CIRCADIAN RHYTHMS

Larger Phase Angle Between Sleep Propensity and Melatonin Rhythms in Sighted Humans with Non-24-Hour Sleep-Wake Syndrome

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Study objectives: This study was aimed to clarify phase angle between sleep propensity and the circadian pacemaker in patients with non-24-hour sleep-wake syndrome (Non-24).

Design and Setting: A case-control study was undertaken.

Participants: Sighted patient with Non-24 (4 males and 1 female, aged 16 to 39 y), and sex- and age-matched healthy controls (12 males and 3 females, aged 19 to 35 y) participated the study.

Measurement and Intervention: Following an actigraphic assessment of the sleep–wake cycle in their homes, the participants entered an ultrashort sleep-wake schedule together with simultaneous measurement of dim light melatonin rhythm after 24-hour sleep deprivation.

INTRODUCTION

SYNCHRONIZATION OF THE CIRCADIAN SYSTEM IN ANIMALS AND HUMANS IS MOST STRONGLY INFLUENCED BY THE LIGHT-DARK CYCLE.1 Individuals living in isolation without a normal 24-h light-dark cycle have a sleep-wake cycle longer than 24 hours.2-5 This cycle results in progressively later bedtimes and wake times. Non-24-hour sleep-wake syndrome (Non-24) is a rare condition which causes a chronic steady pattern of one- to two-hour delays in sleep onset and wake times in an individual living in normal environmental conditions;6 the period of the sufferer’s sleep-wake cycle is longer than 24 hours. The International Classification of Sleep Disorders (ICSD)6 provides following criteria for diagnosing Non-24 in clinical setting: 1) difficulty initiating sleep or difficulty in awakening, 2) progressive delay of sleep phase with inability to maintain entrainment to 24-hour-day, and 3) presence of the sleep pattern for at least six weeks.

Based on the characteristics of previously documented cases, blindness is a strong predisposing factor for development of the disorder.7-10 In blind patients with Non-24, light stimulus is unlikely to be conveyed from the retina to the circadian pacemaker, so their biological clock may not be regulated by normal environmental light-dark cycles. Subsequent reports have described Non-24 in sighted subjects living in normal environments.11-19 In some of these reports, other pathological factors may have had an influence. For example, a patient’s extreme social withdrawal due to psychopathology19 or deviated personality11 may result in less exposure to the regulatory influences of the normal light-dark cycle, and, therefore, a Non-24-hour sleep-wake cycle.

In animal studies, mutations with long tau or short tau (endogenous period length) have been shown to be responsible for alteration of the phase angle between the rest-activity cycle and the 24-hour environmental light-dark cycle.20-22 However, in human Non-24 there is no evidence for such a tau abnormality mutation nor have there been any observations of sleep-wake problems in the early developmental stages that would be an expected consequence of this mutation.6 In other single case reports, the investigators hypothesized that a blunted response to light stimulus or a limited phase advance capacity may have etiological significance for the prolonged sleep-wake period in sighted Non-24 patients.19,23 These hypotheses have not been tested. There have been suggestions that changes in the phase relationship between sleep timing and the circadian pacemaker could be involved in Non-24.11,15,17,18 However, the etiological significance of such abnormal phase relationships has not been elucidated.

These studies indicate that there may be systematic changes of period in the sleep-wake cycle of sighted patients with Non-24.11,14,15,17,18 However, there have been no explanations about

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why sighted and otherwise healthy individuals, who previously had been entrained to a 24-hour day, started to experience prolongation of the sleep-wake cycle and lost the ability to reset it. Here we report a study on sighted Non-24, in which the melatonin rhythm and diurnal sleep propensity fluctuation were investigated using an ultra-short sleep-wake schedule together with simultaneous measurement of dim light melatonin rhythm and compared with those in healthy control subjects. To testify phase angle abnormality in Non-24 was of our particular interest.

**METHODS**

**Subjects**

We studied five patients with Non-24 and 15 age- and sex-matched healthy controls. The patient group consisted of four males and one female, aged 16 to 39 years, in whom Non-24 was diagnosed according to ICSD criteria. Ophthalmologic specialists found no ophthalmologic abnormalities, except for myopia. The study was conducted before the patients had undergone any therapeutic interventions. No patients had been given any medication, drank alcohol before bedtime habitually, or abused alcohol or other psychotropic agents. We conducted a semi-structured psychiatric interview and found no Axis I or II disorders of DSM-IV. Interviews and examinations by general physicians of our hospital, together with blood counts, urine examinations, serum biochemistry, electrocardiography and routine electroencephalography, confirmed that no patient had a diagnosed medical disorder, or a history of developmental problems or severe physical disorders. The patients had experienced a persistent longer-than-24-hour sleep-wake cycle for 2-9 years. Prior to onset of Non-24 no subjects had any history of any sleep disorders and all had been entrained normally to a 24-hour day. Symptoms started when the subjects changed their sleep habits due to night-shift work, job loss, or an evening-type lifestyle preference (Table 1). None of the patient’s repeated attempts to advance or correct their sleep timing by retiring and arising at conventional social times had been successful.

The controls were healthy, paid volunteers (12 males and 3 females, aged 19 to 35 years) without any known sleep, physical or psychiatric disorders, or any histories of using psychoactive drugs. They had regular sleep-wake habits, and no marked weekday-weekend differences in sleep length or remarkable irregularity due to work schedules. None of the controls had difficulty in waking up at the desired time. The basal body temperature of the female participants was used to determine the phases of their sleep-wake cycle.
menstrual cycles and we carried out the study during the follicular phase of their cycle. The study protocol was approved by the Intramural Research Board of National Center of Neurology and Psychiatry, and each subject gave his or her informed consent after the procedures and the possible risks of the experiment had been explained in detail.

Study Design

Each participant was asked to keep a detailed sleep log for more than four weeks prior to the laboratory investigation and to wear a wrist activity-monitoring device (Actigraph, AMI, New York, USA) for the last 14 days (Figure 1). During this period, we found that delayed phase jumps in sleep onset (larger-than-four-hour delay per day) occurred in two patients. The procedure was repeated in those patients. In all the Non-24 patients we obtained records of regular sleep-wake cycles that were longer than 24 hours for at least eight consecutive days prior to the laboratory investigation. In the controls, we confirmed that no marked irregular day-to-day variations in their bed and wake times occurred and that their sleep schedules were not remarkably constrained by their work schedules.

Sleep onset and offset times, as well as sleep length, were determined by two raters who were unaware of each subject’s status; actigrams and automatically generated data (Action3 software, AMI, New York, USA) in five-minute bins and sleep logs were inspected. Regression lines were fitted through the sleep onset times obtained during the 8–10 days prior to the laboratory investigation (Fig. 1). The estimated sleep onset time on the day of laboratory investigation was obtained by adding the slope of the regression line of sleep onset times to that on the prior day. The periods of the sleep-wake cycles were computed by adding the slope of the regression line of SW period onset times to 24 hours. A rhythm was considered to be entrained to a 24-hour day when the 95% confidence intervals of the SW period crossed 24 hours. We confirmed that both the sleep onset and offset times of the control subjects were entrained to a 24-hour day and that those of the Non-24 patients were not entrained to a 24-hour day (Table 2).

In the Non-24 patients, the laboratory investigation was scheduled when the patient’s sleep phase was not completely out of phase, in order to avoid irregular changes of phase angle between sleep timing and the circadian pacemaker due to delayed phase jumps. In all the subjects, the means of sleep length between sleep timing and the circadian pacemaker due to delayed of phase, in order to avoid irregular changes of phase angle scheduled when the patient’s sleep phase was not completely out (Table 2).

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the delayed sleep onset and offset times relative to the circadian
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fundamental biological differences between sighted persons with
Non-24 and healthy controls.
Recently, the authors, using ultra-short sleep-wake regimen
identical to the present study, have revealed that sleep propensity
onset was not delayed, but that the sleep propensity offset was
delayed relative to the melatonin rhythm in DSPS patients com-
pared as controls. This confirmed DSPS patients’ similar phase
angle features observed in less controlled clinical settings. These
previous findings on DSPS sufferers made clear contrast to
the present results with respect to circadian timings of sleep and
sleep propensity onsets, but were comparable to the present
results in respect with those of the offsets. These may provide an
explanation for differences in clinical manifestations of these two
phase-delay syndromes; DSPS sufferers can entrain to a 24-hour
day, but Non-24 sufferers cannot. In addition, Non-24 patients
slept less during their circadian day than controls, but had an ade-
quate ability for sleep during the circadian night determined by
melatonin secretion. This finding, together with our previous
report on DSPS, suggests that patients suffering from these
eight phase delay syndromes lack flexibility to sleep during their
circadian day even after sleep deprivation.
The present laboratory findings on phase relation between
sleep propensity rhythm and the circadian pacemaker may explain
clinical observations reported previously. Oren et al. have reported that Non-24 was provoked after chronotherapy,
suggesting that an enforced sleep phase delay, irrespective of the
circadian pacemaker, may trigger a prolongation of the sleep-
wake cycle. A similar prolongation of the period of the sleep-
wake cycle was reported in animals as a physiologic after effect
following forced lengthening of the activity period. Recent
studies demonstrated that normal artificial illumination as much as,
and even less than 200 lux can phase-shift a human circadian
rhythm. In our study, artificial illumination during the night-
time might have caused a similar delayed phase response, lead-
ing to a sleep-wake cycle that was longer than 24 hours. In sight-
ed Non-24 patients living in a normal day-night cycle, the sleep-
wake cycle longer than 24 hours may be a consequence of
delayed phase response triggered by artificial and/or natural illu-

Table 2—Sleep, sleep propensity, melatonin and phase-angle measures in patients and controls

<table>
<thead>
<tr>
<th>Non-24 SWperiod [95%CI]</th>
<th>HSl</th>
<th>SPdur</th>
<th>MLdur</th>
<th>SPon-MLmid</th>
<th>MLmid-SPoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.09 [24.91-25.27]</td>
<td>9.39</td>
<td>8.56</td>
<td>8.25</td>
<td>3.52</td>
</tr>
<tr>
<td>4</td>
<td>24.91 [24.75-25.07]</td>
<td>8.41</td>
<td>10.11</td>
<td>7.96</td>
<td>4.01</td>
</tr>
<tr>
<td>5</td>
<td>25.73 [25.54-25.92]</td>
<td>11.89</td>
<td>6.99</td>
<td>7.88</td>
<td>3.53</td>
</tr>
<tr>
<td>Mean</td>
<td>25.12</td>
<td>9.58</td>
<td>7.90</td>
<td>8.20</td>
<td>2.90</td>
</tr>
<tr>
<td>SE</td>
<td>0.18</td>
<td>0.60</td>
<td>0.66</td>
<td>0.16</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Controls (n=20)

Mean | 24.02 | 7.33 | 8.37 | 8.01 | 4.77 | 3.61 |
SE | 0.02 | 0.31 | 0.21 | 0.23 | 0.30 | 0.28 |
p | <.0001 | .002 | .35 | .64 | .007 | .02 |

SWperiod = period of sleep-wake cycle (h); HSl = habitual sleep length (h); SPdur = duration of high sleep propensity (h); MLdur = duration of melatonin secretion (h); SPon-MLmid = interval between sleep propensity onset and melatonin midpoint (h); MLmid-SPoff = interval between melatonin midpoint and sleep propensity offset (h)

Not significant. A repeated ANOVA for sleep propensity data revealed a significant main effect of the groups (Non-24, 3.59±0.30 vs. controls, 4.67±0.13 hours) (df=1, p=0.04), a significant effect of time course of the nap trials (df=23, e=0.25, p<0.0001) and interaction (df=23, e=0.25, p=0.001) (Figure 2). A post-hoc test revealed that the hourly sleep propensity values in the patients were significantly smaller during the period before the patients’ MLmid in comparison with those in controls (Figure 2).

The interval from SPon to the MLmid was 1.87 hours shorter in the Non-24 patients than in the controls (p=0.007). The interval from the MLmid to the SPoff was 1.39 hours longer in the Non-24 patients than in the controls (p=0.007). The inter-

DISCUSSION

In the present study, the interval from sleep propensity onset to melatonin midpoint was shortened and that from melatonin midpoint to sleep propensity offset was prolonged in the Non-24 patients compared as that in the controls. Light-induced phase-
delay and phase-advance of the circadian pacemaker in normal humans occurs over several hours in the evening (phase delay portion of phase response curve (PRC)) and in the early morning (phase advance portion of PRC). It has been postulated that the delayed sleep onset and offset times relative to the circadian pacemaker observed in Non-24 sufferers differentially gated the light-induced phase-advance or phase-delay. The later bed-
time relative to the circadian pacemaker may increase the time during which the delay portion of PRC is exposed to light, whereas the later wake time relative to the circadian pacemaker prevented the advance portion of PRC from being properly exposed to morning light. However, the bedtime and wake time may be confounded by psychological status and/or social constraints, which had weakened the hypothesis. We minimized these confounding factors by using the ultra-short sleep-wake schedule, and found that the sleep propensity rhythm of Non-24 sufferers was more delayed relative to the circadian pacemaker in comparison with that of controls. These provided the first evidence of
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Figure 2—Sleep propensity values, percentage serum melatonin values (using each subject's maximum value as 100%), SPon and SPoff are shown for the Non-24 patients and the controls, time-locked to MLmid. The open circles and the gray areas represent sleep propensity and percentage serum melatonin values, respectively. The thick vertical bars represent SPon and SPoff. a: p=0.007, and b: p=0.02 (t-test, vs. controls). A repeated ANOVA revealed that shape of sleep propensity rhythm was different between two groups, while shape of melatonin rhythm did not differ. The asterisks indicated differences of a post-hoc test (vs. controls).

mination rather than a manifestation of the endogenous free-run period that is likely in blind Non-24 sufferers.7,10,26 Since mutations in rodents with short or long tau are associated with phase-advanced or phase-delayed sleep-wake cycles under normal LD conditions20,22 it could be postulated that a human tau (endogenous period length) mutant also exists in Non-24 sighted humans. The present laboratory experiment provides only limited information about tau because of the short period of the investigation (1-1.5 circadian cycle), but our findings that, under strictly controlled conditions, neither the MLduration nor SPduration differ significantly between Non-24 patients and controls indicate that a period mutation may be unlikely in these patients. The clinical history of the patients also suggested that a profound inborn period mutation was unlikely. All the subjects had been entrained normally to a 24-hour day until they changed their sleep habits due to night shift work, an altered vacation schedule, or an evening-type lifestyle preference and no unusual features were observed with respect to sleep-wake habits during childhood in the patients. Recent reports demonstrated that some Non-24 patients had an abnormal melatonin receptor gene38 which expressed low receptor affinity in transfected cells.39 These observation may indicate that coupling between the circadian pacemaker and sleep could have pathogenic significance in Non-24.

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