Supplemental Material

Living in Biological Darkness:

Objective Sleepiness and the Pupillary Light Responses are Affected by Different Metameric Lighting Conditions during Daytime

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Scores on the screening questionnaires

| Questionnaire                             | Mean Score (± SD) |
|-------------------------------------------|-------------------|
| Pittsburgh Sleep Questionnaire Index      | 3.2 (1.2)         |
| Morningness-Eveningness Questionnaire     | 54.5 (6.6)        |
| Munich Chronotype Questionnaire           | 4.4 (0.6)         |
| Seasonal Pattern Assessment Questionnaire | 7.1 (3.4)         |

Table S1. Mean scores of the screening questionnaires. Mean ± SD; n = 72 (24 male, 48 female).

Spectral power distribution

In a separate Excel file we provide the mean spectral power distribution for each light condition, as they are shown in Figure 3. We set negative raw values from the spectrometer to zero since these negative values simply reflect low signal-to-noise-ratios on those wavelengths.

Detailed LED specifications

The light source consisted out of six LED light panels and each panel contained 80 single LED lamps. The 80 LEDs were grouped in 4 LED modules. Each module had 4 LEDs with peak wavelengths at 480 nm (“blue”), 6 LEDs with peak wavelengths at 435 nm (“blue”), 6 LEDs with longer-wavelengths (“orange”) and 4 LEDs with different longer-wavelengths (“yellow”). The lamps for this study were produced by Vossloh-Schwabe GmbH, Urbach, Germany.

Contrast differences between light exposures

| Light Conditions         | $E_{lc}$ | $E_{mc}$ | $E_{lc}$ | $E_{nh}$ | $E_{mel}$ |
|--------------------------|----------|----------|----------|----------|-----------|
| Low illuminance          |          |          |          |          |           |
| High-mel vs. low-mel     | 1.02     | 1.10     | 1.07     | 1.43     | 1.71      |
| Highest-mel vs. low-mel  | 1.86     | 1.36     | 1.06     | 2.33     | 3.01      |
| Highest-mel vs. high-mel | 1.82     | 1.24     | 0.99     | 1.63     | 1.76      |
| Higher illuminance       |          |          |          |          |           |
| High-mel vs. low-mel at 200 lx | 1.56 | 1.05 | 1.04 | 1.52 | 1.91 |
| High-mel vs. low-mel at 600 lx | 1.54 | 1.06 | 1.05 | 1.48 | 1.85 |
| High-mel vs. low-mel at 1200 lx | 1.58 | 1.06 | 1.04 | 1.51 | 1.91 |

Table S2. Contrasts between light conditions as perceived by different photoreceptor classes. This table shows the contrast between two light conditions for each α-opic irradiance ($E_\alpha$ condition 1 / $E_\alpha$ condition 2). A contrast close to 1 means that there is almost no difference while, e.g., the contrast of 1.71 for $E_{mel}$ means that there is a 71% stronger stimulation of melanopsin in the high-mel vs. low-mel condition. The largest contrast differences were found for melanopic irradiance (column 6) while the smallest contrast differences were found for L-cone-opic irradiance (column 4).
Rod saturation threshold

| Photopic Lux | Low-mel Scotopic Retinal Illuminance ($T_s; \text{cd/m}^2 \times \text{mm}^2$) | Log$_{10}$-scotopic Retinal Illuminance ($\log T_s$) | High-mel Scotopic Retinal Illuminance ($T_s; \text{cd/m}^2 \times \text{mm}^2$) | Log$_{10}$-scotopic Retinal Illuminance ($\log T_s$) |
|--------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 100          | 1354                                          | 3.132                                         | 1590                                          | 3.201                                         |
| 200          | 2544                                          | 3.405                                         | 3098                                          | 3.491                                         |
| 600          | 7234                                          | 3.859                                         | 7929                                          | 3.899                                         |
| 1200         | 13424                                         | 4.128                                         | 14601                                         | 4.164                                         |

Table S3. Retinal illuminance. This table shows scotopic retinal illuminance (= scotopic troland) for low-mel and high-mel conditions at all light intensity conditions (second and third columns) as well as the log-transformed scotopic troland values (third and fifth column). According to Adelson (1982), rod saturation occurs when values are greater than 3.0 log-transformed scotopic troland [[Adelson 1982], Fig. 2] and according to Aguilar and Stiles, rod saturation occurs between 2000 and 5000 scotopic troland, which corresponds to a rod saturation threshold between 3.3 and 3.7 log scotopic troland (Aguilar and Stiles 1954). We found that our scotopic retinal illuminance was higher than 1000 scotopic troland for all lighting conditions.

Dose-response curves for pupillometry vs. α-opic lux/irradiance

The averaged pupil recordings of all participants in the higher illuminance group ($n = 48$) were used to create a dose-response curve for the maximum CA and the PIPR, as is reported in the main manuscript for melanopic lux and melanopic irradiance. The same methods were also applied to create dose-response curves for all other α-opic illuminances (Fig. S1 and S2; panels on the left) and α-opic irradiances (Fig. S1 and S2; panels on the right). Significant fits ($p < 0.001$) were found for maximum CA (Fig. S1) and all α-opic intensities and irradiances with similar $R^2$ values as for melanopic lux or melanopic irradiance. Significant fits ($p < 0.05$) were also found for the PIPR (Fig. S2) and all α-opic intensities and irradiance but with lower $R^2$ values as for melanopic lux or melanopic irradiance.
Figure S1. Dose-response curve for maximum CA vs. α-opic illuminance and α-opic Irradiance. Dose-response curve in the higher illuminance group (n = 48) for maximum CA (z-scores) versus α-opic illuminance (a, c, e, g) and α-opic irradiance (b, d, f, h). The lower x-axis of each figure shows absolute α-opic lux or α-opic irradiance, whereas the upper x-axis shows the same values as log₁₀ transformed values. Curve fitting was performed with log₁₀ transformed data. All dose-response curves reached statistical significance (see upright corner of each panel for exact R² and p-values). The pair of data points at each melanopic lux-value represent the pupil response to two different light pulses. The two different colors of symbols represent the two spectral lighting conditions (i.e. low- high-mel). See Figure 3 in the main manuscript for a description of the light conditions. Means ± SEM bars.
Figure S2. Dose-response curve for the PIPR vs. α-opic illuminance and α-opic irradiance. Dose-response curve in the higher illuminance group (n = 48) for the PIPR (z-scores) versus α-opic illuminance (a, c, e, g) and α-opic irradiance (b, d, f, h). The lower x-axis of each figure shows absolute α-opic lux or α-opic irradiance, whereas the upper x-axis shows the same values as log_{10} transformed values. Curve fitting was done with the log_{10} transformed values. All dose-response curves reached statistical significance (see upright corner of each panel for exact R^2 and p-values). The pair of data points at each melanopic lux-value represent the pupil responses to the two different light pulses. The two different colors of symbols represent the two spectral lighting conditions (i.e. low- high-mel). See Figure 3 in the main manuscript for a description of the light conditions. Means ± SEM bars.

**Absolute pupil sizes during the first 0.5 s in darkness**

In low illuminance (100 photopic lux) the absolute light adapted pupil sizes during the first 0.5 s in darkness showed a significant effect of light spectrum (F_{3,80} = 34.464, p < 0.001; Fig. S3a; main effect of “LIGHT SPECTRUM”). Post-hoc analysis showed that baseline pupil size was smaller in all light conditions compared to DL (p < 0.001), and was smaller in highest-mel compared to high-mel and low-mel (p < 0.013). There was a significant difference between the 3 time points during the light exposure (F_{2,188} = 6.287, p = 0.002; main effect of “DURATION”) such that baseline pupil size became smaller over time. There was no significant interaction between these factors (p = 0.577).

In higher illuminance (200, 600 and 1200 photopic lux) the absolute light adapted pupil sizes during the first 0.5 s in darkness showed no significant effect of light spectrum (F_{1,178} = 2.031, p = 0.156; main effect of “LIGHT SPECTRUM”; Fig. S3b). There was a significant effect of light intensity (F_{2,146} = 17.852, p < 0.001; Fig. S3c; main effect of “INTENSITY”). Post-hoc analysis showed that baseline pupil size was smaller in all light intensities compared to DL (p < 0.001), smaller in 600 and 1200 lx compared to 200 lx (p < 0.001), and smaller in 1200 compared to 600 lx (p = 0.025). There was no significant difference between the 3 time points during the light exposure (F_{2,368} = 1.560, p = 0.212; main effect of “DURATION”). There were no significant interactions between these factors (p > 0.284).
Figure S3. Pupillometry: Absolute pupil size during first 0.5 s in darkness. Absolute light adapted pupil size at the start of the each measurement during the first 0.5 s in darkness. Results for low illuminance (100 lx); n = 24: a) Means across participants per spectral light compositions; +/-SEM. Results for higher illuminance (200-1200 lx); n = 48: b) Means across participants per spectral light composition. c) Means across participants for different light intensities. * p < 0.05. See Figure 3 in the main manuscript and text for a description of the light conditions.

References:

Adelson EH (1982) Saturation and adaptation in the rod system. Vision Res 22:1299-1312.
Aguilar M and Stiles WS (1954) Saturation of the Rod Mechanism of the Retina at High Levels of Stimulation. J Mod Opt 1:59-65.