Cortical Excitability is Affected by Light Exposure – Distinct Effects in Adolescents and Young Adults

Roya Sharifpour[1*], Fermin Balda Aizpurua[1], Ilenia Paparella[1], John Read[1], Zoé Leysens[1], Sara Letot[1], Islay Campbell[1], Elise Beckers[1,2], Fabienne Collette[1], Christophe Phillips[1], Mikhail Zubkov[1], Gilles Vandewalle[1*]

[1] GIGA-CRC Human Imaging, University of Liège, Liège, Belgium.

[2] Alzheimer Centre Limburg, Mental Health and Neuroscience Research Institute, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands.

*Corresponding authors: Gilles Vandewalle & Roya Sharifpour; GIGA-CRC Human Imaging, University of Liège, Liège, Belgium.

Key words: Blue-Light, Non-Image-Forming, TMS-EEG, Cortical excitability, Adolescents

Author Contributions: R.S. and G.V. designed the research. R.S, F.B.A, I.P. J.R., Z.L. and S.L. acquired the data. All authors provided valuable insights while acquiring, interpreting, and discussing the data. R.S. analyzed the data supervised by G.V. R.S. and G.V. wrote the paper. All authors edited and approved the final version of the manuscript.

The protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège and all participants received monetary compensation for their participation.

ABSTRACT

Light, particularly blue-wavelength light, exerts a broad range of non-image forming (NIF) effects including the stimulation of cognition and alertness and the regulation of mood, sleep and circadian rhythms. However, its underlying brain mechanisms are not fully elucidated. Likewise, whether adolescents show a different NIF sensitivity to light compared to adults is not established. Here, we investigated whether cortical excitability, a basic aspect of brain function that depends on sleep-wake regulation, is affected by blue light and whether the effect is similar in young adults and adolescents. To do so, we used transcranial magnetic stimulation coupled to high-density electroencephalography (TMS–EEG) in healthy young adults (N=13, 24.2y ±3.4) and in adolescents (N=15, 16.9y ±1.1). Our results showed that, in young adults, blue light affected cortical excitability following an apparent inverted-U relationship, while adolescents' cortical excitability was not significantly impacted by blue light. In addition, although light did not affect performance on a visuomotor vigilance task completed during the TMS-EEG recordings, cortical excitability was positively correlated to task performance in both age groups. This study provides valuable insights into the complex interplay between light, cortical excitability, and behavior. Our findings highlight the role of age in NIF effects of light, suggesting that brain responses to light differ during developmental periods.

INTRODUCTION

Besides enabling vision, light exerts a broad range of physiological, hormonal, and neurobehavioral effects not directly related to vision that are often referred to as non-image forming (NIF) effects of light[1,2]. NIF effects are primarily mediated by a subclass of retinal ganglion cells (RGCs) that act as photoreceptors by expressing the photopigment melanopsin[1,2]. These intrinsically photosensitive RGCs (ipRGCs) combine rods and cones outputs to their own response to light and funnel light signal to many (subcortical) parts of the brain involved in the regulation of the circadian system, sleep need, mood and cognition[3–6]. Since melanopsin is maximally sensitive to short wavelength blue light, at around 480 nm, NIF impacts of light on brain functions are mostly driven by the blue-wavelength content of light[1,2]. A basic aspect of the brain functioning is cortical excitability that can be defined as the responsiveness and response selectivity of cortical neurons[7]. It is therefore fundamental to cognitive brain functions and dictates the impact of an incoming stimulus on cortical activity and on behavior. Cortical excitability depends on prior sleep-wake history and circadian phase[8–10]. It remains relatively stable during the day while well rested and sharply increases if one stays awake[10] such that overnight increase of cortical excitability is correlated to performance decrement[8] and is therefore likely to contribute to the detrimental effect of sleep loss on cognition. The relationship between performance and cortical excitability may follow an inverted U-shape function where an optimal mid-range level of excitability, likely happening during the day when well-rested[11], is associated with optimal performance while lower or higher levels lead to poor cognitive outcomes. Cortical excitability depends on several environmental factors that directly or indirectly affect alertness or sleepiness level, including alcohol and potentially caffeine consumption[12–14]. It is therefore plausible that ambient light could also affect cortical excitability. Findings of a small scale study, carried out close to 3 decades ago on seven young adults(21-25y), suggested in fact, that there might be an inverted U-shape relationship between light-induced increase in arousal and indirect measure of

cortical excitability inferred from an evoked EEG response[15]. Besides this early report, whether exposure to light affects cortical excitability is not established.

Adolescence may be of particular interest when focusing on NIF effects of light. Optimizing the lighting in classrooms has indeed been proposed as a promising simple means to improve alertness and performance at school[16,17]. Research also reported that NIF effects depend on the prior light history, such that prior exposure to high light levels over the preceding hours, days or even weeks, may reduce NIF effects[18,19]. Adolescents are high consumers of LED devices, typically enriched in blue light, and may spend more time outdoors, they may therefore present a reduction in NIF effects of light on brain function. Adolescents, however, are characterized by a late chronotype (i.e. intrinsic time-of-day preference to be physically and/or mentally active), and late chronotypes in adults are typically associated with a higher sensitivity to NIF impact of light[20] that can lead to an increase in NIF effects.

Although it is established that exposure to light affects cognitive brain functions[1], whether spectral composition of light impacts cortical excitability, and whether it happens differently in adolescents versus young adults, is not yet established. Such questions need to be resolved before one can use the flexibility of LEDs to design truly individualized human-centered lighting.

In the present study, we used transcranial magnetic stimulation coupled with high-density electroencephalography (TMS-EEG), as a non-invasive tool to assess cortical excitability *in vivo* in 13 healthy young adults aged 19-30y while they were exposed to light of different illuminance levels (as computed in Melanopic Equivalent Daylight Illuminance - melEDI lux). We further assessed whether cortical excitability was correlated to the performance on a concomitant visuomotor vigilance task. Data were also collected in 15 healthy adolescents aged 15-18y to determine whether sensitivity to the impact of light on cortical excitability was altered in this age group. We hypothesized that in young adults, cortical excitability would be increased by increasing melanopic

illuminance and performance on the task would be correlated with cortical excitability. We further anticipated that the impact of light would be different in adolescents with no prior expectation on the directionality of the difference.

Method

Participants

Between March-2022 and September-2023, thirty-six healthy volunteers participated in the study, 19 adolescents (16.8±1.0 years old, 6 Females) and 17 young adults (24.9±3.9 years old, 9 Females). The study was approved by the Ethics Committee of the University of Liege. Participants gave their written informed consent and received financial compensation for their participation. Exclusion criteria were as follows: BMI>28; recent psychiatric history; severe trauma; sleep disorders; addiction; chronic medication; smoking, excessive alcohol (>14 units/week) or caffeine (>3 cups/day) consumption; night shift work during the last year; transmeridian travel during the past 2 months; anxiety[21] or depression[22]; poor-sleep quality[23]; excessive self-reported daytime sleepiness[24]; extreme late/early chronotypes[25]; and pregnancy.

Seven participants were excluded from the analyses due to poor data quality (i.e. TMS artefact masking the response of interest) and one participant was excluded due to both cortical excitability metrics > 3 standard deviations (SD) compared to the rest of the sample(outlier). Thus, the data presented here includes twenty-eight participants (15 adolescents and 13 young adults). **Table1** summarizes the demographic characteristics of the final sample.

Table 1. *Demographic characteristics of the participants included in the analyses.*

	Adolescents	Adults	Comparison (t-test)
Number of Subjects	15	13	
Age (y)	16.9±1.1	24.2±3.4	
Sex: Female (Male)	4 (11)	5 (8)	P=0.52
Body mass index (kg/m2)	21.8±2.4	22.4±2.3	P=0.45
Right-handed	15/15	13/13	P=1.00
Caffeinated drinks (cup/day)	0.4±0.7	1.1±0.9	**P=0.03**
Alcohol (unit/week)	2.4±4.3	2.2±2.0	P=0.86
Depression (BD-II[a])	7.4±4.7	5.1±3.0	P=0.36
Anxiety Level (BAI[b])	6.5±6.0	5.9±3.0	P=0.77
Chronotype (HO[c])	42.5±8.3	42.6±8.0	P=0.98
Sleep time (night before the TMS session)	23:08±0:41	23:12±0:46	P=0.65
Wake time (day of the experiment)	7:08±0:50	7:46±1:09	P=0.19
Sleep duration (h) (night before the TMS session)	7.6±1.2	8.3±1.2	P=0.13
Sleep Quality (PSQI[d])	4.3±1.5	4.7±1.6	P=0.83
Habitual daytime Sleepiness (ESS[e])	5.9±3.9	6.9±2.8	P=0.45
Insomnia (ISI[f])	6.0±3.0	6.7±5.1	P=0.70
Season of experiment *	-0.2±0.6	-0.2±7	P=0.90
Seasonality (SPAQ[g])	1.1±1.0	1.2±0.8	P=0.83
Eye color: Bright (Dark) **	11 (4)	7 (6)	P=0.30
Electrical Field (Mean±SD)	115.0±16.7	109.3±10.4	P=0.08
Distance (Mean±SD) ***	33.0±8.5	32.1± 8.9	P=0.67

[a]. Beck's Depression Inventory II
[b]. Beck's Anxiety Inventory
[c]. Horne and Östberg Questionnaire
[d]. Pittsburg Sleep Quality Index
[e]. EPWORTH Sleepiness Scale
[f]. Insomnia Severity Index

[g]. Seasonal Pattern Assessment Questionnaire
*Cosine (acquisition day of year*360/365) : 21 December=0°
**Bright: Green and Blue ; Dark: Grey, Brown and Black
*** Distance (in mm) between the electrode of interest and the stimulation spot (maximum electrical field)
Sleep and wake times assessed based on the actimetry data.

Experimental protocol

To avoid excessive sleep restriction while maintaining real-life conditions, participants were asked to keep a regular sleep–wake schedule (±1h), in agreement with their preferred bed and wake-up times, for five days preceding the in-lab experiment. Compliance was verified using sleep diaries and wrist actigraphy (Actiwatch,Cambridge Neurotechnology,UK). Additional methodological information can be found as supplementary method.

On the experiment day, participants arrived at the laboratory between 13:00-14:00. To control for recent light history, participants were maintained in dim-light (<10 lux) for 1:28±0:27, during which the optimal TMS parameters (i.e., location, intensity, coil orientation) providing artifact free TMS-EEG recordings were determined and set. The stimulation target was set to the superior frontal gyrus (SFG) on the dominant hemisphere (left hemisphere as all our participants were right-handed). This brain area was chosen for the following reasons: (1) the SFG is highly sensitive to sleep pressure, including at the neuronal level, just like the entire frontal lobe[26] ; (2) it plays an important role in cognitive performance[27]; and (3) its stimulation does not cause muscle activation, which is a source of EEG signal contamination.

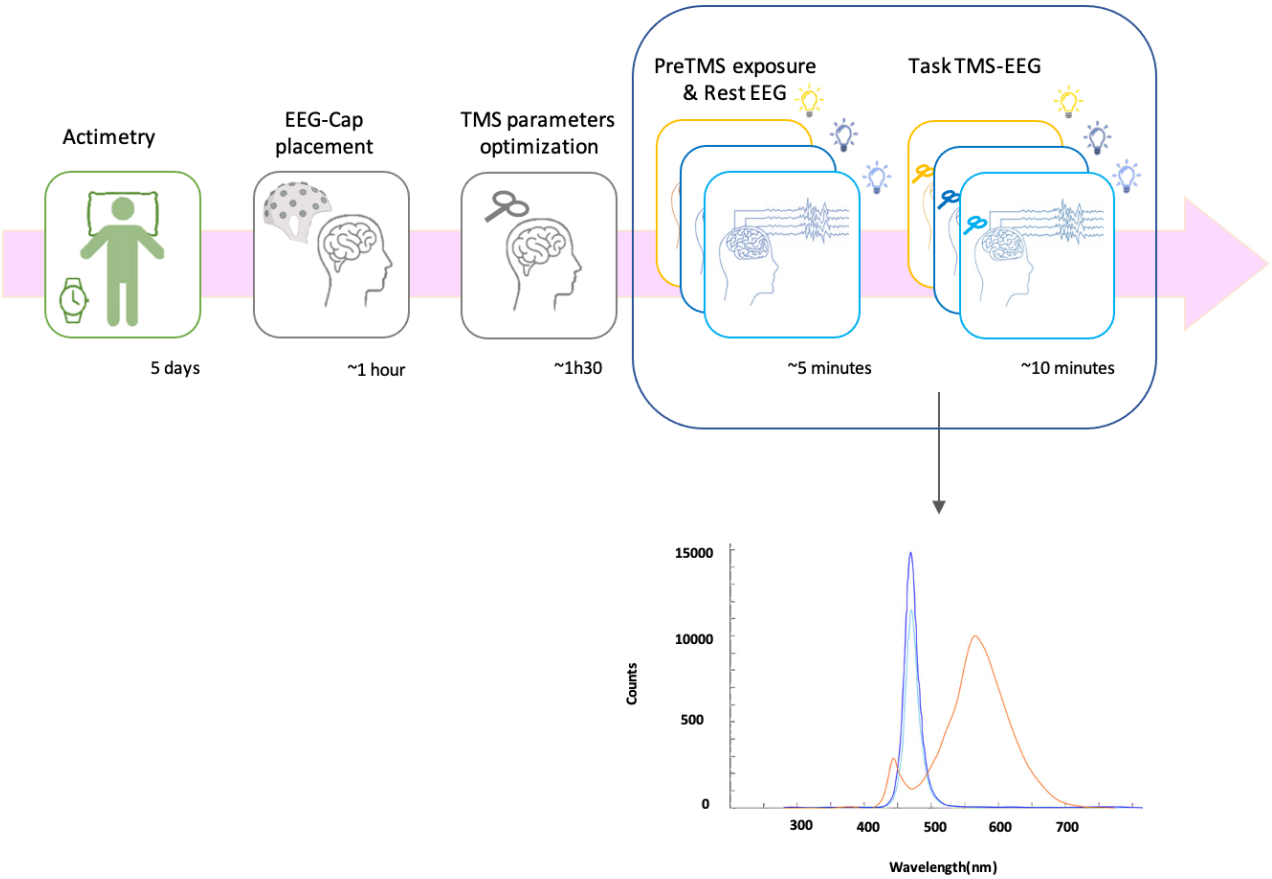

***Figure 1**: Graphical representation of the protocol and the spectrums of light conditions: Polychromatic orange light at 24 melEDI lux; Monochromatic lower-intensity blue light at 312 melEDI lux, and monochromatic higher-intensity blue light at 625 melEDI lux.*

Light set-up and protocol

The light source used in this study was a tunable 35cm×45cm light LED box (EOS,Balder,Huy,Belgium) which allowed us to create three light conditions including orange light served as control light condition, and active lower-intensity and higher-intensity blue light conditions. The illuminance level of each light condition was adjusted manually for each participant such that the orange light and lower-intensity blue light conditions had the same illuminance of 30 lux at the eye level while the illuminance for the higher-intensity blue light condition was increased to 60 lux (**Table S1**). **Figure 1** shows the spectrums of the three light conditions.

Each light session began with 1-2 mins of light adjustment followed by a two-minute eye-open waking EEG recording. In each Light session, TMS-EEG recording pre-exposure time was approximately

5-mins, and the entire TMS-EEG recording time was ~10 mins. The order of the orange and lower-intensity blue sessions was randomized, with the higher-intensity blue session always being last. The main question of this study was to determine the impact of blue light on cortical excitability compared to the orange light with the same photopic illuminance. Therefore, given the long duration of the entire protocol (around 5 hours) and the possibility of teenage volunteers becoming fatigued and withdrawing early, before the last session of the experiment (happened for one subject), the order of the sessions was decided as explained so that we would anyway have the two sessions needed to answer our main research question. The three sessions were separated by at least a 15-minute washout period in dim light (<10 lux).

TMS-EEG acquisition

A Focal Bipulse 8-shape coil (Eximia; Nexstim,Helsinki,Finland) was used to generate TMS pulses (Suppl.Method). The stimulation target (SFG) was located on individual structural MRI using a neuronavigation system (Navigated Brain Stimulation; Nexstim, Helsinki, Finland).

Each session included around 250 TMS stimulations. The interstimulus interval varied randomly from 1900 to 2200ms. The coil recharging time was set at 900ms after TMS. TMS-evoked responses (TEP) were recorded using a 60-channel TMS-compatible EEG amplifier (Eximia; Nexstim, Helsinki, Finland). The reference and ground electrodes were placed on the forehead. Two extra bipolar electrodes were also used to record the electrooculogram (EOG).

Wake EEG acquisition

Prior to each TMS session, eye-open rest-waking EEG was recorded using the same 60-channel TMS-compatible EEG (+2 EOGs) amplifier. Participants were asked to fixate on a black dot, placed on the light box in front of them, for 2 mins while relaxing and avoiding excessive blinking.

Visuospatial vigilance task

Participants were instructed to perform a compensatory tracking task (CTT)[28] during the TMS-EEG recordings. Using a trackball device, the aim was to maintain a constantly moving, randomly positioned cursor on a fixed target in the center of a black background on a computer screen that was set to the lowest brightness (Suppl.Method).

TMS-EEG analysis

TMS-EEG data preprocessing (Suppl.Method) was performed using MNE python package[29] (https://mne.tools/stable/index.html). Independent components of the preprocessed EEG recordings were computed using the fastICA algorithm[30] in order to remove clear TMS-induced artifacts (Suppl.Method).

Cortical excitability was inferred from the amplitude and slope of the first EEG component (0–35 ms) of the TEP measured at the artifact-free electrode closest to the hotspot (i.e. the location with highest TMS-induced electrical field estimated by the neuronavigation system). The latter electrode was always located in the stimulated brain hemisphere. It could vary across participants but remained constant at individual level.

Wake EEG analysis

Power spectral densities were computed using a fast Fourier transform on artifact-free 4s windows, overlapping by 2s, using the Welch's method (pwelch function in MATLAB 7.5.0) (Suppl.Method). EEG activity was computed over frontal region(F3,F1,Fz,F2,F4,FC5,FC3,FC1,FCz,FC2,FC4,FC6) for theta(4.25–8 Hz) and alpha(8.25-12 Hz) frequency bands over the entire 2-min recording.

Statistical analysis

All statistical analyses were performed with SAS version 9.4 (SAS Institute,Cary,NC,USA).

Generalized linear mixed models (GLMM; PROC GLIMMIX SAS procedure) were applied separately in each age group on TEP amplitude, TEP slope, power spectra and task performance as dependent variables according to their distribution (Suppl.Method), with subject (intercept and slope) effect included as a random factor, light conditions (3 illuminance levels) as repeated measures with autoregressive correlation type 1 (ar(1)) and session-order, sex , age, and BMI as covariates. Direct *post hoc* tests of the main analyses were corrected for multiple comparisons using a Tukey adjustment. In each age groups, additional models included the following potential confound variables separately: chronotype, seasonality, depression and anxiety indices, eye color, season, sleep duration (prior night), daytime sleepiness, sleep quality, insomnia index, subjective sleepiness before and after each session, average habitual caffein and alcohol consumption, electrode of interest distance from hotspot or alpha/theta power. For simplicity' sake, GLMMs were first performed on each age group separately and then in another GLMM, age group and illuminance-by-age group interaction terms were included to seek statistical differences between the groups.

Results

Cortical excitability increases under moderate blue light exposure and is correlated to performance on the vigilance task in young adults.

We extracted cortical excitability metrics as both the amplitude and slope of the earliest EEG response evoked by the TMS pulses (TEP; 0–35ms post-TMS)[10], measured at the electrode closest to the hotspot. Statistical analyses employing both metrics separately as dependent variable revealed a significant main effect of the light condition ($p<.02$), and no main effect of the other covariates (**Figure 2; Table 2**). *Post hoc* contrasts showed that compared to the orange light(24 melEDI lux) , both the amplitude and the slope of the TEP were increased during the lower-intensity (312 melEDI lux) blue light exposure (amplitude: $t=-3.05$, $p=.005$; slope: $t=-2.58$, $p=.016$) but not during the higher-intensity (624 melEDI lux) blue light (amplitude: $t=-1.11$, $p=.27$; slope: $t=-1.00$, $p= .33$). TEP

amplitude during the later 624 melEDI lux blue light exposure showed a statistical trend (t=1.94, p=0.06) to be lower compared to 312 melEDI lux blue light, while no difference was detected when comparing TEP slope (t=1.58,p=0.13).

Including one of the following potential confounding factors in separate models did not alter the findings: chronotype, seasonality, depression and anxiety indices, eye color, season (in which the sessions were performed), sleep duration (prior night), daytime sleepiness, sleep quality, insomnia index, subjective sleepiness before and after each session and average habitual caffein and alcohol consumption.

Each TMS-EEG recording was initiated after 5-min of light exposure and lasted about 11 mins (11.5±1.2min). They were preceded by a 2-min recording of the spontaneous EEG activity while quietly awake (1-to-3 mins post-light onset). We computed the power of the EEG over the theta (4.25-8Hz) and alpha (8.25-12Hz) bands as markers of sleepiness/sleep need and alertness, respectively[31,32]. Statistical analyses yielded no significant variation in theta or alpha power across the light conditions (**Table 2**) indicating that baseline brain activity did not differ between the light conditions. Importantly, including theta and alpha power as covariates in our main GLMM did not modify our main statistical outputs (**Table 2**).

Table 2. *Statistical outcomes of GLMMs with the cortical excitability metrics and theta/alpha spectral power versus the light melEDI and covariates.*

Adults

	Light Condition	Session	Age	Sex	BMI
TEP Amplitude	F(1,23)=8.73 **P = 0.007** **R^2 = 0.27**	F(1,23)=0.46 P = 0.50	F(1,9)=0.50 P = 0.50	F(1,9)=0.05 P = 0.83	F(1,9)=0.05 P = 0.82
TEP Slope	F(1,23)=6.37 **P = 0.019** **R^2 = 0.22**	F(1,23)=0.00 P = 0.98	F(1,9)=0.80 P = 0.39	F(1,9)=0.22 P = 0.65	F(1,9)=0.01 P = 0.93
Theta Power	F(1,23)=0.04 P = 0.82	F(1,23)=0.46 P = 0.50	F(1,9)=0.97 P = 0.35	F(1,9)=1.86 P = 0.20	F(1,9)=1.07 P = 0.33
Alpha Power	F(1,23)=2.96 P = 0.10	F(1,23)=2.82 P = 0.11	F(1,9)=0.52 P = 0.49	F(1,9)=0.16 P = 0.70	F(1,9)=0.55 P = 0.48

Adolescents

	Light Condition	Session	Age	Sex	BMI
TEP Amplitude	F(1,26)=0.09 P = 0.76	F(1,26)=0.02 P = 0.90	F(1,11)=1.12 P = 0.31	F(1,11)=0.01 P = 0.93	F(1,11)=9.36 **P = 0.01** **R^2 = 0.46**
TEP Slope	F(1,26)=0.01 P = 0.92	F(1,26)=0.05 P = 0.82	F(1,11)=1.44 P = 0.25	F(1,11)=0.11 P = 0.75	F(1,11)=6.23 **P = 0.03** **R^2 = 0.36**
Theta Power	F(1,26)=0.07 P = 0.79	F(1,26)=0.17 P = 0.68	F(1,11)=1.13 P = 0.31	F(1,11)=0.16 P = 0.70	F(1,11)=0.08 P = 0.78
Alpha Power	F(1,26)=3.01 P = 0.094	F(1,26)=0.67 P = 0.42	F(1,11)=3.12 P = 0.10	F(1,11)=0.04 P = 0.85	F(1,11)=1.28 P = 0.28

In the next step we considered the performance on the visuomotor vigilance task completed during the TMS-EEG recording. All participants performed the task well as indicated by the low number of lapses (1.92±3.1). Statistics indicated that performance was not significantly affected by the light condition ($p>0.1$) and yet it was significantly correlated to both the amplitude ($R=-0.43$, $p<0.03$) and the slope ($R=-0.44$, $p<0.03$) of the TEP (**Figure 2**), such that higher cortical excitability was associated with better performance (shorter distance between the moving and fixed dots).

No changes in cortical excitability under the different light conditions in adolescents.

In adolescents, statistical analyses, using TEP amplitude or slope as dependent variable, did not reveal a significant main effect of light condition ($p>0.7$) (**Figure 3; Table 2**), nor other covariates (except BMI, $p=0.03$). Including the same potential confounding factors in separate models as in adults did not change the statistical outputs. Likewise, theta and alpha immediately prior to the TMS session did not change across the light conditions ($p>0.09$; **Table 2**). Again, similarly to adults, including theta or alpha power in the model seeking for an impact of the light condition on the cortical excitability metrics did not modify the statistical outputs of the models (**Table 2**). Examining performance on the visuomotor vigilance task, the statistics revealed similar findings to those found in young adults. Still similarly to adults, performance on the visuomotor was not significantly affected by light condition ($p>0.2$), but it showed significant correlation with both the amplitude ($R=-0.57$, $p=0.0004$) and the slope ($R=-0.50$, $p<0.0028$) of the TEPs.

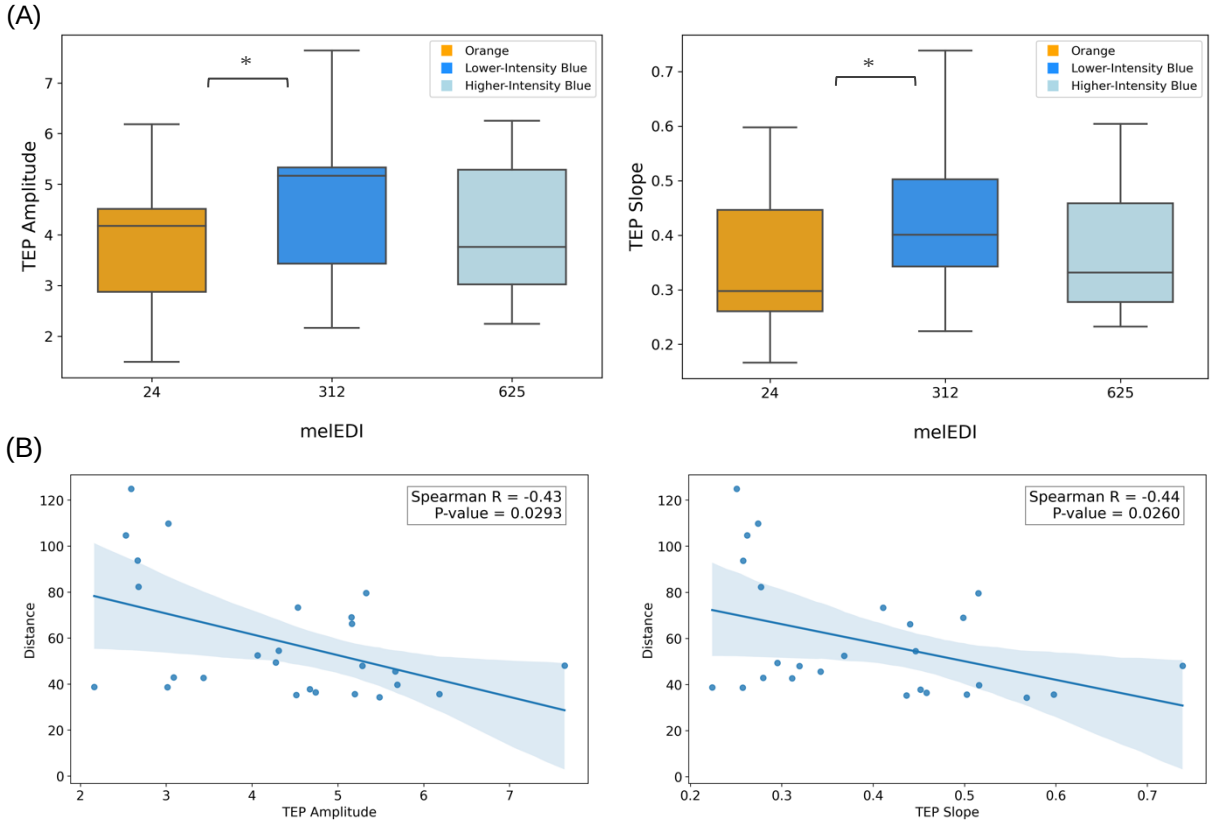

Figure 2. Results in the **young adults** group. TEP amplitude and slope under different light conditions (A). Regression plots of the association between TEP amplitude and slope with performance on the visuomotor task (B). Spearman R is indicative and do not substitute for GLMM outputs.

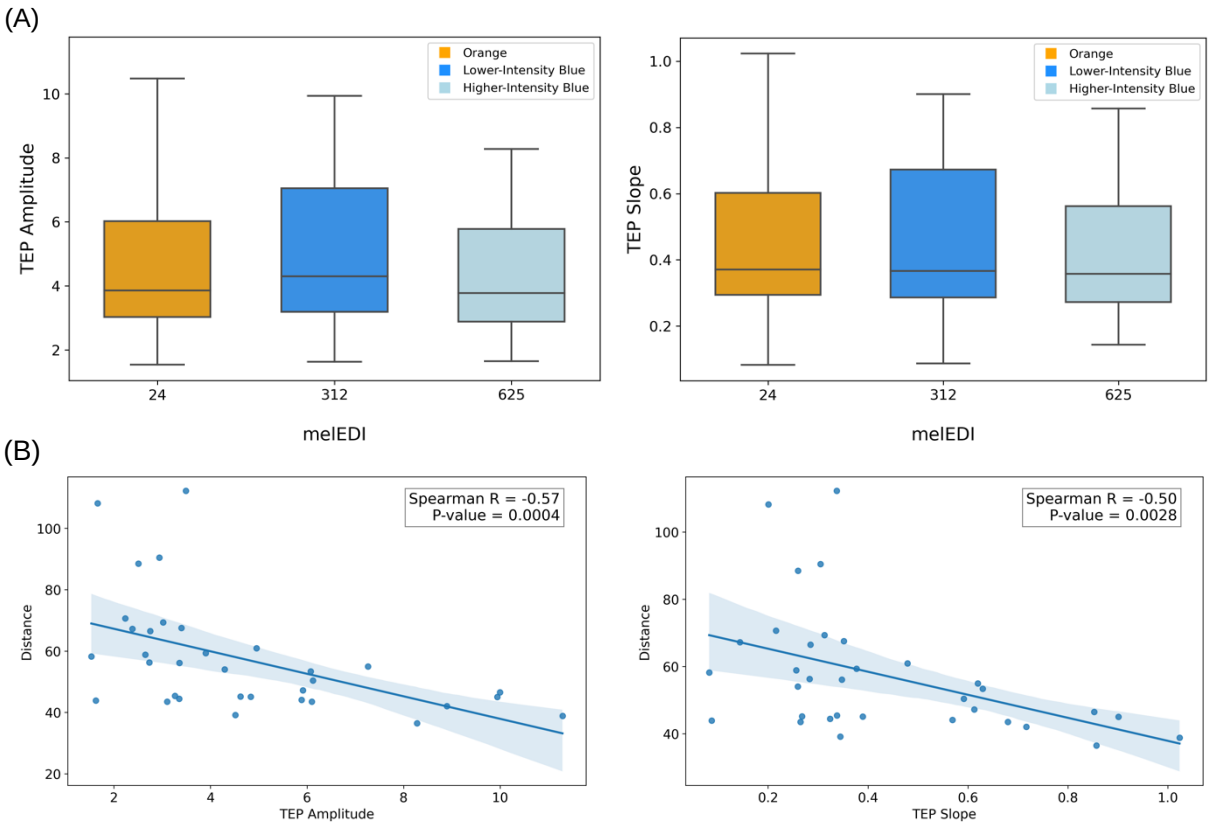

Figure 3. Results in the **adolescents** group. TEP amplitude and slope under different light conditions (A). Regression plots of the association between TEP amplitude and slope with performance on the visuomotor task (B). Spearman R is indicative and do not substitute for GLMM outputs.

In our final set of analyses, we made a direct group comparison to seek for light condition-by-age group interaction controlling for the same covariates. The GLMM including TEP amplitude as dependent variable yielded a statistical trend for both light condition ($F(1,50)=3.81$; $p=0.056$) and light condition-by-age group interaction ($F(2,50)=2.77$; $p=0.072$). Conversely, the GLMM including TEP slope as dependent variable revealed no effect of light condition ($F(1,50)=2.77$; $p=0.10$) or light condition-by-group interaction ($F(2,50)=1.68$; $p=0.20$).

Discussion

We used TMS–EEG to test whether illuminance affected cortical excitability. We showed that, in healthy young adults, the amplitude and slope of TMS evoked potential increased from lower (~24 melEDI lux) to moderate (~320 melEDI lux) melanopic illuminance, while they may decrease if melanopic illuminance is further increased (~620 melEDI lux). Despite the limited age gap between our two age groups, these effects of light were not detected in adolescents, in which light condition did not significantly affect TEPs. Intriguingly, in both age groups, performance on a visuomotor vigilance task was not affected by the light condition and was yet significantly positively related to the amplitude and slope of TEPs. Our findings add to the previously reported multiple NIF effects of light on physiology and behavior and show that cortical excitability, which is a basic and fundamental aspect of brain function, depends on environmental light. Our results also suggest that the sensitivity to NIF impact of light on cortical excitability changes from adolescence to young adulthood. Our findings further suggest that optimal cortical excitability changes over different timescales.

The increase in cortical excitability we detected in young adults between the orange and lower-illuminance blue light can only be attributed to the higher content in blue-wavelength photons of the latter light. Both light conditions were indeed equal in terms of photopic illuminance (in lux) while they differed markedly in NIF illuminance as indexed by the melEDI. This strongly suggests

that ipRGC contribute to the increase in cortical excitability we detected. Their projections to many subcortical structures involved in the regulation of alertness (and sleep), including the hypothalamus, may mediate this impact. These subcortical structure would then pass on light stimulating impact to the cortex, either directly, or indirectly through the locus coeruleus in the brainstem[33] or through the pulvinar in the thalamus[34,35] or a combination of multiple subcortical structures. We are, however, in no position to ascertain that only ipRGCs are involved in the effects we detected and stress that other retinal photoreceptors are most likely mediating at least part of the impact of light on cortical excitability, potentially through their inputs to ipRGCs[36].

Our findings in young adults are further compatible with an inverted U-shape relationship between melanopic illuminance and cortical excitability, where increasing illuminance would initially increase cortical excitability before inducing a decrease if illuminance is further increased (though as statistical trends only). This is in line with an early indirect suggestion based on EEG-evoked potential collected in a small sample, relating the impact of light to an effect on the alpha power of the quietly resting EEG [15]. This suggested that alertness change would underlie the inverted U-shape relationship between light illuminance and cortical excitability, such that an optimal range of alertness would be associated with an optimal range of excitability. We did not observe an impact of light illuminance on alpha or theta EEG power and cannot therefore directly relate our findings to changes in alertness or sleepiness. This may be because quiet resting brain activity was recorded after only 1-min of exposure to light which may have been short to induce detectable changes. The impact of light on alertness during the day has in fact not been consistently reported [37–40] and may depend on the study protocol, light quality and the employed technique[41,42].

To guarantee that at least 2 TMS-EEG sessions were completed by all participants, which allowed for testing of our main research question (i.e. does illuminance affect cortical excitability?), the TMS-EEG session with brighter blue light exposure was always performed last. This limitation of the

protocol was partly addressed by including session order in our statistical models. We cannot exclude, however, that the trend for a decrease in cortical excitability we observed under high melanopic illuminance is not in part due to a change in time of day. Cortical excitability was indeed reported to decrease in the evening hours,2-4 hours later than our protocol[8]. Likewise, although we do not have any indication of this, the brighter blue light exposure might also have triggered more visual discomfort and contributed thereby to the decrease in cortical excitability. Replication of our findings in a larger sample size and a proper session randomization is therefore warranted to definitely establish an inverted U-shape relationship between melanopic illuminance and cortical excitability.

Contrary to our expectation, in adolescents, cortical excitability was not significantly affected by light illuminance. This could be attributed to either higher or lower sensitivity to light. The theoretical greater transparency of crystalline lens and larger pupil size of teenagers might make them more sensitive to light[43–46] such that a ceiling effect would already be induced by the orange light. However, we did not characterize the crystalline or pupil of our participants. Adolescents are also more prone to be later chronotypes when late chronotype has been suggested to increase the sensitivity to NIF impacts of light. Chronotype is however unlikely to have significantly contributed to our findings as it did not significantly differ between young adults and adolescents in our sample. On the other hand, adolescents may be less sensitive to light such that none of the light conditions we administered affected cortical excitability. Adolescents were likely exposed to more outdoor and/or artificial light over the hours or days preceding the experiment. Despite the standardization of the recent history implemented in the protocol (over the 2h30 preceding the first light exposure), this difference in light history may have reduced their sensitivity to light[18,19]. The inclusion of session recorded in dim-light or in darkness could help determining whether the light exposure we administered induced a significant change in cortical excitability or not.

Still contrary to our expectation, we did not observe a significant impact of melanopic illuminance on performance. Similar to alertness, a significant impact of light on behavior during the day is not always reported and may depend on the exact procedures of the protocols[39,40,47]. The difficulty of the visuomotor task we administered was undoubtedly low such that individual performance might have been ceiling across the light conditions. This explanation appears however unlikely to fully explain the absence of light effect on performance since we did find a positive correlation between cortical excitability with vigilance task performance. Performance varied, irrespective of the light condition, in proportion of the variation in cortical excitability. While previous studies reported that the increase in cortical excitability induced by overnight sleep deprivation is associated with poorer performance following a putative inverted U-shape[8], our findings indicate that under well-rested condition in the afternoon, higher cortical excitability is related to better performance.

There may be therefore distinct association between cortical excitability and behavior over different timescales. Sleep deprivation may increase cortical excitability far beyond its optimum level and result in poor performance, while increase in cortical excitability toward an optimum level at a given moment of the normal and well-rested waking day results in improved behavior. These relationships would be equally present in adolescents and young adults. In a smaller time scale, at a given moment of the normal waking day, light exposure, a common environmental factor, can also modulate cortical excitability following an inverted U-shape in young adults and thereby putatively affect behavior, though we provide no evidence in support of this later hypothesis. In adolescents, the impact of light on cortical excitability would be distinct and follows a pattern that remains to be established. This is because of the different sensitivity to light either due to difference in their developing physiology or in light exposure habits. These putative views are summarized in **Figure 4**.

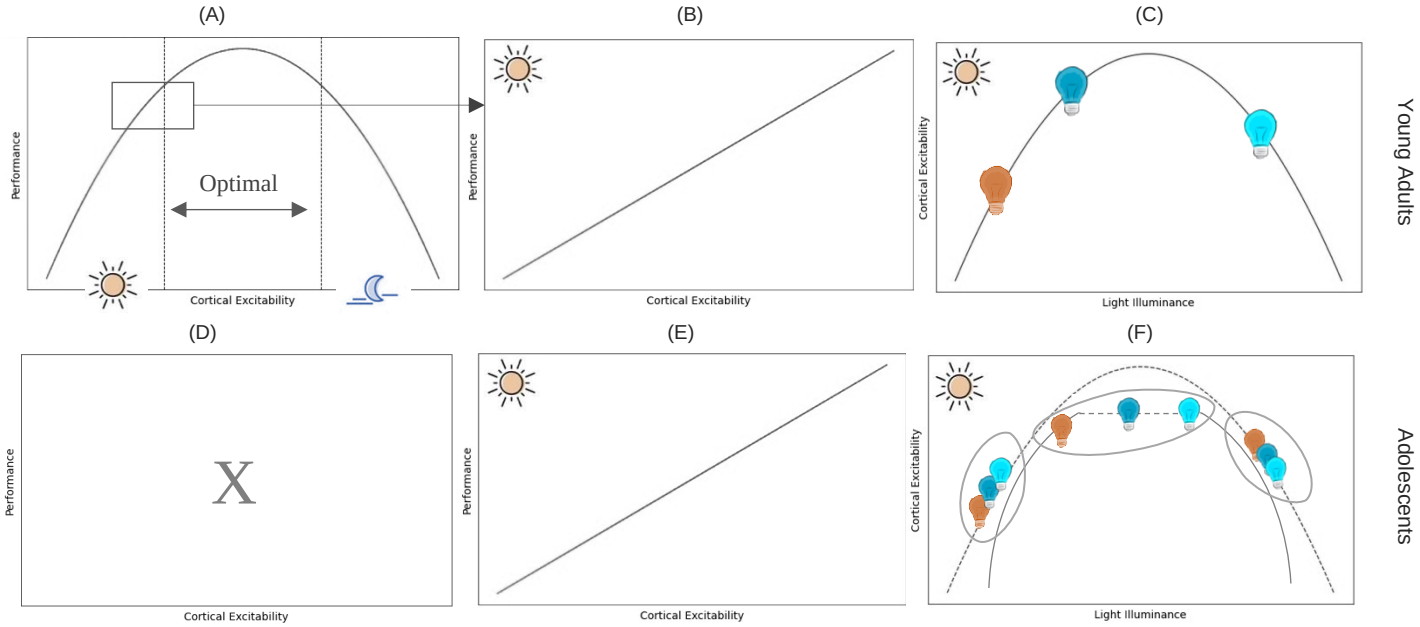

Figure 4. *Putative schematic representation of the relationships between cortical excitability, performance and light illuminance in adults (top row) and adolescents (bottom row).* ***(A)*** *In adults, cortical excitability increases with time awake, and correspondence change in performance follows an inverted-U shaped function. Under well-rested condition (current study) higher cortical excitability leads to better performance* ***(B)*** *whereas under extended wakefulness condition, higher cortical excitability has detrimental effects on performance. Our findings suggest an inverted U-shaped relationship between light illuminance and cortical excitability in adults* ***(C)****. The relationship between cortical excitability and performance is not established in adolescent* ***(D)*** *but our findings confirm a similar relationship to adults under well-rested condition (E). However, our data cannot determine the relationship between cortical excitability and light illuminance in this age group. Some of the possible scenarios have been shown* ***(F)****.*

To conclude, we provide evidence that the quality of environmental light affects brain function down to one of its very basic aspects i.e. cortical excitability. Our findings further suggest that the biological effect of light on cortical excitability is different in adolescents and young adults. and therefore, the development of light interventions in this age group, to alleviate part of their common daytime sleepiness for instance, will need fine-tuning geared toward their specific light sensitivities. The interplay between light, cortical excitability, and behavior leads to complex outcomes and our study lays the foundation for further exploration into the neurobiological effects of light on adolescents and its broader implications for cognitive processes.

Acknowledgments

The study was conducted at the GIGA-CRC technological platform of ULiège, Belgium. The authors thank Christine Bastin, Christina Schmit, Annick Claes, Christian Degueldre, Catherine Hagelstein, Gregory Hammad, Brigitte Herbillon, Patrick Hawotte, Sophie Laloux, Erik Lambot, Benjamin Lauricella, Pierre Maquet, and Eric Salmon for their help over the different steps of the study.

It was supported by the Belgian Fonds National de la Recherche Scientifique (FNRS; CDR J.0222.20), the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 860613, the Fondation Léon Frédéricq, ULiège, and the European Regional Development Fund (Biomed-Hub). None of these funding sources had any impact on the design of the study nor the interpretation of the findings. RS and FB were supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 860613. EB is supported by the Maastricht University - ULiège Imaging Valley. MZ is supported by Fondation Recherche Alzheimer (SAO-FRA 2022/0014), IC, IP, FB, JR, FC, CP and GV are supported by the FRS-FNRS.

References

1. Campbell I, Sharifpour R, Vandewalle G. Light as a Modulator of Non-Image-Forming Brain Functions—Positive and Negative Impacts of Increasing Light Availability. *Clocks and Sleep*. 2023;5(1):116-140. doi:10.3390/clockssleep5010012
2. Mahoney HL, Schmidt TM. The cognitive impact of light: illuminating ipRGC circuit mechanisms. *Nat Rev Neurosci*. 2024;25(3):159-175. doi:10.1038/s41583-023-00788-5
3. Zhang Z, Beier C, Weil T, Hattar S. The retinal ipRGC-preoptic circuit mediates the acute effect of light on sleep. *Nat Commun*. 2021;12(1):1-11. doi:10.1038/s41467-021-25378-w
4. Legates TA, Altimus CM, Wang H, et al. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature*. 2012;491(7425):594-598. doi:10.1038/nature11673
5. Altimus CM, Güler AD, Villa KL, McNeill DS, LeGates TA, Hattar S. Rods-cones and melanopsin detect light and dark to modulate sleep independent of image formation. *Proc Natl Acad Sci U S A*. 2008;105(50):19998-20003. doi:10.1073/pnas.0808312105
6. Alkozei A, Smith R, Pisner DA, et al. Exposure to blue light increases subsequent functional activation of the prefrontal cortex during performance of a working memory task. *Sleep*. 2016;39(9):1671-1680. doi:10.5665/sleep.6090
7. Rosanova M, Casarotto S, Pigorini A, Canali P, Casali AG, Massimini M. Combining transcranial magnetic stimulation with electroencephalography to study human cortical excitability and effective connectivity. *Neuromethods*. 2012;67(November 2011):435-457. doi:10.1007/7657_2011_15
8. Ly JQM, Gaggioni G, Chellappa SL, et al. Circadian regulation of human cortical excitability. *Nat Commun*. 2016;7(May). doi:10.1038/ncomms11828
9. Gaggioni G, Ly JQM, Muto V, et al. Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition. *Neurobiol Aging*. 2019;78:52-63. doi:10.1016/j.neurobiolaging.2019.02.004
10. Huber R, Mäki H, Rosanova M, et al. Human cortical excitability increases with time awake. *Cereb Cortex*. 2013;23(2):332-338. doi:10.1093/cercor/bhs014
11. Valdez P, Ramírez, García A. Circadian rhythms in cognitive performance: implications for neuropsychological assessment. *ChronoPhysiology Ther*. Published online 2012:81. doi:10.2147/cpt.s32586
12. Naim-Feil J, Bradshaw JL, Rogasch NC, et al. Cortical inhibition within motor and frontal regions in alcohol dependence post-detoxification: A pilot TMS-EEG study. *World J Biol Psychiatry*. 2016;17(7):547-556. doi:10.3109/15622975.2015.1066512
13. Kaarre O, Kallioniemi E, Könönen M, et al. Heavy alcohol use in adolescence is associated with altered cortical activity: a combined TMS–EEG study. *Addict Biol*. 2018;23(1):268-280. doi:10.1111/adb.12486
14. Zulkifly MFM, Merkohitaj O, Paulus W, Brockmöller J. The roles of caffeine and corticosteroids in modulating cortical excitability after paired associative stimulation (PAS) and transcranial alternating current stimulation (tACS) in caffeine-naïve and caffeine-adapted subjects. *Psychoneuroendocrinology*. 2021;127(January). doi:10.1016/j.psyneuen.2021.105201
15. Higuchi S, Watanuki S, Yasukouchi A, Sato M. Effects of changes in arousal level by continuous light stimulus on contingent negative variation (CNV). *Appl Human Sci*. 1997;16(2):55-60. doi:10.2114/jpa.16.55
16. Keis O, Helbig H, Streb J, Hille K. Influence of blue-enriched classroom lighting on students' cognitive performance. *Trends Neurosci Educ*. 2014;3(3-4):86-92. doi:10.1016/j.tine.2014.09.001
17. Sleegers PJC, Moolenaar NM, Galetzka M, Pruyn A, Sarroukh BE, Van Der Zande B. Lighting affects students' concentration positively: Findings from three Dutch studies. *Light Res Technol*. 2013;45(2):159-175. doi:10.1177/1477153512446099
18. Hébert M, Martin SK, Lee C, Eastman CI. The effects of prior light history on the suppression of melatonin by light in humans. *J Pineal Res*. Published online 2002. doi:10.1034/j.1600-079X.2002.01885.x
19. Chang AM, Scheer FAJL, Czeisler CA. The human circadian system adapts to prior photic history. *J Physiol*. Published online 2011. doi:10.1113/jphysiol.2010.201194
20. Wright KP, McHill AW, Birks BR, Griffin BR, Rusterholz T, Chinoy ED. Entrainment of the human circadian clock to the natural light-dark cycle. *Curr Biol*. 2013;23(16):1554-1558. doi:10.1016/j.cub.2013.06.039
21. Morin, C.M., Landreville, P., Colecchi, C., McDonald, K., Stone, J. and Ling W. The Beck Anxiety Inventory: psychometric properties with older adults. *Springer*. 1999;1(5):19-29. doi:10.1023/A:1022986728576
22. Beck, A.T., Steer, R.A. and Carbin MG. Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. *Clin Psychol Rev*. 1988;8(1):77-100. doi:10.1016/0272-7358(88)90050-5

23. Buysse, D.J., Reynolds III, C.F., Monk, T.H., Berman, S.R. and Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Elsevier*. 1989;28(2):193-231. doi:10.1016/0165-1781(89)90047-4
24. Johns MW. A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *Sleep*. Published online 1991. doi:10.1093/sleep/14.6.540
25. Horne JA, Ostberg O. A self assessment questionnaire to determine Morningness Eveningness in human circadian rhythms. *Int J Chronobiol*. Published online 1976.
26. Cajochen C, Wyatt JK, Czeisler CA, Dijk DJ. Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. *Neuroscience*. 2002;114(4):1047-1060. doi:10.1016/S0306-4522(02)00209-9
27. Boisgueheneuc F Du, Levy R, Volle E, et al. Functions of the left superior frontal gyrus in humans: A lesion study. *Brain*. 2006;129(12):3315-3328. doi:10.1093/brain/awl244
28. Makeig S, Jolley K. Technical document 96-3C. *Nav Heal Res Cent*. Published online 1996.
29. Gramfort A, Luessi M, Larson E, et al. MEG and EEG data analysis with MNE-Python. *Front Neurosci*. 2013;7(7 DEC):1-13. doi:10.3389/fnins.2013.00267
30. Hyvärinen A. Fast and robust fixed-point algorithms for independent component analysis. *IEEE Trans Neural Networks*. 1999;10(3):626-634. doi:10.1109/72.761722
31. Cajochen C, Foy R, Dijk DJ. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Res Online*. 1999;2(3):65-69.
32. Xavier G, Su Ting A, Fauzan N. Exploratory study of brain waves and corresponding brain regions of fatigue on-call doctors using quantitative electroencephalogram. *J Occup Health*. 2020;62(1):1-8. doi:10.1002/1348-9585.12121
33. Gaggioni, G., Maquet, P., Schmidt, C., Dijk, D.J. and Vandewalle G. Neuroimaging, cognition, light and circadian rhythms. *Front Syst Neurosci*. 2014;8:126. doi:10.3389/fnsys.2014.00126
34. Paparella I, Campbell I, Sharifpour R, et al. Light modulates task-dependent thalamo-cortical connectivity during an auditory attentional task. *Commun Biol*. 2023;6(1):1-10. doi:10.1038/s42003-023-05337-5
35. Vandewalle G, Maquet P, Dijk D-J. Light as a modulator of cognitive brain function. Published online 2005. doi:10.1016/j.tics.2009.07.004
36. Güler AD, Ecker JL, Lall GS, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature*. 2008;453(7191):102-105. doi:10.1038/nature06829
37. Dumont M, Carrier J. Daytime sleep propensity after moderate circadian phase shifts induced with bright light exposure. *Sleep*. 1997;20(1):11-17. doi:10.1093/sleep/20.1.11
38. Smolders KCHJ, Peeters ST, Vogels IMLC, de Kort YAW. Investigation of Dose-Response Relationships for Effects of White Light Exposure on Correlates of Alertness and Executive Control during Regular Daytime Working Hours. *J Biol Rhythms*. 2018;33(6):649-661. doi:10.1177/0748730418796438
39. Lok R, Smolders KCHJ, Beersma DGM, de Kort YAW. Light, Alertness, and Alerting Effects of White Light: A Literature Overview. *J Biol Rhythms*. 2018;33(6):589-601. doi:10.1177/0748730418796443
40. Segal AY, Sletten TL, Flynn-Evans EE, Lockley SW, Rajaratnam SMW. Daytime exposure to short- and medium-wavelength light did not improve alertness and neurobehavioral performance. *J Biol Rhythms*. 2016;31(5):470-482. doi:10.1177/0748730416659953
41. Siraji MA, Kalavally V, Schaefer A, Haque S. Effects of Daytime Electric Light Exposure on Human Alertness and Higher Cognitive Functions: A Systematic Review. *Front Psychol*. 2022;12(January):1-17. doi:10.3389/fpsyg.2021.765750
42. Mu YM, Huang XD, Zhu S, et al. Alerting effects of light in healthy individuals: A systematic review and meta-analysis. *Neural Regen Res*. 2022;17(9):1929-1936. doi:10.4103/1673-5374.335141
43. Van den Berg TJTP, Felius J. Relationship between spectral transmittance and slit lamp color of human lenses. *Investig Ophthalmol Vis Sci*. 1995;36(2):322-329.
44. Najjar RP, Teikari P, Cornut PL, Knoblauch K, Cooper HM, Gronfier C. Heterochromatic flicker photometry for objective lens density quantification. *Investig Ophthalmol Vis Sci*. 2016;57(3):1063-1071. doi:10.1167/iovs.15-18642
45. Eto T, Teikari P, Najjar RP, et al. A Purkinje image-based system for an assessment of the density and transmittance spectra of the human crystalline lens in vivo. *Sci Rep*. 2020;10(1):1-12. doi:10.1038/s41598-020-73541-y
46. Eto T, Ohashi M, Nagata K, Shin N, Motomura Y, Higuchi S. Crystalline lens transmittance spectra and pupil sizes as factors affecting light-induced melatonin suppression in children and adults. *Ophthalmic Physiol Opt*. 2021;41(4):900-910. doi:10.1111/opo.12809
47. Phipps-Nelson J, Redman JR, Dijk DJ, Rajaratnam SMW. Daytime exposure to bright light, as compared to dim light, decreases sleepiness and improves psychomotor vigilance performance. *Sleep*. 2003;26(6):695-700. doi:10.1093/sleep/26.6.695