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IMPACT OF SHORT DARK ADAPTATION PERIODS ON POST-ILLUMINATION PUPIL RESPONSE

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Abstract

The pupil response serves as a proxy for the biological potency of light. A reproducible measurement of the pupil response requires controlled dark adaptation, but it is currently unclear whether shorter dark adaptation periods can be used to reliably determine the biological potency of light through measuring the pupil response. Therefore, we conduct a within-participant experiment, continuously measuring the pupil response for dark adaptation periods between 40 and 120 seconds. Results show a trend in the effect of mEDI on the 6s-PIPR increasing with longer adaptation periods. The dark adaptation period of 120 seconds does not fit this trend, and more research is needed here. Overall, our study shows that a dark adaptation period of 60 to 100 seconds is suitable for repeated measurements of the 6s-PIPR, with the aim of using the pupil response as a proxy for the biological potency of light.

Keywords: pupillometry, post-illumination pupil response, biological potency, dark adaptation

1 Introduction

People are affected in several ways by the non-image forming effects of light, such as changes in circadian rhythm or mood (Vetter et al., 2022). The capacity of light to influence these non-image forming effects can be referred to as the biological potency of light (Houser & Esposito, 2021). Non-image-forming effects of light can have large interpersonal differences (Santhi et al., 2012) and show a slow response to light, ranging from effects on acute alertness over minutes (Souman et al., 2018) to effects on phase delay over days (Chang et al., 2012). It can thus be challenging to determine what the biological potency of light is for an individual.

Proxies of biological potency are, among others, melanopic equivalent daylight illuminance (mEDI), melatonin suppression, or circadian shift. All of these proxies have drawbacks: mEDI is a property of light (CIE, 2018) and cannot, by definition, take personal differences into account; melatonin suppression requires blood or saliva samples and is only available close to habitual bedtime (Kennaway, 2020); and a circadian shift requires multiple days or weeks to be reliably measured.

Another promising proxy is the pupil response (De Zeeuw et al., 2019). Advantages of this proxy are that the pupil responds very quickly to light, and can be measured in an unintrusive way with camera recordings during short light exposures. While the pupil response cannot provide an absolute measure of biological potency, it can provide a measure of the relative biological potency of two light conditions within a person. It can do so by comparing distinct patterns in the return to steady-state pupil size after a short flash of light.

There are several of these patterns (Adhikari, Zele & Feigl, 2015), but an accessible pattern is the time it takes for the pupil to return to steady-state, which can be, among other ways, quantified through the pupil size 6s after a flash of light relative to the pupil size in a steady state (6s-PIPR) (Kelbsch et al., 2019). A common protocol to measure this 6s-PIPR consists of measuring the steady state in dark for periods up to ten minutes, followed by a light flash of one second.

The pupil response is influenced by various factors such as visual effects, cognitive processes, and prior light exposure. Excitement can cause the pupil to enlarge, while sleepiness may result

in a smaller pupil size. Acute light history is often controlled by ensuring the pupil is in a dark-adapted steady state, typically achieved by having individuals spend several minutes in darkness. Adhikari et al. (2016) found that the time for the pupil to return to a steady state after exposure to various narrow-band one-second light pulses depends on the mEDI. Their results suggest a time-to-steady-state ranging from approximately 10 to 90 seconds for mEDI values between 1.6 and 160 lux.

While current methods of ensuring sufficient adaptation by using prolonged dark adaptation periods are reliable, it is impractical to wait for such extended periods before every single measurement of the pupil response, especially when multiple measurements are required. Therefore, it is crucial to determine whether the dark adaptation time between successive measurements can be shortened, while still reliably measuring the biological potency of light.

This study aims to investigate which dark adaptation periods ensure valid measurements of biological potency through the 6s-PIPR. Based on findings by Adhikari et al. (2015), we expect measurements of relative biological potency for adaptation periods above 60 seconds to be reliable.

2 Methods

In order to determine what the required minimum dark adaptation period is for a reliable measure of the biological potency using the 6s-PIPR, we perform a within-participant experiment comparing the 6s-PIPR after exposure to two metameric light conditions across various dark adaptation periods.

2.1 Participants and Design

This study includes fourteen participants recruited through the university participant database. Participants between 18 and 35 years old ($M = 27.1$, $SD = 2.7$) with normal vision are included, while participants with self-reported sensitivity to light flashes or history of epilepsy in their direct family are excluded, as well as participants that do not pass the full Ishihara Test for Color Blindness with a perfect score.

The participant's task is to look at a 1s flash of light after adapting to the dark for a variable amount of time, ranging from 40 to 120 seconds. The light flash consists of either one of two metameric lights. Participants wear a pair of Pupil Labs neon glasses (Pupil Labs, n.d.) to record the pupil diameter from which the 6s-PIPR is derived. The 6s-PIPR after each light stimulus is the dependent variable for this experiment. The independent variable is the adaptation period between subsequent light pulses. The difference in 6s-PIPR between the metameric pairs is determined based on the dependent variable during the analysis.

2.2 Setup

Light stimuli are presented using our custom-built ColorBox. This box is 50 cm deep x 80.4 cm wide x 63 cm high with a circular opening of 21 cm diameter. Participants sit in front of the box and place their head in a chinrest directly in front of the circular opening. This results in a 64 degrees field, which corresponds to a 63 cm diameter view of the backwall.

Two light panels with 11 LED primaries with narrow-band spectra approximately evenly spaced between 380 and 780 nm are placed in the box outside of the field of view of the participants. These panels allow the creation of tuneable light spectra and metameric light stimuli. This light illuminates the white interior of the box. This results in a uniformly illuminated backwall, the light of which is reflected to the participant.

2.3 Stimuli

Participants are shown metameric pairs of light stimuli, where each stimulus is a brief one-second light pulse in an otherwise dark (<0.1 lx) environment. Each metameric pair consists of a pulse with an mEDI of 73 lx and one with an mEDI of 131 lx. The corresponding α -opic EDI values are shown in table 1. The main independent variable in the experiment is the dark adaptation period between subsequent pulses, which varies between 40s, 60s, 80s, 100s or 120s. The full set of ten stimuli (i.e., 2 stimuli for 5 pairs) is shown in a block, and then repeated two more times. The order of all stimuli within each block is fully randomised.

Table 1. α -opic EDI of the light stimuli

	Rod (saturated)	s-cone	m-cone	l-cone	ipRGC
Stimuli 1	73.8 lx	68.9 lx	72.7 lx	74.9 lx	72.5 lx
Stimuli 2	115.1 lx	68.9 lx	72.6 lx	74.8 lx	130.6 lx

2.4 Protocol

Participants take place in front of the experimental setup, and the continuous pupil measurement starts. Participants are then dark adapted for 10 minutes, after which a one-second light pulse is shown as reference to the dark adaptation. This is followed by a block of approximately 10 minutes of measurements for the 10 stimuli in a block. In total, each participant is exposed to 30 stimuli.

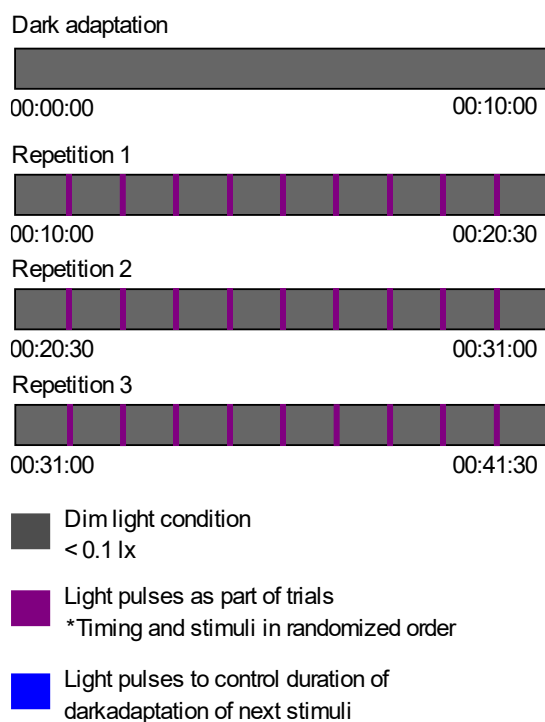


Figure 1 – Overview of experimental protocol

* Light pulses are evenly spaced at 75 seconds in the figure. In the experiment, each pulse within a repetition is randomly spaced at 40, 60, 80, 100, or 120 seconds.

2.5 Analysis

First, blinks are detected in the collected pupillary data using the blink-detection algorithm from Pupil Labs (Pupil Labs, n.d.), and removed a total of 11% of the data. Second, any measured pupil size outside the range from 2 to 8 mm is removed (total of 2% of data), along with any data showing a rate of change above 4000 mm/s (total of 30% of data).

A centred median filter with a window size of 2 seconds is then used to smooth the remaining data. Then, the pupil size averaged over the right and left eye is determined as a function of time. The continuous pupil size (in millimetres) is normalized to the average pupil size calculated from the last three seconds before each light stimulus. This determines the relative

pupil size as a percentage compared to the fully dilated pupil. The 6s-PIPR is determined by averaging the relative pupil size from 5.5 to 6.5 seconds after each light stimulus.

Unfortunately, the pupil detection software does not detect all blinks or eyes-closed events. However, it returns a pupil size of approximately 50-75% during these events. Any instance of the pupil dropping below 60% during steady state or 75% during the 6s-PIPR measurement period is marked and manually checked against the video recording for blinks or eyes-closed events.

One participant indicated they kept their eyes closed during parts of the light stimuli for increased comfort. Two more participants kept their eyes closed for an extended period during the 6s-PIPR measurement in over half of the stimuli. One participant blinked excessively in 60% of the stimuli during or right before the 6s-PIPR measurement. For all these four participants insufficient reliable data are available. For the remaining 10 participants, 7 stimuli are excluded due to blinking during the 6s-PIPR measurement.

Finally, z-scores are determined for each participant, and any data point with a z-score over 2.5 is excluded to account for outliers. Five stimuli have been excluded based on the z-score being larger than 2.5.

A linear mixed model is used to analyse the 6s-PIPR data. The full model is clustered on the participant, and includes the mEDI of the light stimuli, the dark adaptation time before the light stimuli, the time within the experiment, and all their interaction terms. Dark adaptation time is included as a factored variable, to circumvent the assumption of linearity. Furthermore, the age, eye colour (blue or brown), and visual acuity (Landolt-C) of the participants are included. This model is simplified using backward reduction of terms, using the loglikelihood ratio – if this loglikelihood ratio significantly improves, the term is dropped as it does not sufficiently contribute to the performance of the model. Exceptions to this are the effect of mEDI, the effect of dark adaptation period, and the interaction between these two, as these terms are required for evaluation of the research question. Using post-hoc analysis, we test for a significant difference in dark adaptation period on the difference in the 6s-PIPR between the metameric pairs of light.

3 Results

In total, we collected data for fourteen participants. Figure 2 shows an overview of the continuous relative pupil size of the first repetition for the first participant, to illustrate the data used for this experiment.

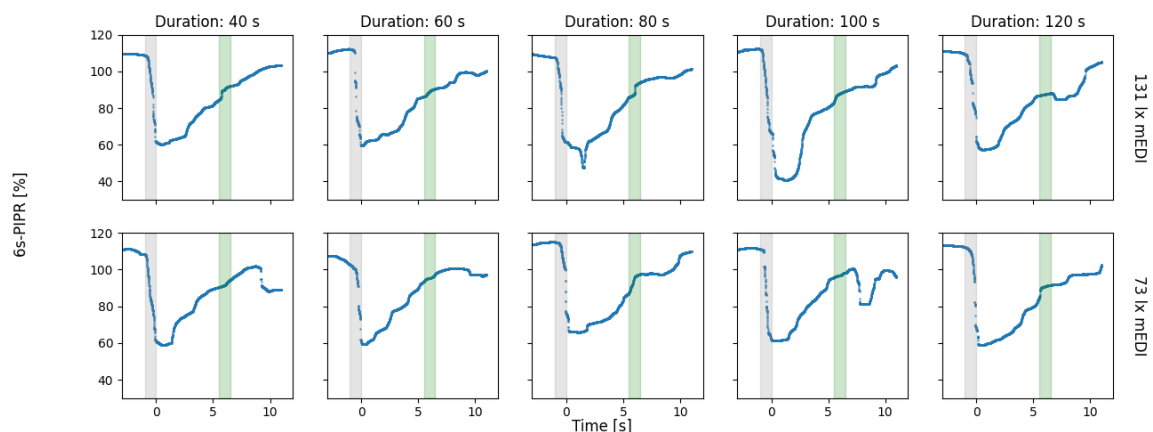


Figure 2 – Example of data

The relative pupil size [%] is shown over time. The time is relative to the light stimulus, in the sense that the light stimulus disappears at $t=0$. The grey vertical section indicates when the light stimulus is turned on, the green vertical section displays the period where the 6s-PIPR is determined. The top row contains data on low mEDI stimuli, while the bottom row contains data on high mEDI stimuli.

An overview of all data is displayed in figure 3. As can be seen from this figure, there are large differences between participants. This highlights the need for taking individual differences of participants into account.

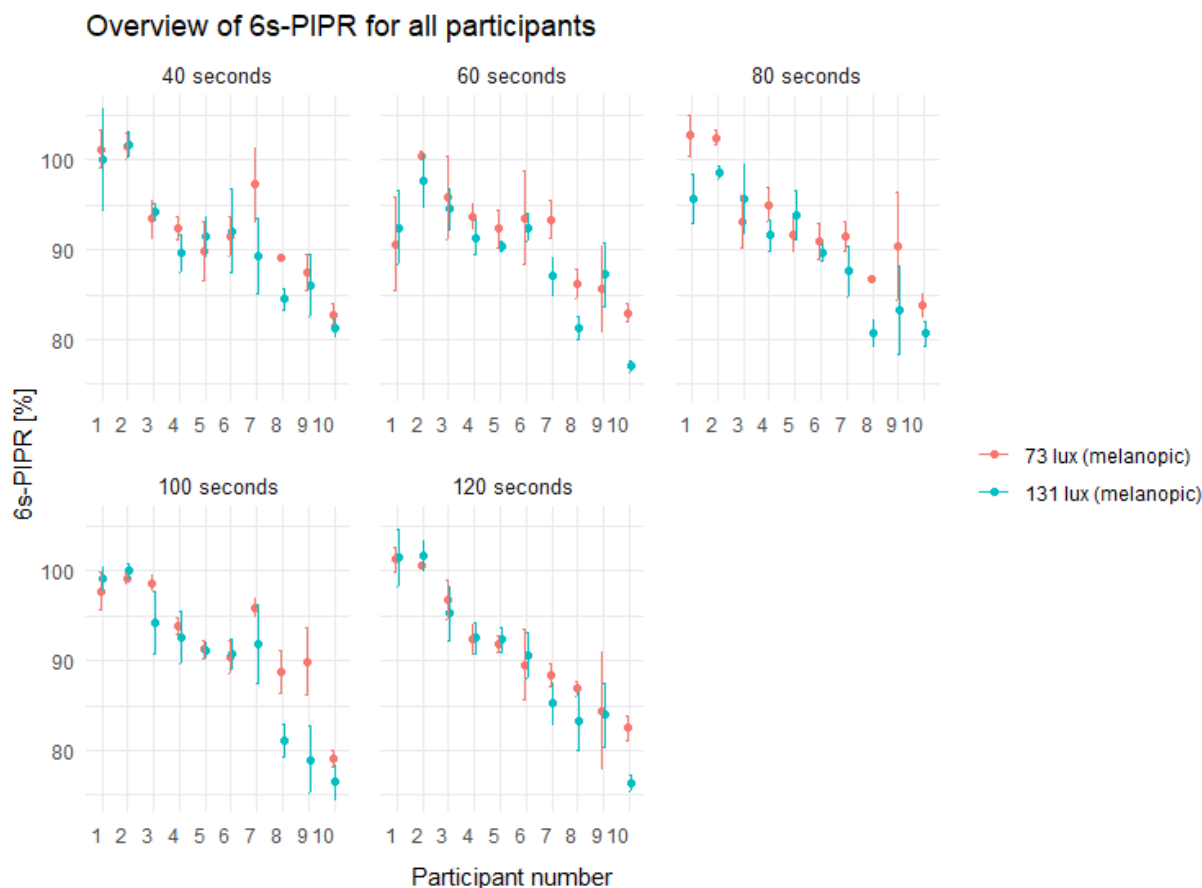


Figure 3 – 6s-PIPR for all trials and participants

The 6s-PIPR [%] of each condition is shown, averaged over each of the three repetitions together with the standard error. Participants are ordered based on their mean 6s-PIPR at 120 seconds.

3.1 Linear Mixed Model

The multilevel linear mixed model as described in the methods is used to analyse the data; doing this clustered per participant allows us to calculate a unique slope and intercept per participant. Using backwards reduction of terms, the best fitting model includes unique (random) intercepts per participant, as well as unique (random) slopes for time within the experiment and mEDI per participant. The best fitting model only includes mEDI and time within the experiment as fixed effects. The dark adaptation period and the interaction between mEDI and dark adaptation period are kept in the model in order to be able to answer our research question. All other interaction terms and terms for age, visual acuity, and eye colour are removed as they do not significantly contribute to the model.

The model shows that intercepts of the 6s-PIPR differ between participants ($M = 93.8$, $SD = 5.6$), as well as the slopes of the effect of mEDI ($M = -1.7$, $SD = 1.4$) and time within the experiment ($M = -0.1$, $SD = 0.1$).

Using analysis of variance on the predictors, we find a significant effect of mEDI. None of the other predictors are statistically significant. Results are shown in table 2.

Table 2. Results of linear mixed model.

Effect	F-value	p-value
Dark adaptation period	$F(4, 269) = 1.16$	0.33
mEDI level	$F(1, 12) = 12.43$	<0.01
Time within experiment	$F(1, 10) = 2.39$	0.15
Interaction mEDI and time within experiment	$F(4, 269) = 0.69$	0.60

3.2 Results of pupil response

We determine the 6s-PIPR based on the model predictions for each dark adaptation period (i.e., 40, 60, 80, 100, and 120s) and for each mEDI level (i.e., 73 and 131 lux). These predicted 6s-PIPR values, the estimated marginal means, are adjusted for other factors such as individual differences. This thus allows us to extract solely the effect of the mEDI and dark adaptation period from the data, to take large individual differences into account.

We calculate the effect of the mEDI of the light on the 6s-PIPR, by subtracting the 6s-PIPR of the high mEDI pulse from that of the low mEDI pulse. This ensures that only the effects of the ipRGCs are included in the pupil response. The rods and cones are stimulated equally between those pulses, and their effect is thus excluded. Figure 4 shows the resulting difference in 6s-PIPR between the low and high mEDI stimulus, for each dark adaptation period (on a logarithmic x-axis scale).

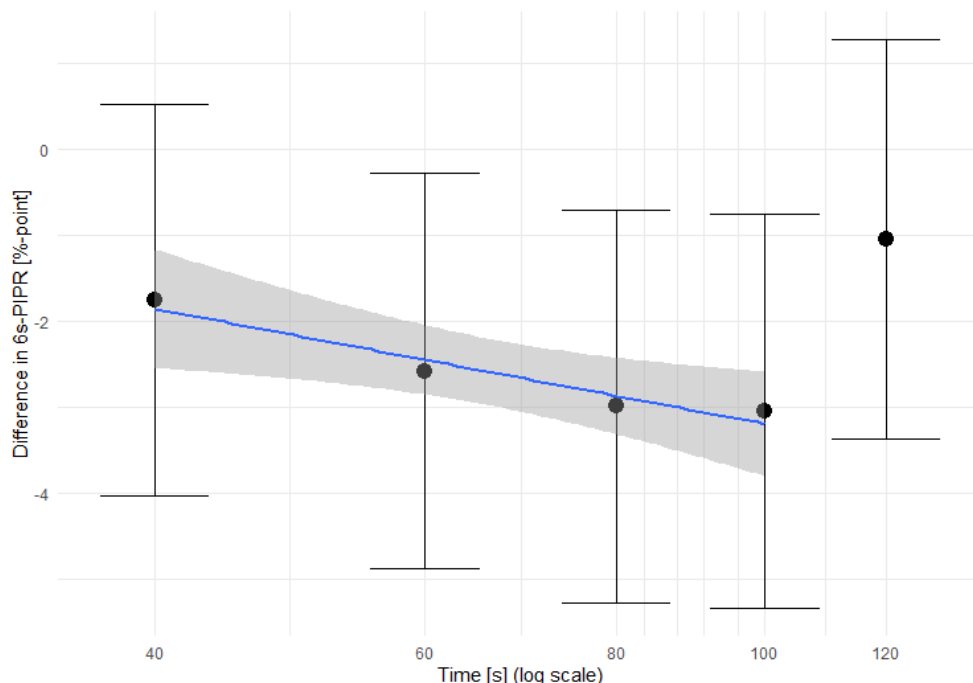


Figure 4 – Effect of mEDI on the 6s-PIPR

The difference between the 6s-PIPR for the low and high mEDI stimuli is shown for each dark adaptation period. Each point includes the confidence interval, and a linear regression line is shown through the data. The confidence intervals do not overlap with the origin for the dark adaptation periods of 60, 80, and 100 seconds, but they do for 40 and 120 seconds.

In Figure 4, we discern a trend of a larger absolute difference in pupil size between the light stimuli with low and high mEDI for larger dark adaptation periods. The data for a dark adaptation period of 120 seconds, however, do not support this trend. When only considering the data from 40 to 100 seconds, we find a significant slope of -3.37 ($t = -5.49$, $p = 0.03$) in the effect of dark adaptation period (logarithmic) on the difference in the 6s-PIPR with mEDI of the light.

4 Discussion

The 6s-PIPR is sensitive to the mEDI of the light pulse, and can be used as a valid proxy for the biological potency of light, as shown in previous literature (Adhikari et al., 2015; Kelbsch et al., 2019). Measuring the 6s-PIPR, however, requires the eye to be in a dark adapted state. Full dark adaptation is not always feasible for each subsequent measurement. Therefore, we studied the effect of varying the dark adaptation period on the 6s-PIPR. We did so by measuring the 6s-PIPR after stimulation with a light pulse of low and high mEDI, and by using different dark adaptation periods between these light pulses.

Similar to other literature, we find an effect of the mEDI on the 6s-PIPR. Analysis shows that the effect size increases logarithmically with the dark adaptation period duration for dark adaptation periods of 40 to 100 seconds, with confidence intervals for dark adaptation periods of 60 to 100 seconds not overlapping zero. However, our data does not follow this trend for the dark adaptation period of 120 seconds. This warrants further analysis of the data, and it would be highly interesting to measure the effect at a duration of 140 seconds or to repeat the measurements at 120 seconds.

The trend we found suggests that there is a tradeoff between the dark adaptation period and effect size. At longer dark adaptation periods, the effect is larger, and the 6s-PIPR measuring method with longer adaptation periods might thus be more suitable for smaller contrasts of mEDI in the light. However, this comes at the cost of increased time required to perform measurements.

Our study shows that a dark adaptation period of 60 to 100 seconds is suitable for repeated measurements of the 6s-PIPR, with the aim of using the pupil as a proxy for the biological potency of light.

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