Cardiovascular control by the biological clock
Scheer, F.A.J.L.

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CHAPTER 9

General Discussion

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A. NEURONAL CONTROL OF CARDIOVASCULAR SYSTEM BY SCN

The heart makes the blood go round. The heart is the driving force for the delivery of oxygen and nutrients, for the disposal of waste and for the distribution of heat. These circulatory demands change greatly over the day and night, and the ability to anticipate these changing demands would obviously be very beneficial to the survival of the individual. The results of the present thesis provide evidence for the means by which the SCN regulates the cardiovascular system, and that allow such anticipation to oncoming demands. Here I will focus on the neuronal mechanisms the SCN uses to regulate the cardiovascular system.

Endogenous circadian rhythm in heart rate

The golden-standard to determine endogenous circadian rhythmicity is the constant routine condition [161, 172], developed initially by Mills et al. [224]. Constant routine conditions require the subjects to remain awake in a supine resting condition, in dim light, with equal-caloric meals at equal time intervals during at least 24 hours. Obviously, this makes the constant routine condition highly laborious, expensive and diffi-
In the experimental setup presented in Chapter 4, we strove to get an optimal estimate of the endogenous circadian rhythm in heart rate (HR) during a normal working day by temporarily ruling out the most important masking factors. Repeated measurements were performed over the day, during which the subjects were awake, in supine resting conditions, in complete darkness, and after at least 2 hours of no food intake, no caffeine or nicotine, and at least 1 hour without physical exercise. By this “repeated routine”, we were able to eliminate the effect of sleep, behavioral activity, body position, light and feeding and replicate exactly, in two separate experiments, the day-night difference in resting HR as demonstrated under constant routine conditions.

In a carefully conducted constant routine experiment with lights below 50 lux, Kräuchi and Wirz-Justice found a day-night difference in HR of 6.4 bpm [172], which was the same as in our first experiment (6.3 bpm) and in our second experiment (6.5 bpm) of Chapter 4. The value of our setup is further supported by two more recent constant routine experiments by Burgess and coworkers [50] and by Kerkhof and coworkers [161], who found a peak-trough difference in resting HR of 6.4 bpm and 6.7 bpm, respectively. Thus, in conclusion, by careful exclusion of masking factors, it is possible to get a reliable measure of endogenous circadian rhythm in HR, even during everyday life conditions.

More generally, with repeated routine, the repeated temporary exclusion of masking factors that interfere with the physiological variable of interest, other endogenous circadian rhythms could also be investigated without the use of constant routine conditions.

The results suggest the involvement of the SCN in the regulation of the heart. The autonomic nervous system is the most important factor in the regulation of HR [21]. The question then arises what is the importance of the sympathetic and parasympathetic nervous system via which the SCN generates the circadian control of HR? To investigate this question, we repeatedly recorded non-invasive indices of both branches of the autonomic nervous system over 24 hours (Chapter 5). As an estimate of sympathetic activity, we measured pre-ejection period (PEP), a measure for cardiac contractility derived from impedance cardiography [52]. As an estimate for parasympathetic activity, we measured the root mean square successive differences (RMSSD), a measure for HR variability derived from electrocardiography [52]. The results suggest that the parasympathetic drive to the heart is the main cause for the daily rhythm in resting HR. Since the HR rhythm in our experiments corresponded tightly with that in studies performed under constant routine protocols, our results further suggest that the actual endogenous circadian rhythm in HR in humans is mainly caused by a circadian rhythm in parasympathetic activity. Previously only a single constant routine experiment had investigated the endogenous circadian rhythm in parasympathetic and sympathetic cardiac activity. This study by Burgess and coworkers, with the same peak-trough difference in HR (6.4 bpm) as in our two experiments (6.3 bpm and 6.5 bpm), demonstrates an endogenous circadian rhythm in respiratory sinus arrhythmia, as an index of parasympathetic car-
diac outflow, and no rhythm in the pre-ejection period [50]. The importance of the para-
sympathetic versus the sympathetic nervous system in the circadian rhythm in HR is
further illustrated by the maintenance of the circadian rhythm in HR in quadriplegic
patients who supposedly have no sympathetic outflow to the heart [174], and in chroni-
cally chained monkeys during blockade of their sympathetic nervous system [322]. In
conclusion, the endogenous circadian rhythm in HR, which prepares us for the activity
of the day, is mainly due to the circadian rhythm in parasympathetic cardiac outflow.

To be able to investigate in more depth the structure responsible for the generation of
a circadian rhythm in HR, we performed lesion studies in rats (Chapter 2). First, we had
to establish if there is a circadian rhythm in HR independent of behavioral activity, the
most important masking factor of HR, and not a result of the rhythm in activity. Obvi-
ously, rats cannot be studied during conscious and voluntary rest continuously over 24
hours. Before, scientists used indirect mathematical techniques on ambulatory data in
an attempt to subtract the effects of different levels of locomotor activity on HR. In con-
trast, we used a direct method. We recorded HR from free moving and unstressed rats by
means of telemetry. By selecting only those periods during which the rat had been vol-
untarily inactive long enough to prevent the effect of any previous activity on HR, we
could determine the actual endogenous daily rhythm in resting HR and perform a simi-
lar approach to the one used in the human studies. That resting HR was free running
during constant light conditions demonstrated that the rhythm was truly a circadian
rhythm. Now we could investigate the role of the SCN in this rhythm by lesioning the
SCN. Firstly, without SCN, the circadian rhythm in HR was completely absent, demon-
strating that the SCN is sine qua non for the circadian rhythm in HR. After lesioning the
SCN, resting HR was between the day and night value of intact rats. This suggests that
the influence of the SCN on resting HR was inhibitory during the day and excitatory
during the night. The mechanism used by the SCN is discussed in “Pathways and trans-
mitters used by SCN in regulation of the heart”, page 141.

Light affects heart rate; importance of the SCN
The effect of light on physiology and behavior is often referred to as ‘masking’, dismiss-
ing it as an unimportant and disturbing factor in the study of the SCN-generated endog-
enous circadian rhythm. However, the circadian rhythm and the response to light may
be part of one and the same system.
Electrical activity of SCN neurons signals the day function. With light as signal of the
day, increasing general SCN activity towards daytime levels in both diurnal (day-active)
and nocturnal (night-active) species (see Introduction of the present thesis), our central
hypothesis was that:

Light induces day-time physiology and behavior via the SCN,
both in nocturnal and diurnal species.
If this would be true, we would expect an opposite effect of light on the physiological state between diurnal and nocturnal species. We demonstrated that light exposure caused an \textit{increase} in HR in human and a \textit{decrease} in rats, both characteristic of daytime levels, in accordance with the central hypothesis (Chapter 2 and 4). Because light only affects the SCN when general SCN-neuronal activity is low (subjective night), we further expected that light would only influence HR at night. Both in human and rat, light exposure affected HR only in the middle of the night and the early morning (end of the night), but not during the day (Chapter 2 and 4), which was in agreement with our second hypothesis. Lesioning the SCN in rats prevented the suppressive effect of light on HR (Chapter 2), the final proof for dependence on the SCN, and thus supporting the central hypothesis.

To investigate the relative importance of sympathetic and parasympathetic nervous system for the effect of light on HR, we measured indices of both branches of the autonomic nervous system in human. The results suggested that the increase in HR by light exposure in the early morning is caused by an increase in sympathetic cardiac activity. Similar experiments performed in a larger population should be performed to characterize further the importance of the cardiac sympathetic and parasympathetic outflow in the stimulatory effect of light on the heart, and thereby the neuronal mechanisms by which the SCN can influence the heart in humans.

Also for cortisol secretion by the adrenal gland in humans, light caused a time-of-the-day dependent \textit{increase} (Chapter 6). Together with the finding that light caused a phase-dependent \textit{decrease} in corticosterone in rats, which was dependent on a functional SCN [47], this suggests that also in human, the adrenal cortex is influenced by the SCN. The influence of light via the SCN on adrenal cortex function in humans (stimulatory) and rats (inhibitory), too, was in accordance with the central hypothesis. The role of the autonomic nervous system in the effect of the SCN on cortisol regulation should be investigated in future experiments (see“\textit{Illuminating autonomic regulation of metabolism by SCN}”, page 159).

What could be the adaptive value of such an immediate effect of nocturnal light for the organism? The immediate response to light by the SCN may be an acute and flexible secondary system of the SCN, besides its endogenous circadian rhythm. The endogenous circadian rhythm can only be shifted slowly, with a maximum close to 1 hour each day (the cause of, e.g., jet lag). An immediate response to changes in light conditions would be valuable if the SCN were incorrectly synchronized to the external demands, either due to endogenous malfunction of the circadian system or to rapid external changes, such as a change in weather conditions with concomitant changes in light conditions. For example, a rapid response of rodents to light, resulting in their returning under ground, could be life-saving, since many predators are visual hunters.
Pathways and transmitters used by SCN in regulation of the heart

In Chapter 2, we demonstrated a multisynaptic projection from the SCN to the heart. The paraventricular nucleus of the hypothalamus (PVN) is the main autonomic relay station of the hypothalamus, and is the most likely target of the SCN in the regulation of the heart [67,326]. In Chapters 2 and 3, we have seen that lesioning of the SCN results in a HR intermediate between the day and night level in intact rats. This suggests that the influence of the SCN on resting HR is inhibitory during the day and excitatory during the night. The ability of the SCN to send out both excitatory and inhibitory signals is supported by the demonstrated secretion from the monosynaptic projections from the SCN to the autonomic PVN of both the inhibitory neurotransmitter GABA and the excitatory neurotransmitter glutamate [67, 129]. The functional involvement of these excitatory and inhibitory SCN-projections has been demonstrated in the regulation of the adrenal cortex and even the pineal gland. After lesioning of the SCN, the levels of corticosterone and melatonin are between the day and night levels, suggesting that the SCN has both inhibitory and excitatory outputs for the regulation of different organs. Indeed, for corticosterone it has been demonstrated that the inhibiting factor is VP [152, 158], and that there is an unknown exciting factor [159]. Also for the autonomic regulation of melatonin secretion by the pineal, the PVN is an essential relay station [324]. It has recently been demonstrated that GABA secreted during the day and during light exposure causes the suppression of melatonin during daytime and during light at night [153], and that glutamate stimulates melatonin release during the night (Perreau, et al., personal communication). In rats, resting HR, similar to melatonin, is also low during the day, high during the night, and suppressed by light at night (Chapter 2). Therefore similar mechanisms could be used in the control of both melatonin and HR. In humans, however, the circadian rhythm of melatonin is the opposite of that of HR. The circadian melatonin rhythm is the same in humans and rats, which is why the projections and transmitters used in the control of melatonin regulation could be the same for both species. However, the circadian rhythm of HR is opposite to that in rats [291, 292], and can thus not be regulated by the same mechanism. In rats, GABA may thus also be used by the SCN to inhibit the autonomic PVN output to suppress resting HR during the day and during light at night, while glutamate may be used to excite the autonomic PVN output to increase resting HR during the night, similar to how the release of melatonin is regulated. Indeed, GABAergic innervation of the PVN seems to play an important role in the regulation of HR via (at least) the sympathetic nervous system. This is demonstrated by the strong rise in HR after disinhibition of the PVN by blocking GABA-inhibition [204], and by the strong attenuation of this by blockade of β1-adrenergic receptors [202]. GABA suppression of the PVN also plays an important role in blood pressure (BP) regulation via sympathetic outflow to the vasculature, since in the same experiments, the blockade of GABA in the PVN increases BP [204], which is completely pre-
vented by blockade of α1-adrenergic receptors [202]. Furthermore, glutamate injection in the PVN increases HR and BP in conscious rats [203]. Future studies should reveal if the glutamatergic projections from the SCN to the PVN are indeed involved in the high nocturnal HR and if the GABAergic projections cause the low HR during the daytime and the low HR induced by light. To get an insight in the projections and neurotransmitters involved in the autonomic regulation of HR by the SCN in humans, experiments are required in diurnal mammals that also have a circadian rhythm in resting HR with the peak during the day, like humans do.

The anatomical basis for (at least) the role of the sympathetic nervous system to relay signals from the SCN to the heart in rats is further demonstrated by PRV-staining in the SCN after injections of PRV in the stellate ganglion, the most important sympathetic ganglion for the heart [336]. In humans, we provided physiological evidence, in Chapter 5, that the parasympathetic nervous system is the most important factor for the generation of the circadian rhythm in resting HR in human, and that the sympathetic nervous system is involved in the phase-dependent increase in resting HR by light. Together, this suggests that the SCN is able to influence the human heart via both the sympathetic and parasympathetic nervous system. Selective lesioning of the sympathetic projections to the heart followed by retrograde PRV tracing of the heart could reveal whether also a parasympathetic route from the SCN can influence the rat heart.

Similar autonomic connections from the SCN may constitute the neural circuit for the circadian regulation of other functions like body temperature, BP, metabolism and hormonal levels. Indeed, the autonomic projections from the SCN to brown adipose tissue [20], vasculature [310], heart [291], liver [176], pancreas [42], white adipose tissue [19, 173], thyroid gland [155] and adrenal cortex [47] support this notion.

**Thermoregulation by the SCN, involvement of vascular system**

Although the SCN is generally acknowledged to regulate circadian rhythms in physiology and behavior, the question whether the SCN is also required for the circadian rhythm in body temperature (BT) is surrounded by controversy and seemingly conflicting results [3, 83, 244, 267, 276, 286, 353].

In Chapter 3, we investigated the role of the SCN in temperature regulation in experimental animals. To study BT independent of LA (resting BT), the same method was used as for resting HR, which is described in Chapters 2 and 3. With this, we demonstrated a circadian temperature rhythm under constant dark conditions independent of the rhythm in activity. Lesioning the SCN abolished the circadian rhythm in core temperature, demonstrating that the SCN is crucial for the endogenous temperature rhythm. The BT level in SCNx rats was in between that of the day and night levels of intact rats, suggesting an inhibiting and a stimulating influence of the SCN on BT, just as for HR, as discussed earlier.
Furthermore, since BT showed a weak response to light in SCNx rats, we investigated whether light may provide a possible explanation for the controversy surrounding the question whether the SCN is required to generate a daily rhythm under a daily light-dark cycle (LD). Firstly, we demonstrated a phase-dependent inhibitory influence of light on BT in intact, but not SCNx animals 2 months after lesioning the SCN. However, under LD conditions, we did see a day-night rhythm in BT 6 months after lesioning, which was absent 2 months after lesioning. This rhythm was completely dependent on light, since under constant darkness (DD), no rhythm was present. Also a weak day-night difference in HR and LA was present, 6 months after lesioning the SCN. However, while the daily rhythm of BT was 70% of that in intact animals, the rhythm of HR and LA were only 25% and 15%, respectively. This demonstrates that, in SCNx animals, light can gradually take over the day signal of the SCN, mainly in the regulation of BT, while other SCN-driven rhythms can be far less influenced by light without the presence of an SCN. We propose that the MPO [63, 184, 285] and/or Edinger-Westphal [96, 101, 248, 310] are likely candidates that might gain in importance after the main relay centre of light effects on body function is destroyed to pass on the effect of light preferentially in the regulation of BT. This gradual increased effect of light on BT may be due to the strengthening of pre-existing projections from the retina to these areas [184, 306] and/or the sprouting of retinal projections to form new connections [148]. In future studies, apart from demonstrating retinal projections by tracing experiments, staining for the putative circadian photoreceptor melanopsin [113] might prove a simple means to follow changes in retinal projections of the RHT after lesioning of the SCN, and may indicate whether sprouting to new target areas occurs.

How does the SCN affect body temperature? Temperature changes are caused by a shift in the balance between heat production and heat loss. In humans, it has been indicated that a circadian rhythm, both in heat production and in heat loss, plays an important role in the production of a circadian rhythm in BT [172]. Changes in heat production during resting conditions in rats, as shown in the present study, are mainly due to changes in the sympathetic innervation of heat production by brown adipose tissue (BAT). In addition, in the rat, the vasculature in the tail is of great importance, as it is responsible for 20% of heat dissipation [310]. Both BAT and tail vasculature receive sympathetic projection from the SCN [20, 310]. Adult humans have no BAT and no tail. However, it is the uncoupling protein (UCP) in BAT that generates the heat. While UCP1 is the main UCP in the BAT, UCP1 and UCP2 are present in white adipose tissue, and UCP3 is present in muscle [104, 271]. UCP in musculature and white adipose tissue could thus be an important means of heat production during rest in humans [271]. Although they have no tail, humans experience heat loss regulated by changes in blood flow through the skin of the distal parts of the body: the skin of the hands, feet and head. Actually, the tips of the fingers contain so-called arteriovenous anastomoses (high den-
thetic vascular shunts) that can regulate blood flow and thus heat dissipation over a great range [25]. SCN outflow via the autonomic nervous system to regulate UCP and distal vasculature in humans may parallel the mechanisms used in rats in the regulation of body temperature.

Consequently, SCN regulation of vasculature could thus also be used in the regulation of BP. The finding that healthy men in constant routine conditions [161] do not show a daily rhythm in BP seems to contradict this hypothesis. However, opposite local changes in vasoconstriction could counteract each other, resulting in a dampened change in total peripheral resistance. That local regulation may differ is demonstrated by the varying daily rhythm of the vascular resistance in the leg as compared to the forearm during forced bed rest [61]. Indeed, the distal vasodilatation in the early night [172] counteracts vasoconstriction of the limb muscles during the night [61]. Furthermore, changes in total peripheral resistance, cardiac output and blood volume over the circadian cycle could compensate for each other, resulting in no net change in BP. Such BP-counteracting changes in total peripheral resistance have already been shown, with total peripheral resistance being high at night when BP is low, during ambulatory measurements [346], but also during 42-days of continuous bed rest [350], and in chronically chained monkeys [89].

Thus the endogenous circadian rhythm in HR and in distal vasodilatation [172, 291, 292] might be based on the anatomical projections from the SCN to the heart, kidney and vasculature, which support a role of the SCN in the circadian distribution of nutrients and heat [291, 309, 310]. Simultaneous 24-h recording of different parts of the vascular system together with changes in cardiac output and blood volume during constant routine (or repeated routine) conditions would have to be performed to reveal further the differential circadian regulation of the different components in the regulation of BP and blood distribution.

B. ENDOCRINE CONTROL BY SCN OF CARDIOVASCULAR SYSTEM

Apart from the autonomic nervous system, endocrine (hormonal) systems, too, play an important role in the regulation of the cardiovascular system. The renin-angiotensin-aldosterone system (RAAS) is one of the central endocrine systems in the regulation of BP, via the effects of angiotensin II on the kidney (via aldosterone) and on the cardiovascular system (via the autonomic nervous system). However, the RAAS is probably not regulated by the SCN, because key players in the RAAS, plasma renin activity [39, 62] and aldosterone [62], show no endogenous circadian rhythm. Therefore, the focus in the present thesis was on two hormones with a clear endogenous circadian rhythm. The first hormone, cortisol, has a known effect on BP. We used light as a tool to investigate
the role of the SCN in the regulation of cortisol, and thereby of BP. For the second hormone, melatonin, the role of the SCN is generally acknowledged and there is a clear effect of light (Fig. 3, Introduction). We investigated the role of melatonin on BP, and thereby a second endocrine route via which the SCN may affect the cardiovascular system.

Effect of single melatonin intake on cardiovascular system

As discussed in the Introduction, melatonin has two effects: a long-term effect, influencing the circadian rhythmicity of the SCN, and an immediate effect, inhibiting SCN-neuronal excitability. First, we will discuss the immediate effects of melatonin on the cardiovascular system, and second, the long-term, synchronizing effects of melatonin on the cardiovascular system and the circadian rhythm.

A single pharmacological dose of melatonin during the daytime can decrease BP in humans [10, 49, 53, 54, 122, 163, 239]. Although melatonin receptors have been demonstrated in human arteries [88, 288], the BP reduction is at least also due to central effects. This is indicated by the absence of a baroreflex-induced increase in HR [10, 49, 54, 163] or even by the presence of a decrease in HR during reduced BP by melatonin [122, 239]. Furthermore, daytime melatonin lowers norepinephrine plasma levels, indicating that melatonin inhibits overall sympathetic activity [10, 53, 239]. The decrease in BP by melatonin seems mainly due to vasodilatation [54]. However, no effect of melatonin on selective sympathetic outflow to the vasculature of the leg muscle was found [163]. The differential effect of melatonin on autonomic output to different vascular beds may underlie this finding. Additionally, melatonin may influence BP by lowering cardiac output. However, the effect of melatonin on non-invasive measures for autonomic regulation of the heart is contradictory, with indications for suppression of sympathetic and stimulation of parasympathetic outflow to the heart [239], while others find no effect [49].

A pharmacological dose of melatonin does not seem to have an additional effect on body temperature or on BP reduction as compared to a physiological dose. This was indicated by comparing the temperature decline in the evening between nights with low (bright light), physiological (dim light) and pharmacological (bright light + 1-5 mg oral melatonin) melatonin concentrations. However, it should be realized that during bright light in the evening, not only the SCN output to the pineal but also to other organs might be affected, although light does not affect HR and cortisol levels in the evening (Chapters 4-6). Suppressing melatonin levels by light attenuated the temperature decline late in the evening [56], suggesting that endogenous melatonin levels in the evening are involved in the nocturnal temperature decline. This is further supported by the fact that supplementation with exogenous melatonin during light exposure could perfectly restore the normal temperature decline at night [56]. Because the melatonin levels reached with supplementation were pharmacological, this further suggests that these pharmacological...
logical melatonin levels have no additional effect on temperature over physiological melatonin levels. The same was shown for systolic BP, with a similar decrease induced by the physiological and pharmacological melatonin levels, as compared to the light-suppressed melatonin night [49]. This corroborates our results (Chapter 8) in which a single melatonin intake 1 hour before sleeping, at a time when endogenous melatonin levels are normally high, does not influence BP. To investigate the effect of physiological melatonin levels on BT and BP without the use of light, future experiments are required comparing conditions, e.g., after the use of a melatonin antagonist with conditions with physiological melatonin concentrations.

These single dose studies suggest that the endogenous melatonin levels of healthy subjects already exert the maximum effect on some biological functions, which could question the benefit of additional melatonin intake in healthy young subjects. However, nighttime melatonin intake could aid subjects with low endogenous melatonin levels or decreased sensitivity.

Effect of long-term melatonin treatment on cardiovascular system

In literature, the effects of long-term melatonin application on BP in humans are ambiguous. Repeated daytime nasal melatonin treatment of patients with essential hypertension was reported to cause a strong reduction of BP [26]. However, the time and method of BP measurement was unclear and the time of melatonin application during the middle of the day would have been disruptive for the endogenous melatonin rhythm [12]. More recently, Lusardi et al. reported an unexplained increase in 24-hour mean BP by 4 weeks 5 mg oral melatonin just before bedtime in hypertensive patients treated with nifedipine [197].

Chapter 8 presents the first double-blind crossover study to investigate the effect of repeated melatonin intake on 24-hour BP rhythm in untreated hypertensive patients. We demonstrated that daily melatonin intake (2.5 mg) over 3 weeks 1 hour before sleep, but not a single intake, lowered nighttime systolic and diastolic BP of patients with essential hypertension with 6 mm Hg and 4 mm Hg, respectively. The reduction of about 6 mm Hg during day and night, which reached significance during the night, due to the higher stability of BP at that time, is of great value, since a 2-3 mm Hg systolic BP reduction has already been shown to be of great clinical importance [66, 315]. Furthermore, since we spend 1/3 of our lives sleeping and since nighttime BP seems to have more predictive value as a cardiovascular risk factor than daytime BP, a reduction of nighttime BP provides great benefit [314]. The cheap and endogenous substance melatonin is effective in lowering BP and promises to be a gentle addition or alternative for other BP-lowering agents.

The fact that a single melatonin intake did not influence BP, suggests that melatonin does not act as a direct vasodilator drug. The reduction of BP was not accompanied by a
change in HR, indicating a resetting of the baroreflex sensitivity. Likewise, repeated, but not single, nighttime melatonin intake improved sleep quality. That single melatonin intake did not affect sleep shows that melatonin did not act as a sleeping pill. With a sleeping pill the reverse would have been expected, with a stronger effect the first time and a decrease in effectiveness (desensitization) after repeated use. The absence of desensitization to melatonin treatment is an advantage over regular sleep-inducing drugs [280, 373]. Although repeated melatonin improved sleep, individual BP changes by repeated melatonin did not correlate with the changes in sleep quality (Fig. 1). This indicates that the effect of melatonin on BP is not just a secondary effect of an improvement of sleep. Together, the role of the SCN in cardiovascular regulation (present thesis), the disturbance of the SCN in hypertensive patients [110], the required time for melatonin to lower BP, to amplify its day-night rhythm and to improve sleep quality (Chapter 8), suggest an important role for the SCN in the BP-lowering effect of melatonin. Alternatively or additionally, melatonin as circadian night signal could also synchronize peripheral oscillators lowering BP (see “Synchronization of peripheral clocks”, page 149).

Cortisol
Corticosteroids, cortisol in humans and corticosterone in rats, have a clear endogenous circadian rhythm. In animal experiments, it could be demonstrated that this rhythm depends on the presence of the SCN [231]. Light, as input to the SCN, inhibits corticos-
terone levels in rats, an effect that is dependent on the phase of the day-night cycle and on the presence of a functional SCN [47]. The projection from the SCN, via the sympathetic nervous system, to the adrenal cortex [47] could be the anatomical route via which the SCN engenders this circadian rhythm and light response of corticosterone. The similar phase-dependent response of cortisol to moderate (800 lux) light in humans, presented in Chapter 6 and later replicated by Leproult and coworkers [182], suggests the involvement of the SCN in the regulation of the adrenal cortex also in humans. The suppression of corticosteroid by light in rat and its stimulation in human is in accordance with the central hypothesis "Light induces day-time physiology and behavior via the SCN, both in nocturnal and diurnal species". It also indicates that the effect of light was not an a-specific stress response. Together with the endogenous circadian rhythm of cortisol in humans [243], a similar phase-dependent effect of light exposure on cortisol levels in humans support the notion that, also in humans, the adrenal cortex is affected by the SCN.

The morning cortisol peak prepares the body for the active period. Cortisol increases glucose output from the liver and increases BP. Cortisol causes the rise in BP by enhancing epinephrine’s vasoconstrictive effect, increasing stroke volume, and enhancing salt and water retention by the kidney, without a change in HR [205, 365]. Interestingly, the morning cortisol peak proved to be important in the circadian rhythm in BP in patients with hypopituitarism and thus endogenous cortisol deficiency, treated with cortisol (also called hydrocortisone) at different times of the day. In these patients, cortisol treatment divided over morning and evening led to an increase in the 24-hour BP mean (by about 10 mm Hg), without day-night rhythm in BP. Only with cortisol intake in the morning (simulating the endogenous circadian rhythm in cortisol concentrations in healthy subjects) the normal day-night difference in systolic and diastolic BP reappeared (about 10 mm Hg) [205], supporting the involvement of cortisol in the daily rhythm in BP. The inability of dexamethasone (glucocorticoid) treatment to affect the diurnal rhythm in BP or HR in healthy controls [255], may be due to the parallel reduction in endogenous cortisol secretion by the negative feedback of dexamethasone onto the hypothalamopituitary-adrenal-axis (HPA-axis). High cortisol levels in the morning not only increase blood pressure, but may also increase coronary blood flow [118], allowing greater cardiac output, needed for the preparation of the body for the activity period.

A question not often addressed, is how corticosteroids can be useful in a rapid stress response if the steroid hormone has only slow-acting, genomic effects. However, more recently, corticosteroids have been demonstrated to also cause rapid, non-transcriptional effects [102]. Binding of corticosteroids to glucocorticoid receptors (GR) causes activation of phosphatidylinositol 3-kinase, which triggers a cascade leading to rapid effects in the cell. For example, high doses of corticosteroids cause non-transcriptional activation of nitric oxide synthase in the heart vasculature, leading to local NO-induced vasorelaxation and protection against myocardial damage after infarction [118]. Simi-
larly, corticosteroids can cause, e.g., rapid release of glucose from the liver [102]. Taken together, the circadian corticosteroid rhythm can rapidly prepare the organism for the circadian activity period, by increasing, i.e., cardiovascular functioning.

The regulation of the human HPA-axis is often investigated in a laboratory or hospital setting, but the impact of such a study environment on experimental outcome has received little attention. The results of the study presented in Chapter 7 plead for caution when performing experiments in a hospital or laboratory setting, especially if small changes in the HPA-axis are subject of study when less stressful alternatives are available. A less stressful and reliable alternative is the repeated routine conditions used in Chapters 4-6. The repeated routine, with repeated temporary exclusion of masking factors to the system of interest, allow experiments to be performed at home during everyday life. The results of Chapter 7 demonstrate that a regular hospital setting leads to a 2 to 5 fold increase of basal cortisol levels, depending on the phase of the circadian cycle. If a hospital or laboratory setting is required for the experiment, one should at least realize the impact of this setting on basal cortisol levels, which could thereby obscure the effect of a modest experimental stimulus on the system under investigation.

**Synchronization of peripheral clocks**

Most components of the transcriptional-translational feedback loop that make up the molecular clockwork of the SCN are also present in tissues outside the SCN. These so-called peripheral clocks were shown not only in lower organisms such as Drosophila [105], but also in vertebrates such as the zebrafish [364], and even in mammals [17, 18, 379]. However, these clock components are unable to maintain an ongoing circadian rhythm, the way they do in the SCN. Without the synchronizing input from the SCN (master oscillator), peripheral oscillators (slave oscillators) show a fading rhythm that only lasts a few cycles. In mammals, outside the SCN, only the retina holds a true circadian pacemaker which can generate a continuing circadian rhythm in isolation [332, 349].

Peripheral clocks are also present in the cardiovascular system. The vasculature expresses molecular clock components [209, 240], and the heart in isolation demonstrates a diurnal variation in contractile function [375]. How does the SCN synchronize these peripheral clocks? Hormonal signals were shown to synchronize the cardiovascular system [209, 240]. Melatonin and cortisol, with a clear circadian rhythm driven by the SCN, are likely candidates. Indeed, the corticosteroid rhythm generated by the SCN couples the rhythm of peripheral oscillators in liver and kidney to that of the SCN [178]. The corticosteroid peak at the beginning of the activity period also prevents the activity rhythm from being shifted rapidly, with a shift in the light-dark cycle (Sage et al., in press). Furthermore, food-related signals are important for setting the time for peripheral oscillators. In mice, daytime restricted feeding (during their normal sleeping pe-
iod) uncouples the circadian rhythm of peripheral oscillators from that of the SCN. Daytime feeding shifts the circadian clock expression in the liver, kidney, heart, and pancreas, while the SCN remains linked to the light-dark cycle [73]. This indicates that feeding time is a dominant Zeitgeber for peripheral circadian oscillators in mice. For organ systems directly related to food intake and processing, such as the liver and pancreas, this seems a beneficial adaptation. However, that the circadian rhythm in clock genes in the heart shifts with food restriction is surprising, because the rhythm in locomotor activity is not shifted by daytime restricted feeding, and remains linked to the rhythm of the SCN. Daytime feeding also blunts the daily body temperature rhythm in mice, with the temperature decreasing during the fasting nights [73]. It should be realized that in larger species the impact of restricted feeding during the sleeping period on body temperature will be less disruptive. Mice have a very small body volume and will thus lose heat and metabolic reserves rapidly, which requires the energy saving measures to kick in earlier. Indeed, leptin levels drop more rapidly in smaller animals, providing faster signaling of a negative energy balance, and providing the required more drastic measures to save energy [131].

What could signal the restricted food availability to the peripheral oscillators? Since they show a peak during the time of restricted feeding, corticosteroids have been proposed. However since the main corticosterone peak is controlled by the SCN and thus does not shift, corticosteroids inhibit, rather than promote, the phase adjustment of the peripheral clocks to daytime feeding [178]. Rather, by the regulation of the circadian corticosteroid rhythm the SCN may prevent the peripheral oscillators from rapidly uncoupling from its circadian influence. It is proposed that nutrients may directly and selectively reset peripheral oscillators. By infusion of glucose, free fatty acids, and proteins in fasting animals the involvement of different nutrients as Zeitgeber of the peripheral oscillators could be tested in the near future.

C. PROSPECTS

Hypertension is one of the main risk factors for the development of cardiovascular disease, the leading cause of death in western countries [66, 314, 315, 345]. The results of the present thesis demonstrate the role of the biological clock in the regulation of the cardiovascular system via the autonomic nervous system and hormones. Together with the recently demonstrated disturbances of the SCN and PVN in patients with hypertension [110, 111], the results of the present thesis provide an insight in possible mechanisms via which disturbances in the SCN could lead to an increased PVN activity and thereby to increased levels of cortisol and sympathetic activity, eventually leading to cardiovascular pathology.
The first new possible treatment, based on amplifying circadian rhythmicity, is presented in the present thesis with the BP reduction in patients with essential hypertension after 3 weeks nighttime melatonin intake. Secondly, light treatment during the day can amplify circadian rhythmicity and could be used to beneficially influence the SCN output in the regulation of the cardiovascular system and other organ systems. Figure 2 illustrates how light and melatonin may affect the cardiovascular system, and in that way BP and BT, via the SCN. In this part (“Prospects”), I will discuss the implications of the results of the present thesis for possible mechanisms involved in the development of cardiovascular disease and the treatment of hypertension (3.1), and for future research on the autonomic regulation of different organ systems using light as a tool (3.2).

Role of SCN in development of cardiovascular disease.

The autonomic nervous system is proposed as one of the multiple factors involved in the development of hypertension [246]. Recent data indicate a functional impairment of the SCN in patients with essential hypertension [110]. In these same patients it was also demonstrated that PVN activity was enhanced, which could lead to increased sympathetic and HPA-axis activity [111]. Data of the present thesis tie together the SCN and the regulation of the cardiovascular system via the autonomic nervous system and hormonal mechanisms, and thereby provide mechanisms via which the SCN could be involved in the development of hypertension.

The risk of stroke, myocardial infarction, and sudden cardiac death shows an incompletely understood peak in the early morning [201, 236]. Although the start of behavioral activity plays an important role [236], there is evidence for an endogenous circadian component [24, 170]. Increasing HR leads to increased cardiac demand, which is a risk factor for ischemia and plaque rupture [236]. Therefore, the endogenous circadian rhythm in HR with the increase in the early morning may form a cardiovascular risk at that moment. The SCN normally synchronizes all different systems of the body to be optimally adapted to each other and to changing external demands. Disturbances of the SCN may thus compromise synchronization of different organ systems, which could lead to maladaptation of, e.g., the cardiovascular system.

There are indeed several indications for a disturbance of the SCN in patients with cardiovascular disease. Patients with hypertension show blunted day-night rhythms in sympathetic and parasympathetic heart tone [117, 237]. The increase in heart rate after a night sleep is far stronger than that after siesta in normotensives, but similar in hypertensives [51], which suggests a blunted endogenous daily rhythm in HR in patients with hypertension. Patients with coronary heart disease - a major complication of chronic hypertension [367] - show a blunted day-night rhythm in vasodilatation [301] and suppressed nighttime melatonin levels [40]. Dysfunctioning of the SCN and reduced
Fig. 2  Hypothesis of the mechanisms via which light and melatonin could influence the autonomic output in the regulation of BP and BT via the SCN in human. In (A) the effect of light via the SCN and autonomic nervous system on the cardiovascular system is depicted, while in (B) the effect of melatonin is shown. Since light suppresses melatonin, which requires a reduction in sympathetic outflow, and since light increases both HR and cortisol, which requires a decreased sympathetic (relative to parasympathetic) outflow, I propose that the SCN in humans sends different projections to different parts of the PVN to inhibit melatonin, while stimulating most other SCN-driven rhythms (e.g. HR and cortisol) after exposure to light. I further hypothesize that melatonin, which can lower SCN neuronal activity, has the opposite effect on SCN output. Also
see “CLOCK OUTPUT” on page 22 of the Introduction for a further explanation of differential SCN-output. BP, blood pressure; BT, body temperature; Cort, cortisol; NA&DMNX, nucleus ambiguous & dorsomedial nucleus of the vagus (together parasympathetic); HR, heart rate; Mel, melatonin; PVN, paraventricular nucleus of the hypothalamus; SCG, superior cervical ganglion; StelG, stellate ganglion; Vasoconst&UCP, vasoconstriction and uncoupling protein. Continuous lines are active pathways and dotted lines are suppressed pathways. ‘Plus’ signs indicate stimulation and ‘minus’ signs indicate inhibition. Small arrow up indicates an increase and small arrow down indicates a decrease. Increases and decreases of encircled endpoints have been demonstrated, while other indicated increases and decreases are hypothesized.
nighttime melatonin secretion could be part of a negative spiral. Reduced nighttime melatonin secretion would provide reduced feedback to the SCN, leading to an attenuated circadian rhythm of the SCN, resulting in reduced nighttime melatonin secretion, etc. In combination with the results of the present thesis, the aforementioned results suggest that reduced nocturnal melatonin levels might actually contribute to the increased BP. Also patients with other cardiovascular conditions, such as cardiac syndrome X, characterized by angina pectoris and normal coronary angiography, show decreased nocturnal melatonin secretion [8]. The most direct evidence for a disturbance of the circadian pacemaker comes from the anatomical demonstration that the neurotransmitter content in the SCN of patients with essential hypertension is reduced by half [110].

**Cause and consequence**

However, which is the chicken and which the egg for the disturbed SCN and hypertension? Does increased BP cause a change in the SCN via baroreceptor feedback [259], or might a disturbance of the SCN be a cause of the development of hypertension? Animal experiments suggest that the SCN of genetically determined hypertensive rats is involved in the development of hypertension.

The spontaneously hypertensive rat (SHR) is a rat strain bred from Wistar Kyoto rats (WKY) for having high BP. This widely used rat model of human essential hypertension shows several disturbances of the circadian system. SHR have a shorter endogenous circadian free running period (tau) than WKY [254]. SHR also have a different phase-shifting response to light, with light in the beginning of the night causing smaller phase delays and light at the end of the night causing stronger phase advances as compared to their normotensive predecessors [254]. Also the anatomy of the SCN in SHR is different from that in WKY. VIP mRNA concentrations are increased in the SCN of SHR compared to that of WKY rats [16, 254]. But do changes in the SCN lead to the development of hypertension, or vice versa? Hints come from transplantation studies. Transplantation of the rostral part of the hypothalamus supposedly containing the SCN from a SHR in the normotensive host results in an increase of BP [86, 87]. Furthermore, the levels of VIP mRNA in the SCN become higher in WKY hosts with SHR graft than in those with WKY graft, and the level of VIP mRNA is positively correlated with BP. Together this suggests a causal role for the SCN in the development of hypertension in the SHR.

The next question is then, how could a change in SCN lead to hypertension? In SHR, sympathetic hyperactivity may be a cause for the development of hypertension. Already at the age of 4 days, SHR pups have increased sympathetic outflow to the heart in comparison to normotensive WKY pups [335]. Then, after 2 weeks, the intrinsic HR is increased without a difference in autonomic control of the heart [335]. This tachycardia precedes the development of hypertension. Indeed, the HR in young SHR (3 weeks) was predictive for the BP at 6 weeks [79], and indicates that pre-hypertensive sympathetic
hyperactivity may be an important first step during the development of hypertension in SHR.

Similarly, human studies in children with a genetic predisposition to later develop hypertension (familial hypertension), suggest that changes in autonomic reactivity precede the development of hypertension. In normotensive humans, a family history of hypertension is associated with increased cardiac (HR) stress reactivity and increased catecholamine (epinephrine and norepinephrine) stress response [99]. Cortisol levels are increased both at rest and during stress in subjects with parental hypertension [99]. That this does not depend on the level of BP, but on familial disposition, is shown in a study by Mehta and coworkers [210]. There was no difference between the HR increase by medical examination (mild stress) in young children with or without borderline hypertension. However, there was a stronger tachycardia when undergoing physical examination in normotensive children with, as compared to without, a positive family history of hypertension [210]. The development of hypertension also seems to be preceded by tachycardia, indicating the importance of HR in hypertension and cardiovascular disease [210].

Interestingly, complete, but also partial, lesioning of the SCN increases the basal corticosterone levels and the corticosterone stress response [46], which suggests that reduced functioning of the SCN, as occurs in patients with essential hypertension, may be a causal factor in the increased stress response in familial hypertension. Further support for a disturbance of the HPA-axis and the autonomic nervous system is the negative correlation between the neurotransmitter content in the SCN and the CRH content in the PVN of patients with essential hypertension [111]. This suggests that reduced functionality of the SCN results in hyperactivity in the PVN, leading to increased sympathetic activity and increased HPA-axis activity, and eventually to hypertension.

With the important role of the SCN in autonomic and endocrine regulation of the cardiovascular system, disturbed SCN function could thus result in increased stress response and increased HR. Chronically increased HR and sympathetic outflow could lead to hypertrophy of the heart and the vasculature and to end organ damage, which together, could maintain hypertension [150]. At present studies are conducted to determine, in SHR, the sequence of appearance of a change in SCN and the appearance of hypertension. This could further help resolve the question of cause and consequence.

Age
In humans, aging is the best known example of decreased circadian functioning. Circadian rhythm amplitude of the melatonin rhythm [189], the cortisol rhythm [337] and the sleep-wake cycle [337], decrease with age, and especially with Alzheimer's disease (AD). In parallel, also the circadian rhythm in VP content of the SCN decreases with age [132] and more strikingly in AD [190]. Also nighttime melatonin levels undergo a con-
tinuing decline after peaking at the age of about 3 years [355]. BP is known to increase with age. Although there are many changes with age, could the change in SCN function and the gradual decline in endogenous melatonin production participate in the increase in BP, and the increased risk in cardiovascular disease? Future research, investigating the correlation between BP and disturbed circadian rhythm in melatonin, cortisol and sleep, in different age groups may provide a first step to investigate this question. Because such a study can only reveal correlation and not cause, a second step, the investigation of changes in BP, together with improvement of the circadian rhythmicity by nighttime melatonin and/or daytime light treatment, would provide insight in both the causal role of a desynchronized or blunted circadian rhythmicity in BP regulation, and could provide further indications for the use of stimuli of the SCN (e.g. melatonin and light) as treatment method for cardiovascular disease.

**Metabolic Syndrome; Clues from fasting**
Hypertension often coincides with central obesity, hyperglycaemia, insulin resistance, high levels of triglyceride and/or low levels of HDL cholesterol and microalbuminuria. The co-occurrence of these symptoms is known as Metabolic Syndrome or Syndrome X. One of the most intriguing mysteries connected with Metabolic Syndrome is the presumed general increase in sympathetic activity. Increased sympathetic activity would fit with the development of hypertension. However, it would be in conflict with central fat accumulation, since sympathetic activity causes lipolysis (breakdown of fat). It is often thought that the sympathetic (and parasympathetic) nervous system is a general system with the same output signal to all organs, and that measuring sympathetic activity, e.g. in the muscle, is indicative of sympathetic outflow to other organs. However, as discussed in the introduction, the autonomic nervous system can send out different signals to different organs ([173], Introduction 3.3). Could local differentiation of autonomic output explain the seemingly conflicting symptoms of Metabolic Syndrome? Metabolic Syndrome is due to excessive energy intake and seems to be a state of overfeeding. Could the knowledge of the processes during underfeeding teach us more about the differential autonomic regulation in Metabolic Syndrome?

During fasting, overall sympathetic activity is decreased, which leads to a lowering of HR, BP, and body temperature, which are required to save energy. However, at the same time, fat reserves have to be used as energy supply. Therefore, increased sympathetic outflow to adipose tissue would be required for lipolysis, the breakdown of fat, to be used as energy supply. Indeed, it has been demonstrated that a selective regional increase in norepinephrine spillover occurs in white adipose tissue [249], illustrating a differential autonomic output to different organ systems. A selective autonomic output could also explain the paradoxical and still unexplained finding in metabolic syndrome (syndrome X) of seemingly overall increased sympathetic activity and increased fat deposition, while
decreased sympathetic output to the fat tissue would be required for the increase in fat accumulation. Such selective down regulation of sympathetic outflow to visceral fat in metabolic syndrome would resemble an inverse fasting state and could explain the autonomic paradox in metabolic syndrome. Future focus on the regional differential output of the autonomic nervous system and its role in metabolic syndrome is thus required.

Future studies
Additional studies should be directed at investigating the long-term application of melatonin in the treatment of hypertension. No adverse effects were found with 3 weeks of 2.5 mg melatonin intake 1 hour before bedtime (Chapter 8). It is expected that treatment with 2.5 mg melatonin during 6 months or shorter may be implemented without serious side effects or risks [11]. In unmedicated men, the only "side-effect" of melatonin at bedtime is enhanced sleepiness at night. Also in untreated patients with essential hypertension 3 weeks melatonin intake at night improved sleep quality (Chapter 8). The results in the present thesis could thus open up a whole new field of antihypertensive treatment aimed at improving circadian rhythm disturbances. The long-term goal would be to use melatonin to improve day-night control of autonomic output, and consequently lower BP in the prevention of stroke, myocardial infarction and sudden cardiac death.

In the study presented in Chapter 8, melatonin treatment seemed the most effective in patients with more severe hypertension (Fig. 3). Like usually with monotherapy of regular anti-hypertensive drugs, monotherapy with melatonin was unable to lower the BP sufficiently into the normotensive range. Therefore, future studies should be directed at investigating the possibilities of combination therapy of melatonin with regular antihypertensive drugs. At the moment, little is known about the effects of such combinations. Combinations with the different types of anti-hypertensive drugs (α-blockers, β-blockers, calcium-antagonist, diuretics, and medication affecting the RAAS: angiotensin II-receptor blockers and angiotensin converting enzyme (ACE)-inhibitors) should be further investigated, since interactions between melatonin and the regular antihypertensives could result either in further BP reduction [377], or in attenuated BP reduction [197] by melatonin.

Furthermore, since repeated daytime light exposure is also able to synchronize the SCN [243], the beneficial effect of light treatment alone or in combination with melatonin on circadian rhythmicity and BP in patients with essential hypertension should be investigated. An amplification of circadian rhythmicity together with a BP-reduction would provide further evidence (next to the results of Chapter 8) for a causal role for improvement of the circadian system in the reduction of BP by melatonin treatment.

A next step in the investigation of a causal role for circadian disturbances in hypertension would be to investigate the effect of nighttime melatonin and/or daytime light in normotensive subjects with familial hypertension, who are prone to develop hyperten-
sion. Since tachycardia and increased stress response seem to characterize normotensive subjects with familial hypertension, the effect of the treatment of melatonin at night and light during the day on this HR and stress response would be the first short-term beneficial signs of the treatment to improve circadian functioning. If successful, long-term treatment might prove able to attenuate or even prevent the development of hypertension.

![Graph showing blood pressure changes](image)

**Fig. 3** All patients with a sleeping systolic blood pressure during placebo above 131 mm Hg showed a lower systolic and diastolic sleeping blood pressure during melatonin. Black lines represent sleeping blood pressure during melatonin for each subject; gray background depicts sleeping blood pressure during placebo.
**Illuminating autonomic regulation of metabolism by SCN**

As demonstrated in the present thesis, light can be used as a suitable tool in both experimental animals and humans to study the regulation by the SCN and by the autonomic nervous system of different organs. Nocturnal light exposure has a direct effect on SCN-neuronal activity [212, 213] and output, as is also known for the suppression of melatonin secretion by the pineal gland [153, 164], and presently demonstrated for the regulation of the heart (Chapter 2, 4, 5) and adrenal cortex (Chapter 6 and [47]). Figure 2 illustrates the mechanisms used by the SCN to modulate both BP and BT via the autonomic nervous system and endocrine routes.

Corticosteroids, thyroid hormones and leptin all play an important role in metabolism. All three show an endogenous circadian rhythm in humans [6, 68, 308] and the SCN is crucial for these rhythms [155, 156, 231]. Classically, the secretion of these hormones by adrenal cortex, thyroid gland and white adipose tissue, are thought to be under endocrine control. However, recently, it was demonstrated that the adrenal cortex [47], the thyroid gland [155], and white adipose tissue [173] receive multi-synaptic autonomic projections from the SCN, and are thus also under neuronal control.

In human, the involvement of autonomic regulation by the circadian pacemaker of these endocrine organs is unknown. There is good reason to believe that also in humans these organs are under control of the SCN. Cortisol, thyroid hormone, and leptin, all show an endogenous circadian rhythm, also in humans, and therefore the endocrine organs are likely targets for the SCN. But future experiments are required to investigate if, also in humans, such regulation by the SCN of cortisol, thyroid hormones, and leptin would act mainly via the autonomic nervous system or via endocrine mechanisms.

How then could the role of 1) the autonomic nervous system and 2) the circadian pacemaker be revealed for aforementioned organs? With light causing a rapid effect (in contrast with the circadian rhythm itself), the distinction could possibly be made between a rapid neuronal communication to the organ and a slower endocrine communication. If a change in the levels of cortisol and thyroid hormone would precede changes of their stimulating hormones, ACTH and TSH, this would indicate that the effect is not mediated by the endocrine route, but probably travels via a rapid neuronal route. For corticosteroids, there is ample evidence that the SCN regulates their secretion via neuronal mechanisms in rats [47] and that light also has a phase-dependent effect on cortisol in humans (Chapter 6), which has been shown to depend on the presence of the SCN in rats [47]. The relative role of an endocrine and autonomic neuronal regulation of the adrenal cortex in human could be investigated by determining the order of increase of ACTH and of cortisol after nocturnal light exposure. Less is known about the effect of light on the regulation of thyroid hormone. In rats, light exposure has been demonstrated to affect deiodinase activity, which converts T4 into the active component T3 [116]. By measuring T3, T4, and TSH during nocturnal light exposure in humans and rats, the
contribution of 1) TSH, 2) the autonomic output to the thyroid gland, and 3) deiodinase activities could be determined. Next, a crucial role of the SCN in the light effect could be determined by lesioning the SCN in experimental animals. The effect of light on leptin levels has never been studied. Long-term nocturnal light exposure could reveal an effect of the SCN on WAT by investigating this in humans and demonstrating the requirement of the SCN in rats. These are just some of the examples by which light at night could be used as a tool to investigate further the role of the SCN and autonomic nervous system in the regulation of different organ systems, which will hopefully also provide us with more understanding on the impact of light in our daily lives.

As mentioned before, light can be used, not only as a tool to investigate organ regulation by the SCN and the autonomic nervous system: light during the active period as well as melatonin supplementation during the inactive period have great potential to amplify and restore blunted circadian rhythmicity [36, 269, 280, 376]. But is amplifying a blunted circadian rhythm desirable? A blunted circadian rhythm could lead to a reduced amount of sleep and reduced alertness during working hours. Reduced sleep leads to reduced memory function, lower growth hormone (GH) levels, and increased cortisol levels at night, all characteristics also of high age [337]. Decreased GH levels and increased cortisol levels are both known to contribute to the development of obesity [147]. A blunted circadian output would also cause reduced melatonin production at night, which could again lead to a further reduced circadian rhythmicity and sleep. Reduced nocturnal melatonin levels could also increase nocturnal BP and BT (see “Effect of single melatonin intake on cardiovascular system”, page 145). Long-term increased nocturnal BP, HR and cortisol levels due to reduced circadian rhythmicity might participate in the development of hypertension (see “Role of SCN in development of cardiovascular disease.”, page 151). Amplification and synchronization of the circadian system and thereby of different organ systems will not only improve the sleep-wake cycle, but, consequently, also improve general health. Specifically those people with demonstrated disturbances of circadian rhythmicity, such as shift-workers [299], elderly people [343, 344], patients with essential hypertension [40, 110, 111, 301], and possibly even patients with obesity and diabetes [179, 242, 253, 338, 369], may benefit greatly from daytime light and nighttime melatonin supplementation. Therapies directed at amplifying circadian rhythmicity may face a bright future.