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

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CHAPTER 5

Light and diurnal cycle affect autonomic cardiac balance in human

In progress

Abstract
The morning shift in cardiac sympato-vagal balance seems involved in the increased risk of cardiovascular incidents in the early morning. To investigate the contribution of the biological clock to the regulation of the heart by the autonomic nervous system, we investigated the presence of a diurnal rhythm independent of external factors, and of a circadian phase-dependent effect of moderate light in healthy subjects. Pre-ejection period (PEP) was used as index of sympathetic outflow, and the root mean square of successive differences of the inter-beat interval (RMSSD) was used as parasympathetic index. Resting heart rate (HR), PEP and RMSSD were measured at different times over the day-night cycle in volunteers between 20 and 40 years of age, during supine, awake, and resting conditions, during exposure to different light intensities. The similarity between the diurnal rhythm in resting HR during complete darkness, and the rhythm measured during constant routine conditions, demonstrated that our setup allowed accurate estimation of the endogenous circadian rhythm in HR. The present study suggests that the circadian rhythm in parasympathetic cardiac activity generates the circadian rhythm in resting HR, and that an increase in sympathetic cardiac activity is responsible for the effect of morning light on HR.

Introduction
The risk of myocardial infarction, stroke, and sudden death shows a peak in the early morning, which seems partially of endogenous origin [170, 201, 236]. The morning shift in cardiac sympato-vagal balance may be involved in this increased risk of cardiovascular incidents at that time [91, 221]. Since the biological clock, located in the suprachiasmatic nucleus (SCN), generates the circadian rhythms in physiology and behavior [45, 316], and shows a circadian phase-dependent response to light [217], we investigated both the circadian rhythm and the circadian phase-dependent light-response of the cardiac sympato-vagal balance.

The SCN sends multisynaptic projections to the heart via the autonomic nervous system, and that it is required for the circadian rhythm in resting heart rate (HR) and the immediate effect of light on HR [291]. The endogenous circadian rhythm in resting HR [161, 172] and the time-of-day-dependent stimulation of resting HR by moderate light
intensity in humans [292], suggests that, also in humans, the heart is under control of the SCN. Heart rate is mainly determined by the autonomic nervous system [21]. However, the relative importance of the sympathetic and parasympathetic nervous system, via which the biological clock brings about the endogenous circadian rhythm in resting HR, has received sparse attention, while this relative importance in the phase-dependent HR response to light in humans is completely unknown. To investigate the importance of the sympathetic and parasympathetic nervous system in the circadian and light-induced changes in resting HR, we repeatedly recorded non-invasive indices of both branches of the autonomic nervous system over 24 hours at different light intensities. As estimate of sympathetic activity, we measured pre-ejection period (PEP), a measure of cardiac contractility derived from impedance cardiography [52]. As estimate for parasympathetic activity, we measured the root mean square of the successive differences of the inter-beat interval (RMSSD), a measure of HR variability derived from electrocardiography [52]. An increase in sympathetic cardiac outflow is indicated by a shortening of the PEP, while an increase in parasympathetic activity is indicated by an increase in RMSSD. The HR data has been published before [292].

Materials and Methods
The details of the experimental procedure have been published before [292]. In short, all subjects were normotensive volunteers between 20 and 40 years of age with regular working weeks and without medication except for oral contraceptives. In Experiment 1, 11 males and 6 females participated, and in Experiment 2, 10 males participated. All measurements were performed after at least 3 regular working days, 2 h no food, caffeine, alcohol or nicotine, and 1 h minimized physical activity. Before each measuring period, subjects walked calmly for 2 min, after which they assumed a supine position while at rest but awake throughout all recordings. During the first 20 min, subjects remained in complete darkness (0 lux), followed by 10-min light periods (Fig. 1). In Experiment 1, 7 measurements were performed at 4-h intervals at home, except for the measurements at ZT4 and ZT8 (ZT stands for “Zeitgeber” Time, i.e. the regular time of awakening), which 14 subjects performed in a quiet room in the institute (Fig 1A). At ZT0, ZT4, ZT8, ZT12 and ZT24, the 20 min 0 lux periods were followed by exposure to indoor light (illumination not determined) for 10 min (indoor-light period) (Fig. 1A). ZT24 is at the same circadian time as ZT0, only 24 hours later. Experiment 2 consisted of 5 40-min measuring periods over the day-night cycle at home, except for that at ZT8, which all subjects performed in a quiet room in the institute (Fig. 1B). The major difference between Experiments 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).
Fig. 1 Experimental design. The axes at the bottom part of the figures indicate a period of two nights and one 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.

Light intensities were obtained by means of a portable light source, the Light visor (MediluX BV, Helvoirt, The Netherlands), in combination with a white diffuse-filter (800 lux), an additional grey filter (100 lux), and a sleeping mask covering their closed eyes (0 lux).

Data Acquisition and Analysis
HR, RMSSD and PEP were recorded with a VU-AMS (Free University, Amsterdam, The Netherlands), an ambulatory monitoring device that combines electrocardiography and thoracic impedance cardiography [103, 366]. HR, RMSSD and PEP were averaged over 30s every 60s for experiment 1 and over 15s every 30s for experiment 2. Because of high electrode resistance for 2 subjects in both experiments, PEP was determined for 15 of the 17 subjects in experiment 1 and for 8 of the 10 subjects in experiment 2. PEP values were determined with VU-AMS-software (AMSIMP). HR, RMSSD and PEP were determined over the last 10 minutes of the 0 lux periods and over the 10 min light periods. Periods from 1 min before to 1 min after each disturbance (as time-marked by the subject), or when increased HR coincided with increased motility (as measured by an accelerometer in the VU-AMS), were excluded from analysis.
The presence of a diurnal rhythm, i.e. the change in absolute value over the day measured during the 0 lux periods, was evaluated 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 light periods and those in the dark periods for each ZT, were analyzed by using a two-way ANOVA, for both ZT-time (5) and illumination condition (2 or 3) as repeated measures. If significance was reached for the ANOVAs, Duncan's Post Hoc Test was used. To investigate the contribution of the sympathetic and parasympathetic cardiac tone in the daily rhythm in HR during the dark, the linear correlation between HR and RMSSD and between HR and PEP for all 0 lux periods was determined for all subjects together and for each subject individually. To compensate for absolute inter-individual differences, difference-scores (Δ) with the mean of all 0 lux periods defined as zero for each subject, were used for these linear correlations. Furthermore, for those circadian times at which there was a significant effect of light on HR and either on RMSSD or on PEP, the linear correlation between these effects of light were analyzed.

Graphical representation
Changes in HR, RMSSD and PEP over time and due to differences in illumination levels are plotted as difference scores (Δ) with the mean of all 0 lux periods defined as zero for each subject to compensate for absolute inter-individual differences (Figs. 2).

Results
Experiment 1
The maximum-minimum difference in HR, between HR at midday (at ZT4, ZT8, and ZT12; average of 58.3 bpm) and that in the middle of the night (at ZT20) at 0 lux was 6.3 beats per min (bpm) (Fig. 2 Exp. 1). There was a highly significant diurnal rhythm in RMSSD during complete darkness (F(6,96) = 5.92; p = 0.0002; one-way ANOVA), while the significance of that in PEP was less strong (F(6,78) = 3.50; p = 0.024; one-way ANOVA). For all measurement in the dark, there was a significant linear correlation between ΔRMSSD and ΔHR, with changes in ΔRMSSD explaining 48% of the variation in HR over the day-night cycle (R² = 0.48; ΔHR = 0.01 - 0.14 * ΔRMSSD; P < 0.0001; n = 119). For all measurements in the dark, there was a significant linear correlation between ΔPEP and ΔHR, however, with ΔPEP explaining only 16% of the variation in HR over the day-night cycle (R² = 0.16; ΔHR = 0.04 - 0.21 * ΔRMSSD; P < 0.0001; n = 105). Although only 7 measurements were performed for each individual, testing for a linear correlation of the 0 lux measurements for each individual revealed a significant correlation between HR and RMSSD for 11 of the 17 subjects, and between HR and PEP for only 3 of the 15 subjects. For RMSSD there was no effect for light or interaction between
diurnal time and light (2-way ANOVA) (Fig. 2 Exp.1). For PEP, there was an effect of light (F(1,13) = 11.65; p < 0.05), but no interaction (Fig. 2 Exp.1). The effect of light on PEP was significant for ZT0, ZT8 and ZT24 (p < 0.05). Furthermore, there was a trend towards a linear correlation between the simultaneous increase in HR and decrease in PEP at ZT24 (p=0.09).

Fig. 2 Changes in resting HR, RMSSD and PEP due to diurnal cycle and light. Symbols are the mean differences relative to the daily mean as measured during 0 lux ± standard error. Solid circles, 0 lux; open circles, indoor-light; gray squares, 100 lux; open triangles, 800 lux; horizontal black bar, night period; significant difference compared to 0 lux: *, P=<0.05; **, P<0.01; significant difference compared to 100 lux: #, P<0.05.
Experiment 2
The difference between HR at the middle of the day (at ZT8; average of 58.4 bpm) and in the middle of the night (at ZT20) during 0 lux was 6.5 bpm (Fig. 2 Exp. 2). RMSSD showed a trend towards a diurnal rhythm in the dark (F(4,36)=2.58; p=0.053; one-way ANOVA), but no effect of light (2-way ANOVA) (Fig. 2 Exp.2). For all measurements in the dark, there was a significant linear correlation between ΔRMSSD and ΔHR, with changes in ΔRMSSD explaining 38% of the variation in HR over the day-night cycle (R^2 = 0.38; ΔHR = 0.08 - 0.20 * ΔRMSSD; P < 0.0001; n = 50). There was no linear correlation between ΔPEP and ΔHR for all measurements in the dark. With only 5 measurements, testing for a linear correlation of the 0 lux measurement for each individual revealed a significant correlation between HR and RMSSD for only 2 of 10 subjects, and between HR and PEP for only 1 of 8 subjects. There were no significant effects of light or interaction between circadian time and light for RMSSD or PEP (Fig. 2 Exp.2).

Discussion
The results of the present study suggest that the daily rhythm in the parasympathetic drive to the heart is the main cause of the daily rhythm in resting HR, and that the increase in sympathetic cardiac drive is the main cause of the HR increase due to morning light.

The present study provides several indications that the daily rhythm in basal HR is due to the rhythm in parasympathetic output to the heart. In Experiment 1, there was a highly significant daily rhythm in RMSSD and only a weak significant rhythm for PEP. In experiment 2, RMSSD showed a trend for a daily rhythm, while there was no rhythm for PEP. Furthermore, RMSSD and HR were highly correlated for both experiments, with changes in RMSSD explaining nearly 50% of the daily changes in HR over the day-night cycle. Changes in PEP explained only 16% of the daily changes in HR in Experiment 1, while there was no correlation in Experiment 2. Also on the individual level, more subjects showed a significant correlation between RMSSD and HR than between PEP and HR for both experiments. Together, these data indicate that the daily rhythm in resting HR is mainly due to the daily rhythm in parasympathetic drive. With the present setup, we were able to exactly replicate, in the two separate experiments, the day-night difference in resting HR as demonstrated under constant routine conditions, the golden-standard for the measurement of the endogenous circadian rhythmicity. 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). 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 differ-
ence in resting HR of 6.4 bpm and 6.7 bpm, respectively. Thus, by careful exclusion of masking factors, as in the present study, it is possible to get a reliable measure of endogenous circadian rhythm in HR, even during every-day-life conditions. Therefore, our results suggest that the actual endogenous circadian rhythm in HR in humans is mainly caused by the circadian rhythm in parasympathetic activity. Previously only a single constant routine experiment 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 index for parasympathetic cardiac outflow, and no rhythm in PEP [50], corroborating our results. The importance of the parasympathetic 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 have no sympathetic outflow to the heart [174] and in chronically chaired monkeys during blockade of the sympathetic nervous system [322]. In conclusion, the endogenous circadian rhythm in HR, that prepares us for the activity of the day, is mainly due to the circadian rhythm in parasympathetic cardiac outflow.

Secondly, the present results suggest that the increase in HR due to light exposure in the early morning is, at least partially, caused by an increase in sympathetic cardiac activity. In the first experiment, light repeatedly caused a significant increase in resting HR in the early morning (ZT0 and ZT24), together with a significant reduction in PEP, indicative of an increase in cardiac sympathetic outflow. In the second experiment, although light caused a significant increase in HR in the early morning, the effects of light on RMSSD and PEP did not reach significance. This is probably due to smaller changes in RMSSD and PEP, and a smaller group size in the second experiment. Although not significant, the results of the second study correspond with those of the first experiment, in that light reduces PEP in the early morning. Future research in a larger population should characterize further the relative importance of sympathetic and parasympathetic cardiac outflow in the phase-dependent stimulation of HR by light.

That the moderate light intensity used in the present study can influence the SCN and SCN-output is demonstrated by the significant phase-shift and immediate melatonin suppression in humans by nighttime light exposure of only 100 lux [378]. In experimental animals, the SCN has been demonstrated to be required for the phase-dependent effect of light on HR and to project to the heart via the autonomic nervous system [291]. We hypothesize that also in humans, light via SCN not only affects the pineal gland in the regulation of melatonin secretion, but also the heart, via the autonomic nervous system.

The present results indicate that, while the parasympathetic nervous system is more important for the endogenous circadian rhythm in HR, the sympathetic nervous system
is more important for the increase in HR caused by light in the early morning. These results suggest that the SCN uses both the sympathetic and parasympathetic nervous system in cardiac regulation.