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# Melanopsin Contribution to Pupillary Light Reflex and Brightness Perception Based on a 65-Inch Four-Primary Projected Display

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**Abstract:** Melanopsin contribution to visual and non-visual effect has drawn widespread concern. However, research about whether this contribution can be applied to display system design is limited. Here, a four-primary display system was designed and constructed based on three projectors with filters to realize isolation control of melanopsin and cones, and a 65-inch uniform display area was achieved. The melanopic luminance metamers (higher and lower) of different colours have been modulated thusly. The effect of melanopic luminance on pupillary light reflex (PLR) and brightness perception was explored under a luminous environment of 300 lx to ensure the saturation of rod. The results showed that the higher melanopic luminance level contributed to delayed contraction maintenance. Moreover, a log relationship was found between melanopic equivalent daylight luminance and pupillary contraction maintenance parameters with coefficient of determination more than 0.85. Furthermore, stimuli of higher melanopic luminance level appeared brighter, indicating that melanopsin contributed to brightness perception.

**Keywords:** photoreceptor isolation; melanopic luminance; pupillary light reflex; brightness perception

# 1. Introduction

In the visual system of humans, there are three classes of photoreceptors, including rods, cones (short (S)-, medium (M)-, and long (L)-wavelength sensitive cones), and intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing photopigment melanopsin, also called melanopsin-dependent retinal ganglion cells (mRGCs) [1]. Each class of photoreceptor features itself with varied spectral sensitive wavelength for rod, S-cone, M-cone, L-cone, and ipRGC at around 507, 420, 535, 565, and 480 nm, respectively [2]. All the photoreceptors cooperate with each other, responding to visual effect as well as non-visual effect by visual pathway and non-visual pathway, respectively. For visual effect, rod and cones in the outer retina dominate light absorption and projection via ipRGCs, participating in image forming and brightness perception [3–5]. In contrast, inner ipRGCs contribute a lot to pupillary light reflex (PLR) and circadian rhythms, which are involved in non-visual effects [6–8].

When ipRGCs were first discovered, researchers refocused on the influence of photoreceptors on visual and non-visual effects—PLR in particular. When the pupil is stimulated by alterations in light intensity, ipRGCs directly project signals to olivary pretectal nucleus



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Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/ licenses/by/4.0/). (OPN), and then the nerve fibre is connected to the iris parasympathetic nerve through the commutator to realize the regulation of PLR [9]. PLR contains the pupillary contraction phase and dilation phase. Rod and cones participate in the contraction phase by the activation of the parasympathetic nervous system and the circular muscles of the iris [10]. In contrast, the dilation phase involves the recovery of the pupil after exposure to bright light, allowing for the entry of more light to facilitate vision in weak light intensity. This dilation phase is under the control of rod, cones, and ipRGCs, regulated by the sympathetic nervous system and the radial muscles of the iris [11].

The contraction phase of the pupillary light reflex is characterized by several parameters: peak contraction amplitude (PCA) representing the maximum reduction in pupil size, time for reaching peak contraction amplitude (Tca), and constriction velocity (Cv) denoting the ratio of PCA to Tca [12]. Conversely, the dilation phase parameters, also called contraction maintenance parameters, include post-illumination pupil response (PIPR) [12] and area under the curve (AUC) [13], indicating the process of pupil dilation to return to its baseline size. During PIPR, melanopsin gradually plays a critical role, especially during later phases of PIPR ( $\geq$ 1.8 s) [14]. Thus, some parameters can be extracted from PIPR to research the effect of ipRGCs such as pupil size at 1.8 s or 6 s after light offset (1.8 s PPIPR or 6 s PIPR) [15].

Studies have been performed to investigate the effect of melanopsin on PLR. Young et al. found that melanopsin contributed to the steady-state pupil size under daylight illuminance [16]. McDougal et al. confirmed that melanopsin plays a substantial role in the maintenance of half PCA when exposed to light stimuli lasting 30 s or more, even under low-photopic-irradiance conditions [10]. Joyce et al. found that Tca was prolonged under high-melanopsin excitation [17]. Apart from melanopsin, cones and rod participated in PLR as well. Adhikari et al. found that the peak sensitivity of PIPR shifted towards longer wavelength during intervals <1.7 s, which predominantly arose from the synergistic influences of melanopsin and rhodopsin. Research by Zele et al. revealed that cones and melanopsin cooperatively participated in cone-initiated pupil responses for faster constriction latencies due to higher velocities [18]. Woelders et al. found that L-cone and M-cone [19]. It has been proven that rod and cones contribute a lot to PLR. As a result, it is important to constrain the contributions of cones and rod to an imperceptible range to emphasize the impact of melanopsin.

Spectral sensitivity overlap renders the impossibility that a single spectrum activates only one photoreceptor class. To realize the independent control of ipRGCs, multi-primary display was designed and the principle of silent substitution was applied [20]. In silent substitution, the presentation of a couple of light stimuli is carefully controlled so that only one type of photoreceptor was selectively activated while the others were minimized. The couple of stimuli were also called metamers [21]. Yang et al. and Delawyer et al. created four-primary display systems based on three projectors with three filters that can independently stimulate melanopsin and cones [22,23]. Further, to enable the independent control of five photoreceptors, Allen et al. established a five-primary display across two projectors with filters installed [24]. Moreover, Hexley et al. designed a highly dynamic range display system with two projectors with filters and a liquid crystal display (LCD) panel to achieve six primaries, and modulated a series of metameric stimuli with melanopsin isolation [25]. Evidently, projectors with filters were widely considered as a method to realize multi-primary display. In contrast, Nugent et al. applied a novel field-programmable gate array control protocol to five displays to split data into five video streams to realize a five-primary display system with high retinal illumination [26]. It has been shown that

compared to complex hardware adjustment, self-designed display systems with filters installed in projectors are convenient to realize photoreceptor isolation.

Aside from the non-visual effect of PLR, the effect of melanopsin on the visual effect of brightness perception has also received more and more attention. IpRGCs can encode irradiance and adjust dorsal lateral geniculate nucleus (dLGN) activity according to the brightness of the environmental background [3,27]. A subjective method such as brightness perception evaluation is always applied for the individual judgement of brightness, which can also correspond to PLR [28–30]. Yamakawa et al. utilized subjective brightness discrimination assessment and PLR to formulate a model where the ratio of melanopsin contribution in brightness discrimination was estimated with different luminance levels of  $5^{\circ}$  white circles as stimuli [31]. Salinas et al. used PLR and brightness perception to quantify the relationship between pupil size and brightness since the results showed that brightness rather than luminance exerted a greater effect on pupil diameter [32].

Most existing studies have explored the effect of melanopsin on PLR or brightness perception with self-designed lighting systems to maximize the relative impact of melanopsin. To explore a new dimension for display performance improvement, here, we investigate the influence of melanopic luminance on PLR parameters and brightness perception on a four-primary display system when the display luminance is kept unchanged between melanopic metamers of circle stimuli.

In our study, a four-primary colour display system was constructed based on three projectors to realize cone isolation, and a 65-inch screen was adopted considering the alignment accuracy and luminance uniformity. We controlled the physical luminance and chromaticity coordinate of modulated melanopic metamers to be the same, and explored the influence of melanopic luminance on PLR and brightness perception. Further, the melanopic luminance effect on PLR parameters was quantified. The results can help to provide evidence for the visual and non-visual effect of ipRGCs and they might be used for display system design to improve display quality from a novel aspect. In the near future, a series of further studies will be conducted on this display system to improve display performance in terms of the visual and non-visual effect of ipRGCs.

## 2. Materials and Methods

#### 2.1. Apparatus

A four-primary display system, similar to that in Delawyer's work [23], was constructed with three identical projectors (X1328WH, ACER,  $1920 \times 1200$ , Suzhou, China), which projected images onto a screen with a 65-inch display area (145 cm  $\times$  81.5 cm). The three projectors were arranged vertically. The two band pass filters and one notch filter were installed into each projector to achieve the four primary colours: yellow (F589 nm, #65-162, BOSON, Shanghai, China), green (F520 nm, #65-154, BOSON, Shanghai, China), and red and blue (NF488 nm, #67-117, BOSON, Shanghai, China), denoted as Y, G, R, and B, respectively. As the display system has four primaries, four classes of photoreceptor isolation can be realized by silent substitution, except the rod since it shows saturation at low light conditions of 2 log units [33].

A uniform  $6 \times 4$  black-and-white checkboard was used to align the three projectors. However, slight edge inconsistencies remained due to the inherent characteristics of the projector's projection onto the screen surface and the relative nonuniformity of luminance compared to an electronic display. The luminance uniformity at full screen was 19.8%, which is below the threshold of 20% as outlined in the display measurement standard [34]. The 65-inch display area was selected as a result of the luminance uniformity and alignment accuracy even if the display system could present an area larger than 65 inches. The luminance uniformity and colour gamut of the full screen were measured by a surface luminance metre after calibration (CA2000, KONICA MINOLTA, Tokyo, Japan).

Gamma curves for each primary colour are presented in Figure 1, showing peak luminance values at 13.14 nits, 17.42 nits, 34.91 nits, and 30.88 nits for blue-, green-, yellow-, and red-primary, respectively. The individual spectra corresponding to the maximum luminance are depicted in Figure 2. Moreover, the enlarged colour gamut was achieved by the four primaries, corresponding to 176.17% coverage of sRGB or 124.01% coverage of NTSC (Figure 3). In contrast, the colour gamut of the original projector without filter was equivalent to 80.84% coverage of sRGB or 57.25% coverage of NTSC (Figure 3). The maximum and minimum luminance of the display system was 499.34 nits and 0.2 nit, correspondingly.



Figure 1. Gamma curves of the four primaries.



Figure 2. Spectra of the four primaries.



Figure 3. Experimental stimuli and colour gamut of the display system and the original projectors.

#### 2.2. Stimuli

In order to research the influence of melanopsin with the display image kept identical, melanopic metamers were modulated to keep the luminance and colour coordinate the same based on the four-primary display system. The 2-degree circular stimuli were utilized in accordance with the photoreceptor distribution in retina [4]. The circle stimuli were displayed on a black background on the 65-inch screen to highlight the effect display stimuli have on PLR. According to ITU-R BT.500, the viewing distance was set for 3.2 times of screen height (approximately 260 cm) [35], and the diameter of the circle was accordingly 9 cm for a 2-degree visual angle.

To quantify the ipRGC-influenced response, equivalent daylight (D65) luminance (EDL) in CIE S 026  $\alpha$ -opic toolbox was employed as the metric, with "cd/m<sup>2</sup>" or "nit" as the unit [36]. Eye sensitivity curves used in the toolbox are depicted in Figure 4, where convolution of the measured spectrum and normalized photoreceptor sensitivity curves under D65 was calculated for EDL. To emphasize the influence of melanopsin on visual and non-visual effect, we designed stimuli with four different colours for the purpose of maximizing Michelson contrast (shorted as MC) [37] of melanopic EDL without constraint on rod. MCs of EDL of S-cone, M-cone, and L-cone were all controlled at 5%. Based on the preliminary experiment, the physical luminance of stimuli was set at 50 nits to ensure display quality as well as larger MC of melanopic EDL, while the MC of luminance between each melanopic metamer was restricted to 2%, and chromatic difference ( $\Delta u'v'$ ) was constrained to 0.004 to guarantee invisible difference [34]. The physical luminance, chromaticity coordinates, and spectra of the melanopic metamers were measured by a spot metre (PR670, Photo Research).



Figure 4. Eye sensitivity curves in toolbox for calculation.

In order to ensure the output stability, the screen was evenly divided into 12 blocks (2 lines  $\times$  6 columns) to conduct measurements repeatedly. Physical luminance and chromaticity coordinate of each area were measured every half hour since the start-up of the display system, with a total of 7 measurements within 3 h. The results showed that the change in physical luminance ranged in 2% of 50 nits and chromatic differences between all melanopic metamers in 0.004, guaranteeing the EDL stability of five photoreceptors.

The measurement values of the melanopic metamers are delineated in Table 1. Four melanopic metamers (skyblue, grassgreen, gold, and orange, plotted in Figure 3) were modulated at two melanopic EDL levels (shorted as MEL-Level), denoted as higher (Mel\_H) and lower (Mel\_L). Apparently, the MC of luminance of the four melanopic metamers were all controlled within 1% and values of  $\Delta u'v'$  were all limited in 0.003 according to Table 1, satisfying the constraints. The EDL and MC of the EDL of five photoreceptors between melanopic metamers are listed in Table 2 and presented in Figures 5 and 6. The maximum

MC of melanopic EDL reached 51.58% for orange, and the MCs of EDL of cones were all limited within 5%.

Colour	MEL- Level	Luminance (Nit)	MC of Lumi- nance (%)	u′	$\mathbf{v}'$	$\Delta u'v'$	
Skyblue	Mel_H	50.28	0 5 4	0.1799	0.4018	0.0020	
	Mel_L	49.74	0.54	0.1787	0.4046	0.0030	
Grassgreen	Mel_H	50.00	0 55	0.1965	0.5198	0.0000	
	Mel_L	49.45	0.55	0.1972	0.5192	0.0009	
Gold	Mel_H	50.01	0.49	0.2486	0.5484	0.0028	
	Mel_L	49.53	0.40	0.2508	0.5502	0.0028	
Orange	Mel_H	50.13	0.20	0.3198	0.5462	0.0012	
	Mel_L	49.84	0.29	0.3205	0.5472	0.0012	

Table 1. Measured data of the four melanopic metamers.

**Table 2.** EDL and the MC of EDL of five photoreceptors (units of EDL and MC of EDL for nit and %, respectively).

Colour	MEL-Level -	S-Cone		M-Cone		L-Cone		Rod		Mel	
		EDL	MC	EDL	MC	EDL	MC	EDL	MC	EDL	MC
Skyblue	Mel_H	99.10	1.24	59.90	2.38	52.50	1.46	80.90	7 71	86.72	7 50
	Mel_L	96.68		57.11		50.98		69.32	/./1	74.48	7.59
Grassgreen	Mel_H	20.83	1.62	48.79	2.26	50.16	1.17	45.26	0.01	41.96	10.00
	Mel_L	21.51		46.63		49.00		37.17	9.81 33.6	33.69	10.93
Gold	Mel_H	4.48	4.02	41.49	2.89	51.24	1.07	30.51	17 40	25.90	<b>31</b> (0
	Mel_L	4.13		39.16		50.15		21.45	17.42	16.67	21.69
Orange	Mel_H	1.82	2.75	32.98	2.61	53.34	0.81	14.72	01 01	10.79	51.58
	Mel_L	1.92		31.31		52.48		7.62	31.81 3.45	3.45	



Figure 5. EDL of five photoreceptors for the four melanopic metamers.



Figure 6. MCs of EDL of five photoreceptors between the four melanopic metamers.

#### 2.3. Experimental Procedure and Setup

To explore the effect of melanopsin on PLR and brightness perception, we divided the experiment into two sessions corresponding to objective PLR measurement and subjective brightness perception. For PLR, pupillary response curves were recorded with an eye tracker (D-LAB, INFO Instruments, 60 Hz,  $1920 \times 1080$  pixels, Shanghai, China) during the observation of the modulated stimuli. Before the experiment, participants went through a 2 min environment adaptation after the explanation of experimental instructions. Then, the first trial of PLR started. In each trial, participants were stimulated for 1 s by a symbol "+" for a hint and then the screen became black for 3 s. After that, the circle stimulus was presented for 1 s, followed by a black screen for 10 s. Finally, subjects had a break for 60 s with a black screen, as shown in Figure 7. The trial was repeated 8 times (4 melanopic metamers  $\times$  2 MEL-Level) with a random order of stimuli per session. The session was repeated three times in the experiment with 10 min intervals. The procedure of each trial was designed to highlight the eye response to the melanopic metamers while black screens before and after the melanopic metamers were designed to calculate the baseline of the pupillary diameter and pupillary recovery, similar to the paradigm in Park's work [38]. The mean values of each individual were calculated and then normalized by the mean baseline values of a 3 s black screen for analysis. Six PLR parameters, including PCA, Tca, Cv, 1.8 s PIPR, 6 s PIPR, and AUC, were extracted from each normalized response curve. For AUC, it was calculated by the logarithm of the area enclosed by the curve at a 9 s interval and the baseline after the stimulus' disappearance.



Figure 7. Procedure of a trial of PLR.

After PLR measurement, the next session of subjective alternative choice was performed. Subjects made choices in terms of brightness between the Melah and Mel\_L metamers. The percentage of choosing either the Mel\_H or Mel\_L stimuli as the brighter one among the three choices of each participant was collected. The procedure of the trial is revealed in Figure 8. To start with, subjects watched one circle of the given melanopic metamer for 5 s, followed by a black screen for 0.5 s, and then the other circle of the given melanopic metamer appeared for 5 s. Finally, the participants had to choose which one was brighter. The trial was repeated 12 times (4 melanopic metamers  $\times$  3 repetitions) with a random sequence.



Figure 8. Procedure of a trial of brightness perception.

The experimental setup is illustrated in Figure 9. The screen centre was 117.5 cm away from the floor and 180 cm away from the display system. The subjects sat on an adjustable chair with circle stimuli right ahead, maintaining eye level in line with the screen centre.



Figure 9. Experimental setup.

The experiment was conducted under a luminous environment, corresponding to a normal environment to watch the display. Specifically, according to suggested daily office illuminance in CIE S 008 [39], a desktop illuminance of 300 lx, equivalent to 100 lx vertical eye-level illuminance, was selected as the luminous environment, ensuring the saturation of rod [31]. The whole procedure lasted approximately within a 70 min timeframe. The experiment was carried out from March 2023 to May 2023.

## 2.4. Participants

The participants were recruited in accordance with CIE TN 011: 2020 [40]. This study included 15 volunteers from Southeast University in Nanjing. All participants were local residents, ensuring a balanced gender distribution, with ages ranging from 23 to 27 years old and an average age of  $24.07 \pm 1.16$  years. To assess the sufficiency of the participant sample, the coefficient of variation (CV) was calculated for all collected data, yielding values ranging from 2.5% to 18.9%, all under 30%, indicating low relative variability and high stability within the data. This suggests that individual observations were consistent or closely aligned with the mean value [41].

Each participant had normal or corrected-to-normal vision ( $\geq$ 1.0) confirmed by a NIDEK RT-500 (Tokyo, Japan) intelligent refractor with Tumbling E tests, and no colour vision deficiencies were identified via Ishihara tests. Participants were instructed to refrain from alcohol and caffeine consumption and ensured sufficient rest 24 h prior to the experiment. Detailed procedures were explained to the participants to ensure comprehension before the formal experiment. Informed consents were obtained from all participants. This study strictly adhered to ethical principles as per the Declaration of Helsinki and received approval from the ethics committee of Southeast University prior to commencement.

# 3. Results

This study conducted an analysis of the impact of MEL-Level on PLR of non-visual effect based on the collected data first. Subsequently, the influence of melanopic EDL was explored to obtain the relationship between melanopic EDL and PLR parameters. Finally, subjective alternative choices were scrutinized to investigate the influence of melanopsin on the brightness of a visual effect.

Considering the normal distribution and homoscedasticity of the PLR data, repeated measures analysis of variance (RM ANOVA) was applied. The analysis of alternative choice utilized a Chi-squared cross table on account of the nominal nature of the independent and dependent variables. In addition, significance level was set at 0.05 (*p* value < 0.05), corresponding to confidence level at 95%. Partial eta square denotes the effect size in RM ANOVA with the following classifications: small for  $0.04 \le \eta^2 < 0.25$ , moderate for  $0.25 \le \eta^2 < 0.64$ , and large for  $\eta^2 \ge 0.64$  in RM ANOVA [42].

#### 3.1. PLR

## 3.1.1. Effect of MEL-Level

Results of the influence of MEL-Level on PLR parameters by RM ANOVA are summarized in Table 3. Significant differences were merely observed in 1.8 s PIPR with a \_

small effect size. In addition, the difference in AUC between MEL-Levels approached the significance level.

Dependent Variable	F	Sig.	$\eta^2$
PCA	1.049	0.310	0.017
Тса	0.113	0.738	0.002
Cv	0.688	0.410	0.012
1.8 s PIPR	4.069	0.048	0.065
6 s PIPR	0.251	0.619	0.004
AUC	3.551	0.064	0.057

Table 3. RM ANOVA results of effect of MEL-Level on PLR parameters.

As shown in Figure 10, Mel\_H witnessed a smaller pupil size of 1.8 s PIPR, corresponding to 0.43% less than Mel\_L. In addition, the AUC was bigger at Mel\_H, 0.14 greater than Mel\_L. Other PLR parameters presented no apparent trend. The PLR curves of Mel\_H and Mel\_L are depicted in Figure 11. The difference between Mel\_H and Mel\_L can be seen clearly, especially during the pupillary contraction maintenance phase.



Figure 10. Cont.



**Figure 10.** Effect of MEL-Level on PLR parameters (\* for p < 0.05). The diamond and triangle with dark colour denote the mean values at Mel\_H and Mel\_L respectively. The scatters for corresponding light colour represented all the data (60 scatters = 15 participants × four colours) at the two MEL-Levels respectively.



**Figure 11.** PLR curves of different MEL-Levels. The shadows of light blue and light orange along with the curves represent standard deviation.

#### 3.1.2. Effect of Melanopic EDL

The modulated four melanopic metamers exhibited eight melanopic EDL values, ranging from 3.45 nits to 86.72 nits. RM ANOVA results of the influence of melanopic EDL on PLR parameters are listed in Table 4. Melanopic EDL exerted no significant impact on pupillary contraction parameters (PCA, Tca, and Cv). In contrast, the effect of melanopic EDL on all contraction maintenance parameters (1.8 s PIPR, 6 s PIPR, and AUC) were or approached significant with small to moderate effect size. Figure 12 demonstrates the trends of all the PLR parameters and the post hoc results of those significant parameters by Tukey's honestly significant difference (HSD). The same label denotes a statistically insignificant group and the different labels represent statistically significant difference between the groups. It can be seen that at 1.8 s PIPR, there was an apparent downward trend as melanopic EDL increased, as well as at 6 s PIPR. In contrast, the AUC underwent reversed tendency with and upward trend. The detailed PLR curves of different melanopic EDL are plotted in Figure 13.



Table 4. RM ANOVA results of the effect of melanopic EDL on PLR parameters.

**Figure 12.** Effect of melanopic EDL on PLR parameters. The four different colours represent the colours of the four melanopic metamers, and the light and dark the fills for each colour denote Mel\_L and Mel\_H respectively. The same label denotes a statistically insignificant group. The different labels represent statistically significant difference between the groups.



Figure 13. PLR curves of the four melanopic metamers.

To further investigate the correlation of melanopic EDL and PLR, a fitting analysis was conducted for pupillary contraction maintenance parameters to show that melanopsin EDL exerted a significant effect. A log relationship between melanopic EDL (Equation (1)) and the parameters was established, and the results are listed in Table 5. It is noticeable that the degrees of fitting for these parameters were high, all of which were over 0.85. With reference to 1.8 s PIPR,  $R^2$  reached 0.977—the maximum. Concerning the AUC,  $R^2$  also attained 0.969. For 6 s PIPR, the minimum  $R^2$  of 0.883 was obtained. The corresponding fitting curves are illustrated in Figure 14.

$$y = a \times \log(x) + b \tag{1}$$

Table 5. Fitting results between melanopic EDL and significant PLR parameters.

PLR Parameters	$R^2$	а	b
1.8 s PIPR	0.977	-1.347	100
6 s PIPR	0.883	-0.540	101
AUC	0.969	0.513	3.24



**Figure 14.** Fitting curves between melanopic EDL and PLR parameters. The dark and light red shadows represent 95% confidence band and 95% prediction band, respectively.

Spearman correlation analysis [43] was conducted to investigate the correlation between melanopic EDL and contraction maintenance parameters with significant effect, and the results are detailed in Table 6. Visibly, all the correlations were significant at the level <0.05 or at the level <0.01. It is worth mentioning that melanopic EDL showed the maximum positive correlation with the AUC, having a correlation coefficient of 0.466, and this was followed by 1.8 s PIPR, having a coefficient of -0.368—corresponding to the two largest values of  $R^2$ . This means that when melanopic EDL rose, the AUC showed a growing trend, but 1.8 s PIPR presented a decreasing tendency, corresponding to the fitting results. Similarly, 6 s PIPR also negatively correlated with melanopic EDL, consistently showing downward trends as melanopic EDL increased. Consequently, 1.8 s PIPR presented strong positive correlation with 6 s PIPR, having a correlation coefficient of 0.192, while showing a reversed, negative correlation with the AUC. In addition, the AUC also showed a negative correlation with 6 s PIPR.

Table 6. Spearman correlation analysis results between melanopic EDL and PLR parameters.

	Melanopic EDL	1.8 s PIPR	6 s PIPR	AUC
Melanopic EDL	1	-0.368 **	-0.247 **	0.466 **
1.8 s PIPR	-0.368 **	1	0.192 *	-0.198 *
6 s PIPR	-0.247 **	0. 192 *	1	-0.352 **
AUC	0.466 **	-0.198 *	-0.352 **	1

\*\* Correlation was significant at level <0.01 (two-tailed). \* Correlation was significant at level <0.05 (two-tailed).

#### 3.2. Brightness Perception

Subjective alternative-choice evaluations between the two MEL-Levels were analyzed by Chi-squared cross table. MEL-Level exerted significant influence (sig. = 0.001) on brightness. As depicted in Figure 15, the percentage of perceiving Mel\_H stimuli as brighter was 17% more than that of Mel\_L stimuli. It means that participants considered Mel\_H stimuli as brighter, in spite of insignificant PCA.



**Figure 15.** Effect of MEL-Level on brightness perception. The diamond and triangle with dark colour denote the mean values at Mel\_H and Mel\_L respectively. The scatters for corresponding light colour represented all the data (60 scatters = 15 participants  $\times$  four colours) at the two MEL-Levels respectively.

## 4. Discussion

Melanopsin, rod, and cones play essential roles in the contraction phase, with rhodopsin and melanopsin making major contributions [14,44]. In terms of rod, saturation was ensured under our experimental environment of 300 lx. For cones, the pupillary contraction phase was induced by melanopsin and L-cone, and inhibited by S- and M-cone specifically [19]. Even if the Michelson contrasts of EDL of cones of the melanopic

metamers are limited to 5%, the EDL of S-cone for skyblue and grassgreen presented much larger values than that of orange and gold. Consequently, cone intrusion should be taken into consideration in our experiment. Fitting curves were established between melanopic EDL and S-cone EDL and PCA, and the values of  $R^2$  were 0.898 and 0.841, respectively, indicating that the pupillary contraction phase was majorly influenced by melanopsin.

For the influence of melanopsin on the pupillary contraction maintenance of PLR, the results indicated that a higher MEL-Level contributed to delayed contraction maintenance. It was supported by the smaller 1.8 s PIPR and larger AUC at Mel\_H. Similar delayed contraction maintenance in Ostrin's finding showed that, compared to red light stimulus, a significant decrement of 6 s PIPR and increment of AUC were observed when exposing the participants to blue stimuli on account of ipRGC activation [45]. Moreover, melanopic EDL also influenced contraction maintenance parameters with log models established. It can be seen that log relationship can principally explain the impact of melanopic EDL on 1.8 s PIPR, 6 s PIPR, and AUC since the coefficients of determination were high, all of which were more than 0.85.

Specifically, melanopsin gradually plays its role at the later contraction maintenance phase [15], supported by insignificant pupillary contraction parameters and the significant contraction maintenance parameter of 1.8 s PIPR as well as AUC in terms of the effect of MEL-Level. In the later phase of PLR, melanopsin emerges as a chief factor in encoding PIPR state under light illumination [16,46], especially during the later phase of PIPR ( $\geq$ 1.8 s) [14]. High  $R^2$  between melanopic EDL and significant contraction maintenance parameters (1.8 s PIPR, 6 s PIPR, and AUC) also implied that melanopsin played a dominant role in later contraction maintenance phases.

Concerning the correlation analysis, melanopic EDL strongly correlated to all the pupillary contraction maintenance parameters, consolidating that the contraction maintenance phase of PLR was mainly influenced by melanopsin. Generally, strong correlations were consistent, with high correlation coefficients between melanopic EDL and all the contraction maintenance parameters, showing that melanopic EDL exerted a significant impact, corresponding to the major contribution of melanopsin to the contraction maintenance phase under the luminous environment.

Furthermore, subjective alternative-choice evaluation revealed that the stimuli of Mel\_H appeared brighter. Under the experimental light condition, melanopsin played its roles. With Mel\_H stimuli presenting a brighter appearance, it can be inferred that melanopsin contributes to brightness perception. It corresponds to the findings of Brown and Salinas that the visual discrimination of humans was supported by inner retinal photoreceptors, and ipRGC response might be the crucial driving mechanism [5,31]. It is noticeable that melanopsin contributed to both non-visual effect and visual effect significantly based on the four-primary display system under normal viewing conditions, reflected by the changes in PLR and brightness perception.

It is important to note that the 300 lx of environment was used to ensure the saturation of the rod since rod was not a set limitation in our experiment. Further research could be conducted to investigate the visual and non-visual effect of melanopsin under different illuminations.

As a basis, the experiment proved that melanopsin exerted a significant impact on PLR and brightness perception with the four-primary 65-inch display system under normal viewing conditions. Moreover, melanopsin plays roles in abundant aspects of visual and non-visual effect such as alertness, cognition, and vision formation [3,24,47]. Consequently, further research may be conducted to provide insight into the visual and non-visual effect of melanopsin based on this 65-inch display system, inducing a novel dimension for display performance evaluation and display system design.

# 5. Conclusions

The influence of melanopsin on PLR and subjective brightness perception was investigated based on a 65-inch four-primary display system under the luminous condition. The system provided a large display area and enlarged colour gamut, and cones and ipRGCs could be independently controlled. The results revealed that melanopsin significantly contributed to both visual and non-visual effect with the four-primary display system under normal viewing conditions. Specifically, a higher MEL-Level induced smaller 1.8 s PIPR and greater AUC, indicating that melanopsin contributed to the pupillary contraction maintenance of PLR. Moreover, a notable log relationship was found between melanopic EDL and contraction maintenance parameters (1.8 s PIPR, 6 s PIPR, and AUC), all with *R*<sup>2</sup> more than 0.85, which was supported by corresponding strong correlations. Concerning brightness perception, the stimuli of higher MEL-Level values appeared brighter, revealing that melanopsin contributes to brightness perception. The findings revealed that melanopsin contribution to the visual and non-visual effect provides a novel dimension for display system design to improve display performance.

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