Summary and general discussion
Chronobiological hypotheses about the pathogenesis of affective disorders have a long history. According to the modern variants, abnormalities of either a sleep-wake cycle dependent process S, or a circadian pacemaker related process C, or an abnormal interaction between these two processes underlie the pathogenesis of depression. However, up till now, research did not yield consistent support for these theories. The development of the forced desynchrony protocol for the study of the human circadian system and the observed effects of extraocular light stimuli on this system offered the opportunity to reinvestigate this issue. In this thesis these opportunities have been utilized to test chronobiological hypotheses in winter-type Seasonal Affective Disorder.

**Chronobiological Aspects of Light Therapy in SAD**

A review of the history of the ideas about the seasonality of affective disorders and the beneficial effects of light on mood shows that both phenomena were already recognized in ancient times (chapter 2). The reappraisal of this ancient knowledge and the subsequent delineation of Seasonal Affective Disorder (SAD) in the 1980s (Rosenthal et al 1984) triggered an increasing amount of research on the effects of light in the treatment of this syndrome. It was demonstrated that the majority of SAD patients responds favorably to light therapy (Terman et al 1989) and nowadays exposure to light is the treatment of first choice.

The seasonality of the syndrome and the efficacy of light therapy have been taken as evidence for the hypothesis that chronobiological disturbances underlie SAD. The phase-delay hypothesis is one of the major hypotheses for the pathogenesis of SAD. It postulates that SAD symptoms are caused by a phase delay of the circadian pacemaker relative to the environmental light-dark cycle and that the exposure to morning light is beneficial due to its phase-advancing properties (Lewy et al 1987a). Several studies have been performed to assess the optimal timing for light therapy. Some authors found evidence for the superiority of morning light over light therapy at other times of day (Eastman et al 1989; Lewy et al 1998; Terman et al 1998), whereas other did not (Wirz-Justice et al 1993; Meesters et al 1995). Strictly speaking, the phase-delay hypothesis predicts a worsening of symptoms after application of evening light therapy, since evening light will shift the circadian pacemaker to an even later phase position. Such a worsening has never been found, on the contrary, evening light has been proven to be beneficial. This has been attributed to other, ‘non-chronobiological’, effects. Evidently, the contribution of these non-chronobiological effects is large. This triggers questions about the contribution of placebo effects to the efficacy of light treatment of SAD. These questions are difficult to answer because no appropriate placebo control for ocular light treatment is available (Eastman 1990).
The finding of the extraocular photoresponsiveness of the human circadian system (Campbell and Murphy 1998) seemed to provide the opportunity to evaluate the effects of light therapy, and particularly the phase-delay hypothesis, in a genuine double-blind placebo-controlled experiment (chapter 3). In this experiment, twenty nine SAD patients received a 5-day experimental treatment with either 13,000 lux of extraocular light by fiber-optic illumination or placebo (no light) in the popliteal fossae, from 8 - 11 AM. Both treatment groups were balanced for age, sex and severity of depression at baseline. The experimental treatment was administered in such a way that neither patients nor staff members knew whether the light was actually applied or not. Clinical state was evaluated before, directly following and one week after treatment. The effects on the circadian system were assessed by means of the dim light melatonin onset (DLMO) of the evening before and after the experimental treatment. Both treatment groups showed a progressive improvement of clinical state over time. Between groups no significant differences were observed in clinical state and in the timing of the DLMO before and directly after treatment. It was therefore concluded that the response to extraocular light therapy in SAD patients did not exceed its placebo effect and that extraocular light did not induce a phase shift of the circadian pacemaker.

The failure to find phase shifts of the circadian pacemaker with extraocular light is in line with results of several studies in healthy subjects simultaneously performed in other laboratories (Lockley et al 1998; Hébert et al 1999; Eastman et al 2000; Jean Louis et al 2000; Lindblom et al 2000a, 2000b). Nevertheless, the exposure to extraocular light as well as the placebo treatment showed effect sizes comparable to those obtained in ocular light therapy studies (Terman et al 1989). Moreover, the percentages of remission were not significantly different in both treatment groups. Depending on the criteria for remission, 27-40% of the patients receiving extraocular light and 21-36% of the patients receiving no light at all showed remission after the experimental treatment. From the evaluation of the effects of pharmacological treatments in major depression, it is known that the placebo response can be as high as 65% (Quitkin 1999), but that the effects are smaller when patients are aware of the fact that they have 50% chance to receive placebo treatment. The conclusion must be that both the effects of extraocular light and placebo can be interpreted as exclusively due to placebo, i.e. non-chronobiological mechanisms.

A recent study of ocular light therapy in SAD revealed a positive correlation between the response to light treatment and the phase advance shifts of the DLMO (Terman et al 2001). Surprisingly, such association was also observed when we combined the data of both experimental treatment groups of our extraocular light study. Ocular light is the most important synchronizer of the circadian system. Thus, the phase advance shifts obtained in the extraocular light experiment are most likely due
to the changes in sleep timing and the resulting changes in natural light exposure induced by the protocol. Like in non-seasonal depressives, sleep deprivation has a mood improving effect in SAD patients (Graw et al. 1998). Thus, the phase shift due to natural light exposure, or the mood improving effects of sleep curtailment, or both might account for the antidepressant effects of the active and placebo treatment. The present study of extraocular light in SAD does not enable to discern which of these two processes might be responsible for the observed effects. The inclusion of a standard light treatment group might have provided the necessary data, because such treatment would have involved a larger phase shift and a similar amount of sleep deprivation. On the other hand, this control treatment would have been insufficient because the subjects would have noted the light.

Apart from the possible direct effects of phase shifts and sleep deprivation on mood, it cannot be ruled out that the relationship between phase advance and mood improvement is due to non-chronobiological mechanisms. It is possible that a more stringent compliance with the protocol resulted in an earlier time of awakening and that higher compliance itself is associated with a higher susceptibility to placebo effects. Such mechanism would lead to the observed relationship. Alternatively, it is also possible that the non-chronobiological effect resulted in higher melatonin values, rather than an earlier timing. The interval in which the melatonin levels have been determined exclusively included the rising part of the curve. Higher melatonin values obtained in such an interval would yield a similar change of the curve, as would an advance shift of melatonin values. Intervals that include peak values of melatonin production are required to discriminate effects on amplitude from effects on phase.

To summarize, the double-blind placebo-controlled study of extraocular light in SAD revealed that extraocular light did not affect the circadian system. The effects of extraocular light and placebo on SAD symptoms were similar. The observed positive relationship between the phase advance shift of the DLMO and the response to treatment can be interpreted as resulting from sleep curtailment, phase advance shifts by natural ocular light and non-chronobiological effects, as well as to any combination of these factors. Hence, this study does not disprove the phase shift hypothesis for SAD, but contributes to the understanding of the mechanisms underlying the efficacy of light treatment.

Chronobiological Aspects of the Pathogenesis of SAD

Like non-seasonal depressives, SAD patients show diurnal mood variations, sleep disturbances and mood improvement after sleep deprivation. These phenomena have resulted in several hypotheses concerning the pathogenetic role of either an abnormality of the homeostatic regulation of the sleep-wake cycle (process S), or of the circadian pacemaker (process C), or of an abnormal interaction between these two pro-
cesses in both seasonal and non-seasonal affective disorders. The seasonal recurrence of symptoms and the efficacy of light therapy represented additional arguments for chronobiological hypotheses about the pathogenesis of SAD. With respect to process S it has been postulated that a deficiency in the homeostatic buildup of sleep pressure underlies the pathogenesis of depression. On the other hand, process C related hypotheses propose an abnormality of circadian phase relative to the timing of the sleep wake cycle, and particularly in SAD a phase delay of the circadian pacemaker (Lewy et al. 1987a). Moreover, a blunted circadian amplitude may be causal to the pathological regulation of mood in SAD (Czeisler et al. 1987). Up to date, studies yielded inconsistent evidence for the chronobiological theories for both seasonal and non-seasonal depression. However, the majority of these chronobiological studies, due to the protocols that were applied, did not provide adequate possibilities to discriminate between sleep-wake cycle related and pacemaker-related processes.

The present thesis addresses the possible involvement of an abnormally functioning circadian pacemaker (chapter 4), a dysfunctioning process S (chapter 5), or a disturbed interaction of process S and process C related mechanisms (chapter 6) in SAD by means of a forced desynchrony protocol. At present, the forced desynchrony protocol offers the most appropriate method to disentangle various aspects of human chronobiological functioning. The studies described in the present thesis are the first in which a forced desynchrony protocol was used in the study of depressed patients.

The Method of Forced Desynchrony

Overt rhythms measured under normal conditions always represent a mixture of process S and process C related influences. Constant routine protocols, in which subjects stay awake for more than 24 hours, aim to reduce the effects of process S and to reveal the influence of process C. However, deprivation of sleep ought to be avoided in the study of mood regulation due to its mood modulating effects. Sleep deprivation may cause a worsening of mood in healthy subjects and an improvement of mood in depressed patients. Forced desynchrony protocols, in contrast, allow the disentanglement of process S and process C by inducing a desynchronization between the sleep-wake cycle and the rhythm of the circadian pacemaker while avoiding sleep deprivation completely or nearly completely. For that reason, the method of forced desynchrony has been applied in this thesis.

Seven SAD patients and eight healthy controls were studied in a 120-hour forced desynchrony protocol. To avoid differences between patients and controls due to differences in sleep timing, subjects had to spent four baseline days at home. During these baseline days sleep was scheduled between midnight and 8 AM. The evening and night of day 4 were spent in the laboratory and served as a habituation period. Subsequently, subjects lived according to a schedule of six 20-hour days in a time isolated apartment. These subjective days each consisted of 13.5 hours of wakefulness in
dim light and a 6.5-hour dark period for sleep. The low light levels are known to be insufficient to influence the period of the rhythm of the circadian pacemaker. As a result the pacemaker continues to have a period close to 24 hours, while sleep and wakefulness alternate with a 20 hours period. Hence the sleep-wake rhythm and the circadian pacemaker are desynchronized.

SAD patients were studied during a depressive episode, while recovered after light therapy and in summer. Healthy controls were matched for age, sex, smoking habits and menstrual cycle phase, and were studied once in winter and once in summer. If possible, all female subjects were studied in the same phase of their menstrual cycle.

All subjects tolerated the protocol rather well. The protocol induced similar effects on sleep in patients and controls and at the termination of each experiment clinical state was similar to the initial state in both groups. Apart from the important advantages of the design, the study has some disadvantages. The relatively small sample size, which is due to the high demands on subjects living in temporal isolation for such a long time, is one of them. The small sample size has consequences for the smallest detectable difference of each variable under study. Another disadvantage of the present forced desynchrony study was the need for the performance of a relatively large number of statistical tests. A large number of statistical tests complicates the interpretation of data, since statistically it is more likely to find differences when more tests are applied. Yet, the most conservative approach to explore all possible differences between SAD patients and controls was applied in order not to miss any of those differences.

The Involvement of Process C in the Pathogenesis of SAD

Core body temperature and melatonin both show a pacemaker- and sleep-wake cycle related modulation (Czeisler et al 1999; Hiddinga et al 1997; Wyatt et al 1999). It is generally assumed that the pacemaker related variation is directly due to the pacemaker. To assess possible abnormalities of process C, body temperature and melatonin were measured during the 120-hour forced desynchrony protocol (chapter 4). Robust sleep-wake cycle related modulations were found in core body temperature, which showed no significant differences, neither between conditions nor between groups. Likewise, no significant differences were observed between patients and controls with respect to the melatonin-derived period (tau) or the circadian phase derived from the timing of the endogenous circadian temperature minimum. However, the amplitude of the endogenous circadian temperature rhythm was significantly smaller in depressed and remitted SAD patients than in controls.

Estimates of tau were based on melatonin secretion profiles instead of core body temperature data. In previous analyses of similar 120-hour forced desynchrony protocols (Hiddinga et al 1997; Koorengevel et al 2000) tau was assessed on the basis of
temperature data only. The fact that the melatonin derived tau values showed much smaller standard deviations than those derived from body temperature led to this other approach. Probably, melatonin secretion at low light levels is less sensitive to all kinds of masking influences than core body temperature. The absence of differences in tau values suggests that the phase delay often observed in SAD patients cannot be attributed to longer endogenous circadian periods.

The absence of differences in the timing of the endogenous circadian temperature minimum contrasts with the results of previous studies that demonstrated a phase delay of the circadian pacemaker in SAD. Both the synchronizing effects of the regular sleep-wake schedule before entering the forced desynchrony protocol and the small sample size might be held responsible for the differences.

The smaller circadian temperature amplitude of the patients in winter can be explained in terms of a disturbance in thermoregulatory processes. A negative correlation between the average temperature level and the circadian temperature amplitude was found in the total data set, as well as in the data of patients and controls separately. In homeothermic organisms body temperature varies within a restricted range of values. Since patients showed a relatively high average level of core body temperature, the blunted circadian amplitude in SAD patients during winter can be interpreted as a consequence of the higher average level of temperature instead of as a result of a disturbance of the circadian system.

Finally, a power analysis showed that the absence of differences in phase position might be caused by a lack of power. Nevertheless, the obtained tau values as well as the values for circadian amplitude appeared to be accurate enough for adequate comparisons between groups and conditions. It was therefore concluded that process C is undisturbed in SAD.

**The Involvement of Process S in the Pathogenesis of SAD**

According to the two-process model of sleep regulation, the timing of sleep is governed by process S and process C related mechanisms. Forced desynchrony studies have demonstrated that the characteristics of sleep are not only determined by homeostatic (process S) and ultradian processes but also by pacemaker related influences (process C) (Dijk and Czeisler 1994, 1995; Wyatt et al 1999). Most of the SAD patients complain of hypersomnia and daytime drowsiness (Anderson et al 1994). Thus, either a dysfunctional process S, or process C or a dysfunction of both can underlie these disturbed sleep patterns. Furthermore, it has been hypothesized that depressed patients show a deficient buildup of sleep pressure during the hours of wakefulness, reflected in a reduced amount of slow wave activity during subsequent sleep.

In the forced desynchrony study described in the present thesis, each period for sleep in the laboratory was evaluated by means of polysomnographic recordings (chapter 5). During forced desynchrony, the consecutive periods for sleep were
subsequently scheduled at circadian phases which were evenly distributed across the circadian cycle. Therefore, the possible pathogenetic role of homeostatic and ultradian mechanisms in SAD could be reliably evaluated, because the influence of process C could be virtually eliminated by averaging the data of all subsequent recordings. Neither homeostatic nor ultradian aspects of sleep showed differences between patients and controls and between the various conditions in which they participated. It is therefore concluded that process S related mechanisms are not involved in the pathogenesis of SAD.

The absence of polysomnographically recorded sleep disturbances is in contrast with the majority of findings of previous studies of sleep in SAD. Differences in design represent the most probable causes for this discrepancy. In none of the previous studies a forced desynchrony design was applied. Thus, these studies did not simultaneously control for the duration of prior wakefulness and for circadian phase. Another advantage of the present study over previous ones, is the fact that in each condition in which a subject participated, polysomnographic data could be averaged across six recordings, thereby reducing intra-individual variance by a factor of approximately 2.5.

The Involvement of Process C and Process S in the Regulation of Mood in SAD

Mood is regulated by a complex interaction between sleep-wake cycle- and pacemaker related processes (Boivin et al 1997). To assess the contributions of the sleep-wake cycle and the circadian pacemaker to the regulation of mood in SAD, self-rating questionnaires on mood were completed at 2-hour intervals during the waking hours of the forced desynchrony protocol (chapter 6).

A modulation of self-rated mood across the protocol was observed in each condition. Polysomnographically evaluated total sleep time revealed that this modulation could not be ascribed to effects of sleep deprivation.

Like in the forced desynchrony study of healthy subjects by Boivin et al (1997), in the present study patients and controls showed robust sleep-wake cycle related and pacemaker related variations of mood. The influences of the duration of wakefulness and of the circadian pacemaker on mood did not differ in patients and controls. After an initial improvement of mood during the first hours of wakefulness, mood deteriorated with the duration of wakefulness in each condition. The circadian pacemaker related variation of mood closely followed the sinusoidal shaped pacemaker related variation of body temperature. Due to the restricted length of the design, the present study was not suitable to study the type of interaction between process S and process C related mechanisms in the regulation of mood. However, for each condition, the reconstruction of the daily course of mood by the addition of the appropriate sleep-wake cycle related and pacemaker related components closely followed the average mood scores obtained during the four baseline days. This suggests that a linear inter-
action between process S and process C is sufficient to explain the course of mood under normal circumstances. From these findings it can be concluded that in SAD the contributions of the pacemaker and the sleep-wake cycle to the regulation of mood are not disturbed. Furthermore, the data might contribute to a better understanding of the mechanisms of light therapy. Many studies report a superiority of morning light over light therapy applied at other times of day. Chronobiological studies in healthy subjects have shown that the phase advances of the circadian pacemaker induced by morning light are accompanied by proportional shifts of circadian rhythms in psychological processes. Therefore, it is likely that the application of morning light in SAD patients will not only result in a phase advance of their circadian system in general, but also specifically of their circadian modulation of mood. As a consequence of this phase advance, the rising limb of the circadian mood variation will shift to an earlier clock time, leading to better mood scores at awakening during morning light treatment.

The Chronobiology of SAD

In many previous studies chronobiological abnormalities were found in SAD. The forced desynchrony data presented in this thesis suggest that process S and process C are undisturbed in SAD. Yet, these data do not disprove that in SAD chronobiological processes are involved in the mechanism of action of light therapy, as postulated by the phase-shift and amplitude hypothesis. To test that aspect of the hypotheses it is necessary to study the effects of light therapy on chronobiological parameters in SAD patients, which is beyond the scope of this thesis. The present thesis might nevertheless contribute to a better understanding of the mechanisms underlying the beneficial effects of light therapy in this disorder. The optimal timing of light therapy for SAD is still controversial. Some studies have shown that the timing of light therapy in SAD is not crucial (Wirz-Justice et al 1993; Meesters et al 1995), others demonstrated a superiority of morning light over evening light (e.g. Lewy et al 1998; Terman et al 2001). The double-blind placebo-controlled study of the effects of extraocular light applied in the morning showed that the application of both light and placebo was followed by changes in mood. Like in a study by Terman et al (2001) in which ocular light was administered, a positive relationship between the phase advance shift of the pacemaker and mood improvement was observed. The present forced desynchrony study revealed that self-rated mood shows a robust pacemaker-related modulation and that a phase advance leads to mood improvement in the morning. The association between phase advance and mood improvement can be interpreted as resulting from sleep curtailment, from phase advance shifts by natural ocular light, from non-chronobiological effects, or from any combination of these factors. Since light therapy applied at other times of day does not result in a phase advance and does not induce sleep deprivation effects, while non-chronobiological effects will probably be similar,
morning light therapy may be expected to be superior to light therapy at other times of day. However, the small size of the differences between the responses to morning light and light at other times of day suggests that the contributions of non-chronobiological factors to the therapeutic results are relatively large compared to those of the induced phase shifts and the induced sleep deprivation. The nature of these contributions is still unknown. These non-chronobiological factors may belong to the domain of ‘aspecific’ ingredients of the working alliance between patients and their doctors.