spot at $R_f$ 0.38. The unreacted strip was cut into sections and eluted

The lightening effect of the test substance on the melanocytes was measured photometrically with transmitted light. This revealed that 95% of recoverable biologic activity was present at the position of the blue spot. Spectrophotofluorometric analysis of the active eluate showed a single fluorescent peak at 3380 Å. which was excited maximally at 2950 Å. Ultraviolet absorption analysis showed a maximum at 2725 Å. with inflections at 2950 and 3080 Å. The fluorescence and ultraviolet absorption were characteristic of hydroxyindoles.

The active material was rechromatographed and eluted in three successive solvent systems. The biologic activity, characteristic fluorescence, and blue color with Ehrlich reagent remained exclusively together as a spot on these chromatograms. The solvent systems were isopropyl alcohol, concentrated ammonium hydroxide, water (16:1:3) $R_f$ = 0.83; 1-butanol, acetic acid, water (4:1:5) $R_f$ = 0.87; isopropyl alcohol, concentrated ammonium hydroxide, water (10:1:1) $R_f$ = 0.86.

In preventing darkening of frog skin by MSH, melatonin, the active pineal gland factor, was at least 100 times as active on a weight basis as adrenaline or noradrenaline, 200 times as active as triiodothyronine and 5,000 times as active as serotonin.[7] Melatonin had no adrenaline nor noradrenaline-like activity on rat uterus and no serotonin-like activity on clam heart. No melatonin activity was detected in beef pituitary, hypothalamus, thymus, thyroid, adrenal, ovary, testis or eye.


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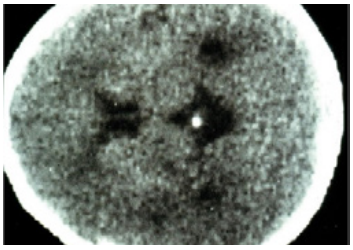
WHAT IS MELATONIN?

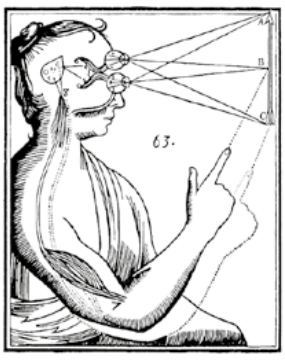
Melatonin is a small molecule discovered in 1958 by a dermatologist (Aaron Lerner, Yale) who was looking for the frog skin lightening factor found in the mammalian pineal gland. The pineal, about the size of a pea in humans, is found in approximately the center of the brain. At the time it had no known function.

The French philosopher René Descartes considered the pineal to be the seat of the soul. But with the advent of melatonin, research interest expanded mightily. Now melatonin itself is of clinical as well as research interest far beyond its ability to lighten frog skin. This is largely due to two factors: its effects on body rhythms and sleep, and its ability to reflect time keeping in the brain by its pattern of secretion.


The melatonin molecule



CAT scan of a calcified human pineal in the center of the brain.



René Descartes thought the pineal was the seat of the soul and was controlled by the eyes.

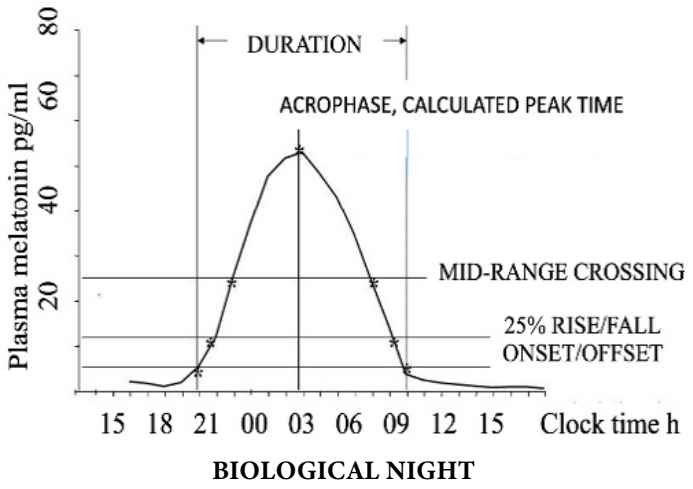



Diagram of a "normal" melatonin rhythm in plasma with the "phase markers" used to characterize circadian status (internal clock time). Onset and offset are also known as DLMO (dim light melatonin onset) and DLMOff. Saliva melatonin and urinary 6-sulphatoxymelatonin can provide the same information.

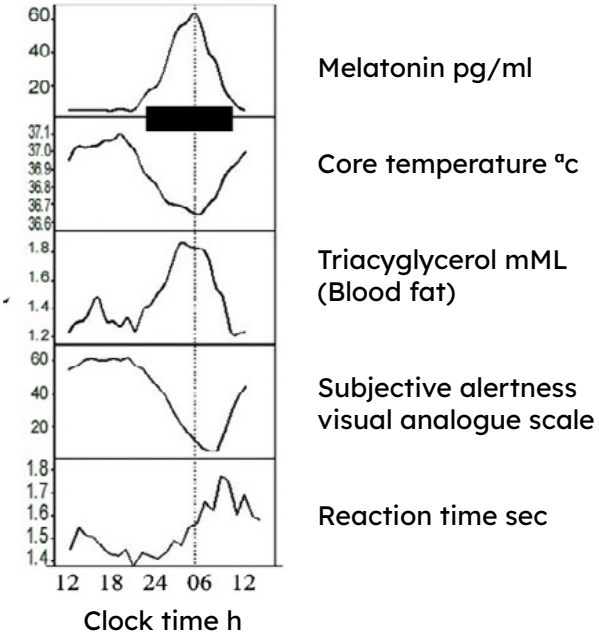

Josephine Arendt, PhD

Measured in blood, saliva or urine (in the latter usually by its metabolite, or breakdown product, 6-sulphatoxymelatonin) melatonin is highly rhythmic with maximum concentrations during the night in a “normal“ environment. The rhythm develops during the first year of life, is maximum before puberty and slowly declines throughout life. Some probably very healthy elderly people do not show low values in old age. This may be due to the large inter-individual differences but very consistent intra-individual production profile (rather like a fingerprint) seen in melatonin production at all ages. The size of the pineal gland has been implicated in these inter-individual differences in melatonin.

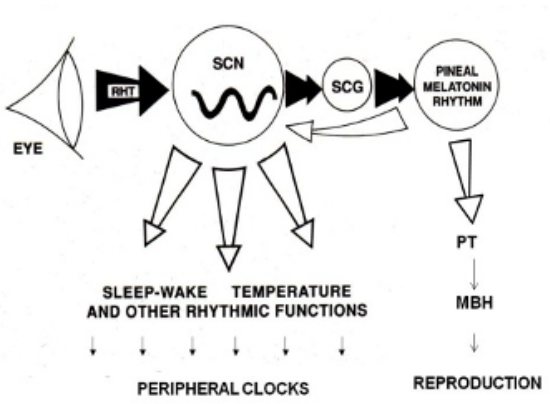
Circulating melatonin derives essentially from its synthesis in the pineal gland which is under neural control by the central “clock” (suprachiasmatic nucleus, SCN) in the brain. The SCN generates approximately 24h circadian rhythms and acts as a master clock for the organization of the circadian system. Melatonin profiles in body fluids reflect the activity of the SCN: it is a “window on the brain.”

The rhythm, as for other circadian rhythms, is synchronized to 24 hours primarily by the light-dark cycle acting via the retina and a specific neural pathway to the SCN. In the absence of a light dark cycle as a strong time cue, circadian rhythms such as melatonin, cortisol, core body temperature, “free-run,” or show an individual‘s intrinsic periodicity, which is genetically determined and usually slightly longer than 24h.

This is very clear in many blind people with no light perception at all, and underlines the importance of light. With weak time cues circadian rhythms may maintain a 24h period but have an abnormal timing within the 24h. For example. instead of peaking at around 3-4 a.m., the melatonin rhythm may peak much later or earlier, and even appear during




The relationship of melatonin to other major circadian rhythms. Note the circadian low points of core temperature, metabolism, alertness and performance close to the melatonin peak, normally at night (black bar).




Light controls the circadian system. RHT, retinohypothalamic tract: specialized neural link from retina to SCN. SCN, suprachiasmatic nucleus: master clock in the brain. SCG, superior cervical ganglion: neural pathway to pineal. PT, pars tuberalis of pituitary. MBH, mediobasal hypothalamus of brain.

## THE CHEMISTRY OF MELATONIN AND ITS SYNTHESIS

Melatonin (N-acetyl-5-methoxytryptamine) is a small molecule derived from the dietary amino-acid tryptophan via conversion of tryptophan to serotonin (5-hydroxytryptamine). Then N-acetylation and O-methylation of serotonin to melatonin. The SCN, via interacting clock genes, generates a self-sustaining approximately 24h rhythm which via a neural pathway controls the activity of the rate-limiting enzyme in synthesis of melatonin, N-acetyl transferase (arylalkylamine N-acetyl transferase, AA-NAT) such that there is a 7- to 150-fold increase in pineal melatonin synthesis and secretion between day and night.

## LIGHT AND MELATONIN

We have seen that a light-dark cycle can maintain the melatonin rhythm synchronized to 24h. Suitable light intensity in the evening will delay the onset of nighttime melatonin production. Similarly, in the early morning sufficient light will advance the timing of the rhythm. Since the tendency of most peoples' rhythm is to delay, the advancing effect of morning light is considered to be the most important factor for synchronizing the rhythm to 24h. At night, light can suppress melatonin production. Turn on a sufficiently bright light at night, and the production of melatonin drops very rapidly.

If this happens during the night, production will resume when the light is turned off. In the early morning after peak production, light will stop further production, thus shortening the time melatonin is made. The difference in duration of secretion may well be important in humans; it certainly is in animals that depend on daylength to time their seasonal functions.

So far, I have just referred to light in general. However, there is much more important detail regarding the circadian system. Both suppression and phase shifting effects of light on melatonin (delay or advance of the rhythm) are at least partly representative of the whole circadian system. These functions are dependent on the intensity and wavelength of the light, with the wavelengths of blue light around 480 nanometers (nm) exerting the most powerful influence. Suppression and phase shifting are dependent on the time of light exposure and photic history (previous light exposure). There are also large individual differences in sensitivity.

Importantly, we may think of the melatonin rhythm as representing "biological night." Light has immediate alerting effects during the biological night, whereas treatment with melatonin with a fast release formulation has rapid soporific effects during the "biological day." Optimizing both circadian synchrony and illumination have powerful beneficial effects on alertness and performance, and probably on health in general. It is well recognized that circadian desynchrony — as, for example, in shift work — is deleterious to health. These observations have led to the design of new types of lighting equipment, and are influencing architectural lighting.

MELATONIN PHYSIOLOGY AND PHARMACOLOGY

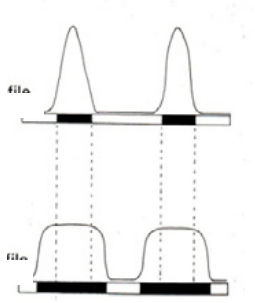
**SEASONAL RHYTHMS**

What is the function of melatonin? Without a doubt, in photoperiodic (daylength dependent) species, its main function is to act as a photoneuroendocrine transducer. By its pattern of secretion — long in short days/long nights, and short in long days/short nights — melatonin provides information about daylength to body physiology. Many species use daylength to time their seasonal functions (for example, reproduction, growth of winter coat, behavior, and the timing of puberty).

Melatonin from the pineal gland provides the essential signal for changing daylength in the vast majority of photoperiodic species. With suitable treatment by melatonin, it is possible in the summer to mimic the long pattern of winter secretion to alter the timing of seasonal functions. Very small amounts are used, which do not normally raise blood melatonin above that normally found at night.

Melatonin has been commercialized to control breeding and coat growth in domestic species such a sheep and goats, taking advantage of the seasonal fluctuation in market prices (as for lamb).

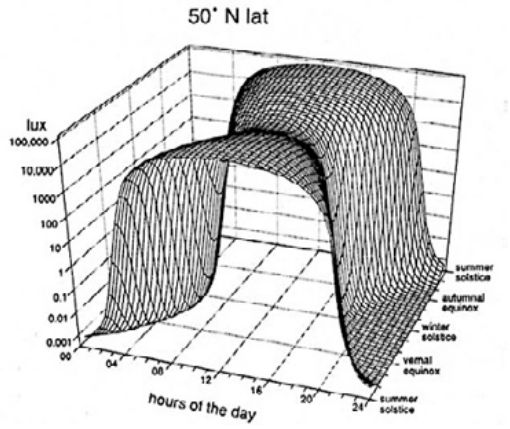
There is also evidence for an influence of daylength on melatonin production in humans, and indeed for effects on human reproduction. In the developed world, with ubiquitous artificial light, we are distanced from natural daylength changes, and in general our seasonality has declined as we control our environment and our fertility. Seasonal affective disorder may provide the best evidence for an effect of daylength on human physiology. However, for the therapeutic use of melatonin in humans —



SUMMER
Long days
Short melatonin profile

WINTER
Short days
Long melatonin profile

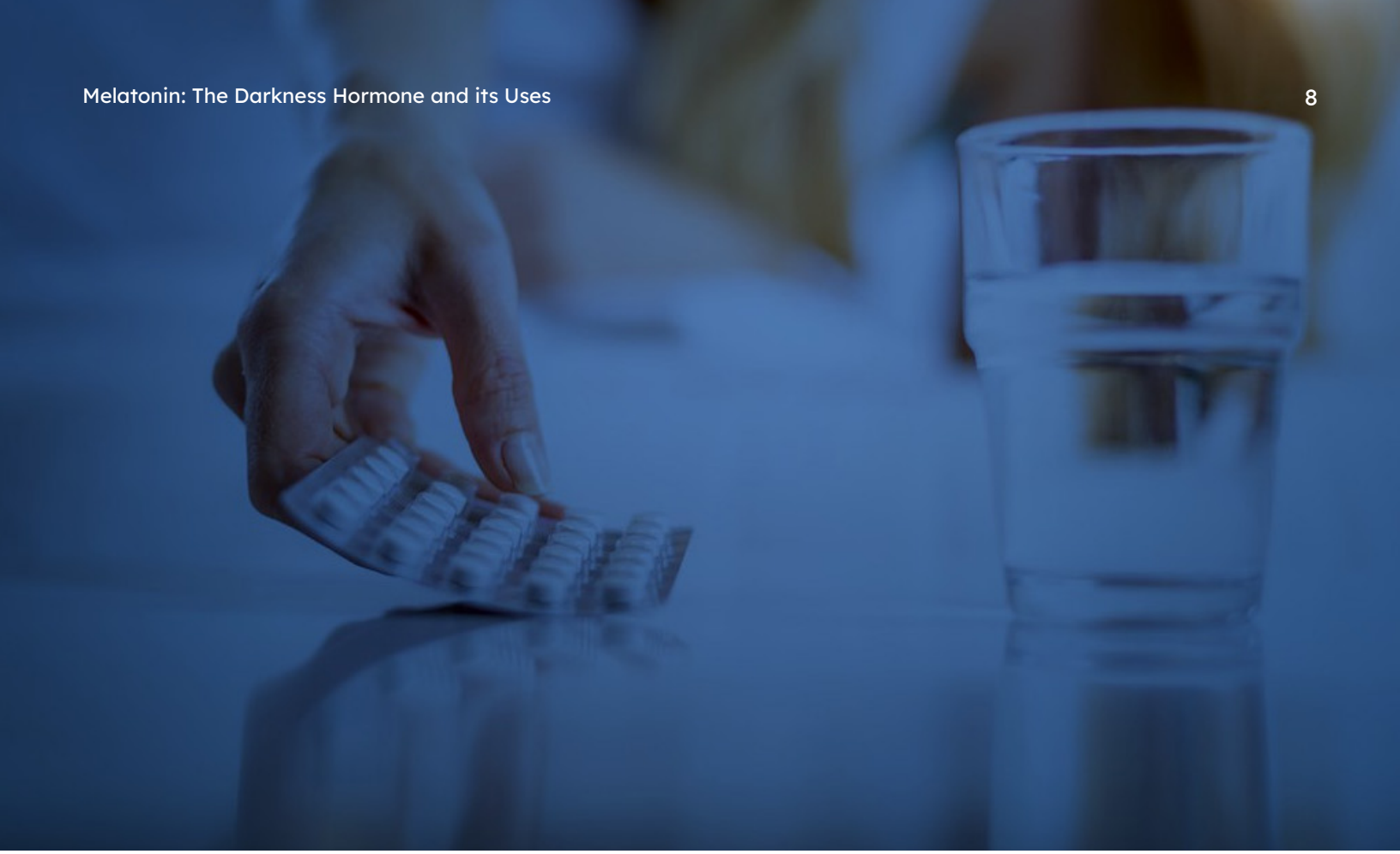
In animals that time seasonal functions by daylength (photoperiod), melatonin provides the timing signal. Melatonin has a higher duration of secretion in winter than in summer, reflecting the change in daylength.




Photoperiod and light intensity calculated at 50 °N latitude over an entire year. (Terman lab, Columbia).

particularly in children — it should never be forgotten that this hormone has profound effects on animal seasonal functions. There is substantial evidence for anti-gonadotrophic effects of high amounts in humans, and an influence on pubertal development is possible, but not yet demonstrated. A serious attempt to develop melatonin as a contraceptive was made some 25 years ago.

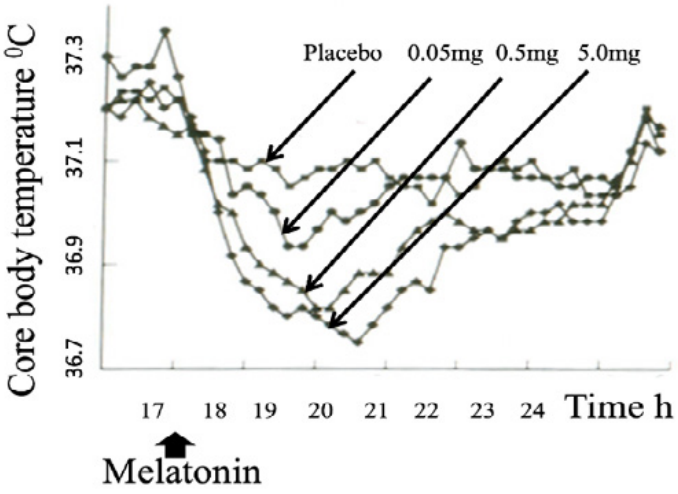
Melatonin treatment indicates darkness in daylength-dependent animals such as sheep and goats.



## CIRCADIAN RHYTHMS

Melatonin is an integral part of the circadian system, but it is not essential to rhythmic functioning. Melatonin is often referred to as a sleep hormone, but in fact it is more correct to call it a darkness hormone. As melatonin rises at night, core body temperature drops, and sleep onset occurs (see Secrets of Falling Asleep. The peak of melatonin is closely associated with the lowest point of the core temperature rhythm and highest sleepiness (see the 2nd graph below, for melatonin, cortisol, thyroid stimulating hormone, and core body temperature).

Treatment with melatonin in the biological evening lowers core temperature and induces sleep. However, it is quite difficult to show major effects of pinealectomy (with removal of circulating melatonin) on circadian rhythms — and even on sleep itself. Removal of the pineal has no effect on sleep in rodents, and also, according to one recent report, on human sleep. However. if pinealectomized animals or melatonin-suppressed humans are subjected to an abrupt phase shift (as in jet lag), they adapt their circadian rhythms faster to the new schedule without melatonin. This suggests that melatonin acts as a brake on adapting to abrupt changes in circadian phase (which are of course undesirable in a natural environment). Ironically, although melatonin treatment is used to hasten circadian adaptation, it is possible that a function of pineal production is to do the opposite. Several investigators have suggested that pineal melatonin acts as a coupling agent across rhythmic systems ("circadian glue"). This fits with the suggested role of melatonin to maintain the status quo.

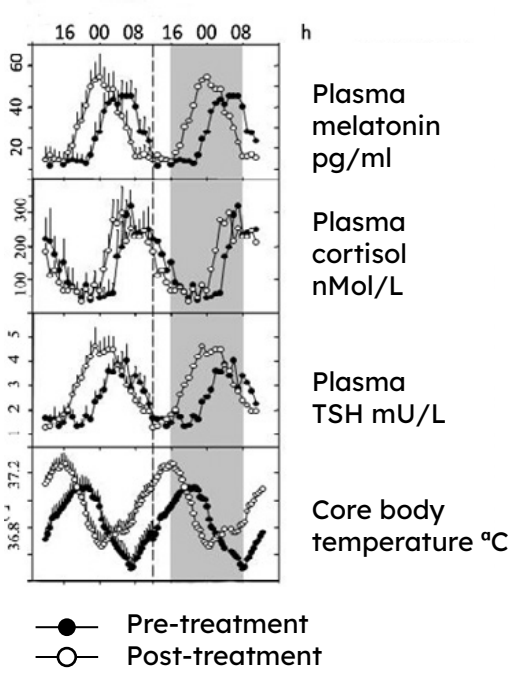



Melatonin lowers core body temperature.

## MELATONIN AS A CHRONOBIOTIC: A DRUG THAT SHIFTS RHYTHMS

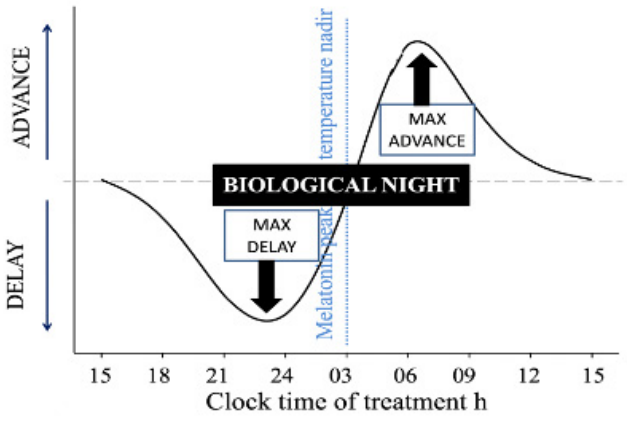
The ability of melatonin treatment to shift seasonal rhythms inspired investigation into its effects on circadian rhythms. There is no doubt that melatonin can shift the phase (timing) of the central brain clock (SCN). This structure can be cultured in a dish. It shows long-term rhythms in electrical activity that can be shifted by timed melatonin treatment.

Timed melatonin treatment in humans phase shifts peripheral SCN-driven rhythms, including its own rhythm, core body temperature, and cortisol. In some respects, it invokes responses opposite to those of light treatment. Taken in small (<5mg) but usually pharmacological (>0.05mg) amounts in the "biological late afternoon," before the evening onset of melatonin secretion, it will advance the circadian system. On the other hand, in the "biological early morning" it will provoke delays. These effects can be formalized as a "phase response curve" (PRC, see diagram). Thus, by knowing the timing of biological night we can judge the time to give either light treatment or melatonin treatment — or indeed a combination of both at opposite times of day — to shift the circadian system to an appropriate phase.

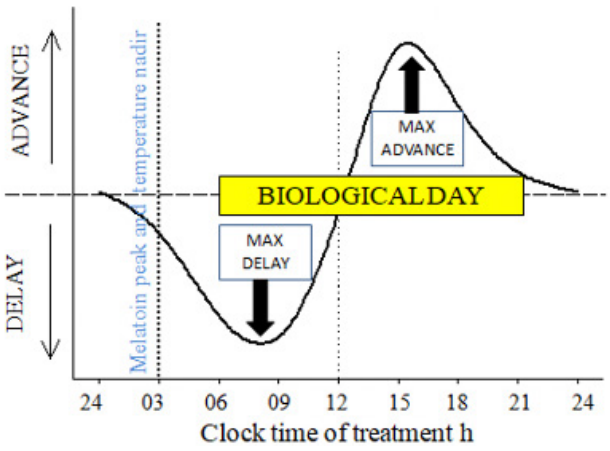



Melatonin 1.5mg daily in the late afternoon shifts all measured circadian rhythms compared with pre-treatment, as shown here, and compared to placebo

Phase response curve to timed light treatment




Phase response curve to timed melatontin treatment



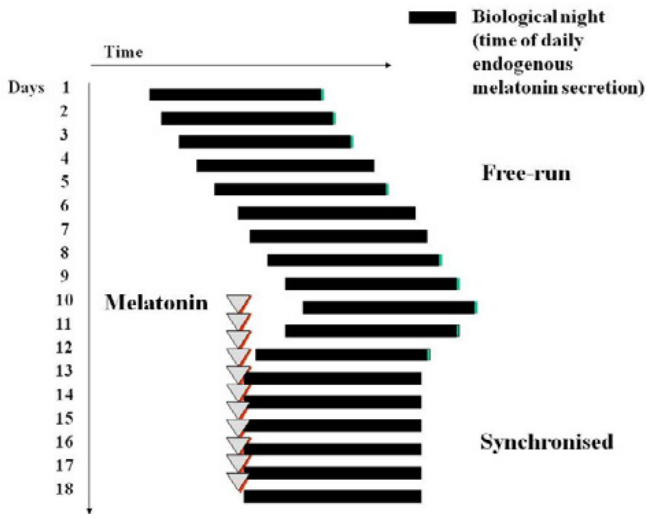

Light and melatonin phase response curves showing the changed timing of the circadian system in response to timed light or melatonin treatment. Biological night is defined as the interval of melatonin secretion.

Clinical situations that can benefit from such treatments, at least in principle, include those with clear circadian abnormalities, such as circadian rhythm sleep disorders (delayed sleep-wake phase disorder, advanced sleep-wake phase disorder, irregular sleep-wake rhythm disorder, non-24h sleep-wake disorder, shift work sleep disorder, and jet lag syndrome); see CET e-book #3. Another group falling into this category are patients with seasonal affective disorder who show an associated circadian rhythm delay or advance. There are likely many other situations where an undetected circadian abnormality exists. Budgetary constraints on research, as well as a lack of recognition regarding the importance of timing, no doubt account for this oversight. A "dipstick" test for circadian abnormality is needed.

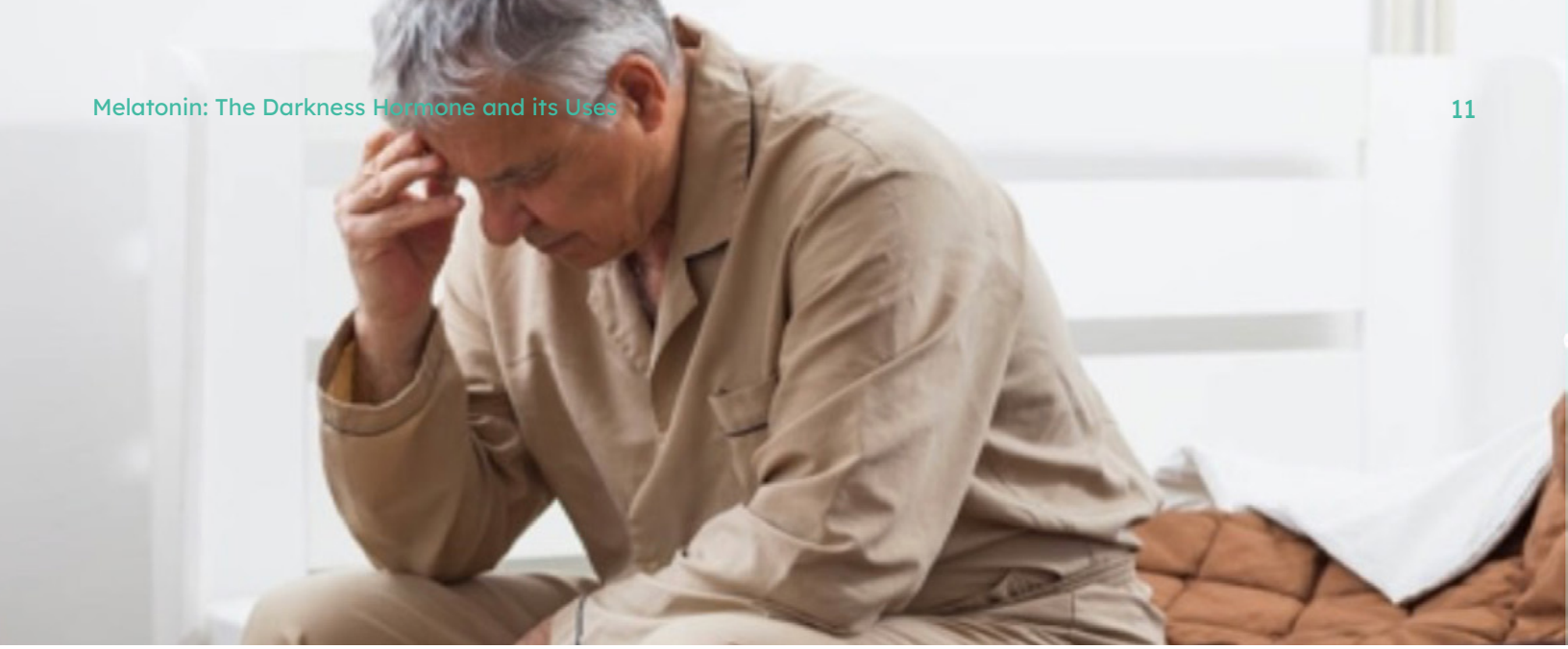
In this context, there is the important case of free-running blind people lacking retinal light perception or signal transmission to the hypothalamus, who experience non - 24h sleep-wake rhythm disorder. In the absence of the light-dark time cue, synchronization by melatonin becomes the treatment of choice. It has proved very successful in several trials. Inevitably, the number of patients studied has been small for this quite rare situation.

The melatonin agonist (a molecule imitating the properties of melatonin) tasimelteon (Hetlioz) was developed specifically for this population and has shown efficacy in a reasonable number of subjects. However, one survey has suggested that conventional hypnotic drugs — which do not work — remain the first line of treatment by general practitioners.

Various literature reviews, including meta-analyses (which combine a set of high- quality studies to derive conclusions) and consensus statements, conclude that melatonin is particularly useful for delayed sleep-wake phase disorder, where timing of treatment is relatively simple. The American Academy of Sleep Medicine recommends its use for circadian rhythm sleep disorders. However, proper timing needs care, dose is still much discussed, and specific formulations need much more research as new formulations continue to be produced. Of particular interest would be delayed release preparations that can be specified for different time zone changes and for advanced sleep-wake phase disorder. Recent data concerning the action of cortisol on peripheral clocks suggest that a combination with melatonin, acting primarily on the central circadian clock in the SCN, would be efficacious.




Melatonin can synchronize free-running rhythms (black bars) in blind and sighted people.



## MELATONIN AND SLEEP

Having discovered melatonin, Aaron Lerner treated himself and first reported its rapid soporific effects when taken during the daytime.

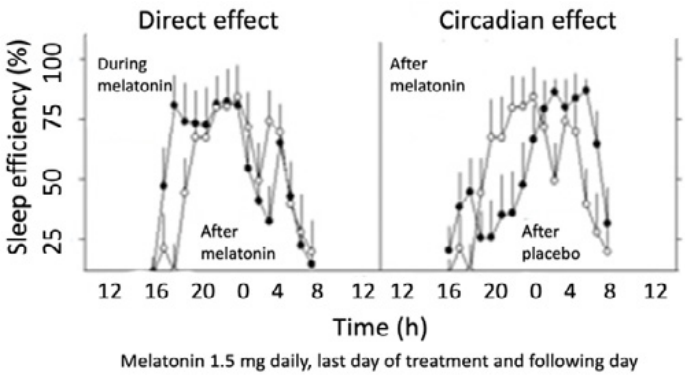
The evolution of sleepiness during the night follows very precisely the profile of melatonin in plasma. Although we can sleep without melatonin (some commonly used drugs such as atenolol suppress melatonin production) there is no doubt that we sleep better when sleep is taken in correct phase with pineal melatonin production and the core body temperature rhythm. The actions of melatonin treatment can be soporific, temperature-lowering, and phase-shifting. If correctly timed, it will both shorten sleep latency and improve sleep quality (and/or duration) by optimizing the timing of sleep in relation to the circadian system.

Development of melatonin formulations and agonists has mainly targeted the "poor sleep" market. As a treatment for nonspecific insomnia, it appears to be quite useful. According to a national health survey published in 2012, some 3,000,000 Americans were using melatonin, with a rising trend.

This popular adoption is likely related to the fact that our circadian rhythms are frequently not in phase in an urban environment, with its insufficient time cues to maintain optimum alignment. In these circumstances most people will delay the circadian system, particularly over the weekend if there is no requirement to get up in the morning. In this way, the social need for sleep takes precedence over the optimum time, and sleep suffers. This has been called "social jet lag." Popping a melatonin pill in the evening has a good chance of lowering core body temperature, increasing sleepiness, and advancing circadian phase to a more appropriate time — and thus, better sleep.

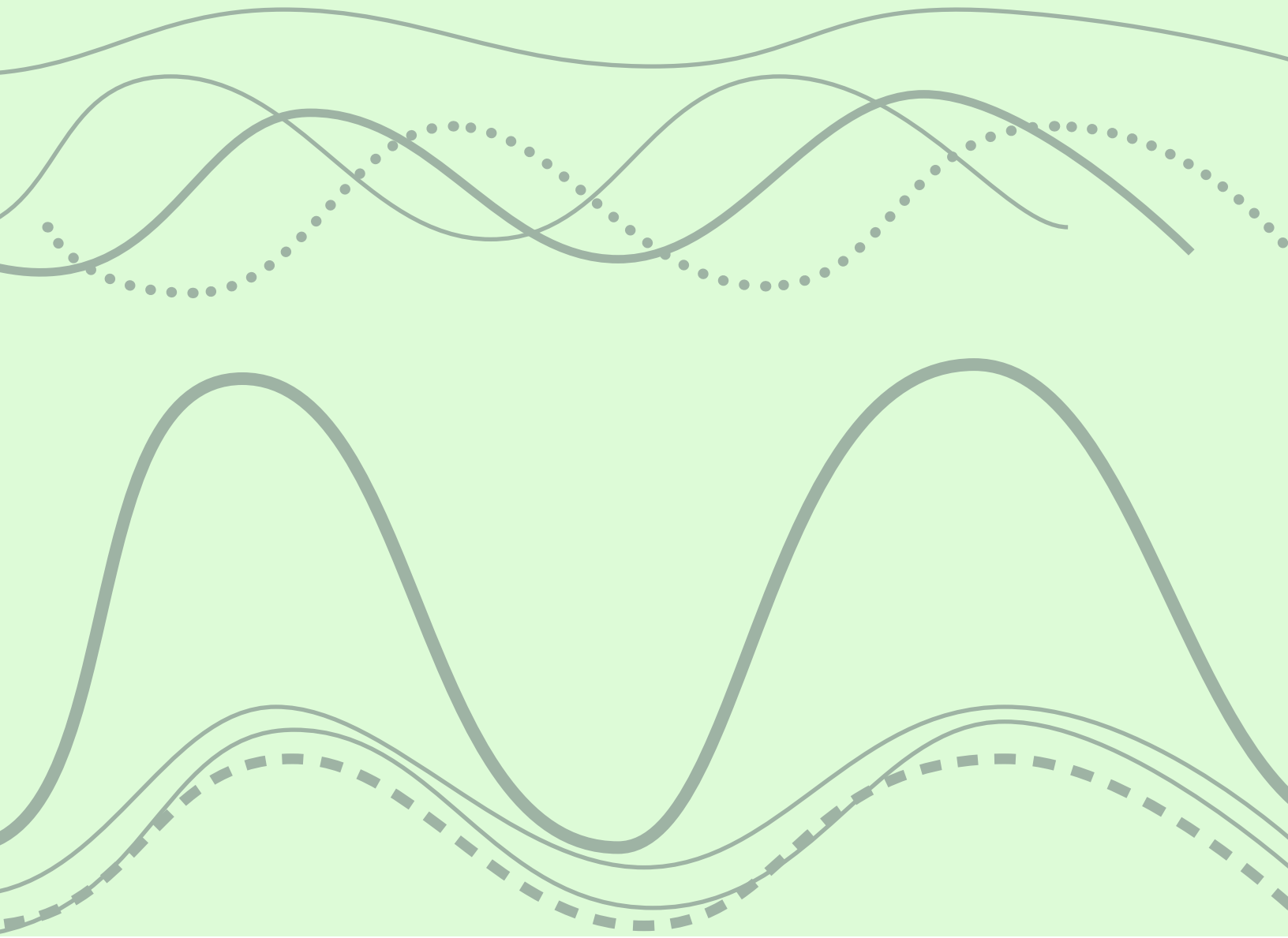
The use of melatonin for sleep in the very elderly and demented has proved somewhat controversial, since lowered mood was recorded in some studies. In the same project, however, extra light was clearly beneficial.

In the UK, the use of melatonin in children has greatly expanded since it was initially applied with success for sleep problems of the neurologically impaired. It is evidently being prescribed off-label, since the only registered preparation is for seniors above age 55.




Melatonin 1.5 mg daily, last day of treatment and following day

Melatonin has both direct and circadian timing effects on sleep. In the left panel, the immediate direct effect of melatonin at 1600h (4 p.m.), shown in black circles, is compared with placebo (white circles). In the right panel, the day after the last dose of melatonin or placebo, the underlying circadian phase advance is seen (white circles) after melatonin compared with after placebo (black circles.)



An assortment of circadian oscillations: in and out of synchrony, low-to-high amplitude.

## CAVEATS

There is real concern about what appears to be indiscriminate use of melatonin in many situations where its value has not been properly assessed. Although there is a large amount of information concerning neuroprotection, antioxidant effects, anti-cancer effects, immunostimulation, relation to glucose metabolism, influence on type 2 diabetes, reduction of blood pressure, and others, no clear clinical consensus as to its usefulness has emerged except with respect to sleep and circadian rhythms. Two recent comprehensive umbrella reviews (covering already published meta-analyses and narrative reviews) provide a good overview of trials in the literature. One, looking at melatonin and general health outcomes, finds significant effects on sleep latency and duration, anxiety and agitation, and an association of low melatonin with breast cancer. It concludes that melatonin is probably beneficial to human health. The second review evaluated trials of melatonin in primary and co-morbid insomnia disorders. Although it finds evidence for improvement in sleep latency and duration, there was no consensus as to whether these effects were clinically meaningful.

Let me conclude by emphasizing that timed treatment with low doses of melatonin is good at changing the timing of sleep, in association with lowering of core body temperature, especially if an advance shift of the internal clock is implicated. Repositioning the clock to an optimum phase at desired sleep time, together with the rapid soporific effect, explains the benefits of melatonin for sleep. And the improvement of sleep has itself a multitude of health benefits.

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## About us

The Center for Environmental Therapeutics is a 501(c)(3) nonprofit based in New York, founded in 1994 in response to international interest in new environmental therapies – drug-free ways to improve mood sleep, and energy. We are leaders in the research and development of light therapies as counterparts to conventional medications. Our program serves health care providers, the consumer public, and industry. CET is made up of a multidisciplinary team of eminent researchers and clinicians committed to pooling their efforts toward the development of effective environmental therapies. We host a popular website, cet.org, with educational material for the general public and clinicians; online, personalized self-assessments of depressive disorders, symptom severity, and circadian rhythm status; and an extensive question library based on inquiries from the public, which offers guidance from academic and clinical experts.

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