

**HOSPITAL FOR CHILDREN AND ADOLESCENTS
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UNIVERSITY OF HELSINKI**

**CIRCADIAN RHYTHMS AND SLEEP
IN NEURONAL CEROID LIPOFUSCINOSES**

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ACADEMIC DISSERTATION

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ABBREVIATIONS

CBT	Core body temperature
EEG	Electroencephalography
EMG	Electromyography
EOG	Electro-oculography
ERG	Electroretinography
FNE	First-night effect
HDSW	High amplitude delta wave activity with intermingled sharp waves
INCL	Infantile neuronal ceroid lipofuscinosis
LINCL	Late infantile neuronal ceroid lipofuscinosis
JNCL	Juvenile neuronal ceroid lipofuscinosis
MEG	Magnetoencephalography
MRI	Magnetic resonance image
NCL	Neuronal ceroid lipofuscinosis
NREM	Non-rapid eye movement
PA	Paroxysmal activity
PSG	Polysomnography
REM	Rapid eye movement
S1	NREM stage 1
S2	NREM stage 2
SCN	Suprachiasmatic nucleus
SEI	Sleep efficiency index
SEP	Somatosensory evoked potential
SPECT	Single photon emission computed tomography
SWA	Slow wave activity
SWS	Slow wave sleep
TST	Total sleep time
VEP	Visual evoked potential

LIST OF 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 Heikkilä E, Hätönen T, Telakivi T, Laakso M-L, Heiskala H, Salmi T, Alila A, and Santavuori P. Circadian rhythm studies in neuronal ceroid-lipofuscinosis (NCL). *Am J Med Gen* 1995, 57:229-234.
- II Hätönen T, Kirveskari E, Heiskala H, Sainio K, Laakso M-L, and Santavuori P. Melatonin ineffective in neuronal ceroid lipofuscinosis patients with fragmented or normal motor activity rhythms recorded by wrist actigraphy. *Mol Gen Met* 1999, 66:401-406.
- III Kirveskari E, Partinen M, Salmi T, Sainio K, Telakivi T, Hämäläinen M, Larsen A, and Santavuori P. Sleep alterations in juvenile neuronal ceroid lipofuscinosis. *Pediatr Neurol* 2000, 22:347-354.
- IV Kirveskari E, Partinen M, and Santavuori P. Sleep and its disturbance in a variant form of late infantile neuronal ceroid lipofuscinosis (CLN5). *Submitted.*

1. INTRODUCTION

The neuronal ceroid lipofuscinoses (NCLs) are among the most common progressive encephalopathies in children, occurring worldwide. They are characterized by accumulation of deposit material in the perikarya of neurons and in numerous other cell types, and by loss of nerve cells, particularly in the cerebral cortex. These diseases lead to severe neurological disability and eventually to premature death. To date, 13 different types of NCL have been described. All childhood types of NCL are inherited in an autosomal recessive fashion, with two types, infantile NCL (INCL, Santavuori-Haltia disease) and the Finnish variant late infantile NCL (vLINCL) being representatives of the Finnish Disease Heritage. In NCL, sleep disorders are common, often being the most disturbing symptom of the disease in the everyday life. The treatment of the sleep disturbances is problematic, since conventional medication is often ineffective in these patients. Many factors may underlie the frequent sleep disorders in NCL, such as neuronal atrophy leading to disturbance of neuronal transmission, epilepsy, nocturnal myoclonia, pain, or psychological problems.

Sleep is an active and highly organized state of the brain. Structural or functional damage to the critical brain regions involved in the generation and maintenance of sleep and wakefulness, and in the alternation between sleep stages may disturb the sleep state and its organization. Disorders of the brain may be associated with abnormalities in sleep EEG and in sleep structure. Although sleep has yet unknown functions, it is obvious that reduction or disruption of sleep hinders an individual's ability to navigate through the waking state.

Linked to the rotation of the planet, humans present with circadian (Latin:

circa=about, dies=a day) rhythms. The most salient behavioral marker of a rhythm with a period of approximately 24 hours is the daily sleep-wake cycle. The endogenous pacemaker generating circadian rhythms is affected by an environmental light-dark cycle, the information of which is thought to be transmitted to body functions by melatonin. Blind individuals may not be entrained by light, and may therefore present with abnormal rhythms of sleep and wake. Coinciding brain damage and mental retardation diminish the ability of an individual to perceive and interpret external time cues, including others besides light, for stabilizing sleep with the environment.

NCL patients with brain damage, blindness, mental retardation, and epilepsy are predisposed to sleep abnormalities. The aim of this study was to explore the circadian rhythms and sleep in NCL and to clarify the pathophysiology underlying the frequent sleep disorders in these patients.

2. REVIEW OF THE LITERATURE

2.1 Neuronal ceroid lipofuscinoses (NCLs)

2.1.1 General

Neuronal ceroid lipofuscinoses (NCLs) are among the most common progressive neurodegenerative diseases in children, occurring worldwide. They are characterized by accumulation of ceroid- and lipofuscin-like material in the perikarya of neurons and in additional numerous intracerebral and extracerebral cell types, and by loss of nerve cells, particularly in the cerebral cortex (Zeman et al. 1970). Different types of NCL are distinguished according to age of onset, clinical phenotype, ultrastructural characterization of the storage material, and chromosomal location of the disease gene. All childhood types of NCL are inherited in an autosomal recessive fashion. At least eight genes underlie these encephalopathies; five of these genes have been isolated and mutations characterized. Two NCL genes encode lysosomal enzymes (Vesa et al. 1995, Sleat et al. 1997), and three encode transmembrane proteins (Ranta et al. 1996, Järvelä et al. 1998, Savukoski et al. 1998). Current evidence indicates that the basic defect in NCLs is associated with lysosomal function. Ultrastructurally, the autofluorescent lipopigments consist of granular, curvilinear, rectilinear, and fingerprint patterns, which can be recognized electron microscopically in circulating lymphocytes, skin and several other tissues. The main component of the storage material is either subunit c of mitochondrial ATP synthase (Hall et al. 1991, Palmer et al. 1992) or saposins A and D (Tyynelä et al. 1993).

Clinical features of NCL include progressive visual failure due to retinal, cortical, and optic nerve degeneration, epilepsy, and psychomotor deterioration,

eventually leading to premature death (Santavuori 1988a). No preventive or curative treatment is available at present.

Three main childhood types of NCL are infantile NCL (INCL, Santavuori-Haltia disease), classical late infantile NCL (LINCL, Jansky-Bielschowsky disease), and juvenile NCL (JNCL, Spielmeyer-Sjögren disease). They have been distinguished on the basis of the age of onset, clinical course, ultrastructural morphology, and separate genetic loci. To date, 13 different types of NCL have been described (Mole 1999), including a Finnish variant LINCL and Northern epilepsy which was recently found in Finland (Hirvasniemi et al. 1995, Ranta et al. 1996).

2.1.2 Juvenile NCL (JNCL, Spielmeyer-Sjögren disease, CLN3)

The incidence of juvenile NCL in Finland is 4.8 per 100 000 live births (Uvebrant 1997). The mutation in a gene for the lysosomal protein responsible for JNCL has been localized to chromosome 16p11.2-12.1 (The International Batten Disease Consortium 1995, Järvelä et al. 1999). The majority of the patients are homozygous for the founder mutation, a 1.02 kb deletion, which causes the disease in about 90% of affected chromosomes in Finland (Järvelä et al. 1996).

In JNCL, the initial symptom is visual failure, detected typically between 4 and 7 years of age, and usually leading to blindness by the age of 8-13 years (Santavuori 1988a, Järvelä et al. 1997). Loss of light perception occurs by the age of 20 years in most patients. Dementia becomes apparent several years after the onset of visual symptoms. During the second decade, speech typically becomes indistinct and rapid and later, severely dysarthric (Järvelä et al. 1997).

Concomitantly, Parkinson-like extrapyramidal dysfunction as well as slight cerebellar disturbance is noticed. Epileptic seizures are typically generalized with onset of seizures around the age of 10 years (Järvelä et al. 1997). Psychological and psychotic symptoms are common, such as fear, aggressive behaviour, restlessness, hallucinations, and depression (Santavuori et al. 1993, Lou and Kristenssen 1973). Death occurs at the mean age of 24 (range 16-35).

The clinical picture and the course of the disease show considerable variability, which may be related to different mutations in the disease gene. Thus, JNCL can manifest as at least three different phenotypes: classic, delayed classic and protracted JNCL (Lauronen et al. 1999).

Sleep disorders are often the most disturbing factor of the everyday life among JNCL patients. An earlier questionnaire study revealed sleep problems, such as settling problems, nocturnal awakenings, and nightmares in 55% of the studied patients (Santavuori et al. 1993). The sleep disturbances became evident at a mean age of 11 years and they usually persisted for several years. Epileptic seizures and tension seemed to aggravate the disturbances. Drugs and/or and sleep hygiene management, such as alterations in sleeping arrangements and daytime activity scheduling, alleviated the symptoms in most of the patients. The sleep problems in JNCL tend to increase during periods with psychotic symptoms or daytime restlessness (Santavuori, personal communication). In JNCL, sleep-related respiratory pattern disturbances, but without sleep apnea, have been reported (Telakivi et al. 1985).

MRI findings in JNCL are non-specific; cerebral and cerebellar atrophy as well as abnormally high signal intensity in the white matter beside the lateral ventricles have been detected (Autti et al. 1996). Hypointense thalami found at an early stage of JNCL seem to coincide with a more rapid progression of the

disease. The atrophy seen in postmortem MRIs has been shown to correlate with neuronal loss. Similarly, high signal intensity in the periventricular white matter has been shown to correlate with loss of myelin and gliosis (Autti et al. 1997b). The most obvious finding in SPECT (single photon emission computed tomography) studies is hypoperfusion in the temporal lobes (Launes et al. 1996).

In the EEG of JNCL patients, runs of high amplitude delta waves intermingled with spikes and/or sharp waves have been described (Lagenstein et al. 1977, Pampiglione and Harden 1977, Westmoreland et al. 1979). The EEG is normal until the age of about 9 years, after which background abnormalities with spike and wave paroxysms progressively appear (Raininko et al. 1990). Westmoreland et al. (1979) mentioned additional activation of the epileptiform abnormalities during sleep, but no detailed description of the EEG during sleep was given. ERG is usually abolished by the time of diagnosis and flash-VEP becomes abolished between by the age of 13-16 years (Santavuori et al. 1988b). A recent magnetoencephalography (MEG) study showed enhancement of the early somatosensory evoked responses in JNCL, suggesting increased excitability of the sensorimotor cortex in these patients (Lauronen et al. 1997).

2.1.3 The Finnish variant late infantile NCL (vLINCL, variant Jansky-Bielschowsky disease, CLN5)

A variant of late infantile NCL is found almost exclusively in Finland where its prevalence is 2.0 per million inhabitants (Uvebrant 1997). The genomic defect causing vLINCL has been localized to chromosome 13q22 (Savukoski et al. 1994). The CLN5 gene encodes a putative transmembrane protein (Savukoski et al. 1998). So far, four mutations are known in vLINCL patients

carrying three different homozygous haplotypes (Savukoski et al. 1998, Holmberg et al. in press). The Finnish major mutation (a 2 bp deletion) has been identified in 94% of the Finnish disease chromosomes.

The first symptoms, visuomotor and concentration difficulties, muscular hypotonia and motor clumsiness, are noticed between 3-6 years of age. Additional symptoms are progressive visual failure and mental retardation. Epilepsy, myoclonia, ataxia and speech difficulties develop between 7-10 years of age (Santavuori et al. 1982, Santavuori et al. 1991a). Epileptic seizures are typically generalized. Myoclonic jerks occur mostly in the extremities and may precipitate seizures. The ability to walk is lost around the ages of 9-13. Death occurs between the ages of 14-36 years. No major differences in the clinical phenotype of patients with different mutations in CLN5 have been reported (Holmberg et al. 2000 in press). In vLINCL, sleep disorders (Santavuori et al. 1993) are often manifested as sleep-wake cycle disturbance, i.e. the patients sleep during daytime and stay awake at night. However, the condition usually improves without any special treatment.

In vLINCL, MRI has revealed cerebellar and cerebral atrophy as well as hypointense thalami and hyperintense periventricular white matter, which, histopathologically, showed loss of myelin and gliosis (Autti et al. 1992, Autti et al. 1997b). SPECT has revealed cerebellar hypoperfusion (Autti et al. 1992). The cerebellar changes appear at an early stage of the disease.

Progressive background abnormality and paroxysmal activity (spikes, spike and wave paroxysms) are typically noticed in the EEGs of vLINCL patients between 4-7 years of age. Posterior spikes to low-frequency photic stimulation, a specific feature of LINCL, appear somewhat later (Santavuori et al. 1982). ERG is abolished around the age of 6-9 years. Giant flash-VEP and SEP, first

appearing around the age of 7-10 years, are specific findings for vLINCL (Santavuori et al. 1991a).

2.1.4 Infantile NCL (INCL, Santavuori-Haltia disease, CLN1)

In global comparison, the occurrence of INCL in the Finnish population is high, the incidence being 1:20 000 (Vesa et al. 1993). The disease gene has been localized to chromosome 1p32 (Järvelä et al. 1991) and it encodes palmitoyl protein thioesterase (PPT), a lysosomal enzyme (Vesa et al. 1995). One point mutation is present in 98% of the Finnish INCL disease chromosomes.

The onset of symptoms occurs at the age of 9-19 months (Santavuori et al. 1973a, Santavuori 1988a). Among the first symptoms are muscular hypotonia, delayed motor development, progressive microcephaly, and ataxia. After some months, these children gradually begin to lose their cognitive and active motor skills, which will be completely lost latest by the age of 3 years. The majority of INCL patients become practically blind at the age of 2 years (Raitta et al. 1973, Vanhanen 1996a). Epileptic seizures may be the presenting symptom of the disease, with the mean onset of epilepsy at the age of 30 months (Vanhanen 1996a). Simple or complex partial seizures are the most common seizure type. Myoclonic jerks may precipitate seizures. Fluctuating irritability, anxiety, and hyperexcitability are present in about 90% of the patients. These symptoms often co-exist with sleep disturbances. Choreoathetosis and dystonic posturing are characteristic. At a later stage of the disease, pain, which may be due to factors such as damage of the central nervous system, musculoskeletal rigidity and gastrointestinal symptoms, is common (Hofman 1990, Vanhanen 1996a). Death usually occurs between 8 and 11 years of age.

Performed in the advanced stage of the disease, MRI has revealed extreme cerebral atrophy and hypointensity of deep gray matter structures, especially thalami (which are hypointense as early as at the preclinical stage (Autti et al. 1997a)) in relation to the white matter, a pattern reverse to normal (Vanhanen et al. 1994, Vanhanen et al. 1995a). These findings reflect the subtotal loss of cortical neurons, axons and myelin sheaths, and the replacement of the normal constituents of the brain with cells filled with storage material, seen in histopathological analysis (Vanhanen et al. 1995b). SPECT shows unspecific cortical hypoperfusion (Santavuori et al. 1991b, Vanhanen 1996b).

In addition to background abnormality and spikes, the EEG in INCL shows some specific features. First there is a lack of attenuation to eye opening and the disappearance of sleep spindles, followed by attenuation of the amplitude leading to inactivity of the EEG by the age of 3 years (Santavuori 1973b, Santavuori et al. 1990b, Vanhanen et al. 1997). ERG abolishes around the age of 3 years and flash-VEP about one year later, with a concomitant progressive attenuation of cortical SEP amplitude (Santavuori et al. 1990b, Vanhanen et al. 1997).

2.1.5 Management of NCL diseases

Since no curative treatment is available for patients with NCL, the treatment is symptomatic. However, in JNCL, patients with antioxidant medication have been reported to have slower progression of the disease than those without medication (Santavuori et al. 1989). Patients with vLINCL or INCL do not benefit from antioxidants.

The symptomatic medication of NCL diseases includes antiepileptic,

antiparkinson, and sedative drugs as well as muscle relaxants, analgesics, antidepressants and antipsychotics. The most widely used antiepileptic drugs in NCLs in monotherapy are lamotrigine and sodium valproate, combined with benzodiazepine later in the advanced stage of the disease (Åberg et al. 1997, Åberg et al. 2000 in press, Holmberg et al. 2000 in press). When Parkinson signs appear, L-dopa in combination with bromocriptin is included in the therapy. In INCL, vLINCL, and in some patients with an advanced stage of JNCL, baclophen and tizanidine may alleviate irritability, choreoathetosis, dystonic posturing, and pain (Santavuori et al. 1993). Additionally, in some of these symptoms levomepromazine and benzodiazepines may be helpful. In patients with severe pain, opiates or opiate-like analgesics are used. In addition, special therapies, such as physio-, ergo- and riding therapy are useful.

The treatment of sleep disturbances in NCL partly overlaps the treatment used to alleviate the other symptoms of the disease. In JNCL, benzodiazepines and antipsychotics are useful. Baclophen and tizanidine often are effective in INCL and vLINCL. In addition, some patients with INCL respond to benzodiazepines.

2.2 Sleep regulation in humans

Three basic processes underlie sleep regulation: 1) a homeostatic process, 2) a circadian process, and 3) an ultradian process.

2.2.1 The two-process model of sleep regulation

Sleep is regulated by both homeostatic and circadian mechanisms (Borbély 1982, Daan et al. 1984, Dijk and Daan 1987, Dijk and Czeisler 1994, Dijk and Czeisler 1995, Dijk and Czeisler 1997). Homeostatic mechanisms stabilize the relation between sleep and wake; they augment sleep propensity when sleep is reduced, and reduce sleep propensity in response to excess sleep (Process S). The circadian clocklike mechanism defines the alternation of periods with high and low sleep propensity and is basically independent of sleep and waking (Process C). This two-process model (Borbély 1982) is a widely accepted model of sleep regulation. However, numerous other models have also been proposed (for review, see Borbély 1992).

Electroencephalographic slow wave activity (SWA) is thought to be a marker of the Process S. This EEG variable can be regarded as an indicator of "sleep intensity" or "sleep depth", which changes as a function of prior sleep and waking. The SWA state is prevailed by a high arousal threshold, and sleep deprivation enhances SWA in a time-dependent way (Dijk et al. 1990).

There is evidence that the homeostatic and circadian facet of sleep can be independently manipulated and thus controlled by separate mechanisms (Dijk and Czeisler 1994). The interaction of these processes govern the timing of sleep; the rising homeostatic sleep pressure during waking is compensated by a declining circadian sleep propensity (Daan et al. 1984, Borbély et al. 1989), and conversely, during sleep the rising circadian sleep propensity may counteract the declining homeostatic sleep pressure and thus ensure the maintenance of sleep. In practise, a circadian rhythm disorder may lead to a disturbance of sleep, which is partially under circadian control.

2.2.2 The ultradian process of sleep

The ultradian process is responsible for the alternation of the two basic states of sleep, that is, REM (rapid eye movement) sleep and NREM (non-REM) sleep. These two separate states exist in virtually all mammals and they can be defined on the basis of electrophysiological parameters. Furthermore, NREM sleep is conventionally subdivided into stages of light (S1-S2) and deep (S3-S4) sleep. The significance and consequences of the REM-NREM organization of sleep are largely unknown.

Among early studies of EEG activity during sleep, Loomis et al. (1936) described five stages of sleep but did not distinguish REM sleep. Later, electro-oculography (EOG) was added to the recording paradigm, and then rapid eye movements and, hence, stage REM could be described (Aserinsky and Kleitman 1953). The modern standard sleep staging system (Rechtschaffen and Kales 1968) was modified from the EEG and EOG categorizations made by Dement and Kleitman in the late 50's (1957). Since muscle atonia during REM sleep had been discovered (Jouvet and Michel 1959, Berger 1961), electromyography (EMG) was included to this recording and scoring system to provide an additional marker of REM sleep.

The main features of the sleep stage classification according to Rechtschaffen and Kales (1968) will be summarized in the following. The four NREM stages (S1-S4) parallel the continuum of arousal threshold, being generally lowest in stage 1 and highest in stage 4. The NREM stages are distinguished principally by changes in EEG pattern. Stage 1 is the transition state between wake and light sleep, and is characterized by relatively low-voltage, mixed-frequency EEG activity, vertex sharp waves and slow eye movements. Stage 2 is distinguished from stage 1 by sporadically occurring spindles and K complexes.

Stages 3-4 are defined as slow wave sleep, according to the presence of high amplitude ($> 75 \mu\text{V}$) slow frequency ($\leq 2 \text{ Hz}$) waves, which predominate in S4. In REM sleep, characteristic activity in all three electrographic measures coincide. EEG shows relatively low voltage, mixed frequency activity and commonly, sawtooth waves. Phasic rapid eye movements are seen in EOG channels, and EMG reveals tonic suppression of skeletal musculature activity interrupted by phasic twitches.

Although part of the criteria by Rechtschaffen and Kales (1968) have been challenged in recent years, it is the only staging system established by a consensus of experts so far. For instance, the length of the epoch (i.e. inability to detect microsleep phenomena) and the fact that the criteria apply most specifically to healthy adults, have raised criticism among sleep researchers.

2.3 Circadian rhythms in humans

2.3.1 General

Linked to the rotation of the planet and the light-dark cycle, humans present with circadian (approximately 24-hour) rhythms. Many physiological events show circadian rhythmicity. The thermoregulation system and most endocrine functions for instance demonstrate diurnal variation. The most salient behavioral marker of an approximately 24-hour rhythm in human adults is the daily sleep-wake cycle.

If isolated from all environmental time cues, the behavioral and physiological rhythms in humans typically free-run with a period of about 25h (Wever 1979). The endogenous pacemaker generating circadian rhythms is located in the

suprachiasmatic nucleus (SCN) of the hypothalamus, and it is thought to drive most, if not all, circadian rhythms. Since the endogenous period of the internal clock is not exactly 24 hours, a daily resetting of the clock by external time cues is necessary. The rhythm of the SCN is reset by environmental light-dark cycles with light signalling via the retinohypothalamic tract (Moore and Klein 1973).

The light-dark cycle appears to be the most important zeitgeber in synchronizing the intrinsic biological cycle with external time (Czeisler 1995). It has been shown that light levels as low as room light can reset the pacemaker (Boivin et al. 1996). External zeitgebers other than light, such as activity, social interaction, meals, and scheduled sleep-wake periods may also contribute to the entrainment of the pacemaker.

2.3.2 Diurnal variation of melatonin, body temperature, and cortisol

The light signal acting through the SCN is transmitted by a multisynaptic pathway to the pineal gland to regulate the secretion of melatonin (Moore 1996). Melatonin levels begin to rise after dusk and cease at dawn, with peak levels occurring during the dark period of the 24-hour day (Lynch et al. 1975, Reiter 1986, Arendt 1988). Light affects melatonin secretion in two principal ways. First, light acutely suppresses melatonin secretion in an intensity- and duration-dependent manner (Lewy et al. 1980, McIntyre et al. 1989). Second, light is able to phase shift the melatonin rhythm, the magnitude of the phase shift varying with the timing, intensity and number of light pulses (Lewy et al. 1987, Dijk et al. 1989).

It has been suggested that in addition to the SCN acting as the primary

pacemaker, the preoptic anterior hypothalamic area is also necessary for the maintenance of homeostasis of core body temperature (CBT). The daily rhythm of CBT displays a nocturnal decline of body temperature with a nadir at 0300 to 0600 (Cagnacci et al. 1992). The patterns of melatonin and CBT are temporally related; the nocturnal decline of CBT is opposite to the nocturnal rise of melatonin.

The SCN drives the basal hypothalamo-pituitary-adrenal rhythmicity and thus the diurnal variation in plasma and urinary cortisol (Moore-Ede et al. 1983a, Moore-Ede et al. 1983b). Glucocorticoid levels are typically elevated in the early morning and then decline to a nadir around midnight (Van Cauter 1990). In a recent study conducted with blind subjects, the rhythms of urinary cortisol paralleled the melatonin rhythms, irrespective of the type of the underlying circadian rhythm (Skene et al. 1999). The authors suggested that this association most likely reflected two output rhythms being generated by a single oscillator.

Melatonin, CBT and cortisol are considered to be markers of the circadian phase. However, there are factors (light, sleep, activity, stress, posture, social cues) known to exert masking effects on circadian waveforms (Aschoff et al. 1971, Lewy et al. 1980, Weitzman et al. 1983, Deacon and Arendt 1994, Buxton et al. 1997), which have to be taken into account when designing experiments.

2.3.3 Properties of melatonin related to sleep

Melatonin has been posited as an 'internal zeitgeber' regulating the entrainment of the circadian system to the geophysical environment (Armstrong, 1989). The

presence of specific melatonin receptors in the SCN (Reppert et al. 1988) gives evidence for a modulatory role of melatonin in circadian time-keeping mechanisms in humans.

The circadian rhythm of sleep propensity refers to sleep tendency within the 24-hour day. A positive correlation between melatonin secretion and sleep propensity has been reported by Nakagawa et al. (1992) and Tzischinsky et al. (1993). Recently, rises in nocturnal melatonin secretion and in sleepiness were found to be phase-locked, with melatonin onset preceding the increase of sleep propensity (Shochat et al. 1997).

As mentioned earlier, the circadian rhythm of melatonin is temporally associated with that of CBT, the patterns being inversely related. Data obtained by increasing melatonin levels during the day as well as by manipulating the melatonin signal at night indicate that melatonin has a capability to reduce the CBT (Cagnacci et al. 1992, Cagnacci et al. 1994). However, the mechanisms mediating this action are unclear. Effects on thermoregulatory centers in the preoptic anterior hypothalamic area, where also melatonin receptors have been detected (Krause and Dubovich 1990), and heat loss are likely to be involved. The CBT decline induced by melatonin is preceded by an increase of heat loss, evaluated as an increase of distal skin temperature, the thermoregulatory effects being evident only in a supine position (Kräuchi et al. 1997a, Kräuchi et al. 1997b). The soporific action of melatonin may result from this hypothermic effect mediated by peripheral vasodilation (Kräuchi et al. 1997a).

Melatonin seems to have the ability to induce phase shifts of the circadian pacemaker. The response to exogenous melatonin depends on the phase of the intrinsic circadian rhythm at the stimulus occurrence, demonstrated as phase-response curves (Lewy et al. 1992, Zaidan et al. 1994). Melatonin

administration in the early subjective evening would advance the phase and delay the phase in the subjective morning. In general, the shifting effect of melatonin appears to be opposite to that of light exposure (Minors et al. 1991, Lewy 1998). Although melatonin has been shown to phase shift the circadian system, the evidence of entrainment of hormonal, CBT and sleep-wake rhythms by melatonin in individuals with free-running rhythms is scarce (Middleton et al. 1997, Hashimoto et al. 1998).

Many studies have shown a sleep promoting effect of exogenous melatonin of different doses when ingested before the endogenous melatonin onset. The sleep promoting effect is manifested as subjective sleepiness, decreased sleep latency, or characteristic EEG changes (Arendt et al. 1984, Lieberman et al. 1984, Tzischinsky and Lavie 1994, Dollins et al. 1994, Cajochen et al. 1996, Hughes and Badia 1997).

Melatonin has a wide diversity of effects in human physiology. Considering the sleep-wake cycle, it has been suggested that in addition to the regulation of the circadian function, melatonin might increase the propensity for physiological processes promoting sleep or processes that occur during the sleep period, such as reduced core temperature, increased heat loss, reduced alertness, and decreased cardiac output (Dawson and van den Heuvel 1998).

2.4 Circadian rhythms and sleep in the blind and in individuals with a diseased brain

2.4.1 Blind individuals

Blind individuals, especially those with no light perception, may not be

entrained by light, the most important external zeitgeber in humans, and may therefore present with desynchronized rhythms of melatonin (Lewy and Newsome 1983, Sack et al. 1992, Nakagawa et al. 1992, Lockley et al. 1997a, Lockley et al. 1997b), cortisol (Orth and Island 1969, Orth et al. 1979, Nakagawa et al. 1992), CBT (Nakagawa et al. 1992, Czeisler et al. 1995b) and the sleep-wake cycle (Miles and Wilson 1977, Nakagawa et al. 1992, Lockley et al. 1997a). However, in some cases with total blindness (i.e. with no light perception) light has shown to have suppressed melatonin secretion (Czeisler et al. 1995a, Hästönen et al. 1998). Thus, in some blind individuals with no conscious light perception, the retinohypothalamic system might remain functional and convey photic input to the SCN, and the entrainment of daily rhythms by light might be possible.

Circadian rhythms in the blind may be normally entrained to 24h, abnormally entrained to 24h (phase advanced or delayed), free-running at a period other than 24h, or they may display no distinguishable rhythm. The incidence of free-running rhythms has been reported to increase with severity of visual loss (Lockley et al. 1997b). Data on the effect of an abnormal circadian phase on sleep are scarce. Tzischinsky et al. (1991) and Lockley et al. (1999) found daytime napping and the overall sleep-activity cycle to reflect the underlying melatonin phase in blind children and adults.

Blind children (Leger et al. 1999) and adults (Miles and Wilson 1977) have a high frequency of sleep disturbance, complaints being more common by individuals with no light perception (Tabandeh et al. 1998). Problems such as short sleep duration and daytime sleepiness have been reported (Leger et al. 1999, Tabandeh et al. 1998). Despite the degree of visual impairment, depression may account for the sleep problems in the blind (Moseley et al. 1996).

2.4.2 Individuals with a diseased brain

Neurological, neurodevelopmental, and neuropsychiatric disabilities predispose children and adolescents to sleep-wake rhythm disorders (Jan and O'Donnell 1996, McArthur and Budden 1998). Coinciding blindness, mental retardation and/or brain damage diminish the ability of these individuals to perceive and interpret external cues for stabilizing their sleep with the environment. Abnormalities of melatonin, cortisol, or body temperature rhythms have been described in various types of brain disease, such as lissencephaly, Rett syndrome, Lennox-Gastaut syndrome, and severe brain damage due to various etiology (Okawa et al. 1986, Laakso et al. 1993, Vogel et al. 1990, Mori et al. 1993, Palm et al. 1997, Pillar et al. 1998, Miyamoto et al. 1999).

The disturbance of the sleep-wake cycle in children with neurological deficits may be manifested as fragmented sleep patterns (frequent nocturnal awakenings and daytime napping) or even as day and night reversals and free-running rhythms, as described by Espezel et al. (1996) in patients with visual loss of ocular or cortical origin and various neurodevelopmental impairment, including mental retardation. Severe brain damage may result in a dispersed-type sleep pattern with sleep periods distributed at indefinite times throughout the 24-hour day (Okawa et al. 1986). In mentally retarded children, many studies have reported frequent sleeping problems, such as settling difficulties and nocturnal awakenings (Clements et al. 1986, Quine 1991). Nocturnal epileptic seizures may underlie or accentuate the sleep disturbance in individuals with primary or secondary epilepsy (Hoepfner et al. 1984, Bruni et al. 1995). However, even in the absence of nocturnal seizures, the sleep architecture may be disrupted by awakenings and stage shifts in epileptic children (Touchon et al. 1991).

2.5 Exogenous melatonin as a sleeping pill

The use of melatonin in sleep disorders is generally based on the observations that melatonin can 1) shift circadian rhythms (Zhdanova et al. 1997), thereby correcting the alignment of the endogenous sleep propensity rhythm with the desired sleep schedule and 2) acutely promote sleep (Wirz-Justice and Armstrong 1996). The doses commonly used (0.5-3 mg p.o.) result in supraphysiological levels of circulating melatonin. The timing of administration depends on the disorder being treated. In humans, the mean half-time of the elimination phase after oral administration of melatonin was recently determined as 47 min (Di et al. 1997). Circulating melatonin can reach all body tissues and it is detectable in body fluids, such as cerebrospinal fluid, saliva, and urine. The toxicity of melatonin is presumably low. The long-term safety data is lacking. There are a few known short-term side-effects of melatonin administration, drowsiness being the primary one. However, inappropriate timing of melatonin treatment may lead to sleep disruption (Middleton et al. 1996).

Several studies have shown stabilization of the sleep-wake rhythm and improved sleep in blind subjects during melatonin treatment (Folkard et al. 1990, Sack et al. 1991, Palm et al. 1991, Tzischinsky et al. 1992, Lapierre and Dumont 1995). However, the entrainment of free-running circadian system in blind individuals using physiological markers of the circadian phase was not reported until recently (Lockley et al. 2000). The timing of melatonin administration in relation to the individual's circadian phase seems to be essential.

The effects of melatonin treatment on sleep disturbances in neurologically impaired children and adolescents (including patients with e.g. cerebral palsy,

congenital rubella, chromosomal abnormalities, head injuries, and Rett syndrome) with normal or impaired vision have been variable (Camfield et al. 1996, Jan and O'Donnel 1996, Palm et al. 1997, McArthur et al. 1998, Pillar et al. 1998, Jan et al. 1999, Miyamoto et al. 1999, O'Callaghan et al. 1999). Melatonin treatment has, however, been predominantly positive in improving sleep. Specifically, in the treatment of fragmented sleep, melatonin has yielded good (Palm et al. 1997), variable (Jan and O'Donnel 1996) and poor (Camfield et al. 1996) results. The more severe the sleep-wake cycle disturbance, the more beneficial melatonin treatment appeared to be (Jan and O'Donnel 1996). The poor responses may have been due to methodological reasons, such as inappropriate timing of melatonin administration and inadequate treatment period, or to complicated disease with pain, psychological symptoms and multiple medication, for instance. Additionally, in sleep disturbances with complex etiology, the responses to melatonin may not become obvious. In experimental conditions with healthy individuals, exogenous melatonin has been reported to cause fragmented sleep pattern in itself (Middleton et al. 1996). The authors speculated the possibility of an uncoupling of evening and morning "oscillators" in the absence of normal environmental light-dark cycle and other time cues when using only the sleep-onset signal (= exogenous melatonin given during the evening). Thus, strengthening of environmental time cues during melatonin therapy seems essential for the synchronization of sleep with environmental time.

3. AIMS OF THE STUDY

The aim of this study was to explore circadian rhythms and sleep in patients with neuronal ceroid lipofuscinosis and to clarify the pathophysiology underlying the sleep disturbances in these patients. Particular aims were:

I To study whether the sleep disorders in neuronal ceroid lipofuscinosis are due to disturbance in circadian rhythms.

II To test the effectiveness of melatonin as a sleeping pill in patients with neuronal ceroid lipofuscinosis.

III To study the sleep structure and sleep EEG in patients with juvenile neuronal ceroid lipofuscinosis and to correlate the findings with the sleep complaints of the patients.

IV To examine the occurrence of the fragmented diurnal sleep-wake pattern in patients with the Finnish variant late infantile neuronal ceroid lipofuscinosis.

4. MATERIALS AND METHODS

4.1 Patients

We studied 35 JNCL patients (age range 6-32 years), 11 vLINCL patients (age range 7-32 years), and 6 INCL patients (age range 3-12 years). In all, 52 NCL patients, five of whom participated in more than one study, were investigated. The diagnosis of all 52 patients had been confirmed by DNA and electron microscopy analyses. Twenty-seven of the JNCL patients were homozygous and 8 compound heterozygous for the major mutation of the CLN3 gene. Most of the patients were blind and neurologically impaired, and had sleep complaints as well as epilepsy treated with antiepileptic drugs. All patients except one had mental impairment varying from borderline to severe. All patients or their parents gave informed consent. The studies were accepted by the Ethical Committee of the Hospital for Children and Adolescents, University of Helsinki.

In **Study I**, 8 JNCL patients (mean age 24 years, range 16-32), 5 INCL patients (mean age 7 years, range 3-10), and one patient with vLINCL (age 12 years) were studied. There were 8 healthy control subjects, age- and sex-matched for the patients with JNCL. **Study II** included 3 JNCL patients (age range 12-19 years), one INCL patient (age 12 years), and one vLINCL patient (age 15 years). In **Study III**, 28 patients with JNCL (mean age 13, range 6-27) were studied. In **Study IV**, 11 patients with vLINCL (age range 7-32) were examined. Age-matched healthy control subjects underwent actigraphic recordings.

4.2. Diurnal measurements and data analysis (melatonin, cortisol, body temperature, and motor activity)

4.2.1. Melatonin, cortisol, and body temperature

Measurement of melatonin and cortisol

Blood samples (5 ml) taken through permanent venous cannulas, were collected from the subjects every 2 hours during a 24-hour period. The nighttime samples were collected in a dimly lit room. Serum melatonin was measured radioimmunologically (Vakkuri et al. 1984) using radioiodinated melatonin as a tracer. This method has been validated for saliva samples (Vakkuri 1985, Laakso et al. 1990). The properties of antiserum raised in rabbits by immunization with bovine thyroglobulin conjugate of N-acetyl-5-methoxytryptophan have been described previously (Laakso et al. 1988). The reference samples were prepared from synthetic melatonin (Sigma, St. Louis, MO, USA) dissolved in the assay buffer over the range 1.95-1,000 pg/ml. The nonspecific binding of the tracer was 5-6%. Serum cortisol was measured radioimmunologically (Dash et al. 1975) as a hospital laboratory routine.

Measurement of body temperature

The axillary temperature was recorded continuously in 10-second epochs by a portable polygraph, Vitalog HMS-5000 (Vitalog Monit. Inc.). Temperature values at 30-minute intervals were used for analysis.

Analysis of melatonin, cortisol, and body temperature patterns

The estimates for the circadian rhythm parameters (acrophase, amplitude, and mesor) for each 24-hour pattern of melatonin, cortisol, and body temperature were calculated by single cosinor analysis (Monk and Fort 1983). To compare the estimates between the JNCL, INCL, and the control group, one-way ANOVA was used ($p < 0.05$ indicating statistical significance).

4.2.2 Motor activity

Diurnal motor activity was recorded by activity monitors (Actigraph Motionlogger, Ambulatory Monitoring Inc.). The motor activity level was recorded continuously in 1-min epochs approximately for a one week period, during which concomitant sleep logs were maintained by the parents or caregivers of the patients. Spectral, cosinor, autocorrelation and harmonic analyses of activity data were used in the identification of the periodicity in the rest-activity rhythms and to test the stability of the period (Action 3.15 software, Ambulatory Monitoring Inc.) (A detailed description of the analyses is given in original articles III and IV). In Study I, each registration minute was scored as either rest or activity by an automatic scoring program (Action 1.23 software, Ambulatory Monitoring Inc.), (Cole and Kripke 1992). Nocturnal sleep was defined as fragmented if the longest continuous period of rest was less than 4 hours during the 24-hour day (up to 5 activity periods with a maximal duration of 10 minutes each, were accepted in the rest period). The sleep phase was defined as irregular if the average sleep onset time, derived from the sleep logs and motor activity data, did not occur within one hour of the sleep onset time on the first night of the recording.

4.3 Administration of melatonin and placebo

The experimental procedure included single-blind administration of immediate-release melatonin (2.5 mg or 5 mg) or placebo with concomitant motor activity recordings (see 4.2.2.) and sleep logs. Melatonin and placebo were ingested 1-2 hours before the intended sleep onset time.

First, a 1-week baseline motor activity recording was performed. Thereafter, successively, i) placebo, ii) 2.5 mg of melatonin, and iii) 5.0 mg of melatonin were administered for 3 weeks each. During the last week of each 3-week period, actigraphic recording with concomitant sleep log was performed. In addition, the parents or caregivers of the patients compared the sleep quality during each treatment period with that of the baseline period.

4.4 Polysomnographic recordings and data analysis

Whole-night polysomnographic recordings were carried out in a sleep laboratory. The recordings included electroencephalography (EEG), electrooculography (EOG), submental electromyography (EMG), electrocardiography (ECG), and additionally, in 3 cases in Study IV, EMG of *m. tibialis anterior* and *m. extensor carpi radialis*, oxygen saturation, nasal air flow, and thoracical and abdominal movements. 5-10 EEG channels were used with the EEG electrodes placed according to the international 10-20 electrode placement system. Horizontal and vertical eye movements were recorded by two channels. Continuous infra-red video recording was used to monitor the posture, movements, and behavior of the subject during sleep.

Sleep was scored visually in 30-second epochs according to the criteria of Rechtschaffen and Kales (1968) by an experienced professional scorer. The measured sleep parameters of the patients were compared with those of healthy age- and sex-matched control subject groups from a normative data set (Williams et al. 1974) including the means, standard deviations, and the numbers of control subjects in distinct age groups. A 95% confidence interval for each sleep parameter of both female and male control subjects was

calculated. Then each sleep parameter of each individual patient was determined to be i) above, ii) within, or iii) below the corresponding 95% confidence interval of the age- and sex-matched control subject group. In Study III, Pearson correlation analysis was used to examine the associations between the numerical clinical data for the patients and the measured sleep parameters, and among both the clinical and the measured data. Only the correlations with a correlation coefficient ≥ 0.5 were taken into consideration. These correlations were further tested by general linear model analysis (Systat 5.0, 1992, SYSTAT, Inc.). The Mann-Whitney U test was used to compare the sleep parameters between homo- and heterozygous patients as well as between patients with and without sleep complaints ($p < 0.05$ indicating statistical significance). A detailed description of the analysis of electroencephalographic features are given in original articles III and IV.

4.5 Sleep questionnaires

Administered to the parents of the patients, the sleep questionnaire included 13 (Study III) or 30 (Study IV) items mainly modified from a previously validated one to assess sleep and its disturbances quantitatively during the preceding 3 months (Partinen and Gislason 1995). Concomitantly, a 2-week sleep log was maintained (Study III). In the statistical analysis, the modal score was used to reveal the category of each item that received the most responses (Study IV).

5. RESULTS

5.1 Diurnal rhythms of melatonin, cortisol, body temperature, and motor activity in NCL (Study I)

The oldest patient with JNCL (age 32 yrs) and the oldest patient with INCL (age 10 yrs) had melatonin patterns with the highest concentrations during the light hours of the day. One JNCL patient (age 27 yrs) had no melatonin secretion. Otherwise the patients and all the control subjects showed a melatonin rhythm with the highest concentrations during nighttime. The melatonin amplitude was significantly higher in INCL patients than in the control subjects. There were no statistically significant differences in the melatonin acrophase or mesor estimates between the groups.

The peak values of cortisol occurred in the morning in all control subjects and patients, except the two oldest JNCL patients and the oldest INCL patient, who showed either peak values in the evening or multiple peaks scattered throughout the day. Cortisol amplitudes were significantly lower in JNCL and INCL patients than in the control group. There were no statistically significant differences in the cortisol acrophase and mesor estimates between the groups.

The temperature data of 4 out of 10 analyzed patients showed temperature maxima between 0200-0500 hours. The rest of the patients and all of the control subjects showed temperature maxima in the afternoon. However, the means of the acrophase, amplitude and mesor did not differ significantly between the groups.

Eleven out of 14 NCL patients had fragmented sleep and an irregular sleep

phase. Two control subjects showed an irregular sleep phase, but none of the controls had fragmented sleep.

In the 12-year-old vLINCL patient, the estimates for the circadian rhythm parameters of melatonin, cortisol or body temperature seemed to be normal. The sleep phase was irregular, but sleep of the patient was not fragmented.

5.2. The effectiveness of melatonin as a sleeping pill in NCL (Study II)

The three JNCL patients with sleep complaints (e.g. settling problems, early awakenings) had normal rest-activity patterns during the baseline recording of diurnal motor activity. The raw data showed distinct periods of rest during the night and activity in the daytime. Moreover, the period analyses revealed a stable, near 24h motor activity rhythm in these patients. Administration of placebo or melatonin did not affect the rest-activity patterns. In all three of the patients, slight improvement of sleep quality during melatonin administration was reported in comparison to the sleep quality of baseline or placebo periods.

The vLINCL and INCL patients with settling problems and nocturnal awakenings had fragmented motor activity patterns during the baseline recordings. In the raw data there were neither distinct periods of rest at night nor distinct periods of activity during daytime, but short rest periods scattered throughout the 24-hour day. The period analyses revealed motor activity rhythms with periods of 12 hours (vLINCL) and 8 hours (INCL). Administration of placebo or melatonin did not normalize the fragmented rhythms. Compared with the baseline period, no changes in sleep quality were reported during the periods of placebo or melatonin administration.

5.3. Polysomnographic findings in JNCL (Study III)

In the recordings of 28 patients with JNCL, all characteristic electroencephalographic features of sleep such as spindles and K-complexes were seen, and the standard sleep stages could all be identified.

In 26 out of 28 patients, the total sleep time (TST) and, in 17 patients the sleep efficiency index (SEI) were below the lower 95% confidence limit of the control population values, i.e. they were significantly below normal. In 25 cases S1% (NREM stage1) and, in 24 cases SWS % (slow wave sleep, NREM stages 3-4) were significantly greater than normal. Furthermore, in 24 cases S2 % (NREM stage 2) and, in 27 cases the REM sleep % were significantly lower than normal. Sleep latency and REM latency were significantly shortened in 12 and 17 patients and lengthened in 5 and 4 patients, respectively. In 18 cases the number of awakenings and, in 27 cases the number of stage shifts were significantly higher than normal. In one patient, some awakenings were preceded by epileptiform activity.

Significant sleep structure alterations occurred across all age groups of the patients. The S1 % and the number of awakenings increased with the progression of the disease, however. The patients with sleep complaints tended to have a lower TST% and SEI, higher S1%, as well as more awakenings and stage shifts than those without sleeping problems. However, the differences in the sleep parameters between the patients with and those without sleep complaints were not statistically significant. The only statistically significant difference in the measured sleep parameters between the homozygous and compound heterozygous patients was in stage REM %, which was smaller among the homozygous patients.

Paroxysmal activity (singly occurring sharp waves) during light sleep (NREM stages 1-2) occurred in 19 patients and during REM sleep in 10 patients. The PA percentage ranged from 0.3 to 7.1 (mean 1.8) during light sleep and from 0.6 to 5.8 (mean 3.3) during REM sleep, respectively. SWS epochs with more than 50% of high amplitude delta wave activity (amplitude typically 300 μ V and at least 300 % of the amplitude of the baseline wake activity, frequency 1-2 Hz) with intermingled sharp waves (HDSW%_{SWS}) was found in 25 recordings. The HDSW%_{SWS} ranged from 3.7 to 75.9 (mean 24.6). In 5 patients, transients of HDSW were found in light sleep (HDSW%_{S1-S2} range 0.4-0.8, mean 0.6). There was no significant difference in the PA% or HDSW% between the homo- vs. heterozygous patients nor the patients with vs. without sleep complaints. Moreover, these parameters did not correlate significantly with any of the sleep structure parameters. Neither clinical nor subclinical epileptic seizures were observed in the recordings.

5.4 Sleep studies in vLINCL (Study IV)

Sleep questionnaires. (Age range 8-19 years). Daily daytime naps and daytime sleepiness were frequently reported (duration of daytime sleep was up to 4 hours). The mean of the usual duration of nighttime sleep was 10.0 hours (range 7.0-11.5 hrs). The shift of the longest sleep period into daytime hours typically occurred less than monthly, but in the three eldest patients, 1-7 days a week. Typically, one short nocturnal awakening per night was observed. Settling problems occurred less than weekly. Transient sleep disturbances were reported as being often associated with concomitant psychological or psychiatric problems such as anxiety, fear, depression, and psychotic symptoms. Nocturnal epileptic seizures or myoclonia, apneas, snoring, and

parasomnia were typically either absent or occurred less than once a month. Narco- or cataleptic symptoms were absent. Hypnotics were usually not used.

Actigraphy. (Age range 6-32 years). The three youngest patients as well as all of the control subjects had a stable motor activity rhythm with a period of about 24 hours, the periods of activity occurring during daytime and rest during nighttime. The other four patients showed multiple spectral peaks. Neither the periods indicated by the spectral peaks nor the 24-hour period showed stability over the measurement days, i.e. the rest-activity pattern was fragmented with no permanent rhythm. In the raw data of these patients, no distinct periods of rest at nighttime hours and activity at daytime hours were seen. The rest-activity data was consistent with the sleep-wake state observations provided in the concomitant sleep logs.

Polysomnography. (Age range 7-12 years). Only the patient with the least progressed stage of the disease showed all of the characteristic electrographic features of sleep. Otherwise, only a few rapid eye movements and morphologically atypical spindles were observed. In every recording, the sleep EEG showed delta waves occurring in all sleep stages. Occasional singly occurring spikes during NREM sleep were seen in the three oldest patients. In the recordings, neither clinical nor subclinical epileptic seizures were observed. Apneas did not occur. In all patients, the total sleep time (TST) was below the lower 95% confidence limit of the control population values, i.e. it was significantly below normal. The awakenings were not preceded by epileptiform activity or twitch movements. Sleep latency was significantly shortened in 4 patients. The percentage of REM sleep for all patients was significantly lower than normal values. The percentages of stage 1, stage 2, and slow wave sleep varied between the patients and were significantly different from those of the control population.

6. DISCUSSION

Light is the most important external zeitgeber in humans. Information about light-dark cycles is thought to be transmitted to body functions by melatonin. Blind individuals, especially those with no light perception, may not be entrained by light, and may therefore present with disrupted rhythms of the sleep-wake cycle (Lockley et al. 1997a). Blind children and adults have a high frequency of sleep problems, such as short sleep duration and daytime sleepiness (Tabandeh et al. 1998, Leger et al. 1999). Neurological, neurodevelopmental, and neuropsychiatric disabilities predispose children and adolescents to sleep disorders (Jan and O'Donnell 1996, McArthur and Budden 1998). Coinciding blindness, mental retardation and/or brain damage diminish the ability of these individuals to perceive and interpret external time cues, including others besides light, for adjusting their sleep with the environment (Palm et al 1997).

Disturbance in sleep architecture can be produced by structural or functional damage to the critical brain regions involved in the generation and maintenance of sleep and wakefulness and in the alternation between NREM and REM sleep. Disorders of the brain may be associated with abnormalities in sleep EEG and in sleep structure (Okawa et al. 1986).

Circadian rhythm studies in NCL

The diurnal patterns of melatonin and cortisol were abnormal only in the two oldest JNCL patients and the oldest INCL patient, all of whom showed abnormal rhythms of body temperature and fragmented rest-activity pattern (Study I). Two of these patients died soon after the measurements and the third

one year later. Thus, the disturbance of melatonin and cortisol rhythms seems to be only a very late phenomenon in the NCL disease. Abnormalities of diurnal hormonal secretion and body temperature have been reported in various types of brain damaged patients, but there is no consistency between the diseases and the incidence of the circadian disorders (Vogel et al. 1990, Laakso et al. 1993, Mori et al. 1993). All of the patients under study had sleep complaints, such as nocturnal awakenings and daytime fatigue, and most of them showed a fragmented rest-activity pattern. Thus, the sleep disturbances in NCL do not seem to be solely due to a general failure of the circadian regulatory system.

The melatonin amplitudes of INCL patients were significantly higher than those of the control subjects, reflecting the younger age of the patients with INCL (Cavallo 1993, Cavallo and Dolan 1996). The cortisol amplitudes in JNCL and INCL were significantly lower than those of the control subjects, a feature observed in brain damaged patients (Braunsdorf et al. 1986).

The so-called "constant routine" conditions (i.e. a regimen of constant recumbency, constant dim light exposure, continuous wakefulness, and constant caloric intake under the form of an intravenous glucose infusion) have been designed to avoid the masking effects on the markers of the circadian rhythms and to reveal the endogenous period of the biological clock. With the exception of light (Lewy et al. 1980), melatonin secretion is less susceptible to environmental and behavioral masking influences (Deacon and Arendt 1994) than cortisol secretion and core body temperature, both of which are influenced by sleep, activity, stress, and social cues, such as meals (Aschoff et al. 1971, Weitzman et al. 1983). Our study was conducted in normal settings (sleep at night, activity in the daytime) in order to explore the output of the pacemaker, the daily rhythms, in normal conditions. To avoid the suppressive effect of light on melatonin secretion, the night serum samples were collected in dim light.

Blind individuals may fail to maintain the diurnal rhythms of hormonal secretion and body temperature (Orth et al. 1979, Lewy and Newsome 1983, Sack et al. 1992, Nakagawa et al. 1992, Czeisler et al. 1995b, Lockley et al. 1997a, Lockley et al. 1997b). In our study, patients with abnormal hormonal rhythms as well as most of the patients under study had no conscious light perception. Thus, despite blindness, light may entrain the diurnal hormonal rhythms in some patients with NCL. Ophthalmoscopical studies and neuropathological autopsies of patients with NCL have revealed optic atrophy, degeneration of retina, and loss of photoreceptors (Haltia et al. 1973, Goebel et al. 1974, Tarkkanen et al. 1977, Traboulsi et al. 1987, Goebel et al. 1988, Santavuori 1988a, Santavuori et al. 1990, Santavuori et al. 1991a). Still, it is obscure what type of retinal receptors are involved in the circadian system. There are studies showing circadian responses to light without rods, without cones, with mere fragments of photoreceptors (Ruberg et al. 1996, Lucas and Foster 1999), or with non-rod, non-cone, yet unidentified photoreceptors (Lucas et al. 1999). Recently, it has been found that despite the degenerated retinas and the lack of conscious light perception in patients with JNCL and INCL, light can penetrate their visual system to the hypothalamic and pineal levels and suppress melatonin secretion (Hätönen et al. 1998) thus possibly regulating the sleep-wake rhythms of these patients. A prerequisite to this is that the circadian regulatory system otherwise functions adequately. However, our patients with abnormal melatonin rhythms were at an advanced stage of the disease, during which disturbance of the pathways between the retina and the pineal gland may finally develop. Also dysfunction of the pineal gland itself might cause abnormalities in the melatonin pattern. Nevertheless, in the JNCL patient with no melatonin secretion, the autopsy revealed a normal pineal gland.

Our circadian rhythm study revealed no abnormalities in melatonin, cortisol, or body temperature rhythms in the 12-year-old patient with vLINCL who had

some light perception (Study I). Moreover, this patient had no sleep-wake rhythm disturbances until four years later (Study IV); at the age of 14 the rest-activity pattern was still normal but fragmentation occurred two years later. In our questionnaire study on vLINCL patients, the primary sleep complaint seemed to be a fragmentation of the 24-hour sleep-wake cycle, becoming more frequent with age. In the diurnal activity studies, the oldest patients had no distinct rest-activity rhythm, but short sleep periods scattered throughout the 24-hour day. Data on the effect of an abnormal circadian phase on sleep are scarce. Tzischinsky et al. (1991) and Lockley et al. (1999) found daytime napping and the overall sleep-activity cycle in blind children and adults to reflect the underlying melatonin phase. In the older vLINCL patients, one cannot exclude the possibility of diurnal melatonin pattern abnormality. Also this form of NCL, like the infantile and juvenile forms, might lead to failure of the circadian regulatory system.

Another feature revealed by the sleep questionnaires was the frequent naps and daytime sleepiness in patients with vLINCL. Baclophen and tizanidine used to treat myoclonia and to alleviate spasticity, and benzodiazepines as antiepileptics may cause fatigue in these patients. However, the youngest patients not receiving any medication also had daily daytime sleepiness and naps. Sleep fragmentation has been found to be an important cause of daytime sleepiness both in experimental studies with normal subjects (Stepanski et al. 1987), and in clinical studies with various diseases (Stepanski et al. 1984, Zamir et al. 1998). Also shortened nighttime sleep might cause daytime sleepiness. However, sleep fragmentation by awakenings or short sleep were not evident in patients with daytime sleepiness (the questionnaires were obtained from the patients under the age of 20 years). Comparing the questionnaire data with the available normative data on sleep length in healthy children and adolescents (Williams et al. 1974), all except the youngest patient

with vLINCL clearly slept longer than their healthy peers. In vLINCL, gradual loss of perception of external stimuli may cause apathy, predisposing the patients to daytime sleepiness. Whether some other specific factors underlie the daytime sleepiness, remains obscure.

Otherwise, sleep problems were transient and often temporally associated with prevailing psychological symptoms, such as anxiety, fear, or restlessness. Nocturnal seizures or myoclonia seemed to occur and disturb sleep only infrequently. This may reflect the effectiveness of the drug combination (i.e. an antiepileptic drug combined with baclophen and tizanidine) used in the treatment of vLINCL. Prior to the present treatment protocol, epileptic seizures and myoclonia were more frequent (Santavuori, personal communication).

Melatonin administration did not distinctly affect the rest-activity rhythm in the patients with NCL (Study II). Neither a sleep-inducing nor a synchronizing effect of melatonin was observed. The regulatory effect of light on activity rhythms seemed to be evident in the JNCL patients with normal rest-activity rhythms. In addition, there is evidence that light is a stronger rhythm regulator than exogenous melatonin (Hätönen et al. 1996). In these patients, a slight positive effect of melatonin on sleep quality was reported. The parents wanted to continue to administer melatonin after the study. However, the positive effect faded, and within 6 months all families discontinued the administration. In the patients with INCL and vLINCL, the neurological impairment was more severe than that in the JNCL patients. In the INCL and vLINCL patients with fragmented rest-activity rhythms, melatonin failed to synchronize the rhythm. Neither any other effects of melatonin on sleep were noted. These patients had probably undergone failure of the circadian system due to the advancing disease and the pathways of the circadian regulatory system or targets of melatonin may have been damaged. They may not have been able to derive benefit from melatonin to synchronize rhythms.

In the treatment of fragmented sleep in neurologically impaired children and adolescents with normal or impaired vision, melatonin has yielded both good (Palm et al. 1997), and poor (Camfield et al. 1996) results. The more severe the sleep-wake cycle disturbance, the more beneficial melatonin treatment appeared to be (Jan and O'Donnel 1996). Despite the variability in the effects of melatonin treatment, an increase in sleep quality seems to be a rather consistent finding (Jan and O'Donnel 1996, Palm et al. 1997, McArthur et al. 1998, Pillar et al. 1998, Jan et al. 1999, Miyamoto et al. 1999, O'Callaghan et al. 1999). The poor responses in some studies may have been due to methodological reasons, such as inappropriate timing of melatonin and an inadequate treatment period. Furthermore, in experimental conditions, exogenous melatonin itself has been reported to cause fragmented sleep patterns in healthy individuals (Middleton et al. 1996). In NCL, the sleep disturbances may have a complex etiology, and therefore the responses to melatonin may not easily become obvious. Various physical and psychological symptoms coupled to the disease may enhance sleep disturbances in these patients.

Oral melatonin is easily absorbed (Vakkuri et al. 1985), and circulating melatonin penetrates the brain (Vitte et al. 1988). However, a great interindividual variability in peak melatonin concentrations, possibly due to variation in hepatic first-pass extraction, has been reported (Di et al. 1997). Thus, there may have been variable bioavailability of melatonin among the NCL patients of this study. Light exposures of common indoor intensity have been shown to suppress melatonin secretion (Laakso et al. 1993) and to shift the endogenous melatonin rhythm (Boivin and Czeisler 1998). However, we found it reasonable to carry out the melatonin administration study in the natural settings of the patients even though the effects of environmental light could

thus not be eliminated. The possible overestimation of the amount of sleep by the actigraphy method (Cole et al. 1992) and the robustness of the data analysis used may have hampered the identification of minor changes in the sleep onset and the sleep continuity especially in the recordings of JNCL patients.

Polysomnographic studies in JNCL and vLINCL

In the polysomnographic recordings of the JNCL patients, all standard sleep stages could be identified (Study III). Each sleep stage showed its typical EEG patterns, making the classification of the stages uncomplicated. The PSG study revealed that the sleep structure is generally abnormal in patients with JNCL. In most of the patients, the total sleep time and sleep efficiency were significantly less, and NREM stage 1%, the number of awakenings and stage shifts more, than the normal values, reflecting poor sleep. REM % was generally decreased. These findings are consistent with studies on mental retardation (reviewed by Grubar 1983), epilepsy (Baldy-Moulinier et al. 1984), and some other progressive encephalopathies, for instance, tuberous sclerosis (Bruni et al. 1995). In patients with epilepsy, sleep organization has been reported to be permanently altered even in the absence of nocturnal seizures (Touchon et al. 1991). The relation between intellectual ability and REM sleep has been discussed for decades.

Sleep deprivation enhances SWS, the enhancement depending on the duration of prior waking (Borbély et al. 1981, Dijk et al. 1990). On the other hand, generation of “sleep satiety” by increasing sleep time in the morning or daytime reduces SWS in the subsequent night (Feinberg et al. 1980). The greater SWS % in JNCL may have been due to an attempt to maintain the restorative function of sleep, despite the shorter total sleep time and fragmented,

unrefreshing sleep. The possibility of a subjective over-estimation of SWS is minor since there were practically no delta waves during sleep stages other than SWS. Moreover, increases in NREM stage 4 have been reported in some studies of mentally retarded individuals (Petre-Quadens and Jouvet 1967, Clausen et al. 1977).

Sleep structure abnormalities were evident throughout all age groups, irrespective of whether the patient had sleep complaints. Nevertheless, the patients with complaints tended to have more fragmented sleep and less sleep on the whole. However, fragmented sleep increased with age and with the progression of JNCL. Half of the patients under study had daytime sleepiness, at least partly due to sleep fragmentation. These patients tended to have lower SWS% than those with no daytime sleepiness (concomitantly, there was no difference in the TST or in the number of awakenings). Thus, some patients may have been able to compensate the shorter sleep time by greater SWS%. However, reduced or disrupted sleep does not necessarily result in daytime sleepiness, but rather irritability, learning and behavior difficulties, and even hyperactivity (Quine 1991).

The first-night effect (FNE) is a well-known phenomenon in sleep recordings of healthy individuals, especially when performed in sleep laboratories. It is characterized by longer sleep and REM latencies, as well as lower sleep efficiency and a lower percentage of REM sleep. For practical reasons, it was only possible to carry out one-night recordings for the handicapped patients of this study. A pleasant setting (comfortable laboratory and friendly staff) has been reported to have reduced the first-night effect (Coble et al. 1974, Browman and Cartwright 1980). Accompanied by her/his parent at the recording, our patients adapted themselves easily to the home-like laboratory environment as well as to the application of electrodes. They generally fell

asleep easily and slept well according to both observations and their own opinion. Studies of the first night effect in some groups of clinical patients have revealed a less marked FNE in depressed and insomniac patients (Toussaint et al. 1995), an absence of FNE in depression with psychotic features (Rotenberg et al. 1997), and even a reverse FNE in insomnia (patients sleep better than usual on their first night in the laboratory) (Hauri and Olmstead 1989). In addition, no obvious FNE was found in normal subjects with anxiety-related personality traits (Kajimura et al. 1998). Patients with JNCL often share some features with the above groups, including depression, psychotic symptoms, anxiety, sleep deprivation due to sleep disturbances, and settling problems in the home environment (Santavuori et al. 1993). Considering the decisive differences in sleep habits and in the mental status between JNCL patients and healthy individuals, it could be assumed that also patients with JNCL have a less marked FNE.

The results of the PSG recordings of the patients with vLINCL were less uniform than those of the JNCL patients. It should be noted, however, that the studied population was also much smaller (Study IV). In vLINCL, the sleep EEG was altered in all but one young patient, making it difficult to classify the sleep stages according to the criteria of Rechtschaffen and Kales (1968) in contrast with the uncomplicated sleep stage classification in JNCL. Also the routine EEG is generally more abnormal in vLINCL (Santavuori et al. 1982) compared to that in JNCL (Raininko et al. 1990), which may reflect the more fatal course of the disease in vLINCL. Thus, lack of rapid eye movements (REMs) and spindles may have caused some under-estimation of the REM stage and stage 2, respectively. The occurrence of delta waves during all sleep stages may have caused some over-estimation of slow-wave sleep. In patients with vLINCL, atypical sleep stages with decreased or absent spindles and REMs, reduced REM percentage and also excessive slow wave activity,

features observed in mental retardation and developmental brain disease (Clausen et al. 1977, Shibagaki 1980, Okawa 1986, Diomedi 1999), probably reflect the underlying encephalopathy.

The findings of the sleep structure parameters and the sleep complaints revealed by the questionnaires were partly contradictory. An unexpectedly high number of nocturnal awakenings and early wakings in the PSGs may have been due to difficulties in adapting to the recording, reported as poor sleep quality by some patients. These sleep alterations may reflect the FNE, which may be more marked in vLINCL compared to JNCL. In contrast to the JNCL patients with frequent sleep disorders, the recorded vLINCL patients had infrequent, if any, settling or awakening problems. Considering this, one might assume that the magnitude of FNE in vLINCL would rather be similar to that of individuals not suffering of sleep problems (Browman and Cartwright 1980) than that of individuals with sleep problems (Hauri and Olmstead 1989).

Antiepileptic drugs may affect sleep. Sodium valproate is regarded as a sleep stabilizer (Declercke et al. 1991). Also lamotrigine appears to stabilize sleep (Placidi et al. 2000). Benzodiazepines have been reported to cause a decrease in REM sleep and SWS. However, these effects were not evident in NCL patients using benzodiazepines.

Nocturnal epileptic seizures may underlie the sleep disturbance in individuals with epilepsy (Hoepfner et al. 1984). In the recordings of the patients with JNCL and vLINCL, no nocturnal epileptic seizures were observed. Only occasional single spikes or sharp waves were found. This epileptiform activity seemed not to be associated with any sleep structure alterations or sleep complaints in these patients.

Taken together, failure of the circadian regulatory system at a level yet unknown, seems only to occur at an advanced stage of NCL, and does thus not seem to explain the sleep disorders in younger patients with NCL. The advancing disease damaging the brain, may disturb the internal circadian timing system, and the coinciding blindness and mental retardation may impair the ability of the patients to use external time cues for the synchronization of their sleep with environmental time. On one hand, the ineffectiveness of melatonin as a sleeping pill in NCL supports the idea of a failure of the circadian system, which might prevent the patient to benefit from melatonin. On the other hand, it also suggests that the sleep disturbances in NCL may have a multifactorial etiology, and therefore the responses to melatonin may not have become obvious.

By disturbing the organization of the sleep state, the underlying progressing encephalopathy might also lead to changes in sleep structure and sleep continuity. Although changes in sleep structure typical for epileptic patients were discovered, distinct epileptic phenomena during sleep seemed not to be associated with sleep fragmentation. Myoclonia (mainly in vLINCL) seemed to disturb sleep only infrequently, possibly due to effective drug therapy. In INCL, pain may be one cause for nocturnal awakenings (Santavuori et al. 1993). Psychological factors may also have an important role in the sleep disturbances of especially the younger patients with NCL. In these patients, varying psychological symptoms are common (Santavuori et al. 1993) and seem to coincide with sleep problems.

To conclude, the present findings suggest that the sleep disorders in NCL have a complex multifactorial etiology. The disturbed sleep of a single NCL patient with the progressive brain disease and multiple disabilities may result from

even more than one cause, depending on the prevailing stage and symptoms of the disease.

7. CONCLUSIONS

1. The sleep disturbances in NCL do not seem to be due solely to a general failure of the circadian regulatory system.
2. Exogenous melatonin seems to be ineffective both in synchronizing fragmented rest-activity rhythms and as a sleep-inducing agent in NCL.
3. The sleep of JNCL patients is fragmented by frequent awakenings, which are generally not associated with epileptic events. The underlying progressive encephalopathy may lead to disturbances in the alternation between the states of arousal, waking, and sleep in JNCL.
4. The patients with vLINCL seem to develop a fragmented, non-24-hour sleep-wake pattern toward the end of their second decade. This may be due to the advancing disease which might damage the internal circadian timing system and impair the ability of these patients to use external time cues for the synchronization of their sleep with environmental time.

8. SUMMARY

The environmental light-dark cycle plays an important role in the regulation of the daily sleep-wake rhythm in humans. Blind individuals may not be entrained by light, and may therefore present with disrupted rhythms of the sleep-wake cycle. Coinciding mental retardation and/or brain damage diminish the ability of an individual to perceive and interpret external time cues, including others besides light, for adjusting the sleep with the environment. In addition, disorders of the brain may disturb the sleep state itself, its structure and continuity.

In neuronal ceroid lipofuscinoses (NCLs), the most common progressive encephalopathies in children, sleep disorders occur frequently. These patients with brain damage, blindness, mental retardation, and epilepsy are predisposed to sleep abnormalities. We explored the circadian rhythms and sleep in patients with infantile (INCL), Finnish variant late infantile (vLINCL), and juvenile (JNCL) forms of NCL to clarify the pathophysiology underlying the sleep disturbances in these patients.

The present investigations revealed that the diurnal hormonal patterns were abnormal in the oldest patients with JNCL or INCL. Thus, failure of the circadian regulatory system at a level yet unknown, seems to occur only at an advanced stage of NCL.

The patients with vLINCL were found to develop a fragmented, non-24-hour sleep-wake pattern with age. The advancing encephalopathy may impair the ability of these patients to use external time cues for the synchronization of their sleep with environmental time. In addition, also in this form of NCL, the disease might damage the internal circadian timing system.

The ineffectiveness of melatonin as a sleeping pill in NCL partly supports the idea of a failure of the circadian system, which might prevent the patients to benefit from melatonin. On the other hand, it also suggests that the sleep disturbances in NCL may have a multifactorial etiology, and therefore the responses to melatonin may not have become obvious.

In patients with JNCL, the sleep structure was altered and sleep was fragmented by frequent awakenings, which were generally not associated with epileptic events. The underlying progressive encephalopathy might lead to disturbances in the alternation between the states of arousal, waking, and sleep in JNCL.

The present findings suggest that the sleep disorders in NCL have a complex multifactorial etiology.

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