A forced desynchrony study of circadian pacemaker characteristics in Seasonal Affective Disorder

Kathelijne M. Koorengevel\textsuperscript{1}, Domien G.M. Beersma\textsuperscript{1,2}, Johan A. den Boer\textsuperscript{1}, Rutger H. van den Hoofdakker\textsuperscript{1}

\textsuperscript{1}Department of Psychiatry
\textsuperscript{2}Zoological Laboratory of the University of Groningen, The Netherlands
ABSTRACT

Background
The circadian pacemaker is an endogenous clock, which regulates oscillations in most physiological and psychological processes with a near-24-hour period. In many species this pacemaker triggers seasonal changes in behavior. The seasonality of symptoms and the efficacy of light therapy suggest involvement of the circadian pacemaker in Seasonal Affective Disorder, winter-type (SAD). In this study circadian pacemaker characteristics of SAD patients are compared with those of controls.

Methods
Seven SAD patients and 8 matched controls were subjected to a 120-hour forced desynchrony protocol, in which core body temperature and melatonin secretion profiles were measured for the characterization of circadian pacemaker parameters. During this protocol, which enables the study of unmasked circadian pacemaker characteristics, subjects were exposed to 6 20-hour days in time isolation. Patients participated twice in winter (while depressed and while remitted after light therapy), and once in summer. Controls participated once in winter and once in summer.

Results
Between the SAD patients and controls no significant differences were observed in the melatonin-derived period nor in the phase of the endogenous circadian temperature rhythm. The amplitude of this rhythm was significantly smaller in depressed and remitted SAD patients than in controls.

Conclusions
No abnormalities of the circadian pacemaker were observed in SAD patients. A disturbance in thermoregulatory processes might explain the smaller circadian temperature amplitude in SAD patients during winter.
INTRODUCTION

The remarkable seasonal fluctuation of mood and the efficacy of bright light therapy in patients with Seasonal Affective Disorder, winter type (SAD), have suggested involvement of the circadian pacemaker in the pathogenesis of this disorder. The present study is devoted to the search for possible circadian abnormalities in SAD patients.

The circadian pacemaker is localized in the suprachiasmatic nuclei (SCN) of the brain and operates as an endogenous timekeeping mechanism (Van Esseveldt et al 2000). This pacemaker generates oscillations in many physiological and psychological processes (Aschoff 1965) with a near-24-hour period (Hiddinga et al 1997; Czeisler et al 1999; Carskadon et al 1999). By its sensitivity to light, the circadian pacemaker is able to respond to seasonal changes in day length, or ‘photoperiod’. Throughout the day, retinal light exposure synchronizes this ‘biological clock’ with the environmental light dark cycle (Jewett et al 1997; Honma and Honma 1988; Minors et al 1991; Boivin et al 1996).

In human circadian pacemaker research, the variations of body temperature and of melatonin secretion are often used to study pacemaker output. However, despite the relative ease with which these variables can be measured, the interpretation of these measures in terms of circadian output is complicated by several masking factors. For instance, the nightly secretion of melatonin, which is dependent on the timing and duration of the photoperiod (Lewy et al 1985), can be directly suppressed by light exposure (Lewy et al 1980). Therefore, the most accurate estimates of circadian pacemaker characteristics derived from the melatonin secretion profile are obtained in dim light. The endogenous circadian variation of body temperature, in turn, is modulated by the effects of activity, meals and sleep. The circadian component in temperature data can be obtained by experimentally controlling for masking factors in a constant routine- or a forced desynchrony protocol. In a constant routine procedure masking factors are avoided by keeping subjects in a supine posture and feeding them with frequent small meals and exposing them to a prolonged period of wakefulness (Mills et al 1978; Czeisler et al 1985). However, the sleep deprivation induced by the constant routine procedure elicits a worsening of mood in healthy subjects (Brendel et al 1990) and an improvement of mood in depressed patients (Graw et al 1998). Therefore this procedure has limitations for studies in which mood regulation is examined. The forced desynchrony (FD) protocol is more suitable because it enables control over the relevant masking factors without eliminating them, while sleep deprivation effects are avoided. During FD participants are subjected to a sleep-wake cycle which is shorter or longer than 24 hours, i.e., either 20 hours or 28 hours. The subjective days are spent in dim light (<10 lux) and have a fixed temporal structure. The circadian pacemaker is not able to adapt to this unusual alternation of wakefulness and sleep and starts to oscillate with its own endogenous period. Consequently,
the pacemaker and the sleep-wake cycle desynchronize (Kleitman and Kleitman 1953; Czeisler et al 1986; Dijk et al 1992). Assuming that circadian and masking factors contribute additively to the overt rhythms measured, a mathematical procedure enables the distinction between these two contributors to the measurements (Hiddinga et al 1997; Czeisler et al 1999), without the need for prolonged sleep deprivation. Therefore, the method of FD was used in the present study.

**Circadian Rhythm Studies in Affective Disorders**

Theories about the pathogenetic role of abnormalities in the functioning of the circadian system in affective disorder - either seasonal or non-seasonal - have a long history (cf. the classical review by Papoušek (1975)). Nevertheless, up till now research did not consistently support these theories.

Patients with non-seasonal depression have been studied in time isolation (Dirlich et al 1981; Wehr et al 1985; Pollak et al 1989). The variation of body temperature showed a near-24-hour period in one unipolar depressed patient (Dirlich et al 1981), whereas a longer-than-24-hour free-running temperature rhythm was observed in another (Wehr et al 1985). In a third free-running experiment the average level of body temperature was higher in depressed outpatients than in matched controls, but no differences in shape and amplitude of the circadian temperature curve were found (Pollak et al 1989).

In other studies addressing the circadian variation of core body temperature, patients in the depressed state showed a higher nocturnal temperature (Avery et al 1982; Von Zerssen et al 1985; Souèbre et al 1988, 1989) and a lower temperature during the day (Souèbre et al 1988, 1989) than when recovered and than controls. Consequently, the amplitude of the circadian temperature curve has been reported to be decreased during depression as shown by comparisons of patients with controls (Avery et al 1982; Von Zerssen et al 1985; Souèbre et al 1988, 1989) and patients with themselves in the depressed state and after recovery (Souèbre et al 1988, 1989). In none of the aforementioned studies were significant disturbances in circadian phase detected (Avery et al 1982; Von Zerssen et al 1985; Souèbre 1988, 1989). Finally, although melatonin secretion profiles have been reported to be not significantly different between non-seasonal depressives and controls (Voderholzer et al 1997; Thompson et al 1988; Rubin et al 1992), a diminished amplitude has also been shown in patients in the depressed state when compared with the remitted state and with controls (Souèbre et al 1989).

To summarize, circadian rhythm studies in non-seasonal affective disorder do not provide a clear picture.

The hypotheses concerning the involvement of the circadian pacemaker in SAD, have mainly focused on circadian phase and amplitude. It has been postulated that a
phase delay of the circadian pacemaker relative to the timing of the habitual sleep-wake cycle underlies the pathogenesis of SAD and that the phase-advancing properties of morning light account for the efficacy of light treatment (Lewy et al 1987a). Alternatively, a diminished circadian amplitude has been hypothesized to be involved in the pathogenesis of SAD. According to this hypothesis the amplitude-enhancing effect of light applied in daytime might explain the beneficial effects of light treatment in SAD (Czeisler et al 1987).

Like in the studies on non-seasonal affective disorders, the majority of studies addressing the circadian hypotheses in SAD are ‘naturalistic’, i.e., carried out under normal sleep timing, which means that masking effects could be involved. Therefore the results of these studies sometimes provide limited information about the circadian pacemaker. A few studies addressed the issue with a constant routine protocol.

**Naturalistic Studies in SAD**

Several studies have been published in which the melatonin secretion patterns of SAD patients in dim light are compared to those found in controls. Some of these revealed a delay of the DLMO in SAD patients (Lewy et al 1987b; Sack et al 1990; Lewy et al 1998), whereas others did not (Checkley et al 1993; Thompson et al 1997). Phase advance shifts of the DLMO have been reported after morning light therapy and phase delay shifts after evening light therapy in both patients (Lewy et al 1987b; Lewy et al 1998; Terman et al 2001) and controls (Lewy et al 1987b; Lewy et al 1998). However, in two studies a phase advance of the DLMO after morning light therapy was observed in patients only (Sack et al 1990; Thompson et al 1997). Studies in which entire melatonin secretion profiles were reported demonstrated similar amounts of melatonin production and amplitudes in patients before (Checkley et al 1993; Thompson et al 1997) and after light therapy (Thompson et al 1997). Likewise, a similar total melatonin production was found in patients before and after morning or evening light therapy (Terman et al 2001). Comparisons of temperature data have demonstrated no differences in circadian temperature parameters between SAD patients and controls in both winter (Rosenthal et al 1990; Eastman et al 1993; Schwartz et al 1997) and summer (Levendosky et al 1991; Schwartz et al 1997). The mean level of body temperature was significantly lower in summer than in winter in both patients and controls in one of these studies (Levendosky et al 1991), whereas another study revealed a significant lowering of the nocturnal body temperature in summer in patients only (Schwartz et al 1997). Following light therapy, an increase in amplitude of the circadian temperature profile has been found when patients received a combination of morning and evening light (Rosenthal et al 1990). However, in a morning light therapy study it was observed that the circadian temperature amplitude of patients and controls remained unaffected (Eastman et al 1993). Both groups showed similar phase-shifting responses.
**Constant Routine Studies in SAD**

In the two constant routine studies done so far, lasting 27 and 40 hours respectively, the DLMO and the circadian rhythms in core body temperature were measured in female SAD patients and controls. (Dahl et al 1993; Wirz-Justice et al 1995). From all investigated circadian parameters in the 40-hour study only the midrange crossing of the rising morning limb of the temperature curve showed a delayed phase position of patients relative to controls. Mid-day light therapy did not change salivary melatonin parameters but resulted in phase advances of some aspects of the temperature curves in patients only (Wirz-Justice et al 1995). In the 27-hour constant routine study, the DLMO and the circadian rhythm in core body temperature were measured (Dahl et al 1993). The DLMO and the circadian temperature minimum were significantly phase delayed in patients in comparison with those in controls. Patients showed an advance after morning light therapy. In both studies no differences in the amplitude of the melatonin secretion patterns and of the body temperature curves were observed between patients and controls, nor did the amplitudes change significantly in response to treatment. Constant routine protocols have the disadvantage that they entail sleep deprivation and therefore potentially yield a change of mood. Indeed, visual analogue scale data on mood revealed an antidepressant effect during the 40-hour constant routine study (Wirz-Justice et al 1995). No changes of mood were found in the 27-hour constant routine experiment, but after finishing the protocol, some subjects experienced improvement (Avery et al 1997).

In conclusion, the data do not yield a consistent picture of the circadian system in SAD patients. The reason for the lack of consistency probably is that masking factors could not be avoided in those studies. As argued before, an alternative way to examine the circadian system is to apply a forced desynchrony protocol. To the best of our knowledge, the present study is the first one in which this was done in SAD patients.

**METHODS AND MATERIALS**

**Recruitment and Selection Criteria**

SAD patients of our outpatient clinic, who were known to be good responders to light therapy in previous years, received written general information on the study. Healthy controls were recruited by advertisements in local newspapers and on local television. Patients and healthy subjects who were interested first received a detailed description of the study by letter and were then invited for a screening procedure if they considered participation. Patients were diagnosed according to the DSM-IV criteria for recurrent major depression with seasonal pattern (American Psychiatric Association...
1994) and had to meet the Rosenthal criteria for SAD (Rosenthal et al 1984). Controls had to have no psychological complaints or sleeping problems. To evaluate general psychological health, depressed mood, seasonality and morningness/eveningness, both patients and healthy subjects completed a number of questionnaires: the Beck Depression Inventory (BDI) (Beck et al 1979), the Structured Interview Guide for the Hamilton Depression Rating Scale - Seasonal Affective Disorder - Self Rating version (SIGH-SAD-SR) (Williams et al 1992), consisting of the 21-item Hamilton Depression Rating Scale (HDRS) and an 8-item atypical symptom scale (ATYP), the General Health Questionnaire (GHQ) (Goldberg and Williams 1988), the Seasonal Personality Assessment Questionnaire (SPAQ) (Rosenthal et al 1987) and the Morningness Evenness questionnaire (M/E) (Horne and Ostberg 1976). Criteria for participation as a control subject were: a BDI score <9, a SIGH-SAD-SR score <8, a GHQ score <3, and a SPAQ score <8 (Kasper et al 1989). All subjects were examined by a physician and were declared physically fit. In females, the menstrual cycle was assessed through self report. With the exception of oral contraceptive pills and NSAIDs, neither patients nor controls used any medication at least one month prior to the experiment. Nobody was dependent on alcohol or caffeine. During 6 months before the study, SAD patients did not use psychoactive drugs. After a verbal explanation of the main characteristics of the protocol, subjects gave written informed consent. The study was approved by the Medical Ethics Committee of the Groningen Academic Hospital. Patients and controls were matched for age, sex, phase of the menstrual cycle and smoking habits. SAD patients were engaged in the study once during a depressive episode, once when remitted after light therapy and once in summer. Controls participated once in winter and once in summer. Both patients and controls were paid for their participation.

During the winter season, BDI and SIGH-SAD-SR ratings were obtained weekly from SAD patients. Participation during a depressive episode required a BDI score \( \geq 16 \). Light therapy thereafter consisted of 45 minutes of bright light once a day with an intensity of 10,000 lux for 5 consecutive days. After recovery, a BDI score <6 was required for participation in the next step in the project. If appropriate, female patients were studied in the follicular phase of their menstrual cycle.

Subjects

From November 1997 until December 1999, 7 SAD patients (1 male and 6 females) and 8 control subjects (1 male and 7 females) were studied. Table 4.1 lists the respective scores (mean ± SD) of patients and controls on the GHQ, SPAQ and M/E completed at the introduction meeting. For the winter studies, subjects entered the time isolation unit in the months October - March. The summer studies started in May - August. One female control subject dropped out after finishing the protocol in summer and was replaced by another matched control for the winter experiment. Three patients and three controls smoked cigarettes.
Of the female patients, one had a regular natural menstrual cycle, three used combined oral contraceptives, one used a depot progesterone, and one was post-menopausal and used hormone replacement therapy during the winter. Consequently, four female patients could consistently be studied in the (pseudo-) follicular phase and one in the pseudo-luteal phase of the menstrual cycle. The post-menopausal patient was studied in the pseudo-luteal phase while depressed and in the pseudo-follicular phase while remitted. Of the female controls, two had a regular natural menstrual cycle, three used combined oral contraceptives and two were post-menopausal (one used estrogen replacement therapy). Except for the winter condition of the control subject who was matched with the postmenopausal patient, the times of participation of female controls were carefully matched for menstrual cycle phase. The mean baseline scores (± SD) on the BDI, SIGH-SAD-SR, HDRS and ATYP are summarized for each condition in Table 4.1. During their stay in the time isolation unit, four patients used NSAIDs (i.e., paracetamol): two while depressed (1 and 4 x 500 mg respectively), one while remitted (3 x 500 mg) and one in summer (1 x 500 mg).

**Design of the Protocol**

In each condition, patients and controls were subjected to a 10-day protocol. During the first 4 days, subjects adhered to a regular sleep-wake schedule at home. Adherence was verified by actometry. The actometer was worn continuously on the non-dominant arm and was connected to a portable recorder (Bakker and Beersma

---

**Table 4.1** Characteristics (mean ± SD) of SAD patients and controls

<table>
<thead>
<tr>
<th></th>
<th>patients</th>
<th>controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>age</strong></td>
<td>36.3±13.9</td>
<td>38.1±12.8</td>
</tr>
<tr>
<td><strong>GHQ</strong></td>
<td>1.4±2.3</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td><strong>SPAQ</strong></td>
<td>16.7±3.6</td>
<td>3.5±1.8</td>
</tr>
<tr>
<td><strong>M/E</strong></td>
<td>44.7±10.4</td>
<td>51.4±13.7</td>
</tr>
</tbody>
</table>

**mood scores at baseline**

<table>
<thead>
<tr>
<th></th>
<th>depressed (n = 7)</th>
<th>remitted (n = 7)</th>
<th>summer (n = 7)</th>
<th>winter (n = 7)</th>
<th>summer (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BDI</strong></td>
<td>20.3±6.1</td>
<td>1.7±1.8</td>
<td>0.6±0.8</td>
<td>0.3±0.8</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td><strong>SIGH-SAD-SR</strong></td>
<td>32.3±11.8</td>
<td>3.7±4.3</td>
<td>1.1±1.9</td>
<td>1.4±2.1</td>
<td>1.6±1.6</td>
</tr>
<tr>
<td><strong>21-item HRSD</strong></td>
<td>21.3±7.9</td>
<td>2.0±2.0</td>
<td>0.9±1.5</td>
<td>0.9±0.9</td>
<td>1.3±1.5</td>
</tr>
<tr>
<td><strong>ATYP</strong></td>
<td>11.0±5.4</td>
<td>1.7±2.2</td>
<td>0.3±0.5</td>
<td>0.6±1.5</td>
<td>0.3±0.8</td>
</tr>
</tbody>
</table>

*a Age at the start of the first experiment.

*b M/E score <30 and >70 correspond with evening- and morning types respectively.
Sleep was scheduled from midnight till 8 AM. Subjects limited their coffee consumption to 4 cups a day and abstained from alcoholic beverages, heavy physical exercise and daytime naps.

On day 4 at 6 PM, subjects entered the time isolation unit located at the department of Psychiatry of the Groningen Academic Hospital. The unit is shielded from all possible information on time of day. It comprises a living room with a bed and a bathroom. After a period of familiarizing with the standard procedures during wake time and a habituation night, the 120-hour FD protocol started on day 5 at 8 AM. Subjects were blind to the experimental time schedule. The schedule consisted of 6 20 hour days. These artificial 20-hour days were subdivided in epochs of 13.5 hours of wakefulness in dim light (<10 lux) and 6.5 hours of darkness in which the subjects had to stay in bed. Each 20-hour day had the same temporal structure. Staff members who were trained to communicate no information on time of day, announced the moments of rising, showering, meals, completing psychometric test batteries, and of going to bed. Besides the scheduled activities, subjects could engage in all kinds of activities such as watching videos, listening to music, etcetera. A maximum of 4 caffeine-containing drinks was allowed only during the subjective morning. Very rarely, subjects were allowed to take an NSAID. Throughout their stay in the time isolation unit, subjects were continuously observed through an infrared television camera.

**Melatonin**

On day 4 and 9 of the protocol, from 7 PM until midnight and from 7 PM until 2 AM respectively, saliva was sampled hourly for the determination of melatonin concentration. In 2 patients saliva was collected in small plastic containers. In all other subjects, it was sampled by means of Salivettes with a cotton swab (Sarstedt, Nümbrecht, Germany). Subjects were asked to remain seated during the 15 minutes prior to the saliva collection. All samples were frozen at ≤ -18°C immediately afterwards. After each saliva sample, eating (with the exception of chocolate and bananas), drinking and smoking were allowed for 15 minutes. Then subjects thoroughly rinsed their mouth with water.

Melatonin concentrations were measured by means of radioimmunoassay (RIA) (Bühlmann, Allschwil, Switzerland) which was performed at the laboratory of the hospital ‘De Gelderse Vallei’, Bennekom, The Netherlands. In this laboratory, the following assay parameters were obtained: a limit of detection of 0.5 pg/mL; an intra assay variation of covariance of 8.78% (mean melatonin concentration 8.65 pg/mL, \(n = 26\)) and an inter-assay variation of covariance in samples with a low melatonin concentration of 9.6% (mean 1.98 pg/mL, \(n = 30\)) and in samples with a high concentration of 9.8% (mean 14.76 pg/mL, \(n = 30\)). For each experiment, melatonin values were expressed as a percentage of the maximum value observed on day 4. The
dim light melatonin onset (DLMO) was defined as the time at which the normalized melatonin curve crossed the 25% level. As the body temperature recordings confirmed that 5 circadian cycles had passed between the 2 DLMOs, an estimate of the circadian rhythm period ($\tau$) could be obtained by dividing the epoch between the two DLMOs by 5.

**Core Body Temperature**

Throughout the entire stay in the time isolation unit, core body temperature was measured continuously with a calibrated rectal thermometer (Yellow Springs Instrument Company, Yellow Springs, OH, US). The data were stored at 1-minute intervals by a portable recorder (Bakker and Beersma 1991). Those parts of the temperature curve that were considered unreliable due to technical failures were not used for further analysis. Intervals of missing data shorter than 1.5 hours in duration were linearly interpolated, intervals longer than 1.5 hours were regarded as missing data.

To identify the endogenous circadian temperature curve, the following mathematical procedure was used. It is based on the assumption that the raw temperature data obtained during the 120-hour forced desynchrony protocol represent the sum of the exogenous sleep-wake induced curve and the endogenous circadian curve. First, the raw data of the 6 consecutive 20-hour subjective days were averaged to obtain an estimation of the mean sleep-wake related course of body temperature. With this procedure the circadian contribution to the raw temperature data is roughly averaged out, because each subjective 20-hour day has the same temporal structure and all scheduled activities occur at all circadian phases. To obtain an estimation of the endogenous circadian temperature curve, the obtained mean sleep-wake related temperature curve was subtracted from the raw curve. Subsequently, the resulting curve was divided in epochs with the length of the melatonin-derived -value and then averaged (starting from 8 AM on day 4 of the protocol). Due to the fact that the melatonin-derived -values differ from 24 hours, the pacemaker-related contribution was not averaged out completely during the first step of the mathematical procedure. To establish a better estimation of the sleep-wake-related contribution to the temperature curve, the obtained average circadian temperature curve was subtracted from the original raw data. The resulting curve was again divided in 20-hour epochs and averaged, etcetera. This procedure was repeated until a stable estimation of the circadian temperature curve was obtained (see Hiddinga et al 1997).

**Polysomnography**

From each subjective night, a polysomnographic recording (PSG) was obtained. Locations of electrodes and scoring criteria were according to the standards defined by Rechtschaffen and Kales (Rechtschaffen and Kales 1968). The PSGs were scored
in 30 second intervals with the aid of the VitaPort software (TEMEC Instruments, Kerkrade, Netherlands) by 3 raters. The average agreement between raters was 95.4% (range 94.1% - 96.3%) with an average largest disagreement interval of 3 minutes (range 1.5 - 4 minutes). Total sleep time (TST) per sleeping period was calculated by adding the total amount of minutes in sleep stages 1, 2, 3, 4 and REM.

Statistical Procedures

Power analyses were performed to estimate the smallest detectable differences by calculating $\delta$ from the formula $n \geq 2(\sigma/\delta)^2\left(t_{\alpha[v]}+t_{2(1-P)[v]}\right)^2$, with a power $P = 0.80$. In this formula $n$ represents the number of replications, $\sigma$ the true sd, $\delta = $ the smallest desired difference to detect, $\alpha$ the level of significance, $P$ the intended power of the test, $v$ the degrees of freedom ($v = a(n-1)$), $a$ the number of populations, and $t_{0.05[12]} = 2.179$ and $t_{0.4[12]} = 0.873$ (Sokal and Rohlf 1981).

The data derived from melatonin and temperature recordings were analyzed statistically by means of paired t tests. Analysis of variance with repeated measures (ANOVA) was applied to evaluate TST. The Spearman's correlation coefficient was computed to evaluate the association between the average level of core body temperature and the amplitude of the observed circadian temperature component. The analyses were two tailed and statistical significance was established at $p < .05$. With this procedure, no correction is applied to account for multiple testing. As a consequence the study is more likely to find significant relationships than statistically justified. We nevertheless adopted this conservative approach to avoid missing any possible difference in pacemaker characteristics between patients and controls.

RESULTS

The Circadian Period, $\tau$, Based on Melatonin Data

In each condition, melatonin concentrations showed a gradual increase. As mentioned in the method section, DLMO was defined as the time at which melatonin production reached 25% of the maximal production on day 4. In 2 of the remitted patients the initial melatonin production in the second sampling period (day 9) exceeded this 25% criterion. Therefore, in these subjects the criterion was raised to 50%. Since the choice of the criterion value influences the assessment of phase, the DLMO times were exclusively used for the estimation of the circadian period ($\tau$). All $\tau$-values approximated 24 hours. Between conditions, no significant differences in $\tau$-values were found (Table 4.2).
Table 4.2  Circadian and sleep-wake related characteristics derived from core body temperature and melatonin (mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Melatonin</th>
<th>Core body temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>τ(^a)</td>
<td>Mean(^b)</td>
</tr>
<tr>
<td>Patients (depressed; (n=7))</td>
<td>24.03±0.07</td>
<td>37.23±0.10</td>
</tr>
<tr>
<td>Patients (remitted; (n=7))</td>
<td>24.13±0.08</td>
<td>37.22±0.07</td>
</tr>
<tr>
<td>Patients (summer; (n=7))</td>
<td>24.17±0.10</td>
<td>37.16±0.11</td>
</tr>
<tr>
<td>Controls (winter; (n=7))</td>
<td>24.22±0.08</td>
<td>37.00±0.11</td>
</tr>
<tr>
<td>Controls (summer; (n=7))</td>
<td>24.27±0.10</td>
<td>36.95±0.15</td>
</tr>
</tbody>
</table>

Paired t tests\(^f\)

<table>
<thead>
<tr>
<th></th>
<th>Pat (dep vs. rem)</th>
<th>Pat (dep vs. sum)</th>
<th>Pat (rem vs. sum)</th>
<th>Pat (dep) vs. Con (win)</th>
<th>Pat (rem) vs. Con (win)</th>
<th>Pat (sum) vs. Con (sum)</th>
<th>Con (win) vs. Con (sum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.282</td>
<td>.984</td>
<td>.431</td>
<td>.240</td>
<td>.621</td>
<td>.813</td>
<td>.327</td>
</tr>
</tbody>
</table>

\(^a\) The circadian rhythm period (\(τ\)) in hours (mean ± SEM) derived from the timing of the DLMO obtained on the first (day 4) and the last (day 9) evening in time isolation.

\(^b\) Mean level (± SEM) of core body temperature (°C) calculated by averaging the temperature data of the last 6.5 hours of wakefulness and the 6.5-hour sleeping period.

\(^c,d\) Amplitude (mean ± SEM) of the sleep-wake related\(^c\) and the circadian\(^d\) variation of body temperature (°C) defined as the standard deviation calculated over the last 6.5 hours of wakefulness and the 6.5-hour sleeping period, and the total circadian variation of body temperature respectively.

\(^e\) Timing of the circadian temperature minimum (mean ± SEM) on the first morning in time isolation.

\(^f\) p-values obtained by two-tailed paired t tests between the various conditions in which patients (Pat) and controls (Con) participated (dep = depressed state; rem=remitted; sum=in summer; win=in winter).

**Evaluation of the sleep-wake- and pacemaker-related temperature components**

9.61% of the core body temperature data were missing due to technical problems and matters of hygiene. The analysis described in the method section provided the contributions of the circadian pacemaker and those of the sleep-wake cycle to the raw temperature curves. Figure 4.1 displays these contributions per condition.

The sleep-wake induced temperature curve shows a remarkable resemblance in the various conditions. After waking-up, body temperature shows a peak induced by showering. Subsequently, it drops and stays relatively stable during the remaining...
Figure 4.1  Sleep-wake- and pacemaker-related variation of core body temperature obtained in a 120-hour forced desynchrony experiment. In each condition 7 SAD patients and 7 matched controls participated. The curves, plotted as deviation from the mean, depict the mean (± SEM) temperature variation in each condition. The shaded area indicates the 6.5-hour sleeping period within the 20-hour subjective day. SAD = Seasonal Affective Disorder.
hours of wakefulness. At the start of the 6.5-hour period in bed, a sharp decrease in temperature can be observed. The mean level of body temperature (listed in Table 4.2) was calculated by averaging the data of the last 6.5 hours of wakefulness and the 6.5 hours for sleep. In this way, the highly variable influence of showering on the mean was excluded. Although not significantly, mean body temperature of the patients was slightly higher during the winter than during the summer and also slightly higher than that of controls.

The amplitudes of the sleep-wake induced variation of body temperature (see Table 4.2), a measure of which is obtained by computing the standard deviation of the data of the last 6.5 hours of wakefulness and those of the 6.5 hours for sleep, showed no differences between patients and controls.

Figure 4.1 also shows the circadian variation of body temperature plotted against circadian time (CT). CT0 indicates the moment at which the endogenous circadian temperature reaches its minimum. A measure proportional to the amplitude of the (almost sinusoidal) curves was obtained by calculating the standard deviation of the pacemaker-induced variation of body temperature (Table 4.2). Only comparisons of the depressed patients and the remitted patients with the controls in winter showed statistically significant differences ($p = .050$ and $p = .031$). The patients’ amplitude turned out to be smaller.

The phase of the circadian pacemaker (circadian time 0 (CT0)) was defined as the timing of the first circadian temperature minimum in time isolation and ascertained as follows. The obtained average endogenous circadian temperature curve was double plotted around the start of the forced desynchrony protocol (8 AM). The timing of the temperature minimum in the early morning of this first day in forced desynchrony was determined by computing the midpoint between the upward and downward crossings through the average value of the temperature curve by means of a moving average procedure. In each condition, CT0 was located in the early morning (Table 4.2). Between conditions, no significant differences in the timing of CT0 were observed.

In Figure 4.2, the standard deviations of the circadian modulations (which are proportional to the amplitudes of the pacemaker induced temperature curves) are plotted against the average levels of body temperature for all subjects in all conditions. The data reveal a significant correlation between the average level of body temperature and the amplitude of the pacemaker related temperature component (Spearman’s rho = 0.627, $p = .000$). A significant correlation between these variables was also found in the patients and controls separately (Spearman’s rho = -0.508 and -0.604, $p = .019$ and $p = .022$). This suggests that the higher the average level of body temperature, the smaller is the amplitude of the endogenous circadian temperature component.
POLYSOMNOGRAPHY

The mean TSTs in the subjective nights in forced desynchrony are summarized in Table 4.3. In the data of all conditions combined, ANOVA with repeated measures yielded a significant effect of night number in forced desynchrony on TST (F(5,26) = 6.872, p = .000). However, no significant interaction between night number and condition was detected (F(20,116) = 0.667, p = .851). Additionally, separate ANOVAs to compare the various conditions in which patients and controls were studied did not reveal significant differences either.

DISCUSSION

The aim of the present forced desynchrony study was to compare the characteristics of the endogenous circadian pacemaker of SAD patients with those of matched controls. It is concluded that the circadian pacemakers of patients and controls have a similar period, in both winter and summer and irrespective of the symptomatological state of the patients. The same applies to the phase of the endogenous circadian cycle after 4 days of restricting sleep to the interval between midnight and 8 AM. The

Figure 4.2  The standard deviation (SD) of the circadian modulation (see Figure 4.1, right panel) as a function of the average level of body temperature. The SD of the circadian modulation is proportional to circadian amplitude (for a sine function the SD equals $\frac{1}{2}\sqrt{2}$ times circadian amplitude). Filled circles depict the data obtained in patients (n = 21), open circles those obtained in controls (n = 14). The diagonal line represents the regression line. Correlation coefficient (Spearman’s rho) of all conditions combined: -0.627 (p = .000), of the patient group: -0.508 (p = .019), and of the control group: -0.604 (p = .022).

Polysomnography

The mean TSTs in the subjective nights in forced desynchrony are summarized in Table 4.3. In the data of all conditions combined, ANOVA with repeated measures yielded a significant effect of night number in forced desynchrony on TST (F(5,26) = 6.872, p = .000). However, no significant interaction between night number and condition was detected (F(20,116) = 0.667, p = .851). Additionally, separate ANOVAs to compare the various conditions in which patients and controls were studied did not reveal significant differences either.

DISCUSSION

The aim of the present forced desynchrony study was to compare the characteristics of the endogenous circadian pacemaker of SAD patients with those of matched controls. It is concluded that the circadian pacemakers of patients and controls have a similar period, in both winter and summer and irrespective of the symptomatological state of the patients. The same applies to the phase of the endogenous circadian cycle after 4 days of restricting sleep to the interval between midnight and 8 AM. The
only possible difference detected in our study concerns the amplitude of the endoge-
nous circadian temperature component. Compared with that found in controls, the
circadian amplitude appeared to be slightly smaller in SAD patients in winter, both
during a depressive episode and in the recovered state.

Previously, we have reported on the circadian and sleep-wake cycle related varia-
tions of core body temperature observed in one 54-year-old male SAD patient studied
in a 120-hour forced desynchrony protocol both while depressed and while remitted
after light therapy (Koorengevel et al 2000). This subject, who is included in the pre-
sent analysis, provided evidence for a phase delay of the circadian pacemaker during a
depressive episode. Compared to recovery, a phase delay in the timing of the circa-
dian temperature minimum of 57 minutes was observed in the depressed state. This
is in contrast with the enlarged data set in which no evidence for pacemaker distur-
bances in SAD was found.

The Endogenous Circadian Period

The DLMOs obtained in the first and last evening in time isolation, showed an endo-
genous circadian period (τ) of approximately 24 hours in each condition. Just like
body temperature, salivary and plasma melatonin concentrations exhibit a distinct
circadian rhythm. Yet, apart from an endogenous circadian modulation, the secretion
of melatonin has also been found to depend on the timing of the sleep-wake cycle (or
the dim light-darkness cycle) (Wyatt et al 1999; Ritz-De Cecco et al 1999; Gordijn et
al 1999). In the present study, a small difference occurred in elapsed hours of wake-
fulness on the two days of saliva collection. This may have induced a small overesti-
mation of τ in all conditions. However, the τ-values observed in SAD patients and con-
trols are similar to those computed with temperature or melatonin data in other FD

<table>
<thead>
<tr>
<th></th>
<th>night 1</th>
<th>night 2</th>
<th>night 3</th>
<th>night 4</th>
<th>night 5</th>
<th>night 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (depressed; n=7)</td>
<td>322±28</td>
<td>279±37</td>
<td>310±26</td>
<td>316±31</td>
<td>356±16</td>
<td>356±2</td>
</tr>
<tr>
<td>Patients (remitted; n=7)</td>
<td>346±15</td>
<td>314±28</td>
<td>319±18</td>
<td>302±27</td>
<td>349±13</td>
<td>351±8</td>
</tr>
<tr>
<td>Patients (summer; n=7)</td>
<td>352±5</td>
<td>308±31</td>
<td>308±23</td>
<td>336±16</td>
<td>334±14</td>
<td>362±5</td>
</tr>
<tr>
<td>Controls (winter; n=7)</td>
<td>347±12</td>
<td>326±19</td>
<td>311±25</td>
<td>348±15</td>
<td>339±14</td>
<td>356±6</td>
</tr>
<tr>
<td>Controls (summer; n=7)</td>
<td>349±10</td>
<td>289±25</td>
<td>315±25</td>
<td>338±20</td>
<td>332±20</td>
<td>359±9</td>
</tr>
</tbody>
</table>

*aTotal time in stage 1, 2, 3, 4, and REM (mean ± SEM) per subjective night in forced desyn-
chrony did not differ between conditions (ANOVA: F(20,116) = 0.667, p = .851).
studies on healthy subjects in 20-hour-day protocols (24.30 h (Hiddinga et al 1997); 24.1-24.2 h (Wyatt et al 1999)) as well as in 28-hour-day protocols (24.18 h (Czeisler et al 1999); 24.3-24.35 h (Carskadon et al 1999)).

The phase position of a circadian process during entrainment depends on the intrinsic period length, a long period leading to a late phase position. In the absence of a difference in $\tau$ between SAD patients and controls, the suggested phase delay of SAD (Lewy et al 1987a) cannot be attributed to a longer intrinsic period.

**Circadian Phase**

Unlike data from some treatment- (Lewy et al 1987b; Sack et al 1990; Lewy et al 1998) and constant routine studies (Dahl et al 1993; Avery et al 1997), our data did not show abnormalities of circadian phase. No differences were found between the various conditions with respect to the timing of the circadian temperature minimum. While this may be due to the synchronizing effects of the instructions concerning the timing of sleep on the days prior to FD, it is also possible that the small sample size of the present study is responsible. This will be discussed below. Nevertheless, the data do not confirm a phase delay in depressed SAD patients, as postulated by the phase-delay hypothesis (Lewy et al 1987a). The implications of the present study for the phase-shift hypothesis (i.e., whether a shift in phase of the circadian pacemaker relative to the sleep-wake cycle influences mood) will be discussed in chapter 6.

**Circadian Amplitude**

In winter, a smaller endogenous circadian amplitude was observed in depressed and remitted patients than in controls. Average level of body temperature and circadian amplitude were negatively correlated in the total data set as well as in the separate data sets on patients and controls. Consequently, the relatively small circadian amplitude in SAD patients might be explained by the relatively high average body temperature. Since body temperature in homeothermic organisms is regulated within a restricted range of values, it is conceivable that a higher body temperature level automatically leads to a smaller variation, because of ceiling effects. Therefore, a decrease of the endogenous circadian amplitude of body temperature in depressed and remitted patients could be the consequence of a higher level of body temperature instead of the result of a disturbance of the circadian system.

**Influence of Menstrual Cycle and NSAIDs on Body Temperature**

To control for the influences of gender and for the influences of gonadal hormones and oral contraceptives on body temperature specifically (Wright and Badia 1999), in each experiment, female subjects were studied in the same phase of the menstrual cycle (which was the follicular phase if appropriate). Apart from the sporadic use of NSAIDs by female patients in all three conditions, all subjects were free of medica-
tion. Studies on the effects of NSAIDs on the body temperature have been inconclusive. NSAIDs have been reported to yield no effects on the circadian variation of body temperature (Scales et al. 1988). However, another study has observed an attenuation of the decrease in tympanic temperature and a suppression of melatonin secretion for some hours after nighttime administration of an NSAID (Murphy et al. 1996). Hence, small effects of the incidentally used NSAIDs cannot be excluded.

**Power of the Study**

A disadvantage of the present study is the rather small sample size. This is due to the high demands on the subjects living isolated from their normal environment for such a long time. Small sample sizes have consequences for the smallest detectable difference ($\delta$) (Sokal and Rohlf 1981) of each variable under study. A post-hoc power analysis of the obtained results, under the assumption of a power value of 0.8, revealed a smallest detectable difference ($\delta$) of (1) 0.45 °C in the average core body temperature level, (2) 22 minutes in the melatonin-derived tau values, (3) 0.059 °C in the amplitude of the endogenous circadian temperature component and (4) 123 minutes in the timing of the circadian temperature minimum. Thus, the absence of differences in phase position might be attributed to a lack of power, whereas the obtained values for $\tau$ and amplitude might be accurate enough to enable adequate comparisons.

Besides the small sample size, another weakness of the present study is the relatively large number of statistical tests performed which increases the chance of finding significant results. Only the data on circadian amplitude yielded statistical significant differences between SAD patients (both when depressed and remitted) and controls in winter. While SAD patients might generally exhibit a smaller circadian amplitude in winter, it is also possible that this result is due to chance variations in our sample. However, we have decided to perform multiple tests in order to avoid missing possible differences in pacemaker characteristics between patients and controls.

**Clinical State**

In all experiments, both patients and controls tolerated the protocol quite well. Comparison of the average scores on the BDI and SIGH-SAD-SR completed prior to and directly following the 10-day protocol revealed a small deterioration of mood in both patients and controls. Thus, the FD protocol did not induce a strong effect on mood in patients. In one FD study, an interaction between pacemaker and sleep-wake cycle related variation has been demonstrated in the regulation of mood in healthy subjects (Boivin et al. 1997). The present study revealed a similar modulation of self rated mood in the course of the experiment in all conditions (see chapter 6).

PSG data did show an effect of night sequence number on TST. This effect did not differ between conditions. Like the TST data from the present study, also PSG data obtained in another FD study using 20-hour subjective days have shown a highest
and lowest sleep efficiency when sleep was centered around CT0 and 16 respectively (Wyatt et al 1999).

Thus, the 120-hour FD protocol did not only induce similar effects on sleep in patients and controls, but it also hardly affected the overall clinical state. It can therefore be concluded that FD provides an adequate tool in the study of the circadian system in depressed patients.

In summary, this 120-hour FD study on SAD revealed that the circadian pacemaker characteristics assessed by measuring core body temperature and melatonin, do not differ in (1) depressed and remitted SAD patients versus controls in winter, (2) in SAD patients versus controls in summer and (3) in SAD patients and controls in winter versus summer. In addition, the significant correlation between average level of temperature and endogenous circadian amplitude suggests that in SAD patients, thermoregulation might be disturbed in the winter season.

ACKNOWLEDGMENTS

The authors are grateful to the subjects for their participation, to Iteke te Riet, Ybe Meesters, Gerda Bloem, Marijke Gordijn, Theodoor van Maaren, Maarten Langenberg, Jan van Dijken, Marieke Kienhuis, Wendy Blom, Louise Dols and Jacqueline Reisel for their contribution to the performance of the study, to Camiel Elsinga for his help in scoring the PSG recordings, and to J.J. Veeken and co-workers of the laboratory of the Hospital ‘De Gelderse Vallei’, Bennekom, The Netherlands for the melatonin analysis.

This research was supported by the Nederlandse Gasunie B.V., the Medical Faculty of the University of Groningen, the Academic Hospital Groningen, the Ministry of Health, and the National Fund of Mental Health.