Psychoneuroendocrinology 108 (2019) 28–34

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

The effects of post-awakening light exposure on the cortisol awakening response in healthy male individuals

Katja Petrowski[a,*], Bjarne Schmalbach[b], Matthias Niedling[c], Tobias Stalder[d]

[a] Medical Psychology & Medical Sociology, University Medical Center of the Johannes Gutenberg - University Mainz, Mainz, Germany
[b] Department of Psychology, University of Muenster, Germany
[c] Energy and Automation Technology, Technical University Berlin, Germany
[d] Department of Educational Science and Psychology, University of Siegen, Germany

ARTICLE INFO

Keywords:
Non-visual light-effects
Cortisol
Melanopsin-expressing-Retinal-Ganglion-Cells
Cortisol-awakening-response

ABSTRACT

Background: It is assumed that the expression of the cortisol awakening response (CAR) is modulated by light exposure during the peri-awakening period. While initial evidence supports this principal effect, the specific role of the spectral composition of light (brightness and wavelength) is still incompletely understood.
Method: Two counterbalanced within-subject experiments were conducted in a standardized sleep laboratory setting to investigate the effect of light intensity (study I; two days: dim vs. bright light) and spectral composition (study II; three days: red vs. blue vs. green light) on the CAR. Across studies, light exposure was conducted for one-hour post-awakening and the accuracy of CAR assessments (based on eight saliva samples) was well-controlled in line with recent guidelines.
Results: The two studies revealed consistent effects of light exposure on the CAR. Specifically, an increased CAR was found after exposure to bright (vs. dim) light (study I; ($F_{(3.7,\ 106.4)} = 11.93$, $p < .001$, $\eta^2_p = .29$) and following blue and green (vs. red) light exposure (study II; $F_{(4.9,\ 194.6)} = 2.49$, $p = .037$, $\eta^2_p = .10$).
Conclusion: Our findings illustrate the crucial role of light intensity and wavelength for expression of the CAR, in line with current theoretical knowledge of underlying neurobiological mechanisms.

1. Introduction

The cortisol awakening response (CAR), the sharp increase in cortisol levels over the first 30–45 min following morning awakening, describes a unique aspect of circadian cortisol rhythmicity (Clow et al., 2004, 2010; Wilhelm et al., 2007). Over the past two decades, the CAR has increasingly been investigated as a biomarker in psychoneuroendocrinological stress-related research (Stalder et al., 2016). However, despite extensive data relating the CAR to psychosocial, physical, and mental health parameters (Chida and Steptoe, 2009; Clow et al., 2004; Fries et al., 2009; Kudielka and Wüst, 2010), there is a relative scarcity of fundamental research seeking to understand the principal determinants and regulating mechanisms of the CAR (Clow et al., 2010).

In this context, a relevant influence on the CAR is assumed to be light exposure during the pre and/or post-awakening period (Clow et al., 2010). Light-induced effects on cortisol secretion are assumed to be mediated by retinal projections to the body's central circadian pacemaker, the hypothalamic suprachiasmatic nucleus (SCN), which, in turn, affects cortisol secretion from the adrenal cortex via different routes (Dickmeis, 2009; Ishida et al., 2005; Ulrich-Lai et al., 2006). Light information for this circadian system is detected by a subset of melanopsin-expressing retinal ganglion cells (mRGCs; Berson et al., 2002; Brainard et al., 2001; Hattar, 2002; Hattar et al., 2003; Provencio et al., 2002) with a characteristic spectral sensitivity pattern peaking at ~480 nm, i.e., in the short-wavelength (blue) light spectrum (Berson et al., 2002; Dacey et al., 2005; Enezi et al., 2011; Hankins et al., 2008; Tu et al., 2005). Several studies have shown that exposure to light of this wavelength, or to bright light of broader wavelengths, has a stimulatory effect on the hypothalamic-pituitary-adrenal (HPA) axis activity (Hatanaka et al., 2008; Ishida et al., 2005; Niijima et al., 1992), which is dependent on the time of day and the circadian phase (e.g. Leproult et al., 2001). Furthermore, besides the intrinsic photo-transduction cascade of the mRGCs, there is also evidence suggesting that the mRGCs receive synaptic input from visual photoreceptors (chiefly cones) which might also be important for the detection of light information of circadian systems (e.g. Hattar et al., 2003; Lall et al., 2010; Tähkämö et al., 2019). Furthermore, light-induced circadian effects

* Corresponding author.
E-mail address: katja.petrowski@tu-dresden.de (K. Petrowski).

https://doi.org/10.1016/j.psyneuen.2019.05.016
Received 9 July 2018; Received in revised form 16 May 2019; Accepted 16 May 2019

have also been described for other biological systems besides the HPA axis, e.g. the autonomic nervous system (e.g., Scheer and Buijs, 1999) but will not be dealt with further, here.

Research directly examining the CAR has overall supported a stimulatory effect of light exposure on the CAR, although with some inconsistency: In an earlier ambulatory sleep study, exposure to bright light (800 lx, fluorescent light) during the first hour post-awakening resulted in 35%-higher cortisol levels at 20 and 40 min post-awakening as compared to waking up in complete darkness (Scheer and Buijs, 1999). In another ambulatory study, waking up by using a dawn simulator (˜250 lx, fluorescent), i.e. mainly pre- and peri-awakening light exposure, was associated with a 12.8%-increased magnitude of the CAR (Thorn et al., 2004). In a sleep laboratory study on sleep-restricted adolescents, exposure to a blue/short-wavelength (470 nm) LED light (40 lx) for 80 min post-awakening at 6:00 am resulted in an increased CAR as compared to dim light exposure over the same time (Figueiro and Rea, 2012). Conversely, in an earlier sleep laboratory study, bright light exposure (2000 lx) for 2 h after an early awakening had no effect on peak cortisol levels 1 h post-awakening (Touitou et al., 1992). Finally, in a sleep laboratory study which did not explicitly study the CAR but light exposure during the ascending (and descending) phases of the circadian cortisol rhythm (without a sleep-wake transition), 6 h bright light exposure (10000 lx, fluorescent) during the ascending phase was even found to result in a reduction of cortisol secretion (Jung et al., 2010).

The above-reviewed research portrays a relatively mixed picture. Whereas the first three studies, which investigated the CAR explicitly, suggest that light exposure over the pre- and post-awakening period has a stimulatory effect on the CAR, two of these studies comprised ambulatory research (Scheer and Buijs, 1999; Thorn et al., 2004) conducted at a time when there was still limited knowledge on critical methodological factors to be considered in ambulatory CAR research (see Stalder et al., 2016). While the study by Figueiro and Rea (2012) was conducted in a sleep laboratory setting, allowing more experimental control, further research corroborating this principal finding is warranted. In addition, such research might also investigate the question whether the nature of light to which individuals are exposed, e.g. its spectral composition, is important in regard to effects on the CAR. Previous research suggests that blue fluorescent light exerts a particularly strong influence on different biological rhythms in humans (Morita and Tokura, 1998), and this corresponds to the notion that mRGCs are most sensitive to the short-wavelength (blue) light spectrum (˜460 nm; Brainard et al., 2001; Thapan et al., 2001 or ca. 480 nm Berson et al., 2002; Dacey et al., 2005; Tu et al., 2005). However, given that the CAR forms a distinct component of circadian rhythmicity with unique regulatory mechanisms (Clow et al., 2010; Stalder et al., 2011), a direct transfer of data on another biological system to the CAR, without any specific empirical testing, does not seem justifiable. While the findings by Figueiro and Rea (2012) suggest a principal stimulatory effect of blue/short-wavelength (470 nm) light on the CAR, to the best of our knowledge, no research to date has compared the effects of light of different spectral compositions on the CAR.

The current study thus sets out to corroborate as well as to extend previous fundamental research on the effects of post-awakening light exposure on the CAR. For this, we conducted two independent within-subject experimental sleep laboratory studies examining the principal influence of 1 h post-awakening bright light vs. dim light exposure on the CAR (*study I*) and the differential effects of blue, red, and green light exposure on the CAR (*study II*). Across both studies, we strove to obtain particularly high-quality endocrine data, seeking to comply with recently published guidelines for best practice CAR assessment (Stalder et al., 2016). Following the findings by Figueiro and Rea (2012) in particular, we hypothesized that in study I bright light exposure as compared to dim light exposure leads to a greater CAR increase. Hypotheses for study II are based on data on the spectral sensitivity of mRGCs. Blue light exposure matches most closely the peak sensitivity of mRGCs. Green light exposure is also likely to activate mRGCs (although less than blue light), whereas this is not the case for red light exposure. Hence, we predict stimulatory effects of post-awakening light exposure on the CAR in the order of blue light > green light > red light.

Table 1
Sociodemographic information for both samples.

	Study 1 (n = 30)	Study 2 (n = 23)	Comparison
Age	24.62 (3.32)	22.83 (3.33)	$t(48) = 1.88$, $p = .066$, $d = 0.54$
Gender			$\chi^2(1) = 0.19$, $p = .342$
Female	0	0	
Male	26	23	
Family status			$\chi^2(3) = 2.16$, $p = .460$
Single	15	12	
Committed relationship	10	10	
Married	1	0	
Separated	0	1	
Education			$\chi^2(2) = 2.36$, $p = .692$
≤ 9 years	0	1	
10 years	0	1	
≥ 12 years	26	21	
Training qualification			$\chi^2(4) = 3.81$, $p = .568$
Still training	13	13	
Completed Apprenticeship	3	2	
University	7	2	
No training completed	2	4	
Other	1	2	
Missing	4	0	

2. Method

2.1. Participants

Two samples of 30 (study I) and 23 (study II) healthy adults were included. Descriptive sociodemographic information on the included participants is provided in Table 1. The participants were recruited and tested between December 2016 and May 2017 at the German Sport University Cologne (GSU). They were recruited through an online advertisement published on the GSU website. Participation was compensated with 50 euros per night. Prior to participation, the participants were screened for potential exclusion criteria by telephone. Exclusion criteria included: psychological disorders, acute and chronic illnesses, such as auto-immune diseases, coronary heart disease, disorders with chronic inflammation, metabolic disorders, blood disorders, or allergies. Further exclusion criteria were any use of psychoactive drugs, smoking of more than ten cigarettes per day, and a BMI of < 27 kg/m. The study protocol was approved by the Ethics Committee of the Medical Faculty of the Technical University of Dresden, Germany (No #EK353092014) and was conducted in accordance with the Declaration of Helsinki (1964). All the participants provided written informed consent prior to participation.

2.2. Procedure

Both studies used within-subject experimental designs in a sleep laboratory setting, with the independent variables being two light conditions (bright vs. dim) in *study I* and three light conditions (red vs. blue vs. green) in *study II.* The order of the light conditions was counterbalanced in both studies. Cortisol data were collected as central biological outcome measures across the post-awakening period in both studies.

Testing took place at the university's sleep laboratory. The

participants were advised via e-mail to refrain from alcohol and any strenuous physical activity/exercise on the days prior to the testing nights and on subsequent mornings. Upon arrival, the participants completed the provided questionnaires, were tested for color vision, fitted with the motion sensor, and introduced to the testing procedure of the following day. The participants went to bed in a darkened room at 11 pm. As pre-announced, the participants were woken up the next morning at 5am. This relatively early wake-up time corresponds to the methodology of an early work schedule (e.g. Figueiro and Rea, 2012) and was chosen in the present study to minimize the likelihood of participants waking up prior to the pre-specified time. Sleep quality as well as earlier wake-up was monitored by actigraphy. The participants were instructed to wear dark sunglasses when using the restrooms during the night and after wake-up. Directly after wakeup, a first saliva sample was taken and followed by seven further samples taken every 15 min. The experimental light exposure started 5 min after morning awakening. During the 60 min of light exposure and after the light exposure, the collection of saliva samples continued for 30 min. The testing took place on consecutive days during one week.

2.3. Light exposure

The instruments used for light exposure were two half Ulbricht spheres that were indirectly illuminated through LEDs positioned equally on the inside around the opening. The LEDs were covered with a spectral selective diffusor to ensure a homogeneous illumination of the participants' retina. The LEDs were regulated by computer (USB to DMX Controller) and powered by electrical DC-dimming. Across the two experiments, four different light exposure settings were used, producing white, blue, red, and green light. Table 2 shows the light settings and intensities that were used as well as the luminance in the sphere and the illuminance at the eye. The light exposure consisted of the following lighting conditions: narrow-band LEDs, blue (201 lx; peak wavelength 470–480 nm); green (806 lx; peak wavelength 520 nm); red (235 lx; peak wavelength 635 nm); bright white light (414 lx; mix of blue, green and red); dim white light (< 2 lx) see Fig. 3. The four light conditions (bright white, blue, green, and red) were set up in such a way that each condition used the same number of photons, resulting in the above mentioned illuminances at the subject's eye. Standardization by photon density, instead of standardization by illuminance (lux), was chosen since the present study aimed to target the non-visual circadian system/mRGCs rather than the visual system. Hence, in order to investigate whether mRGCs respond differentially to light of different wavelengths and consequently induce a differential influence on the CAR, it was deemed critical to standardize light conditions by the unit that actually mediates the photo-biological response at the receptor level, i.e. photon density (see also Brainard et al., 2001). This differentiates from luminance perception, which is a phenomenon which is dependent on the spectral sensitivity of the visual system and is thus of less interest in this context.

The light exposure took place in a darkened room with stray light levels below 1 lx (at the eye). Intensity of illuminance (lux) was measured at eye-level before and after light exposure each day using an illumination meter. The participants were positioned in a chair in front of the light sources with their chin resting on a chinrest so that their faces reached into a half- sphere (2PI-Geometry) and the eye-level was the same for every participant.

2.4. Cortisol awakening response

Directly after awakening at 5:00 am, the first saliva sample was taken and followed up by seven more samples at 15- minute-intervals. The saliva samples were obtained using Salivette swabs (Sarstedt, Nümbrecht, Germany). The samples were kept frozen at −20 °C until assay. Before analysis, the samples were centrifuged at 3000 rpm for five minutes to produce a clear supernatant of low viscosity. 50 µl were removed for cortisol analysis using a commercially available immunoassay with chemi-luminescence detection. The lower detection limit of this assay is 0.43 nmol/l. The intra- and inter-assay coefficients of variation were below 8% for low (3 nmol/l) and high (25 nmol/l) cortisol levels, respectively.

A MotionloggerWatch and the MotionloggerWatchWare by Ambulatory Monitoring Inc. (Motionlogger® Watch by Ambulatory Monitoring Inc., Ardsley, USA) was used to monitor the participants' activity and rest phases as well as to obtain an objective verification of awakening times during the night or the morning in order to eliminate early wake-up (in line with Stalder et al., 2016). Awakening was defined as the transition from sleep to wake-up after applying the UCSD sleep algorithm to the collected PIM-data, which is implemented in the AW2.7 software. The algorithm applies a weighted sum of the activity in a seven- minute- interval, i.e., the preceding four and the subsequent two minutes of the minute to be scored. When the value is below 1, the respective minute is scored as sleep and when it is above 1, it is scored as being wake (see Jean-Louis et al., 2001). This algorithm was used to eliminate three participants who woke up before 5 pm out of the studies.

2.5. Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 23 (IBM, Chicago, Illinois). First, outlying cortisol values of more than three standard deviations above or below the mean for each sampling point were excluded and subsequently replaced by multiple imputation (study I: 12 data points; study II: 24 data points). In the initial analyses, the differences in baseline cortisol (S1) values between conditions were examined using paired t-tests (study I) or repeated-measures ANOVA (study II). For the main analyses, we calculated a 2 (light condition: dim, bright) x 8 (measurement points as described in procedure) repeated-measures ANOVA for study I and a 3 (light condition: red, blue, green) x 8 (measurement points) repeated-measures ANOVA for study II. Besides reporting on the first cortisol value upon awakening (S1), we further calculated the area under cortisol curve with respect to increase (AUC_I) and ground (AUC_G) using the formula described by Pruessner et al. (2003).

3. Results

3.1. Study I: bright vs. dim light

The baseline cortisol levels (S1) did not differ between bright and

Table 2
Light exposure characteristics.

		Red	Green	Blue	White 1/3 R + G + B
Number of photons		4.26E + 14	4.26E + 14	4.26E + 14	4.26E + 14
Spectral irradiance	W/m^2	1.341	1.598	1.760	1.566
Illuminance at the eye	Lux	235	806	201	414
(narrow band) Spectra with different peak wavelength	nm	635	520	475	Combination of RGB
Luminance within the half Ulbricht sphere [cd/sqm]	cd/m^2	74.8	256.6	64.0	131.8

Table 3
Means and standard deviations for cortisol levels across measurement points by light condition.

Light condition					
	Study I		Study II		
	dim	bright	red	blue	green
1	3.36 [a] (2.94)	2.69 [a] (1.86)	2.84 [a] (2.07)	2.82 [a] (2.46)	3.38 [a] (2.86)
2	5.77 [a] (4.93)	5.05 [a] (3.08)	6.40 [a] (5.65)	6.44 [a] (4.68)	7.37 [a] (5.39)
3	11.93 [a] (6.80)	13.91 [a] (7.01)	14.68 [a] (9.03)	15.78 [a] (8.66)	17.27 [a] (11.78)
4	12.24 [a] (7.99)	17.89 [b] (9.24)	16.35 [a] (11.22)	20.25 [a] (11.62)	19.73 [a] (11.84)
5	9.81 [a] (6.25)	16.83 [b] (9.55)	12.10 [a] (9.21)	21.30 [b] (11.91)	17.38 [a, b] (10.76)
6	8.19 [a] (5.61)	15.03 [b] (8.36)	9.58 [a] (7.27)	16.84 [b] (10.65)	14.88 [b] (7.87)
7	7.82 [a] (4.66)	11.27 [b] (5.81)	7.49 [a] (4.93)	11.47 [a] (8.44)	9.40 [a] (5.58)
8	8.10 [a] (4.77)	8.63 [a] (4.81)	6.01 [a] (3.85)	9.29 [a] (6.81)	7.78 [a] (3.98)
Mean	8.40 [a] (4.54)	11.41 [b] (5.07)	9.43 [a] (5.53)	13.02 [b] (5.97)	12.15 [b] (5.96)
AUC_G	922.32 [a] (507.32)	1284.43 [b] (574.25)	1065.30 [a] (632.20)	1472.03 [b] (675.51)	1374.17 [b] (685.79)
AUC_I	569.78 [a] (383.93)	1002.03 [b] (482.62)	766.98 [a] (552.50)	1175.98 [b] (713.74)	1019.26 [b] (551.04)

Note. Means with different superscripts differ at $p < .05$ between light conditions.

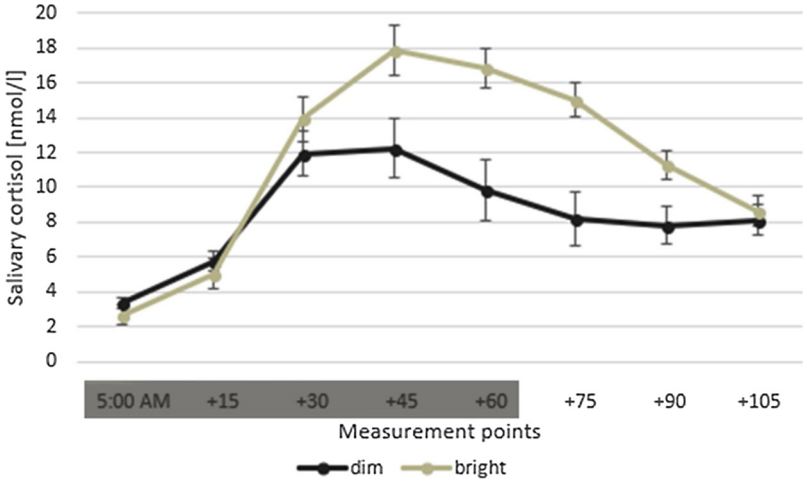

Fig. 1. Mean (± *SE*) salivary cortisol levels across measurement points for dim and bright light conditions.

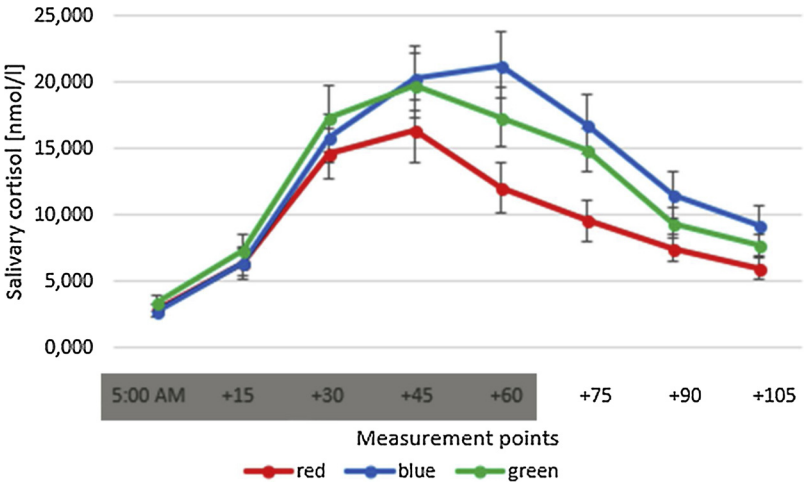

Fig. 2. Mean (± *SE*) salivary cortisol levels across measurement points for colored light conditions.

dim light conditions ($t(29) = 1.41$, $p = .170$, $d = .23$). The repeated-measures ANOVA revealed a main effect of light exposure condition (bright vs. dim light) on overall cortisol levels ($F(1, 106.37) = 11.55$, $p = .002$, $\eta^2_p = .29$). Across measurement points, the bright light condition was associated with higher cortisol levels than the dim light condition (see Table 3 for descriptive statistics). There was also a significant interaction of measurement point and light exposure condition ($F(3.67, 106.37) = 11.93$, $p < .001$, $\eta^2_p = .29$). Fig. 1 illustrates this effect, which was characterized by a more pronounced increase in cortisol levels in the bright light condition. The analyses for individual measurement points revealed significant differences in the cortisol levels between light exposure conditions on measurement points 4–7 ($ps \leq .003$), but not for the remaining four points ($ps \geq .159$). There were significant effects for both, AUC_G, $F(1, 29) = 13.10$, $p = .001$, $\eta^2_p =$

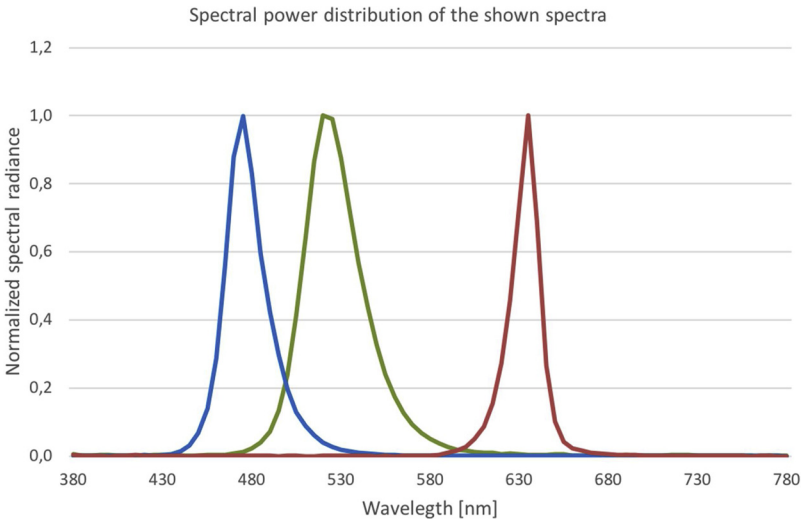

Fig. 3. Spectral power distribution of the shown spectra.

.311, and AUC_I, $F(1, 29) = 28.40$, $p < .001$, $\eta^2_p = .495$.

3.2. Study II: red vs. blue vs. green light

There were no differences in baseline values (S1) across the three light conditions ($F(2, 44) = .387$, $p = .681$. $\eta^2_p = .03$). The repeated-measures ANOVA revealed a main effect of the light condition ($F(2, 44) = 3.60$, $p = .036$, $\eta^2_p = .14$). Post-hoc-tests showed that blue and green light exposure were associated with significantly higher overall cortisol levels than the red light exposure (blue vs. red: p = .009; green vs. red: p = .042), whereas there was no significant difference between the cortisol levels of the blue and green light exposure ($p = .600$).

Furthermore, a significant interaction effect of the light exposure condition with measurement point was present ($F(4.87, 107.16) = 2.49$, $p = .037$, $\eta^2_p = .10$). Fig. 2 illustrates this effect which was characterized by a more pronounced increase in cortisol levels in the blue and green light exposure conditions compared to the red light exposure condition. Pairwise comparisons revealed differences between blue and red light for the 5th measurement point ($p = .007$), as well as for blue and red light ($p = .005$), and green and red light for the 6th measurement point ($p = .005$). There were significant differences in the AUC_G ($F(2, 44) = 3.60$, $p = .036$, $\eta^2_p = .141$) and AUC_I ($F(2, 44) = 4.39$, $p = .018$, $\eta^2_p = .166$). These were mainly due to lower values in the red light condition.

4. Discussion

The present study reports the results of two well-controlled, within-subject experimental sleep laboratory experiments with male participants on the differential effects of light exposure on the CAR. The results consistently reveal that post-awakening light exposure has a stimulatory effect on the CAR and further show that this effect is dependent on the spectral composition of light. Specifically, our data from study I reveal that one-hour post-awakening bright light exposure leads to a 76% increased CAR (AUC_I) as compared to dim light exposure. This finding is in line with previous data from an ambulatory CAR study (Scheer and Buijs, 1999) and from a sleep laboratory study (Figueiro and Rea, 2012), which also suggested positive effects of post-awakening light exposure on the CAR. The magnitude of the present effect is broadly within the same range than those reported by Scheer and Buijs (1999; i.e. ˜35% raised cortisol levels at 20 and 40 min vs. 31.5% raised cortisol levels at 30 and 45 min post-awakening) and by Figueiro and Rea (2012; i.e. 94% raised CAR-AUC_I vs. 76% raised CAR-AUC_I). Overall, these data contradict findings from another early sleep laboratory study, reporting no significant effect of two-hour bright light exposure on plasma cortisol levels 1 -h post-awakening (Touitou et al., 1992). However, the latter only had a small sample (N = 6) and did not explicitly focus on the CAR, thus only collecting one relevant post-awakening endocrine sample. Hence, it seems fair to conclude that the study by Touitou et al. (1992) was most likely underpowered and that the majority of evidence suggests that post-awakening light exposure indeed exerts a sizeable stimulatory effect on the expression of the CAR.

Next to this principal finding of study I, current study II data provided first empirical support for the notion that light-induced effects on the CAR are dependent on the spectral composition of light. Based on previous data showing peak sensitivity of mRGCs around 480 nm, we predicted that the strongest stimulatory influence on the CAR would be observed for blue light exposure, followed by weaker effects of green light, and no or only negligible effects of red light. This hypothesis was not fully supported by the present findings: although numerically the predicted order of stimulatory effects on the CAR was observed (blue light (AUC_I: 1176) > green light (AUC_I: 1019) > red light: (AUC_I: 767), statistical testing failed to reveal significant differences between the blue and green light condition but showed that both the blue *and* the green light conditions were associated with larger CARs than the red light condition. Given that at least numerically the expected trend was observed, it could be speculated that the current study may have lacked statistical power to detect an existent, but rather small difference in the CAR-stimulatory effects of blue and green light exposure. Indeed, although the peak sensitivity of mRGCs of ˜480 nm was most closely reflected by our blue light condition, mRGCs exhibit a relatively broad range of spectral sensitivity (e.g. Berson et al., 2002; Brainard et al., 2001; Hattar, 2002; Hattar et al., 2003; Lall et al., 2010; Provencio et al., 2002), meaning that they are still substantially activated by light of longer wavelengths, such as our green light condition peaking at 520 nm. By contrast, the peak wavelength of the current red light condition of 635 nm is likely to elicit no or only a negligible mRGC response (Berson et al., 2002; Brainard et al., 2001; Hattar, 2002; Hattar et al., 2003; Lall et al., 2010; Provencio et al., 2002). Combined, although our hypothesis was only partly confirmed statistically, we still view the present results as principally in line with the notion that the magnitude of post-awakening light-induced effects on the CAR correspond to the spectral sensitivity of mRGCs. However, this clearly is only at tentative interpretation which would profit from further empirical support.

An interesting aspect of the current findings relates to the relatively late time of peak cortisol concentrations in the light exposure conditions. In study I, cortisol levels in the bright light condition peaked at

45 min (and were still high at 60 min) post-awakening, whereas in study II, cortisol levels in the blue light condition only peaked at 60 min post-awakening. This relatively late CAR peak timing is surprising, particularly since the current research was conducted with male participants who have often been associated with earlier CAR peaking, around the 30 min post-awakening sample (e.g. Wüst et al., 2000). Different factors may have contributed to this finding.

First, it must be stated that most previous knowledge on the temporal pattern of the CAR originates from ambulatory research which unfortunately often failed to employ much needed control for participant sampling non-compliance (review: Stalder et al., 2016). There is clear evidence suggesting that under such conditions, a sizeable number of participants delay the beginning of post-awakening saliva sampling relative to their true awakening (e.g. Griefahn and Robens, 2011; Kupper et al., 2005), which results in false estimations of a smaller CAR *and* an earlier peak time (Stalder et al., 2016). Second, some recent CAR research in females, which employed objective methods to verify times of awakening and sampling, reported rather late peak timings around 45 to 60 post-awakening (e.g. Smyth et al., 2015, 2016). This could suggest that the true peak of the CAR might indeed occur somewhat later than previously expected.

While this might explain a part of our findings, it is important to note that late CAR peak timing was found particularly in conjunction with the stimulatory effects of light-exposure, i.e. occurring mainly under bright light (study I) and blue light (study II) exposure. This suggests that light-exposure may have exerted a stimulatory effect rather late during the post-awakening phase, i.e. after ˜30 min. In line with this, the current analyses of individual sampling time data revealed significant differences between light conditions only after 45 min (study I) and 60 min (study II) post-awakening. Since light exposure only commenced 5 min after waking up in the present study, the may have been a slight mismatch/delay between the awakening-induced stimulatory effect and the stimulatory effect due to light exposure. The slightly later commencement of light-induced effects could have further contributed to the late CAR peak timing under light conditions as well as to the fact that differences between conditions emerged rather late. This latter pattern differs from the results by Scheer and Buijs (1999) and Figueiro and Rea (2012), both reporting light-induced differences from 20 min post-awakening onwards.

Concerning these different patterns of the results, the question about the temporal course of light-induced CAR effects has potential implications with regard to the assumed underlying mechanisms. Whereas an involvement of the mRGC/retinohypothalamic tract in this effect seems evident, the route by which subsequent SCN activation stimulates cortisol secretion from the adrenal cortex might potentially involve two pathways: (i) direct or indirect effects of the SCN on the medio-parvo-cellular paraventricular nucleus which then lead to activation of the classical HPA axis pathway (e.g. Dickmeis et al., 2009) or (ii) a faster extra-pituitary, non-HPA axis-dependent mechanism through sympathetic innervation of the adrenal gland via the splanchnic nerve that increases adrenal sensitivity to ACTH (Bornstein et al., 2008; Engeland and Arnold, 2005). Particularly this latter, extra-pituitary route has been suggested to be implicated in mediating light-induced effects on the CAR (review: Clow et al., 2010). However, since this is a neural pathway it would be assumed to act more quickly than the time course seen in the present study. Conversely, the neuroendocrine cascade of the classical HPA axis has a slower time course (e.g. Sapolsky et al., 2000) which concurs more closely with the temporal pattern of the present light-induced effects for blue and bright light. While we are thus tempted to interpret the present findings as tentative, indirect support for the notion that the main pathway by which light exposure induces a stimulatory influence on the CAR involves the activation of the classical HPA axis, the fact that previous research has also reported quicker effects (Figueiro and Rea, 2012; Scheer and Buijs, 1999) calls for a more cautious interpretation and highlights the need for further research in this area. Similarly, further speculation about the underlying neurobiological mechanisms of light intensity and wavelength for expression of the CAR is difficult based on the present evidence.

The present study has some strengths and limitations, which need to be considered. Strengths include the high level of standardization of the test protocol, such as sleep in the laboratory, standardized wake-up, monitored CAR and use of sleep actigraphy. The sleep laboratory setting also allowed us to control for non-experimental light sources; i.e. before experimental light exposure, the participants were in a completely dimmed room (1 lx) which, amongst other things, minimizes the possibility of seasonal effects. Limitations of the present study include the fact that we only investigated the effects of post-awakening light exposure but cannot make inferences with regard to pre-awakening light exposure. A previous study has indicated stimulatory effects of dawn simulation, i.e. mainly involving pre- and peri-awakening light exposure, on the CAR (Thorn et al., 2004). It has been argued that the mechanisms that regulate cortisol secretion during the pre-awakening period differ from those that regulate post-awakening secretion (Clow et al., 2010). This suggests that a systematic investigation of the effects of pre-awakening light exposure on the CAR in carefully conducted future research would be of interest. Next, the interpretation of the current study II data is somewhat limited by the fact that we did not employ a dim light condition in study II. This means that we can only speculate about the question how the magnitude of the CAR expression in the blue, green, and red light conditions compares to the dim and bright light conditions. While we chose to split up the current investigation in order to reduce the participant burden in our within-subject design, future research may decide to combine these light conditions in order to extend knowledge on their differential effects. Another limitation of the present study design relates to the fact that we did not include an acclimatization night for participants. This could have led to influences on the CAR data, e.g. due to novelty effects during the first night. Although the fact that we used a counterbalanced design makes it unlikely that this induced any systematic influences on our results, it may have increased the error variance. Hence, future research may be well advised to include an acclimatization night in the sleep laboratory, if possible. Finally, besides the influences of circadian rhythmicity, the dosage of light might further be investigated to more clearly understand dose-response relationships and to elucidate whether a point of receptor saturation might be reached when even more intense light stimulation leads to no additional effect on the CAR.

The present findings have some implications for future research on the CAR. First, the principal finding that light levels influence expression of the CAR provides further support for the previously raised notion that ambient light levels need to be considered as a potential source of confounding in CAR research (Stalder et al., 2016). Second, the knowledge arising from the present study that differential light exposure provides an effective way to manipulate expression of the CAR could be used in future experimental research seeking to investigate the effects of the CAR later during the day, e.g. on cognitive functioning. Previously, such research may have used a dim light vs. bright light comparison, which is effective in manipulating the CAR but is problematic since it also induces differential visibility during the post-awakening phase, i.e. creating a potential confounding influence. Following the present findings, researchers may choose to compare the effects of blue light vs. red light exposure, which would allow the provision of adequate illumination under both conditions while still effectively manipulating the CAR.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This research received funding from the Federal Ministry of

Education and Research (BMBF), Germany. This study was conducted in the subproject: “Einfluss von Licht auf die hormonelle Stressverarbeitung” [influence of light on hormonal stress management] as part of the joint project: “Nicht-visuelle Lichtwirkungen” [non-visual effects of light] (Funding code: 13N13397).

References

Berson, D.M., Dunn, F.A., Takao, M., 2002. Phototransduction by retinal ganglion cells that set the circadian clock. Science 295 (5557), 1070–1073. https://doi.org/10.1126/science.1067262.

Bornstein, S.R., Engeland, W.C., Ehrhart-Bornstein, M., Herman, J.P., 2008. Dissociation of ACTH and glucocorticoids. Trends Endocrinol. Metab. 19, 175–180.

Brainard, G.C., Hanifin, J.P., Greeson, J.M., Byrne, B., Glickman, G., Gerner, E., Rollag, M.D., 2001. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. J. Neurosci. 21 (16), 6405–6412.

Chida, Y., Steptoe, A., 2009. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol. Psychiatry 80 (3), 265–278.

Clow, A., Hucklebridge, F., Stalder, T., Evans, P., Thorn, L., 2010. The cortisol awakening response: more than a measure of HPA axis function. Neurosci. Biobehav. Rev. 35, 97–103.

Clow, A., Thorn, L., Evans, P., Hucklebridge, F., 2004. The awakening cortisol response: methodological issues and significance. Stress 7, 29–37.

Dacey, D.M., Liao, H.W., Peterson, B.B., Robinson, F.R., Smith, V.C., Pokorny, J., Yau, K.W., Gamlin, P.D., 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature 433 (7027), 749–754. https://doi.org/10.1038/nature03387.

Dickmeis, T., 2009. Glucocorticoids and the circadian clock. J. Endocrin. 200, 3–22.

Enezi, J., Revell, V., Brown, T., Wynne, J., Schlangen, L., Lucas, R.A., 2011. "Melanopic" spectral efficiency function predicts the sensitivity of melanopsin photoreceptors to polychromatic lights. J. Biol. Rhythms 26 (4), 314–323. https://doi.org/10.1177/0748730411409719.

Engeland, W.C., Arnold, M.M., 2005. Neural circuitry in the regulation of adrenal corticosterone rhythmicity. Endocrine 28, 325–332.

Figueiro, M.G., Rea, M.S., 2012. Short-wavelength light enhances cortisol awakening response in sleep-restricted adolescents. Int. J. Endocrinol. 16, 301935.

Fries, E., Dettenborn, L., Kirschbaum, C., 2009. The cortisol awakening response (CAR): facts and future directions. Int. J. Psychophysiol. 72, 67–73.

Griefahn, B., Robens, S., 2011. Cortisol awakening response: are sampling delays of 15 minutes acceptable? Int. J. Psychophysiol. 82 (2), 202–205. https://doi.org/10.1016/j.ijpsycho.2011.08.005.

Hankins, M.W., Peirson, S.N., Foster, R.G., 2008. Melanopsin: an exciting photopigment. Trends Neurosci. 31, 27–36.

Hatanaka, M., Tanida, M., Shintani, N., Isojima, Y., Kawaguchi, C., Hashimoto, H., et al., 2008. Lack of light-induced elevation of renal sympathetic nerve activity and plasma corticosterone levels in PACAP-deficient mice. Neurosci. Lett. 444 (2), 153–156.

Hattar, S., 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295, 1065–1070. https://doi.org/10.1126/science.1069609.

Hattar, S., Lucas, R.J., Mrosovsky, N., Thompson, S., Douglas, R.H., Hankins, M.W., et al., 2003. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424 (6944), 76–81. https://doi.org/10.1038/nature01761.

Ishida, A., Mutoh, T., Ueyama, T., Bando, H., Masubuchi, S., Nakahara, D., et al., 2005. Light activates the adrenal gland: timing of gen expression and glucocorticoid release. Cell Metab. 2, 297–307.

Jean-Louis, G., Kripke, D.F., Mason, W.J., Elliott, J.A., Youngstedt, S.D., 2001. Sleep estimation from wrist movement quantified by different actigraphic modalities. J. Neurosci. Methods 105 (2), 185–191.

Jung, C.M., Khalsa, S.B., Scheer, F.A., Cajochen, C., Lockley, S.W., Czeisler, C.A., Wright Jr., K.P., 2010. Acute effects of bright light exposure on cortisol levels. J. Biol. Rhythms 25 (3), 208–216. https://doi.org/10.1177/0748730410368413.

Kudielka, B.M., Wüst, S., 2010. Human models in acute and chronic stress: assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. Stress 13, 1–14.

Kupper, N., De Geus, E.J.C., Van Den Berg, M., Kirschbaum, C., Boomsma, D.I., Willemsen, G., 2005. Familial influences on basal salivary cortisol in an adult population. Psychoneuroendocrinology 30, 857–868.

Lall, G.S., Revell, V.L., Momiji, H., Al Enezi, J., Altimus, C.M., Güler, A.D., et al., 2010. Distinct contributions of rod, cone, and melanopsin photoreceptors to encoding irradiance. Neuron 66 (3), 417–428. https://doi.org/10.1016/j.neuron.2010.04.037.

Leproult, R., Colecchia, E.F., L'Hermite-Balériaux, M., Van Cauter, E., 2001. Transition from dim to bright light in the morning induces an immediate elevation of cortisol levels. J. Clin. Endocrinol. Metab. 86, 151–157.

Morita, T., Tokura, H., 1998. The influence of different wavelengths of light on human biological rhythms. Appl. Human Sci. 17 (3), 91–96.

Niijima, A., Nagai, K., Nagai, N., Nakagawa, H., 1992. Light enhances sympathetic and suppresses vagal outflows and lesions including the suprachiasmatic nucleus eliminate these changes in rats. J. Auton. Nerv. Syst. 40, 155–160.

Provencio, I., Rollag, M.D., Castrucci, A.M., 2002. Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. Nature 415 (6871), 493.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28 (7), 916–931.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.

Scheer, F.A., Buijs, R.M., 1999. Light affects morning salivary cortisol in humans. J. Clin. Endocrinol. Metab. 84 (9), 3395–3398.

Smyth, N., Thorn, L., Hucklebridge, F., Evans, P., Clow, A., 2015. Detailed timecourse of the cortisol awakening response in healthy participants. Psychoneuroendocrinology 62, 200–203. https://doi.org/10.1016/j.psyneuen.2015.08.011.

Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J., Wüst, S., et al., 2016. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432.

Stalder, T., Evans, P., Hucklebrige, F., Clow, A., 2011. Associations between the cortisol awakening response and heart rate variability. Psychoneuroendocrinology 36, 454–462.

Tähkämö, L., Partonen, T., Pesonen, A.K., 2019. Systematic review of light exposure impact on human circadian rhythm. Chronobiol. Internat. 36, 151–170.

Thapan, K., Arendt, J., Skene, D.J., 2001. An action spectrum for melatonin suppression: evidence for a novel non-rod non-cone photoreceptor system in humans. J. Physiol. (Paris) 535, 261–267.

Thorn, L., Hucklebridge, F., Esgate, A., Evans, P., Clow, A., 2004. The effect of dawn simulation on the cortisol response to awakening in healthy participants. Psychoneuroendocrinology 29, 925–930.

Touitou, Y., Benoit, O., Foret, J., Aguirre, A., Bogdan, A., Clodore, M., Touitou, C., 1992. Effects of a two-hour early awakening and of bright light exposure on plasma patterns of cortisol, melatonin, prolactin and testosterone in man. Acta Endocrinol. 126, 201–205.

Tu, B.P., Kudlicki, A., Rowicka, M., McKnight, S.L., 2005. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science 310 (5751), 1152–1158. https://doi.org/10.1126/science.1120499.

Ulrich-Lai, Y.M., Arnold, M.M., Engeland, W.C., 2006. Adrenal splanchnic innervation contributes to the diurnal rhythm of plasma corticosterone in rats by modulating adrenal sensitivity to ACTH. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, 1128–1135.

Wilhelm, I., Born, J., Kudielka, B.M., Schlotz, W., Wüst, S., 2007. Is the cortisol awakening rise a response to awakening? Psychoneuroendocrinology 43 (4), 358–366.

Wüst, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., 2000. The cortisol awakening response - normal values and confounds. Noise Health 2 (7), 79–88.