Cardiovascular control by the biological clock
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CHAPTER 6

Light affects morning salivary cortisol in humans

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Abstract
The effect of light on the morning-cortisol peak in humans was investigated in fourteen healthy men by exposing them to darkness and to light of 800 lux during a 1-h period on two subsequent mornings. In the early morning, we demonstrated a temporary increase of salivary cortisol levels after awakening, while light exposure resulted in a \( \pm 35\% \) further increase in cortisol levels. Cortisol levels 20 and 40 min after waking were significantly higher during 800 lux exposure than during darkness. In order to investigate the time-dependency, the experiment was repeated in the late evening. In the evening, light had no effect on cortisol levels. These results demonstrate that light conditions in the early morning have a strong impact on the morning-cortisol peak, but that evening cortisol levels are unaffected by light. The possible role of the circadian pacemaker as mediator of the light effect on cortisol level is discussed.

Introduction
HUMAN cortisol levels follow an endogenous circadian (about 24 h) rhythm with a peak in the early morning [93, 313, 339]. In animal experiments it was demonstrated that the endogenous circadian rhythm in corticosteroid level [95], like other diurnal rhythms in physiology and behavior [316], is generated by the circadian pacemaker, which is located in the hypothalamic suprachiasmatic nucleus (SCN) [231]. Because the organization of the human SCN in the hypothalamus is similar to that in the rat in many aspects [71], because the human SCN, like that of the rat [302], shows a diurnal rhythm in neuronal activity [133] and appears to be necessary for expression of circadian rhythmicity [64], and because light can phase-shift the circadian rhythm in cortisol [29], it is likely that the human SCN generates this circadian rhythm in cortisol.

Light is the most important 'Zeitgeber', or time-marker, and entrains the circadian rhythm of SCN activity and thereby couples most circadian rhythms in physiology and behavior to the light-dark cycle [28, 29, 68, 339, 359]. Light furthermore has an instantaneous effect on the SCN and SCN-output, as has been demonstrated in experimental animals, e.g. by an increase in firing rate of SCN neurons and by the suppression of me-
latonin by nocturnal light [153, 164, 218]. In the present study we investigated whether light also has an instantaneous effect on cortisol levels in humans, possibly via the SCN. The circadian cortisol peak in the early morning is thought to prepare us for the active period. We expect that light, as signal of the active period, will further increase cortisol levels at the beginning, but not at the end, of the active period. Firstly, the morning-cortisol peak after waking in the presence and absence of light was studied (Experiment 1). And secondly, as a control against any effect of stress, the effect of morning light exposure was compared with evening light exposure (Experiment 2). Cortisol levels were measured in saliva, a convenient and reliable measure for the unbound, and therefore active, plasma cortisol levels [162].

Subjects, Materials and Methods

**Subjects**

Fourteen males, aged 24 - 41 yr (32.5 ± 5.6, mean ± SD), voluntarily participated in Experiment 1. Twelve of these males also participated in Experiment 2. The habitual time of awakening for the subjects was between 0545 h and 0730 h (0653 ± 38 min, mean ± SD). The subjects were students and collaborators from our institute. All subjects had regular working weeks and did not use any corticosteroid treatment. Morning measurements were done after a habitual night's sleep at home. On measuring days of Experiment 2, subjects did not consume alcohol and did not consume coffee or caffeine containing soft-drinks after 1800 h, thus making measuring conditions more similar to those just after awakening in Experiment 1. All subjects gave informed consent.

**Methods**

**GENERAL EXPERIMENTAL SETUP**

The effect of light on cortisol levels in humans was determined by exposing volunteers to 0 lux and 800 lux for 1 h on two consecutive days and measuring salivary cortisol levels every 20 min (Fig. 1). Subjects performed the experiments under 'normal environmental conditions' as much as possible: i.e. on regular working days, at home, in their own bed after waking by their own alarm clock at their habitual time of waking, at the same time as on three regular working days before. This habitual time of awakening is defined 'Zeitgeber Time 0' (ZT0) for each individual.

**EXPOSURE TO LIGHT**

During both the 0 and the 800 lux exposure, the subjects wore a light device, a Light visor (Bio-Brite, Bethesda, Maryland). During the 0 lux exposure, the Light visor was not turned on and subjects wore a dark cap over their closed eyes, which reduced light intensity to 0 lux. During the 800 lux exposure, the Light visor was turned on and subjects wore white plastic glasses (diffuse filter), which diffused the light from the Light
A. Protocol for morning cortisol sampling in saliva

B. Protocol for evening cortisol sampling in saliva

Fig. 1 Experimental design of assessment group 0 - 800 (as example).

A. Experiment 1. Subjects took a total of 12 samples during this experiment, with 1 sample on both evenings (ZT16, 16 h after habitual waking), 4 samples during the first hour after waking and 1 sample two hours after waking on both mornings. The subjects took the evening samples (1) 10 min after lying down with a dark mask over closed eyes. After these evening samples the subjects went to bed at their habitual bed time, wearing the dark mask for the entire night, both nights, to prevent light from influencing hormone levels. The following mornings, the subjects woke up at ZT0 and remained lying down with the dark mask covering their eyes. Within 5 min after waking the subjects took the first morning sample (2) after which the 1-h exposure to either 800 lux or 0 lux started (see 'Illumination'). After the 1-h exposure, during which a sample was taken every 20 min (3 to 5), the subjects got up. After another hour, during which samples were occupied with their habitual morning activities and were exposed to daylight, the subjects took the last sample (6) of that morning.

B. Experiment 2. The subjects took a total of 8 samples during this experiment, with 4 samples on both evenings. On both these evenings, the subjects took the first evening sample (1), 10 min (timed by sound signal of stopwatch) after lying down with the dark mask covering their eyes. After this first sample, the 1-h exposure to either 800 lux or 0 lux started (see 'Illumination'), during which a sample was taken every 20 min (2 to 4).

The axes at the bottom part of the figures indicate periods of two nights and one day. Horizontal black bars, night periods; black vertical bars, 0 lux exposures; white vertical bars, 800 lux exposures; vertical lines, saliva-sample points.

visor, resulting in an even illumination of 800 lux at eye distance (6 - 8 cm). The subjects were assigned randomly to either 'assignment group 0-800', with 0 lux exposure on the first day and 800 lux on the second day (n = 6 and n = 5; for Experiment 1 and 2 respectively), or to 'assignment group 800-0', with the reversed order of exposure (n = 8 and n = 7; for Experiment 1 and 2).

Cortisol sampling and determination

Saliva samples were taken by chewing on a cotton stick (Salivette, Sarstedt, Nümbrecht, Germany) for 1.5 min. Before producing each sample subjects did not eat or drink for at
least 30 min. The moments and duration of chewing during the 1 h measuring period were announced by instructions on a cassette player. Saliva samples taken during the evening were stored at ± 4 °C during the night. The day after evening samples and the day of morning samples, the Salivette tubes were centrifuged at 1000 G for 2 min and the supernatant was frozen at -20 °C. Within 2 months after freezing the cortisol levels were determined by coated tube Radio Immuno Assay (Coat-A-Count Cortisol, DPC, Los Angeles, USA). The intra-assay variance was 8.7 % and 11.9 % at a mean cortisol concentration of respectively 12 ng/ml and 1.2 ng/ml. The detection limit was 0.5 ng/ml cortisol. Cortisol levels during 0 lux and 800 lux exposure were analyzed within the same assay.

**STATISTICS**  
Because cortisol values were not normally distributed for Experiment 2 (only 33 of the 96 cortisol assessments were above detection level), cortisol data was rank-transformed and non-parametric statistical tests were used. For comparison between Experiment 1 and 2, the data of Experiment 1 was also rank-transformed and tested with the same non-parametric tests as those of Experiment 2. First it was ascertained with Wilcoxon Matched Pairs Test that cortisol levels at the 0 min sample points before the 0 and 800 lux exposure were not different. If not different, the effects of light on cortisol levels in the 20, 40 and 60 min samples were analyzed with non-parametric two-way multivariate analysis of variance (MANOVA) on the rank-transformed data (cf. [65], p 337), for three samples and two illumination conditions as repeated measures. The time effect, i.e. the change in cortisol level between each sample during the same illumination condition, was evaluated with non-parametric one-way analysis of variance (ANOVA) for repeated measures on the rank-transformed data (cf. [65], p 337), for the 0 lux exposure (4 samples).

If significance was reached for the (M)ANOVAs, contrasts were investigated using Duncan's Post Hoc Test. In order to evaluate possible confounding of the effects in Experiment 1 by induced phase-shift, sequence effects (darkness or 800 lux first) were tested with MANOVAs, with group assignment as between factor and as repeated factor either the difference in cortisol levels between the 0 and 800 lux condition, the cortisol levels at the 0 lux condition, or the cortisol levels at the 800 lux condition.

**Results**

**EXPERIMENT 1**

There was no difference between cortisol level in the 0 min samples before 0 lux exposure and that before 800 lux exposure in the early morning (p = 0.31). There was a significant effect of light (p < 0.05), time (p < 0.001) and interaction (p < 0.05) on cortisol levels 20, 40 and 60 min after awakening. During the light exposure cortisol levels were higher than during the dark exposure for the 20 min and 40 min samples (p < 0.005 and
p < 0.001, respectively) (Fig. 2, A). For the 1-h 0 lux exposure in the early morning there was a significant change of cortisol level over time (p < 0.05), with levels at the 20 min and 40 min sample being higher than those at the 0 min sample (p < 0.005 and p < 0.05, respectively). There was no effect of assignment group on the difference between cortisol levels during 800 and 0 lux conditions (p = 0.73), on the cortisol levels over the 0 lux conditions (p = 0.71), or over the 800 lux conditions (p = 0.62), making it unlikely that results were confounded by any phase shifts.

**EXPERIMENT 2**

There was no difference between cortisol level in the 0 min samples before 0 lux exposure and that before 800 lux exposure in the evening (p = 0.31). There was no effect of light (p = 0.35), time (p = 0.20) or interaction (p = 0.83) on the 3 cortisol levels in sample 2, 3 or 4 (Fig. 2, B). For the 1-h 0 lux exposure in the evening, there was no change of cortisol level over time (p = 0.60).

**Fig. 2** Effect of diurnal cycle and light on salivary cortisol levels.

A. Salivary cortisol levels (mean ± SEM) in the evening (E) and early morning (0, 20, 40, 60, and 120 min after waking) are depicted for Experiment 1. Cortisol levels were low in the evening and high in the early morning. In the dark (0 lux), cortisol levels increased after waking, to reach a peak at the 20 min sampling point. Cortisol levels during light exposure (800 lux) were significantly higher compared to those in the dark at the 20 and 40 min sampling point.

B. The salivary cortisol levels (mean ± SEM) in the evening (10, 30, 50, and 70 min after lying down) are depicted for Experiment 2. As in Experiment 1, salivary cortisol levels are low in the evening. However, unlike in the early morning, in the evening cortisol levels during light exposure were not different compared to those in the dark.

Difference between cortisol levels during 800 and 0 lux exposure are depicted: * , p < 0.05. Filled triangles, 0 lux assessment; open circles, 800 lux assessment; arrow, getting up of subjects after 1-h exposure to 0 or 800 lux.
Discussion

The results of the present study demonstrate I) a temporary increase of salivary cortisol levels after awakening in the early morning, II) a further increase of cortisol levels by exposure to light in the early morning and III) no effect of light exposure on cortisol levels in the evening.

We showed a temporary cortisol increase after awakening when subjects remain lying down, which is therefore not caused by the transition from a horizontal and resting to a vertical and active condition, which is in agreement with the study of Späth-Schwalbe and coworkers [313]. In the present study however, we demonstrate that, also without increasing light intensities, cortisol levels increase just after waking in the early morning.

Secondly, we demonstrate that light further increases early morning cortisol levels in humans. Also in experimental animals, it has been shown that light affects corticosteroid levels at the beginning of their active period [47]. But unlike the increase in human cortisol level by light as demonstrated in the present study, light decreased corticosterone level in rat. Because the corticosteroid peak precedes the activity period in both diurnal and nocturnal species, and light is the signal for the active period for diurnal humans and for the inactive period for nocturnal rats, it is also to be expected that light in humans will stimulate corticosteroid secretion while light in rats will inhibit corticosteroid secretion. This effect of light on corticosteroids in the rat is dependent on the integrity of the SCN [47], and could be caused by the increase of SCN-neuronal activity toward day-time levels by light exposure [218].

There are many similarities in circadian corticosteroid regulation in experimental animals and humans: I) corticosteroid levels follow an endogenous circadian rhythm [94, 95]; II) this rhythm is phase-shifted by light [28]; and III) the anatomical organization of the projections from the SCN to the paraventricular nucleus regulating the circadian corticosteroid secretion [47, 48, 71]. Moreover, several experiments have shown that the light intensity of 800 lux that was used can influence the human circadian timing system [28, 29, 208, 359]. Consequently, we suggest that the observed effect of light on cortisol levels in humans may be mediated by the SCN. In favor of this hypothesis is our third finding that light exposure in the evening does not affect cortisol levels, indicating a phase-dependent effect of light on cortisol secretion. Recently, we demonstrated in rats that the circadian peak of corticosterone is shaped by the combination of stimulating and inhibiting factors from the SCN, and that during the peak the inhibitory factor is low or not in effect [158]. We would like to propose that light is only able to cause a stimulation of cortisol in the early morning because then the inhibitory factor is not in effect. This phase-dependency is not due to a lower sensitivity of the pituitary to CRH or of the adrenal cortex to ACTH in the evening as is demonstrated by increases in cortisol levels by CRH, which are even larger in the evening than in the early morning [76]. The
absence of an effect of light on cortisol levels in the evening is in agreement with a study by McIntyre and coworkers [207], who exposed subjects to 600 lux for 3 h. Also in experimental animals, it has been shown that the effect of light on corticosteroid secretion is phase-dependent, and dependent on the integrity of the SCN [47], demonstrating an SCN mediated effect.

Because cortisol levels in saliva are unaffected by saliva flow rate [162], the increase in cortisol levels by light are not due to changes in saliva flow rate. Although there is a clear increase in cortisol by exposure to light in the early morning, this increase is only temporary. That the effect of light on cortisol is absent after 60 min might be due to the aforementioned circadian changes in inhibitory and stimulatory factors on the HPA axis [158] and/or due to the circadian rhythm in light sensitivity of the SCN [218], which both show rapid changes. It is unlikely that the exposure to 800 lux caused any discomfort or movements because the light intensity was relatively low (compared to ± 10,000 lux during a cloudy day) and did not induce any movement in a study during similar resting conditions at home [292]. In agreement with this idea, is the absence of an effect of light exposure in the evening.

In humans as well as rats, it was shown that the diurnal rhythm in corticosteroid secretion is not only determined by changing ACTH levels, but also by the diurnal rhythm in adrenal sensitivity [47, 72, 76, 94, 95]. The SCN is known to affect ACTH secretion, but recently also a multi-synaptic autonomic pathway between the SCN and the adrenal cortex has been demonstrated in rats, mediating the effect of light on adrenal sensitivity [47]. Consequently, both the observed rise in cortisol levels after waking and the phase-dependent stimulation by light might be mediated via autonomic modulation of adrenal sensitivity to ACTH by the SCN.

Recently Born and coworkers suggested that surprise awakening may increase cortisol levels [31]. However, because light intensities are not mentioned, it cannot be ruled out that exposure to light caused this increase in cortisol levels, which show a similar peak as in our experiment at about 30 min after awakening. The effect of light on cortisol levels in the early morning stresses the importance of controlling lighting conditions for those in the field, investigating cortisol regulation.

The present results expand the range of acute effects of light on physiological parameters mediated by the SCN, as is demonstrated by SCN lesions in rats: not only does light influence melatonin levels and autonomic activity in rats [153, 164, 238] it also affects melatonin levels, autonomic activity and heart rate in humans [208, 283, 292], and furthermore, like in the rat [47], the present study shows that light affects corticosteroid levels also in humans. We propose that light mediated by the SCN is the day-signal, not only serving to shift the endogenous circadian rhythm, but also to acutely prepare the physiology of the organism for the beginning of the day.