Dark adaptation for one- and two-photon visual stimuli

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1. Main Text

Human visual system has the ability to adapt to different light intensities [1]. Photopic vision occurs when cones are active and when the luminance level exceeds 5 cd/m². Below this threshold, another receptor type is active - rods, and this is referred to as scotopic vision [2]. After exposure to intense light, photoreceptors' pigments become bleached. The eye's sensitivity to light is disrupted, and readaptating vision to darkness takes up to several dozen minutes. Cones readapt faster than rods due to differences in visual pigment cycle for rhodopsin and cones' opsins [1]. The changes in visual sensitivity during readaptation process can be measured and analysed to distinguish between cones and rods sensitivity for a given retinal location [2]. In this study we present results of measuring visual threshold after bleaching of 60% of rhodopsin in 6 deg temporal location of retina of healthy participant. The measurements were conducted for two stimuli: visible (VIS: 520 nm) and infrared (IR: 1040 nm), both of which were perceived as green due to perception of infrared by two-photon vision process [3]. The observed differences between both mechanism of vision are in the cone's part of readaptation to darkness which is in agreement with our previous results [2,3].

2. Methods and results

In the experiment, a HighQ "FemtoTrain" femtosecond laser with a frequency of 76 MHz and a pulse width of 260 fs was used to display a circular stimulus of 2 deg radius, due to fast scanning. The optical scheme of the system is presented in Fig. 1.The stimulus was placed 6 deg temporally from the fovea center. At the fovea center a red fixation point was projected, on which the subject focused their gaze. In the pupil plane, the stimulating laser beams had a Gaussian profile with a radius of 0.58 mm (VIS) and 0.42 mm (IR). The square image of a bleaching LED diode formed in pupil plane had a diagonal of 1.1 mm. The visual thresholds were determined by method of adjustments with a step of 0.5 dB in stimulus intensity.

The participant was 47 years old healthy female, dark adapted for 30 minutes before measurements. The measurements were performed on her right eye which was myopic (-1.5 D). The correction of refraction error for both stimulating beams was achieved by moving lenses: L2 and L9. To enlarge pupil and block accommodation response 1% tropicamide drops were applied; thus, the pupil diameter during whole experiment, including bleaching flash, was larger than 7 mm. The bleaching flash had an intensity of $4.55 \cdot 10^7$ Td (scotopic) and lasted 200 ms, corresponding to bleaching of 60% rhodopsin molecules [4].

First, the average visual threshold from five consecutive measurements after the eye had adapted to darkness was determined and further referred as P_{ref}. The logarithm of the threshold level was calculated as the ratio of the instantaneous threshold power to the reference threshold power:

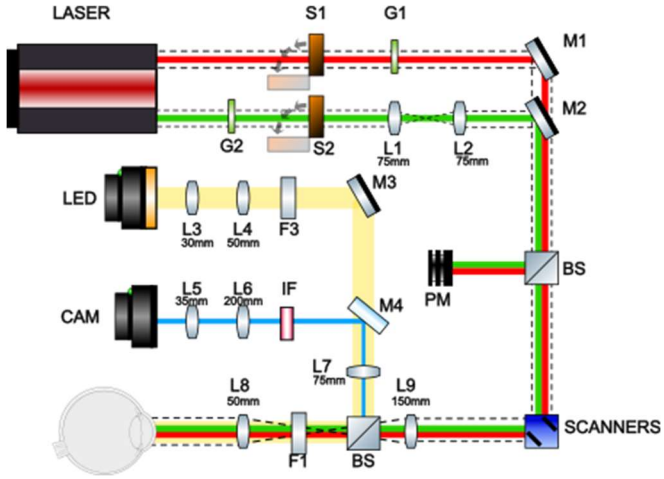

Fig. 1. Scheme of the setup. The laser beam size and intensity is properly prepared by lenses (L) and filters (F-grey filter, G-gradient filter). Laser beams can be covered with shutters (S1 and S2). Power is being measured constantly by power meter (PM) connected with the computer. There is also bleaching led diode (LED) and camera (CAM) that record eye picture.

$$\log_{10}(\text{VIS threshold level}) = \log_{10}\frac{P_t}{P_{ref}}, \quad (1)$$

where P_t is the power of threshold level in W.

The brightness of two-photon stimuli depends quadratically on the beam power [3]. Therefore, to present both visual processes on the same scale, the logarithm values for 1040 nm were multiplied by a factor of 2.The initial visual threshold was reached after more than half an hour. The log elevation of threshold against time is presented in fig. 2. Two independent exponential functions of the form were fitted to the collected data for both stimuli:

$$y = a \cdot \exp\left(-\frac{x}{b}\right) + c. \quad (2)$$

Each exponent refers to a different type of photoreceptors. The first exponential function (IR 1, VIS 1) corresponds to process of readaptation of cones, while the second one corresponds to rods (IR 2, VIS 2). Parameters of fitted function for data are presented in Fig. 2 and listed in the Tab. 1. The rods exponents for both processes seem to be similar, while cones readaptation probed with one- and two-photon stimuli id significantly different. Particularly, the cones plateau for two-photon stimuli is less elevated (the difference between parameter c for both exponents equals 1.21 vs 0.68 for IR and VIS, respectively), resulting in delayed incidence of rod-cone break. The rode-cone break occurred 788 s and 538 s after bleaching for IR and VIS stimuli, respectively. The results are in accordance with previously obtained ones for the similar stimuli [2,3]. The observed differences may result from the smaller difference in the sensitivity of rods and cones for the two-photon stimulus than for one-photon stimulus of similar colour [3]. Further experiments are planned to test this hypothesis.

Tab. 1. Parameters of fitted functions.

	a (a. u.)	b (s)	c (a. u.)
IR 1	1.75± 0.16	48±3	0.68±0.15
VIS1	0.92±0.11	232±111	1.21±0.11
IR 2	2.17±0.15	681±52	0.00±0.02
VIS 2	3.82±0.11	483±11	0.04±0.01

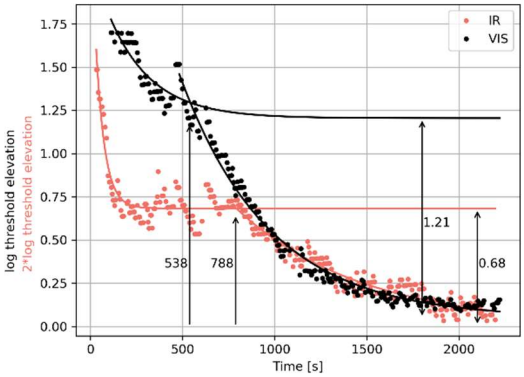

Fig. 2. Two independent exponential functions were fitted to collected data for both typed of stimuli.

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4. References

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