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Prior Light Exposure Enhances the Pupil Response to Subsequent Short Wavelength (Blue) Light

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Abstract

Background and Purpose: The photo pigment melanopsin initiates cell depolarization in response to high-intensity, short-wavelength light. Antecedent long-wavelength light may potentiate regeneration of the melanopsin photo pigment, We investigated the influence of red or blue exposure on the pupil response to subsequent blue light.

Methods: Nine healthy subjects were examined using chromatic pupillometry. With a sequence of 3 consecutive blue exposures or a sequence in which the middle exposure was red light, both sequences repeated in the dark-adapted state. The summed pupil response during light was obtained as the area under the curve and the percentage difference (diff %) between the first and last blue stimulus was calculated for each sequence.

Findings: The pupil response to the third blue exposure was greater than to first blue light. No significant difference was seen in the diff% when comparing a sequence with a blue intervening versus red intervening light, in the light adapted ($P = 0.39$) or dark adapted state ($P = 0.58$).

Conclusion: Prior light exposure enhances the pupil response to subsequent blue light stimulation, no differential effect was found between blue and red light. This study suggests that antecedent light history is important when designing protocols and evaluating results of chromatic pupillometry.

Keywords: Pupil light reflex; Melanopsin; Intrinsically photosensitive retinal ganglion cells; Pupillometry; Bistability

Introduction

A subset of retinal ganglion cells that contains the photo pigment melanopsin is sensitive to photons and these cells have been collectively termed intrinsically photosensitive retinal ganglion cells (ipRGCs). The sensitivity of melanopsin-mediated phototransduction is in the blue region of the light spectrum, and peaks around 470 ± 10 nm [1,2,3]. Though melanopsin is expressed in vertebrates including humans, its structure has greater homology to invertebrate rhodopsin photo pigments [4]. This similarity may extend to functional properties as well.

Invertebrate photopigments Are bistable and recent studies Have suggested that vertebral melanopsin shows the same characteristic [3,5,6]. Bistability refers to a dual state of photosensitivity in which photon absorption at one wavelength initiates phototransduction and cell depolarization via a conformational change in the melanopsin chromophore, and subsequent photon absorption at another wavelength regenerates the chromophore via isomerization back to the photosensitive states. The photo pigments of human rods and cones are not bistable as they depend on the retinal pigment epithelium for their chromophore regeneration. It is not yet established if melanopsin-mediated phototransduction in mammals is a bistable system, as current studies addressing this issue are conflicting [5,6,7].

One of the major functions of the ipRGCs is signaling the pupil light reflex. In a study exploring the hypothesis of melanopsin bistability in vivo in humans, Mure et al., used the pupil light reflex as a behavioural marker of in vivo melanopsin action [8]. To allow recovery of rods or cones to the same baseline state for the human study, the authors included a 40 minute dark period before each blue light exposure. Using a paradigm of successive light exposures, these authors found that 5 minutes of retinal exposure to long wavelength light caused an increase in the sustained pupil response to subsequent short wavelength (blue) light whereas preceding darkness or a previous exposure to short wavelength decreased the pupil response to blue light. Because of the long interval between light exposures the authors suggested that ipRGCs might have a sort of "photic memory" for prior light exposure. Such an adaptive feature of ipRGCs had not been observed previously. It indicate that long term temporal and spectral light changes govern photic entrainment through the ipRGCs.

The pupil light reflex has long been used in clinical practice as an objective marker of neuroretinal integrity, particularly for detecting a difference in light sensitivity between the two eyes [9]. Typically, a brief, white light (less than 5 seconds) has been the standard stimulus for such clinical pupil testing. More recently, the pupil light reflex to chromatic Light stimulation has been investigated to assess individual photoreceptor function [2,10,11,12]. Therefore, it would be important to determine if enhanced responsiveness and ipRGC "photic memory" play a role in pupillary responsiveness under conditions of clinical pupil testing. The purpose of the present study is to investigate if there is an effect of previous red or blue light exposure of short duration on the pupil response to a bright blue light stimulus.

Methods

Subjects

Nine healthy volunteers participated in the study. Participants did

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not have any past or present ocular or systemic conditions involving the retina or optic nerve (such as trauma, glaucoma, macular degeneration, and familial colour blindness and diabetes mellitus.) or any history of depression or psychiatric diseases as it is suggested that certain of them may be related to dysregulation of circadian rhythm.

Visual function was tested with ETDRS charts. All subjects had normal vision on the examined left eye, the mean best corrected visual acuity being log MAR -0.15 SD 0.1 (snellen equivalent 1.25). Mean refraction on the examined eye was -1.89 D, SD 2.86 (range –7.0 to 0.25 D).

Visual field were obtained using automated perimetry (programme 24-2, Humphrey Visual Field Analyzer, San Diego, CA), all subjects had normal visual fields with mean defect on the examined eye of -0.40 dB, SD 1.08 (range – 1.54 to 1.54). Subjects were colour testet with Farnsworth D15 hues. No subject had a significant colour defect (1 subject had a single error on one eye). The swinging-flashlight test was performed with a handheld light source to look for a relative afferent pupillary defect (RAPD). Retinal thickness and retinal nerve fiber layer thickness was examined with Spectral-domain OCT (Cirrus, Zeiss Humphrey, San Diego, CA). All scans were within normal limits, on the examined eye with a mean retinal thickness of 275 µm, SD 34.39 µm and a mean retinal nerve fiber of 86 µm, SD 5.66.

Colour fundus photographs were obtained with Topcon non-mydriatic camera (Topcon, Tokyo, Japan). Informed consent was obtained from all participants after a full description of the procedure, in compliance with the Declaration of Helsinki. The study was approved by the local ethics committee.

Chromatic pupillometer

We used a prototype pupillometer which combined a computerized Pupil video recorder and a chromatic light stimulator (Idea Medical, Copenhagen, Denmark) Full-field light stimulation was obtained with blue (470 nm) and red (660 nm) monochromatic, narrow bandwidth light-emitting diodes (LEDs). The colour, initiation and timing of the light stimulus were fully automated and controlled by a computer. For this study, the light intensity was fixed at 300 cd/m^2 for each wavelength and confirmed with a spectral photometer. In compliance with safety regulations of light use, the light intensity was chosen well below the recommendations of American National Standard (ANSI-2007) and International Commission on Non-Ionizing Radiation Protection (ICNIRP) for red, blue and IR illumination. The prototype has been described earlier [12]. In short, the examined eye is illuminated from a telecopic tube, preventing light scattering to the fellow eye. The LED's are placed appr. 10 cm in front of the eye behind a spherical diffuser, to ensure uniform illumination. The non-stimulated eye was situated in front of a video camera placed at the same distance as the illumination. An infrared video camera (850 nm, 470 lines, Sony, Japan) recorded the pupillary movement from the non-stimulated eye continuously at 20 Hz. The video signal was converted to graphic using a customized program. Care was taken to avoid any visual clues to accomodation and also, the subjects were instructed not to fixate at near.

Light source

Chromatic light stimulation was obtained by means of an array of blue (470 nm – Kingbridge type L-7104QBC-D) and red (660 nm – LED Technology type LURR 3000 G3) monochromatic narrow bandwidth LEDs with 20-22 nm full width at half maximum.

Test protocol

The pupillary test consisted of a triplet of light stimulations of 30 seconds each flanked by 30 seconds recovery in the dark – called a sequence (Figure 1). The dark periods allowed a separation of the response to light exposure before the next light stimulus was presented. The first and third light exposure of the sequence was identical blue light stimulations whereas the middle (intervening) light stimulus was either a blue or red light. Two light sequences were used: the first sequence was a triplet of blue-blue-blue light stimulations and the second sequence consisted of blue-red-blue light stimulations. In other words, the only difference between the two sequences was the second light stimulus which was either a blue light or a red light. For comparable terminology as used in the Mure study [6], the first light exposure, or stimulation, is termed the "reference stimulus" and the third is termed the "test stimulus".

The test protocol was performed under mesopic conditions in the light-adapted subject. The testing protocol commenced with a pre-stimulus recording in darkness for 10 seconds followed by presentation of the first light sequence (blue-blue-blue). After a 60 second pause in darkness the second sequence (blue-red-blue) was presented.

The entire test protocol as described in the preceding paragraph was then repeated in the same subjects under dark-adapted conditions

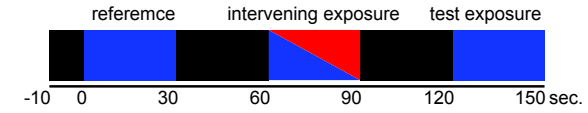

Figure 1: Schematic presentation of the testing protocol. With either a light sequence of blue-blue-blue or a sequence of blue-red-blue. The identical first and third blue light stimulations are termed the reference and test stimulus, respectively and the middle light stimulus is termed the intervening light exposure. Each light stimulus lasts 30 seconds and is separated by a 30 second dark period. Before presentation of the light stimulus sequence, the pupil is recorded for 10 seconds in darkness in order to obtain a baseline pupil size.

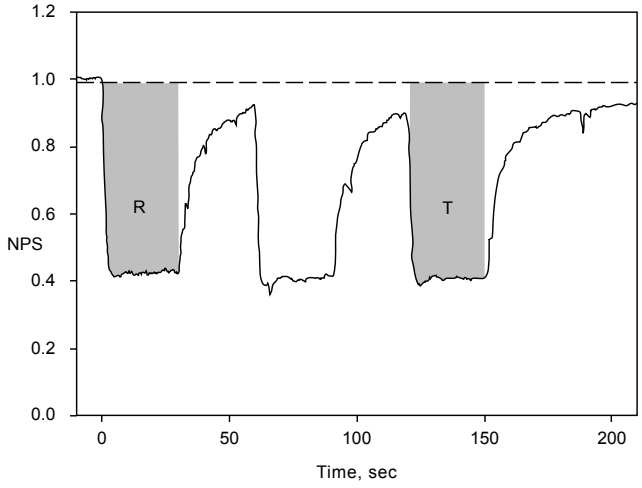

Figure 2: Graphic representation of the normalized pupillary size (NPS). NPS to 3 successive blue light exposures of 30 seconds each. The baseline pupil size is represented as a dotted line, and extrapolated throughout the graph. The summed pupillary response to light stimulus (area under the curve, AUC) is shown in grey for the first reference and third test light stimulus. R=reference blue light stimulus and T=test blue light stimulus.

		Sequence 1 with intervening blue light	Sequence 2 With intervening red light	P-value diff% in sequence 1 compared diff% in sequence 2
Light adapted	**$Blue_{reference}$** mean (95% CI)	**AUC** 16.63 15.93 – 17.33	**AUC** 16.52 15.94 – 17.09	
	$Blue_{Test}$ mean (95% CI)	**AUC** 17.40 16.70 – 18.11	**AUC** 17.08 16.59 – 17.55	
	$Blue_{Test}$- $Blue_{Reference}$ mean (95%CI)	**diff %** 4.7 2.1 - 7.4	**diff%** 3.4 1.0 - 5.9	0.39
Dark adapted	**$Blue_{Reference}$** mean (95% CI)	**AUC** 15.63 14.97 – 16.30	**AUC** 15.46 14.50 – 16.43	
	$Blue_{test}$ mean (95% CI)	**AUC** 17.04 16.37 – 17.72	**AUC** 17.04 16.39 – 17.69	
	$Blue_{Test}$- $Blue_{Reference}$ mean 95 %CI	**diff%** 9.2 5.0 – 13.3	**diff%** 10.7 4.3 – 17.1	0.58

Table1: AUC results. Pupil response to the reference and the test blue stimulus for 2 light sequences The area under the curve (AUC) of the pupillary response (mean and 95 % CI) is shown in the upper part of the table for the light adapted and in the lower part of the table for the dark adapted state. Sequence 1 refers to a triplet of 3 successive, equiluminant blue light exposures and sequence 2 refers to a similar triplet of light stimulation, except that the intervening (middle) exposure is a red light of similar intensity. For simplicity, only data from the reference and test stimuli are shown. Results are given as the relative difference between the reference and test stimuli for each sequence (diff%). P-values are calculated with a paired t-test comparing the relative difference with intervening blue light and intervening red light.

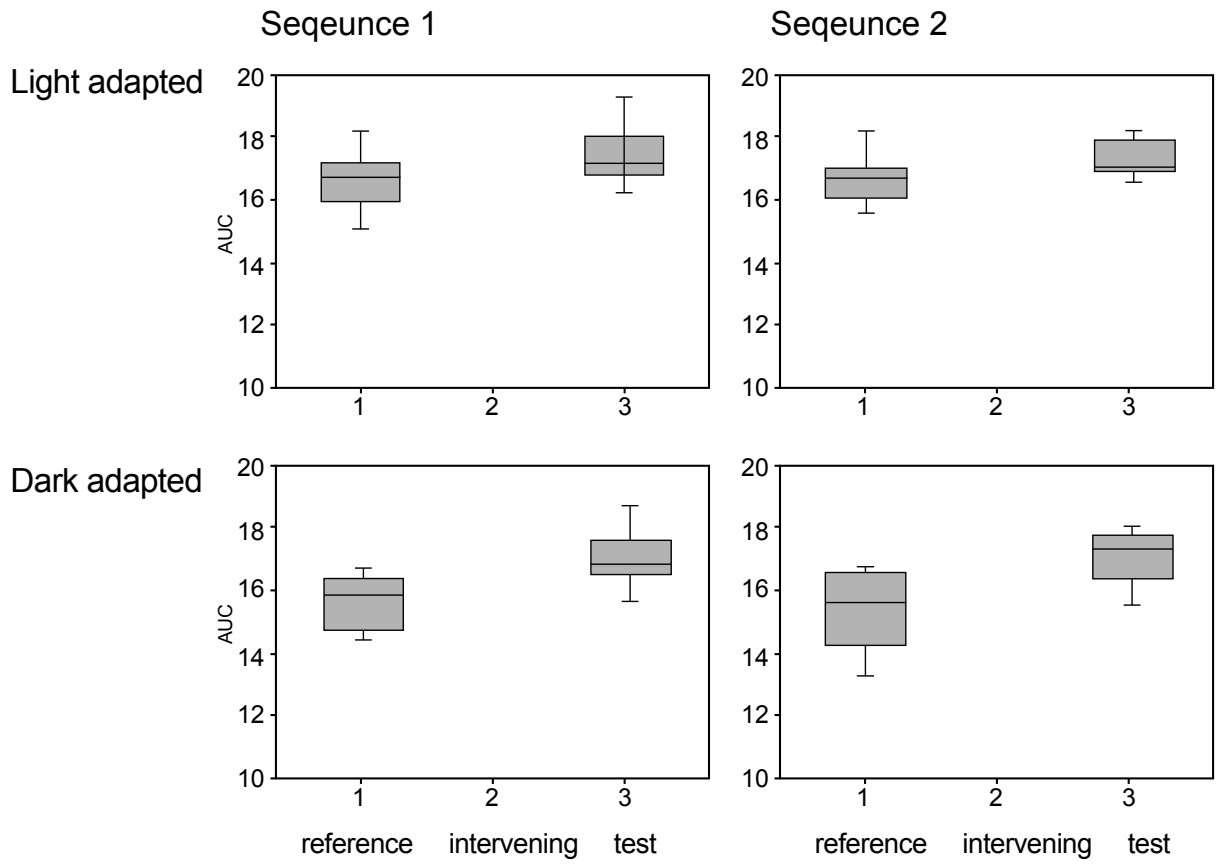

Figure 3: Box plot showing AUC. AUC during exposure to blue light in the light adapted state (upper panel) and after 30 minutes of dark adaptation initial to the reference stimulus (lower panel) for the two test sequences. Data are given as area under the curve (AUC) for the first reference blue stimulation and the third test stimulus, intervening stimulation of either blue or red are not shown. Both for sequence 1 (intervening blue) and sequence 2 (intervening red) the AUC for the final test stimulus was significantly increased compared to the initial stimulation, in particular for the dark adapted state. The AUC of the first reference blue stimulus following light adaptation was larger than the AUC for first reference blue stimulus following dark adaptation for both sequences 1 ($P = 0.08$) and 2 ($P = 0.05$).

on the same day. A 30 minute period of dark adaptation was added before presentation of each light sequence.

Data processing

The baseline pupil size was calculated as the mean pupil diameter during 10 seconds of darkness before presentation of the first blue light stimulus. The normalized pupillary size (NPS) at any given time point was calculated as the actual pupil size divided by the baseline pupil size (Figure 2). A customized smoothing program was applied to the raw tracing (data not shown) using a nearest neighbour approach, i.e., each data point was re-calculated as the mean of the initial value and the three adjacent points at each side.

The artefacts generated by single eyelid blinks were removed by an internal algorithm, which evaluated the recorded signal for 5 successive points, thus most (but not all) blinks were removed.

Analysis of data

In order to quantify the overall pupil response to continuous light exposure, the summed response during the 30 seconds of light stimulation was calculated from the response graph as the area between the line extrapolated from the baseline pupil size and the actual pupil size from 0 to 30 seconds (measured at 20 Hz) as the area under the curve, AUC= ($\sum_{0}^{30} 1 - NPS$), Figure 2.

Statistical methods

The difference in pupil response between the first blue exposure (reference stimulus) and the last blue exposure (test stimulus) for each sequence was expressed as diff% = (T-R) /R*100%, where R and T are the AUC values for the reference and test stimuli. In order to examine for a possible differential effect of an intervening blue light versus red light exposure on a subsequent blue light stimulus, paired tests were used to compare the diff % between sequence 1 and 2 for the two protocols. A p-value < 5% was considered significant and due to the limited sample size, results were confirmed with Wilcoxon rank-sum tests.

Results

The nine healthy participants had a mean age of 37 years with a range from 26 to 51 years and a normal ophthalmologic examination.

The blue light stimulus (reference, intervening and test) consistently evoked a pupil contraction that was sustained during the 30 seconds of light exposure (Figure 2). In contrast, the magnitude of pupil contraction during exposure to red light was maximal immediately following onset of the light stimulus but thereafter diminished during the 30 seconds of continuous stimulation (not shown). Similar curves were obtained after 30 minutes of dark adaptation.

The pupil response to blue light increased within any given test sequence (Table 1 and Figure 3). In the light adapted and dark adapted test protocol, the AUC of the test stimulus was larger then the AUC of the reference stimulus (light adapted state: diff% 4.5, $P = 0.003$ and diff% 9.2, $P = 0.012$ for sequence 1 and 2 respectively; dark adapted state: diff% 9.2, $P = 0.0007$ and diff% 10.7, $P = 0.0026$ for sequence 1 and 2 respectively).

There was no differential effect of the colour of the intervening light exposure on the test stimulus, i.e., the percentage difference of AUC (% diff) did not change significantly between sequences with intervening blue versus intervening red (Table 1 and Figure 3), P = 0.39 and P=0.58 for the light- and dark adapted state respectively.

The data also indicated an increased pupillary response in the light adapted state compared to the dark adapted state. The AUC of the reference blue stimulus following light adaptation was larger than the AUC for reference blue stimulus following dark adaptation for both sequences but did not reach statistical significance, ($P = 0.08$ and $P = 0.05$ for sequence 1 and 2, respectively, Table 1 and Figure 3).

Discussion

Several studies have described the use of colour light stimulus for pupil testing in clinical patients [10,11,13,14]. In such clinical test protocols, a bright blue light stimulus is believed to activate the melanopsin-mediated phototransduction of the ipRGCs based on the finding of persistent pupillary contraction during and after light termination [15]. In comparison, a red light stimulus is interpreted as a reference stimulus in which the pupil response to red light reflects ipRGC activity driven primarily by synaptic signalling from the outer photoreceptors. Recent studies examining bistability of the melanopsin photopigment suggest that the ipRGC response, as measured by pupil contraction, to blue light is influenced by previous light exposure. Specifically, Mure et al. has been demonstrated that a preceding exposure to long wavelength (red) light can potentiate the subsequent pupil response to blue light by up to 30% [8]. Such an enhancing effect was not found with exposure to light of shorter wavelengths.

In the present study, any previous light exposure seems to enhance the pupil response to bright blue light but no differential effect was found between red and blue light. In the study by Mure, the stimulation and intervening dark periods were substantially longer then in the present study. In an in vitro study by Mawad, [5] a multielectrode array recording was applied to the murine retina, recording the cell-firing from ipRGC during light stimulation. This study did not find any potentiating effect with intervening red light (620 nm) on ipRGC firing to blue light (480 nm) for various time settings.

The lack of consistency in the literature is probably due to differences in methodology and lack of optimal protocols , especially as it is not yet established whether melatonin is a bistable pigment or not.

Chromatic pupillometry test protocols frequently use red and blue light stimuli within a single examination and the effect of preceeding light conditions on the subsequent pupil response to blue light would certainly bias the results of any clinical pupil test. The present study is primarily aimed at understanding if a differential effect of preceding blue versus red light exposure on subsequent pupil response to blue light might exist under clinical testing conditions. We selected intensity bright enough to activate melanopsin-mediated phototransduction and yet remained within safety regulations and subject comfort. We selected 30 second duration of light stimulation as clinical pupil tests, from a practical standpoint, would not likely exceed this duration.

Under the light stimulus conditions used in our study, we did see an increase in pupil response from the first (reference) to the subsequent test blue exposure in each sequence but did not find that the intervening That the colour of the intervening light had any differential effect. Specifically, the pupillary light response to bright blue light was not significantly different following a red as compared to a blue light exposure. Additionally, we noted that the pupil response to first (reference) blue light was slightly decreased after 30 minutes of dark adaptation, as compared to the response in the light adapted state.

A major drawback of our study is the limited number of subjects. Further studies on the effect of the state of light adaptation and the amount of preceding light exposure are needed to clarify and optimize

the stimulus conditions appropriate for clinical chromatic pupillometry, in assessing neuroretinal function from the pupil response to red colour light.

The sampling frequency of 20 Hz is approximately 4 times higher than the frequency for pupillary unrest, thus no significant loss of information should be present due to pupillary oscillations.

In conclusion, our results indicate that an antecedent history of light exposure can be an important influence on the pupil response to a bright blue light and land further support to the notion of photic memory of the melanopsin ganglion cells. Our study shows that even a brief (30 second) exposure to a bright light of either short or long wavelength, can enhance the pupil response to a subsequent bright blue light. Furthermore light adaptation may have an influence as we found that light adaptation using ambient lighting conditions can augment the pupil response to a blue light stimulus compared to a dark adapted state. Thus, chromatic pupillometry protocols should consider the patient´s antecedent light history when evaluating the pupil responses to blue light.

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References

1. Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, et al. (2005) Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. Nature 433: 749-754.
2. Gamlin PD, McDougal DH, Pokorny J, Smith VC, Yau KW, et al. (2007) Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. Vision Res 47: 946-954.
3. Terakita A, Tsukamoto H, Koyanagi M, Sugahara M, Yamashita T, et al. (2008) Expression and comparative characterization of Gq-coupled invertebrate visual pigments and melanopsin. J Neurochem 105: 883-890.
4. Kiselev A, Subramaniam S (1994) Activation and regeneration of rhodopsin in the insect visual cycle. Science 266: 1369-1373.
5. Mawad K, Van Gelder RN (2008) Absence of long-wavelength photicpotentiation of murine intrinsically photosensitive retinal ganglion cell firing *in vitro*. J Biol Rhythms 23: 387-391.
6. Mure LS, Rieux C, Hattar S, Cooper HM (2007) Melanopsin-dependent nonvisual responses: evidence for photopigment bistability *in vivo*. J Biol Rhythms 22: 411-424.
7. Rollag MD (2008) Does melanopsin bistability have physiological consequences? J Biol Rhythms 23: 396-399.
8. Mure LS, Cornut PL, Rieux C, Drouyer E, Denis P, et al. (2009) Melanopsinbistability: a fly's eye technology in the human retina. PloS One Jun 24; 4(6): e5991.
9. Kardon R, Kawasaki A, Miller NR (2006) Origin of the Relative Afferent Pupillary Defect in Optic Tract Lesions. Ophthalmology 113: 1345-1353.
10. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, et al. (2009) Chromatic pupil responses: preferential activation of the melanopsin-mediated versus outer photoreceptor-mediated pupil light reflex. Ophthalmology 116: 1564-1573.
11. Kawasaki A, Kardon RH (2007) Intrinsically photosensitive retinal ganglion cells. J Neuroophthalmol. 27: 195-204.
12. Herbst K, Sander B, Milea D, Lund-Andersen H, Kawasaki A (2010) Test-retest repeatability of the pupil light response to blue and red light stimuli in normal human eyes using a novel pupillometer. Front Neurol. 2011 Feb 22;2:10.
13. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, et al. (2011) Chromatic pupillometry in patients with retinitis pigmentosa. Ophthalmology 118: 376-381.
14. Kawasaki A, Herbst K, Sander B, Milea D (2010) Selective wavelength pupillometry in Leber hereditary optic neuropathy. Clin Experiment Ophthalmol 38: 322-324.
15. Kankipati L, Girkin CA, Gamlin PD (2010) Post-illumination pupil response in subjects without ocular disease. Invest Ophthalmol Vis Sci 51: 2764-2769.