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The inflammatory immune system after wake up in healthy male individuals: A highly standardized and controlled study

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ABSTRACT

This study investigates the effects of two light conditions on innate proinflammatory IL-6 cytokines and the cortisol awakening response. The between-subject experiment was conducted with 55 healthy adult-males (aged $M_{bright} = 24.40$, SD = 4.58; $M_{dim} = 25.47$, SD = 4.96) in a standardized sleep laboratory setting with 60-min light exposure post-awakening. Cortisol significantly increased with bright light exposure as compared to dim light (significant interaction effect). As for IL-6, the main effects of time and light condition were significant, however, the interaction effect between light and time was insignificant. Results replicate stimulatory effects of bright light on cortisol. In general, IL-6 concentrations decreased in both light conditions; however, bright light graphically showed higher concentrations 45–90 min after exposure in comparison to dim light, thus bright light has a potential stimulatory effect on IL-6 production.

1. Introduction

The circadian rhythm is the synchronization of physiological processes over a period of 24 h and regulates the sleep-wake rhythm. With the help of special photoreceptors in the outer granular layer of the retina, it can align itself in a relatively constant rhythm with time and the periodically listed activities, such as sleep, food intake, hibernation, reproduction, etc. Therefore, light exposure represents a relevant regulatory factor for the circadian rhythm (Clow et al., 2010).

Light-induced effects by retinal projections can affect the body's central circadian pacemaker, the hypothalamic suprachiasmatic nucleus (SCN) and initiate cortisol secretion from the adrenal cortex (Dickmeis, 2009; Ishida et al., 2005; Jung et al., 2010; Ulrich-Lai et al., 2006). This circadian system receives light information through a subgroup 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 that peaks at 480 nm, i.e. in the short-wave (blue) light spectrum (Berson et al., 2002; Dacey et al., 2005; Enezi et al., 2011; Hankins et al., 2008; Tu, 2005). Furthermore, there is evidence that mRGCs receive synaptic input from visual photoreceptors (mainly cones) in addition to the intrinsic phototransduction cascade, which is part of the detection of light information for circadian systems (e.g. Hattar et al., 2003; Lall et al., 2010; Tähkämö et al., 2019; Temme and Frumkes, 1977).

As a unique effect of the circadian rhythm, there is a strong increase in the cortisol level in the first 30–40 min after waking up in the morning, which reaches its peak here in the cortisol-awakening response (CAR) (Clow et al., 2004, 2010; Wilhelm et al., 2007). Glucocorticoids exhibit broad anti-inflammatory properties (Coutinho and Chapman, 2011) by suppressing the synthesis of proinflammatory factors on the one hand and by strengthening anti-inflammatory mediators (Scheiermann et al., 2013) on the other hand. Here, the production of cytokines and transmigration of leukocytes is initiated (Coutinho and Chapman, 2011). The inflammatory system thus fluctuates in a circadian cycle of about 24 h, therefore the underlying pathways of these circadian effects on the inflammatory system is of particular interest.

Although glucocorticoids are typically known for their ability to suppress the production of proinflammatory cytokines (e.g. (Coutinho and Chapman, 2011), this effect cannot be over-generalized and requires a more differentiated view. For example, a study by DeRijk et al. (1997)

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in rats presented a different picture. Herein, the IL-6 production was shown to be resistant to suppression by cortisol levels within the physiological range, in contrast to TNFα and IL-1β. Furthermore, a study by Steensberg et al. (2003), conducted with humans, found that the infusion of IL-6 did not induce enhanced levels of the proinflammatory cytokine TNFα, but instead enhanced plasma levels of the two anti-inflammatory cytokines IL-1 receptor agonist (IL-1ra) and IL-10, as well as cortisol.

Therefore, despite the proinflammatory effect of IL-6, there is a synergistic effect between IL-6 and glucocorticoids here, suggesting that IL-6 may have an anti-inflammatory effect in synergy with cortisol. Indeed, IL-6 has been shown to assume anti-inflammatory tasks, given specific circumstances (Scheller et al., 2011).

Empirical results on the mechanisms of fluctuating immune function after wake-up are quite diverse due to methodological differences. Circadian rhythmicity of inflammatory parameters has been empirically investigated particularly during nocturnal sleep, where elevated IL-6 and TNFα levels were observed (Brodin and Davis, 2017; Dickstein and Moldofsky, 1999; Gudewill et al., 1992; Moldofsky, 1994; Petrovsky, 2001). More specifically, IL-6 levels rise during sleep, peak in the early morning between 1:00 a.m. and 4:00 a.m., and fall to their lowest between 8:00 a.m. and 10:00 a.m. (Brodin and Davis, 2017; Petrovsky, 2001). The IFN-y/IL-10 ratio of anti-inflammatory cytokines peaks at 4:00 a.m., correlating negatively with plasma cortisol, positively with plasma melatonin levels, and reaches a minimum value at about 3:00 p.m. (Petrovsky and Harrison, 1997). However, the bi-directional pattern between peripheral cellular immune function and cytokines is associated with patterns of sleep and wakefulness, where disorganization or loss of sleep inhibit the diurnal patterns of cytokine-immune-neuroendocrine functions (Dickstein and Moldofsky, 1999).

Experiments on the effect of **light after wake up** on the circadian rhythmicity showed that blue fluorescent, blue LED light and bright LED light have a particularly strong influence on cortisol (Figueiro and Rea, 2012; Morita and Tokura, 1998; Petrowski et al., 2019, 2020). Concerning immune function there are, to our knowledge, no empirical studies published on the processes after wake up. In animals, exposure to green-wave LED light generally led to improved immune function (Jung et al., 2016; Kim et al., 2014). Low-dose LED light exposure at 880 nm caused a decrease in inflammatory cells and mRNA expression of IL-1β, IL-6 and COX-2 after collagenase-induced tendinitis (Xavier et al., 2010). In vitro human applications of LED light exposure of different LED wavelengths on human T-cell cultures demonstrated that LED light exposure at 415 nm, 850 nm, and 830 nm significantly decreased mRNA expression of IL-2, IL-4, and IFN-gamma (Cheong et al., 2012). T-cell--derived cytokines could be suppressed by lower doses of LED light exposure (850 nm), and IL-10 expression rates could be reduced by irradiating $CD3^+$ T lymphocytes with 850 nm light (Cheong et al., 2012). In addition to light exposure in vitro, two white light intensities with correlated colour temperatures of 2954K and 7378K were compared in a human experiment, which showed that 7378K light induced an upregulation of IL-6, whereas 2954K did not (Shen et al., 2016).

Thus, light exposure seems to influence the inflammatory system, but the exact mechanism of the circadian rhythm, light conditions at different intensities, and the changes they induce in immune parameters have not yet been investigated. Therefore, this study investigates the effects of two light conditions (bright-white, and dim-white light) on the innate immune parameter IL-6 cytokines.

H1. The literature on the cycles of pro- and anti-inflammatory cytokines shows that IL-6 peaks in early morning before 5:00 a.m. (Petrovsky and Harrison, 1997; Scheiermann et al., 2013). Therefore, we expect high concentrations of IL-6 at 5:00 a.m. and a steeper decrease between 5:00 a.m. (T0) and 7:00 a.m. (T8) under the dim-white light condition compared to the bright light condition (and the same for cortisol, respectively; Petrowski et al., 2019, 2020).

2. Method

2.1. Participants

The sample includes 55 healthy male adults (aged $M_{\text{bright}} = 24.40$, $SD = 4.58$; $M_{\text{dim}} = 25.47$, $SD = 4.96$). Females were excluded from this first experimental study in efforts to reduce potential mediating factors of the menstruation cycle on cytokine and cortisol levels, however, a follow up study is expected to include female participants. Descriptive sociodemographic information on the included participants is provided in Table 1. The participants were recruited and tested between October 2019 and March 2020 at the Johannes Gutenberg University in Mainz, Germany, in which recruitment was carried out through online and flyer advertisements. Participation was compensated with 50 euros. Prior to participation, volunteers were screened for potential exclusion criteria by telephone. Exclusion criteria were defined based on factors that can influence HPA-axis activation or are known to have a lasting impact on the reactivity of the HPA-axis. As such, exclusion criteria included: Colorblindness, psychological disorders, acute and chronic illnesses, such as auto-immune diseases, coronary heart disease, disorders with chronic inflammation, metabolic disorders, blood disorders and 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 (see e.g., Kudielka, Hellhammer & Kirschbaum, 2007; Herhaus et al., 2020). 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). Each participant provided written informed consent prior to participation.

2.2. Procedure

The study used a between-subjects experimental design in a sleep laboratory setting, with the independent variable being the light condition (bright vs. dim). This sleep laboratory setting as well as CAR being the DV were chosen in order to standardize and eliminate all previous light exposure on the system. Light condition was randomly assigned from a computer algorithm to eliminate potential bias, with $N = 25$ participants in the bright light and $N = 30$ in the dim light condition. Blood samples were collected via intravenous (IV) catheter as the central biological outcome measure to analyze blood cortisol and pro-inflammatory cytokine (IL-6) across the post-awakening period.

Testing took place at the university's sleep laboratory and was conducted after wake-up on the following day. The participants were advised to refrain from alcohol and any strenuous physical activity/

Table 1
Sociodemographic information for both samples.

	Bright light ($n = 25$)	Dim light ($n = 30$)	Comparison
Age	24.40 (4.58)	25.47 (4.96)	$t(52.40) = -0.83$, $p = .412$, $d = -0.223$
Family status			$\chi^2(1) = 0.03$, $p = .871$
Single	23	29	
Married	2	1	
Education			$\chi^2(2) = 3.59$, $p = .166$
≤ 9 years	0	3	
10 years	0	1	
≥ 12 years	25	26	
Training qualification			$\chi^2(4) = 3.90$, $p = .420$
Still training	11	8	
Completed Apprenticeship	4	5	
University	3	10	
No training completed	4	4	
Other	1	1	
Missing	2	2	

exercise on the days prior to the testing nights and on subsequent mornings via e-mail. 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 IV catheter was set the night before testing (between 10:30 and 11:00 p.m.) to eliminate an effect on stress levels. The participants went to bed in a darkened room at 11:00 p.m. and woken up the next morning at 5:00 a.m. This relatively early wake-up time corresponds to the methodology of an early work schedule (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 blood sample was taken and followed by seven additional samples taken every 15 min. The experimental light conditions started 10 min after awakening and lasted 60 min each. After light exposure, participants sat in a dim light room (<3 Luminance (lux)) for the remaining three blood samples. Testing ended by 7 a.m. (see Fig. 1 for study design).

2.3. Light conditions

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. The experiment used dim and bright light conditions. Bright light (1241 lux) exposure was produced by simultaneously setting narrow-band LEDs of blue (201 lux; peak wavelength 470–480 nm), green (806 lux; peak wavelength 520 nm), and red (235 lux; peak wavelength 635 nm) together; while dim white light was standardized at less than 3 lux.

The light exposure took place in a darkened room with stray light levels below 1 lux (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. Biological markers: blood cortisol and cytokines

Directly after awakening at 5:00 a.m., the first blood sample was taken and followed up by seven additional samples at 15 min-intervals. The blood samples were obtained using Serum-Gel-Monovette® (Sarstedt, Nümbrecht, Germany) and set to coagulate for 30 min at room temperature. The blood samples were then centrifuged at 20 °C for 10 min at 2500xG RCF. The serum was stored at −80 °C before being assayed for cytokines and cortisol levels.

Serum cortisol levels were determined using a commercially available radioimmunoassay kit with the Solid Phase Antigen Linked Technique (SPALT) with the LIAISON-Analyzer® (DiaSorin, S.p.A., Italy). The lower detection limit of this assay is 0.43 nmol/l. Serum cytokine concentrations were analyzed using a using highly-sensitive ELISA enzyme-linked immunosorbent assays (IBL International GmbH, Germany). The detection limit was <0.08 pg/mL serum.

A Motionlogger® Watch and the MotionloggerWatchWare by Ambulatory Monitoring Inc. (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 2 min of the minute to be scored. When the value is below 1, the respective minute is scored as sleep, when it is above 1, as being awake (see Jean-Louis et al., 2001). In case such a value above 1 was present, the respective participant's data was excluded from further analysis. Accordingly, three participants were excluded from this study.

2.5. Statistical analysis

All statistical analyses were performed in R using the packages *bnstruct*, *ez*, *ggplot2*, and *MBESS* (Franzin et al., 2016; Kelley, 2020; Lawrence, 2016; Wickham, 2016). First, outlying values were deleted and replaced with the average of the preceding and succeeding value. Outliers were identified as values that were more than three standard deviations above or below the mean for each sampling point and light condition. In this fashion, a total of 15 cortisol (3.4%) and 23 IL-6 values (5.2%) were replaced by the average score of the preceding and succeeding values. Next, the baselines were compared for the two light conditions via *t*-test. The main analyses were then conducted via a mixed-measures ANOVAs with two factors: light condition (2 levels: bright vs dim light) and time (8 measurement points). Following the main analyses, we also compared light conditions on a per-timepoint-basis. We complemented these analyses by calculating the summary statistics area-under-the-curve with respect to the ground (AUC_G) and with respect to the increase (AUC_I; Pruessner et al., 2003). In addition to significance levels, we report standardized effect size measures d and η_p^2 with their 95% and 90% confidence intervals, respectively.

3. Results

3.1. Cortisol

Descriptive statistics of both groups and measurement points are reported in Table 2 and Fig. 2. There were no significant differences with regard to the baseline measurement, $t(52.74) = -1.62$, $p = .111$, $d = -0.440$ [−0.975; 0.100]. In the subsequent mixed-measures ANOVA across all eight measurement points, we found a highly significant main effect for time, $F(2.62, 139.02) = 66.21$, $p < .001$, $\eta_p^2 = .555$ [0.495; 0.593]. There was no significant main effect for light condition, $F(1, 53) = 0.48$, $p = .491$, $\eta_p^2 = .009$ [0.000; 0.090], but we found a significant interaction term, $F(2.62, 139.02) = 6.22$, $p < .001$, $\eta_p^2 = .105$ [0.047; 0.140]. AUC_G, $t(41.26) = 0.22$, $p = .824$, $d = 0.060$ [−0.472; 0.590] was not significantly different between light conditions, but AUC_I was, $t(45.25) = 2.88$, $p = .006$, $d = 0.779$ [0.225; 1.327].

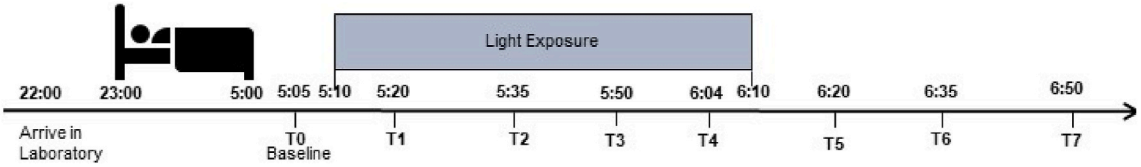

Fig. 1. Study design.

Table 2
Means and standard deviations for cortisol levels across measurement points by light condition.

	Bright light (n = 25)		Dim light (n = 30)		Comparison	
Time	*M*	*SD*	*M*	*SD*	*p*	*d*
0	59.42	33.44	74.96	37.52	.111	−0.435
+15	**121.54**	**31.83**	**140.81**	**38.79**	**.048**	**−0.538**
+30	152.84	34.52	151.99	39.24	.932	0.023
+45	153.92	33.55	133.47	42.54	.051	0.528
+60	**144.2**	**39.44**	**121.1**	**41.18**	**.039**	**0.572**
+75	128.98	41.74	108.38	36.53	.060	0.528
+90	113.59	43.21	101.49	38.87	.284	0.296
+105	106.44	39.04	102.65	35.97	.712	0.101

Note: bold lines indicate significant effects p < .05.

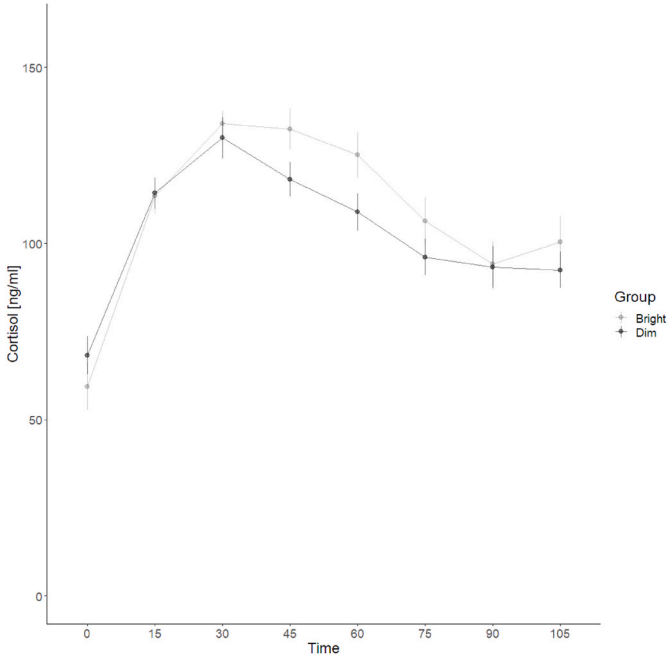

Fig. 2. Mean (±*SE*) cortisol levels across measurement points for dim and bright light conditions.

3.2. IL-6

Descriptive statistics of both groups and measurement points are displayed in Table 3 and Fig. 3. There was a significant difference with regard to the baseline measurement, $t(48.76) = 2.46$, $p = .018$, $d = 0.665$ [0.117; 1.208]. First and foremost, light condition, $F(1, 53) = 5.86$, $p = .019$, $\eta_p^2 = .100$ [0.009; 0.234], and time, $F(3.73, 197.83) = 3.16$, $p < .001$, $\eta_p^2 = .056$ [0.011; 0.081], had significant main effects.

Table 3
Means and standard deviations for IL-6 levels across measurement points by light condition.

	Bright light (n = 25)		Dim light (n = 30)		Comparison	
Time	*M*	*SD*	*M*	*SD*	*p*	*d*
0	**7.7**	**4.5**	**4.84**	**4.03**	**.018**	**0.672**
+15	**6.44**	**4.56**	**3.65**	**3.01**	**.013**	**0.733**
+30	**5.8**	**3.56**	**3.74**	**3.07**	**.028**	**0.623**
+45	**6.16**	**4.59**	**3.59**	**3.06**	**.021**	**0.671**
+60	6.12	4.38	4.01	3.63	.061	0.529
+75	**6.48**	**4.46**	**3.97**	**3.3**	**.024**	**0.648**
+90	6.08	3.85	4.02	3.87	.054	0.533
+105	6.55	4.54	5.29	5.74	.368	0.241

Note: bold lines indicate significant effects p < .05.

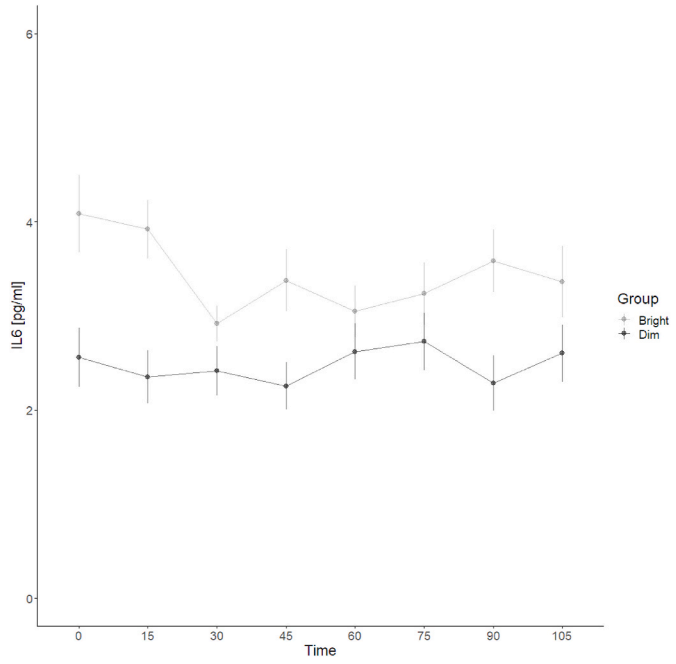

Fig. 3. Mean (±*SE*) IL-6 levels across measurement points for dim and bright light conditions.

The interaction term was not significant, $F(3.73, 197.83) = 0.75$, $p = .553$, $\eta_p^2 = .014$ [0.000; 0.019]. AUC_G was significantly higher in the bright light condition, $t(46.39) = 2.42$, $p = .019$, $d = 0.656$ [0.108; 1.199], whereas there was no effect for AUC_I, $t(52.73) = -0.650$, $p = .519$, $d = -0.176$ [−0.707; 0.357].

4. Discussion

The circadian rhythm is the synchronization of physiological processes over a period of 24 h and regulates the sleep-wake rhythm. Special photoreceptors in the outer granular layer of the retina help to synchronize the circadian rhythm during exposure to light and regulate the respective hormone, cortisol and melatonin production.

Here, a bi-directional pattern between peripheral cellular immune function and cytokines is associated with patterns of sleep and wakefulness. However, the effects of light conditions (such as bright-white and dim-white light) on the innate immune parameter IL-6 is still unclear. Literature referring to the cycles of inflammatory cytokines show that IL-6 peaks in early morning before 5:00 a.m.(Petrovsky and Harrison, 1997; Scheiermann et al., 2013). Therefore, we expected high concentrations of IL-6 at 5:00 a.m. and a steeper decrease between 5:00 a.m. (T0) and 7:00 a.m. (T8) under a dim-white light condition compared to a bright light condition (and the same for cortisol, respectively; Petrowski et al., 2019, 2020).

The present study showed the expected cortisol awakening response and the previously found effect of the light intensity condition on cortisol (Petrowski et al., 2019, 2020), where the bright light condition shows a steep increase after waking when compared to the dim light condition. Regarding pro-inflammatory cytokine levels, average IL-6 levels significantly decreased in both conditions as expected. Furthermore, IL-6 showed significant differences between light conditions at timepoints 1 (+15) and 6 (+90), with the bright light condition showing higher levels of pro-inflammatory cytokines when compared to the dim light condition. These results suggest a circadian rhythmicity of inflammatory parameters during nocturnal sleep and replicate findings of elevated IL-6 levels early in the morning (Brodin and Davis, 2017; Dickstein and Moldofsky, 1999; Gudewill et al., 1992; Moldofsky, 1994; Petrovsky, 2001) and their ensuing fall to the lowest values later in the

morning (Brodin and Davis, 2017; Petrovsky, 2001).

Concerning the effect of light conditions **after wake up,** bright LED light has a particularly strong influence on cortisol (Figueiro and Rea, 2012; Morita and Tokura, 1998; Petrowski et al., 2019, 2020). Glucocorticoids exhibit broad anti-inflammatory properties (Coutinho and Chapman, 2011) by suppressing the synthesis of proinflammatory factors on the one hand and strengthening anti-inflammatory mediators (Scheiermann et al., 2013) on the other hand. In the present study, the natural peak of the cortisol awakening response starts at +30 min and corresponds with the trough of IL-6 levels, suggesting an interplay between cortisol levels and pro-inflammatory cytokine production. Petrovsky et al. (1998) reported the inverse relationship between plasma cortisol and various pro-inflammatory cytokines including IFN-g, TNFα, IL-1 and IL-12, IL-6 however was unfortunately not included as a parameter in their study. Based on the present results, the increase in cortisol during the cortisol awakening response is accompanied by a decrease in IL-6, which is in line with the proposed mechanism in the literature. However, the bright light effect with a significant increase in cortisol concentrations as well as in IL-6 concentrations does not show this inhibiting mechanism of cortisol on IL-6. In contrast, the bright light effect led to an associated increase in the concentration of both parameters in the present study. This may be due to the synergistic effect of cortisol and IL-6 already demonstrated, where there is no decrease in concentration of IL-6 as is the case in other proinflammatory cytokines, with IL-6 in contrast resistant to this kind of negative feedback (DeRijk et al., 1997). In regard to light effects in immune parameters, a contrasting effect was observed in the literature. Comparing two white light intensities with correlated colour temperatures of 2954K and 7378K, 7378K (bright) light induced an upregulation of IL-6, which 2954K did not (Shen et al., 2016).

It should be noted that the study presented here has both strengths and limitations. The high level of standardization of the test protocol, sleep in a laboratory setting, standardized waking schedule, supervised CAR and the use of sleep actigraphy count as strengths. Prior to the experimental light exposure performed in the study, the participants were in a completely darkened room with values below one lux, which allowed for control of non-experimental light sources and minimized the possible influence of seasonal effects.

Limitations are evident in the IL-6 concentration before awakening, which are not measured in this study, since the parameters were measured only after awakening. There is already one study that has shown the stimulating effects of dawn simulation, mainly with pre- and peri-awakening light exposure, on CAR (Thorn et al., 2004). In terms of the inflammatory system, no study has been conducted that investigates whether the mechanisms regulating cortisol secretion during the pre-awakening period are different from those regulating secretion after awakening (Clow et al., 2010). Therefore, in future research, it would be of interest to conduct a systematic investigation of the effects of light exposure on the inflammatory system before awakening.

Additionally, the sample excluded female participants in an attempt to isolate the relationship between light exposure and the CAR and IL-6 response. There is however a larger discussion of the immunological differences between males and females. While evidence of sex differences in IL-6 concentrations is inconsistent (Chapman et al., 2009; Lockwood et al., 2016), there is a fundamental difference in hormones that may contribute to female susceptibility to increased levels of IL-6, specifically under stressful conditions (Chapman et al., 2009). Lockwood et al. (2016) further show significant differences among acute and systemic inflammation between males and females. While this study is a first step in understanding the mechanisms behind light exposure and IL-6 cytokine response, there is much more to explore, in particular the effects in female participants.

A further limitation of the study design is the fact that no acclimatization night was planned for the participants. Thus, novelty effects during the first night might have influenced the CAR and cytokine concentrations. Although a balanced design was used, it is unlikely that this led to systematic influences on the study results, however, an increase in error variance would be possible. This point should be considered in future research. Additionally, while there was no significant difference in baseline cortisol measurements between the dim and bright light conditions, baseline IL-6 measurements did however differ significantly between both groups, even though participants were randomly assigned to them. This finding could be explained by participants in the bright light condition already being exposed to bright light before the first blood sample was taken, a circumstance that could have influenced their baseline IL-6 values. As individual trends were of interest for this study and baseline measurements were statistically controlled for as part of the relevant analyses, it is not to be expected that this difference in baseline IL-6 values had any impact disadvantageous to the reported findings. Still, researchers of future studies should consider factors that might influence baseline values and their importance in the respective research context carefully. For instance, one such factor might be individuals' chronotype: In a laboratory setting with controlled bed and wake-up times, participants with different inclinations towards morningness or eveningness might be at a different stage in their circadian rhythm at the time of testing. Again, as changes in CAR and IL-6 as a result of light exposure were of interest for this study, such a potential influence was not deemed relevant to the study findings. Furthermore, the chronotype of participants were collected but these results are a part of a forthcoming publication with a separate analysis focus. This interplay between different pro-inflammatory parameters as well as anti-inflammatory parameters should be an object of future research.

In conclusion, the study results replicate expected effects of bright and dim light exposure on cortisol concentrations after waking, where bright light has a stimulatory effect on cortisol in comparison to a dim light condition. In regard to the pro-inflammatory cytokine response, the IL-6 concentrations showed the expected decrease over time in both light conditions, however the effects of bright and dim light are less clear. Bright light exposure had a potential stimulatory effect on IL-6 production, which contradicts the expectations expressed beforehand. Further research is required to understand the interplay between cortisol and IL-6 in addition to light exposure.

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Declaration of competing interest

None.

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References

Berson, D.M., Dunn, F.A., Takao, M., 2002. Phototransduction by retinal ganglion cells that set the circadian clock. Science 295, 1070–1073. https://doi.org/10.1126/science.1067262 (80-.).

Brainard, G.C., Hanifin, J.R., 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, 6405–6412. https://doi.org/10.1523/jneurosci.21-16-06405.2001.

Brodin, P., Davis, M.M., 2017. Human immune system variation. Nat. Rev. Immunol. 17, 21–29.
Chapman, B.P., Khan, A., Harper, M., Stockman, D., Fiscella, K., Walton, J., Duberstein, P., Talbot, N., Lyness, J.M., Moynihan, J., 2009. Gender, race/ethnicity, personality, and interleukin-6 in urban primary care patients. Brain Behav. Immun. 23, 636–642. https://doi.org/10.1016/j.bbi.2008.12.009.
Cheong, K.A., Kim, C., Choi, Y., Park, C. Do, Lee, A.-Y., 2012. Irradiation of light emitting diode at 850nm inhibits T cell-induced cytokine expression. J. Dermatol. Sci. 65, 27–37. https://doi.org/10.1016/j.jdermsci.2011.10.005.
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. https://doi.org/10.1016/j.neubiorev.2009.12.011.
Clow, A., Thorn, L., Evans, P., Hucklebridge, F., 2004. The awakening cortisol response: methodological issues and significance. Stress 7, 29–37. https://doi.org/10.1080/10253890410001667205.
Coutinho, A.E., Chapman, K.E., 2011. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol. Cell. Endocrinol. https://doi.org/10.1016/j.mce.2010.04.005.
Dacey, D.M., Liao, H.W., Peterson, B.B., Robinson, F.R., Smith, V.C., Pokomy, 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, 749–754. https://doi.org/10.1038/nature03387.
DeRijk, R., Michelson, D., Karp, B., Petrides, J., Galliven, E., Deuster, P., Paciotti, G., Gold, P.W., Sternberg, E.M., 1997. Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNFα) production in humans: high sensitivity of TNFα and resistance of IL-6. J. Clin. Endocrinol. Metab. 82, 2182–2191. https://doi.org/10.1210/jcem.82.7.4041.
Dickmeis, T., 2009. Glucocorticoids and the circadian clock. J. Endocrinol. 200, 3–22. https://doi.org/10.1677/JOE-08-0415.
Dickstein, J., Moldofsky, H., 1999. Sleep, cytokines and immune function. Sleep Med. Rev. 3, 219–228. https://doi.org/10.1016/S1087-0792(99)90003-5.
Enezi, J. al, Revell, V.L., Brown, T., Wynne, J., Schlangen, L., Lucas, R.J., 2011. A "melanopic" spectral efficiency function predicts the sensitivity of melanopsin photoreceptors to polychromatic lights. J. Biol. Rhythm. 26, 314–323. https://doi.org/10.1177/0748730411409719.
Figueiro, M.G., Rea, M.S., 2012. Short-wavelength light enhances cortisol awakening response in sleep-restricted adolescents. Internet J. Endocrinol. https://doi.org/10.1155/2012/301935, 2012.
Franzin, A., Sambo, F., Di Camillo, B., 2016. bnstruct: an R package for Bayesian Network structure learning in the presence of missing data. Bioinformatics btw807. https://doi.org/10.1093/bioinformatics/btw807.
Gudewill, S., Pollmächer, T., Vedder, H., Schreiber, W., Fassbender, K., Holsboer, F., 1992. Nocturnal plasma levels of cytokines in healthy men. Eur. Arch. Psychiatr. Clin. Neurosci. 242, 53–56.
Hankins, M.W., Peirson, S.N., Foster, R.G., 2008. Melanopsin: an exciting photopigment. Trends Neurosci. 31, 27–36. https://doi.org/10.1016/j.tins.2007.11.002.
Hattar, S., 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295, 1065–1070. https://doi.org/10.1126/science.1069609 (80-.).
Hattar, S., Lucas, R.J., Mrosovsky, N., Thompson, S., Douglas, R.H., Hankins, M.W., Lem, J., Biel, M., Hofmann, F., Foster, R.G., Yau, K.W., 2003. Melanopsin and rod–cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424, 75–81. https://doi.org/10.1038/nature01761.
Herhaus, B., Ullmann, E., Chrousos, G., Petrowski, K., 2020. High/low cortisol reactivity and food intake in people with obesity and healthy weight. Transl. Psychiatry 10 (1). https://doi.org/10.1038/s41398-020-0729-6.
Ishida, A., Mutoh, T., Ueyama, T., Bando, H., Masubuchi, S., Nakahara, D., Tsujimoto, G., Okamura, H., 2005. Light activates the adrenal gland: timing of gene expression and glucocorticoid release. Cell Metabol. 2, 297–307. https://doi.org/10.1016/j.cmet.2005.09.009.
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, 185–191. https://doi.org/10.1016/S0165-0270(00)00364-2.
Jung, C.M., Khalsa, S.B.S., Scheer, F.A.J.L., Cajochen, C., Lockley, S.W., Czeisler, C.A., Wright, K.P., 2010. Acute effects of bright light exposure on cortisol levels. J. Biol. Rhythm. 25, 208–216. https://doi.org/10.1177/0748730410368413.
Jung, S.J., Kim, N.N., Choi, Y.J., Choi, J.Y., Choi, Y.-U., Heo, Y.S., Choi, C.Y., 2016. Effects of melatonin and green-wavelength LED light on the physiological stress and immunity of goldfish, Carassius auratus, exposed to high water temperature. Fish Physiol. Biochem. 42, 1335–1346. https://doi.org/10.1007/s10695-016-0221-7.
Kelley, K., 2020. MBESS: the MBESS R Package.
Kim, N.N., Choi, Y.J., Shin, H.S., Lim, J.R., Han, J.M., Cho, J.H., Lee, J., Kil, G.S., Choi, C. Y., 2014. The effect of LED light spectra on antioxidant system by thermal stress in goldfish, Carassius auratus. Mol Cell Toxicol 10, 47–58. https://doi.org/10.1007/s13273-014-0006-y.
Lall, G.S., Revell, V.L., Momiji, H., Al Enezi, J., Altimus, C.M., Güler, A.D., Aguilar, C., Cameron, M.A., Allender, S., Hankins, M.W., Lucas, R.J., 2010. Distinct contributions of rod, cone, and melanopsin photoreceptors to encoding irradiance. Neuron 66, 417–428. https://doi.org/10.1016/j.neuron.2010.04.037.
Lawrence, M.A., 2016. ez: Easy Analysis and Visualization of Factorial Experiments.
Lockwood, K.G., Marsland, A.L., Cohen, S., Gianaros, P.J., 2016. Sex differences in the association between stressor-evoked interleukin-6 reactivity and C-reactive protein. Brain Behav. Immun. 58, 173–180. https://doi.org/10.1016/j.bbi.2016.07.001.
Moldofsky, H., 1994. Central nervous system and peripheral immune functions and the sleep-wake system. J. Psychiatry Neurosci. 19, 368–374.
Morita, T., Tokura, H., 1998. The influence of different wavelengths of light on human biological rhythms. APPLIED HUMAN SCIENCE Journal of Physiological Anthropology (Appl Human Sci.
Petrovsky, N., 2001. Towards a unified model of neuroendocrine-immune interaction. Immunol. Cell Biol. 79, 350–357. https://doi.org/10.1046/j.1440-1711.2001.01029.x.
Petrovsky, N., Harrison, L.C., 1997. Diurnal rythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type1/T helper cell type 2 balance. J. Immunol. 158, 5163–5168.
Petrovsky, N., McNair, P., Harrison, L.C., 1998. Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. Cytokine 10, 307–312. https://doi.org/10.1006/cyto.1997.0289.
Petrowski, K., Buehrer, S., Niedling, M., Schmalbach, B., 2020. The effects of light exposure on the cortisol stress response in human males. Stress 1–20. https://doi.org/10.1080/10253890.2020.1741543.
Petrowski, K., Schmalbach, B., Niedling, M., Stalder, T., 2019. The effects of post-awakening light exposure on the cortisol awakening response in healthy male individuals. Psychoneuroendocrinology 108, 28–34. https://doi.org/10.1016/J.PSYNEUEN.2019.05.016.
Provencio, I., Rollag, M.D., Castrucci, A.M., 2002. Photoreceptive net in the mammalian retina. Nature 415. https://doi.org/10.1038/415493a, 493–493.
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, 916–931. https://doi.org/10.1016/S0306-4530(02)00108-7.
Scheiermann, C., Kunisaki, Y., Frenette, P.S., 2013. Circadian control of the immune system. Nat. Rev. Immunol. 13 https://doi.org/10.1038/nri3386.
Scheller, J., Chalaris, A., Schmidt-Arras, D., Rose-John, S., 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim. Biophys. Acta Mol. Cell Res. 1813, 878–888. https://doi.org/10.1016/j.bbamcr.2011.01.034.
Shen, Y., Xie, C., Gu, Y., Li, X., Tong, J., 2016. Illumination from light-emitting diodes (LEDs) disrupts pathological cytokines expression and activates relevant signal pathways in primary human retinal pigment epithelial cells. Exp. Eye Res. 145, 456–467. https://doi.org/10.1016/j.exer.2015.09.016.
Stalder, T., Kirschbaum, C., Kudielka, Brigitte M., et al., 2016. Psychoneuroendocrinology 63. https://doi.org/10.1016/j.psyneuen.2015.10.010.
Steensberg, A., Fischer, C.P., Keller, C., Møller, K., Pedersen, B.K., 2003. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am. J. Physiol. Endocrinol. Metab. 285, E433–E437. https://doi.org/10.1152/ajpendo.00074.2003.
Tähkämö, L., Partonen, T., Pesonen, A.-K., 2019. Systematic review of light exposure impact on human circadian rhythm. Chronobiol. Int. 36, 151–170. https://doi.org/10.1080/07420528.2018.1527773.
Temme, L.A., Frumkes, T.E., 1977. Rod-cone interaction in human scotopic vision—III: rods influence cone increment thresholds. Vis. Resour. 17, 681–685. https://doi.org/10.1016/S0042-6989(77)80002-3.
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. https://doi.org/10.1016/j.psyneuen.2003.08.005.
Tu, B.P., 2005. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science 310, 1152–1158. https://doi.org/10.1126/science.1120499 (80-.).
Ulrich-Lai, Y.M., Arnhold, 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. Integr. Comp. Physiol. 290, R1128–R1135. https://doi.org/10.1152/ajpregu.00042.2003.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag.
Wilhelm, I., Born, J., Kudielka, B.M., Schlotz, W., Wüst, S., 2007. Is the cortisol awakening rise a response to awakening? Psychoneuroendocrinology 32, 358–366. https://doi.org/10.1016/j.psyneuen.2007.01.008.
Xavier, M., David, D.R., de Souza, R.A., Arrieiro, A.N., Miranda, H., Santana, E.T., Silva, J.A., Salgado, M.A.C., Aimbire, F., Albertini, R., 2010. Anti-inflammatory effects of low-level light emitting diode therapy on achilles tendinitis in rats. Laser Surg. Med. 42, 553–558. https://doi.org/10.1002/lsm.20896.