Investigation of steady-state pupil responses to test stimuli defined in photoreceptor stimulation

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The pupillary light reflex has been known to be useful in medical and clinical applications and the understanding its mechanisms is of great importance. Previous studies have shown that factors such as the luminance and colours of light stimuli are related to the pupillary light reflex. Light information entering the retina is first encoded by retinal photoreceptors and signals from each photoreceptor are input to the pupil control mechanism via post-receptoral processes and complex neural circuits in higher brain functions. Test stimuli of varying colour and luminance seem to be insufficient to investigate the pupil control mechanism. This is because multiple photoreceptors respond to colour and/or luminance stimuli, and the outputs of multiple photoreceptors are delivered to the pupil control mechanism or other higher brain functions. To understand the pupil control mechanism, it seems important to consider the physiological processes such as photoreceptors and post-receptoral mechanisms and possibly higher brain functions that contribute to the pupil control mechanism. The aim of this study was to investigate how each retinal photoreceptor affects the pupillary light reflex, rather than changing the colour or luminance of the test stimulus. To this end, a four-primary light stimulator was used to independently stimulate three types of cones and melanopsin photoreceptors. We measured steady pupillary responses to the test stimuli which stimulated each retinal photoreceptor independently. The test stimuli increased or decreased the stimulation to four types of photoreceptors, melanopsin, L cone, M cone and S cone. It was found how the retinal photoreceptors, melanopsin, L cone, M cone and Scone, contribute to the pupillary light reflex. The use of test stimuli defined in photoreceptor stimulation is expected to advance future pupil research.

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Introduction

In general, the pupil constricts in bright light and dilates in dark light. This pupil response is called the pupillary light reflex and is known to be useful for both basic research such as the study of brain functions, and medical/clinical applications such as disease detection and preventive medicine. It is therefore considered very important to investigate the mechanisms that drive the pupil. Previous studies have shown that the pupil light reflex varies with stimulus luminance, colour, spatial frequency and motion [1-9].

In the literature although there are variety of pupil studies for many years the mechanism that drives the pupil is still poorly understood. The human retina contains cone and rod photoreceptors, as well as the recently discovered melanopsin photoreceptors [10]. Melanopsin photoreceptors are capable of photoreception themselves. They are also well known to transmit signals of light information to the pupil control mechanism. Light information entering the retina is first encoded by retinal photoreceptors and signals from each photoreceptor are input to the pupil control mechanism via post-receptoral processes and complex neural circuits in higher brain functions (Figure 1).

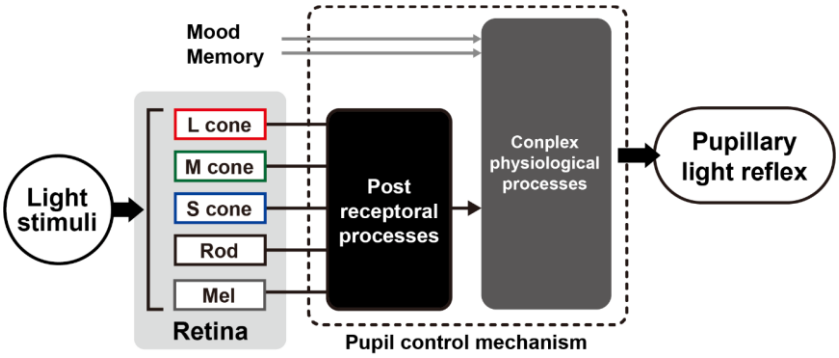

Figure 1: Schematic diagram of the pupil control mechanism.

Test stimuli of varying colour and luminance seem to be insufficient to investigate the pupil control mechanism associated with the pupillary light reflex. This is because multiple photoreceptors respond to colour and/or luminance stimuli, and the outputs of multiple photoreceptors are delivered to the pupil control mechanism or other higher brain functions. To understand the pupil control mechanism, it seems important to consider the physiological processes such as photoreceptors and post-receptoral mechanisms and possibly higher brain functions that contribute to the pupil control mechanism.

The aim of this study was to investigate how each retinal photoreceptor affects the pupillary light reflex, rather than changing the colour or luminance of the test stimulus. To this end, a four-primary light stimulator was used to independently stimulate three types of cones and melanopsin photoreceptors. Using this device, it is possible to stimulate only the target photoreceptors and not change the amount of stimulation to the other photoreceptors. Such a technique has long been used as the silent-substitution technique in physiology and psychophysics [11-12]. By using this technique, it is possible, for example, to distinguish pupillary light reflex to a metameric pair of stimuli with equal stimulation of L, M and S cones; the photometric luminance and chromaticity are the same whereas the melanopsin stimulation is different. This study clarified how light flux affects the pupillary light reflex in relation to the amount of photoreceptor stimulation. The use of test stimuli defined in photoreceptor stimulation is expected to advance future pupil research.

Methods

Apparatus

We used a multi-primary stimulation system for our experiments [13]. This stimulation system can independently stimulate each photoreceptor using silent-substitution technique, based on the amount of stimulation to each photoreceptor.

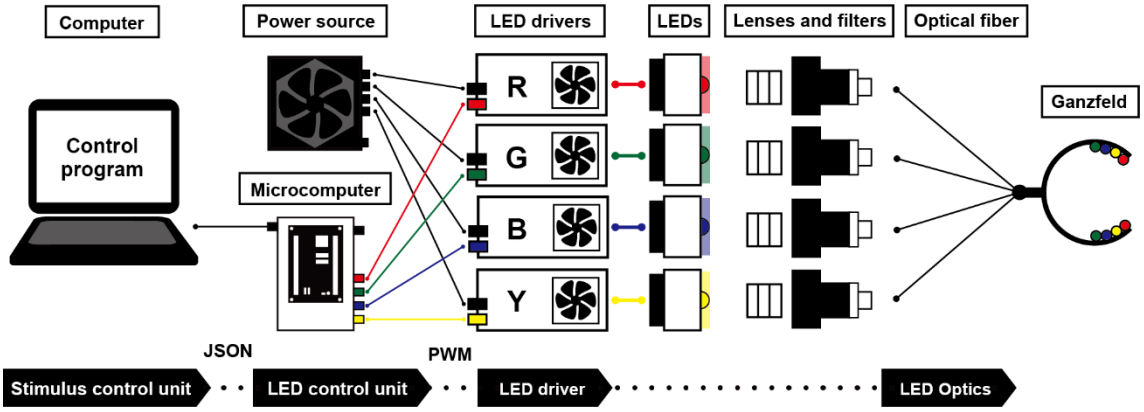

Figure 2: A multi-primary stimulation system.

Figure 2 shows the multi-primary stimulation system. The stimulation system consisted of a stimulus control unit, LED control units, LED drivers, interreference filters, etc. The stimulus control unit calculated the luminance of LEDs which stimulate to each photoreceptor. The output of stimulus control unit was sent to the LED control unit via USB with JSON (JavaScript Object Notation). In the LED control unit, the microcomputer (STM32F407G, STMicroelectronics, USA) calculated a Pulse Width Modulation (PWM) for each LED. The outputs of the LED light sources were controlled by PWM. The LED drivers send a power to the four LED light sources. The lights from the LED light sources reaches the semi-integrating sphere through lenses, interference filters and optical fibres.

The stimulation system had four primary colours: red, yellow, green and blue, with peak wavelengths of 633 nm, 569 nm, 532 nm and 463 nm, respectively. Figure 3 shows these spectral radiances.

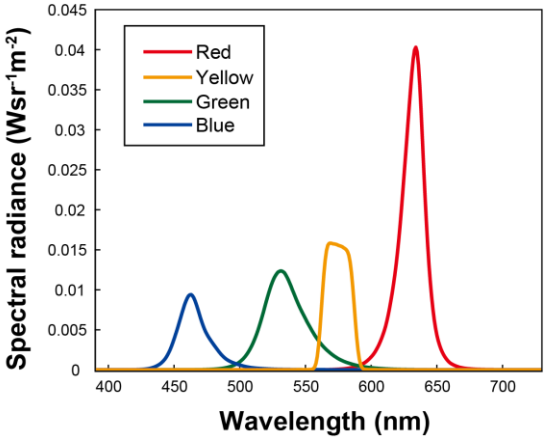

Figure 3: Primary of each LED.

We chose these four primary colours to maximise the contrast of melanopsin modulation on a white control stimulus and to minimise individual differences. The four-primary stimulation system enables us to independently control L-, M-, S-cones and melanopsin stimulations. To minimise the effect of rods, we used the control stimulus with high luminance level to prevent rod contamination. In order to independently control the five photoreceptors it is straightforward to use five primaries for the independent control of the stimulation. However, bandwidths of the primaries in five-primary stimulation system are usually narrower than those of a four-primary stimulation system that might cause a large individual difference. When the bandwidths of the primary are narrow there is a large individual difference in the colour matching functions [14]. This is because the photoreceptor stimulation is based on the inner product of the spectral radiance of the test stimulus and the spectral

sensitivity of each photoreceptor, so-called the principle of univariance [15]. To ensure accuracy, it is important to consider these individual differences. The Full Width Half Maximum (FWHM) of the four primaries were 65 nm for red, 27 nm for green, 25 nm for yellow, and 10 nm for blue, respectively.

Calibration

The light stimulus consists of the four primaries. The relationship between luminance from each primary and PWM ratio is slightly away from the linearity due to physical characteristics of LED in the real environment. Although PWM is an efficient technique that provides high LED output linearity, small deviations from linearity in luminance were observed, which were probably caused by thermal effects of the LEDs [16]. We used a fourth-order polynomial fit to consider deviations in each LED.

Test stimuli

We used nine test stimuli that can stimulate each photoreceptor independently. The silent-substitution paradigm was used for the independent stimulations [17-19]. The test stimuli' s amounts of stimulation to each photoreceptor were modulated from the control stimulus.

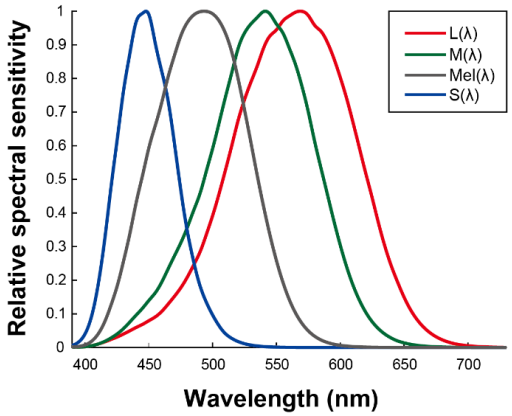

Figure 4: Relative spectral sensitivities of cone and melanopsin photoreceptors.

The amount of stimulation to each photoreceptor was calculated from the spectral radiance of the test stimuli and the spectral sensitivity of each photoreceptor. Figure 4 shows the spectral sensitivities of L, M, S cones and melanopsin. The amount of cone stimulation was calculated based on cone fundamentals at the peripheral visual field in human (L, M and S) [20-21], and that of melanopsin stimulation was calculated based on the sensitivity curve of melanopsin (Mel) [22-24] that has a peak sensitivity at 493 nm for young adult. The amount of stimulation to each photoreceptor L, M, S and Mel are calculated by the following Equations (1-4); $I(\lambda)$ represents the spectral radiance of the test stimulus. $L(\lambda)$, $M(\lambda)$, $S(\lambda)$ and $Mel(\lambda)$ represent the spectral sensitivity of each photoreceptor; L cone, M cone, S cone and melanopsin.

$$Mel = K_{mel} \int Mel(\lambda)\, I(\lambda) d\lambda \quad (1)$$

$$L = K_m \int L(\lambda)\, I(\lambda) d\lambda \quad (2)$$

$$M = K_m \int M(\lambda)\, I(\lambda) d\lambda \quad (3)$$

$$S = K_s \int S(\lambda)\, I(\lambda) d\lambda \quad (4)$$

K_m represents the conversion factor from the radiant power (watt) to the luminous power (lumen); The conversion factor K for the L- and M-cone stimulations was 683 lmW^{-1}. Since we assume that S-cone and melanopsin do not contribute to the photopic luminance, we used the conversion factor for S cone [25]; the stimulation to S cone 1 cdm^{-2} was defined as the amount of stimulation to S cone produced by equal energy white with luminance of 1 cdm^{-2}. Similarly, we defined the conversion factor for melanopsin. The conversion factor, Ks for S cones is 1466 lmW^{-1} and the conversion factor for melanopsin, K_{mel}, was 872 lmW^{-1}, respectively.

The nine test stimuli were used for the experiment. These test stimuli were increased or decreased the amount for each photoreceptor by 20 %, compared to the control stimulus (Figure 5). Summaries of cone, and melanopsin stimulations for the stimuli and their spectra for all conditions in the present study are in Figure 6 and Table 1.

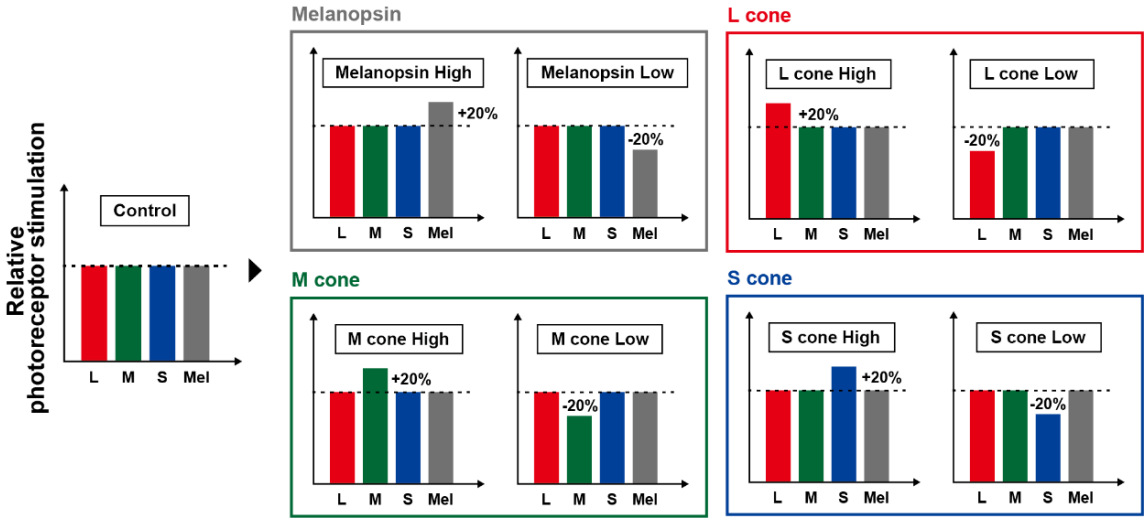

Figure 5: Relative modulation of photoreceptor stimulation from the control stimulus.

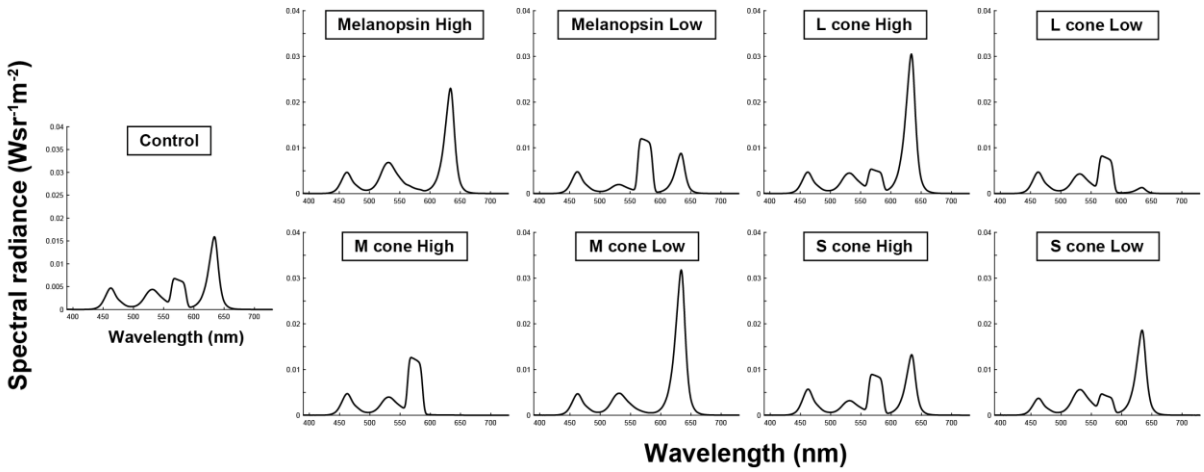

Figure 6: Spectra of each test stimulus.

A white control stimulus was used with photoreceptor stimulations of 206 cdm^{-2} for L cone, 76 cdm^{-2} for M cone, 132 cdm^{-2} for S cone, and 174 cdm^{-2} for melanopsin. We used the melanopsin high stimulus, MelH, and melanopsin low stimulus, MelL, which only modulated the amount of melanopsin stimulation by ±20% from the control stimulus. Similarly, we modulated the amount of stimulation to L, M and S cone by ±20%. Those stimuli were LH, LL, MH, ML, SH and SL. The five test stimuli, Control,

MelH, MelL, SH, and SL stimuli, have the same luminance of 282 cdm^{-2}. In particular, Control, MelH, and MelL stimulus were metamers with the same tristimulus values.

Stimulus	Photoreceptor stimulation (cdm^{-2})				Luminance	Contrast	x	y
	Mel	L	M	S	(cdm^{-2})		CIE2006	
Control	174	206	76	132	282	0	0.43	0.39
Melanopsin High	209	206	76	132	282	+20%	0.43	0.39
Melanopsin Low	140	206	76	132	282	-20%	0.43	0.39
L cone High	174	248	76	132	324	+20%	0.48	0.37
L cone Low	174	165	76	132	241	-20%	0.34	0.42
M cone High	174	206	91	132	297	+20%	0.37	0.44
M cone Low	174	206	61	132	267	-20%	0.48	0.35
S cone High	174	206	76	159	282	+20%	0.42	0.37
S cone Low	174	206	76	106	282	-20%	0.44	0.41

Table 1: Photoreceptor stimulation for each test stimulus.

We calculated the photoreceptor stimulation from the measured spectra of the test stimuli and the spectral sensitivity of each photoreceptor. The contrast of photoreceptor stimulation, calculated from the spectra to the control stimulus, was approximately ±20% (Table 2). These results indicate that the exposed test stimuli met our conditions for stimulus presentation.

Stimulus	Contrast of each measured photoreceptor stimulation			
	L	M	S	Mel
Melanopsin High	0.2%	0.3%	0.2%	20.5%
Melanopsin Low	-0.2%	-0.3%	-0.1%	-20.4%
L cone High	20.3%	0.3%	0.4%	0.4%
L cone Low	-20.2%	-0.2%	-0.2%	-0.1%
M cone High	-0.6%	19.6%	0.0%	0.1%
M cone Low	0.2%	-19.7%	0.6%	0.4%
S cone High	0.0%	-0.1%	19.7%	-0.3%
S cone Low	0.3%	0.3%	-19.4%	0.6%

Table 2: Contrast of each measured photoreceptor stimulation to the control stimulus.

Procedure

The right eye was exposed to full-field stimuli and the pupillary response of the left eye was measured with an infrared camera. The observers were exposed to the test stimulus for approximately 5 minutes (Figure 7). The steady pupil diameter to the test stimuli was recorded for the last 100 seconds. The order of a pair of the test stimuli were counterbalanced. The eight observers participated in the experiment. They were 3 males and 5 females aged between 21 and 25 years old, with a mean age of 22.1.

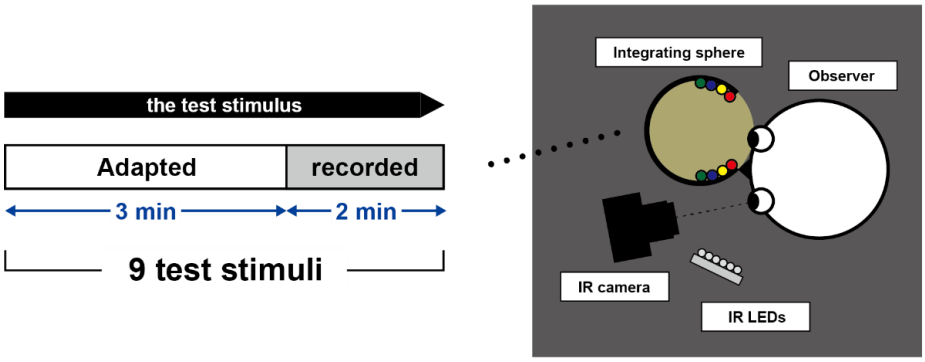

Figure 7: Experimental environment and presentation of the test stimuli.

Analysis

A time series of pupil data for the last 100 seconds during which the observer was exposed to the test stimulus was used for analysis. The video was captured at 30 frames per second, resulting in 3000 frames of pupil images for each test stimulus. The pupil diameters during the 100 seconds were averaged. The observers took at least four measurements for each test stimulus.

Results and discussion

The averaged pupil diameters from 8 observers were shown in Figure 8. Error bars represent standard deviations. The significant differences in the results of melanopsin and S cone were found. The steady-state pupil diameter to the MelH stimulus was significantly smaller than that to the MelL stimulus (t (7) = 2.36, p = .004). The pupil diameter to the LH stimulus was smaller than that to the LL stimulus (t (7) = 2.36, p = .210). The pupil diameter to the ML stimulus was smaller than that to the MH stimulus (t (7) = 2.36, p = .144). The pupil diameter to the SH stimulus was significantly smaller than that to the SL stimulus (t (7) = 2.36, p = .014).

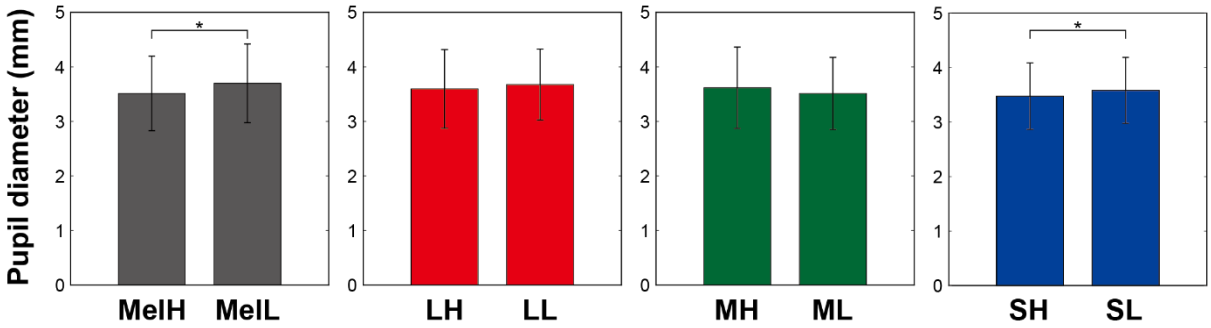

Figure 8: Averaged pupil diameters to the test stimuli.

It was found that the stimulation to melanopsin photoreceptors affects the steady-state pupil diameter which is consistent with the previous studies [19,26-29]. In addition, L-cone stimulation decreases a pupil diameter whereas M-cone stimulation increases it, suggesting that there is a contribution of L-M cone-opponent mechanism to the pupil control mechanism that is also consistent with previous studies [9,29-30]; the L-M cone opponent mechanism contributes to the pupil responses.

Conclusions

In this study, we measured steady pupillary responses to the test stimuli modulating the amount of stimulation to each photoreceptor independently, rather than changing the colour or luminance of the test stimulus. This is because multiple photoreceptors respond to colour and/or luminance stimuli. To understand the pupil control mechanism, it seems important to consider the physiological processes such as photoreceptors, post-receptoral mechanisms and possibly higher brain functions that contribute to the pupil control mechanism. It was found how the retinal photoreceptors, melanopsin, L cone, M cone and Scone, contribute to the pupillary light reflex. The use of test stimuli defined in photoreceptor stimulation is expected to advance future pupil research.

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