

Institute of Biomedicine  
Department of Physiology  
and  
Hospital for Children and Adolescents  
Neurology

University of Helsinki  
Helsinki, Finland

# **THE IMPACT OF LIGHT ON THE SECRETION OF MELATONIN IN HUMANS**

**Taina Hätönen**

ACADEMIC DISSERTATION

*To be presented, with the permission of the Faculty of Medicine of the University of Helsinki,  
for public discussion in the auditorium of the Institute of Biomedicine, Department of  
Physiology (Siltavuorenpenger 20 J), Helsinki, on April 28th, 2000, at 12 noon.*

**Helsinki 2000**

**Supervisors:**

Docent Maija-Liisa Laakso  
Institute of Biomedicine  
Department of Physiology  
University of Helsinki  
Helsinki, Finland

Professor Pirkko Santavuori  
Hospital for Children and Adolescents  
Neurology  
University of Helsinki  
Helsinki, Finland

**Reviewers:**

Research Professor Mikko Härmä  
Finnish Institute of Occupational Health  
Helsinki, Finland

Docent Markku Partinen  
Haaga Neurological Research Centre  
Department of Clinical Neurosciences  
University of Helsinki  
Helsinki, Finland

**Official opponent:**

Professor Juhani Leppäluoto  
Institute of Physiology  
University of Oulu  
Oulu, Finland

ISBN 951-45-9171-2 (PDF version)  
<http://ethesis.helsinki.fi/>

Helsingin yliopiston verkkojulkaisut  
Helsinki 2000

# TABLE OF CONTENTS

<b>LIST OF ORIGINAL PUBLICATIONS .....</b>	<b>5</b>
<b>ABBREVIATIONS .....</b>	<b>6</b>
<b>SUMMARY .....</b>	<b>7</b>
<b>INTRODUCTION .....</b>	<b>8</b>
<b>REVIEW OF THE LITERATURE .....</b>	<b>9</b>
CIRCADIAN RHYTHMS .....	9
CIRCADIAN TIMING SYSTEM .....	10
Input pathways .....	10
Evidence for the pacemaker in the suprachiasmatic nucleus .....	11
Mechanisms of the pacemaker in the suprachiasmatic nucleus .....	11
Output pathways .....	12
CIRCADIAN PHOTORECEPTION .....	13
Retinal photoreception .....	13
Extraocular photoreception .....	14
LIGHT AND THE DAILY RHYTHMS .....	15
Light as a synchronizer of the circadian timing system .....	15
MELATONIN AND THE PINEAL GLAND .....	19
The regulation of pineal melatonin .....	21
Effects of light on melatonin .....	22
Melatonin as a circadian effector .....	24
Other effects of melatonin .....	26
Extrapineal melatonin .....	27
BLINDNESS AND CIRCADIAN RHYTHMS .....	27
Patients with neuronal ceroid lipofuscinosis and blindness .....	28
PRACTICAL CONSIDERATIONS OF LIGHT AND MELATONIN .....	31
Circadian rhythm sleep disorders .....	32
Seasonal affective disorder .....	33
Conclusions of light and melatonin treatment .....	34
<b>AIMS OF THE STUDY .....</b>	<b>35</b>

<b>MATERIALS AND METHODS</b> .....	<b>36</b>
ETHICAL CONSIDERATIONS .....	36
SUBJECTS AND GENERAL PROCEDURES .....	36
Suppression of melatonin without conscious light perception .....	36
Suppression of melatonin through closed eyelids .....	36
Phase shift of melatonin rhythm by 500-lux light .....	37
Phase shift of melatonin rhythm by light with concomitant exogenous melatonin administration .....	37
LIGHT SOURCES AND EXPOSURE PROCEDURES .....	38
MEASUREMENT OF MELATONIN AND CHARACTERIZATION OF THE PATTERNS .....	38
Radioimmunoassay .....	38
Calculations of individual phase marker times and phase shifts .....	39
STATISTICS .....	39
<b>RESULTS</b> .....	<b>40</b>
SUPPRESSING EFFECT OF LIGHT ON MELATONIN SECRETION .....	40
Bright light exposure in patients without conscious light perception .....	40
Bright light exposure in subjects with closed eyelids .....	40
PHASE SHIFTING EFFECT OF LIGHT ON MELATONIN RHYTHM .....	41
Phase shift of melatonin rhythm by moderate illuminance .....	41
Phase shift of melatonin rhythm by bright light with concomitant exogenous melatonin administration .....	41
<b>DISCUSSION</b> .....	<b>43</b>
SUPPRESSING EFFECT OF LIGHT ON MELATONIN SECRETION .....	43
Bright light exposure in patients without conscious light perception .....	43
Bright light exposure in subjects with closed eyelids .....	45
PHASE SHIFTING EFFECT OF LIGHT ON MELATONIN RHYTHM .....	47
Phase shift of melatonin rhythm by moderate illuminance .....	47
Phase shift of melatonin rhythm by bright light with concomitant exogenous melatonin administration .....	48
<b>CONCLUSIONS</b> .....	<b>50</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>51</b>
<b>REFERENCES</b> .....	<b>52</b>

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which will be referred to in the text by their Roman numerals I-IV.

- I) Hätönen T, Laakso M-L, Heiskala H, Alila-Johansson A, Sainio K & Santavuori P. Bright light suppresses melatonin in blind patients with neuronal ceroid-lipofuscinoses. *Neurology* 1998;50:1445-1450.
  
- II) Hätönen T, Alila-Johansson A, Mustanoja S & Laakso M-L. Suppression of melatonin by 2000-lux light in humans with closed eyelids. *Biological Psychiatry* 1999;46:827-831.
  
- III) Laakso M-L, Hätönen T, Stenberg D, Alila A & Smith S. One-hour exposure to moderate illuminance (500 lx) shifts the human melatonin rhythm. *Journal of Pineal Research* 1993;15: 21-26.
  
- IV) Hätönen T, Alila A & Laakso M-L. Exogenous melatonin fails to counteract the light-induced phase delay of human melatonin rhythm. *Brain Research* 1996;710:125-130.

## ABBREVIATIONS

ANOVA	analysis of variance
cAMP	cyclic adenosine 3',5'-monophosphate
<i>CLNI-8</i>	neuronal ceroid lipofuscinosis gene symbols 1-8
EEG	electroencephalogram
ERG	electroretinogram
IGL	intergeniculate leaflet
INCL	infantile neuronal ceroid lipofuscinosis
JNCL	juvenile neuronal ceroid lipofuscinosis
LD	light-dark
MRI	magnetic resonance imaging
NAT	<i>N</i> -acetyltransferase
NCL	neuronal ceroid lipofuscinosis
PRC	phase response curve
PVN	paraventricular nucleus
RHT	retinohypothalamic tract
SAD	seasonal affective disorder
SCN	suprachiasmatic nucleus
SD	standard deviation
VEP	visual evoked potential

## SUMMARY

The machinery that generates the daily temporal organization of the body functions is collectively known as the circadian clock and the output of the clock is called circadian rhythms. The clock generally requires daily resetting by external time cues. The dominant synchronizing signal for the rhythmicity is provided by the light-dark cycles of a 24-hour solar day. One of the most clearly fluctuating functions is melatonin secretion from the pineal gland. Photic information is transmitted as multisynaptic neural inputs by the central nervous system *via* peripheral nerves to the pineal gland. Melatonin is secreted rhythmically with increased synthesis during the dark period of the day.

Light has dual effects on melatonin secretion: it decreases melatonin levels acutely and shifts the phase of the melatonin rhythm. In the present investigations, light-induced changes in the level and rhythm of melatonin were investigated in order to examine the importance of light in the regulation of human circadian rhythms.

In the first study, a bright light exposure was found effective in suppressing melatonin secretion in blind neuronal ceroid lipofuscinosis patients with degenerated retinas. This result indicates that an intact retina is not required for the transmission of light to the hypothalamus or for the regulation of neuroendocrine functions.

In the second study, a bright light exposure induced a decrease in melatonin concentrations only in a minority of volunteers with closed eyelids. Together with other studies, this finding suggests that the eyes are in a dominant role in the transduction of photic information to the circadian system, and closed eyelids (*e.g.* during sleep) can significantly limit the hypothalamic effects of light.

In the third study, a bright light pulse of relatively low intensity and short duration induced an interruption of melatonin secretion and shifted the phase of the melatonin rhythm. Thus, melatonin acts as a sensitive marker of environmental lighting even in artificial indoor conditions.

In the fourth study, a bright light pulse during the rising phase of the melatonin synthesis delayed the melatonin rhythm irrespective of concomitant placebo or melatonin administration. This indicates that suppression of melatonin is not essential for the phase shifting mechanisms of light.

Not long ago it was thought that light had no impact on the circadian rhythms in humans. The present findings together with other studies indicate that light is the primary external cue regulating our circadian functions. Melatonin acts as a reliable neuroendocrine messenger of environmental lighting conditions, but the phase shifting effect of light on circadian rhythms can be independent of the melatonin concentration in the circulation.

## INTRODUCTION

All biological functions, in cells, tissues, organs and the entire body, run on a cycle of alternating activity and rest. In the world of science, this relatively new discipline is termed chronobiology, meaning that time-related events shape our daily physiological responses.

The rhythms of biological functions with a period of approximately 24 hours are called circadian rhythms (Latin, *circa*=about, *dies*=a day). Most of these rhythms are under the control of an internal clock. This timepiece is affected by events in the environment, and especially by the light-dark cycle.

Besides its function in vision, light has a fascinating role in the modulation of the circadian rhythms. One of the well-known action of light is its dual effects on pineal neurohormone melatonin synthesis: light decreases melatonin levels acutely and shifts the phase of the melatonin rhythm.

Researchers all over the world try eagerly to find out the physiological importance of melatonin in the human body. In mass media, melatonin has been introduced as a life-giving miracle agent. At the moment, indications for melatonin treatment are lacking. The long-term adverse effects of exogenous melatonin are not known, and its use has not been proven safe by usual clinical trial criteria either.

The best-described action of melatonin is its function as a circadian marker and chemical expression of darkness. Light and melatonin may serve complementary effects: light provides a daytime and melatonin a nighttime signal to an organism. Indeed, light could be an exogenous timing agent and melatonin an endogenous one. However, there is still a lot to be learned about melatonin's role in neuroendocrine phototransduction and its interaction with light and dark alterations in humans.

The topic of this thesis was chosen with physiological aspects of light and melatonin in view. The present series of investigations focuses on the regulation of human melatonin by light, and light-induced changes in the secretion of the hormone were performed in order to shed light on the mechanisms underlying this phenomenon.

# REVIEW OF THE LITERATURE

## CIRCADIAN RHYTHMS

The French astronomer Jean Jacques d'Ortous de Mairan discovered in 1729 that when a *Mimosa* plant was placed in the constant darkness of a closet, the opening and closing of the leaves still occurred on the basis of an intrinsic rhythm (de Mairan 1729, referred by Meijer & Rietveld 1989). Thus, he pointed out for the first time the independence of biological rhythms of the light-dark cycle.

All organisms generally have the capacity for endogenous temporal organization of biological processes over the course of day. The cellular, neural and humoral machinery that generates this ability is collectively known as the biological clock. This timing device functions as a pacemaker of a complex oscillation network. The endogenous period of the clock is not exactly 24 hours. Thus, the clock generally requires daily resetting by external time cues. This process is called entrainment.

The output of the pacemaker is called circadian rhythms. These rhythms have two principal properties: they are normally entrained to external time cues, but in the absence of synchronizing signals, they free-run with a period slightly different from 24 hours (Aschoff 1965a). The light-dark (LD) cycle of a 24-hour solar day is the main environmental cue entraining the clock and the rhythms driven by it.

The existence of a circadian or daily rhythm is apparent in physiological, pharmacological and pathological events and parameters. These include physical activity, body temperature, plasma levels of hormones (*e.g.* cortisol, renin, angiotensin, aldosterone, noradrenaline, insulin, prolactin, growth hormone, thyrotropin, atrial natriuretic peptide, vasopressin), response to a glucose-tolerance test, performance variables (*e.g.* reaction time, reading error, subjective alertness, working memory speed, self-chosen work-rate), birth and death rate, pharmacokinetics and effects of drugs, and risk for cardio- and cerebrovascular attacks (Wever 1979, Reilly *et al.* 1997, Schwartz 1997, Lemmer 1999). Moreover, various functions of the heart and circulation (*e.g.* cardiac output, heart rate, blood pressure), of the respiratory system (*e.g.* minute volume, oxygen consumption, carbon dioxide production) and of the kidneys (*e.g.* glomerular filtration rate, urine flow rate, electrolyte excretion) can vary with the time of day (Wever 1979, Reilly *et al.* 1997).

In addition to the action of light on the period of the circadian rhythms, light exposures are able to affect the phase of the rhythms. There are three possible effects: no shift, delay, or advance. The magnitude and direction of the phase shift depend on the phase of the rhythm at the time of stimulus application. This relationship can be illustrated by a phase-response curve (Aschoff 1965b).

Temporal regulation of behavioral and internal homeostatic events is a fundamental feature of mammalian adaptation. It helps to optimize the economy of biological systems, and prepares an organism to foresee and cope with the 24-hour solar day resulting from the rotation of our planet. Although normally the biological clock and the rhythms driven by it are entrained by environmental time cues, in certain situations the rhythms are not synchronized with the time cues (external desynchronization) or with each other (internal desynchronization). For example, time zone transitions, night work or blindness may cause deficient entrainment and desynchronized circadian rhythms, namely circadian disorders.

## CIRCADIAN TIMING SYSTEM

In vertebrates, the master biological clock system is located in the hypothalamic suprachiasmatic nucleus (SCN). The paired nucleus consists of thousands of neurons, for instance in rats of about 8000 neurons occupying 0.07 mm<sup>3</sup> and in humans of about 10 000 neurons occupying 0.25 mm<sup>3</sup> (van den Pol 1980, Swaab *et al.* 1993, Hastings 1997). In humans, the SCN is situated on either side of the brain midline on the top of the optic chiasma and about 3 cm behind the eyes in the basal part of the anterior hypothalamus. The SCN is involved in the generation and expression of physiological functions with circadian properties. These include *e.g.* water and food intake, motor activity, sleep-wake rhythm, corticosterone release, activity of pineal *N*-acetyltransferase enzyme, and body temperature (reviewed by Rusak & Zucker 1979). In addition, lesions of the SCN abolish the nocturnal increase of melatonin synthesis (Reppert *et al.* 1981, Lehman *et al.* 1984, Kalsbeek *et al.* 1996).

The circadian timing system consists of three essential components: entrainment pathways (inputs), oscillation mechanisms generating the rhythmicity (pacemaker) and efferent pathways mediating the expression of circadian rhythms (output).

### Input pathways

The best-described entrainment pathways to the SCN originate in retinal ganglion cells. The retinohypothalamic tract (RHT) is the main route responsible for photoentrainment (Hendricson *et al.* 1972, Moore & Lenn 1972). The RHT originates from a distinct and homogenous group of ganglion cells (Moore *et al.* 1995). It leaves the optic nerve mainly in the anterior part of the optic chiasm and terminates predominantly within the ventrolateral part of the SCN (Levine *et al.* 1991, Speh & Moore 1993, Reuss 1996). It has been shown that the RHT has several components; the largest projects to the SCN, but there are additional projections to the lateral and anterior hypothalamic area, and to the retrochiasmatic area which is a complex zone immediately caudal to the SCN (Johnson *et al.* 1988b). Glutamate appears to be the main transmitter of the RHT (de Vries *et al.* 1993, Shirakawa & Moore 1994, Mintz *et al.* 1999).

The retinal pathway responsible for the projection of the circadian system to the SCN branches off the projection to the visual centers of the brain (Klein & Moore 1979, Moore *et al.* 1995). In fact, the section of visual pathways beyond the RHT does not affect stable entrainment (Klein & Moore 1979), whereas ablation of the RHT (Johnson *et al.* 1988a) or blinding (Wurtman *et al.* 1964, Klein & Weller 1970, Klein & Weller 1972, Nelson & Zucker 1981, Lucas & Foster 1999) results in the loss of the ability of light to entrain circadian rhythmicity.

In addition to the RHT, the SCN also receives input from the intergeniculate leaflet (IGL), raphe nuclei, the paraventricular thalamus and the limbic telencephalon (reviewed by Moga & Moore 1997). The pathway *via* the IGL (Pickard 1985) of the lateral geniculate complex of the thalamus through the geniculohypothalamic tract (Rusak *et al.* 1993, Moore & Card 1994) to the SCN is suggested to modulate photic and nonphotic information to the circadian system (Johnson *et al.* 1989, Moore & Card 1994, Moga & Moore 1997). In this tract, gamma-aminobutyric acid, neuropeptide Y and enkephalin are likely candidates for being the chemical transmitters (Moore & Speh 1993, Moga & Moore 1997). Midbrain raphe nuclei also project to the SCN (Rusak *et al.* 1993, Miller *et al.* 1996) and serotonergic input from these nuclei is suggested to play a role in the circadian system, but not to

be primarily involved in the entrainment to the LD cycle (Meijer & Rietveld 1989, Meyer-Bernstein & Morin 1999). The specific functions of the thalamus, the limbic region and other unexplored tracts in the circadian timing machine await clarifying.

### **Evidence for the pacemaker in the suprachiasmatic nucleus**

The late Curt Richter deserves the credit for pinpointing the hypothalamus as the home of the biological clock, even though he did not specifically identify the SCN. Richter (1967) investigated the function of the hypothalamus by making lesions, and he discovered that lesions in the region of the ventral median nucleus eliminated eating and drinking rhythms in rats.

The SCN seems not to be just a simple hourglass timer directly driven by external signals. In addition to the SCN being the major site for the RHT input, there are five lines of evidence indicating that the SCN acts as a circadian pacemaker. First, ablation of the SCN eliminates circadian functioning as shown originally by two teams of investigators working independently. Moore and Eichler (1972) found that SCN lesions destroyed circadian adrenal corticosterone rhythms in the rat, and that in the absence of the SCN not even the preservation of the optic tracts was sufficient to maintain synchronization. In the same year, Stephan and Zucker (1972) demonstrated that electrolytic lesions in the SCN permanently eliminated circadian rhythms of drinking behavior and locomotor activity of the rats.

The second line of evidence suggesting a pacemaker function for the SCN comes from the studies in which circadian functioning was found to be maintained in isolated SCN, both *in vivo* (Inouye & Kawamura 1979) and *in vitro* (Shibata & Moore 1988). In addition, Green and Gillette (1982) showed that single cells, removed from the SCN and put in culture, could persist in their rhythms for up to 60 hours.

Third, electrical stimulation of the SCN in hamsters and rats resulted in phase-dependent shifts of the free-running activity cycle (Rusak & Groos 1982). Phase-shifting effects are also observed after local stimulation of the SCN with a cholinergic agonist, glutamate, or a glutamate agonist (Zatz & Herkenham 1981, Meijer *et al.* 1988, Mintz & Albers 1997).

The fourth line of evidence for the role of the SCN as a pacemaker is provided by the findings on the circadian rhythm of metabolic activity in the SCN. Indeed, in the absence of periodic environmental light cues, glucose utilization of the nucleus was found to be high during the subjective day and low during the subjective night (Schwartz *et al.* 1980, Reppert & Schwartz 1984).

Fifth, transplantation of the fetal anterior hypothalamus containing the SCN into the third ventricle of arrhythmic, SCN-lesioned animals was demonstrated to restore rhythmicity (Lehman *et al.* 1987); the period of the restored rhythm being determined by the graft (Ralph *et al.* 1990).

### **Mechanisms of the pacemaker in the suprachiasmatic nucleus**

There is no point having a watch unless it keeps time. The timekeeping mechanisms underlying the oscillating action of the SCN have been the focus of many recent studies. To date, molecular clock models of similar structure have been proposed for cyanobacteria, fungi and animals, but the key

proteins for these models do not share sequence similarity (Dunlap 1999). Thus, it appears likely that the clock systems have developed independently in different organisms, and by alternating environment natural selection has apparently led to the evolvement of clock systems which have only some physiological properties in common (reviewed by Kondo & Ishiura 1999).

The fruit fly *Drosophila* has autonomous circadian clocks throughout the body (Plautz *et al.* 1997), suggesting that individual cells are capable of supporting their own independent clocks. In mammals, however, experimental findings suggest that SCN neurons are born as circadian oscillators coupled within the nucleus to form a complex network pacemaker (Moore & Bernstein 1989). Support for this hypothesis is provided by the observations that the SCN contains many autonomous, single-cell circadian oscillators (Welsh *et al.* 1995), that isolated glands and pieces of tissue can continue to show circadian oscillations in culture (reviewed by Takahashi & Zatz 1982) and that various rhythms of an organism sometimes free-run with different circadian periods during constant environmental conditions (Wever 1979, Turek *et al.* 1982). In addition, this hypothesis of a multioscillator system is supported by the report of Moore-Ede (1983) as well as by the study of Illnerová and Vanecek (1982), indicating a two-oscillator pacemaking system with an evening oscillator coupled to dusk and a morning oscillator coupled to dawn.

Indeed, the light-induced immediate increase in the multiple unit activity of SCN neurons was found to be independent of the timing of the exposure (Inouye 1984), whereas the shift of the activity of the neurons was found to be depend on the timing of the exposure (Inouye & Kawamura 1982). Thus, while the direct effect of a light stimulus at the input side of the SCN shows no circadian variation, the output of the SCN shows circadian variation, indicating that the state and internal process of the pacemaker dictate the outcome of the circadian event.

In addition, researchers are trying to assign molecular clock components by investigating different genes involved in or capable of affecting the operation of the biological clock. Until 1997, clock researchers had only three clock components to work on: two proteins from a fruit fly (*Drosophila*) and one from a bread mold (*Neurospora*) (reviewed by Dunlap 1999). Takahashi and coworkers (Antoch *et al.* 1997, King *et al.* 1997) demonstrated the existence of the first mammalian clock gene, *Clock*, from mice. In addition, common elements of oscillation are likely to be present up and down the evolutionary tree. For instance, in the mouse there are three different gene relatives (*Per1*, *Per2* and *Per3*) that are related to the *Drosophila per* gene (reviewed by Dunlap 1999). Transcription of clock genes and synthesis of the proteins they encode form the basis of the timekeeping function. Interactions among these proteins result in feedback inhibition of gene transcription. With degradation of the protein products, gene transcription is again initiated to reestablish the cycle (Dunlap 1999). The major elements of the cycle have been identified, but exact functional roles of the molecular components await revealers.

## **Output pathways**

The function of the different projections of the pacemaker and transmitters communicating circadian information to the rest of the brain is not well understood. In addition, the morphological organization of SCN projections is rather difficult to study because of technical difficulties combined with the very small size of the SCN. The human SCN can be apportioned into five chemoarchitectonic subdivisions of different kinds of neurons (Mai *et al.* 1991); vasopressin and vasoactive intestinal polypeptide containing neurons are the common ones in the human SCN (Hofman *et al.* 1996, Dai *et al.* 1997).

Efferent projections leave the SCN *via* ventrocaudal, lateral, dorsal and rostral pathways (Stephan *et al.* 1981) and many of these projections terminate in other hypothalamic nuclei as well as in the thalamus and midbrain (Berk & Finkelstein 1981) in rats. On the basis of immunological studies, there is general agreement that the SCN project to a number sites in the basomedial hypothalamus and the midline thalamus in rodents (Watts *et al.* 1987, Watts & Swanson 1987, Kalsbeek *et al.* 1993). In humans, the efferent projections of the SCN appear to be comparable to those described in rats and hamsters (Dai *et al.* 1998). The human SCN was found to be connected with the nuclei in the hypothalamus that are involved in hormone secretion, cardiovascular regulation and integration of autonomic information. It is suggested that through these projections the SCN may influence brain areas that regulate *e.g.* thirst, food intake, metabolism, sleep, sexual behavior, and body temperature (reviewed by Dai *et al.* 1998).

The best-described efferent pathway of the SCN emerges from the pineal gland. This route from the SCN runs *via* the paraventricular nucleus (PVN) of the hypothalamus, the intermediolateral cell column of the spinal cord, the superior cervical ganglion, and sympathetic efferents to the pineal gland (Moore 1996).

## **CIRCADIAN PHOTORECEPTION**

The visual and circadian light detection systems have been shown to differ from each other (Chase *et al.* 1969, Frost *et al.* 1979). Eyes provide the primary source of circadian photoreception because eye loss in both humans and other mammals abolishes photoentrainment (Wurtman *et al.* 1964, Klein & Weller 1970, Klein & Weller 1972, Nelson & Zucker 1981, Lucas & Foster 1999). However, the nature of the photoreceptors that provide input from the retina to the circadian timekeeping system remains elusive.

### **Retinal photoreception**

Originally, orthodox photoreceptor cells, *i.e.* retinal rods and cones capturing light for vision, were thought to represent also the photoreceptive elements in light-to-circadian-clock transmission. The light sensitivity of the mammalian pineal gland has been demonstrated with single cell recordings to be contributed by both rods and cones (Thiele & Meissl 1987). On the other hand, it was suggested that a rhodopsin-like pigment mediate circadian vision, because both the spectral sensitivity of the phase-shifting effect and the suppression of pineal *N*-acetyltransferase enzyme by light resemble the absorption spectrum of the pigment (Takahashi *et al.* 1984, Bronstein *et al.* 1987). These studies are in line with the study on the wavelength sensitivity of melatonin suppression in humans (Brainard *et al.* 1985) reporting that rods arbitrate the effects of light in the mammalian circadian timing system.

More recently, studies in strains of mice with hereditary retinal degeneration suggest that the hypothalamic effects of light might be mediated rather by the remaining fragments of cone photoreceptors than by rods (Provencio *et al.* 1994, Lucas & Foster 1999). In a study by Rutkowska and colleagues (1998), the rhythm of core temperature in color-deficient subjects was found to be phase-delayed as compared with normal sighted subjects, suggesting a role of retinal cones in the mediation information between environment and the circadian clock. On the other hand, in aged mice, circadian responses to light do not correlate with the number of surviving cones (Provencio *et al.*

1994), and at least in humans, a normal trichromatic visual system is not necessary for light-mediated melatonin regulation (Ruberg *et al.* 1996).

It has been suggested that there may be some unidentified retinal light-sensitive cells that send information to the lower brain centers (Foster *et al.* 1993, Provencio *et al.* 1994, Huerta *et al.* 1999, Lucas & Foster 1999). In a strain of retinally degenerate mice with remaining but high-threshold circadian responses to light, the spectral sensitivity of the phase-shifting effect was different from the sensitivity of sighted animals, indicating that various photopigments may be involved in the response (Yoshimura & Ebihara 1996). More recently, it has been shown that neither rods nor cones are required for light-induced melatonin suppression (Lucas *et al.* 1999) or for photoentrainment of wheel-running activity (Freedman *et al.* 1999) in mice. In addition, Provencio and coworkers (2000) have identified a novel human opsin which is expressed in cells of the inner retina, but not in retinal photoreceptor cells involved in image formation. The question remains whether there are still uncharacterized photoreceptors in the retina for the transduction of light stimulus to the circadian system.

Besides the studies conducted on opsin/retinal-based photopigments, the possible involvement of cryptochrome blue-light photoreceptors in circadian photic responses has recently become a topic of interest. Cryptochrome proteins are light-sensitive, putative vitamin-B2 based pigments, and cryptochromes 1 and 2 are expressed in the mouse retina and SCN (Miyamoto & Sancar 1998). The observation that the expression of cryptochrome 1 gene exhibits circadian oscillations in the mice SCN (Miyamoto & Sancar 1998) proposes that cryptochromes have a role in circadian photoreception in mammals. Recently, it was shown that the mammalian cryptochrome 1 and 2 are essential for the circadian clockwork (van der Horst *et al.* 1999). However, the cryptochrome 1 and 2 genes are not essential for the light-induced phase shifting of the clock (Thresher *et al.* 1998, Okamura *et al.* 1999).

### **Extraocular photoreception**

Extraocular photoreceptors are capable of providing sufficiently light to the circadian timing system in nonmammalian vertebrates (Underwood & Groos 1982, Yoshikawa & Oishi 1998), and some researchers have also demonstrated nonocular photoreception in mammals. For instance, the neonatal rat pineal has been shown to be photosensitive (Blackshaw & Snyder 1997) and extraretinal mechanisms are reported to mediate light-induced changes in the regulation of pineal *N*-acetyltransferase enzyme in newborn rats blinded by bilateral orbital enucleation (Torres & Lytle 1989). In addition, other investigators have obtained evidence suggesting that light can directly affect hypothalamic neurons in enucleate adult rats (Lisk & Kannwischer 1964).

In 1998, a surprising finding about nonocular phototransduction in humans was reported. The results of this study suggested that a bright light exposure to the back of the knee could phase shift human body temperature and melatonin secretion rhythms without any transmission of light through the eyes (Campbell & Murphy 1998). A photosensitive property of the skin has also been proposed by the finding that the LD alterations synchronize melatonin levels in genetically mutant anophthalmic rats lacking a complete visual system (Jagota *et al.* 1999).

One possible mechanism for extraocular photoreception has been attributed to chronobiological photoreceptors in blood (Oren & Terman 1998). In fact, Oren (1996, 1997) has hypothesized that heme moieties and bile pigments contained by blood could serve as photoreceptors. This humoral phototransduction model postulates that tetrapyrrole-based pigments, *e.g.* primary light-sensitive plant

pigments of chlorophyll and phytochrome, and mammalian hemoglobin and bilirubin, mediate light-induced circadian effects.

The skin is an interesting candidate for being a photoreceptive element. Two research reports of Iyengar (1994, 1998) have suggested that the melanocyte network in cultured human skin senses light, indicating that cells in the skin might be able to take part in chronobiological events.

In contrast to the above studies on nonocular phototransduction, an earlier study by Nelson and Zucker (1981) demonstrated that the activity rhythms of blinded diurnal ground squirrels and nocturnal grasshopper mice failed to entrain to the LD cycle. In line with this, the study in bilaterally anophthalmic rats showed no evidence of extraocular photoreception (Ibuka 1987). Recent studies by Meijer and coworkers (1999) and Yamazaki and associates (1999) report that illumination of the skin of blinded and shaved hamsters did not result in phase shifting effects on activity rhythms. In addition, light responsiveness of metabolic activity and gene expression of the SCN have been found to be mediated only through the eyes in preterm infant baboons (Hao & Rivkees 1999). Indeed, the absence of nonocular photic regulation of the circadian rhythms in humans has been suggested by our recent study indicating that a bright light exposure on the skin of the abdomen and chest does not induce phase shifting of melatonin, cortisol and thyrotropin rhythms (Lindblom *et al.* 2000).

Although a phase shifting effect of extraocular light exposure on the melatonin rhythm has been suggested (Campbell & Murphy 1998), there is no evidence of suppression of melatonin levels by skin illumination either in humans (Lockley *et al.* 1998, Hébert *et al.* 1999, Lindblom *et al.* in press) or hamsters (Yamazaki *et al.* 1999).

It seems that there are still discrepancies in the findings on the photoreceptive elements of circadian events. Several possible mechanisms and pathways could be involved in the effect that light has on the melatonin synthesis and other circadian rhythms. In conclusion, more research is needed before the nature of retinal photoreceptors and the role of extraocular phototransduction in the function of the circadian timing system are solved.

## **LIGHT AND THE DAILY RHYTHMS**

The visible portion of the electromagnetic spectrum covers the wavelength range from 380 to 760 nm, and the eye discriminates between different wavelengths within this range by sensation of color. Light is used to generate a visual image of the environment and to provide time-of-day information. In addition to the timing effects of light on the circadian system, light has also some direct neural effects. For instance, light exposures are able to increase body temperature (Strassman *et al.* 1991), enhance alertness (Campbell *et al.* 1995) and suppress melatonin (Brainard *et al.* 1997).

### **Light as a synchronizer of the circadian timing system**

A temporal structure is needed for circadian timekeeping. The internal circadian clock generally requires daily resetting by external time cues (Wever 1979, Czeisler *et al.* 1980). Several environmental and behavioral stimuli have been shown to act as circadian synchronizers. These include the timing of food availability, social interaction and physical activity (Stephan 1981, Mrosovsky &

Salmon 1987, Mrosovsky *et al.* 1989, Van Reeth & Turek 1989, Edgar & Dement 1991, Marchant & Mistlberger 1996).

However, in animals the dominant synchronizing signal for circadian rhythmicity is provided by environmental LD cycles. In many species of animals, light plays an important role in the regulation of circadian rhythms, *e.g.* motor activity, hormone secretion and temperature (McGuire *et al.* 1973, Wurtman 1975, Elliott 1976). Phase response curves (PRCs) are constructed by exposing the organism to an external signal or by administering a compound at different phases of the endogenous rhythm and measuring the resulting effect on the phase of the cycle (Aschoff 1965b). The first PRC to light was demonstrated by DeCoursey (1960) 40 years ago in flying squirrels. In 1978, Honma and associates (1978) reported the PRC of the locomotor activity rhythm to light pulses in rats which showed that phase delays occurred in the early subjective night followed by phase advances.

The influences of light and darkness on circadian rhythms can also be demonstrated by studies conducted in constant environmental lighting conditions. In constant darkness the rhythms free-run in rats (Redman *et al.* 1983, Thomas & Armstrong 1988). Continuous light treatment induces suppression of melatonin biosynthesis (Klein & Weller 1970, Laakso *et al.* 1994a) and the circadian rhythmicity of locomotor activity is lost (Honma & Hiroshige 1978, Chesworth *et al.* 1987) in rats. Several other circadian rhythms in rats (*e.g.* behavioral, temperature and some humoral rhythms) may persist for several weeks depending on the intensity of light (Honma & Hiroshige 1978, Eastman & Rechtshaffen 1983, Deprés-Brummer *et al.* 1995).

Based on current knowledge, the circadian rhythms of humans are also sensitive to light, although earlier findings of temporal isolation experiments proposed that social contacts are more effective than light in the entrainment of human circadian rhythms (Wever 1979). Indeed, as early as in 1960, support for the capacity of light and darkness to synchronize the human circadian system was provided by Sharp (1960) who reported a phase delay in the plasma levels of leucocytes in response to extension of darkness following normal wake time. A similar kind of finding was reported by Orth and Island (1969) and by Osterman (1974), who demonstrated that the circadian rhythm of plasma corticosteroids could be phase shifted by prolongating the dark period of the day.

In addition, more recent studies have shown that the effects of a single light pulse on the phase of human circadian rhythm markers can be observed after only a single day (Honma *et al.* 1987, Burešová *et al.* 1991, Minors *et al.* 1991, Van Cauter 1994) and in primates effective entrainment can be induced by a 1-s light pulse (Sulzman *et al.* 1981). In fact, the importance of the LD cycle in human circadian resetting was shown in the study of Czeisler and associates (1981) and Middleton and coworkers (1996a) underlining the lesser role of social cues and knowledge of clock time.

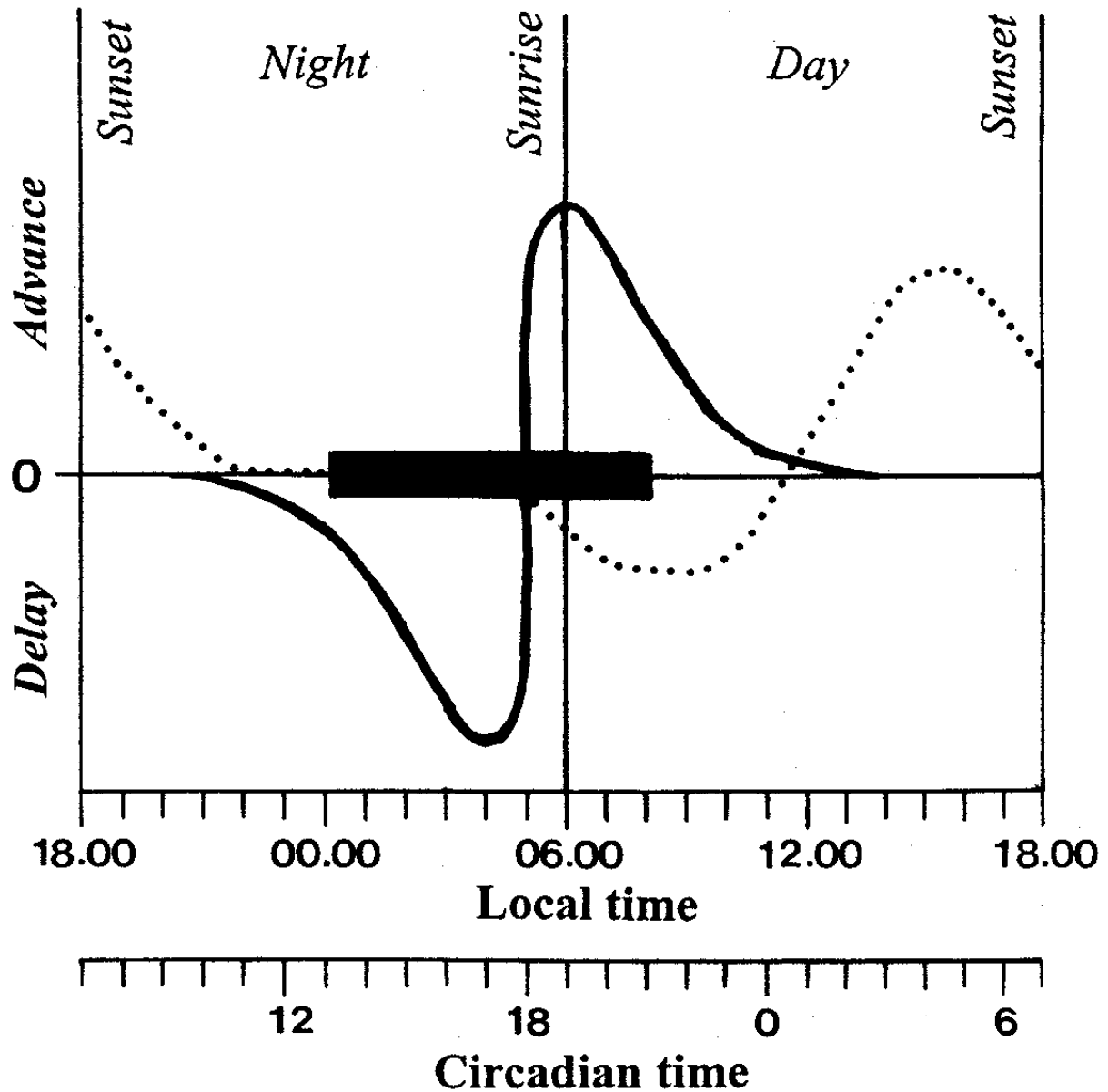
In humans, light has been found to be a stronger synchronizer of circadian rhythms than the sleep-wake rhythm. For instance, light-induced phase shifts of body temperature, cortisol, melatonin and sleep propensity rhythms could be seen even when the timing of the sleep-wake cycle was held constant (Czeisler *et al.* 1986, Drennan *et al.* 1989, Dijk *et al.* 1987, Lewy *et al.* 1987, Dijk *et al.* 1989). Clodoré and associates (1990) and Foret and coworkers (1993) have reported that the rhythms of cortisol, alertness and performance can be phase advanced by repeated morning exposure to bright light.

The theoretical basis of the PRC to light in humans was postulated by Lewy and collaborators (1983). The first contribution toward the findings of light-induced phase advances and delays were provided by Honma and colleagues (1987) who showed that phase shifts of both sleep-wake and temperature

rhythms could be induced by a bright light pulse. In 1989, the PRC to a non-24-hour LD cycle for body temperature was demonstrated (Wever 1989). More recently, Minors and coworkers (1991), Dawson and colleagues (1993) and Van Cauter and associates (1994) established the human PRCs to single bright light pulses for body temperature, melatonin and thyrotropin rhythm (Figure 1). In addition, a strong resetting of the human circadian timing system by multiple light pulses has been demonstrated (Czeisler *et al.* 1989, Shanahan *et al.* 1999).

Together, circadian PRCs produced in response to light have been found to share the following time-dependent properties: light stimuli early in the subjective night induce phase delay shifts, light stimuli late in the subjective night induce phase advance shifts, and light stimuli during the subjective day induce no or minimal phase shifts (Figure 1). Dose-response relationships have also been established between light intensity and phase shifting of the human temperature rhythm (Boivin *et al.* 1996).

Constant environmental lighting conditions also influence the circadian rhythms in nonhuman primates and humans. For instance, in many blind people the circadian rhythms free-run (Miles *et al.* 1977, Orth *et al.* 1979, Smith *et al.* 1981, Lewy & Newsome 1983, Nakagawa *et al.* 1992, Sack *et al.* 1992b, Klein *et al.* 1993, Skene *et al.* 1999). In the sighted, the period of human melatonin, body temperature and cortisol rhythms has been demonstrated to average 24.18 hours in controlled conditions of low light levels (Czeisler *et al.* 1999). Continuous light treatment induces suppression of melatonin rhythms in primates (Perlow *et al.* 1980, Perlow *et al.* 1981, Tetsuo *et al.* 1982). On the other hand, constant light conditions have variable disturbing effects on other circadian rhythms. For instance, the daily rhythmicity of cortisol and urinary potassium excretion in humans (Krieger *et al.* 1969) and cortisol secretion in primates are not altered (Perlow *et al.* 1981) in constant illumination.



**Figure 1.** SCHEMATIC HUMAN PHASE-RESPONSE CURVES TO LIGHT AND MELATONIN.

The y-axis of the phase-response curve (PRC) shows the direction and relative magnitude of the phase shift of the body temperature rhythm induced by light exposure (—) and the phase shift of the melatonin rhythm produced by melatonin administration (...) at various times shown on the x-axis as local or circadian times. The circadian time 18 corresponds roughly to the minimum of the body temperature. The black bar indicates a typical time for sleep relative to the minimum of the body temperature when the circadian system is entrained to the 24-hour day. For orientation purposes, sunrise and sunset are drawn in for the time of year when the night and day are of equal length. The PRC to light is about 12 hours out of phase with the PRC to melatonin (modified from Minors et al. 1991, Lewy et al. 1998a, Eastman & Martin 1999).

## MELATONIN AND THE PINEAL GLAND

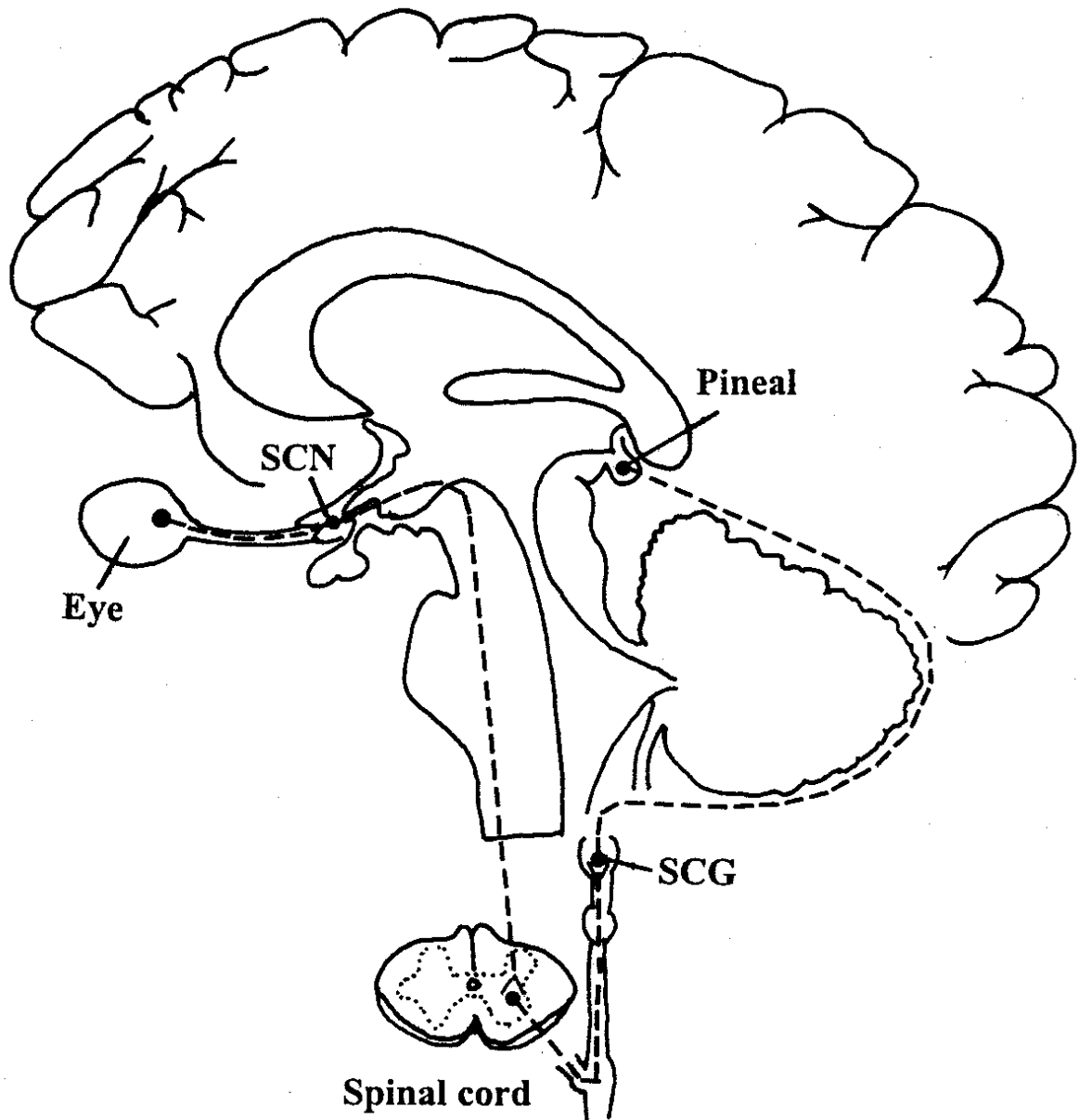
Over two millennia ago, Herophilos (325-280 B.C.), who was a famous anatomist at the University of Alexandria in Egypt, was probably the first researcher to discover the pineal organ in humans. Nothing is left of his writings, which have been cited by a Greek physician Galen ( $\pm$ 130-300 A.D.). Galen termed the pineal *konareion* (Latin, *conarium*) after its pineconelike shape, and described its possible glandular function (reviewed by Kappers 1979).

In the 17th century, the French philosopher and mathematician René Descartes (1596-1650) regarded the pineal organ as the soul's right hand, meaning that the soul exercises its functions to a great degree in the unpaired pineal organ. He likened pineal functions to a valve that regulates the direction and volume of the animal spirits flowing to and from the brain. Thus, he suggested that the pineal initiates the motor stimulus by sending the spirits into the tubular motor nerves (reviewed by Kappers 1979).

In 1917, McCord and Allen (1917) demonstrated that extracts of the pineal gland cause the skin of tadpoles to lighten. A major breakthrough in pineal studies occurred in 1958 when the dermatologist Aaron Lerner and coworkers (1958) extracted bovine pineal glands and were able to isolate a skin lightening compound, and determine its structure (1959) after 4 years of work and the use of more than 250 000 pineal glands (Lerner 1999). The compound was called melatonin (Greek, *melas*=black and *tosos*=labor). Axelrod and associates (Axelrod & Weissbach 1960, Weissbach *et al.* 1960, Weissbach *et al.* 1961) demonstrated that melatonin could be synthesized within the pineal gland. In later studies, in which it was shown that pinealectomy abolished the circadian rhythm of melatonin, it was confirmed that the pineal gland is the main source of melatonin in rats (Ozaki & Lynch 1976, Lewy *et al.* 1980a), rhesus monkeys (Tetsuo *et al.* 1982) and humans (Neuwelt & Lewy 1983, Petterborg *et al.* 1991).

The pineal gland (Latin, *epiphysis cerebri*, *glandula pinealis*) is a part of the brain derived from the caudal portion of the embryonic dorsal diencephalic cell column, the epithalamus. The morphology of the pineal changes dramatically with phylogenetic development. In mammals, the pineal has lost virtually all of its photoreceptor elements and instead contains parenchymal cells whose appearance suggests an entirely secretory function (Korf & Oksche 1986). According to current knowledge, the mammalian pineal (weight, 100 mg in humans, 1 mg in rats; Axelrod 1974) is considered a neuroendocrine organ. In humans, it is located at the posterior wall of the third ventricle near the geometric center of the brain. In addition, different indoleamines, including melatonin, and also several peptides are found in the pineal (Vaughan 1984, Ebels & Balemans 1986).

Previously, a widely held view was that the human pineal is a calcified vestigial organ which provides only a useful landmark for neuroradiologists. According to present knowledge, the physiological significance of the pineal gland in humans is mainly related to the function of melatonin. Melatonin is regulated by the circadian timing system, and LD cycles control the timing of melatonin secretion. Indeed, elevated melatonin levels are associated with nighttime, and melatonin has become to be known as the chemical expression of darkness (Reiter 1991a). In addition to the function of melatonin as a marker of environmental lighting conditions, melatonin may have some other physiological effects *e.g.* on reproduction and immune system.



**Figure 2. THE REGULATION PATHWAY OF THE PINEAL MELATONIN.**

*Sagittal view of human brain showing the pineal and its innervation. Retinohypothalamic fibers synapse in the suprachiasmatic nuclei (SCN), and there are connections from the SCN to the intermediolateral gray column in the spinal cord. Preganglionic neurons pass from the spinal cord to the superior cervical ganglion (SCG), and the postganglionic neurons project from this ganglion to the pineal in the nervi conarii (modified from Ganong 1997).*

## The regulation of pineal melatonin

### *Synthesis of melatonin*

Melatonin is a small (molecular weight 232.3) indoleamine secreted rhythmically with increased synthesis during the dark period of day. Alterations in environmental lighting are signaled as multisynaptic neural inputs by the central nervous system *via* peripheral nerves into a hormonal output of the pineal gland (Figure 2). In mammals, circadian photoreceptors of the retina convert light and darkness into signals that are sent directly to the SCN through the main pathway, the RHT (Hendrickson *et al.* 1972, Moore & Lenn 1972).

From the SCN, neuronal projections make synaptic connections in the PVN of the hypothalamus descending onward through the medial forebrain bundle to the intermediolateral cell column of the spinal cord from where preganglionic fibers reach the superior cervical ganglia (Swanson & Cowan 1975, Saper *et al.* 1976, Rando *et al.* 1981, Moore 1996). Sympathetic postganglionic noradrenergic fibers from the superior cervical ganglia innervate the pineal gland through the *nervi conarii* (Kappers 1960). Interruption of this regulation pathway by a lesion of the SCN or PVN, or by superior cervical ganglionectomy abolishes the pineal gland synthesis (Wurtman *et al.* 1964, Hastings & Herbert 1986, Bittman *et al.* 1989). Thus, production of pineal melatonin occurs in response to noradrenergic stimulation which produces a cascade of biochemical events within the pinealocytes.

The *N*-acetyltransferase (NAT) activity represents a key regulatory step in melatonin synthesis. Noradrenaline release from the sympathetic nerves that innervate the pineal gland is normally high at night and low during the day (Brownstein & Axelrod 1974). In most species, noradrenaline interacts with both beta<sub>1</sub>- and alpha<sub>1</sub>-adrenergic receptors present in the pineal gland (Vanecek *et al.* 1985). In the rat pinealocyte, stimulation of adrenergic receptors induces a rise in adenylate cyclase, and the cyclic adenosine 3',5'-monophosphate (cAMP) signaling pathway activates the NAT enzyme that catalyses the rate limiting step of melatonin synthesis (Deguchi & Axelrod 1972, Axelrod 1974, Sugden 1989). Simultaneous activation of alpha<sub>1</sub>-receptors potentiates the effects mediated through the beta<sub>1</sub>-receptors (Klein *et al.* 1983). Locally in the pineal gland, the rhythmic melatonin synthesis is ensured by the oscillating cAMP-dependent transcriptional control mechanism (Stehle *et al.* 1993, Foulkes *et al.* 1996).

Melatonin's biosynthetic pathway involves tryptophan, one of the 9 essential aminoacids in humans, as a precursor. Tryptophan is hydroxylated and decarboxylated to serotonin, and then serotonin is acetylated by the rate-limiting enzyme NAT and further methylated by hydroxyindole-*O*-methyltransferase to melatonin (5-methoxy-*N*-acetyltryptamine) in the pineal gland (Reiter 1991b). After the synthesis, melatonin is secreted into the blood and cerebrospinal fluid (Smith *et al.* 1976a, Smith *et al.* 1976b, Arendt *et al.* 1977). Although melatonin is found to be a very lipophilic compound which readily crosses the blood-brain barrier (Pardridge & Mietus 1980, Le Bars *et al.* 1991), there is also evidence of high hydrophilicity of the molecule (Shida *et al.* 1994).

Irrespective of whether a person is asleep or awake in dim light, melatonin is usually secreted between 2100 and 1000 h, with peak levels occurring between 0200 and 0600 h (Laakso *et al.* 1990, Laakso *et al.* 1994b). The circulating daytime serum levels of melatonin in healthy adults do not normally exceed 20 ng/l, while the range of the nighttime values may be about 20-170 ng/l (Laakso *et al.* 1990, Brzezinski 1997).

In fullterm newborn infants, the rhythm of a melatonin urinary metabolite is not apparent but develops between the ninth and twelfth week after birth (Kennaway *et al.* 1992). The highest nocturnal levels are reached at the age of one to three years (Waldhauser *et al.* 1993). These peak levels of nighttime blood melatonin levels and the urinary excretion of melatonin metabolites decrease during puberty (Waldhauser *et al.* 1993, Cavallo & Dolan 1996). After the attainment of sexual maturity, there seems to be a moderate decline of melatonin synthesis until old age (Hartmann *et al.* 1982, Sack *et al.* 1986), although in recent studies the difference in the amplitude of melatonin levels between old and young subjects was not significant (Luboshitzky *et al.* 1998, Zeitzer *et al.* 1999).

### *Metabolism and pharmacokinetics of melatonin*

Melatonin in the circulation is quickly metabolized in the liver primarily to a watersoluble hydroxy derivative followed by conjugation with sulfate and glucuronic acid (Kopin *et al.* 1961). The remaining melatonin is excreted into urine (Lynch *et al.* 1975, Ozaki & Lynch 1976) or converted into some other metabolites (Hirata *et al.* 1974).

Waldhauser and associates (1984) examined the pharmacokinetic profile of exogenously administered melatonin in healthy humans. After oral administration of melatonin (80 mg) in gelatin capsules during daytime, the absorption half-life was 0.40 h with an elimination half-life of 0.80 h, and the melatonin levels ranged between 350 and 10 000 times those occurring endogenously at nighttime. Those findings are in line with the experiments in which the mean half-time of the elimination phase after oral administration was 47 min (Di *et al.* 1997). In both studies a great interindividual variability was observed in the peak melatonin concentrations. In the study by Di and coworkers (1997) it was suggested that the variable bioavailability of oral melatonin (intervariability from 10 to 56 %) is a consequence of variation in hepatic first-pass extraction.

### **Effects of light on melatonin**

Light and dark alterations constitute the principal timing signal of melatonin secretion from the pineal gland. Light influences melatonin synthesis in three ways in humans. First, light exposure acutely suppresses elevated melatonin levels. Second, light is able to phase shift the melatonin rhythm. Third, changes in the photoperiod can alter the melatonin secretion.

#### *Suppressing effects of light*

The first piece of evidence for the suppressing effect of light on the enzymatic capacity of the pineal gland to synthesize melatonin came from the study by Wurtman and coworkers (1963b) in rats. The early studies in human were unsuccessful in their attempts to suppress melatonin synthesis by light (Vaughan *et al.* 1976, Jimerson *et al.* 1977, Vaughan *et al.* 1979, Åkerstedt *et al.* 1979), probably because neither the exposure conditions nor the light stimulus were optimized.

However, in 1980, Lewy and associates (1980b) showed that also in humans this suppressing effect takes place if an exposure to bright light occurs during the abundant synthesis of melatonin. The discovery that bright light ( $\geq 2500$  lux) could suppress melatonin secretion in humans (Lewy *et al.* 1980b) led to the assumption that only high-intensity ( $> 500$  lux) light is able to affect on human circadian rhythms. Although human melatonin synthesis is less sensitive to light than that of nocturnal rodents (Minneman *et al.* 1974, Illnerová & Vanecek 1979), an interruption or a significant decrease in the synthesis has been produced by intensities as low as 300 lux (Bojkowski *et al.* 1987), 350-400

lux (McIntyre *et al.* 1989), 500 lux (Laakso *et al.* 1994c, Hashimoto *et al.* 1996) or 650 lux (Laakso *et al.* 1991). In addition, it has been shown that melatonin is suppressed by light in an intensity- and duration-dependent manner (Brainard *et al.* 1988, McIntyre *et al.* 1989, Aoki *et al.* 1998).

A study of Brainard and coworkers (1993) suggests that the peak sensitivity for melatonin suppression is in the blue-green range (509 nm). In fact, in specially controlled conditions when the pupils have been dilated, the volunteers' heads kept motionless and the light beam directed uniformly on the retina the mean threshold illuminance of monochromatic light of 509 nm for producing a statistically significant melatonin suppression is between 6 and 17 lux (photopic) or 28 and 86 lux (scotopic) in normal volunteers (Brainard *et al.* 1988); a level of illuminance equal to civil twilight and well below typical indoor light. This means that under optimal conditions much lower intensities of light can suppress melatonin than was originally believed.

#### *Phase delaying and advancing effects of light*

Studies in humans (Hashimoto *et al.* 1996) and in golden hamsters (Nelson & Takahashi 1991) suggest that the threshold intensity of light needed to phase shift the circadian system is greater than that needed for suppression of the melatonin level. After the suppressing effects of light on human melatonin secretion (Lewy *et al.* 1980b) and the theoretical prediction of light PRC (Lewy *et al.* 1983) were reported, it was shown that the onset of nocturnal melatonin production could be phase shifted depending on the timing of the bright light (~2500 lux) exposure (Lewy *et al.* 1987). Furthermore, the PRC to single light pulses of high intensity (~5000 lux, 3 hours) for human melatonin rhythm was constructed by Van Cauter and coworkers (1994). This study demonstrated delays or advances of the melatonin rhythm, indicating that the response to light is phase dependent.

In addition, the magnitude of the phase shifts of melatonin rhythms vary with the number and timing of light pulses. For example, in a study of Burešová and colleagues (1991), one day after a single exposure to bright light (~3000 lux, 6 hours) in the morning, melatonin onset and offset phase advanced by 0.6-2.6 hours. Dijk and coworkers (1989) reported that an exposure to bright light during three consecutive mornings (2000 lux, 3 hours) advanced melatonin rhythms by about an hour. Deacon and Arendt (1994) found melatonin phase delays of about 2-3 hours when exposing the subjects to light (1200 lux, 6 hours) during three consecutive nights.

Recently, light exposures as weak as the common indoor intensity (150-500 lux) have been shown to shift the endogenous circadian rhythm of plasma melatonin (Zeitler *et al.* 1997, Boivin & Czeisler 1998) and body temperature (Waterhouse *et al.* 1998) in humans.

#### *Effect of photoperiod*

In nonhuman mammals, the photoperiod participates in the regulation of annual rhythms, *e.g.* breeding, renewal of fur and body weight rhythms (Martinet & Allain 1985, Zucker *et al.* 1991). Humans are not generally considered to be as photoperiodic as other species. A study by Van Dongen and coworkers (1998) demonstrated the absence of seasonal variation in the phase of circadian rhythms. However, data from Northern temperate (Beck-Friis *et al.* 1984, Kivelä *et al.* 1988) and Polar regions (Broadway 1987, Makkison & Arendt 1991), and from simulated long and short photoperiod experiments (Wehr 1991, Burešová *et al.* 1992, Van Dongen *et al.* 1997, Vondrašová *et al.* 1997) indicate that we retain a number of photoperiodic responses as a function of daylength. For instance, changes in melatonin levels, in duration of nocturnal melatonin secretion, and in the phase of the melatonin rhythm have been induced by seasonal variation of natural lighting conditions (Illnerová

*et al.* 1985, Martikainen *et al.* 1985, Kauppila *et al.* 1987, Kivelä *et al.* 1988, Laakso *et al.* 1994b, Stokkan & Reiter 1994, Luboshitzky *et al.* 1998). These findings suggest that the regulation of melatonin is influenced by the photoperiod in humans as in other mammals.

### **Melatonin as a circadian effector**

Melatonin is evolutionarily well preserved and present in most organisms, from unicell algae to humans (Pelham *et al.* 1973, Smith *et al.* 1976a, Hardeland & Fuhrberg 1996). Melatonin's role as a phylogenetically conservative signal might be associated with its involvement in the adaptation of early living cells to the demands of climate. According to a hypothesis of Paitta (1982), the increased level of free oxygen in the early eukaryote evolution could have pushed the organisms to minimize the deleterious effects of the diurnal photooxidative exposures by developing circadian rhythmicity of metabolic activities. In addition, melatonin can detoxify highly reactive oxygen radicals (Poeggeler *et al.* 1993, Reiter *et al.* 1993, Tan *et al.* 1993). Therefore, melatonin as a natural oxidant may represent a property which during evolution has made this molecule a suitable indicator of the dark period (Hardeland 1993).

#### *The effects of pinealectomy and melatonin administration on rhythms in animals*

Pinealectomy, in which the lack of clear rhythmical production of melatonin is induced surgically, results in the elimination of the normal circadian rhythm of locomotor activity in birds (Gaston and Menaker 1968), whereas in rats pinealectomy has little effect on free-running circadian activity patterns (Richter 1964, Richter 1967, Quay 1968, Karppanen *et al.* 1973). However, pinealectomized rats do re-entrain to the phase-shifted photoperiods more rapidly than their sham-operated controls (Quay 1970).

A major step was taken in the studies of the role of melatonin in mammalian circadian organization in 1983 when Redman, Armstrong and Ng (1983) reported that daily injections of melatonin entrained the circadian locomotor rhythms of rats in constant dim red light. Somewhat later, it was shown that exogenous melatonin was able to phase shift activity rhythms (Redman & Armstrong 1988). In constant light, however, melatonin injections are not always able to repair a light-induced disruption of the rhythms (*e.g.* wheel-running or locomotor activity, drinking, and body temperature), while a clear synchronization occurs in some individuals (Chesworth *et al.* 1987, Thomas & Armstrong 1988, Marumoto *et al.* 1996, Deprés-Brummer *et al.* 1998, Witte *et al.* 1998). Thus, the mammalian pineal seems not to be essential for the generation of the circadian rhythm of locomotor activity, and the action of melatonin as a circadian synchronizer may be somewhat limited in strength (reviewed by Redman 1997).

The relationship between intrinsic rhythms and external signals is classically defined by phase response curves. The first PRC to melatonin in a vertebrate was conducted by Underwood (1986). Exogenous melatonin injected into free-running lizards induced phase delays (injections were administered late in the subjective night or in the first half of the subjective day) or phase advances (injections were administered between midsubjective day or early subjective night) of circadian activity rhythms. Afterwards, the PRC to melatonin was also constructed in other animals, *e.g.* in rats (Armstrong 1989).

### *The effects of exogenous melatonin on rhythms in humans*

The first finding on a substance which had melanin aggregating and chromatographic properties similar to those of melatonin and a cyclic pattern was published in 1973 by Pelham and coworkers (1973) in humans. After that study, several studies have yielded evidence about melatonin's role as a rhythm modulator. For example, Arendt and colleagues (1985) first suggested that exogenous melatonin administration might be able to phase advance the endogenous melatonin profile. After it had been shown that daily melatonin administration induced a phase advance of the endogenous melatonin rhythm in blind subjects (Sack *et al.* 1991), Lewy and coworkers (1992) provided the first piece of evidence for a human PRC to melatonin (Figure 1). Melatonin administration in the morning elicited phase delays, and administration in the afternoon or early evening induced phase advances of the endogenous melatonin onset. In addition to the phase shifting effects of exogenous melatonin, Sack and associates (1991) have demonstrated a normally entrained endogenous melatonin rhythm in one blind person after about a year of melatonin treatment.

Compared to the human phase-response curve to light (Minors *et al.* 1991), PRC to exogenous melatonin (Lewy *et al.* 1992, Zaidan *et al.* 1994, Lewy *et al.* 1998a) has been described to be nearly opposite in phase (Figure 1). In practice, the optimal time to produce a phase delay by exogenous melatonin is near the offset of endogenous secretion (at about 0700 h) and the optimal time of day to administer melatonin to produce a phase advance is 4-8 h before the onset of endogenous melatonin (at about 1700 for people on a conventional schedule) (Lewy & Sack 1997, Lewy *et al.* 1998a). Other circadian hormonal rhythms (*e.g.* cortisol, prolactin) and temperature rhythms can also be affected by melatonin administration (Arendt *et al.* 1987, Mallo *et al.* 1988, Kräuchi *et al.* 1997b).

### *Melatonin binding sites and feedback to the pacemaker*

Although an intracellular function and a direct gene regulatory action of melatonin have been suggested (Carlberg & Wiesenberg 1995, Steinhilber & Carlberg 1999), many of the established effects of physiological concentrations of melatonin have been shown to be mediated *via* high-affinity cell membrane receptors belonging to the superfamily of G-protein-coupled receptors (reviewed by Kokkola & Laitinen 1998).

Based on sequence dissimilarities, melatonin receptors are classified into three subtypes (Mel<sub>1a</sub>, Mel<sub>1b</sub> and Mel<sub>1c</sub>), and two of the subtypes (Mel<sub>1a</sub> and Mel<sub>1b</sub>) have been found in mammals (Kokkola & Laitinen 1998). The regions which show specific melatonin-binding in most mammals studied are the SCN and the hypophyseal pars tuberalis (Morgan *et al.* 1994). In humans, melatonin-binding sites are found in various regions of the brain, including the SCN (Weaver *et al.* 1993, Weaver & Reppert 1996), the temporal cortex (Fauteck *et al.* 1995), the cerebellum (Fauteck *et al.* 1994) and possibly in the hypophyseal pars tuberalis (Weaver *et al.* 1993), and also in peripheral sites such as the kidney (Song *et al.* 1995), granulosa cells (Yie *et al.* 1995) and prostate (Laudon *et al.* 1996).

There is also evidence of circadian oscillators within the SCN which are sensitive to the pineal hormone melatonin, raising the possibility that the circadian organization may be modulated by pineal feedback *via* melatonin. First, specific melatonin receptors in the SCN (Vanecek *et al.* 1987, Reppert *et al.* 1988, Morgan *et al.* 1994), also in humans (Weaver *et al.* 1993), provide a critical link for a functional feedback loop whereby circulating melatonin can influence the circadian pacemaker. Second, pinealectomy, *i.e.* suppressed melatonin production, increases the density of these binding sites (Gauer *et al.* 1992), and can alter the firing-rate rhythm in the SCN (Rusak & Yu 1993).

Third, the pinealectomy-induced increase in the density of binding sites can be inverted by a single melatonin injection (Gauer *et al.* 1993). In addition, exogenous melatonin inhibits the spontaneous electrical activity of the SCN of the rat when melatonin is administered near the transition to subjective night (Mason & Brooks 1988, Shibata *et al.* 1989, Stehle *et al.* 1989). In fact, the circadian rhythm of the discharge rate of the SCN can be reset by melatonin within two windows of sensitivity corresponding to dusk and dawn (McArthur *et al.* 1991, McArthur *et al.* 1997).

Fourth, melatonin alters the metabolic activity of the rat SCN (Cassone *et al.* 1988). Administration of melatonin to rats induced expression of Fos, the protein product of the *c-fos* proto-oncogene, in the SCN (Kilduff *et al.* 1992), and *in vivo* a single melatonin administration phase advanced the evening rise in the light-induced SCN *c-fos* expression (Sumová & Illnerová 1996). In conclusion, it seems that melatonin may modulate circadian overt rhythms probably by coupling individual oscillators to form an internal synchronization and affecting the photic sensitivity of the circadian timing system.

## **Other effects of melatonin**

### *Biological calendar of reproduction*

Previous classical studies on melatonin have mainly focused on its action on the hypothalamic-hypophyseal-gonadal axis, whereby it controls seasonal reproduction (Reiter 1980, Tamarkin *et al.* 1985). Because melatonin can act as a signal of photoperiod to the body, a long duration of melatonin secretion at night may cause gonadal atrophy in spring and summer breeders (*e.g.* hamsters), and gonadal growth in species which breed in the autumn and winter (*e.g.* sheep) (Bartness *et al.* 1993). In addition, melatonin was found to have antigonadal effects on rodent species that are not seasonal breeders (*e.g.* rats, mice) (Wurtman *et al.* 1963a, Glass & Lynch 1981).

In the late 19th century, Otto Heubner (1898) observed precocious puberty in a child with a pineal tumour. Since then, there have been several reports about the relationship between sexual maturation disorders and abnormal melatonin levels (Berga *et al.* 1988, Karasek *et al.* 1990, Puig-Domingo *et al.* 1992, Luboshitzky *et al.* 1995). Although correlations between melatonin and reproductive hormones have been observed in humans, the functional relationship remains to be determined (reviewed by Luboshitzky & Lavie 1999).

### *Melatonin as a protective agent*

Melatonin has been found to be a potent intracellular scavenger of hydroxyl and peroxy free radicals when administered at pharmacological doses both *in vivo* and *in vitro* (Reiter 1995, Reiter 1998), suggesting that it has a protective role against oxidative damage. For instance, melatonin treatment may be beneficial in some neurodegenerative diseases, *e.g.* Alzheimer's and Parkinson's diseases (Reiter 1998, Reiter *et al.* 1998).

Melatonin may also function as an immunomodulator, and the immunoenhancing action seems to be mediated by certain T-lymphocytes and lymphokines (Maestroni 1993, Neri *et al.* 1998). In addition, some antitumoral effects of melatonin have also been reported (Buswell 1975, Wilson *et al.* 1992, Lissoni *et al.* 1995, Lissoni *et al.* 1996). In contrast to these findings, melatonin has been shown to be able to promote melanoma growth in hamsters (Stanberry *et al.* 1983) and induce suppression of human lymphocyte natural killer cell activity *in vitro* (Lewinski *et al.* 1989).

Furthermore, it has been suggested that melatonin has a role in the modulation of brain excitability. An anticonvulsant potential of exogenous melatonin has been proposed on the basis of *in vivo* studies in humans (Anton-Tay 1974, Brueske *et al.* 1981, Molina-Carballo *et al.* 1997, Jan *et al.* 1999) and an *in vitro* experiment in which epileptiform activity in human temporal slices was reduced by melatonin (Fauteck *et al.* 1995). On the other hand, Sheldon (1998) reported that melatonin induced an increased seizure frequency in 4 of 6 neurological patients. Taken together, it seems that the effects of melatonin may not be completely protective.

### **Extrapineal melatonin**

The pineal gland is not the only source of melatonin hormone. The suggestion that extrapineal melatonin exists in humans was made by Raikhlin and coworkers (1975) when they blanched the frog's skin with extracts of human intestinal mucosa. In fact, the gastrointestinal tract represents the most abundant extrapineal source of melatonin, for it contains over 80 times more melatonin than the pineals of chicks and rats (Huether *et al.* 1992). Arrhythmic melatonin levels have been observed in alligators (*Alligator mississippiensis*) lacking pineal bodies (Roth *et al.* 1980). Melatonin is also found in the blood of surgically pinealectomized rats (Ozaki & Lynch 1976, Pang *et al.* 1977). In vertebrate retinas, melatonin is thought to act locally to regulate various dark-adaptive functions (Cahill & Besharse 1995). Melatonin is also synthesized in small amounts in the human retina (Leino 1984), but the role of extrapineal melatonin in circadian functions in humans is not known.

## **BLINDNESS AND CIRCADIAN RHYTHMS**

The entry of the amount of light needed for the resetting of the rhythms to the circadian timing system may be prevented by blindness. Thus, blind people may fail to maintain the circadian rhythms of sleep and wake, body temperature, melatonin and cortisol synchronized with the LD cycles (Miles *et al.* 1977, Orth *et al.* 1979, Smith *et al.* 1981, Lewy & Newsome 1983, Okawa *et al.* 1987, Nakagawa *et al.* 1992, Sack *et al.* 1992b, Klein *et al.* 1993, Palm *et al.* 1997, Klerman *et al.* 1998, Skene *et al.* 1999, Lockley *et al.* 2000). They may have sleep-wake disruption and may develop either free-running circadian rhythms or disorganized sleep-wake cycles (Arendt *et al.* 1988, Lockley *et al.* 1997a, Lockley *et al.* 1997b, Tabandeh *et al.* 1998, Lockley *et al.* 1999). In addition, periodic insomnia and daytime sleepiness (Sack *et al.* 1992b, Tzischinsky *et al.* 1992) may occur when the internal clock becomes desynchronized from the solar and social 24-hour cycle.

Although in a study by Czeisler and coworkers (1995), a 3-hour, 10000-lux facial light exposure in patients with different origins of blindness (*e.g.* retinopathy or optic neuropathy) did not suppress melatonin synthesis in most of the 11 blind subjects with no conscious perception of light, a clear decrease of melatonin was found in 3 subjects who had totally lost the pupillary reflexes. The light suppressing effect was only found when the patients' eyes were uncovered. This work indicates that in a subgroup of patients without conscious light perception the photic pathway through the eyes used by the circadian system may be functional.

## Patients with neuronal ceroid lipofuscinosis and blindness

### *Classification of NCL diseases*

Neuronal ceroid lipofuscinoses (NCLs) are the most common group of progressive neurodegenerative diseases of childhood in the Western world. The disorders were first described by Stengel, Batten and others in the 19th and early 20th centuries (reviewed by Goebel *et al.* 1999), and termed neuronal ceroid lipofuscinoses by Zeman and Dyken (1969) because the neural and extraneural accumulation of storage material resembled ceroid and lipofuscin. Based on recent findings on molecular genetics, NCLs can be classified as atypical lysosomal storage diseases (Mole 1998, Mole *et al.* 1999).

Classically, NCLs have been divided into four main forms: the infantile (INCL, Santavuori-Haltia disease), late infantile (LINCL, Jansky-Bielschowsky or early onset Batten disease), juvenile NCL (JNCL, Spielmeyer-Vogt-Sjögren or late onset Batten disease) and adult onset NCL (Kufs' disease and Parry disease). In addition, a congenital NCL and Northern Epilepsy Syndrome (Progressive Epilepsy with Mental Retardation) have been reported (Hirvasniemi *et al.* 1994, Goebel *et al.* 1999).

To date, depending on the age of onset, clinical course of the disease, characterization of the storage material, presence of vacuolated lymphocytes, and chromosomal location of the disease gene, at least 11 childhood and two adult types are recognized (Mole 1999). All childhood and most adult types are inherited in an autosomal recessive fashion. According to present knowledge, eight genes (symbols from *CLN1* to *CLN8*) underlie NCLs (Goebel *et al.* 1999). Five of the genes have been identified: *CLN1* (INCL), *CLN2* (classical LINCL), *CLN3* (JNCL), *CLN5* (Finnish variant LINCL) and *CLN8* (Northern Epilepsy Syndrome) (Vesa *et al.* 1995, Sleat *et al.* 1997, The International Batten Disease Consortium 1995, Savukoski *et al.* 1998 and Ranta *et al.* 1999, respectively). In addition, 86 different disease-causing mutations have been reported (<http://www.ucl.ac.uk/ncl>, accessed on Mar 23, 2000).

### *Incidence and common symptoms of NCL diseases in Finland*

The most common NCL types in Finland are INCL and JNCL, whereas LINCL is rarer (Santavuori 1999). One adult form of NCL has been diagnosed in Finland (Haltia, personal communication). At present, the incidence of INCL is 1:20000, approximating three new INCL patients per year (Santavuori 1999, Santavuori personal communication). Altogether 158 INCL patients have been diagnosed. The number of diagnosed LINCL patients is 31, consisting of five classic LINCL (gene symbol *CLN2*) and 26 variant LINCL (*CLN5*) patients. 185 JNCL patients have been diagnosed, with an incidence of 1:21000.

NCL children are healthy at birth with normal development until the onset of the disease. In the most common Finnish NCL types, including INCL (gene symbol *CLN1*) and JNCL (*CLN3*), the clinical course is characterized by loss of vision, epilepsy, progressive cognitive impairment, and psychomotor disturbances leading to premature death.

### *Clinical picture of INCL*

In INCL patients the development is normal until the age of 0.5-1 year, and in some children until 1.5 years (Santavuori 1988). The onset of epilepsy occurs at the mean age of 30 months (Vanhanen 1996). INCL patients suffer from anxiety and hyperexcitability (Santavuori 1988) causing sleep problems, which are among the most disturbing factors in the everyday life of the patients and their

families. In our earlier study a fragmented activity rhythm was observed in 4 of 5 INCL patients, but disturbances in the daily melatonin and cortisol rhythms occurred only in the minority of patients, and only at an advanced stage of the disease (Heikkilä *et al.* 1995).

In neurophysiological examination of INCL patients, electroencephalogram (EEG) shows posterior low activity to eye opening and closing starting at a mean age of 1.8 (range 1.4-2.3) years, and the activity in the EEG disappears after the age of 2.7 years (Vanhanen *et al.* 1997). Neuroradiological findings by magnetic resonance imaging (MRI) may show hypointensity of the thalamus as compared with the white matter and basal ganglia, and cerebral and cerebellar atrophy (Autti *et al.* 1997, Vanhanen *et al.* 1994).

The condition of the patient deteriorates rapidly and before the age of 3 years the child has lost all cognitive and active motor skills (Santavuori *et al.* 1974). Death usually takes place at the age of 8-13 years (Santavuori *et al.* 1999).

The characteristic neuropathological finding in INCL patients is extreme atrophy of the brain, whereas the brain stem and particularly the spinal cord are less affected (Haltia *et al.* 1973, Santavuori *et al.* 1974). The consistency of the brain is tough and rubberlike, and all cerebral gyri are narrowed and sulci widened (Haltia *et al.* 1973).

#### *Pathology of visual system in INCL*

Blindness is one of the main symptoms in INCL patients, and visual disturbance is usually observed between 12 and 24 months of age (Santavuori *et al.* 1974). Perception of light can be lost as early as at the age of 18 months, but most often a few months later (Kohlschütter & Goebel 1997). Visual failure leads rapidly to blindness. INCL children are usually practically blind at the age of two (Raitta & Santavuori 1973). Pupillary reactions are slow or absent after the age of two years (Santavuori *et al.* 1974) but, surprisingly, may reestablish during a later stage of the disease (Santavuori *et al.* 1999).

The earliest ophthalmoscopic findings are hypopigmentation of the fundus without distinct macular changes (Raitta & Santavuori 1981). Macular changes appear by the age of 12-18 months as depigmentation and mottling of the pigment layer. In fluorescein angiography the changes can be distinguished by the age of 18-24 months and are parallel to the general dystrophy of the retina. Retinal vessels become extremely narrow. They are hardly visible by the age of 3 years when the optic disc appears atrophic (Raitta & Santavuori 1981). In addition to retinal degeneration, clearly visible choroidal vessels are seen (Santavuori *et al.* 1974). Pigment aggregation of the fundus periphery is not usually found in INCL patients (Raitta & Santavuori 1973, Kohlschütter & Goebel 1997).

In neurophysiological examination, visual evoked potential (VEP) and electroretinogram (ERG) abnormalities appear between 22 and 25 months of age (Vanhanen *et al.* 1997). The mean age of abolition of ERG is 3.1 (range 2.3-4.1) years and that of VEP 3.8 (2.2-5.4) years.

Neuropathological autopsy of 5 INCL patients showed that the retinas were completely destructed (Tarkkanen *et al.* 1977). The retina was severely atrophic with complete loss of photoreceptors, bipolar and ganglion cells which were replaced by marked glial proliferation. Loss of pigment in the retinal pigment epithelium had taken place to some extent. The optic nerve was atrophic and gliosed with a complete loss of myelin sheets. In addition, there was accumulation of granular material in the nonpigmented ciliary epithelium, the retina, the retinal pigment epithelium, and the optic nerve.

### *Clinical picture of JNCL*

In JNCL patients the disease usually becomes manifest at early school age. The first symptom is usually visual failure detected between 4 and 7 years of age (Santavuori 1988). At first, mental impairment is slight and apparent only in school performance.

Some time after the onset of visual loss, a personality change may be noted which can be accompanied by various types of psychiatric disturbances (Boustany 1992, Wisniewski *et al.* 1992, Hofman *et al.* 1999). The greatest decline in motor functions and intelligence usually takes place between 11 and 15 years of age (Järvelä *et al.* 1997). The onset of epilepsy occurs, on the average, at the age of 11 years (Järvelä *et al.* 1997).

Sleep problems are reported in more than half of JNCL patients (Santavuori *et al.* 1993). The most common problems include frequent awakenings, difficulties in falling asleep, nightmares and night terrors. Despite the disturbances observed in the sleep-wake rhythm in 7 of 8 JNCL patients, most of the patients had normal melatonin, cortisol and temperature rhythms (Heikkilä *et al.* 1995).

In neurophysiological recordings, EEG is usually found normal at the preclinical stage, and normal or slightly abnormal by the age of 5-6 years in most patients (Santavuori *et al.* 1988). In MRI, abnormal periventricular white matter may be found in all age groups, and the thalamus, caudate nucleus and putamen usually give low signal in patients above the age of ten years (Autti *et al.* 1996). During the progression of the disease, movement difficulties develop (Boustany 1992, Wisniewski *et al.* 1992, Hofman *et al.* 1999). The disease leads to death at the mean age of 24 years (Järvelä *et al.* 1997).

In neuropathological examination of JNCL patients, the macroscopic appearance of the brain may be nearby normal (Zeman & Dyken 1969, Zeman *et al.* 1970). The cerebral cortex is slightly atrophic, whereas the number of neurons is usually not markedly reduced. The most marked changes in the white matter are seen in periventricular areas (Vanhanen *et al.* 1995).

### *Pathology of the visual system in JNCL*

In JNCL patients the leading initial symptom is visual failure. The visual problems are noticed at a mean age of 5.8 (range 4-10) years, and the patients become practically blind between 6 and 20 years of age (Järvelä *et al.* 1997, Kohlschütter & Goebel 1997). The characteristic ophthalmological findings are retinal degeneration, macular dystrophy, optic atrophy and thinning of vessels (Spalton *et al.* 1980, Traboulsi *et al.* 1987, Santavuori 1988, Seeliger *et al.* 1997, Neppert & Kemper 1998). In addition, the typical pigment aggregation is usually seen in the peripheral retina (Raitta & Santavuori 1981, Kohlschütter & Goebel 1997).

In neurophysiological examination, ERG is usually abolished around 6 years of age (Santavuori *et al.* 1988, Kohlschütter & Goebel 1997, Seeliger *et al.* 1997, Weleber 1998). VEP is pathological when clinical signs are apparent and usually become abolished between 13 and 16 years. In some patients VEP was still recordable, although abnormal, between the age of 18 and 21 years (Santavuori *et al.* 1988). The abnormality of the ERG is usually seen as a severe loss of rod and cone responses (Horiguchi & Miyake 1992, Seeliger *et al.* 1997, Weleber 1998).

Pathological examination of one JNCL patient revealed a near-complete loss of photoreceptors (Traboulsi *et al.* 1987). The autopsy of 3 JNCL patients showed that two of them had lost all retinal photoreceptor cells, whereas in one patient with the shortest clinical course of the disease there were

still photoreceptors present in the periphery of the retina (Goebel *et al.* 1974). Changes were observed in the optic nerves, ganglion cell layers and the pigment epithelium (Goebel *et al.* 1974, Traboulsi *et al.* 1987) but the choroid and sclera were normal (Traboulsi *et al.* 1987).

### *Treatment of NCL diseases*

At present, the treatment of NCLs is merely palliative, including medication (*e.g.* antiepileptics and antiparkinsonian drugs, analgetics, antidepressants, antipsychotic therapy, antioxidants and hormonal treatment) combined with family guidance, psychological support, social help, artificial feeding, physiotherapy, speech and occupational therapy (Hofman *et al.* 1999, Santavuori *et al.* 1999). Thus, there is no cure yet for this devastating group of diseases. However, new forms of treatment will hopefully emerge as the understanding of the pathological processes increases.

## **PRACTICAL CONSIDERATIONS OF LIGHT AND MELATONIN**

The current knowledge about the effects of light and melatonin on human physiology is partly based on unsystematic observations. The indications for phototherapy and melatonin administration are not yet conclusively established, neither are the long-term benefits and risks thoroughly known.

Patients treated with bright light have reported some temporary side effects including headache, eyestrain, irritability, nausea and insomnia (Levitt *et al.* 1993). There is also evidence that symptoms of mania may emerge as a consequence of phototherapy (Schwitzer *et al.* 1990). However, short- or long-term daily treatment with 10 000 lux light has not been observed to result in ocular changes in patients suffering from seasonal affective disorder (Gallin *et al.* 1995).

Melatonin is available as a nutritional supplement in the US without a premarket approval from the FDA (Food and Drug Administration), and it is widely sold in health-food and drug stores. In Canada, Finland, Great Britain and some other European countries melatonin is classified as a medicine and available only on prescription. However, the standard clinical trial methodologies for judging the safety of a drug have not been applied to melatonin. Indeed, very little is known about the long-term adverse effects of melatonin in humans. The results of experiments in rodents indicate low toxicity (Sudgen 1983, Jahnke *et al.* 1999) and no mutagenicity (Neville *et al.* 1989). This is consistent with the human studies in which doses ranging from 0.1 mg to several grams have not been found to result in medical catastrophes (Lerner & Nordlund 1978, Wirtz-Justice & Armstrong 1996, Sack *et al.* 1997, Zhdanova *et al.* 1997).

The potential side effects of melatonin are drowsiness (Arendt *et al.* 1984, Dollins *et al.* 1993, Dollins *et al.* 1994) and phase shifting of circadian rhythms (Dawson & Armstrong 1996). In addition, sleep could be disrupted if melatonin is not properly timed (Middleton *et al.* 1996b). The possible adverse effects of melatonin also include cutaneous flushing, gastrointestinal disorders, headache and vascular constriction (Papavasiliou *et al.* 1972, Guardiola-Lemaître 1997, Mahle *et al.* 1997).

The most common group of diseases or symptoms treated with light or exogenous melatonin comprises circadian disorders, which are linked to poor resetting of the circadian timing system and usually associated with free-running or abnormal overt rhythms. Disturbances in the circadian control of various body functions have been found to be related to working during unusual hours (Sack *et al.*

1992a, Weibel *et al.* 1997) and jet lag (Désir *et al.* 1981, Fevre-Montange *et al.* 1981, Désir *et al.* 1982, Goldstein *et al.* 1983, Härmä *et al.* 1993, Suvanto *et al.* 1993). In addition, abnormal circadian rhythms have been suggested to underly the sleep difficulties of blind people (Arendt *et al.* 1988, Nakagawa *et al.* 1992, Lockley *et al.* 1997a, Lockley *et al.* 1997b, Tabandeh *et al.* 1998, Lockley *et al.* 1999), of some neurologically disabled children (Laakso *et al.* 1993, Palm *et al.* 1997, McArthur & Budden 1998, O'Callaghan *et al.* 1999, Zhdanova *et al.* 1999) and of the elderly (Haimov *et al.* 1994). Circadian pathology has been suggested to be related to some affective disorders (Wehr & Goodwin 1983).

### **Circadian rhythm sleep disorders**

The sleep-wake cycle is one of the most obvious rhythms regulated by the circadian clock. The disorders that are related to the timing of sleep within the 24-hour day are named circadian rhythm sleep disorders (American Sleep Disorders Association 1997). In most of the disorders, the underlying problem is that the person cannot sleep or be awake when desired, needed, or expected.

The phase shifts of circadian rhythms are the probable mechanism by which light improves sleep (Dijk *et al.* 1995). The alerting and activating property of bright light exposures has also been proposed to be one possible way in which light affects arousal and sleep onset (Campbell *et al.* 1995). Administration of melatonin might improve sleep by producing corrective circadian phase shifts (Zhdanova *et al.* 1997), thereby improving the alignment of the endogenous sleep propensity rhythm with the desired sleep schedule (Sack & Lewy 1997). In addition, melatonin may increase sleepiness by a soporific effect (Wirz-Justice & Armstrong 1996).

After rapid air travel across several time zones, the endogenous circadian timing system is desynchronized and begins to adapt to new environmental time cues (American Sleep Disorders Association 1997). Because the adjustment process of the circadian system is slow, jet lag symptoms, *e.g.* disturbed sleep, daytime sleepiness and impaired performance, can last for several days after the flight. Scheduled exposure to bright light can alleviate the symptoms (Daan & Lewy 1984, Boulos *et al.* 1995). During the last ten years, evidence has accumulated about the ability of melatonin administration to decrease jet lag symptoms (Arendt & Marks 1983, Arendt *et al.* 1986, Arendt *et al.* 1987, Petrie *et al.* 1989, Claustrat *et al.* 1992, Petrie *et al.* 1993), although in a recent extensive study by Spitzer and coworkers (1999) melatonin did not show any beneficial effects as compared with placebo.

Shift work is a common cause of circadian rhythm sleep disorders. The symptoms, *e.g.* difficulties in sleep maintaining and reduced alertness and performance, can be explained by a mismatch between the work-sleep schedule and the internal circadian rhythms (American Sleep Disorders Association 1997). There is evidence indicating that maladaptation to shift work can be treated with properly timed exposures to light (Dawson & Campbell 1991, Bjorvatn *et al.* 1999). Also, the use of artificial nocturnal bright light combined with enforced daytime dark periods can phase shift circadian rhythms effectively despite an exposure to conflicting 24-hour time cues (Czeisler *et al.* 1990, Eastman *et al.* 1994, Koller *et al.* 1994, Eastman & Martin 1999). Application of exogenous melatonin has been tested as therapy in sleep disorders of night workers (Folkard *et al.* 1993).

The delayed sleep phase syndrome is a disorder in which the major sleep episode is delayed in relation to the desired clock time, resulting in symptoms of sleep-onset insomnia or difficulty in awakening at a desired time (American Sleep Disorders Association 1997). Unlike the advanced sleep phase

syndrome, which is rather rare, the delayed sleep phase syndrome is quite a common sleep schedule disorder. Lewy and coworkers (1985) were the first ones to recommend the use of a morning light exposure to treat the delayed sleep phase syndrome. After that study, more evidence has been obtained about the beneficial implications of light (Rosenthal *et al.* 1990) and melatonin (Dahlitz *et al.* 1991, Tzischinsky *et al.* 1993, Oldani *et al.* 1994, Dagan *et al.* 1998) in the therapy of the delayed sleep phase syndrome.

Blind people may have a non-24-hour sleep cycle that reflects a nonentrained intrinsic rhythm of the circadian pacemaker (American Sleep Disorders Association 1997), resulting in recurrent insomnia and daytime sleepiness (Sack *et al.* 1992b, Tabandeh *et al.* 1998). Several reports indicate beneficial effects of melatonin administration in these patients (Arendt *et al.* 1988, Folkard *et al.* 1990, Sarrafzadeh *et al.* 1990, Sack *et al.* 1991, Tzischinsky *et al.* 1992, Lockley *et al.* 2000).

Melatonin has also been found to promote sleep in patients with neurological disabilities, especially in those with circadian sleep disorders (Palm *et al.* 1991, Jan *et al.* 1994, Lapierre & Dumont 1995, Palm *et al.* 1997, McArthur & Budden 1998, O'Callaghan *et al.* 1999, Zhdanova *et al.* 1999). On the other hand, controversial effects have also been observed in children with mental retardation and fragmented sleep (Camfield *et al.* 1996), and melatonin was also ineffective in our previous study in neuronal ceroid lipofuscinosis patients with fragmented or normal motor activity rhythms (Hätönen *et al.* 1999). In addition, bright light therapy has been demonstrated to have some positive effects in neurologically disabled children with sleep-wake rhythm problems (Guilleminault *et al.* 1993).

The study of Haimov and associates (1994) has documented lower melatonin levels in elderly people with poor sleep compared with the elderly with no sleep complaints. It is suggested that exogenously administered melatonin may be beneficial in elderly insomniacs (Garfinkel *et al.* 1995, Haimov *et al.* 1995), and in sleep problems that are related to melatonin deficiency (Etzioni *et al.* 1996, Lehmann *et al.* 1996). On the other hand, a study of Youngstedt and coworkers (1998) showed no association between low melatonin and insomnia. Furthermore, bright light treatment has been shown to have beneficial effects on rest-activity rhythm disturbances and sleep maintaining in old demented patients and elderly subjects (Murphy & Campbell 1996, Van Someren *et al.* 1997).

### **Seasonal affective disorder**

Seasonal affective disorder (SAD) was originally defined as a syndrome in which depression developed during the autumn or winter and remitted in the spring or summer (Marx 1946, Rosenthal *et al.* 1984). At present, it is classified as a form of recurrent depressive or bipolar disorder, and it is characterized by episodes that vary in severity (Partonen & Lönnqvist 1998).

Bright light exposure treatment has been used successfully in SAD (Lewy *et al.* 1987, Eastman *et al.* 1992, Partonen 1994, Eastman *et al.* 1998, Lewy *et al.* 1998c, Terman *et al.* 1998). However, the mechanism by which light reduces depressive symptoms is unknown, and even nonphotic high-density negative air ionization has been shown to act as an antidepressant in patients with SAD (Terman *et al.* 1998). This rhythmic blue disorder was also proposed to be linked to melatonin secretion (Lewy *et al.* 1987), but later findings showed that winter depression patients do not have abnormal melatonin levels (Checkley *et al.* 1993), and melatonin suppression does not appear to be causally involved in the antidepressant effects of bright light therapy on the affective illness (Dietzel *et al.* 1985, Wehr *et al.* 1986). Exogenous melatonin has been demonstrated to decrease (Lewy *et al.* 1998b) or have no effect on (Witz-Justice *et al.* 1990) the depressive symptoms of SAD patients. However, because alternative

treatment is available for SAD, such as nondrug therapy by bright light, melatonin is not considered a first-choice treatment at the present time.

### **Conclusions of light and melatonin treatment**

Light may well affect mood (Lewy *et al.* 1987, Eastman *et al.* 1992, Partonen 1994, Eastman *et al.* 1998, Lewy *et al.* 1998c, Terman *et al.* 1998). In fact, there is now general agreement that the treatment of choice in patients suffering from seasonal affective disorder is bright light exposures (Partonen & Lönnqvist 1998). In addition, light therapy can be useful in the treatment of delayed and advanced sleep phase syndromes (Chesson *et al.* 1999). However, the benefits of light treatment are less clear in jet lag, shift work, and in non-24-hour sleep-wake syndrome of blind people. Above all, further applied research in everyday conditions is required, because it is important to find out how alterations in indoor and outdoor lighting conditions and avoidance of bright light exposures affect the entrainment of circadian timing system.

Although melatonin promotes sleep in humans (Zhdanova *et al.* 1997), it is clear that melatonin is not primarily a sleep hormone, because in nocturnal species melatonin is associated with wake and activity (Mendelson *et al.* 1980, Huber *et al.* 1998). The majority of published data indicates that melatonin has a therapeutic potential in circadian rhythm-related sleep disorders (Arendt *et al.* 1997, Arendt & Deacon 1997). As yet, however, its mechanism of action remains unclear, the appropriate dose, timing and method of delivery with respect to any given condition and individual are uncertain, the contraindications remain to be defined, and there are virtually no data on the long-term safety or about the use with concomitant medication or organic disease.

Although melatonin functions in mammalian physiology as an important photoperiodic messenger molecule (Wehr 1997), very little information is available concerning its function as a neuroendocrine phototransducer in humans. Indeed, much further research is needed on melatonin's interaction with light and LD alterations.

## AIMS OF THE STUDY

This thesis was focused to examine the significance of light in the regulation of human melatonin hormone.

The results of the investigations performed before the present studies give rise to the following conclusions. Blindness may prevent the entry of light to the circadian system and the circadian rhythms are free-running in many blind people. In our earlier study, however, it was found that the cortisol and melatonin rhythms in blind neuronal ceroid lipofuscinosis patients were disturbed only in a minority of the patients. The transduction of light declines also in sighted people when the eyes are closed, but there was no former evidence of light-induced reduction of melatonin levels through closed eyelids. Further, it was thought that shifting the phase of the melatonin rhythm requires higher illuminances than the suppression of melatonin levels. There was no earlier information about the phase shifting effect of low intensity light in humans. Furthermore, light acutely suppresses melatonin levels during the abundant melatonin synthesis and both light and melatonin are able to reset the circadian timing system, suggesting that the direct suppressing effect of light on the secretion of melatonin can be one of the factors related to the phase shifting of the melatonin rhythm.

Based on prior knowledge we formulated four hypotheses presented below. Light-induced changes in the secretion of melatonin were investigated to gain the following specific objectives.

**I) *Light suppresses melatonin synthesis in blind neuronal ceroid lipofuscinosis patients.***

The ability of bright light to suppress melatonin synthesis in blind neuronal ceroid lipofuscinosis patients with degenerated retinas was investigated in order to find out whether an intact retina is needed for the transmission of light to the human circadian timing system.

**II) *Closed eyelids limit the entry of photic information to the hypothalamic level.***

The ability of bright light to suppress melatonin synthesis through closed eyelids in healthy people was investigated in order to find out how much closed eyelids (*e.g.* during sleep) limit the transmission of light to the circadian timing system.

**III) *Moderate illuminance induces a phase delay of the human melatonin rhythm.***

The ability of ordinary room illuminance to induce a phase shift of the melatonin rhythm in healthy people was investigated in order to find out whether the melatonin rhythm of urbanized people is adjusted by light in everyday conditions.

**IV) *Exogenous melatonin counteracts the light-induced phase delay of the melatonin rhythm.***

The ability of exogenous melatonin to nullify the phase shifts of the endogenous melatonin rhythm induced by bright light was investigated in order to find out whether the effect of light on the human melatonin rhythm is mediated by a mechanism involving a decrease of melatonin concentration.

## **MATERIALS AND METHODS**

More detailed descriptions of the materials and methods are presented in the original publications.

### **ETHICAL CONSIDERATIONS**

All volunteers (II,III,IV), the control subjects (I), one patient and the relatives of the mentally retarded patients (I) gave written informed consent after the nature of the experiments had been explained (Declaration of Helsinki). The study plans (I,IV) were approved by the Ethical Committees of the Institutions. The oral administration of melatonin (IV) was permitted by the Finnish National Agency for Medicines, Ministry of Social Affairs and Health.

### **SUBJECTS AND GENERAL PROCEDURES**

#### **Suppression of melatonin without conscious light perception**

The penetration of light information to the hypothalamic level and pineal gland of clinically blind NCL patients and sighted control subjects were examined (I). The experiments were carried out in three INCL patients (one girl and two boys, ages 6-11 years), four JNCL patients (one female and three males, ages 13-28 years), and seven healthy drug-free control subjects (two women and five men, ages 18-27 years). All patients had been practically blind for years and all had neurological impairment. All patients, except one man (patient 7), were mentally retarded and had epilepsy. All patients received antiepileptic drugs, and JNCL patients received antioxidant therapy (Santavuori *et al.* 1988). Pupillary light reflexes, noncorneal ERG, and VEPs to flashes of light were studied.

Each patient and control subject participated in two sessions. During the dim-light control session, the individual melatonin profiles were determined. The samples were collected before midnight in ordinary room light (50 to 200 lux) and after midnight in a dimly lit room (<10 lux). During the second session, a 60-min light exposure (3000 lux) was applied during the latter half of the rising phase of the melatonin production. The blood samples were collected every 60 minutes from 1900 to 0600 h and an additional sample was taken 30 minutes after the beginning of the light exposure.

#### **Suppression of melatonin through closed eyelids**

The effect of bright light on melatonin through closed eyelids was studied by exposing the volunteers to 2000-lux light during the rising phase of the melatonin secretion (II). Salivary melatonin levels were measured in 8 healthy drug-free volunteers (3 males, 5 females, ages 20-53 years). The volunteers came to the laboratory for 3 sessions: first for the determination of the control pattern of melatonin in dim light with the eyes open, then for the 2000-lux light exposure either with the eyes open or closed. The samples were collected every hour from 1900 to 0200 or 0300 h, before 2400 h in ordinary room

light (50-200 lux) and after 2400 h in dim light (<10 lux). During the second and third sessions, the 2000-lux light for 60 min was applied during the rising phase of the melatonin synthesis determined individually according to the control melatonin profiles. When the exposure occurred with the eyelids closed, the eyelashes of the subjects were taped to the skin under the eyes so that the tape did not cover the upper eyelids. The light exposure times were between 2400 and 0200 h, and during the exposures the samples were collected at 15-min intervals.

### **Phase shift of melatonin rhythm by 500-lux light**

In the first experiment the effect of moderate illuminance level on the melatonin rhythm was studied by exposing the volunteers to 500-lux light at 2300 h for 60 min (III). Salivary melatonin levels were measured in 7 volunteers (3 males and 4 females, ages 21-37 years). They were healthy and drug-free students or personnel of the institute. On day 1 the control melatonin profiles were determined, day 2 was for rest, on day 3 the melatonin production was interrupted by a 60-min light exposure (from 2300 to 2400 h), and on day 4 the samples were collected as on day 1. Lighting was <100 lux until 2400 h. During the sleeping period (from 2400 to 0700 h) lighting was <0.1 lux. From 0700 to 1000 h the illuminance was <100 lux. During the sessions, samples were collected every hour from 2000 to 1000 h, except at 0300 and 0500 h.

In the second experiment the effect of a moderate illuminance level on the melatonin rhythm was studied by exposing the subjects to 500-lux light at matched exposure times during the latter half of the rising phase of the melatonin production (III). Salivary melatonin levels were measured in 5 healthy volunteers (1 female and 4 male students, ages 18-19 years). On day 1, the samples were collected every hour from 1800 to 1100 h under constant illumination of <10 lux. After a week, on day 8, the 500-lux light exposure was from 2300 to 2400, from 2400 to 0100, or from 0100 to 0200 h according to the individual melatonin profiles measured on the experimental control day 1. On day 8, the samples were collected immediately before and after the light exposure and 0.5, 1 and 2 hours before and after the exposure. On day 9, the samples were collected every hour from 1800 to 1100 h as during day 1.

### **Phase shift of melatonin rhythm by light with concomitant exogenous melatonin administration**

The effect of bright light on the human melatonin rhythm was examined by exposing the subjects to 2000-lux light at matched exposure times during the latter half of the rising phase of the melatonin production. The light-induced decrease of melatonin concentration was replaced with exogenous melatonin in a placebo-controlled, single-blind, randomized study (IV). Salivary melatonin levels were measured in 6 volunteers (1 male and 5 females, ages 20-52 years). They were healthy and drug-free students or personnel of the institute. Both the placebo and melatonin parts of the study consisted of three sessions: a control session, a light-exposure session and an after-light session. The lighting was <10 lux in the laboratory. During the control and after-light session, samples were collected every hour from 1800 to 1100 h. One week after the control session, the subjects came to the laboratory for the light-exposure session. Placebo or 0.5 mg melatonin (supplied by Sigma Warrants, St. Louis, US; prepared by the Pharmacy at Helsinki University Hospital, Helsinki, Finland) in gelatin capsules were taken 60 min before the light exposure. The 2000-lux light exposure occurred from 2400 to 0100 or from 0100 to 0200 h according to the control melatonin profiles.

The effect of prolongation of dim morning light on the melatonin rhythm was also tested (IV). Salivary melatonin levels were measured in 6 volunteers (2 males and 4 females, ages 20-55 years). They were healthy and drug-free students or personnel of the institute. The subjects were in the laboratory two consecutive nights from 1800 to 1100 h, and samples were collected every hour. The illuminance was <10 lux during the experiment.

## **LIGHT SOURCES AND EXPOSURE PROCEDURES**

In the patient study (I), take-along lamps were used to facilitate the experimental procedures involving the NCL patients. The highest intensity achieved by these lamps was 3000 lux, measured at the forehead of each subject (Chroma Meter CL-100, Minolta Camera Co., Ltd., Osaka, Japan). During the exposures, the patients and control subjects were lying in bed and the lamps (fluorescent tubes, 11W, 2700K, Lival, Helsinki, Finland), forming a reflection area of 20 x 10 cm<sup>2</sup>, were placed in the correct position (about 20 cm from the eyes). The penetration of light to the eyes was controlled as follows. During the whole exposure time a researcher inspected the patient's eyes. If the eyes were not open, the experimenter opened them by hand every 2 minutes for 2 seconds. Two of seven patients kept their eyes open all the time and thus two control subjects were exposed to light with the eyes open. The eyes of the other five patients were opened by the researchers and five control subjects opened their eyes by themselves similarly every 2 minutes by request.

In the other studies (II-IV), the light source was a panel on a wall. It was composed of 16 True-Lite fluorescent tubes (40W, 5500K), mounted vertically on a wall, and forming a luminant area 2.4 m wide and 1.3 m high. In front of the tubes there was a white flashed opal glass for diffusing light evenly over the panel. In order to achieve the desired illuminance at the eye level, the subjects were sitting at the correct distance from the panel and they were instructed to gaze the light source all the time during the exposures.

## **MEASUREMENT OF MELATONIN AND CHARACTERIZATION OF THE PATTERNS**

### **Radioimmunoassay**

Melatonin was measured from saliva (II,III,IV) or serum (I) samples by the radioimmunological method developed by Vakkuri and co-workers (1984) using radioiodinated melatonin as a tracer. This method has earlier been validated for saliva samples, and the salivary levels have been 30-40% of the levels in serum (Vakkuri 1985, Laakso *et al.* 1990). The properties of antiserum raised in rabbits by immunization with bovine thyroglobulin conjugate of *N*-acetyl-5-methoxytryptophan as described by Arendt and coworkers (1975) have been published previously (Laakso *et al.* 1988). The reference samples were prepared from synthetic melatonin (Sigma, St. Louis, MO, US) dissolved in the assay buffer over the range 1.95-1,000 pg/ml.

The nonspecific binding of the tracer was 5 to 6%. The least detectable concentration, defined as apparent concentration at two standard deviations (SDs) from the counts at maximum binding, was

smaller than the lowest standard. The intra-assay variability, calculated from the duplicate measurements, was 4 to 10%. During the assays of this study the interassay variability, calculated as SDs of the quality control samples, was 5 to 19% depending on the concentration.

### **Calculations of individual phase marker times and phase shifts**

In order to characterize the individual melatonin patterns and to calculate the phase shifts (III,IV), the half-rise and half-decline times were determined as follows: (i) peak level was calculated as the mean of the three highest values of the pattern, (ii) baseline level was the mean of the three lowest values of the pattern, (iii) half-maximal level was defined as the half-way point between the peak and baseline level, and (iv) half-rise and half-decline times were estimated graphically from the drawings of the individual raw data without smoothing the curves. They were the time points at which the half-maximal level was reached in the ascending or descending part of the pattern, respectively. The individual phase shifts were the differences of half-rise times or half-decline times between the two patterns.

### **STATISTICS**

The melatonin patterns were evaluated with two-way analysis of variance (ANOVA) for repeated measures followed by parametric post tests (Tukey-Kramer or Bonferroni for selected pairs). Logarithmic values of the data were used when Barlett's test showed heterogeneity of variances. Student's t-test or paired t-test was used in evaluations of two groups of samples. Nonparametric Wilcoxon test was chosen for calculations if the data were not normally distributed and did not fulfil the criteria for parametric statistical tests. Spearman's rank order correlation coefficients were calculated to evaluate relationship between two groups of samples.

## RESULTS

### SUPPRESSING EFFECT OF LIGHT ON MELATONIN SECRETION

#### Bright light exposure in patients without conscious light perception

The effect of a 3000-lux, 60-min light pulse on melatonin levels was studied in control subjects and NCL patients (I). The serum melatonin concentrations of all seven control subjects and six of seven patients increased during the night. One patient's (patient 6) melatonin pattern was abnormal, without a clear maximum at nighttime. The data from this patient are included in the calculations. The ranges of the highest levels were 67 to 181 ng/l (control subjects) and 21 to 164 ng/l (patients). In the control subjects, the suppression of the average melatonin level by light seemed to be quite modest, but in the patients, the effect of light was more evident. However, the average melatonin pattern during the dim-light session differed significantly from that during the light-exposure session in both groups (controls: session NS, time  $p < 0.001$ , session x time  $p < 0.02$ ; patients: session NS, time  $p < 0.001$ , session x time  $p < 0.001$ ; two-way ANOVA). Bonferroni multiple comparisons test for selected pairs did not show any differences in the dim-light melatonin levels between the control subjects and patients, but the concentrations at the end of the light exposure were significantly lower in patients than control subjects ( $p < 0.05$ ). The postlight level and the corresponding dim-light level of the patients were significantly different from each other ( $p < 0.01$ ).

The 3000-lux light exposure suppressed melatonin in three of seven control subjects (in the two control subjects with the eyes open during the exposure and in one who opened his eyes every 2 minutes for 2 seconds). The average postlight level was equal to the prelight level, but tended to be lower than the corresponding control level (80%). The light exposure reduced melatonin concentrations in all seven patients. The average level after the light pulse was 61% of the prelight level and 51% of the corresponding level in the control session. The decrease of melatonin level by light tended to be more marked in patients than control subjects ( $p = 0.058$ , t-test). After one hour of recovery in darkness, the average melatonin levels were close to the prelight values both in control subjects and patients. When expressed in percentages of prelight values, they did not differ significantly from each other. However, in the patients, the concentration remained clearly lower than the corresponding control level (58%), but in the healthy subjects, the level was almost the same as during the control session (88%). The recovered levels, expressed in percentages of the dim-light control melatonin concentrations, were significantly lower in patients than control subjects ( $p = 0.032$ , t-test).

#### Bright light exposure in subjects with closed eyelids

The effect of a 2000-lux, 60-min light pulse on melatonin secretion was examined in volunteers with the eyes closed (II). The salivary melatonin concentrations of all 8 subjects increased during the dim-light control session. The range of the highest melatonin levels was 18-65 ng/l. The suppressive effect of 2000-lux light was evident in all subjects when they were exposed to light with their eyes open (control *versus* eyes open session: session  $p < 0.02$ , time  $p < 0.001$ , session x time  $p < 0.001$ ; two-way

ANOVA). The average remaining concentration at the end of exposure was  $45\pm 14\%$  ( $\pm$ SD) of the prelight level and  $30\pm 18\%$  of the corresponding control concentration. When the subjects kept their eyes closed, the light exposure diminished the melatonin levels only in 2 of the 8 volunteers. In the other 6 subjects they remained at the prelight level or continued to increase. The average remaining concentration at the end of exposure with closed eyes was  $121\pm 61\%$  of the prelight level and  $78\pm 24\%$  of the corresponding control concentration. The overall melatonin level, however, was lower during the eyes-closed session than dim-light control session (session  $p<0.05$ , time  $p<0.005$ , session x time NS; two-way ANOVA).

## **PHASE SHIFTING EFFECT OF LIGHT ON MELATONIN RHYTHM**

### **Phase shift of melatonin rhythm by moderate illuminance**

The effect of moderate illuminance (500 lux) for 60 min on the melatonin rhythm was examined in healthy volunteers (III). The salivary melatonin concentration of all subjects increased during the dark period, but the interindividual variation of peak levels was great (range 7-51 ng/l). In the first experiment in which the light exposure occurred at the early or late rising phase of the pattern, the average melatonin profile during the night following the exposure did not differ significantly from the control profile (session, NS; time,  $p<0.001$ ; session x time, NS; two-way ANOVA). In the second experiment in which the exposure time was individually adjusted to the late rising phase, two-way ANOVA suggested that the melatonin levels at some time points were significantly different, but the overall melatonin levels did not differ (session, NS; time,  $p<0.001$ ; session x time,  $p<0.01$ ).

By combining the results of both experiments, the difference between the control and experimental onset times was statistically significant in parametric ( $p<0.01$ , t-test) as well as in nonparametric ( $p<0.005$ , Wilcoxon) testing. The delay of offset occurred in 7 of 12 subjects, but it was not statistically significant (NS, t-test; NS, Wilcoxon test). The suppressive effect of the 500-lux light exposure varied individually between 21 and 83% of the prelight level; the average suppression was about half of the prelight level ( $56\pm 18\%$ ). No significant correlation was found between the percentage of the suppression and the magnitude of the delay of the melatonin onset ( $r_s = -0.112$ , Spearman's correlation coefficient), but the delay of the melatonin offset was the greater the more the melatonin level was suppressed ( $r_s = +0.775$ ,  $p<0.01$ ).

### **Phase shift of melatonin rhythm by bright light with concomitant exogenous melatonin administration**

The effect of exogenous melatonin on a bright light pulse (2000 lux, 60 min) induced phase shift of the melatonin rhythm was studied in healthy volunteers (IV). The salivary melatonin concentration of all volunteers increased during the night. The interindividual variation of the highest levels was 18-71 ng/l. The mean melatonin profiles of the control sessions in the placebo and melatonin experiments were similar (two-way ANOVA). The suppressive effect of the 2000-lux light exposure varied individually between 44 and 80% of the prelight level; the average suppression was  $64\pm 14\%$  of the prelight level and  $72\pm 11\%$  of the corresponding level in the control session. There was great interindividual variation among the peak melatonin concentrations reached after the oral dosage of

melatonin (range 39-1096 ng/l; mean 407 ng/l). There was no difference between the half-rise times of the two control sessions (placebo *versus* melatonin session); nor did the half-decline times differ between those sessions (NS, paired t-test). In the placebo experiment, there was a delay (mean delay 0.5 h) between the half-rise times of the control and after-light session in 5 of the 6 subjects, whereas the half-rise times did not differ significantly between the sessions. In the melatonin experiment, the half-rise times of the control and after-light session differed significantly from each other ( $p < 0.005$ , t-test; mean delay 0.8 h). The half-decline times (control *versus* after-light session) differed significantly in both placebo ( $p < 0.05$ , t-test; mean delay 0.7 h) and melatonin experiment ( $p < 0.02$ , t-test; mean delay 0.7 h).

The effect of the prolongation of dim morning light on the melatonin rhythm was studied in healthy volunteers (IV). The interindividual variation of the highest melatonin levels was 24-52 ng/l. The mean melatonin profiles of the two consecutive nights were similar (two-way ANOVA). The half-rise and half-decline times did not differ between those nights (NS, t-test).

## DISCUSSION

### SUPPRESSING EFFECT OF LIGHT ON MELATONIN SECRETION

#### **Bright light exposure in patients without conscious light perception**

Study I shows that bright light is able to suppress melatonin secretion in INCL and JNCL patients despite their degenerated retinas and visual failure, indicating that the photic pathway used by the circadian system may be able to function in NCL patients without conscious light perception. This is in line with the results of Czeisler and coworkers (1995) who described three patients who despite different origins of blindness (retinopathy or optic neuropathy) and totally lost pupillary light reflexes responded positively in a melatonin suppression test (~10 000-lux light exposure for 90 to 100 minutes). Furthermore, alterations in melatonin concentrations, body temperature, subjective sleepiness and mood have been reported after a 2-week morning bright light intervention in a group of blind as well as sighted subjects (Partonen *et al.* 1995).

In our study the suppression of melatonin by bright light (3000 lux, 60 minutes) was more distinct in the patients than control subjects. It is possible that NCL patients are exceptionally sensitive to the hypothalamic effects of light for some unknown reason. For instance, the degeneration of the retinas induced by the NCL disease (Goebel *et al.* 1974, Tarkkanen *et al.* 1977) may affect neural and pigment layers allowing more light to enter ocular vessels. Indeed, Oren (1996, 1997) has hypothesized that the retina within its prominent blood vessels may be a primary site of phototransduction by the aid of some blood-borne light-sensitive compounds.

However, the difference in the suppression between the patients and sighted subjects could be explained by methodological factors as well: the eyes of the patients were opened by the researchers whereas the control subjects opened their eyes themselves. We did not force the uncooperative patients to keep their eyes closed during the intervals between the eye openings. Thus, some patients might have opened their eyes during the intervals and received more light during the whole exposure than the control subjects who kept their eyes closed and opened them only by request.

Another reason for the greater suppression in the patients might be their abnormal pupillary reflexes. The pupil diameter may be a factor in the effectiveness of light stimuli to suppress melatonin (Gaddy *et al.* 1993). Because of the slow pupillary reflex in six of the seven patients, more light may have entered their retinas.

A third explanation for the difference might be provided by the different age ranges of the groups. For ethical reasons, all control subjects were young adult volunteers, whereas four of seven patients were children between 6 and 13 years of age. The transmission of light through the optic structures of the eye changes somewhat with age (Lerman 1987, Brainard *et al.* 1993, Brainard *et al.* 1997). The lenticular transmission of short wavelengths is especially reduced. Although the age difference between the groups was not great, it is, at least theoretically, a source of error.

A few studies suggest that extraretinal photoreception can participate in rhythm regulation in humans (Campbell & Murphy 1998) and in rats (Jagota *et al.* 1999). However, there are also contradictory results proposing that neither nonhuman mammals (Nelson & Zucker 1981, Ibuka 1987, Meijer *et al.* 1999, Yamazaki *et al.* 1999) nor humans (Lindblom *et al.* 2000) have nonocular circadian photoreception. In any case, there is evidence against suppression of melatonin levels by skin illumination in humans (Lockley *et al.* 1998, Hébert *et al.* 1999, Lindblom *et al.* in press) and in hamsters (Yamazaki *et al.* 1999), indicating that in our study in INCL and JNCL patients the suppression of melatonin was not induced by light exposure to the facial skin. This is supported by a study of Czeisler and coworkers (1995) in which bright light suppressed melatonin secretion in two blind subjects only when their eyes were uncovered.

The average suppression of melatonin in our healthy control subjects was quite slight compared with the findings that illuminances as low as 300-500 lux were sufficient for a significant suppression of melatonin levels (Bojkowski *et al.* 1987, McIntyre *et al.* 1989, Hashimoto *et al.* 1996, Aoki *et al.* 1998). Our result was probably due to five of the seven subjects opening their eyes only every 2 minutes, because closed eyelids may limit the amount of light entering to the hypothalamus and pineal gland (II).

Blindness is one of the main symptoms in NCL patients. Degeneration of the retina with loss of photoreceptors has been found in both the INCL and JNCL disease (Haltia *et al.* 1973, Goebel *et al.* 1974, Tarkkanen *et al.* 1977, Spalton *et al.* 1980, Traboulsi *et al.* 1987, Goebel *et al.* 1988). Based on ophthalmological and neurophysiological findings, the blindness in INCL and JNCL patients can be both of retinal and cortical origin (Raitta & Santavuori 1973, Pampiglione & Harden 1977, Santavuori 1988, Santavuori *et al.* 1988, Horiguchi & Miyake 1992, Seeliger *et al.* 1997, Vanhanen *et al.* 1997, Weleber 1998). All our patients responded to light with pupillary constriction, although the reflex could be considered normal only in one patient. Despite these deficiencies and abnormalities in visual function, the suppression of melatonin by light was clearly apparent in each patient. The findings could be interpreted as evidence for intact retinohypothalamic pathways and sympathetic connections to the pineal gland in spite of the affected visual pathways.

Although the retinal damage induced by the NCL disease can be determined on the basis of autopsies, the present study does not provide information about the quality of the retinal cells mediating light information to the melatonin regulating system, because we have no knowledge of the patients' retinal structure at the time of the experiment.

In fact, it is not at all clear what kinds of retinal receptors are involved in circadian timekeeping. Originally they were thought to be classical photoreceptors, rods and cones (Thiele & Meissl 1987) or only rhodopsin-like pigments (Takahashi *et al.* 1984, Brainard *et al.* 1985, Bronstein *et al.* 1987). More recently, the role of traditional photoreceptors have been challenged by studies showing circadian responses to light without rods, or without cones, or only with fragments of photoreceptors (Foster *et al.* 1993, Provencio *et al.* 1994, Ruberg *et al.* 1996, Lucas & Foster 1999). Nevertheless, it seems that there might be some unidentified retinal light-sensitive elements involved in circadian function (Yoshimura & Ebihara 1996, Freedman *et al.* 1999, Huerta *et al.* 1999, Lucas *et al.* 1999).

Furthermore, compensatory structures subserving photic functions (Cooper *et al.* 1993) might be recruited by blindness. Perhaps a distinct subset of retinal ganglion cells working as a unique communicator for the circadian timing system (Moore *et al.* 1995) elaborates some kinds of coping mechanisms generated by the degenerative niche.

In our recent study in NCL patients, disturbances in the daily melatonin and cortisol rhythms occurred only in a minority of the patients and only at an advanced state of the disease (Heikkilä *et al.* 1995), suggesting that light may entrain the hormonal rhythms despite the blindness. Although the sleep-wake rhythms were not normal in the patients, it seems that light may have an impact on other rhythmical outputs. This may be due to a direct effect of light on the hypothalamus and autonomic functions as proposed by studies showing *e.g.* that the heart rate (Warren *et al.* 1994, Scheer *et al.* 1999) or the menstrual cycle length (Rex *et al.* 1997) respond to light.

Taken together, the present results propose that the ability of light to reach the hypothalamus and regulate neuroendocrine functions may persist long after a visual failure appears. The quality of the photoreceptive cells needed for the transmission of light from the eyes to the circadian timing system is not known. However, it could be proposed on the basis of the present study that the traditional retinal photoreceptors are not essential or that only a small number of these cells are needed for the circadian function in humans, as is also suggested by animal studies. Moreover, there may be some unidentified pathways through which the circadian effects of light may be transmitted to the human body. Thus, taking care of proper environmental lighting conditions may enhance the well-being of those blind people in whom light is able to penetrate, at least to some extent, the optical structures.

### **Bright light exposure in subjects with closed eyelids**

Study II shows that melatonin concentrations decreased only in 2 of the 8 volunteers during the light-exposure sessions (2000 lux, 60 min) with the eyes closed, although the suppressive effect was evident in all subjects with their eyes open. To my knowledge, a light-induced reduction of melatonin levels through closed eyelids has not been reported earlier. However, it has been observed that 6-17-lux (photopic) or 28-86-lux (scotopic) illuminance of monochromatic light of 509 nm is sufficient to suppress melatonin within 60 min in humans examined in specially controlled conditions when the pupils have been dilated, the volunteers' heads kept motionless and the light beam directed uniformly on the retina (Brainard *et al.* 1988). In other studies, higher illuminances (300 to 500 lux) have been needed to suppress melatonin levels significantly (Bojkowski *et al.* 1987, McIntyre *et al.* 1989, Hashimoto *et al.* 1996, Aoki *et al.* 1998) and in earlier studies light has not been observed to suppress melatonin (Vaughan *et al.* 1976, Jimerson *et al.* 1977, Vaughan *et al.* 1979, Åkerstedt *et al.* 1979). The variation of the results is probably caused by differences in the gaze control of subjects during the light exposures. Indeed, the direction of gaze is one of the main factors influencing the amount of light reaching the retina, and gaze aversion can reduce both the illuminated corneal and retinal area (Dawson & Campbell 1990, Gaddy 1990).

The transmittance of light through human eyelids has been found to be 10-15% in the red end of the spectrum at 700 nm declining to approximately 3% below 600 nm in measurements conducted *in vivo* (Moseley 1988, Robinson *et al.* 1991). In our study, the varying results could be explained so that in the two responders the transmitted amount of light produced a retinal illuminance that exceeded the lowest effective limit for suppression whereas in the other subjects less light was transmitted or their individual thresholds to respond were higher.

Some methodological reasons may also explain the different responses in the subjects. Our subjects were asked to gaze directly at the light panel during the entire 60-min exposure. When exposed to light with the eyes open, the behavior of the subjects could be controlled by the researcher. When the exposure occurred with closed eyelids, the head position could be controlled but the gaze behavior could not. For instance, the so called Bell's phenomenon (palpebro-oculogyric reflex), in which forcible closing of the eyelids causes the eyes to turn up and out (Newell & Ernest 1974), might have affected the admission of light to the retina in some subjects.

The degree of skin pigmentation could not have caused any major variation in the penetration of light because all subjects were blond northern people. Both responders were young females with relatively high peak levels of melatonin. Therefore, it could be suggested that melatonin suppression through closed eyelids occurred also in the low-secretors but it was so small that it was not detected with the measurement method applied. However, this explanation does not seem valid since the young female with the highest melatonin peak did not respond. The interindividual variation might also have resulted from the subjects being exposed at different circadian phases. This explanation is not convincing either, because it has been shown that sensitivity of the human melatonin synthesis to light remains unchanged during the rising phase (Laakso *et al.* 1994c).

The present study shows that closed eyelids can induce interindividual variation in melatonin suppression induced by 2000-lux light. In ambulatory measurements, usual room illumination has been reported to be 150-180 lux (Koller *et al.* 1993) and people in the modern industrialized society spend >90% of their time at ambient illuminance levels below 1000 lux (Savides *et al.* 1986). This indicates that the common indoor conditions, where the illuminance is usually much lower than 2000 lux, do not provide enough light to suppress melatonin secretion in humans with closed eyelids. However, it is possible that high illuminances (*e.g.* 10 000 lux) can be effective also through closed eyelids, for instance during sleeping.

The present finding is in agreement with the studies indicating that light exposure without penetration of light *via* the eyes has no effect on melatonin levels in humans (Czeisler *et al.* 1995, Lockley *et al.* 1998, Hébert *et al.* 1999, Lindblom *et al.* in press). However, light may influence performance, daytime alertness and mood (Campbell *et al.* 1995, Daurat *et al.* 1996, Partonen & Lönnqvist 1998) even when applied to sleeping subjects (Avery *et al.* 1994, Wirz-Justice *et al.* 1997), most probably by some unknown mechanisms.

## PHASE SHIFTING EFFECT OF LIGHT ON MELATONIN RHYTHM

### Phase shift of melatonin rhythm by moderate illuminance

Study III indicates that a single light pulse of relatively low intensity (500 lux) and short duration (60 min) induces interruption of melatonin secretion and delays the melatonin rhythm one day after the exposure. Furthermore, the increase of background illuminance from <1 lux to <100 lux in the morning suppressed or stopped the melatonin synthesis, and the mean melatonin offset time was not delayed. The intensity of the exposure used in the present study was clearly lower than the intensities previously thought to be required to phase shift or entrain the circadian pacemaker in humans (Lewy *et al.* 1984, Lewy *et al.* 1985, Lewy *et al.* 1987, Wever 1989).

More recently, it has been confirmed that light of domestic intensity (150-500 lux) produces phase shifts of the human melatonin (Zeitzer *et al.* 1997, Boivin & Czeisler 1998) and body temperature rhythm (Waterhouse *et al.* 1998), proposing together with the present results that ordinary indoor room light could exert physiological effects on circadian functions of individuals living most of the time in artificial lighting conditions. If this proposition holds true, the melatonin signal can be considered a sensitive marker of environmental lighting conditions, even in urbanized people.

In the present study, there is a methodological factor which has to be considered when assessing the results. Repeated (during 3 consecutive days) light exposures of high intensity (~5000-10000 lux) and of long duration (5-6 hours) have been shown to induce changes in the melatonin rhythm even during daytime (Hashimoto *et al.* 1997, Jewett *et al.* 1997). In the present study the volunteers were allowed to leave the laboratory during the intervals of the sessions. However, they were instructed to avoid exceptionally bright light exposures and asked not to take naps during daytime hours. In the PRCs to light, the phase shifts of the human melatonin or temperature rhythm have been small or absent during daytime (Minors *et al.* 1991, Van Cauter *et al.* 1994). Moreover, if any shifts occur during daytime hours, light may induce phase advances rather than delays of the melatonin rhythm (Dijk *et al.* 1989, Burešová *et al.* 1991, Van Cauter *et al.* 1994), indicating that the phase delays of the melatonin rhythm in the present study were induced by the experimental light pulses. This is supported by the results of Study IV of this thesis, in which the conditions were similar to those of the present study, showing that the mean melatonin patterns of two consecutive nights were equal despite the subjects being away from the laboratory during the day between the sessions. In addition, in Study IV no shifts of the melatonin rhythm were found between the two control sessions with intervals of 4 to 9 weeks. Taken together, the above findings could be interpreted as evidence that extraneous light did not significantly influence the results, and that the changes observed in the melatonin levels and rhythms were induced by the 500-lux moderate illuminance.

The present study and other studies conducted later (Zeitzer *et al.* 1997, Boivin & Czeisler 1998, Waterhouse *et al.* 1998) propose that human body temperature and melatonin rhythms are influenced by light of moderate intensity. At present, it is not known whether other human rhythms, *e.g.* sleep-wake rhythms, are adjusted by light of domestic intensity. It is possible that the simple rhythms of temperature and melatonin are mainly adjusted by lighting, while in the regulation of more complex rhythms, *e.g.* performance and rest-activity rhythms, repeated or stronger signals of light are required.

## **Phase shift of melatonin rhythm by bright light with concomitant exogenous melatonin administration**

In Study IV a 60-min, 2000-lux light pulse during the rising phase of melatonin synthesis delayed the average half-decline and half-rise times equally in the placebo and melatonin replacement experiments. This is consistent with the study of Yellon and Hilliker (1994) who used a similar paradigm and showed that a melatonin injection to hamsters before the light pulse failed to alter the effect of light on the melatonin rhythm. The inefficacy of melatonin to counteract the light-induced phase shift in humans has been confirmed by Kräuchi and coworkers (1997a) who showed that the hypothermic effect of late evening exogenous melatonin does not block the phase delay induced by a concurrent 5000-lux light exposure in humans. Quite recently, in a study by Kennaway and Rowe (2000), stimulation of endogenous melatonin production by isoproterenol failed to block the phase shifting effect of a light exposure on melatonin metabolite excretion in rats. These findings suggest that the suppression of melatonin by light stimulation is not responsible for the phase delay in melatonin production.

On the other hand, slightly different experimental paradigms have disclosed some interactions between melatonin and light exposure. For instance, the phase advance of melatonin offset was inhibited and the phase advance of melatonin onset was enhanced by a 3000-lux light stimulus with concomitant melatonin administration in humans (Cagnacci *et al.* 1997). In addition, Benloucif and coworkers (1999) found that combined administration of melatonin and light in mice affected circadian timing in a complex manner which could not be predicted by summing the effects of melatonin and light treatments given separately.

Melatonin has binding sites in the circadian clock of the SCN (Vanecek *et al.* 1987, Reppert *et al.* 1988, Weaver *et al.* 1993, Morgan *et al.* 1994) and melatonin is able to reset the discharge rate rhythm of the pacemaker within two windows of sensitivity (McArthur *et al.* 1991, McArthur *et al.* 1997). On the basis of the PRC to exogenous melatonin, the optimal time to produce a phase delay and advance varies depending on the circadian time (Lewy & Sack 1997, Lewy *et al.* 1998a). Thus, the efficacy of melatonin action depends on the phase of the circadian timing system. In the above studies, the variable circadian time of melatonin administration and other methodological factors (*e.g.* dose, administration route, interval between melatonin and light, species) may explain the discrepancies between the results. However, on the basis of the current knowledge there is insufficient evidence to determine the precise conditions under which melatonin interacts with light exposure. Therefore, further studies are needed to clarify the possible influences that the absence or presence of interactions between melatonin and light might have on the rhythm regulation.

There were two intervening factors that might have influenced the results of the present study: the prolongation of dim light in the morning and the abnormally high melatonin levels still in the morning due to the ingestion of the melatonin capsule. Theoretically, both factors could have potentiated the effect of the light exposure. The prolongation of dim light was necessary to avoid a premature cessation of melatonin secretion by light. In the additional control experiment, however, the prolongation of dim light in the morning did not produce any systematic phase shift within one day. Thus, the dim morning light did not seem to be a source of error in the main experiment of the present study.

The dose of melatonin used in the present study (0.5. mg) is considered 'physiological' (Lewy *et al.* 1992) in the sense that it produces plasma levels and a peak duration comparable to the endogenous levels and peak duration. This dose was used to ensure that the suppression caused by the light exposure would be sufficiently replaced. In all subjects, the peak levels of melatonin after the oral dosage were higher than the endogenous peak levels. In the morning, 10-12 hours after the ingestion, the melatonin concentrations were still somewhat higher than the values measured after the placebo. Based on the PRC to melatonin (Lewy *et al.* 1992, Lewy *et al.* 1998a), it seems that this oral dose of melatonin does not produce any major phase shifts when ingested during the period corresponding to the rising phase of the endogenous melatonin synthesis. Therefore, it could be proposed that the delay of the melatonin rhythm was not a consequence of the somewhat elevated melatonin levels in the morning. This indicates that the light exposure preceding the peak time of melatonin secretion was responsible for the phase delay both in the placebo and melatonin experiments.

In conclusion, the present study and the study by Kräuchi and coworkers (1997a) with similar paradigms indicate that the delaying effect of light on the human circadian rhythms do not depend on the melatonin concentration in the circulation. Thus, light is able to influence the circadian rhythms independently of melatonin levels in the body, indicating that melatonin suppression is not an essential part of the mechanism by which light modulates our circadian timing system.

## CONCLUSIONS

A few decades ago it was still thought that light had no impact on the circadian rhythms in humans. The present findings together with other studies indicate that light is the primary external cue regulating our circadian functions. Melatonin acts as a reliable neuroendocrine messenger of environmental lighting conditions, but the phase shifting effect of light on circadian rhythms can be independent of the melatonin concentration in the circulation.

- I) A bright light exposure was able to suppress melatonin secretion in blind neuronal ceroid lipofuscinosis patients with degenerated retinas, proposing that the ability of light to reach the hypothalamus and regulate neuroendocrine functions can persist independently of the visual failure.
- II) A bright light exposure induced a decrease in melatonin concentrations only in a minority of volunteers with closed eyelids. Thus, closed eyelids (*e.g.* during sleep) significantly limit the transmission of light to the circadian timing system.
- III) A single light pulse of moderate room illuminance induced an interruption of melatonin secretion and shifted the phase of the melatonin rhythm. Thus, melatonin acts as a sensitive marker of environmental lighting in everyday conditions.
- IV) A bright light pulse during the rising phase of the melatonin synthesis delayed the melatonin rhythm irrespective of concomitant placebo or melatonin administration. This indicates that suppression of melatonin is not essential for the phase shifting mechanisms of light.

## ACKNOWLEDGEMENTS

This study was carried out during 1993-1998 at the Institute of Biomedicine, Department of Physiology, and at the Hospital for Children and Adolescents, Department of Neurology, University of Helsinki. I wish to thank Professors Olli Jänne, Dag Stenberg, Ismo Virtanen, Jaakko Perheentupa and Matti Iivanainen for providing research facilities at my disposal.

I wish to express my deepest gratitude to my supervisors, Maija-Liisa Laakso and Pirkko Santavuori for their patience, encouragement, and guidance throughout this project. Their enthusiastic attitude towards science will have an everlasting impact on me.

I owe thanks to the reviewers of this thesis, Mikko Härmä and Markku Partinen for their valuable comments and constructive criticism.

I wish to express my thanks to all the personnel of the Institute of Biomedicine, especially to Anne Nenonen for secretarial assistance, Seija Turunen, Nina Katajamäki and Ulla Lähdesmäki for their assistance in obtaining the literature, and Ilkka Linnankoski for the revision of the English language of the manuscript.

I also wish to thank my research colleagues Satu Mustanoja, Mikael Peder, Niki Lindblom, Fredrik Lindroos and Lea Leinonen for their support, help and collaboration, as well as Aino Alila-Johansson for measuring the melatonin samples with excellent accuracy. My sincere thanks to Erika Kirveskari and Kimmo Sainio at the Hospital for Children and Adolescents, and Hannu Heiskala at the Rinnekoti Foundation for fruitful collaboration.

I am very grateful to the NCL families for participating in this study, and to the personnel of the Hospital for Children and Adolescents, Rinnekoti Foundation, Pääjärvi Joint Municipal Authority Rehabilitation Center and Mäntsälä Health Center for their help in the collection of the samples. I also want to thank warmly all the volunteers who made this work possible.

All my friends and relatives are warmly acknowledged for balancing the research work with delightful moments of leisure.

I wish to express my deepest gratitude to my parents Anna-Liisa and Lauri, my brother Timo and his wife Merja, and my parents-in-law Riitta and Eero for their understanding, support and help in many practical ways.

Finally, I am grateful to my dearest husband Pasi for continuous encouragement, love, humour and never-failing cooperation in our everyday life.

The financial support from the Finnish Medical Society Duodecim, the Arvo and Lea Ylppö Foundation, the Finnish Sleep Research Society, the Emil Aaltonen Foundation and the Counselor of the University of Helsinki are gratefully acknowledged.

Helsinki, March 2000

Taina Hätönen

## REFERENCES

- American Sleep Disorders Association. International classification of sleep disorders, revised: Diagnostic and coding manual. Minnesota: American Sleep Disorders Association; 1997. p. 117-140.
- Antoch M P, Song E-J, Chang A-M, Vitaterna M H, Zhao Y, Wilsbacher L D, Sangoram A M, King D P, Pinto L H, Takahashi J S. Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. *Cell* 1997;89:655-667.
- Anton-Tay F. Melatonin: effects on brain function. *Adv Biochem Psychopharmacol* 1974;11:315-324.
- Aoki H, Yamada N, Ozeki Y, Yamane H, Kato N. Minimum light intensity required to suppress nocturnal melatonin concentration in human saliva. *Neurosci Lett* 1998;252:91-94.
- Arendt J, Aldhous M, English J, Marks V, Marks M, Folkard S. Some effects of jet-lag and their alleviation by melatonin. *Ergonomics* 1987;30:1379-1393.
- Arendt J, Aldhous M, Marks V. Alleviation of jet-lag by melatonin: preliminary results of controlled double blind trial. *BMJ* 1986;292:1170.
- Arendt J, Aldhous M, Wright J. Synchronization of a disturbed sleep-wake cycle in a blind man by melatonin treatment. *Lancet* 1988;I(8588):772-773.
- Arendt J, Bojkowski C, Folkard S, Franey C, Marks V, Minors D, Waterhouse J, Wever R A, Wildgruber C, Wright J. Some effects of melatonin and the control of its secretion in humans. In: *Photoperiodism, melatonin and the pineal*. London: Pitman; 1985. p. 266-283.
- Arendt J, Borbely A A, Franey C, Wright J. The effects of chronic, small doses of melatonin given in the late afternoon on fatigue in man: a preliminary study. *Neurosci Lett* 1984;45:317-321.
- Arendt J, Deacon S. Treatment of circadian rhythm disorders - melatonin. *Chronobiol Int* 1997;14:185-204.
- Arendt J, Marks V. Can melatonin alleviate jet lag? *BMJ* 1983;287:426.
- Arendt J, Paunier L, Sizonenko P. Melatonin radioimmunoassay. *J Clin Endocrinol Metab* 1975;40:347-350.
- Arendt J, Skene D J, Middleton B, Lockley S W, Deacon S. Efficacy of melatonin treatment in jet lag, shift work, and blindness. *J Biol Rhythms* 1997;12:604-617.
- Arendt J, Wetterberg L, Heyden T, Sizonenko P C, Paunier L. Radioimmunoassay of melatonin: human serum and cerebrospinal fluid. *Horm Res* 1977;8:65-75.
- Armstrong S M. Melatonin: The internal zeitgeber of mammals. *Pineal Reseach Reviews* 1989;7:157-202.
- Aschoff J. Circadian rhythms in man. *Science* 1965a;148:1427-1432.
- Aschoff J. Response curves in circadian periodicity. In: Aschoff J, ed. *Circadian Clocks*. Amsterdam: North-Holland; 1965b. p. 95-111.
- Autti T, Raininko R, Vanhanen S-L, Santavuori P. MRI of neuronal ceroid lipofuscinosis. 1. Cranial MRI of 30 patients with juvenile neuronal ceroid lipofuscinosis. *Neuroradiology* 1996;38:476-482.
- Autti T, Raininko R, Vanhanen S-L, Santavuori P. Magnetic resonance techniques in neuronal ceroid lipofuscinoses and some other lysosomal diseases affecting the brain. *Curr Opin Neurol* 1997;10:519-524.

- Avery D H, Bolte M A P, Wolfson J K, Kazaras A L. Dawn simulation compared with a dim red signal in the treatment of winter depression. *Biol Psychiatry* 1994;36:181-188.
- Axelrod J. The pineal gland: a neurochemical transducer. *Science* 1974;184:1341-1348.
- Axelrod J, Weissbach H. Enzymatic O-methylation of N-acetylserotonin to melatonin. *Science* 1960;131:1312.
- Bartness T J, Powers J B, Hastings M H, Bittman E L, Goldman B D. The timed infusion paradigm for melatonin delivery - what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? *J Pineal Res* 1993;15:161-190.
- Beck-Friis J, Von Rosen D, Kjellman B F, Ljungén J G, Wetterberg L. Melatonin in relation to body measures, sex, age, season and the use of drugs in patients with major affective disorders and healthy subjects. *Psychoneuroendocrinology* 1984;9:261-277.
- Benloucif S, Masana M I, Yun K, Dubocovich M L. Interactions between light and melatonin on the circadian clock of mice. *J Biol Rhythms* 1999;14:281-289.
- Berga S L, Mortola J F, Yen S S C. Amplification of nocturnal melatonin secretion in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab* 1988;66:242-244.
- Berk M L, Finkelstein J A. An autoradiographic determination of the efferent projections of the suprachiasmatic nucleus of the hypothalamus. *Brain Res* 1981;226:1-13.
- Bittman E L, Crandell R G, Lehman M N. Influences of the paraventricular and suprachiasmatic nuclei and olfactory bulbs on melatonin responses in the golden hamster. *Biol Reprod* 1989;40:118-126.
- Bjorvatn B, Kecklund G, Åkerstedt T. Bright light treatment used for adaptation to night work and re-adaptation back to day life. A field study at an oil platform in the North Sea. *J Sleep Res* 1999;8:105-112.
- Blackshaw S, Snyder S H. Developmental expression pattern of phototransduction components in mammalian pineal implies a light-sensing function. *J Neurosci* 1997;17:8074-8082.
- Boivin D B, Czeisler C A. Resetting of circadian melatonin and cortisol rhythms in humans by ordinary room light. *Neuroreport* 1998;9:779-782.
- Boivin D B, Duffy J F, Kronauer R E, Czeisler C A. Dose-response relationships for resetting of human circadian clock by light. *Nature* 1996;379:540-542.
- Bojkowski C J, Aldhous M E, English J, Franey C, Poulton A L, Skene D J, Arendt J. Suppression of nocturnal plasma melatonin and 6-sulphatoxymelatonin by bright and dim light in man. *Horm Metab Res* 1987;19:437-440.
- Boulos Z, Campbell S S, Lewy A J, Terman M, Dijk D-J, Eastman C I. Light treatment for sleep disorders: Consensus report. VII. Jet lag. *J Biol Rhythms* 1995;10:167-176.
- Boustany R-M. Neurology of the neuronal ceroid-lipofuscinoses: late infantile and juvenile types. *Am J Med Gen* 1992;42:533-535.
- Brainard G C, Gaddy J R, Barker F M, Hanifin J P, Rollag M D. Mechanisms in the eye that mediate the biological and therapeutic effects of light in humans. In: Wetterberg L, ed. *Light and Biological Rhythms in Man*. New York: Pergamon Press; 1993. p. 29-53.
- Brainard G C, Lewy A J, Menaker M, Fredrickson R H, Miller L S, Weleber R G, Cassone V, Hudson D. Effect of light wavelength on the suppression of nocturnal plasma melatonin in normal volunteers. In: Wurtman R J, Baum M J, Potts J T J, eds. *The Medical and Biological Effects of Light*. New York: The New York Academy of Sciences; 1985. p. 376-378.

- Brainard G C, Lewy A J, Menaker M, Fredrickson R H, Miller L S, Weleber R G, Cassone V, Hudson D. Dose-response relationship between light irradiance and the suppression of plasma melatonin in human volunteers. *Brain Res* 1988;454:212-218.
- Brainard G C, Rollag M D, Hanifin J P. Photic regulation of melatonin in humans - ocular and neural signal transduction. *J Biol Rhythms* 1997;12:537-546.
- Broadway J, Arendt J, Folkard S. Bright light phase shifts the human melatonin rhythm during the Antarctic winter. *Neurosci Lett* 1987;79:185-189.
- Bronstein D M, Jacobs G H, Haak K A, Neitz J, Lytle L D. Action spectrum of the retinal mechanism mediating nocturnal light-induced suppression of rat pineal gland N-acetyltransferase. *Brain Res* 1987;406:352-356.
- Brownstein M, Axelrod J. Pineal gland: 24-hour rhythm in norepinephrine turnover. *Science* 1974;184:163-165.
- Brueske V, Allen J, Kepic T, Meissner W, Lee R, Vaughan G, Weinburg U. Melatonin inhibition of seizure activity in man. *EEG Clin Neurophysiol* 1981;51:20P.
- Brzezinski A. Melatonin in humans. *N Engl J Med* 1997;336:186-195.
- Burešová M, Dvoráková M, Zvolský P, Illnerová H. Early morning bright light phase advances the human circadian pacemaker within one day. *Neurosci Lett* 1991;121:47-50.
- Burešová M, Dvoráková M, Zvolský P, Illnerová H. Human circadian rhythm in serum melatonin in short winter days and in simulated artificial long days. *Neurosci Lett* 1992;136:173-176.
- Buswell R S. The pineal and neoplasia. *Lancet* 1975;1:34-35.
- Cagnacci A, Soldani R, Yen S S C. Contemporaneous melatonin administration modifies the circadian response to bright light stimuli. *Am J Physiol* 1997;272:R482-R486.
- Cahill G M, Besharse J C. Circadian rhythmicity in vertebrate retinas: regulation by a photoreceptor oscillator. *Prog Retin Eye Res* 1995;14:267-291.
- Camfield P, Gordon K, Dooley J, Camfield C. Melatonin appears ineffective in children with intellectual deficits and fragmented sleep: six "N of 1" trials. *J Child Neurol* 1996;11:341-343.
- Campbell S S, Dijk D-J, Boulos Z, Eastman C I, Lewy A J, Terman M. Light treatment for sleep disorders: Consensus report. III. Alerting and activating effects. *J Biol Rhythms* 1995;10:129-132.
- Campbell S S, Murphy P J. Extraocular circadian phototransduction in humans. *Science* 1998;279:396-399.
- Carlberg C, Wiesenberg I. The orphan receptor family RZR/ROR, melatonin and 5-lipoxygenase: An unexpected relationship. *J Pineal Res* 1995;18:171-178.
- Cassone V M, Roberts M H, Moore R Y. Effects of melatonin on 2-deoxy[I-14C]glucose uptake within rat suprachiasmatic nucleus. *Am J Physiol* 1988;255:R332-R337.
- Cavallo A, Dolan L M. 6-Hydroxymelatonin sulfate excretion in human puberty. *J Pineal Res* 1996;21:225-230.
- Chase P A, Seiden L S, Moore R Y. Behavioral and neuroendocrine responses to light mediated by separate visual pathways in the rat. *Physiol Behav* 1969;4:949-952.
- Checkley S A, Murphy D G M, Abbas M, Marks M, Winton F, Palazidou E, Murphy D M, Franey C, Arendt J. Melatonin rhythms in seasonal affective disorder. *Br J Psychiatry* 1993;163:332-337.
- Chesson A L Jr, Littner M, Davilla D, MacDowell Anderson W, Grigg-Damberger M, Hartse K, Johnson S, Wise

- M. Practice parameters for the use of light therapy in the treatment of sleep disorders. *Sleep* 1999;22:641-660.
- Chesworth M J, Cassone V M, Armstrong S M. Effects of daily melatonin injections on activity rhythms of rats in constant light. *Am J Physiol* 1987;253:R101-R107.
- Claussen M, Heim P, Knispel J, Goebel H H, Kohlschütter A. Incidence of neuronal-ceroid lipofuscinoses in West-Germany: variation of a method for studying autosomal recessive disorders. *Am J Med Gen* 1992;42:536-538.
- Claustrat B, Brun J, David M, Sassolas G, Chazot G. Melatonin and jet lag - confirmatory result using a simplified protocol. *Biol Psychiatry* 1992;32:705-711.
- Clodoré M, Foret J, Benoit O, Touitou Y, Aguirre A, Bouard G, Touitou C. Psychophysiological effects of early morning bright light exposure in young adults. *Psychoneuroendocrinology* 1990;15:193-205.
- Cooper H M, Herbin M, Nevo E. Ocular regression conceals adaptive progression of the visual system in a blind subterranean mammal. *Nature* 1993;361:156-159.
- Czeisler C A, Allan J S, Strogatz S H, Ronda J M, Sánchez R, Ríos C D, Freitag W O, Richardson G S, Kronauer R E. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* 1986;233:667-671.
- Czeisler C A, Duffy J F, Shanahan T L, Brown E N, Mitchell J F, Rimmer D W, Ronda J M, Silva E J, Allan J S, Emens J S, Dijk D-J, Kronauer R E. Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 1999;284:2177-2181.
- Czeisler C A, Johnson M P, Duffy J F, Brown E N, Ronda J M, Kronauer R E. Exposure to bright light and darkness to treat physiologic maladaptation to night work. *N Engl J Med* 1990;322:1253-1259.
- Czeisler C A, Kronauer R E, Allan J S, Duffy J F, Jewett M E, Brown E N, Ronda J M. Bright light induction of strong (type 0) resetting of the human circadian pacemaker. *Science* 1989;244:1328-1332.
- Czeisler C A, Richardson G S, Zimmerman J C, Moore-Ede M C, Weitzman E D. Entrainment of human circadian rhythms by light-dark cycles: a reassessment. *Photochem Photobiol* 1981;34:239-247.
- Czeisler C A, Shanahan T L, Klerman E I B, Martens H, Brotman D J, Emens J S, Klein T, Rizzo J F (1995): Suppression of melatonin secretion in some blind patients by exposure to bright light. *N Engl J Med* 332:6-11.
- Czeisler C A, Weitzman E D, Moore-Ede M C, Zimmerman J C, Knauer R S. Human sleep: its duration and organization depend on its circadian phase. *Science* 1980;210:1264-1267.
- Daan S, Lewy A J. Scheduled exposure to daylight: a potential strategy to reduce "jet lag" following transmeridian flight. *Psychopharmacol Bull* 1984;20:566-568.
- Dagan Y, Yovel I, Hallis D, Eisenstein M, Raichik I. Evaluating the role of melatonin in the long-term treatment of delayed sleep phase syndrome (DSPS). *Chronobiol Int* 1998;15:181-190.
- Dahlitz M, Alvarez B, Vignau J, English J, Arendt J, Parkes J D. Delayed Sleep Phase Syndrome Response to Melatonin. *Lancet* 1991;337:1121-1124.
- Dai J, Swaab D F, Buijs R M. Distribution of vasopressin and vasoactive intestinal polypeptide (VIP) fibers in the human hypothalamus with special emphasis on suprachiasmatic nucleus efferent projections. *J Comp Neurol* 1997;383:397-414.
- Dai J, Swaab D F, van der Fliet J, Buijs R M. Postmortem tracing reveals the organization of hypothalamic projections of the suprachiasmatic nucleus in the human brain. *J Comp Neurol* 1998;400:87-102.

- Daurat A, Foret J, Touitou Y, Benoit O. Detrimental influence of bright light exposure on alertness, performance, and mood in the early morning. *Clin Neurophysiol* 1996;26:8-14.
- Dawson D, Armstrong S M. Chronobiotics - drugs that shift rhythms. *Pharmacol Ther* 1996;69:15-36.
- Dawson D, Campbell S S. Bright light treatment: Are we keeping our subjects in the dark? *Sleep* 1990;13:267-271.
- Dawson D, Campbell S S. Timed exposure to bright light improves sleep and alertness during simulated night shifts. *Sleep* 1991;14:511-516.
- Dawson D, Lack L, Morris M. Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiol Int* 1993;10:94-102.
- Deacon S J, Arendt J. Phase-shifts in melatonin, 6-sulphatoxymelatonin and alertness rhythms after treatment with moderately bright light at night. *Clin Endocrinol* 1994;40:413-420.
- DeCoursey P J. Daily light sensitivity rhythm in a rodent. *Science* 1960;131:33-35.
- Deguchi T, Axelrod J. Control of circadian change of serotonin N-acetyltransferase activity in the pineal organ by the beta-adrenergic receptor. *Proc Natl Acad Sci USA* 1972;69:2547-2550.
- De Mairan J. Observation botanique. *Hist Acad R Sci Paris* 1729:35-36.
- Deprés-Brummer P, Lévi F, Metzger G, Touitou Y. Light-induced suppression of the rat circadian system. *Am J Physiol* 1995;37:R1111-R1116.
- Deprés-Brummer P, Metzger G, Lévi F. Pharmacologic restoration of suppressed temperature rhythms in rats by melatonin, melatonin receptor agonist, S20242, or 8-OH-DPAT. *Eur J Pharmacol* 1998;347:57-66.
- Désir D, van Cauter E, Fang V S, Martino E, Jadot C, Spire J-P, Noël P, Refetoff S, Copinschi G, Golstein J. Effects of "jet lag" on hormonal patterns. I. Procedures, variations in total plasma proteins, and disruption of adrenocorticotropin-cortisol periodicity. *J Clin Endocrinol Metab* 1981;52:628-641.
- Désir D, van Cauter E, L'Hermite M, Refetoff S, Jadot C, Caufriez A, Copinschi G, Robyn C. Effects of "jet lag" on hormonal patterns. III. Demonstration of an intrinsic circadian rhythmicity in plasma prolactin. *J Clin Endocrinol Metab* 1982;55:849-857.
- Di W L, Kadva A, Johnston A, Silman R. Variable bioavailability of oral melatonin. *N Engl J Med* 1997;336:1028-1029.
- Dietzel M, Waldhauser F, Lesch O M, Musalek M, Walter H. Bright light treatment success not explained by melatonin. *J Interdiscip Cycle Res* 1985;16:165.
- Dijk D J, Beersma D G M, Daan S, Lewy A J. Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *Am J Physiol* 1989;256:R106-R111.
- Dijk D-J, Boulos Z, Eastman C I, Lewy A J, Campbell S S, Terman M. Light treatment for sleep disorders: Consensus report. II. Basic properties of circadian physiology and sleep regulation. *J Biol Rhythms* 1995;10:113-125.
- Dijk D J, Visscher C A, Bloem G M, Beersma D G M, Daan S. Reduction of human sleep duration after bright light exposure in the morning. *Neurosci Lett* 1987;73:181-186.
- Dollins A B, Lynch H J, Wurtman R J, Deng M H, Kischka K U, Gleason R E, Lieberman H R. Effect of pharmacological daytime doses of melatonin on human mood and performance. *Psychopharmacology* 1993;112:490-496.

- Dollins A B, Zhdanova I V, Wurtman R J, Lynch H J, Deng M H. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc Natl Acad Sci USA* 1994;91:1824-1828.
- Drennan M, Kripke D F, Gillin J C. Bright light can delay human temperature rhythm independent of sleep. *Am J Physiol* 1989;257:R136-R141.
- Dunlap J C. Molecular bases for circadian clocks. *Cell* 1999;96:271-290.
- Eastman C I, Lahmeyer H W, Watell L G, Good G D, Young M A. A placebo-controlled trial of light treatment for winter depression. *J Affect Disord* 1992;26:211-222.
- Eastman C I, Martin S K. How to use light and dark to produce circadian adaptation to night shift work. *Ann Med* 1999;31:87-98.
- Eastman C, Rechtschaffen A. Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiol Behav* 1983;31:417-427.
- Eastman C I, Stewart K T, Mahoney M P, Liu L W, Fogg L F. Dark goggles and bright light improve circadian rhythm adaptation to night-shift work. *Sleep* 1994;17:535-543.
- Eastman C I, Young M A, Fogg L F, Liu L, Meaden P M. Bright light treatment of winter depression: A placebo-controlled trial. *Arch Gen Psychiatry* 1998;55:883-889.
- Ebels I, Balemans M G B. Physiological aspects of pineal functions in mammals. *Physiol Rev* 1986;66:581-605.
- Edgar D M, Dement W C. Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *Am J Physiol* 1991;261:R928-R933.
- Elliott J A. Circadian rhythms and photoperiodic time measurement in mammals. *Fed Proc* 1976;35:2339-2346.
- Etzioni A, Luboshitzky R, Tiosano D, Benharush M, Goldsher D, Lavie P. Melatonin replacement corrects sleep disturbances in a child with pineal tumor. *Neurology* 1996;46:261-263.
- Fauteck J D, Bockmann J, Böckers T M, Wittkowski W, Köhling R, Lücke A, Straub H, Speckmann E-J, Tuxhorn L, Wolf P, Pannek H, Oppel F. Melatonin reduces low-Mg<sup>2+</sup> epileptiform activity in human temporal slices. *Exp Brain Res* 1995;107:321-325.
- Fauteck J D, Lerchl A, Bergmann M, Moller M, Fraschini F, Wittkowski W, Stankov B. The adult human cerebellum is a target of the neuroendocrine system involved in the circadian timing. *Neurosci Lett* 1994;179:60-64.
- Fevre-Montagne M, Van Cauter E, Refetoff S, Désir D, Tourniaire J, Copinschi G. Effects of "jet lag" on hormonal patterns. II. Adaptation of melatonin circadian periodicity. *J Clin Endocrinol Metab* 1981;52:642-649.
- Folkard S, Arendt J, Aldhous M, Kennett H. Melatonin stabilises sleep onset time in a blind man without entrainment of cortisol or temperature rhythms. *Neurosci Lett* 1990;113:193-198.
- Folkard S, Arendt J, Clark M. Can melatonin improve shift workers' tolerance of the night shift? - some preliminary findings. *Chronobiol Int* 1993;10:315-320.
- Foret J, Aguirre A, Tuitou Y, Clodoré M, Benoit O. Effect of morning bright light on body temperature, plasma cortisol and wrist motility measured during 24 hour of constant conditions. *Neurosci Lett* 1993;155:155-158.
- Foster R G, Argamaso S, Coleman S, Colwell C S, Lederman A, Provencio I. Photoreceptors regulating circadian behavior: a mouse model. *J Biol Rhythms* 1993;8(Suppl):S17-S23.

- Foulkes N S, Borjigin J, Snyder S H, Sassone-Corsi P. Transcriptional control of circadian hormone synthesis *via* the CREM feedback loop. *Proc Natl Acad Sci USA* 1996;93:14140-14145.
- Freedman M S, Lucas R J, Soni B, von Schantz M, Munoz M, David-Gray Z, Foster R. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 1999;284:502-504.
- Frost D O, So K-F, Schneider G E. Postnatal development of retinal projections in syrian hamsters: A study using autoradiographic and anterograde degeneration techniques. *Neuroscience* 1979;4:1649-1677.
- Gaddy J R. Sources of variability in phototherapy. *Sleep Res* 1990;19:394.
- Gaddy J R, Rollag M D, Brainard G C. Pupil size regulation of threshold of light-induced melatonin suppression. *J Clin Endocrinol Metab* 1993;77:1398-1401.
- Gallin P F, Terman M, Reme C E, Rafferty B, Terman J S, Burde R M. Ophthalmologic examination of patients with seasonal affective disorder, before and after bright light therapy. *Am J Ophthalmol* 1995;119:202-210.
- Ganong W F. *Review of Medical Physiology*. A Lange medical book. 18th ed. Stamford, Connecticut: Appleton & Lange; 1997. p. 433.
- Garfinkel D, Laudon M, Nof D, Zisapel N. Improvement of sleep quality in elderly people by controlled-release melatonin. *Lancet* 1995;346:541-544.
- Gaston S, Menaker M. Pineal function: the biological clock in the sparrow? *Science* 1968;160:1125-1127.
- Gauer F, Masson-Pévet M, Pévet P. Effect of constant light, pinealectomy and guanosine triphosphate gamma-S on the density of melatonin receptors in the rat suprachiasmatic nucleus - A possible implication of melatonin action. *J Neuroendocrinol* 1992;4:455-459.
- Gauer F, Masson-Pévet M, Pévet P. Melatonin receptor density is regulated in rat pars tuberalis and suprachiasmatic nuclei by melatonin itself. *Brain Res* 1993;602:153-156.
- Glass J D, Lynch G R. Melatonin: Identification of sites of antigonadal action in mouse brain. *Science* 1981;214:821-823.
- Goebel H H. The neuronal ceroid-lipofuscinoses. *J Child Neurol* 1995;10:424-437.
- Goebel H H, Fix J D, Zeman W. The fine structure of the retina in neuronal ceroid-lipofuscinosis. *Am J Ophthalmol* 1974;77:25-39.
- Goebel H H, Klein H, Santavuori P, Sainio K. Ultrastructural studies of the retina in infantile neuronal ceroid-lipofuscinosis. *Retina* 1988;8:59-67.
- Goebel H H, Mole S E, Lake B D. *The Neuronal Ceroid Lipofuscinoses (Batten Disease)*. Biomedical and Health Research. Vol. 33. Amsterdam: IOS Press; 1999.
- Golstein J, van Cauter E, Désir D, Noël P, Spire J-P, Refetoff S, Copinschi G. Effects of "jet lag" on hormonal patterns. IV. Time shifts increase growth hormone release. *J Clin Endocrinol Metab* 1983;56:433-440.
- Green D J, Gillette R. Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic brain slice. *Brain Res* 1982;245:198-200.
- Guardiola-Lemaître B. Toxicology of melatonin. *J Biol Rhythms* 1997;12:697-706.
- Guilleminault C, McCann C C, Querasalva M, Cetel M. Light therapy as treatment of dyschronosis in brain impaired children. *Eur J Pediatrics* 1993;152:754-759.

- Haimov I, Laudon M, Zisapel N, Souroujon M, Nof D, Shlitner A, Herer P, Tzischinsky O, Lavie P. Sleep disorders and melatonin rhythms in elderly people. *BMJ* 1994;309:167.
- Haimov I, Lavie P, Laudon M, Herer P, Vigder C, Zisapel N. Melatonin replacement therapy of elderly insomniacs. *Sleep* 1995;18:598-603.
- Haltia M, Rapola J, Santavuori P. Infantile type of so-called neuronal ceroid-lipofuscinosis. Histological and electron microscopic studies. *Acta Neuropathol* 1973;26:157-170.
- Hao H, Rivkees S A. The biological clock of very premature primate infants is responsive to light. *Proc Natl Acad Sci USA* 1999;96:2426-2429.
- Hardeland R. The presence and function of melatonin and structurally related indoleamines in a dinoflagellate, and a hypothesis on the evolutionary significance of these tryptophan metabolites in unicellulars. *Experientia* 1993;49:614-623.
- Hardeland R, Fuhrberg B. Ubiquitous melatonin - presence and effects in unicells, plants and animals. *Trends Comp Biochem Physiol* 1996;2:25-45.
- Hartmann L, Roger M, Lemaître B J, Massias J F, Chaussain J L. Plasma and urinary melatonin in male infants during the first 12 months of life. *Clin Chim Acta* 1982;121:37-42.
- Hashimoto S, Kohsaka M, Nakamura K, Honma H, Honma S, Honma K. Midday exposure to bright light changes the circadian organization of plasma melatonin rhythm in humans. *Neurosci Lett* 1997;221:89-92.
- Hashimoto S, Nakamura K, Honma S, Tokura H, Honma K-I. Melatonin rhythm is not shifted by lights that suppress nocturnal melatonin in humans under entrainment. *Am J Physiol* 1996;270:R1073-R1077.
- Hastings M H. Central clocking. *Trends Neurosci* 1997;20:459-464.
- Hastings M H, Herbert J. Neurotoxic lesions of the paraventriculo-spinal projection block the nocturnal rise in pineal melatonin synthesis in the syrian hamster. *Neurosci Lett* 1986;69:1-6.
- Hébert M, Martin S K, Eastman C I. Nocturnal melatonin secretion is not suppressed by light exposure behind the knee in humans. *Neurosci Lett* 1999;274:127-130.
- Heikkilä E, Hätönen T H, Telakivi T, Laakso M-L, Heiskala H, Salmi T, Alila A, Santavuori P. Circadian rhythm studies in neuronal ceroid-lipofuscinosis (NCL). *Am J Med Gen* 1995;57:229-234.
- Hendrickson A E, Wagoner N, Cowan W N. An autoradiographic and electronmicroscopic study of retino-hypothalamic connections. *Z Zellforsch Mikrosk Anat* 1972;135:1-26.
- Heubner O. Tumor der Glandula pinealis. *Deutsche Medizinische Wochenschrift* 1898;24:214-215.
- Hirata F, Hayaishi O, Tokuyama T, Senoh S. *In vitro* and *in vivo* formation of two new metabolites of melatonin. *J Biol Chem* 1974;249:1311-1313.
- Hirvasniemi A, Lang H, Lehesjoki A-E, Leisti J. Northern epilepsy syndrome: an inherited childhood onset epilepsy with associated mental deterioration. *J Med Genet* 1994;31:177-182.
- Hofman I, Kohlschütter A, Santavuori P, Gottlob I, Goebel H H, Lake B D, Schutgens R B H, Greene N D E, Leung K-Y, Mitchison H M, Munroe P B, Taschner P E M. CLN3 Juvenile NCL. In: Goebel H H, Mole S E, Lake B D, eds. *The Neuronal Ceroid Lipofuscinoses (Batten Disease)*. Amsterdam: IOS Press; 1999. p. 55-76.
- Hofman M A, Zhou J-N, Swaab D F. Suprachiasmatic nucleus of the human brain: an immunocytochemical and morphometric analysis. *Anat Rec* 1996;244:552-562.

- Honma K, Hiroshige T. Endogenous ultradian rhythms in rats exposed to prolonged continuous light. *Am J Physiol* 1978;235:R250-R256.
- Honma K, Honma S, Wada T. Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. *Experientia* 1987;43:1205-1207.
- Honma K, Katabami F, Hiroshige T. A phase response curve for the locomotor activity rhythm of the rat. *Experientia* 1978;34:1602-1603.
- Horiguchi M, Miyake Y. Batten disease - deteriorating course of ocular findings. *Jap J Ophthalmol* 1992;36:91-96.
- van der Horst G T J, Muijtjens M, Kobayashi K, Takano R, Kanno S, Takao M, de Wit J, Verkerk A, Eker A P M, van Leenen D, Buijs R, Bootsma D, Hoeijmakers J H J, Yasui A. Mammalian *Cry1* and *Cry2* are essential for maintenance of circadian rhythms. *Nature* 1999;398:627-630.
- Huber R, Deboer T, Schwierin B, Tobler I. Effect of melatonin on sleep and brain temperature in the Djungarian hamster and the rat. *Physiol Behav* 1998;65:77-82.
- Huerta J J, Llamosas M M, Cernuda-Cernuda R, García-Fernández J M. Spatio-temporal analysis of light-induced Fos expression in the retina of rd mutant mice. *Brain Res* 1999;834:122-127.
- Huether G, Poeggeler B, Reimer A, George A. Effect of tryptophan administration on circulating melatonin levels in chicks and rats - evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. *Life Sci* 1992;51:945-953.
- Härmä M, Laitinen J, Partinen M, Suvanto S. The effects of four-day round trip flights over 10 time zones on the circadian variation of salivary melatonin and cortisol in airline flight attendants. *Ergonomics* 1993;37:1479-1489.
- Hätönen T, Kirveskari E, Heiskala H, Sainio K, Laakso M-L, Santavuori P. Melatonin ineffective in neuronal ceroid lipofuscinosis patients with fragmented or normal motor activity rhythms recorded by wrist actigraphy. *Mol Gen Metab* 1999;66:401-406.
- Ibuka N. Circadian rhythms in sleep-wakefulness and wheel-running activity in a congenitally anophthalmic rat mutant. *Physiol Behav* 1987;39:321-326.
- Illnerová H, Vanecek J. Effect of one-minute exposure to light at night on rat pineal serotonin N-acetyltransferase. *Prog Brain Res* 1979;52:241-243.
- Illnerová H, Vanecek J. Two-oscillator structure of the pacemaker controlling the circadian rhythm of N-acetyltransferase in the rat pineal gland. *J Comp Physiol A* 1982;145:539-548.
- Illnerová H, Zvolský P, Vanecek J. The circadian rhythm in plasma melatonin concentration of the urbanized man: the effect of summer and winter time. *Brain Res* 1985;328:186-189.
- Inouye S T. Light responsiveness of the suprachiasmatic nucleus within the island with the retino-hypothalamic tract spared. *Brain Res* 1984;294:263-268.
- Inouye S-I, Kawamura H. Persistence of circadian rhythmicity in mammalian hypothalamic 'island' containing the suprachiasmatic nucleus. *Proc Natl Acad Sci USA* 1979;76:5962-5966.
- Inouye S T, Kawamura H. Characteristics of a circadian pacemaker in the suprachiasmatic nucleus. *J Comp Physiol A* 1982;146:153-160.
- Iyengar B. Indoleamines and the UV-light-sensitive photoperiodic responses of the melanocyte network: a biological calendar? *Experientia* 1994;50:733-736.

- Iyengar B. The UV-responsive melanocyte system: A peripheral network for photoperiodic time measurements - A function of indoleamine expression. *Acta Anat* 1998;163:173-178.
- Jagota A, Olcese J, Rao S H, Gupta P D. Pineal rhythms are synchronized to light-dark cycles in congenitally anophthalmic mutant rats. *Brain Res* 1999;825:95-103.
- Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. *Toxicol Sci* 1999;50:271-279.
- Jan J E, Connolly M B C, Hamilton D, Freeman R D, Laudon M. Melatonin treatment of non-epileptic myoclonus in children. *Dev Med Child Neurol* 1999;41:255-259.
- Jan J E, Espezel H, Appleton R E. The treatment of sleep disorders with melatonin. *Dev Med Child Neurol* 1994;36:97-107.
- Jewett M E, Rimmer D W, Duffy J F, Klerman E B, Kronauer R E, Czeisler C A. Human circadian pacemaker is sensitive to light throughout subjective day without evidence of transients. *Am J Physiol* 1997;273:R1800-R1809.
- Jimerson D C, Lynch H J, Post R M, Wurtman R J, Bunney W E J. Urinary melatonin rhythms during sleep deprivation in depressed patients and normals. *Life Sci* 1977;20:1501-1508.
- Johnson R F, Moore R Y, Morin L P. Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Res* 1988a;460:297-313.
- Johnson R F, Moore R Y, Morin L P. Lateral geniculate lesions alter circadian activity rhythms in the hamster. *Brain Res Bull* 1989;22:411-422.
- Johnson R F, Morin L P, Moore R Y. Retinohypothalamic projections in the hamster and rat demonstrated using cholera toxin. *Brain Res* 1988b;462:301-312.
- Järvelä I, Autti T, Lamminranta S, Åberg L, Raininko R, Santavuori P. Clinical and magnetic resonance imaging findings in Batten disease: analysis of the major mutation (1.02-kb deletion). *Ann Neurol* 1997;42:799-802.
- Kalsbeek A, Teclemariam-Mesbah R, Pévet P. Efferent projections of the suprachiasmatic nucleus in the golden hamster (*Mesocricetus auratus*). *J Comp Neurol* 1993;332:293-314.
- Kalsbeek A, Drijfhout W-J, Westerink B H C, van Heerikhuizen J J, van der Woude T P, van der Vliet J, Buijs R M. GABA receptors in the region of the dorsomedial hypothalamus of rats are implicated in the control of melatonin and corticosterone release. *Neuroendocrinology* 1996;63:69-78.
- Kappers J A. The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. *Z Zellforsch* 1960;52:163-215.
- Kappers J A. Short history of pineal discovery and research. *Prog Brain Res* 1979;52:3-22.
- Karasek M, Pawlikowski M, Nowakowskajankiewicz B, Kolodziejmaciejewska H, Zieleniewski J, Cieslac D, Leidenberger F. Circadian variations in plasma melatonin, FSH, LH, and prolactin and testosterone levels in infertile men. *J Pineal Res* 1990;9:149-157.
- Karppanen H, Airaksinen M M, Särkimäki J. Effects in rats of pinealectomy and oxypertine on spontaneous locomotor activity and blood pressure during various light schedules. *Ann Med Exp Biol Fenn* 1973;51:93-103.
- Kaupilla A, Kivelä A, Pakarinen A, Vakkuri O. Inverse seasonal relationship between melatonin and ovarian activity in humans in a region with a strong seasonal contrast in luminosity. *J Clin Endocrinol Metab* 1987;65:823-828.
- Kennaway D J, Rowe S A. Effect of stimulation of endogenous melatonin secretion during constant light exposure

- on 6-sulphatoxymelatonin rhythmicity in rats. *J Pineal Res* 2000;28:16-25.
- Kennaway D J, Stamp G E, Goble F C. Development of melatonin production in infants and the impact of prematurity. *J Clin Endocrinol Metab* 1992;75:367-369.
- Kilduff T S, Landel H B, Nagy G S, Sutin E L, Dement W C, Heller H C. Melatonin influences Fos expression in the rat suprachiasmatic. *Mol Brain Res* 1992;16:47-56.
- King D P, Zhao Y, Sangoram A M, Wilsbacher L D, Tanaka M, Antoch M P, Steeves T D L, Vitaterna M H, Kornhauser J M, Lowrey P L, Turek F W, Takahashi J S. Positional cloning of the mouse circadian clock gene. *Cell* 1997;89:641-653.
- Kivelä A, Kauppila A, Ylöstalo P, Vakkuri O, Leppälüoto J. Seasonal, menstrual and circadian secretions of melatonin, gonadotropins and prolactin in women. *Acta Physiol Scand* 1988;132:321-327.
- Klein T, Martens H, Dijk D J, Kronauer R E, Seely E W, Czeisler C A. Circadian sleep regulation in the absence of light perception - chronic non-24-hour circadian rhythm sleep disorder in a blind man with a regular 24-hour sleep-wake schedule. *Sleep* 1993;16:333-343.
- Klein D C, Moore R Y. Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: control by the retinohypothalamic tract and the suprachiasmatic nucleus. *Brain Res* 1979;174:245-262.
- Klein D C, Sugden D, Weller J L. Postsynaptic alpha-adrenergic receptors potentiate the beta-adrenergic stimulation of pineal serotonin N-acetyltransferase. *Proc Natl Acad Sci USA* 1983;80:599-603.
- Klein D C, Weller J L. Indole metabolism in the pineal gland: A circadian rhythm in N-acetyltransferase. *Science* 1970;169:1093-1095.
- Klein D C, Weller J L. Rapid light-induced decrease in pineal serotonin N-acetyltransferase activity. *Science* 1972;177:532-533.
- Klerman E B, Rimmer D W, Dijk D J, Kronauer R E, Rizzo J F, Czeisler C A. Nonphotic entrainment of the human circadian pacemaker. *Am J Physiol* 1998;274:R991-R996.
- Kohlschütter A, Goebel H H. Die Neuronalen Ceroid-Lipofuszinosen. Neurodegenerative Krankheiten des Kindesalters auf dem Weg zur Aufklärung. *Deutsches Ärzteblatt* 1997;94:B-2581-B-2586.
- Kokkola T, Laitinen J T. Melatonin receptor genes. *Ann Med* 1998;30:88-94.
- Koller M, Härmä M, Laitinen J T, Kundi M, Piegler B, Haider M. Different patterns of light exposure in relation to melatonin and cortisol rhythms and sleep of night workers. *J Pineal Res* 1994;16:127-135.
- Koller M, Kundi M, Stidl H G, Zidek T, Haider M. Personal light dosimetry in permanent night and day workers. *Chronobiol Int* 1993;10:143-155.
- Kondo T, Ishiura M. The circadian clocks of plants and cyanobacteria. *Trends Plant Sci* 1999;4:171-176.
- Kopin I J, Pare C M B, Axelrod J, Weissbach H. The fate of melatonin in animals. *J Biol Chem* 1961;236:3072-3075.
- Korf H-W, Oksche A. Photoneuroendocrine aspects of the pineal gland: phylogeny and ontogeny. In: Gupta D, Reiter R J, eds. *The Pineal Gland during Development: from Fetus to Adult*. London: Croom Helm; 1986. p. 1-13.
- Kräuchi K, Cajochen C, Danilenko K V, Wirz-Justice A. The hypothermic effect of late evening melatonin does not block the phase delay induced by concurrent bright light in human subjects. *Neurosci Lett* 1997a;232:57-61.
- Kräuchi K, Cajochen C, Möri D, Graw P, Wirz-Justice A. Early evening melatonin and S-20098 advance

- circadian phase and nocturnal regulation of core body temperature. *Am J Physiol* 1997b;272:R1178-R1188.
- Krieger D T, Kreuzer J, Rizzo F A. Constant light: effect on circadian pattern and phase reversal of steroid and electrolyte levels in man. *J Clin Endocrinol Metab* 1969;29:1634-1638.
- Laakso M-L, Hätönen T, Alila A. Uncoupling of the pineal melatonin synthesis of rats from the circadian regulation. *Neurosci Lett* 1994a;179:5-8.
- Laakso M-L, Leinonen L, Hätönen T, Alila A, Heiskala H. Melatonin, cortisol and body temperature rhythms in Lennox-Gastaut patients with or without circadian rhythm sleep disorders. *J Neurol* 1993;240:410-416.
- Laakso M-L, Porkka-Heiskanen T, Alila A, Peder M, Johansson G. Twenty-four-hour patterns of pineal melatonin, and pituitary and plasma prolactin in male rats under "natural" and artificial lighting conditions. *Neuroendocrinology* 1988;48:308-313.
- Laakso M-L, Porkka-Heiskanen T, Alila A, Stenberg D, Johansson G. Correlation between salivary and serum melatonin: dependence on serum melatonin levels. *J Pineal Res* 1990;9:39-50.
- Laakso M-L, Porkka-Heiskanen T, Alila A, Stenberg D, Johansson G. Twenty-four-hour rhythms in relation to the natural photoperiod: a field study in humans. *J Biol Rhythms* 1994b;9:283-293.
- Laakso M-L, Porkka-Heiskanen T, Stenberg D, Alila A. Interindividual differences in the responses of serum and salivary melatonin to light. In: Fraschini F, Reiter R J, eds. *Role of Melatonin and Pineal Peptides in Neuroimmunomodulation*. New York: Plenum Press; 1991. p. 307-311.
- Laakso M-L, Porkka-Heiskanen T, Stenberg D, Alila A, Hätönen T. Suppression of human melatonin by light over the course of the rising phase of the synthesis. *Biol Rhythm Res* 1994c;25:37-50.
- Lapierre O, Dumont M. Melatonin treatment of a non-24-hour sleep-wake cycle in a blind retarded child. *Biol Psychiatry* 1995;38:119-122.
- Laudon M, Gilad E, Matzkin H, Braf Z, Zisapel N. Putative melatonin receptors in benign human prostate tissue. *J Clin Endocrinol Metab* 1996;81:1336-1342.
- Le Bars D, Thivolle P, Vitte P A, Bojkowski C, Chazot G, Arendt J, Frackowiak R S J, Claustrat B. PET and plasma pharmacokinetic studies after bolus intravenous administration of [<sup>11</sup>C]melatonin in humans. *Nucl Med Biol Int J Radiat Appl Instrum B* 1991;18:357-362.
- Lehman M N, Bittman E L, Newman S W. Role of the hypothalamic paraventricular nucleus in neuroendocrine responses to daylength in the golden hamster. *Brain Res* 1984;308:25-32.
- Lehman M N, Silver R, Gladstone W R, Kahn R M, Gibson M, Bittman E L. Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. *J Neurosci* 1987;7:1626-1638.
- Lehmann E D, Cockerell O C, Rudge P. Somnolence associated with melatonin deficiency after pinealectomy. *Lancet* 1996;347:323.
- Leino M. The occurrence, distribution and function of 5-methoxyindoles with special reference to retinal 6-methoxy-tetrahydro-beta-carboline. Doctoral thesis. Kuopio, Finland: University of Kuopio; 1984.
- Lemmer B. Chronopharmacokinetics: implications for drug treatment. *J Pharm Pharmacol* 1999;51:887-890.
- Lerman S. Chemical and physical properties of the normal and aging lens: Spectroscopic (UV, fluorescence, phosphorescence, and NMR) analyses. *Am J Optometry Physiol Optics* 1987;64:11-22.
- Lerner A B. My 60 years in pigmentation. *Pigment Cell Res* 1999;12:131-144.

- Lerner A B, Case J D, Heinzelman R V. Structure of melatonin. *J Am Chem Soc* 1959;81:6084-6085.
- Lerner A B, Case J D, Takahashi Y, Lee T H, Mori W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc* 1958;80:2587.
- Lerner A B, Nordlund J J. Melatonin: Clinical pharmacology. *J Neural Transm* 1978;13(Suppl):339-347.
- Levine J D, Weiss M L, Rosenwasser A M, Miselis R R. Retinohypothalamic tract in the female albino rat - A study using horseradish peroxidase conjugated to cholera toxin. *J Comp Neurol* 1991;306:344-360.
- Levitt A J, Joffe R T, Moul D E, Lam R W, Teicher M H, Lebegue B, Murray M G, Oren D A, Schwartz P, Buchanan A, Glod C A, Brown J. Side effects of light therapy in seasonal affective disorder. *Am J Psychiatry* 1993;150:650-652.
- Lewinski A, Zelazowski P, Sewerynek E, Zerek-Melen G, Szkudlinski M, Zelazowska E. Melatonin-induced suppression of human lymphocyte natural killer activity *in vitro*. *J Pineal Res* 1989;7:153-164.
- Lewy A J, Ahmed S, Jackson J M L, Sack R L. Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiol Int* 1992;9:380-392.
- Lewy A J, Bauer V K, Ahmed S, Thomas K H, Cutler N L, Singer C M, Moffit M T, Sack R L. The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. *Chronobiol Int* 1998a;15:71-83.
- Lewy A J, Bauer V K, Cutler N L, Sack R L. Melatonin treatment of winter depression: a pilot study. *Psychiatry Res* 1998b;77:57-61.
- Lewy A J, Bauer V K, Cutler N L, Sack R L, Ahmed S, Thomas K H, Blood M L, Jackson J M. Morning vs evening treatment of patients with winter depression. *Arch Gen Psychiatry* 1998c;55:890-896.
- Lewy A J, Newsome D A. Different types of melatonin circadian secretory rhythms in some blind subjects. *J Clin Endocrinol Metab* 1983;56:1103-1107.
- Lewy A J, Sack R L. Exogenous melatonin's phase shifting effects on the endogenous melatonin profile in sighted humans - a brief review and critique of the literature. *J Biol Rhythms* 1997;12:588-594.
- Lewy A J, Sack R L, Fredrickson R H, Reaves M, Denney D D, Zielske D R. The use of bright light in the treatment of chronobiologic sleep and mood disorders: the phase response curve. *Psychopharmacol Bull* 1983;19:523-525.
- Lewy A J, Sack R L, Miller L S, Hoban T M. Anti-depressant and circadian phase-shifting effects of light. *Science* 1987;235:352-354.
- Lewy A J, Sack R L, Singer C L. Assessment and treatment of chronobiologic disorders using plasma melatonin levels and bright light exposure: the clock-gate model and the phase response curve. *Psychopharmacol Bull* 1984;20:561-565.
- Lewy A J, Sack R L, Singer C M. Immediate and delayed effects of bright light on human melatonin production: shifting "dawn" and "dusk" shifts the dim light melatonin onset (DLMO). In: Wurtman R J, Baum M J, Potts J T J, eds. *The Medical and Biological Effects of Light*. New York: The New York Academy of Sciences; 1985. p. 253-259.
- Lewy A J, Tetsuo M, Markey S P, Goodwin F K, Kopin I J. Pinealectomy abolishes plasma melatonin in the rat. *J Clin Endocrinol Metab* 1980a;50:204-205.
- Lewy A J, Wehr T A, Goodwin F K, Newsome D A, Markey S P. Light suppresses melatonin secretion in humans.

Science 1980b;210:1267-1269.

Lindblom N, Heiskala H, Hätönen T, Mustanoja M, Alfthan H, Alila-Johansson A, Laakso M-L. No evidence for extraocular light induced phase shifting of human melatonin, cortisol and thyrotropin rhythms. *Neuroreport* 2000;11:713-717.

Lindblom N, Hätönen T, Laakso M-L, Alila-Johansson A, Laipio M-L, Turpeinen U. Bright light exposure of a large skin area does not affect melatonin or bilirubin levels in humans. *Biol Psychiatry*, in press.

Lisk R D, Kannwischer L R. Light: Evidence for its direct effect on hypothalamic neurons. *Science* 1964;146:272-273.

Lissoni P, Barni S, Tancini G, Mainini E, Piglia F, Maestroni G J M, Lewinski A. Immunoendocrine therapy with low-dose subcutaneous interleukin-2 plus melatonin of locally advanced or metastatic endocrine tumors. *Oncology* 1995;52:163-166.

Lissoni P, Brivio O, Brivio F, Barni S, Tancini G, Crippa D, Meregalli S. Adjuvant therapy with the pineal hormone melatonin in patients with lymph node relapse due to malignant melanoma. *J Pineal Res* 1996;21:239-242.

Lockley S W, Skene D J, Arendt J, Tabandeh H, Bird A C, DeFrance R. Relationship between melatonin rhythms and visual loss in the blind. *J Clin Endocrinol Metab* 1997a;82:3763-3770.

Lockley S W, Skene D J, Butler L J, Arendt J. Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 1999;22:616-623.

Lockley S W, Skene D J, James K, Thapan K, Wright J, Arendt J. Melatonin administration can entrain the free-running circadian system of blind subjects. *J Endocrinol* 2000;164:R1-R6.

Lockley S W, Skene D J, Tabandeh H, Bird A C, DeFrance R, Arendt J. Relationship between napping and melatonin in the blind. *J Biol Rhythms* 1997b;12:16-25.

Lockley S W, Skene D J, Thapan K, English J, Ribeiro D, Haimov I, Hampton S, Middleton B, von Schantz M, Arendt J. Extraocular light exposure does not suppress plasma melatonin in humans. *J Clin Endocrinol Metab* 1998;83:3369-3372.

Luboshitzky R, Lavi S, Thuma I, Lavie P. Increased nocturnal melatonin secretion in male patients with hypogonadotropic hypogonadism and delayed puberty. *J Clin Endocrinol Metab* 1995;80:2144-2148.

Luboshitzky R, Lavie P. Melatonin and sex hormone interrelationships - A review. *J Ped Endocrinol Metab* 1999;12:355-362.

Luboshitzky R, Yanai D, Shen-Orr Z, Israeli E, Herer P, Lavie P. Daily and seasonal variations in the concentration of melatonin in the human pineal gland. *Brain Res Bull* 1998;47:271-276.

Lucas R J, Foster R G. Neither functional rod photoreceptors nor rod or cone outer segments are required for the photic inhibition of pineal melatonin. *Endocrinology* 1999;140:1520-1524.

Lucas R J, Freedman M S, Munoz M, Garcia-Fernandez J-M, Foster R G. Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* 1999;284:505-507.

Lynch H J, Wurtman R J, Moskowitz M A, Archer M C, Ho M H. Daily Rhythm in Human Urinary Melatonin. *Science* 1975;187:169-171.

Maestroni G J M. Mini-review - the immunoneuroendocrine role of melatonin. *J Pineal Res* 1993;14:1-10.

Mahle C D, Goggins G D, Agarwal P, Ryan E, Watson A J. Melatonin modulates vascular smooth muscle tone. *J*

Biol Rhythms 1997;12:690-696.

Mai J K, Kedziora O, Teckhaus L, Sofroniew M V. Evidence for subdivisions in the human suprachiasmatic nucleus. *J Comp Neurol* 1991;305:508-525.

Makkison, Arendt. Melatonin secretion in humans on two different Antarctic bases (68 and 75 S). *J Interdiscip Cycle Res* 1991;22:149-150.

Mallo C, Zaidan R, Faure A, Brun J, Chazot G, Claustrat B. Effects of a four-day melatonin treatment on the 24 h plasma melatonin, cortisol and prolactin profiles in humans. *Acta Endocrinol (Copenh)* 1988;119:474-480.

Marchant E G, Mistlberger R E. Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. *Physiol Behav* 1996;60:657-663.

Martikainen H, Tapanainen J, Vakkuri O, Leppäluoto J, Huhtaniemi I. Circannual concentrations of melatonin, gonadotropins, prolactin and gonadal steroids in males in a geographical area with a large annual variation in daylight. *Acta Endocrinologica (Copenh)* 1985;109:446-450.

Martinet L, Allain D. Role of the pineal gland in the photoperiodic control of reproductive and non-reproductive functions in mink (*Mustela vison*). In: *Photoperiodism, Melatonin and the Pineal*. London: Pitman, Ciba Foundation Symposium 117; 1985. p. 170-187.

Marumoto N, Murakami N, Katayama T, Kuroda H, Murakami T. Effects of daily injections of melatonin on locomotor activity rhythms in rats maintained under constant bright or dim light. *Physiol Behav* 1996a;60:767-773.

Marx H. 'Hypophysäre Insuffizienz' bei Lichtmangel. *Klin Wochenschr* 1946;24/25:18-21.

Mason R, Brooks A. The electrophysiological effects of melatonin and a putative melatonin antagonist (N-acetyltryptamine) on rat suprachiasmatic neurones *in vitro*. *Neurosci Lett* 1988;95:296-301.

McArthur A J, Budden S S. Sleep dysfunction in Rett syndrome: a trial of exogenous melatonin treatment. *Dev Med Child Neurol* 1998;40:186-192.

McArthur A J, Gillette M U, Prosser R A. Melatonin directly resets the rat suprachiasmatic circadian clock *in vitro*. *Brain Res* 1991;565:158-161.

McArthur A J, Hunt A E, Gillette M U. Melatonin action and signal transduction in the rat suprachiasmatic circadian clock: activation of protein kinase C at dusk and dawn. *Endocrinology* 1997;138:627-634.

McCord C P, Allen F P. Evidences associating pineal gland function with alterations in pigmentation. *J Exp Zool* 1917;23:207-224.

McGuire R A, Rand W M, Wurtman R J. Entrainment of the body temperature rhythm in rats: Effect of color and intensity of environmental light. *Science* 1973;181:956-957.

McIntyre I M, Norman T R, Burrows G D, Armstrong S M. Human melatonin suppression by light is intensity dependent. *J Pineal Res* 1989;6:149-156.

Meijer J H, Rietveld W J. Neurophysiology of the the suprachiasmatic circadian pacemaker in rodents. *Physiol Rev* 1989;69:671-707.

Meijer J H, Thio B, Albus H, Schaap J, Ruijs A C J. Functional absence of extraocular photoreception in hamster circadian rhythm entrainment. *Brain Res* 1999;831:337-339.

Meijer J H, van der Zee E A, Dietz M. Glutamate phase shifts circadian activity rhythms in hamsters. *Neurosci Lett* 1988;86:177-183.

- Mendelson W B, Gillin J C, Dawson S D, Lewy A J, Wyatt R J. Effects of melatonin and propranolol on sleep of the rat. *Brain Res* 1980;201:240-244.
- Meyer-Bernstein E L, Morin L P. Electrical stimulation of the median or dorsal raphe nuclei reduces light-induced FOS protein in the suprachiasmatic nucleus and causes circadian activity rhythm phase shifts. *Neuroscience* 1999;92:267-279.
- Middleton B, Arendt J, Stone B M. Human circadian rhythms in constant dim light (8 lux) with knowledge of clock time. *J Sleep Res* 1996a;5:69-76.
- Middleton B A, Stone B M, Arendt J. Melatonin and fragmented sleep patterns. *Lancet* 1996b;348:551-552.
- Miles L E M, Raynal D M, Wilson M A. Blind man living in normal society has circadian rhythm of 24.9 hours. *Science* 1977;198:421-423.
- Miller J D, Morin L P, Schwartz W J, Moore R Y. New insights into the mammalian circadian clock. *Sleep* 1996;19:641-667.
- Minneman K P, Lynch H J, Wurtman R J. Relationship between environmental light intensity and retina-mediated suppression of rat pineal serotonin-N-acetyl transferase. *Life Sci* 1974;15:1791-1796.
- Minors D S, Waterhouse J M, Wirz-Justice A. A human phase response curve to light. *Neurosci Lett* 1991;133:36-40.
- Mintz E M, Albers H E. Microinjection of NMDA into the SCN region mimics the phase shifting effect of light in hamsters. *Brain Res* 1997;758:245-249.
- Mintz E M, Marvel C L, Gillespie C F, Price K M, Albers H E. Activation of NMDA receptors in the suprachiasmatic nucleus produces light-like phase shifts of the circadian clock *in vivo*. *J Neurosci* 1999;19:5124-5130.
- Miyamoto Y, Sancar A. Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proc Natl Acad Sci USA* 1998;95:6097-6102.
- Moga M M, Moore R Y. Organization of neural inputs to the suprachiasmatic nucleus in the rat. *J Comp Neurol* 1997;389:508-534.
- Mole S E. Batten disease: four genes and still counting. *Neurobiol Dis* 1998;5:287-303.
- Mole S E. Batten's disease: eight genes and still counting? *Lancet* 1999;354:443-445.
- Mole S E, Mitchison H M, Munroe P B. Molecular basis of the neuronal ceroid lipofuscinoses: mutations in CLN1, CLN2, CLN3, and CLN5. *Hum Mutat* 1999;14:199-215.
- Molina-Carballo A, Munoz-Hoyos A, Reiter R J, Sanchez-Forte M, Moreno-Madrid F, Rufo-Campos M, Molina-Font J A, Acuna-Castroviejo D. Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic epilepsy: two years' experience. *J Pineal Res* 1997;23:97-105.
- Moore R Y. Neural control of the pineal gland. *Behav Brain Res* 1996;73:125-130.
- Moore R Y, Bernstein M E. Synaptogenesis in the rat suprachiasmatic nucleus demonstrated by electron microscopy and synapsin I immunoreactivity. *J Neurosci* 1989;9:2151-2162.
- Moore R Y, Card J P. Intergeniculate leaflet: an anatomically and functionally distinct subdivision of the lateral geniculate complex. *J Comp Neurol* 1994;344:403-430.
- Moore R Y, Eichler V B. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in

the rat. *Brain Res* 1972;42:201-206.

Moore R Y, Lenn N J. A retinohypothalamic projection in the rat. *J Comp Neurol* 1972;146:1-14.

Moore R Y, Speh J C. GABA is the principal neurotransmitter of the circadian system. *Neurosci Lett* 1993;150:112-116.

Moore R Y, Speh J C, Card J P. The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. *J Comp Neurol* 1995;352:351-366.

Moore-Ede M C. The circadian timing system in mammals: two pacemakers preside over many secondary oscillators. *Fed Proc* 1983;42:2802-2808.

Morgan P J, Barrett P, Howell H E, Helliwell R. Melatonin receptors -localization, molecular pharmacology and physiological significance. *Neurochem Int* 1994;24:101-146.

Moseley M J. Light transmission through the human eyelid: *in vivo* measurement. *Ophthalm Physiol Optics* 1988;8:229-230.

Mrosovsky N, Reeb S G, Honrado G I, Salmon P A. Behavioural entrainment of circadian rhythms. *Experientia* 1989;45:696-702.

Mrosovsky N, Salmon P A. A behavioural method for accelerating re-entrainment of rhythms to new light-dark cycles. *Nature* 1987;330:372-373.

Murphy P J, Campbell S S. Enhanced performance in elderly subjects following bright light treatment of sleep maintenance insomnia. *J Sleep Res* 1996;5:165-172.

Nakagawa H, Sack R L, Lewy A J. Sleep propensity free-runs with the temperature, melatonin and cortisol rhythms in a totally blind person. *Sleep* 1992;15:330-336.

Nelson D E, Takahashi J S. Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. *Brain Res* 1991;554:272-277.

Nelson R J, Zucker I. Absence of extraocular photoreception in diurnal and nocturnal rodents exposed to direct sunlight. *Comp Biochem Physiol* 1981;69A:145-148.

Neppert B, Kemper B. Juvenile Neuronale Ceroid-Lipofuszinose (M. Spielmeyer-Vogt). *Klin Monatsbl Augenheilkd* 1998;213:362-366.

Neri B, de Leonardi V, Gemelli M T, di Loro F, Mottola A, Ponchiatti R, Raugi A, Cini G. Melatonin as biological response modifier in cancer patients. *Anticancer Res* 1998;18:1329-1332.

Neuwelt E A, Lewy A J. Disappearance of plasma melatonin after removal of a neoplastic pineal gland. *N Engl J Med* 1983;308:1132-1135.

Neville S, Arendt J, Ioannides C. A study of the mutagenicity of melatonin and 6-hydroxymelatonin. *J Pineal Res* 1989;6:73-76.

Newell F W, Ernest J T. *Ophthalmology. Principles and Concepts*. 3rd ed. Saint Louis: Mosby; 1974.

O'Callaghan F J K, Clarke A A, Hancock E, Hunt A, Osborne J P. Use of melatonin to treat sleep disorders in tuberous sclerosis. *Dev Med Child Neurol* 1999;41:123-126.

Okamura H, Miyake S, Sumi Y, Yamaguchi S, Yasui A, Muijtjens M, Hoeijmakers J H J, van der Horst G T J. Photoc induction of *mPer1* and *mPer2* in *Cry*-deficient mice lacking a biological clock. *Science* 1999;286:2531-2534.

- Okawa M, Nanami T, Shimizu T, Hishikawa Y, Sasaki H, Nagamine H, Takahashi H. Four congenitally blind children with circadian sleep-wake rhythm disorder. *Sleep* 1987;10:101-110.
- Oldani A, Ferini-Strambi L, Zucconi M, Stankov B, Fraschini F, Smirne S. Melatonin and delayed sleep phase syndrome: ambulatory polygraphic evaluation. *Neuroreport* 1994;6:132-134.
- Oren D A. Humoral phototransduction: blood is a messenger. *Neuroscientist* 1996;2:207-210.
- Oren D A. Bilirubin, REM sleep, and phototransduction of environmental time cues. A hypothesis. *Chronobiol Int* 1997;14:319-329.
- Oren D A, Terman M. Tweaking the human circadian clock with light. *Science* 1998;279:333-334.
- Orth D N, Besser G M, King P H, Nicholson W E. Free-running circadian plasma cortisol rhythm in a blind human subject. *Clin Endocr* 1979;10:603-617.
- Orth D N, Island D P. Light synchronization of the circadian rhythm in plasma cortisol (17-OHCS) concentration in man. *J Clin Endocrinol Metab* 1969;29:479-486.
- Osterman P O. Light synchronization of the circadian rhythm of plasma 11-hydroxycorticosteroids in man. *Acta Endocrinologica* 1974;77:128-134.
- Ozaki Y, Lynch H J. Presence of melatonin in plasma and urine of pinealectomized rats. *Endocrinology* 1976;99:641-644.
- Paietta J. Photo-oxidation and the evolution of circadian rhythmicity. *J Theor Biol* 1982;97:77-82.
- Palm L, Blennow G, Wetterberg L. Correction of non-24-hour sleep/wake cycle by melatonin in a blind retarded boy. *Ann Neurol* 1991;29:336-339.
- Palm L, Blennow G, Wetterberg L. Long-term melatonin treatment in blind children and young adults with circadian sleep-wake disturbances. *Dev Med Child Neurol* 1997;39:319-325.
- Pampiglione G, Harden A. So-called neuronal ceroid lipofuscinosis. Neurophysiological studies in 60 children. *J Neurol Neurosurg Psych* 1977;40:323-330.
- Pang S F, Brown G M, Grotta L J, Chambers J W, Rodman R L. Determination of N-acetylserotonin and melatonin activities in the pineal gland, retina, Harderian gland, brain and serum of rats and chickens. *Neuroendocrinology* 1977;23:1-13.
- Papavasiliou P S, Cotzias G C, DUBY S E, Steck A J, Bell M, Lawrence W H. Melatonin and parkinsonism. *JAMA - J Am Med Assoc* 1972;221:88-89.
- Pardridge W M, Mietus L J. Transport of albumin-bound melatonin through the blood-brain barrier. *J Neurochem* 1980;34:1761-1763.
- Partonen T. Effects of morning light treatment on subjective sleepiness and mood in winter depression. *J Affect Disord* 1994;30:47-56.
- Partonen T, Lönnqvist J. Seasonal affective disorder. *Lancet* 1998;352:1369-1374.
- Partonen T, Vakkuri O, Lamberg-Allardt C. Effects of exposure to morning bright light in the blind and sighted controls. *Clin Physiol* 1995;15:637-646.
- Pelham R W, Vaughan G M, Sandlock K L, Vaughan M K. Twenty-four-hour cycle of a melatonin-like substance in the plasma of human males. *J Clin Endocrinol Metab* 1973;37:341-344.

- Perlow M J, Reppert S M, Boyar R M, Klein D C. Daily rhythms in cortisol and melatonin in primate cerebrospinal fluid. Effects of constant light and dark. *Neuroendocrinology* 1981;32:193-196.
- Perlow M J, Reppert S M, Tamarkin L, Wyatt R J, Klein D C. Photic regulation of the melatonin rhythm: monkey and man are not the same. *Brain Res* 1980;182:211-216.
- Petrie K, Conaglen J V, Thompson L, Chamberlain K. Effect of melatonin on jet-lag after long haul flights. *BMJ* 1989;298:705-707.
- Petrie K, Dawson A G, Thompson L, Brook R. A double-blind trial of melatonin as a treatment for jet lag in international cabin crew. *Biol Psychiatry* 1993;33:526-530.
- Petterborg L J, Thalen B E, Kjellman B F, Wetterberg L. Effect of melatonin replacement on serum hormone rhythms in a patient lacking endogenous melatonin. *Brain Res Bull* 1991;27:181-185.
- Pickard G E. Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suprachiasmatic nucleus and the intergeniculate leaflet of the thalamus. *Neurosci Lett* 1985;55:211-217.
- Plautz J D, Kaneko M, Hall J C, Kay S A. Independent photoreceptive circadian clocks throughout *Drosophila*. *Science* 1997;278:1632-1635.
- Poeggeler B, Reiter R J, Tan D X, Chen L D, Manchester L C. Melatonin, hydroxyl radical-mediated oxidative damage, and aging - a hypothesis. *J Pineal Res* 1993;14:151-168.
- van den Pol A N. The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy. *J Comp Neurol* 1980;191:661-702.
- Provencio I, Rodriguez I R, Jiang G, Hayes W P, Moreira E F, Rollag M D. A novel human opsin in the inner retina. *J Neurosci* 2000;20:600-605.
- Provencio I, Wong S Y, Lederman A B, Argamaso S M, Foster R G. Visual and circadian responses to light in aged retinally degenerate mice. *Vision Res* 1994;34:1799-1806.
- Puig-Domingo M, Webb S M, Serrano J, Peinado M A, Corcoy R, Ruscalleda J, Reiter R J, Deleiva A. Melatonin-related hypogonadotropic hypogonadism - brief report. *N Engl J Med* 1992;327:1356-1359.
- Quay W B. Individuation and lack of pineal effect in the rat's circadian locomotor rhythm. *Physiol Behav* 1968;3:109-118.
- Quay W B. Precocious entrainment and associated characteristics of activity patterns following pinealectomy and reversal of photoperiod. *Physiol Behav* 1970;5:1281-1290.
- Raikhlin N T, Kvetnoy I M, Tolkachev V N. Melatonin may be synthesised in enterochromaffin cells. *Nature* 1975;255:344-345.
- Raitta C, Santavuori P. Ophthalmological findings in infantile type of so-called neuronal ceroid lipofuscinosis. *Acta Ophthalmol* 1973;51:755-763.
- Raitta C, Santavuori P. Ophthalmological findings and main clinical characteristics in childhood types of neuronal ceroid-lipofuscinosis. In: Huber A, Klein D, eds. *Neurogenetics and Neuro-ophthalmology*. Amsterdam: Elsevier; 1981. p. 307-316.
- Ralph M R, Foster R G, Davis F C, Menaker M. Transplanted suprachiasmatic nucleus determines circadian period. *Science* 1990;247:975-978.
- Rando T A, Bowers C W, Zigmond R E. Localization of neurons in the rat spinal cord which project to the superior cervical ganglion. *J Comp Neurol* 1981;196:73-83.

- Ranta S, Zhang Y H, Ross B, Lonka L, Takkunen E, Messer A, Sharp J, Wheeler R, Kusumi K, Mole S, Liu W C, Soares M B, Bonaldo M D, Hirvasniemi A, de la Chapelle A, Gilliam T C, Lehesjoki A E. The neuronal ceroid lipofuscinoses in human EPMP and mnd mutant mice are associated with mutations in CLN8. *Nature Gen* 1999;23:233-236.
- Redman J R. Circadian entrainment and phase shifting in mammals with melatonin. *J Biol Rhythms* 1997;12:581-587.
- Redman J R, Armstrong S M. Reentrainment of rat circadian activity rhythms: Effects of melatonin. *J Pineal Res* 1988;5:203-215.
- Redman J, Armstrong S, Ng K T. Free-running activity rhythms in the rat: Entrainment by melatonin. *Science* 1983;219:1089-1091.
- Reilly T, Atkinson G, Waterhouse J. *Biological Rhythms and Exercise*. Oxford: Oxford University Press; 1997.
- Reiter R J. The pineal and its hormones in the control of reproduction in mammals. *Endocr Rev* 1980;1:109-131.
- Reiter R J. Melatonin - the chemical expression of darkness. *Mol Cell Endocrinol* 1991a;79:C153-C158.
- Reiter R J. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr Rev* 1991b;12:151-180.
- Reiter R J. Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J* 1995;9:526-533.
- Reiter R J. Oxidative damage in the central nervous system: protection by melatonin. *Prog Neurobiol* 1998;56:359-384.
- Reiter R J, Garcia J J, Pie J. Oxidative toxicity in models of neurodegeneration: responses to melatonin. *Restor Neurol Neurosci* 1998;12:135-142.
- Reiter R J, Poeggeler B, Tan D, Chen L D, Manchester L C, Guerrero J M. Antioxidant capacity of melatonin - a novel action not requiring a receptor. *Neuroendocrinol Lett* 1993;15:103-116.
- Reppert S M, Perlow M J, Ungerleider L G, Mishkin M, Tamarkin L, Orloff D G, Hoffman H J, Klein D C. Effects of damage to the suprachiasmatic area of the anterior hypothalamus on daily melatonin and cortisol rhythms in the rhesus monkey. *J Neurosci* 1981;1:1414-1425.
- Reppert S M, Schwartz W J. The suprachiasmatic nucleus of the fetal rat: characterization of a functional circadian clock using <sup>14</sup>C-labeled deoxyglucose. *J Neurosci* 1984;4:1677-1682.
- Reppert S M, Weaver D R, Rivkees S A, Stopa E G. Putative melatonin receptors in a human biological clock. *Science* 1988;242:78-81.
- Reuss S. Components and connections of the circadian timing system in mammals. *Cell Tissue Res* 1996;285:353-378.
- Rex K M, Kripke D F, Cole R J, Klauber M R. Nocturnal light effects on menstrual cycle length. *J Alternat Compl Med* 1997;3:387-390.
- Richter C P. Biological clocks and endocrine glands. *Proceedings of the 2nd International Congress of Endocrinology*. Excerpta Medica 1964;83:119-123.
- Richter C P. Sleep and activity: their relation to the 24-hour clock. In: Kety S S, Evarts E V, Williams H L, eds. *Sleep and Altered States of Consciousness*. Baltimore: The Williams and Wilkins Company; 1967. p. 8-29.

- Robinson J, Bayliss S C, Fielder A R. Transmission of light across the adult and neonatal eyelid *in vivo*. *Vision Res* 1991;31:1837-1840.
- Rosenthal N E, Joseph-Vanderpool J R, Levendosky A A, Johnston S H, Allen R, Kelly K A, Souetre E, Schultz P M, Starz K E. Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. *Sleep* 1990;13:354-361.
- Rosenthal N E, Sack D A, Gillin J C, Lewy A J, Goodwin F K, Davenport Y, Mueller P S, Newsome D A, Wehr T A. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. *Arch Gen Psychiatry* 1984;41:72-80.
- Roth J J, Gern W A, Roth E C, Ralph C L, Jacobson E. Nonpineal melatonin in the alligator (*Alligator mississippiensis*). *Science* 1980;210:548-550.
- Ruberg F L, Skene D J, Hanifin J P, Rollag M D, English J, Arendt J, Brainard G C. Melatonin regulation in humans with color vision deficiencies. *J Clin Endocrinol Metab* 1996;81:2980-2985.
- Rusak B, Abe H, Mason R, Piggins H D, Ying S-W. Neurophysiological analysis of circadian rhythm entrainment. *J Biol Rhythms* 1993;8(Suppl):S39-S45.
- Rusak B, Groos G. Suprachiasmatic stimulation phase shifts rodent circadian rhythms. *Science* 1982;215:1407-1409.
- Rusak B, Yu G D. Regulation of melatonin-sensitivity and firing-rate rhythms of hamster suprachiasmatic nucleus neurons - pinealectomy effects. *Brain Res* 1993;602:200-204.
- Rusak B, Zucker I. Neural regulation of circadian rhythms. *Physiol Rev* 1979;59:449-526.
- Rutkowska D, Tokura H, Morita T. Deviations in circadian rhythms of the core temperature in color-deficient subjects. *Naturwissenschaften* 1998;85:130-132.
- Sack R L, Blood M L, Lewy A J. Melatonin rhythms in night shift workers. *Sleep* 1992a;15:434-441.
- Sack R L, Hughes R J, Edgar D M, Lewy A J. Sleep-promoting effects of melatonin: at what dose, in whom, under what conditions, and by what mechanisms. *Sleep* 1997;20:908-915.
- Sack R L, Lewy A J. Melatonin as a chronobiotic - treatment of circadian desynchrony in night workers and the blind. *J Biol Rhythms* 1997;12:595-603.
- Sack R L, Lewy A J, Blood M L, Keith L D, Nakagawa H. Circadian rhythm abnormalities in totally blind people: incidence and clinical significance. *J Clin Endocrinol Metab* 1992b;75:127-134.
- Sack R L, Lewy A J, Blood M L, Stevenson J, Keith L D. Melatonin administration to blind people - phase advances and entrainment. *J Biol Rhythms* 1991;6:249-261.
- Sack R L, Lewy A J, Erb D L, Vollmer W M, Singer C M. Human melatonin production decreases with age. *J Pineal Res* 1986;3:379-388.
- Santavuori P. Neuronal ceroid-lipofuscinoses in childhood. *Brain Devel* 1988;10:80-83.
- Santavuori P. NCL in different European countries. Finland. In: Goebel H H, Mole S E, Lake B D, eds. *The Neuronal Ceroid Lipofuscinoses (Batten Disease)*. Amsterdam: IOS Press; 1999. p. 130.
- Santavuori P, Gottlob I, Haltia M, Rapola J, Lake B D, Tynnelä J, Peltonen L. CLN1. Infantile and other types of NCL with GROD. In: Goebel H H, Mole S E, Lake B D, eds. *The Neuronal Ceroid Lipofuscinoses (Batten Disease)*. Amsterdam: IOS Press; 1999. p. 16-36.

- Santavuori P, Haltia M, Rapola J. Infantile type of so-called neuronal ceroid-lipofuscinosis. *Dev Med Child Neurol* 1974;16:644-653.
- Santavuori P, Heiskala H, Westermarck T, Sainio K, Moren R. Experience over 17 years with antioxidant treatment in Spielmeier-Sjögren disease. *Am J Med Gen* 1988;5:265-274.
- Santavuori P, Linnankivi T, Jaeken J, Vanhanen S-L, Telakivi T, Heiskala H. Psychological symptoms and sleep disturbances in neuronal ceroid-lipofuscinoses (NCL). *J Inher Metab Dis* 1993;16:245-248.
- Saper C B, Loewy A D, Swanson L W, Cowan W M. Direct hypothalamo-autonomic connections. *Brain Res* 1976;117:305-312.
- Sarrafzadeh A, Wirz-Justice A, Arendt J, English J. Melatonin stabilises sleep onset in a blind man. In: Horne J, ed. *Sleep '90*. Bochum: Pontenagel Press; 1990. p. 51-54.
- Savides T J, Messin S, Senger C, Kripke D F. Natural light exposure of young adults. *Physiol Behav* 1986;38:571-574.
- Savukoski M, Klockars T, Holmberg V, Santavuori P, Lander E S, Peltonen L. CLN5, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroid lipofuscinosis. *Nature Genet* 1998;19:286-288.
- Scheer F A J L, van Doornen L J P, Buijs R M. Light and diurnal cycle affect human heart rate: possible role for the circadian pacemaker. *J Biol Rhythms* 1999;14:202-212.
- Schwartz W J. Understanding circadian clocks: from c-fos to fly balls. *Ann Neurol* 1997;41:289-297.
- Schwartz W J, Davidsen L C, Smith C B. *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *J Comp Neurol* 1980;189:157-167.
- Schwitzer J, Neudorfer C, Blecha H G, Fleischhacker W W. Mania as a side effect of phototherapy. *Biol Psychiatry* 1990;28:532-534.
- Seeliger M, Rütther K, Apfelstedt-Sylla E, Schlote W, Wohlrab M, Zrenner E. Juvenile neuronale Ceroid-lipofuszinose (Batten-Mayou). *Augenärztliche Diagnostik und Befunde. Ophthalmologie* 1997;94:557-562.
- Shanahan T L, Kronauer R E, Duffy J F, Williams G H, Czeisler C A. Melatonin rhythm observed throughout a three-cycle bright-light stimulus designed to reset the human circadian pacemaker. *J Biol Rhythms* 1999;14:237-253.
- Sharp G W G. The effect of light on diurnal leucocyte variations. *J Endocrin* 1960;21:213-218.
- Sheldon S H. Pro-convulsant effects of oral melatonin in neurologically disabled children. *Lancet* 1998;351:1254.
- Shibata S, Cassone V M, Moore R Y. Effects of melatonin on neuronal activity in the rat suprachiasmatic nucleus *in vitro*. *Neurosci Lett* 1989;97:140-144.
- Shibata S, Moore R Y. Electrical and metabolic activity of suprachiasmatic nucleus neurons in hamster hypothalamic slices. *Brain Res* 1988;438:374-378.
- Shida C S, Castrucci A M L, Lamyfreund M T. High melatonin solubility in aqueous medium. *J Pineal Res* 1994;16:198-201.
- Shirakawa T, Moore R Y. Glutamate shifts the phase of the circadian neuronal firing rhythm in the rat suprachiasmatic nucleus *in vitro*. *Neurosci Lett* 1994;178:47-50.
- Skene D J, Lockley S W, James K, Arendt J. Correlation between urinary cortisol and 6-sulphatoxymelatonin

- rhythms in field studies of blind subjects. *Clin Endocrinol* 1999;50:715-719.
- Sleat D E, Donnelly R J, Lackland H, Liu C-G, Sohar I, Pullarkat R K, Lobel P. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. *Science* 1997;277:1802-1805.
- Smith I, Mullen P E, Silman R E, Snedden W, Wilson B W. Absolute identification of melatonin in human plasma and cerebrospinal fluid. *Nature* 1976a;260:718-719.
- Smith J A, Mee T J, Barnes N D, Thorburn R J, Barnes J L. Melatonin in serum and cerebrospinal fluid. *Lancet* 1976b;2:425.
- Smith J A, O'Hara J, Schiff A A. Altered diurnal serum melatonin rhythm in blind men. *Lancet* 1981;2:933.
- Song Y, Tam P C, Poon A M S, Brown G M, Pang S F. 2-[<sup>125</sup>I]iodomelatonin-binding sites in the human kidney and the effect of guanosine 5'-O-(3-thiotriphosphate). *J Clin Endocrinol Metab* 1995;80:1560-1565.
- Spalton D J, Taylor D S I, Sanders M D. Juvenile Batten's disease: an ophthalmological assessment of 26 patients. *Br J Ophthalmol* 1980;64:726-732.
- Speh J C, Moore R Y. Retinohypothalamic tract development in the hamster and rat. *Dev Brain Res* 1993;76:171-181.
- Spitzer R L, Terman M, Williams J B W, Terman J S, Malt U F, Singer F, Lewy A J. Jet lag: clinical features, validation of a new syndrome-specific scale, and lack of response to melatonin in a randomized, double-blind trial. *Am J Psychiatry* 1999;156:1392-1396.
- Stanberry L R, Das Gupta T K, Beattie C W. Photoperiodic control of melanoma growth in hamsters: influence of pinealectomy and melatonin. *Endocrinology* 1983;113:469-475.
- Stehle J H, Foulkes N S, Molina C A, Simonneaux V, Pévet P, Sassone-Corsi P. Adrenergic signals direct rhythmic expression of transcriptional repressor CREM in the pineal gland. *Nature* 1993;365:314-320.
- Stehle J, Vanecek J, Vollrath L. Effects of melatonin on spontaneous electrical activity of neurons in rat suprachiasmatic nuclei: an *in vitro* iontophoretic study. *J Neural Transm* 1989;78:173-177.
- Steinhilber D, Carlberg C. Melatonin receptor ligands. *Exp Opin Ther Patents* 1999;9:281-290.
- Stephan F K. Limits of entrainment to periodic feeding in rats with suprachiasmatic lesions. *J Comp Physiol A* 1981;143:401-410.
- Stephan F K, Berkley K J, Moss R L. Efferent connections of the rat suprachiasmatic nucleus. *Neuroscience* 1981;6:2625-2641.
- Stephan F K, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci USA* 1972;69:1583-1586.
- Stokkan K A, Reiter R J. Melatonin rhythms in arctic urban residents. *J Pineal Res* 1994;16:33-36.
- Strassman R J, Qualls C R, Lisansky E J, Peake G T. Elevated rectal temperature produced by all-night bright light is reversed by melatonin infusion in men. *J Appl Physiol* 1991;71:2178-2182.
- Sudgen D. Psychopharmacological effects of melatonin in mouse and rat. *J Pharmacol Exp Ther* 1983;227:587-591.
- Sugden D. Melatonin biosynthesis in the mammalian pineal gland. *Experientia* 1989;45:922-932.
- Sulzman F M, Fuller C A, Moore-Ede M C. Effects of phasic and tonic light inputs on the circadian organization

of the squirrel monkey. *Photochem Photobiol* 1981;34:249-256.

Sumová A, Illnerová H. Melatonin instantaneously resets intrinsic circadian rhythmicity in the rat suprachiasmatic nucleus. *Neurosci Lett* 1996;218:181-184.

Suvanto S, Härmä M, Ilmarinen J, Partinen M. Effects 10 h time zone changes on female flight attendants' circadian rhythms of body temperature, alertness, and visual search. *Ergonomics* 1993;36:613-625.

Swaab D F, Hofman M A, Lucassen P J, Purba J S, Raadsheer F C, Vandenes J A P. Functional neuroanatomy and neuropathology of the human hypothalamus. *Anat Embryol* 1993;187:317-330.

Swanson L W, Cowan W M. The efferent connections of the suprachiasmatic nucleus of the hypothalamus. *J Comp Neurol* 1975;160:1-12.

Tabandeh H, Lockley S W, Buttery R, Skene D J, DeFrance R, Arendt J, Bird A C. Disturbance of sleep in blindness. *Am J Ophthalmol* 1998;126:707-712.

Takahashi J S, DeCoursey P J, Bauman L, Menaker M. Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 1984;308:186-188.

Takahashi J S, Zatz M. Regulation of circadian rhythmicity. *Science* 1982;217:1104-1111.

Tamarkin L, Baird C J, Almeida O F X. Melatonin: a coordinating signal for mammalian reproduction? *Science* 1985;227:714-720.

Tan D-X, Chen L-D, Poeggeler B, Manchester L C, Reiter R J. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocr J* 1993;1:57-60.

Tarkkanen A, Haltai M, Merenmies L. Ocular pathology in infantile type of neuronal ceroid-lipofuscinosis. *J Pediatr Ophthalmol* 1977;14:235-241.

Terman M, Terman J S, Ross D C. A controlled trial of timed bright light and negative air ionization for treatment of winter depression. *Arch Gen Psychiatry* 1998;55:875-882.

Tetsuo M, Perlow M J, Mishkin M, Markey S P. Light exposure reduces and pinealectomy virtually stops urinary excretion of 6-hydroxymelatonin by rhesus monkeys. *Endocrinology* 1982;110:997-1003.

The International Batten Disease Consortium. Isolation of a novel gene underlying Batten disease, CLN3. *Cell* 1995;82:949-957.

Thiele G, Meissl H. Action spectra of the lateral eyes recorded from mammalian pineal glands. *Brain Res* 1987;424:10-16.

Thomas E M V, Armstrong S M. Melatonin administration entrains female rat activity rhythms in constant darkness but not in constant light. *Am J Physiol* 1988;255:R237-R242.

Thresher R J, Vitaterna M H, Miyamoto Y, Kazantsev A, Hsu D S, Petit C, Selby C P, Dawut L, Smithies O, Takahashi J S, Sancar A. Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 1998;282:1490-1494.

Torres G, Lytle L D. Extraretinal mechanisms mediate light-induced changes in neonatal rat pineal gland N-acetyltransferase activity. *J Pineal Res* 1989;7:211-220.

Traboulsi E I, Green W R, Luckenbach M W, de la Cruz Z C. Neuronal ceroid lipofuscinosis. Ocular histopathologic and electron microscopic studies in the late infantile, juvenile, and adult forms. *Graefes Arch Clin Exp Ophthalmol* 1987;225:391-402.

- Turek F W, Earnest D J, Swann J. Splitting of the circadian rhythm of activity in hamsters. In: Aschoff J, Daan S, Groos G, eds. *Vertebrate Circadian Systems*. Berlin Heidelberg: Springer-Verlag; 1982. p. 203-214.
- Tzischinsky O, Dagan Y, Lavie P. The effects of melatonin on the timing of sleep in patients with delayed sleep phase syndrome. In: Touitou Y, Arendt J, Pévet P, eds. *Melatonin and the Pineal Gland - From Basic Science to Clinical Application*. Amsterdam: Elsevier; 1993. p. 351-354.
- Tzischinsky O, Pal I, Epstein R, Dagan Y, Lavie P. The importance of timing in melatonin administration in a blind man. *J Pineal Res* 1992;12:105-108.
- Underwood H. Circadian rhythms in lizards: phase response curve for melatonin. *J Pineal Res* 1986;3:187-196.
- Underwood H, Groos G. Vertebrate circadian rhythms: retinal and extraretinal photoreception. *Experientia* 1982;38:1013-1021.
- Vakkuri O. Diurnal rhythm of melatonin in human saliva. *Acta Physiol Scand* 1985;124:409-412.
- Vakkuri O, Leppäluoto J, Vuolteenaho O. Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin as tracer. *Acta Endocrinol (Copenh)* 1984;106:152-157.
- Van Cauter E, Sturis J, Byrne M M, Blackman J D, Leproult R, Ofek G, L'Hermite-Balériaux M, Refetoff S, Turek F W, Van Reeth O. Demonstration of rapid light-induced advances and delays of the human circadian clock using hormonal phase markers. *Am J Physiol* 1994;266:E953-E963.
- Van Dongen H P A, Kerkhof G A, Klöppel H-B. Seasonal covariation of the circadian phases of rectal temperature and slow wave sleep onset. *J Sleep Res* 1997;6:19-25.
- Van Dongen H P A, Kerkhof G A, Souverijn J H M. Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiol Int* 1998;15:623-632.
- Vanecek J, Pavlik A, Illnerová H. Hypothalamic melatonin receptor sites revealed by autoradiography. *Brain Res* 1987;435:359-362.
- Vanecek J, Sudgen D, Weller J, Klein D C. Atypical synergistic alpha1- and beta-adrenergic regulation of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in rat pinealocytes. *Endocrinology* 1985;116:2167-2173.
- Vanhanen S-L. *Neuroradiological and neurophysiological findings in infantile neuronal ceroid-lipofuscinosis (INCL)*. Doctoral thesis. Helsinki, Finland: University of Helsinki; 1996.
- Vanhanen S-L, Raininko R, Autti T, Santavuori P. MRI evaluation of the brain in infantile neuronal ceroid-lipofuscinosis. Part 2: MRI findings in 21 patients. *J Child Neurol* 1995;10:444-450.
- Vanhanen S-L, Raininko R, Santavuori P. Early differential diagnosis of infantile neuronal ceroid lipofuscinosis, Rett syndrome, and Krabbe disease by CT and MR. *AJNR Am J Neuroradiol* 1994;15:1443-1453.
- Vanhanen S-L, Sainio K, Lappi M, Santavuori P. EEG and evoked potentials in infantile neuronal ceroid-lipofuscinosis. *Dev Med Child Neurol* 1997;39:456-463.
- Van Reeth O, Turek F W. Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. *Nature* 1989;339:49-51.
- Van Someren E J W, Kessler A, Mirmiran M, Swaab D F. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. *Biol Psychiatry* 1997;41:955-963.
- Vaughan G M, Bell R, de la Peña A. Nocturnal plasma melatonin in humans: episodic pattern and influence of light. *Neurosci Lett* 1979;14:81-84.

- Vaughan G M, Pelham R W, Pang S F, Loughlin L L, Wilson K M, Sandock K L, Vaughan M K, Koslow S H, Reiter R J. Nocturnal elevation of plasma melatonin and urinary 5-hydroxyindoleacetic acid in young men: Attempts at modification by brief changes in environmental lighting and sleep and by autonomic drugs. *J Clin Endocrinol Metab* 1976;42:752-764.
- Vaughan M K. Pineal peptides: an overview. In: Reiter R J, ed. *The Pineal Gland*. New York: Raven Press; 1984. p. 39-81.
- Vesa J, Hellsten E, Verkruyse L A, Camp L A, Rapola J, Santavuori P, Hofmann S L, Peltonen L. Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature* 1995;376:584-587.
- Vondrašová D, Hájek I, Illnerová H. Exposure to long summer days affects the human melatonin and cortisol rhythms. *Brain Res* 1997;759:166-170.
- de Vries M J, Nunes Cardozo B, van der Want J, de Wolf A, Meijer J H. Glutamate immunoreactivity in terminals of the retinohypothalamic tract of the brown Norwegian rat. *Brain Res* 1993;612:231-237.
- Waldhauser F, Ehrhart B, Forster E. Clinical aspects of the melatonin action - impact of development, aging, and puberty, involvement of melatonin in psychiatric disease and importance of neuroimmunoendocrine interactions. *Experientia* 1993;49:671-681.
- Waldhauser F, Waldhauser M, Lieberman H R, Deng M H, Lynch H J, Wurtman R J. Bioavailability of oral melatonin in humans. *Neuroendocrinology* 1984;39:307-313.
- Warren W S, Champney T H, Cassone V M. The suprachiasmatic nucleus controls the circadian rhythm of heart rate *via* the sympathetic nervous system. *Physiol Behav* 1994;55:1091-1099.
- Waterhouse J, Minors D, Folkard S, Owens D, Atkinson G, MacDonald I, Reilly T, Sytnik N, Tucker P. Light of domestic intensity produces phase shifts of the circadian oscillator in humans. *Neurosci Lett* 1998;245:97-100.
- Watts A G, Swanson L W. Efferent projections of the suprachiasmatic nucleus: II. Studies using retrograde transport of fluorescent dyes and simultaneous peptide immunohistochemistry in the rat. *J Comp Neurol* 1987;258:230-252.
- Watts A G, Swanson L W, Sanchez-Watts G. Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. *J Comp Neurol* 1987;258:204-229.
- Weaver D R, Reppert S M. The MEL1A melatonin receptor gene is expressed in human suprachiasmatic nuclei. *Neuroreport* 1996;8:109-112.
- Weaver D R, Stehle J H, Stopa E G, Reppert S M. Melatonin receptors in human hypothalamus and pituitary - implications for circadian and reproductive responses to melatonin. *J Clin Endocrinol Metab* 1993;76:295-301.
- Wehr T A. The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod). *J Clin Endocrinol Metab* 1991;73:1276-1280.
- Wehr T A. Melatonin and seasonal rhythms. *J Biol Rhythms* 1997;12:518-527.
- Wehr T A, Goodwin F K. *Circadian Rhythms in Psychiatry. Psychobiology and Psychopathology, Vol. 2*. Pacific Grove, CA: Boxwood Press; 1983
- Wehr T A, Jacobsen F M, Sack D A, Arendt J, Tamarkin L, Rosenthal N E. Phototherapy of seasonal affective disorder. Time of day and suppression of melatonin are not critical for antidepressant effects. *Arch Gen Psychiatry* 1986;43:879-5.
- Weibel L, Spiegel K, Gronfier C, Follenius M, Brandenberger G. Twenty-four-hour melatonin and core body temperature rhythms: their adaptation in night workers. *Am J Physiol* 1997;272:R948-R954.

- Weissbach H, Redfield B G, Axelrod J. Biosynthesis of melatonin: enzymic conversion of serotonin to N-acetylserotonin. *Biochim Biophys Acta* 1960;43:352-353.
- Weissbach H, Redfield B G, Axelrod J. The enzymic acetylation of serotonin and other naturally occurring amines. *Biochim Biophys Acta* 1961;54:190-192.
- Weleber R G. The dystrophic retina in multisystem disorders: the electroretinogram in neuronal ceroid lipofuscinoses. *Eye* 1998;12:580-590.
- Welsh D K, Logothetis D E, Meister M, Reppert S M. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 1995;14:697-706.
- Wever R A. The circadian system of man. Results of experiments under temporal isolation. In: Schaefer K E, ed. *Topics in Environmental Physiology and Medicine*. New York: Springer-Verlag; 1979.
- Wever R A. Light effects on human circadian rhythms. A review of recent Andechs experiments. *J Biol Rhythms* 1989;4:161-184.
- Wilson St, Blask D E, Lemus-Wilson A M. Melatonin augments the sensitivity of MCF-7 human breast cancer cells to tamoxifen *in vitro*. *J Clin Endocrinol Metab* 1992;75:669-670.
- Wirz-Justice A, Armstrong S M. Melatonin - nature's soporific. *J Sleep Res* 1996;5:137-141.
- Wirz-Justice A, Graw P, Kräuchi K, Gisin B, Arendt J, Aldhous M, Poldinger W. Morning or night-time melatonin is ineffective in seasonal affective disorder. *J Psychiatr Res* 1990;24:129-137.
- Wirz-Justice A, Terman M, Terman J S, Boulos Z, Remé C E, Danilenko K V. Dawn stimulation in animals and humans: Effects on melatonin secretion, depression and sleep. *Chronobiol Int* 1997;14(Suppl 1):184.
- Wisniewski K E, Kida E, Patxot O F, Connell F. Variability in the clinical and pathological findings in the neuronal ceroid lipofuscinoses: review of data and observations. *Am J Med Gen* 1992;42:525-532.
- Witte K, Grebmer W, Scalbert E, Delagrangé P, Guardiola-Lemaître B, Lemmer B. Effects of melatonergic agonists on light-suppressed circadian rhythms in rats. *Physiol Behav* 1998;65:219-224.
- Wurtman R J. The effects of light on man and other mammals. *Am Rev Physiol* 1975;37:467-483.
- Wurtman R J, Axelrod J, Chu E W. Melatonin, a pineal substance: effect on the rat ovary. *Science* 1963a;141:277-278.
- Wurtman R J, Axelrod J, Fischer J E. Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system. *Science* 1964;143:1328-1330.
- Wurtman R J, Axelrod J, Phillips L S. Melatonin synthesis in the pineal gland: control by light. *Science* 1963b;142:1071-1073.
- Yamazaki S, Goto M, Menaker M. No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *J Biol Rhythms* 1999;14:197-201.
- Yellon S M, Hilliker S. Influence of acute melatonin treatment and light on the circadian melatonin rhythm in the Djungarian hamster. *J Biol Rhythms* 1994;9:71-81.
- Yie S M, Niles L P, Younglai E V. Melatonin receptors on human granulosa cell membranes. *J Clin Endocrinol Metab* 1995;80:1747-1749.
- Yoshikawa T, Oishi T. Extraretinal photoreception and circadian systems in nonmammalian vertebrates. *Comp Biochem Physiol* 1998;119B:65-72.

- Yoshimura T, Ebihara S. Spectral sensitivity of photoreceptors mediating phase-shifts of circadian rhythms in retinally degenerate CBA/J (rd/rd) and normal CBA/N (+/+) mice. *J Comp Physiol A* 1996;178:797-802.
- Youngstedt S D, Kripke D F, Elliott J A. Melatonin excretion is not related to sleep in the elderly. *J Pineal Res* 1998;24:142-145.
- Zaidan R, Geoffriau M, Brun J, Taillard J, Bureau C, Chazot G, Claustrat B. Melatonin is able to influence its secretion in humans: description of a phase-response curve. *Neuroendocrinology* 1994;60:105-112.
- Zatz M, Herkenham M A. Intraventricular carbachol mimics the phase-shifting effect of light on the circadian rhythm of wheel-running activity. *Brain Res* 1981;212:234-238.
- Zeitler J M, Daniels J E, Duffy J F, Klerman E B, Shanahan T L, Dijk D-J, Czeisler C A. Do plasma melatonin concentrations decline with age? *Am J Med* 1999;107:432-436.
- Zeitler J M, Kronauer R E, Czeisler C A. Photopic transduction implicated in human circadian entrainment. *Neurosci Lett* 1997;232:135-138.
- Zeman W, Donahue S, Dyken P, Green J. The neuronal ceroid-lipofuscinoses (Batten-Vogt syndrome). In: Vinken P J, Bruyn G W, eds. *Handbook of Clinical Neurology*. Amsterdam: North-Holland Publishing Co.; 1970. p. 588-679.
- Zeman W, Dyken P. Neuronal ceroid-lipofuscinosis (Batten's disease): Relationship to amaurotic family idiocy? *Pediatrics* 1969;44:570-583.
- Zhdanova I V, Lynch H J, Wurtman R J. Melatonin: a sleep promoting hormone. *Sleep* 1997;20:899-907.
- Zhdanova I V, Wurtman R J, Wagstaff J. Effects of a low dose of melatonin on sleep in children with Angelman syndrome. *J Ped Endocrinol Metab* 1999;12:57-67.
- Zucker I, Lee T M, Dark J. The suprachiasmatic nucleus and annual rhythms of mammals. In: Klein D C, Moore R Y, Reppert S M, eds. *Suprachiasmatic Nucleus: the Mind's Clock*. New York: Oxford University Press; 1991. p. 246-259.
- Åkerstedt T, Fröberg J E, Friberg Y, Wetterberg L. Melatonin excretion, body temperature and subjective arousal during 64 hours of sleep deprivation. *Psychoneuroendocrinology* 1979;4:219-225.