Light and Diurnal Cycle Affect Human Heart Rate: Possible Role for the Circadian Pacemaker

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Abstract Humans and animals demonstrate diurnal rhythms in physiology and behavior, which are generated by the circadian pacemaker, located in the suprachiasmatic nucleus (SCN). The endogenous diurnal rhythm of the SCN is synchronized to the diurnal cycle most effectively by light. However, light also influences the SCN and its output instantaneously, as is demonstrated for the immediate effects of light on SCN neuronal firing frequency and on the output of the SCN to the pineal, inhibiting melatonin secretion. In addition to this, the circadian pacemaker modulates neuronally also other organs such as the adrenal. Therefore, the authors investigated the effect of this light input to the SCN on human heart rate, using light at different phases of the day-night cycle and light of different intensities. Resting heart rate (HR) was measured in volunteers between 20 and 40 years of age during supine, awake, resting conditions, and after 2 hours of fasting. In Experiment 1, HR was measured at different times over the day-night cycle at 0 lux and at indoor light. In Experiment 2, HR was measured at different times over the day-night cycle at controlled light intensities of 0 lux, 100 lux, and 800 lux. The authors demonstrate a clear diurnal rhythm in resting HR in complete darkness, similar to that measured under constant routine conditions. Second, it is demonstrated that light increases resting HR depending on the phase of the day-night cycle and on the intensity of light. These data strongly suggest that the circadian pacemaker modulates human HR.

Key words cardiovascular, circadian rhythm, constant routine, endogenous, hypertension, illumination, masking, SCN

The number of cardiovascular incidents follows a circadian rhythm with highest risk in the early morning, which cannot be totally explained by a circadian rhythm in exogenous factors such as activity and body position. This could be attributed rather to circadian changes in blood pressure, vascular tone, catecholamines, platelet aggregation, and heart rate (HR) (Kranz et al., 1996).

For the expression of circadian rhyhms, such as the rhythm in drinking behavior and locomotor activity, the suprachiasmatic nucleus (SCN) is essential, as was demonstrated by the disappearance of these rhythms after lesioning of the SCN in mammals (Stephan and Zucker, 1972). In humans also, lesions of the suprachiasmatic region of the hypothalamus have been shown to result in the disruption of circadian rhythms

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Table 1. Subject information for Experiment 1.

Subject	HR_{Lux0}	*Gender*	*SBP*	*DBP*	*age*	*ZT0*
1	52.9	Male	126	72	22	7:00
2	57.5	Male	145	97	29	6:00
3	45.8	Male	142	78	37	6:45
4	57.9	Male	142	88	24	5:00
5	53.8	Male	114	71	22	6:30
6	54.7	Male	145	92	40	6:30
7	51.7	Male	131	81	33	6:30
8	55.2	Male	120	77	35	8:30
9	48.3	Male	128	70	27	8:00
10	55.8	Male	137	57	22	7:00
11	50.9	Male	120	75	31	7:30
12	55.5	Female	136	84	22	7:00
13	63.6	Female	103	65	30	6:30
14	60.3	Female	118	81	25	7:00
15	50.8	Female	—	—	23	6:15
16	56.7	Female	122	65	32	6:00
17	69.3	Female	116	72	23	5:00

Table 2. Subject information for Experiment 2.

Subject	HR_{Lux0}	*Gender*	*SBP*	*DBP*	*age*	*ZT0*
1	73.2	Male	128	87	38	6:15
2	41.7	Male	129	74	38	6:45
3	57.8	Male	112	74	23	7:15
4	58.5	Male	114	70	34	7:00
5	56.7	Male	140	87	23	6:30
6	49.8	Male	113	66	29	7:00
7	45.6	Male	117	76	32	7:30
8	52.4	Male	113	73	25	8:30
9	57.4	Male	118	84	28	7:00
10	43.5	Male	113	79	33	7:30

(Cohen and Albers, 1991). SCN neurons maintain their circadian rhythm in firing frequency and in metabolic activity independent of external day-night influences (Bos and Mirmiran, 1990; Inouye and Kawamura, 1979; Schwartz et al., 1983). This endogenous circadian rhythm of the SCN, which is not precisely 24 h, is also in humans synchronized by the external light-dark rhythm (Boivin et al., 1996). Herein, light is the strongest zeitgeber, or time marker, for the SCN. Moreover, light during the (subjective) dark period also influences the SCN instantaneously, increasing neuronal firing frequency (Meijer et al., 1996) and mRNA levels of early genes in the SCN of both diurnal (Abe et al., 1995) and nocturnal animals (Romijn et al., 1996; Rusak et al., 1992). Anatomical and functional studies support the notion that light via the SCN not only is able to affect the output of the pineal (Kalsbeek et al., in press; McIntyre et al., 1989; Moore and Klein, 1974; Teclemariam-Mesbah et al., 1998) but also that of the adrenal cortex (Buijs et al., 1997, 1999), indicating that the SCN is able to transmit its time-of-the-day signal to different organs of the body.

As one of the most prevalent and powerful contributors to cardiovascular disease (Kannel, 1996), hypertension seems associated with changes in SCN morphology and function not only in rodents (Avidor et al., 1989; Eilam et al., 1994; Peters et al., 1994) but also in humans (Buijs et al., 1998; Guzzetti et al., 1991). These disturbances in circadian regulation associated with hypertension and the occurrence of tachycardia before the development of hypertension (Dickhout and Lee, 1998) stimulated us to investigate the role of the SCN in the regulation of HR in healthy subjects.

We hypothesized that the SCN modulates HR via (multisynaptic) neural pathways. If the SCN indeed controls HR, then changes in basal HR over the diurnal cycle would be expected, which we investigated during constant routine-like conditions, minimizing "masking" of the HR by rhythms in activity and posture (Mann et al., 1979), food intake (Hayano et al., 1990), state of wakefulness (van de Borne et al., 1994) and light (this study). Second, if the SCN controls HR, changes in HR in response to light would also be expected as animal studies had indicated that light has a strong influence on the SCN and its output to the heart (Amir, 1992), which we investigated using different light intensities at different phases of the diurnal cycle.

SUBJECTS, MATERIALS, AND METHODS

Subjects

Experiment 1. Eleven males ages 22 to 40 years and 6 females ages 22 to 32 years (see Table 1) participated voluntarily in this experiment (performed in the Netherlands in the summer of 1996). The habitual times of awakening for the subjects were between 5:00 and 8:30 (Table 1). The average afternoon systolic and diastolic blood pressures were 132 ±11 mmHg and 78 ± 11 mmHg for males and 119 ± 12 mmHg and 73 ± 9 mmHg for females (Table 1). Two subjects were light smokers.

Experiment 2. Ten males ages 23 to 38 years (Table 2) participated voluntarily in this experiment (performed in the Netherlands in the autumn of 1997). The

habitual times of awakening for the subjects were between 6:15 and 8:30 (Table 2). The afternoon systolic and diastolic blood pressures were 119 ± 8.9 mmHg and 77 ± 6.8 mmHg, respectively (Table 2). None of the subjects was a smoker.

The subjects were students and collaborators from our institute. All subjects had regular working weeks and did not use medication except for oral contraceptives for the female subjects in Experiment 1.

Experimental Protocol

General experimental setup. Subjects in both experiments started after at least 3 regular and habitual working days. Both experiments consisted of measurements at different zeitgeber times. "Zeitgeber Time 0" (ZT0) is defined as the habitual time of awakening during working days for each individual. For at least 2 h before each measurement, no food, caffeine, or nicotine was consumed, and for 1 h before each measuring period, physical exercise was minimized. For at least 2 h before each measurement in Experiment 1 and 24 h in Experiment 2, no alcohol was consumed. Just before the ZT0 and ZT24 measurements, each subject was woken by his or her morning alarm after a habitual night's sleep at home and started the measurements 2 to 10 min after getting up.

Each measuring period started with the subject walking calmly for about 2 min and then lying down resting but awake (Fig. 1). Hence, the subjects started each measurement with the same change from a vertical and active condition to a horizontal and resting condition, thus creating similar conditions for the measurements during the day and for those in the middle of the night and the early morning. After having walked, the subjects lay down with a dark cap over their eyes (illumination at eye level < 1 lux) and eyes closed (0 lux period) for 20 min for all measuring periods. The 0 lux periods were followed by light periods in most of the measurements of Experiment 1 and in all of Experiment 2 (see Experiments 1 and 2).

That a subject had not fallen asleep during a measuring period used for analysis was verified in two ways. A measuring period was discarded from analysis if a subject had written down to have fallen asleep or if a subject had not pushed the button on an ambulatory measuring system (AMS) (see Data Acquisition) by estimation every 10 min for Experiment 1 and every 5 min for Experiment 2.

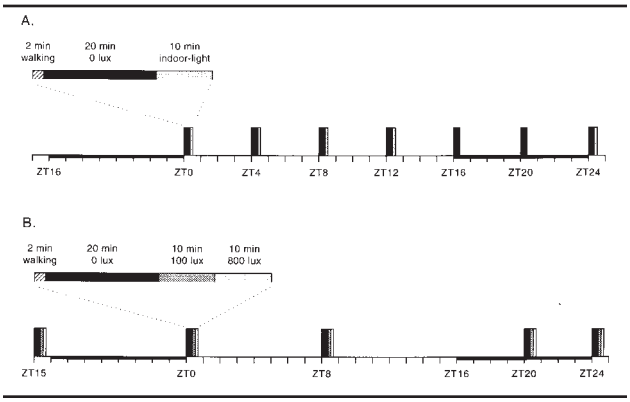

Figure 1. Experimental design. The axes at the bottom part of the figures indicate a period of 2 nights and 1 day, with the horizontal black bars indicating the night periods. The vertical bars indicate the measuring periods. (A) Experimental design of Experiment 1. (B) Experimental design of Experiment 2.

An alarm clock (Experiment 1) or a microcassette player (Experiment 2) was used to time the transitions in illumination levels. The subjects marked the beginning and end of the measuring period and the transitions in illumination level during the measuring period by pushing the button on the AMS. Any disturbances during the measuring period (sounds and movements) were signaled by the subject by pushing the button on the AMS twice.

Experiment 1. This experiment consisted of seven measuring periods at 4-h intervals (± 0.5 h), with the first measurement in the early morning just after waking (ZT0) (Fig. 1A). During the night period (ZT16-ZT24) and during the evening and night measurements (at ZT16 and ZT20), light was avoided to prevent possible phase-shifting effects. The measurements were done at home, except for the measurements at ZT4 and ZT8, which 14 subjects did in a quiet room in the institute and 3 did at home.

After the 20 min of lying down at rest in the dark (0 lux period) at ZT0, ZT4, ZT8, ZT12, and ZT24, the subjects took off the dark cap, opened their eyes, and remained at rest for 10 min with exposure to indoor light (indoor light period), while the 0 lux period was not followed by an indoor light period at ZT16 and ZT20 (Fig. 1A). The measurements during an indoor light period at home (at ZT0, ZT12, and ZT24 for all subjects and at ZT4 and ZT8 for 3 subjects) were done in a room illuminated by daylight supplemented with artificial light (illumination level not measured). The measurements in a room at the institute (at ZT4 and ZT8 for 14 subjects) were done with tube lighting (150 lux, at eye level).

Experiment 2. This experiment consisted of five 40-min measuring periods: the first at ZT15, the second at ZT0, the third at ZT8, the fourth at ZT20, and the last at ZT24 (Fig. 1B). During the night period (ZT16-ZT24), light was avoided except for the night measuring period (at ZT20). The measurements were done at home, except for the measurement at ZT8, which all subjects did in a quiet room in the institute.

The major difference between Experiment 1 and 2 was that in Experiment 2, the light intensities were controlled and equal for all five measurements. The 20-min 0 lux period was followed by 10 min of exposure to 100 lux (100 lux period) and then by 10 min to 800 lux (800 lux period) (Fig. 1B). Light was generated by a portable light source, the light visor (MediluX BV, Helvoirt, the Netherlands), which consists of small krypton halogen lamps mounted in a head holder, beaming light at the eyes at a short distance (6-8 cm). An eye mask with white plastic (diffuse filter) diffused the light from the light visor, resulting in even illumination of the eyes. With the diffuse filter over the eyes and the light visor at maximum capacity, the light intensity was 800 lux at eye distance (6-8 cm) (Mavolux-digital, Gossen, Bogen Photo Corp., Ramsey, USA). This light intensity was reduced to 100 lux with an extra gray filter (three f/stop light reduction, Neutral Density, Hama, Germany). The light was reduced to near complete darkness (< 1 lux at eyes) with a dark cap over this diffuse filter plus gray filter and the light visor turned off (0 lux with eyes closed). In this way, reproducible exposure to the same light intensities was ensured during the different phases of the day-night cycle.

Data Acquisition

Heart rate and motility. HR was determined from electrocardiography recordings by an ambulatory measuring system (AMS, Psychonomy, Free University, Amsterdam, the Netherlands) (de Geus and van Doornen, 1996; Willemsen et al., 1996). With an accelerometer built into the AMS, bodily movements, called *motility*, also were determined (de Geus and van Doornen, 1996). Both parameters were averaged over 30 sec every 60 sec for Experiment 1 and over 15 sec every 30 sec for Experiment 2. Because of the exclusion criteria (see Data Analysis), some values of labeled measuring periods were missing. For each labeled measuring period, a maximum of 1 subject was allowed to have a missing value, which was substituted by the group mean. Motility was measured to verify that the subject, wearing the AMS, was at rest.

Blood pressure. Blood pressure was measured twice with an arm cuff in sitting posture. The average of these two measurements was used as the individual blood pressure. For Experiment 1, blood pressure was measured in the week of the experiment at two moments in the afternoon at least 5 min apart; for Experiment 2, blood pressure was measured on the day of the experiment, also in the afternoon, once just before and once just after the ZT8 measurement. For Experiment 1, blood pressure was recorded with Spacelabs model 90207 ABPM (Spacelabs, Redmond, WA) and for Experiment 2 with a Profilomat (Disetronic, Medical Systems, Burgdorf, Switzerland). These two different blood pressure measuring devices were chosen solely for the practical reason of availability.

Data Analysis

AMS software was used to initialize the AMS, to read out the data, and to label periods. Labeled periods were those periods of interest over which the values were averaged. Periods with disturbances were excluded from these labeled periods as mentioned for Experiments 1 and 2.

Experiment 1. Periods from 1 min before to 1 min after each recorded time stamp and periods when increased HR coincided with increased motility were excluded from a labeled period. The remainder of the last 10 min of a 0 lux period (10-20 min after lying down) was labeled as "Lux0," and the remainder of the 10 min of an indoor light period (20-30 min after lying down) was labeled as "Lux-indoor." HR and motility were averaged over both Lux0 and Lux-indoor.

An effect of light was defined as the difference between the value during Lux-indoor and that during Lux0. During at least the first 10 min of the period at 0 lux, HR was allowed to stabilize. To test whether HR had come to a constant rate at the end of the stabilizing period, mean HR during the period from 5 to 10 min after lying down, the stabilization period, was compared with the HR during Lux0.

Experiment 2. Periods from 1 min before to 1 min after each disturbance (as time-marked by the subject) were excluded from a labeled period. The 5-min periods up to 2 min before each transition to the next light

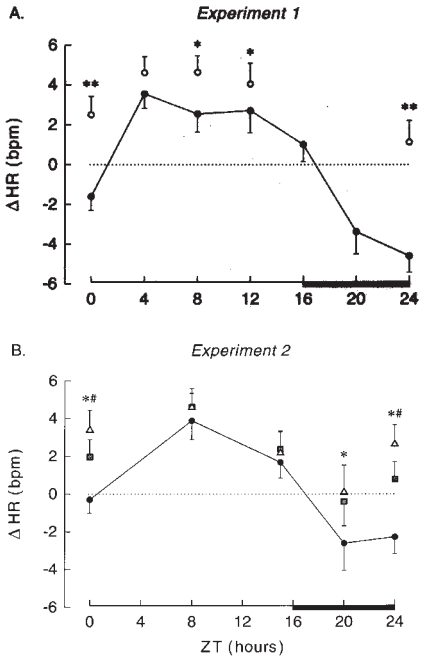

Figure 2. Change in HR due to diurnal cycle and light intensity. (A) The differences between HR during Lux0 and HR during Lux-indoor compared to the average HR over the seven levels at the Lux0s are depicted for Experiment 1 (n = 17, mean ± SEM). Basal HR during darkness shows a clear diurnal rhythm with high levels during the daytime (ZT4, ZT8, and ZT12) and low levels during the middle of the night (ZT20) and the early morning (ZT0 and ZT24). HR levels increased by indoor light most strongly in the early morning. Two significant levels for the difference between the value during Lux-indoor and the value during Lux0 are depicted: *p < 0.05; **p < 0.001. Filled circles = Lux0s; open circles = Lux-indoors; horizontal black bar = night period from ZT16 to ZT24. (B) The differences between HR during Lux0, Lux100, and Lux800 compared to the average over the five levels at the Lux0s are depicted for Experiment 2 (n = 10, mean ± SEM). Notice that the measurements were not done in the same order as depicted in the figure, as the measurement at ZT15 was done first, followed by that at ZT0, ZT8, ZT20, and ZT24. As in Experiment 1, basal HR during darkness shows a clear diurnal rhythm with high levels during the daytime (ZT8) and low levels during the middle of the night (ZT20) and the early morning (ZT0 and ZT24). HR increased by light in the middle of the night and early morning, and this increase by light was dependent on the intensity of light in the early morning. Significant differences between HR during Lux0 compared to that during both Lux100 and Lux800 are depicted: *p < 0.05. Significant differences between HR during Lux100 and HR during Lux800 are depicted: #p < 0.05. Filled circles = Lux0s; gray squares = Lux100s; open triangles = Lux800s; horizontal black bar = night period from ZT16 to ZT24.

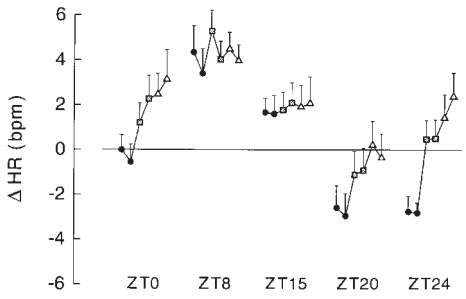

Figure 3. Change in HR over the 40-min measuring period. The differences between HR during the eight 5-min periods for each ZT-time compared to the average over the last 10 min of the 5 Lux0s are depicted for Experiment 2 (n = 10, mean ± SEM). Filled circles = 5-min 0 lux periods; gray squares = 5-min 100 lux periods; open triangles = 5-min 800 lux periods.

period were labeled: a 5-min period during 0 lux illumination was labeled as "Lux0," that during 100 lux as "Lux100," and that during 800 lux as "Lux800." In this way, Lux0 was from 13 to 18 min after lying down, Lux100 from 23 to 28 min after lying down, and Lux800 from 33 to 38 min after lying down. HR and motility were averaged over Lux0, Lux100, and Lux800.

An effect of light was defined as the difference between the value during Lux100 or Lux800 and that during Lux0. The effect of light intensity was defined as the difference between the value during Lux800 and that during Lux100.

Also, the last 30 min of all measuring periods were divided into six 5-min periods to test the influence of the experimental setup on the outcome. A change in HR during the 10-min 0 lux, 100 lux, and 800 lux periods was defined as the difference between the value over the first 5-min period compared with that over the second 5-min period.

Statistics

The time effect—that is, the change in absolute value over the day measured under the same light condition (Lux0, Lux-indoor, Lux100, or Lux800)—was evaluated (for HR and motility) with a one-way analysis of variance (ANOVA) for repeated measures (7 or 5; for Experiment 1 and 2, respectively). The light effect (i.e., the differences between the absolute values in the labeled light periods and those in the labeled dark periods for a ZT) was analyzed by using a two-way multivariate ANOVA (MANOVA) for both ZT-time (5) and illumination condition (2 or 3) as repeated measures. The change in HR within each measuring period in Experiment 2 was analyzed by three two-way MANOVAs: one for the last 10 min of the 0 lux periods, with ZT-time (5) against the 5-min period (2), and one for the 100 lux periods and one for the 800 lux

periods, both with ZT-time (5) against the 5-min period (2). If significance was reached for the (M)ANOVAs, Duncan's post hoc test was used.

Graphical Representation

Changes in HR over time and changes due to differences in illumination levels are plotted as difference scores (Δ HR) with the mean of all Lux0s defined as zero for each subject to compensate for absolute inter-individual differences (Figs. 2 and 3).

RESULTS

General Subject Data

In Tables 1 and 2, the mean resting HR over the seven Lux 0s, gender, systolic blood pressure (SBP), diastolic blood pressure (DBP), age, and the ZT0 time for each subject is depicted for Experiment 1 (Table 1) and Experiment 2 (Table 2).

Experiment 1

Effect of diurnal phase on HR. One-way ANOVA of the seven Lux0s revealed a significant diurnal rhythm in basal HR, $F(6, 96) = 8.37$ ($p < 0.001$); HR at Lux0s at midday (at ZT4, ZT8, or ZT12) was significantly higher than that in the middle of the night (at ZT20) or in the early morning (at ZT0 and ZT24) ($p < 0.05$; Fig. 2A). The maximum-minimum difference in HR between HR at Lux0s at midday (at ZT4, ZT8, and ZT12; average of 58.3 bpm) and that in the middle of the night (at ZT20) was 6.3 beats per min (bpm). There were no significant differences between the HR at Lux0s in the early mornings 24 h apart (i.e., between ZT0 and ZT24).

Effect of light on HR at different diurnal phases. Two-way MANOVA of the HR at the five Lux-indoors (at ZT0, ZT4, ZT8, ZT12, and ZT24) revealed a significant light effect, $F(1, 16) = 39.33$ ($p < 0.001$); a significant time effect, $F(4, 64) = 7.39$ ($p < 0.001$); and a significant time versus light interaction, $F(4, 64) = 7.59$, ($p < 0.001$). Light exposure increased HR most strongly at ZT0 ($p < 0.001$) and ZT24 ($p < 0.001$) (Fig. 2A). A less strong increase in HR by light was found at ZT8 ($p < 0.05$) and at ZT12 ($p < 0.05$), and no increase was found at ZT4. There was a trend for a diurnal rhythm in HR during Lux-indoors ($p = 0.056$), whereas for the Lux0s tested at the same five ZT-time points, there was a clear significant diurnal rhythm ($p < 0.001$). We found no diurnal rhythm for motility over the Lux0s or an effect of light on motility.

Tests for stabilization of HR. HR remained stable after the first 5 min of stabilization for most ZTs: there were no differences in HR during the periods 5 to 10 min after lying down and the labeled periods 10 to 20 min after lying down (i.e., Lux0s), except at ZT8.

In summary, in Experiment 1, we demonstrated a clear diurnal rhythm in HR independent of activity or light. We also demonstrated that light induced an increase in HR especially in the early morning, but because the indoor light was not of equal intensity at the different ZTs, no conclusions could be drawn with regard to a diurnal rhythm in sensitivity of HR to light stimulation. In Experiment 2, we therefore compared the effect of fixed-light intensities on HR at different ZTs, enabling us to compare the HR responses to a light intensity at different phases of the diurnal rhythm. Also, in Experiment 2, the effect of the intensity of light was investigated by using three intensities (0, 100, and 800 lux) instead of two (0 lux and indoor light).

Experiment 2

Effect of diurnal phase on HR. One-way ANOVA of the 5 Lux0s revealed a significant diurnal rhythm in basal HR, $F(4, 36) = 10.06$ ($p < 0.001$) (Fig. 2B); the difference between HR at Lux0 at the middle of the day (at ZT8; average of 58.4 bpm) and in the middle of the night (at ZT20) was 6.5 bpm. No significant differences in HR at Lux0s in the early mornings 24 h apart (i.e., between ZT0 and ZT24) were present.

Effect of light intensity on HR at different diurnal phases. Two-way MANOVA of the five measurements with three light intensities revealed a significant light effect, $F(2, 18) = 20.50$ ($p < 0.001$); a significant time effect, $F(4, 36) = 5.51$ ($p < 0.05$); and a significant time versus light interaction, $F(8, 72) = 5.72$, ($p < 0.001$) (Fig. 2B). The HR at Lux800 was significantly different from that at Lux0 at ZT0 ($p < 0.001$), ZT20 ($p < 0.001$), and ZT24 ($p < 0.001$). Also, the HR at Lux100 was significantly different from that at Lux0 at ZT0 ($p < 0.001$), ZT20 ($p < 0.001$), and ZT24 ($p < 0.001$). The HR at Lux800 was significantly different from that at Lux100 at ZT0 ($p < 0.05$) and ZT24 ($p < 0.05$). A significant diur-

nal rhythm in HR, although weaker than at Lux0s, was seen at Lux100s, $F(4, 36) = 4.80$ ($p < 0.05$), and at Lux800s, $F(4, 36) = 2.70$ ($p < 0.05$). No effects of time or light were found for motility.

Change in HR over the different light periods. Two-way MANOVA of the last 10 min of the 0 lux periods (ZT-time [5] against 5-min period [2]) revealed a significant effect for ZT-time, $F(4, 36) = 9.68$ ($p < 0.001$), but not for the 5-min period or for the interaction between both (Fig. 3). HR at 0 lux between 10 and 15 min and 15 and 20 min after lying down was only different for the measuring period at ZT8. For HR during the 10-min 100 lux periods, there was an effect of ZT-time, $F(4, 36) = 18.95$ ($p < 0.05$); there was no effect of the 5-min period; and there was an interaction between both, $F(4, 36) = 4.82$ ($p < 0.05$). For 100 lux, HR at 25 to 30 min after lying down was higher at ZT0 ($p < 0.05$) and lower at ZT8 ($p < 0.05$) in comparison to HR at 20 to 25 min after lying down. For HR during the 10-min 800 lux periods, there was no effect of ZT-time, no effect of the 5-min period, but an interaction between both, $F(4, 36) = 2.89$ ($p < 0.05$), with a higher HR during 25 to 30 min after lying down than during 20 to 25 min after lying down at ZT24 ($p < 0.05$).

DISCUSSION

In the course of this study, we demonstrated that (1) there is a diurnal rhythm in basal HR, (2) light increased HR, (3) the effect of light on HR depended on diurnal phase, and (4) the effect of light on HR was dose dependent. These data indicate an influence of light on human HR, probably mediated via the circadian pacemaker.

Animal experiments and mathematical and statistical methods have been used to investigate the role of the circadian pacemaker in the modulation of HR. However, animal experiments have the limitation that (SCN-driven) activity patterns preclude an unambiguous determination of the direct effect of the SCN on HR. Lesioning the SCN does result in the disappearance of the diurnal rhythm in HR (Warren et al., 1994); however, because all other diurnal rhythms, such as that of activity, also disappear after lesioning the SCN, a direct modulation of HR by the SCN cannot be concluded. Mathematical and statistical methods do indicate the presence of an endogenous rhythm in HR (Degaute et al., 1991; Lemmer et al., 1995; Reynolds et al., 1995), but these methods are indirect, relying on the assumptions that masking effects are constant over the diurnal cycle and that a cosine function is the best fit for an endogenous rhythm. Therefore, animal experiments and mathematical and statistical investigation of ambulatory HR data cannot demonstrate a direct SCN-generated diurnal rhythm in HR. However, by conducting measurements during constant routine-like conditions, we aimed at measuring the endogenous diurnal rhythm in HR directly.

First, we demonstrated a clear diurnal rhythm in HR in both experiments, as measured during awake, horizontal, and resting conditions at 0 lux after 2 h of fasting (see Subjects, Materials, and Methods). This basal HR was highest during the middle of the day (ZT4, ZT8, and ZT12) and lowest during the middle of the night (ZT20) and the early morning (ZT0 and ZT24) (Figs. 2 and 3), illustrating an endogenous diurnal rhythm in HR. As expected, basal HR at Lux0 between ZT0 and ZT24 did not differ significantly. The HR seemed to be stabilized to a resting level during Lux0 in both experiments, as indicated by similar HR between the last 5 min of the stabilization periods and the Lux0 periods for Experiment 1 and by the stable HR over the Lux0 period in Experiment 2, except for ZT8. To induce a similar level of arousal during day and night measurements, the volunteers walked around calmly for 2 min before each measurement, also in the night measurements.

We propose that the diurnal rhythm in resting HR at 0 lux we observed is an endogenous rhythm since constant routine conditions with light intensity below 50 lux, as described by Kräuchi and Wirz-Justice (1994), resulted in a difference of 6.4 bpm between HR in the middle of the night and in the middle of the day, which is similar to the differences of 6.3 bpm in our Experiment 1 and 6.5 bpm in our Experiment 2. Also, a recent constant routine experiment by Kerkhof and coworkers (1998) supports this range in HR between the middle of the night and the middle of the day, which they found was 6.7 bpm. Consequently, these observations and our present data indicate that constant routine-like unmasking conditions are possible even under “field” conditions.

In conclusion, our experimental setup seems to give a good estimate of the endogenous diurnal rhythm in basal HR.

Second, in agreement with our hypothesis that the SCN modulates HR, we demonstrated in both Experiments 1 and 2 that HR can be increased by light (Figs. 2 and 3). Light influences the output of the SCN to the pineal, adrenal, and autonomic nervous system in

experimental animals (Buijs et al., 1997, 1999; Kalsbeek et al., in press; Niijima et al., 1992; Teclemariam-Mesbah et al., 1998) and melatonin plasma levels and the phase setting of the SCN in humans (Boivin et al., 1996; McIntyre et al., 1989). Furthermore, light has been shown to influence the autonomic nervous system in humans (Saito et al., 1996). In experimental animals, it has been demonstrated that for the effect of light on HR, a functional SCN is necessary (Amir, 1992). Both diurnal (daytime active) animals and nocturnal (nighttime active) animals have an endogenous diurnal rhythm in metabolic activity of SCN neurons with a peak in the subjective day period (Schwartz et al., 1983). As nocturnal light increases the activity of the SCN (Meijer et al., 1996), thus bringing the activity of the SCN toward the daytime level, it was expected that light would also bring HR toward a (higher) daytime level in humans. Indeed, the observed increase in HR by light is in agreement with this expectation. The increase in human HR by light observed in both our experiments is also in agreement with the increase in HR during a 20-min exposure to bright light reported by Saito and coworkers (1996).

The observed phase response curve (PRC) of the increase in HR by light supports the hypothesis that this increase in HR by light is mediated by the SCN, the third argument that the SCN modulates HR. The PRC of the increase in HR by light we observed is similar to the PRC of the increase in firing frequency of SCN neurons by light, of the expression of mRNAs for the early gene in the SCN by light, and to the PRC in shifting the circadian rhythm of the SCN by light, all with the strongest response in the night period (Meijer et al., 1996; Rusak et al., 1992).

The increase in HR by light in the middle of the night and early morning, but not during the day, is unlikely to be the result of HR having reached a maximum during the day, as HR at 0 lux during the day had a resting level of only 58.4 bpm for Experiment 1 and 58.4 bpm for Experiment 2, far from the maximum capacity of the heart. The role of retinal sensitivity in the diurnal phase dependency of light on the circadian system has been proposed. Studies indicate that the retinal sensitivity follows an endogenous circadian rhythm, although it is not indicated that the same mechanism for retinal sensitivity is also involved in the effect of light on the circadian system (Rosenwasser et al., 1979; Terman and Terman, 1985; Terman et al., 1991). Just as for all other human studies on the PRC of the effect of light on the circadian system, we cannot completely exclude a role of a PRC in retinal sensitivity in the PRC of the increase in HR to light in our experiment. However, that a circadian rhythm in retinal sensitivity is not necessary for a PRC of the SCN to light exposure is demonstrated in animal studies in which the PRC of behavioral rhythms in rats and hamsters to electrical stimulation of the SCN or of the optic nerve (thereby bypassing the retina) is similar to the PRC of these behavioral rhythms to light exposure (Rusak and Groos, 1982; de Vries et al., 1994).

The dose dependency of the effect of light on HR also supports the hypothesis that the increase in HR by light is mediated via the SCN, the fourth argument that the SCN modulates HR. Similar to our finding that at ZT0 and ZT24 a higher light intensity increased HR more strongly, a higher light intensity induced a larger phase shift (Boivin et al., 1996) and a stronger decrease in nocturnal melatonin secretion (McIntyre et al., 1989), both of which are mediated via the SCN (Boivin et al., 1996; Moore and Klein, 1974; Rusak and Groos, 1982). The dose dependency we observed indicates that it is not the presence of visual information or the complexity of visual information but the intensity of light, which influences HR. This indicates that the visual cortex probably does not mediate the effect of light on HR. This is also demonstrated in Experiment 2, in which visual information from the surrounding was reduced to a minimum by light exposure through a diffuse filter, which clearly increased HR in the early morning and middle of the night. The dose-dependent increase in HR by light in the early morning, at the habitual time of awakening but not in middle of the night, is in parallel with the study of Campbell and Dawson (1990), who showed that alertness is increased more by a higher light intensity in the early morning than by a higher light intensity in the middle of the night.

That low light intensities of 100 lux and 800 lux, as used in Experiment 2, are able to affect the SCN and its output, is also demonstated by the phase-shifting ability of normal indoor illumination levels described by Boivin and coworkers (1996) and by the suppression of melatonin secretion by 100 lux white light described by Gaddy and coworkers (1993). Both phase shifting and melatonin suppression by light are mediated by the SCN (Boivin et al., 1996; Moore and Klein, 1974; Rusak and Groos, 1982).

In Experiment 2, the increase in HR by 100 lux and 800 lux was not reduced in the second 5-min period in the light compared to the first 5-min period in the light but remained at a high level or was even enhanced (at ZT24) (Fig. 3). Consequently, it is unlikely that the effect of light on HR in day-active humans is induced

by arousal. Interestingly in this respect and arguing also against an increase in HR by visual arousal, we observed that light in night-active rats decreases HR (unpublished results). Furthermore, the increase in HR by light is also unlikely to be caused by (even small) movements of the subject, as there was no increase in motility by light. We also demonstrated that the higher levels of HR during exposure to light were not a result of an endogenous increase in HR in the middle of the night or the early morning due to awakening and activity, as basal HR over the last 10-min period at 0 lux did not already start to increase before light exposure (Fig. 3).

Previous studies in rodents have demonstrated that the SCN affects the pineal and the adrenal neuronally via the paraventricular nucleus and the intermediolateral cell column of the spinal cord (Buijs et al., 1997, 1999; Teclemariam-Mesbah et al., 1998). Furthermore it was demonstrated in humans that the anatomical organization of the hypothalamus is largely similar to that in rodents (Dai et al., 1998). Consequently, we propose the most likely pathway to mediate the effect of light on HR to be via the retinohypothalamic tract to the SCN and from the SCN via paraventricular nucleus, brain stem, and spinal cord to the heart (Amir, 1992; Hermes et al., 1996; Niijima et al., 1992; Saito et al., 1996; Ter Horst et al., 1996; Teclemariam-Mesbah et al., 1998).

In conclusion, we demonstrated that light increases HR, probably via the SCN. This increase depends on the intensity of light and on the phase in the diurnal rhythm, with the strongest effects in the middle of the night and early morning. We also found a clear diurnal rhythm in HR under constant routine-like conditions at 0 lux.

Changes in SCN morphology and function seem associated with development of hypertension in rats (Eilam et al., 1994; Peters et al., 1994) and also in humans (Buijs et al., 1998; Guzzetti et al., 1991). Our experimental setup could be used to investigate the effect of these changes in the circadian pacemaker on the response of resting HR to light and diurnal phase in patients with hypertension, which may help to understand the role of circadian disturbances in hypertension.

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