Illumination characteristics

Table S1. Illumination characteristics.

	Experiment 1 and 2		Experiment 3		
	Red light	Blue light	Red light	Green light	Blue light
Peak wavelength [FWHM] (nm)	635 [20]	465 [20]	630 [20]	520 [30]	460 [20]
Log_{10} photon flux ($1/cm^2/s$)	14.8	14.6	15.6	15.5	15.8
Irradiance ($\mu W/cm^2$)	178	159	1286	1108	2501
Photopic illuminance (photopic lux)	327	121	2514	5350	1574
S cone illuminance (cyanopic lux)	12	1132	11	297	20855
Melanopsin illuminance (melanopic lux)	8	1061	14	5575	15533
Rod illuminance (rhodopic lux)	13	752	62	6666	10859
M cone illuminance (chloropic lux)	115	378	892	5998	5260
L cone illuminance (erythropic lux)	420	199	3207	4441	2737

The characteristics were estimated from the spectral irradiance at eye level (AvaSpec-3648-USB2 spectrometer, Avantes, Apeldoorn, The Netherlands) and described according to standard procedures [S1]. FWHM, full width at half maximum; S cone, short-wavelength cone; M cone, mid-wavelength cone; L cone, long-wavelength cone.

Statistical analysis

Experiment 1

Mixed-effects regression models were used to estimate the post-illumination effects on pupil diameter and response speed (Table S2). The data were structured in a three-level hierarchy: two dark blocks were nested within two trials, which in turn were nested within 12 participants. Outcome parameters were pupil diameter and response speed. Post-red and post-blue covariates were included as dichotomous variables, which flagged the dark block following either red or blue light exposure (1 for the post-red or post-blue block and 0 for the baseline dark block).

Table S2. Mixed model estimates of the post-illumination effects on pupil diameter and response speed (Experiment 1).

	Pupil diameter (mm)	Response speed (s^{-1})
Intercept	5.67 ± 0.25	4.12 ± 0.15
Post-red	0.57 ± 0.23*	-0.09 ± 0.05
Post-blue	-2.15 ± 0.3***	0.07 ± 0.05

Mean values ± SE are displayed. *p < 0.05, ***p < 0.001.

Experiment 2

Mixed-effects regression models were used to estimate the post-illumination effects on pupil diameter, response speed, and the cardiac parameters (Table S3 and S4; Figure S1). The data represented a four-level hierarchy: 12 participants came to the lab twice on two separate days and performed two trials per day (i.e., one trial in sitting position and one trial in lying position) with each trial consisting of seven blocks (i.e., five dark blocks and two illumination blocks). Since we aimed to assess post-illumination changes during darkness, we included only the dark blocks in the analysis. Pupil diameter, response speed, R-R interval, ln SDNN, ln RMSSD, and ln SDNN/RMSSD were the outcome parameters.

The distributions of heart rate variability parameters are right-skewed. Logarithm and inverse functions are the most commonly used data transformations to normalize right-skewed distributions. The Shapiro-Wilk test showed that the data were closer to a normal distribution when applying a natural logarithmic instead of a reciprocal transformation. Hence, we selected the natural logarithm of the R-R variability parameters as outcome measures in the analysis, which is common practice in studies on heart rate variability [S2], although for ln RMSSD and ln SDNN/RMSSD deviation from normality could still not be rejected.

The post-red covariate was included as a dichotomous variable, which flagged the dark block immediately following red-light exposure (1 for the post-red block and 0 for the other dark blocks). The post-blue covariate was added to mark the dark blocks subsequent to the blue light. Since pupil diameter returned linearly to baseline within the three consecutive dark blocks after blue-light exposure, post-blue was included as a weighted variable in the model for pupil diameter (1 for the block immediately following blue light exposure, ⅔ for the second consecutive post-blue dark block, and ⅓ for the third consecutive post-blue dark block, and 0 for the dark blocks preceding the blue-light exposure). In the models for response speed and the cardiac parameters, the post-blue covariate was dummy coded with 1 for the dark block immediately following the blue-light exposure and 0 for the other dark blocks. We moreover added covariates for time of day (1 for afternoon and 0 for morning) and posture (1 for supine and 0 for upright).

In the model for response speed, we added block, ranging from 1 to 7, as a time-varying covariate in order to take the time-on-task effect on response speed into account. In line with our previous work [S3, S4], we selected the best fitting model from a first order polynomial, a second order polynomial and a square-root time course. Likelihood ratio tests and visual inspection revealed that the time-on-task effect was best captured by the square root of the block covariate, which was therefore selected for inclusion in the model for response speed.

Where a post-illumination effect was observed, we introduced the interaction between time of day and posture and the corresponding post-illumination variable in order to assess possible differences in post-illumination changes across the day and across body postures.

Table S3. Mixed model estimates of the post-illumination effects on pupil diameter and response speed (Experiment 2).

	Pupil diameter (mm)	Response speed (s^{-1})
Intercept	4.75 ± 0.21	4.62 ± 0.23
Post-red	0.65 ± 0.08***	-0.10 ± 0.04**
Post-blue	-1.72 ± 0.08***	-0.002 ± 0.04
Time of day (afternoon vs. morning)	-0.003 ± 0.13	-0.04 ± 0.06
Posture (supine vs. upright)	-0.13 ± 0.08	0.05 ± 0.05
√Block		-0.26 ± 0.03***

Mean values ± SE are displayed. **p < 0.01, ***p < 0.001.

Table S4. Mixed model estimates of the post-illumination effects on R-R interval and its variability (Experiment 2).

	R-R interval (ms)	ln SDNN (ln ms)	ln RMSSD (ln ms)	ln SDNN/RMSSD
Intercept	877.3 ± 29.9	4.28 ± 0.07	4.10 ± 0.13	0.18 ± 0.07
Post-red	-9.3 ± 4.4*	0.05 ± 0.03*	-0.04 ± 0.02	0.09 ± 0.02***
Post-blue	-0.4 ± 4.4	0.04 ± 0.03	0.01 ± 0.02	0.03 ± 0.02
Time of day (afternoon vs. morning)	-23.1 ± 24.0	-0.004 ± 0.05	0.04 ± 0.08	-0.04 ± 0.04
Posture (supine vs. upright)	102.1 ± 14.2***	-0.24 ± 0.04***	-0.39 ± 0.06***	0.15 ± 0.03***

Mean values ± SE are displayed. *p < 0.05, ***p < 0.001. SDNN, standard deviation of the normal R-R intervals; RMSSD, square root of the mean squared differences of successive normal R-R intervals.

Ancillary mixed-effect models were conducted to evaluate whether the post-illumination effects on pupil diameter and response speed might both be parallel indices of an underlying process. In case the R-R interval was altered after red or blue light exposure, we correlated the post-illumination change in R-R interval with the post-illumination alteration in pupil diameter and response speed. The pupil dilation after red light was larger when the R-R interval shortening was stronger ($\beta = -0.06$, $p = 0.04$), suggesting that the increase in pupil size and shortening of R-R interval could in part reflect a common underlying process. On the other hand, the change in R-R interval was not associated with the change in response speed ($\beta = 0.004$, $p = 0.83$).

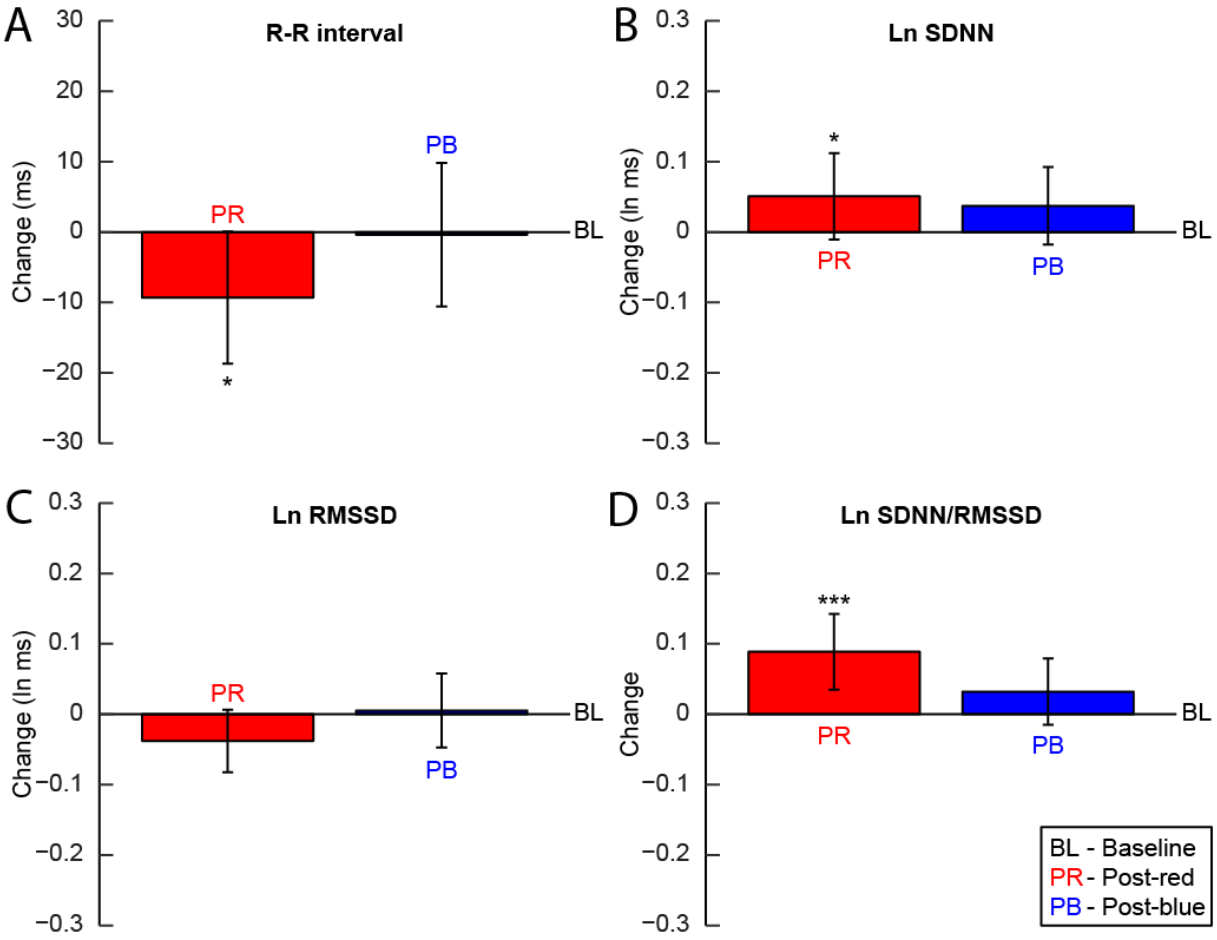


Figure S1. Post-illumination changes in R-R interval and its variability (Experiment 2). The bars represent the post-red (PR) and post-blue (PB) changes in (A) R-R interval which is inversely associated with mental effort, (B) the natural logarithm (ln) of the standard deviation of the normal R-R intervals (SDNN), (C) the natural logarithm of the square root of the mean squared differences of successive normal R-R intervals (ln RMSSD), and (D) the natural logarithm of the ratio between SDNN and RMSSD ratio (ln SDNN/RMSSD), relative to the baseline (BL) blocks. Error bars represent the within-subject 95% confidence interval. Asterisks indicate within-subject differences between the post-illumination blocks and the baseline blocks (*$p < 0.05$, ***$p < 0.001$).

Experiment 3

Mixed-effects regression models were used to estimate the post-illumination effects on sleep propensity (Table S5), which was defined as the inverse of sleep onset latency. Sleep onset latency distributions are right-skewed. Logarithm and inverse functions are the most commonly used data transformations to normalize right-skewed distributions. To compare their performance, the Shapiro-Wilk test was used to inspect deviation from normality for the within-subject distributions of sleep onset latency data transformed with either a logarithm or an inverse function. For the reciprocally transformed data, deviation from normality could be rejected for all of the 16 within-subject distributions, whereas this was not the case for the logarithmically transformed data. Because the reciprocal transformation performed better in normalizing the distributions and is preferable with respect to power [S5, S6], the inverse of sleep onset latency was used as outcome measure and referred to as sleep propensity.

The data were structured in a two-level hierarchy: the outcome parameter sleep propensity was estimated during eight consecutive blocks, which were nested in 16 participants. Post-red, post-green, and post-blue were included as dummy variables (1 for the block containing either red, green or post light exposure and 0 for the block containing dark exposure). In line with the mixed model analysis of the time-on-task effect in Experiment 1, we added block, ranging from 1 to 8, as a time-varying second order-modelled covariate in order to account for the time-of-day modulation of daytime sleep propensity [S7]. We likewise compared between models with the inclusion of block as a first order polynomial, a second order polynomial or a square-root variable. Likelihood ratio tests and visual inspection revealed that the time-of-day effect was best captured by the second order polynomial of the block covariate, which was therefore selected for inclusion in the model for sleep propensity. We aimed to acquire a data set with 16 participants in a counterbalanced experimental design with each illumination condition applied twice in a fully randomized order. In total, we recruited 18 participants, of which two participants were excluded from the analysis: one participant did not comply with the instructed sleep restriction and offline sleep scoring revealed that another participant did not reach sleep onset in any of the blocks.

Table S5. Mixed model estimates of the post-illumination effects on sleep propensity (Experiment 3).

	Sleep propensity ($min^{-1} \cdot 10^{-1}$)
Intercept	1.91 ± 0.73
Post-red	0.85 ± 0.41*
Post-green	-0.62 ± 0.42
Post-blue	-0.03 ± 0.40
Block	0.91 ± 0.29**
$Block^2$	-0.11 ± 0.03***

Mean values ± SE are displayed. *p < 0.05, **p < 0.01, ***p < 0.001.

Supplemental references

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