Pupillary light reflex associated with melanopsin and cone photorecetors

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ABSTRACT

Retinal ganglion cells containing the photopigment melanopsin are intrinsically photosensitive in primates. Several studies have shown that the intrinsically photoreceptive retinal ganglion cells project to the pupillary control center in the pretectum. Here, we independently stimulated human ipRGCs and cones, and investigated how signals driven by ipRGCs and cone-mediated signals contribute to the pupillary control mechanism. A four-primary illumination system that enables independent stimulation of each photoreceptor class was used to present the following three types of test stimuli. The transient pupil responses to these stimuli were measured. It was found that the transient pupil response to ipRGC stimuli had a longer latency than the responses to the LMS-cone and light flux stimuli. The longer latency suggests that signals from ipRGCs in the non-image forming pathway travel more slowly than that of the LMS achromatic mechanism in the image forming pathway.

1. INTRODUCTION

The intrinsically photoreceptive retinal ganglion cells (ipRGCs), which contains photopigment melanopsin, mediate signals to the pupillary control center in the pretectum. The ganglion cell is photosensitive and receives signals from classical photoreceptors. Although both cone- and ipRGC-mediated signals contribute to pupillary light reflex it is difficult to investigate how these signals are summed. Here, we independently stimulated human cones and ipRGCs, and investigated how cone- and ipRGC-mediated signals contribute to the pupillary control mechanism.

2. METHODS

2.1 Apparatus

An eight-channel, four-primary illumination system (Brown *et al*., 2012) that enables independent stimulation of each photoreceptor class was used to present the following three types of test stimuli: one varying L-, M- and S-cone stimulation only without change in stimulation of ipRGCs (LMS-cone stimulus), another varying radiant flux of the stimuli without change in spectral composition which reduced/increased the radiant flux uniformly at all wavelengths (Light flux stimulus) and the other varying ipRGC stimulation without change in stimulation of L-, M- and S-cones (ipRGC stimulus). The intense test and adapting fields were used which minimized the involvement of rods. The test and adapting fields had a CIE coordinate of (0.57, 0.36) and a luminance of 1,221 cd m^{-2} for the test field and 355 cd m^{-2} for the adapting field, respectively. The transient pupil responses to these stimuli were measured.

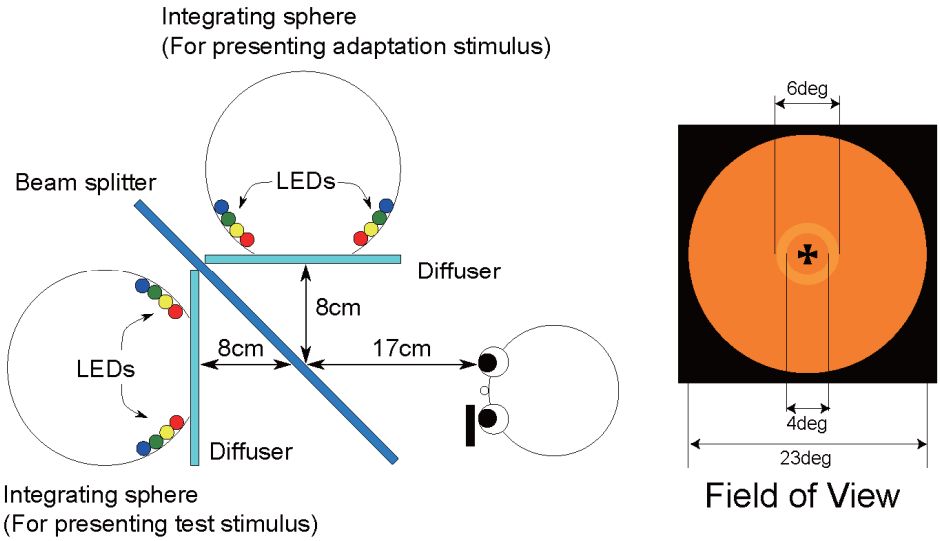

Figure 1 Eight channel, four-primary stimulation system

2.2 Spectral sensitivity curve for melanopsin ganglion cell

The test stimuli were generated based on the cone and ipRGC spectral sensitivities. The 10-deg cone fundamentals proposed by Stockman *et al.* (Stockman *et al*., 1999, 2000) were used to calculate the stimulation of Long-wavelength sensitive cone (L cone), Middle-wavelength sensitive cone (M cone) and Short-wavelength sensitive cone (S cone). We estimated the spectral sensitivity curve of ipRGCs based on a pigment template nomogram with a peak wavelength, λ_{max}, of 480 nm and ocular optical properties. The lens and macular pigment density spectra were those of Stockman *et al*. The fraction of incident light absorbed by the receptor depends on peak axial optical density (D_{peak}). We tentatively chose 0.1 as the D_{peak} for ipRGC. We assumed that neither the S cones nor the ipRGC affect the photopic luminance efficiency function (*i.e.*, luminance), despite using photopic luminance units (cd m^{-2}). Similar to S-cone stimulation (Boynton and Kambe, 1980), one ipRGC stimulation was defined as the level of ipRGC stimulation produced by an equal energy spectrum of luminance 1 cd m^{-2}. The resultant spectral sensitivity function of ipRGC in a 10-deg field displayed a peak of 872 at a wavelength of 493 nm. The shape of spectral sensitivity curve we estimated is similar to that proposed by Lucas and his colleagues (Enezi *et al.*, 2011). We further considered the human macular pigment density at 10-deg for the estimation.

2.3 Procedure

Five visually corrected observers (age range 21–23 years) participated in the experiment. All observers had normal color vision according to the Ishihara color blindness test. All observers gave their written informed consent, and the study was approved by the local research ethics committee. The observers were seated 25 cm from the diffuser and monocularly fixated upon a black Maltese cross, which subtended 1.8° and was always present at the center of the diffuser. After an initial adaptation period of 5 min, we began a session of experimental trials. We used a ramp stimulus presented for 500 ms.

The pupil of the right eye was imaged using a video camera (Dragonfly, Point Grey Research, Canada) located 0.5 m from the observer and 28° nasal to the visual axis. The video image was fed into a personal computer and analyzed using LabVIEW and IMAQ Vision software (National Instruments) at a frequency of 60 Hz. The pupil was located using thresholding and edge detection techniques, allowing the pupil diameter to be analyzed.

3. RESULTS AND DISCUSSION

3.1 Influence of a modulation of ipRGC on amplitude of pupil response

Since both Light flux and LMS-cone stimuli modulated cones in the same way these stimuli were indistinguishable for cones. Therefore, the difference could be attributed to the difference in stimulation with or without ipRGC modulation. Pupil responses to the Light flux stimulus and to the LMS-cone stimulus were shown in Fig 2. It was found that the amplitude elicited by LMS-cone stimulus was significantly higher than that by the Light flux stimulus. The average pupil response was 0.24±0.06 mm for the LMS-cone stimulus, 0.20±0.07 mm for the Light flux stimulus and 010±0.04 mm for the ipRGC stimulus. In other words, the modulation of ipRGC in Light flux stimulus influenced amplitude of the pupil response, suggesting that the ipRGC stimulation suppresses pupillary amplitude response.

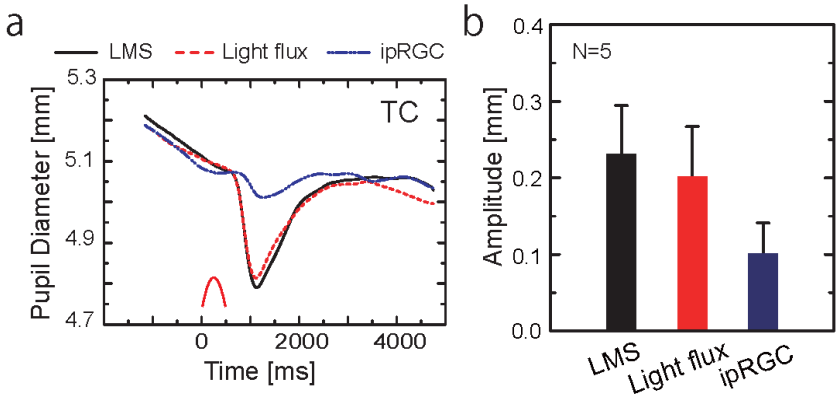

Figure 2 Pupillary responses and its amplitudes to the LMS-cone stimulus (black), to Light flux stimulus (red) and to the ipRGC stimulus (blue).

3.2 Sluggish pupillary response to the ipRGC stimulus

Typical normalized pupil responses are shown in Fig. 3. The horizontal axis represents a time and the vertical axis represents pupil diameters in mm to the test stimuli. The black curve represents curves for the LMS-cone stimulus, the red curve for the Light flux stimulus and the blue curve for the ipRGC stimulus. It was found that the transient pupil response to the ipRGC stimulus had a longer latency than those to the LMS-cone and to the Light flux stimuli. The average pupil latencies were 814±39 ms for the LMS-cone stimulus, 821±41 ms for the Light flux stimulus and 1,377±618 ms for the ipRGC

stimulus. The longer latency to the ipRGC stimulus was consistent with those in the previous study (Lucas *et al*., 2001; Tsujimura *et al*., 2011), suggesting that the ipRGC-mediated signals in the non-image forming pathway travel more slowly than the LMS cone-mediated achromatic signals in the image forming pathway. These results suggested that the pupil responses to the LMS-cone stimulus and to the Light flux stimulus are mediated by cones and those to the ipRGC stimulus are mediated by ipRGCs.

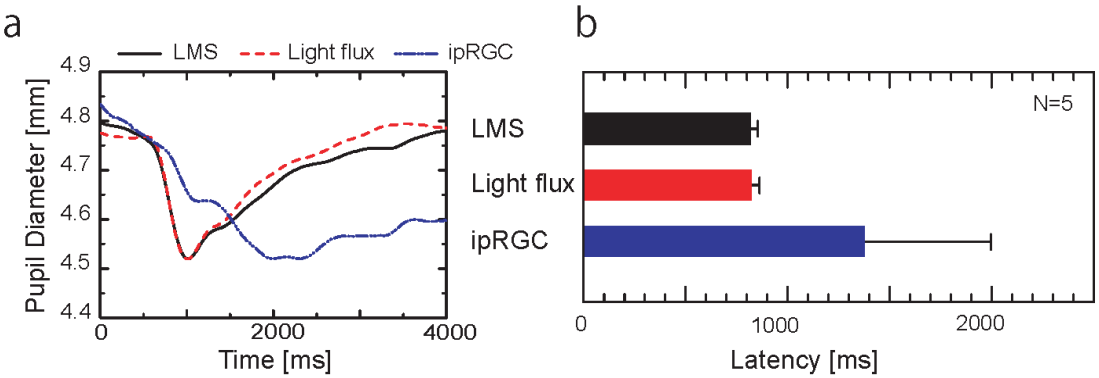

Figure 3 Normalised pupillary responses to the LMS-cone stimulus (black), to Light flux stimulus (red) and to the ipRGC stimulus (blue).

4. CONCLUSIONS

It was found that the transient pupil response to ipRGC stimuli had a longer latency than the responses to the LMS-cone stimulus and to the Light flux stimulus. The results indicate that we successfully demonstrated the pupillary response to ipRGCs under conditions where ipRGCs are isolated in humans. The longer latency suggests that signals from ipRGCs in the non-image forming pathway travel more slowly than that of the LMS-cone mediated signals in the image forming pathway.

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