Sustained pupillary constrictions mediated by an L- and M-cone opponent process

Eiji Kimura [a,*], Rockefeller S.L. Young [b]

[a] Department of Psychology, Faculty of Letters, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522, Japan
[b] Department of Ophthalmology & Visual Sciences, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

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ABSTRACT

We show that irradiance-coding alone cannot explain the sustained pupillary constrictions evoked by chromatic and luminance variations in a stimulus. For example, stimulus modulations that decremented the contrasts in L- and M-cones as well as rods and melanopsin photoreceptors produced sustained constrictions rather than the predicted dilations. Although the sustained responses are unidirectional, we confirm that they are at least partially mediated by an L- and M-cone opponent interaction. We discuss the implications of sustained unidirectional chromatic responses in view of the function of the pupil to improve the clarity of vision.

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1. Introduction

The afferent process mediating the human pupillary light response appears to be rich in functional capability. The pupil reacts not only to changes in the irradiance of light but also to changes in chromaticity, spatial composition, and motion of the retinal image (Barbur, Harlow, & Sahraie, 1992; Kohn & Clynes, 1969; Saini & Cohen, 1979; Slooter & van Norren, 1980; Ukai, 1985; Young & Alpern, 1980; Young, Han, & Wu, 1993; Young & Kennish, 1993; Young, Kimura, & DeLucia, 1995). Such capability raised questions about the neurons and neural pathways involved. Previous studies entertained the possibility that the afferent pathways might be functionally similar to or anatomically shared with the neurons in the parvo (P-) and magno (M-) streams (e.g., Krastel, Alexandridis, & Gertz, 1985; Kimura & Young, 1995, 1996, 1999; Young et al., 1995; Tsujimura, Wolffsohn, & Gilmartin, 2001, 2006). Like the activity in the P- and M-streams, the shape of the pupillary response waveform was associated with specific stimulus features (Kimura & Young, 1995; Young & Kennish, 1993; Young et al., 1993). For example, whereas the waveform was temporally sustained in response to high spatial frequency gratings, it was purely transient in response to low spatial frequency grating (Ukai, 1985; Young & Kennish, 1993). But the parallel between the pupillary response waveform and the temporal activity in the P- and M-streams appeared to break down when examining the temporal responses to stimulus irradiance and wavelength (Young et al., 1993). Just opposite to the temporal nature of the activity in the P- and M-streams, the pupillary responses to isoluminant color

exchanges were characterized as unidirectional and transient, whereas those to the stimulus irradiance were bidirectional and sustained. Other studies using a two-color (flash-on-background) paradigm showed that the pupillary responses to the flash onset had a sustained constriction component (Kimura & Young, 1995; Kohn & Clynes, 1969). But as the stimulus always incremented the photon absorptions in the underlying photoreceptors in this paradigm, there was no easy way to decide whether the sustained response could be mediated by a wavelength- as well as an irradiance-coding process.

More information has since emerged about the retinal physiology underlying the pupillary response. First, the pupillary light response is mediated by five photoreceptors. In addition to the L-cone, M-cone, S-cone, and rod, the pupillary response is also driven by a light-sensitive pigment called *melanopsin*, which has been shown in rodents and monkeys (e.g., Dacey et al., 2005; Hattar et al., 2003; Lucas, Douglas, & Foster, 2001; Lucas et al., 2003; Panda et al., 2003) and has been implicated in humans (Gamlin et al., 2007; Kawasaki & Kardon, 2007; Young & Kimura, 2008). Second, the pathway for the melanopsin response begins with the transduction of light at the membrane surface of certain retinal ganglion cells. Light striking the retinal ganglion cells itself could produce a sustained pupillary constriction (Gamlin et al., 2007). Third, the same ganglion cells can also receive strong input from cones as well as rods. Moreover, some of the cones formed chromatic opponent interactions as evidenced by the finding of a rare S-OFF, (L + M)-ON type of cone opponency (Dacey et al., 2005).

In view of these recent findings, we now wondered whether, in humans, chromatic signals generated in the eye might lead to sustained pupillary responses. The fact that no one has yet observed either sustained chromatic constrictions or dilations is not compelling evidence for their absence. But it does suggest that such

* Corresponding author. Fax: +81 43 290 2281.
E-mail addresses: kimura@L.chiba-u.ac.jp (E. Kimura), rocky.young@suddenlink.net (R.S.L. Young).

responses are probably small or, in some other way, not easily recognized. So the success of future studies to investigate such responses might depend on refinements in the previous methods. Perhaps, the experimental methods for detecting chromatic opponent effects can be optimized, confounding factors that reduce or mask the effects of interest can be eliminated, and independent methods for verifying claims of opponent interactions might be used.

The objectives of the present study were to refine the method of analysis, to investigate whether an L- and M-cone opponent interaction produces sustained pupillary responses and, if such responses are found, to determine whether the pupillary responses are bidirectional, like those of chromatic opponent neurons. Our decision to study the L–M opponency was based on the strength of the previous evidence for its effect on the pupillary (transient) light response (Kimura & Young, 1995, 1996), the abundance of L- and M-cones in the eye, and the availability of established paradigms for confirming whether a response is mediated by an L–M opponent interaction.

2. Methods

2.1. Observers

Two observers participated in this study. They had normal color vision as assessed by the SPP (the standard pseudo–isochromatic plates, Igaku–shoin) and normal or corrected to normal visual acuity. Neither was on any medication during the testing period. Written informed consent was obtained from each observer prior to the start of the study.

2.2. Apparatus and stimulus

The stimuli were generated by a Cambridge Research Systems VSG 2/5 graphic card and displayed on a 21-in. Sony color monitor (GDM F500R) with a pixel resolution of 1024×768 and a frame rate of 100 Hz. The intensity of each phosphor could be varied with 15-bit resolution. Spectroradiometric calibration was performed on three phosphors of the monitor with a Minolta CS-1000 spectroradiometer and an LS-100 luminance meter. The Psychophysics toolbox extensions for Matlab were used in the phosphor calibrations and colorimetric calculations (Brainard, 1997; Pelli, 1997).

The test field was a homogeneous $5°$ circular area centered on a $19 \times 14°$ white background. The background chromaticity coordinates and luminance were ($x = 0.308$, $y = 0.322$) and 28 cd/m², respectively. The observer fixated the center of the test field which was circumscribed by a narrow black contour of 4′ width. The test stimulus was presented as a 3 s step-change in chromatic and luminance contrast from the white background. Stimulus changes were produced by decrementing as well as incrementing the phosphor intensities from the background white on the Sony monitor. Several stimulus parameters helped to control for possible confounding factors. The stimulus changes were limited to the portion of the monitor's color gamut that was effective to L- and M-cones in order to reduce, if not eliminate, the contributions of S-cones. The test stimulus was chosen to be small in size and brief in duration to minimize the contribution of rods and of melanopsin photoreceptors, while maximizing the contribution of L–M opponent processes at the central retina. The large white background was used to reduce possible effects of stray light.

The stimulus changes on the monitor were quantified as a Weber contrast for each cone and described as vectors in L-cone contrast ($\Delta L/L$) versus M-cone contrast ($\Delta M/M$) space (Fig. 1). The cone contrast metric takes into account Weberian sensitivity scal-
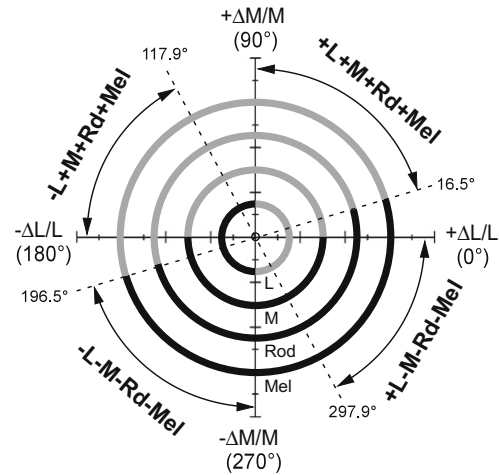
Fig. 1. Stimulus directions for testing the hypothesis that the sustained pupillary constrictions are solely mediated by an irradiance-coding process. Chromatic contrast from white is described as a vector in L-cone contrast ($\Delta L/L$) versus M-cone contrast ($\Delta M/M$) space. The origin represents the white color in the background field. Stimuli producing an incremental change in L-cone contrast alone are represented by a vector pointing in the $0°$ direction. Stimuli creating an incremental change in M-cone contrast alone are represented by a vector in the $90°$ direction. To consider the more general possibility that there is also contribution from rods and melanopsin photoreceptors, we located the intersections between their stimulus null planes and the L- and M-cone contrast space. The intersections for melanopsin photoreceptors (16.5–196.5° line) and rods (15.6–195.6° line) are very close, so only the former is shown for clarity. The stimulus vector directions for the more general case are labeled as **+L + M + Rd + Mel**, **+L−M−Rd−Mel**, **−L + M + Rd + Mel**, and **−L−M−Rd−Mel** conditions where the "+" and "−" symbols refer to incremental and decremental contrast directions, respectively, in each photoreceptor (see text for more detail). The gray and black arcs in the figure illustrate these contrast changes for each photoreceptor. Gray represents incremental, while black represents decremental changes. The intersection of the isoluminant plane (i.e., $\Delta L + \Delta M = 0$) is computed by assuming that the photopic luminous efficiency of the pupil is identical to that derived psychophysically (Alpern & Campbell, 1962) and located at an azimuth of $117.9°$. This is used to confine the conditions producing mixed photoreceptor contrasts (**+L−M−Rd−Mel** and **−L + M + Rd + Mel** conditions).

ing due to adaptation to a background and this normalization has the effects of equating the stimulus efficacy for each type of cones (Brainard, 1996; Eskew, McLellan, & Giulianini, 1999).

To accurately measure minute pupillary responses, the observer's head was stabilized on a chin and forehead rest. An infrared light positioned below the observer's line of sight illuminated the iris surface. The position of the light was adjusted to optimize the contrast of the pupil image before every measurement. A Pulnix video camera magnified and recorded the pupil image continuously over time. The signals from the camera were fed into a pupil tracking system (ISCAN model RK-716) where the pupil diameters were digitized with 9-bit resolution and sampled at 60 Hz. The size of the observer's entrance pupil was calibrated with images of black spots placed in the plane of the observer's pupil. The diameter of the calibrating spots ranged from 2 to 9 mm in diameter. The precision in the averaged recording was estimated to be about 28 μm.

2.3. Experimental design and method of analysis

To investigate the nature of visual processes underlying the sustained pupillary responses, we posed the hypothesis that irradiance-coding alone accounts for the sustained pupillary responses to all stimulus changes from white. Then we investigated its truth or falsehood by examining the predicted effects on the pupil produced by incremental and/or decremental contrasts in the L- and M-cones (Fig. 1 and Table 1). Stimuli producing only incremental

Table 1
Stimulus directions and the pattern of predicted responses assuming the pupil is mediated by L- and M-cones.

Stimulus direction (Fig. 1)	Perceptual appearance	Effects on cones	Predicted effects on the pupil		
			Irradiance-coding	Wavelength-coding	Joint processing
Quadrant 1	Bright yellow	Increase L- and M-cone signals	Constriction	Constriction	Constriction
Quadrant 3	Dark blue	Decrease L- and M-cone signals	Dilation	Constriction	Reduced response
Quadrant 2	Greenish	Decrease L- but increase M-cone signals	Minimal or no response[a]	Constriction	Constriction
Quadrant 4	Reddish	Increase L- but decrease M-cone signals	Minimal or no response[a]	Constriction	Constriction

[a] The exact direction of the effects predicted are unknown as they vary depending upon the relative weights with which the signals from L- and M-cones are combined. However, the additive interaction between L- and M-cone signals is expected to produce a response null within quadrants 2 and 4.

contrasts (i.e., quadrant 1 in the L- and M-cone contrast space) would be expected to produce pupillary constrictions, whereas those producing only decremental contrasts (i.e., quadrant 3) would produce dilations. Stimuli producing mixed incremental-decremental contrasts (i.e., quadrants 2 and 4) would produce minimal, if any, responses (Table 1).

The possibility that rods and melanopsin photoreceptors contributed in addition to L- and M-cones cannot be entirely discounted. Our strategy to consider their possible contribution was to organize the stimulus conditions into those corresponding to the purely incremental, purely decremental, and mixed contrast conditions. The stimulus conditions are illustrated by the arcs labeled **+L + M + Rd + Mel**, **+L−M−Rd−Mel**, **−L + M + Rd + Mel**, and **−L−M−Rd−Mel** in Fig. 1. Note that if we later found that there was no rod and melanopsin contribution, the same stimuli would still satisfy the requirement of the hypothesis test. That is, **+L + M + Rd + Mel**, **+L−M−Rd−Mel**, **−L + M + Rd + Mel**, and **−L−M−Rd−Mel** stimuli would reduce to **+L + M**, **+L−M**, **−L + M**, and **−L−M** stimuli, respectively. The abbreviations **L**, **M**, **Rd**, and **Mel** stand for L-cones, M-cones, rods, and melanopsin photoreceptors. Labels such as **+L−M** do not stand for photoreceptor opponency. They simply describe the contrast direction in each photoreceptor produced by the stimulus. The "+" and "−" symbols designate incremental and decremental contrast directions, respectively, for each photoreceptor.

If the evidence did not support the irradiance-coding hypothesis, our plan was to investigate the contribution of wavelength-coding. The wavelength-coding hypothesis predicts the pupillary constrictions to chromatic changes (e.g., Young & Alpern, 1980) and thus a different pattern of responses (Table 1). Moreover, we would investigate whether there is any evidence that the sustained responses were mediated by an L- and M-opponent interaction. We used the classical psychophysical threshold approach for investigating how L- and M-cones interact to determine the detection threshold (e.g., Cole, Hine, & McIlhagga, 1993; Eskew et al., 1999; Sankeralli & Mullen, 1996) and analyzed the thresholds for the pupillary response.

2.4. Derivation of photoreceptor contrasts and magnitude of contrast changes

Contrasts for each photoreceptor were calculated using the spectral power distributions of three phosphors of the color monitor and the spectral sensitivity curves of the photoreceptors, i.e., the fundamentals by Smith and Pokorny (1975) for cones, the scotopic luminous efficiency function for rods (Wyszecki & Stiles, 1982), and the A1 photopigment nomogram (Govardovskii, Fyhrquist, Reuter, Kuzmin, & Donner, 2000) with a peak at 482 nm for melanopsin. Corrections were made for the lens (Stockman, Sharpe, & Fach, 1999) and the macular pigment (Bone, Landrum, & Cains, 1992; Stockman et al., 1999) density to derive the spectral sensitivity curve for melanopsin photoreceptors. To specify the effects of the present stimulus on rods and melanopsin photorecep-

tors, we located the intersection of their stimulus null planes in the L- and M-cone contrast space. The intersection occurred at an azimuth of 15.6° and 16.5° for rods and melanopsin photoreceptors, respectively (Fig. 1).

To quantify the magnitude of contrast changes produced by stimulus changes from white, we followed the psychophysical convention and used a measure based on the Euclidean distance. When only L- and M-cones were concerned, the test contrast, c, was derived by the Euclidean distance from the origin on the L- and M-cone contrast space, i.e., $c = [(\Delta L/L)^2 + (\Delta M/M)^2]^{1/2}$. When four photoreceptors mediate the response, the contrast is derived similarly but with all four photoreceptors, i.e., $c = [(\Delta L/L)^2 + (\Delta M/M)^2 + (\Delta Rd/Rd)^2 + (\Delta Mel/Mel)^2]^{1/2}$, where $\Delta Rd/Rd$ and $\Delta Mel/Mel$ represent the rod and melanopsin contrast, respectively.

2.5. Testing procedure

Each observer dark-adapted for at least 5 min and then pre-adapted to the white field for 2 min. The pupillary responses were recorded from the left eye while the observer viewed the stimulus with both eyes. The test contrast used in the pupil recordings was varied from several tenths of a log unit below the psychophysical threshold to the highest contrast available. The test conditions (16 directions for observer MI and 18 directions for observer SM) were divided into blocks of two to four directions depending upon the number of contrast levels available. The different direction and contrast combinations within each block were tested in a pseudo random order in 3-h sessions. Each stimulus condition was repeated at least 30 times. As there were many chromatic directions and contrasts, the study took more than 6 months for each observer to complete.

Psychophysical detection thresholds were obtained using a two-alternative forced-choice paradigm involving the left and right halves of the test field. The two halves were divided vertically by a narrow black border (4′). On each trial, the observer had to report the side on which the test stimulus was presented. The observer's response terminated the stimulus presentation and produced an auditory feedback. The test contrast was varied using a double random staircase procedure, while the test direction in the L- and M-cone contrast space remained fixed. An incorrect response raised the test contrast by 0.1 log units, and two consecutive correct responses lowered the contrast by 0.05 log units. The detection threshold was determined by the average of the last 6 of the 10 staircase reversals. The measurement was repeated at least twice in different daily sessions. The thresholds were averaged across different sessions.

3. Results

3.1. Sustained pupillary constriction

The waveforms of the pupillary responses are illustrated for representative stimulus directions (Fig. 2). The prestimulus pupil
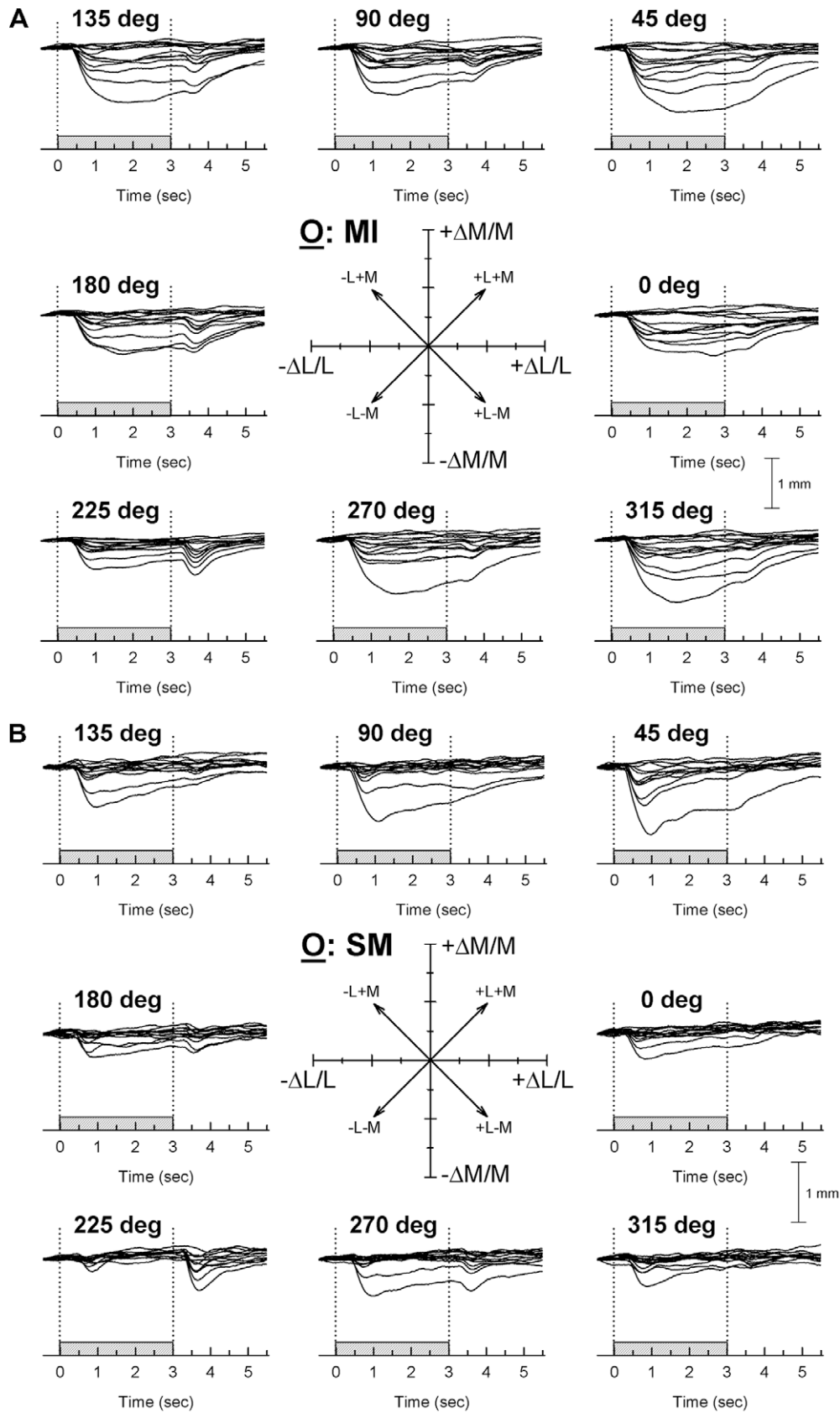
Fig. 2. Pupillary responses evoked by representative test stimuli. The waveforms are grouped with respect to the direction of the chromatic change in the L- and M-cone contrast space. Waveforms stacked on top of one another represent the responses to different test contrasts. The stimulus onset occurred at time zero and the offset at 3 s. The pupillary response is generally characterized by an initial transient constriction overshoot following the stimulus onset (ON response), a steady-state constriction phase (sustained response), and a transient constriction overshoot and dilation following the stimulus offset (OFF response). We quantified the sustained amplitude as the difference from the baseline in the pupil size averaged over the time from 2.5 s to 3.0 s. (A) The results for observer MI. (B) The results for observer SM.

diameter was arbitrarily set to zero. The sustained aspect of the response refers to the persistence of a pupil diameter change following the stimulus onset. Sustained constrictions (or dilations) are represented by a downward (or upward) deflection. The results for both observers showed that the stimuli in quadrants 1, 2, and 4 produced sustained constrictions. The results for quadrant 3 appeared to differ between observers. For observer MI, the stimuli in quadrant 3 produced distinct, but relatively small, sustained constrictions. For observer SM, there is uncertainty whether any response occurred.

Because the time-averaged pupillary responses are typically confounded with a dilatory trend in the pupil diameter, i.e., a "task-related" effect (Richer & Beatty, 1985; Young & Kimura, 2008), it was important to remove such trends prior to quantifying the sustained pupillary amplitudes. The method used was to first estimate the slope of the baseline from the averaged pupil diameters derived over the first and last 0.5 s of the recording and then subtract the estimated trend from the actual pupil recordings. The sustained amplitude was taken as the average difference from the baseline during the time from 2.5 s to 3.0 s. The sustained amplitudes for the decremental, incremental, and mixed contrast conditions are plotted as a function of test contrast (Fig. 3). The different symbols in each panel represent the data from various test directions in the L- and M-cone contrast space.

Measurements of the sustained amplitudes provided evidence that the decremental contrast condition produced a pupillary constriction (Fig. 3, top). Furthermore, the statistical analysis (Appendix) provided little or no support for the hypothesis that the sustained amplitudes were identical for all test contrasts ($p < 0.001$ with $df = 16$ for observer MI and $p < 0.05$ with $df = 16$ for observer SM, respectively). Rather the constriction amplitude was contrast-dependent. Larger constrictions are found with higher stimulus contrast. We considered the possibility that the observed constrictions might not be a true sustained response but rather a carryover from the initial ON-transient constriction. So in ancillary recordings from observer MI, we examined the pupillary responses to stimuli of a longer (6-s) duration. The results, however, provided little, if any, evidence for believing that the observed constrictions were related to the slow recovery of the pupil diameter following the initial transient constriction. Instead, the results for stimuli in quadrant 3 showed fairly constant sustained constrictions 2.5 s after the stimulus onset.

To further test the irradiance-coding hypothesis, we examined the prediction that the incremental contrast condition should produce larger constrictions than the mixed contrast conditions (Fig. 3, bottom). The results for observer MI were clearly contrary to the expectation (left). The results of observer SM, while less convincing, were consistent with those of MI (right).

3.2. Identification of underlying photoreceptor interactions

Psychophysical experiments confirmed the classical chromatic opponent relationships in detection thresholds expected under the present stimulus conditions and apparatus. So to investigate the nature of the interactions mediating the sustained pupillary responses, we performed a comparable analysis using a criterion sus-
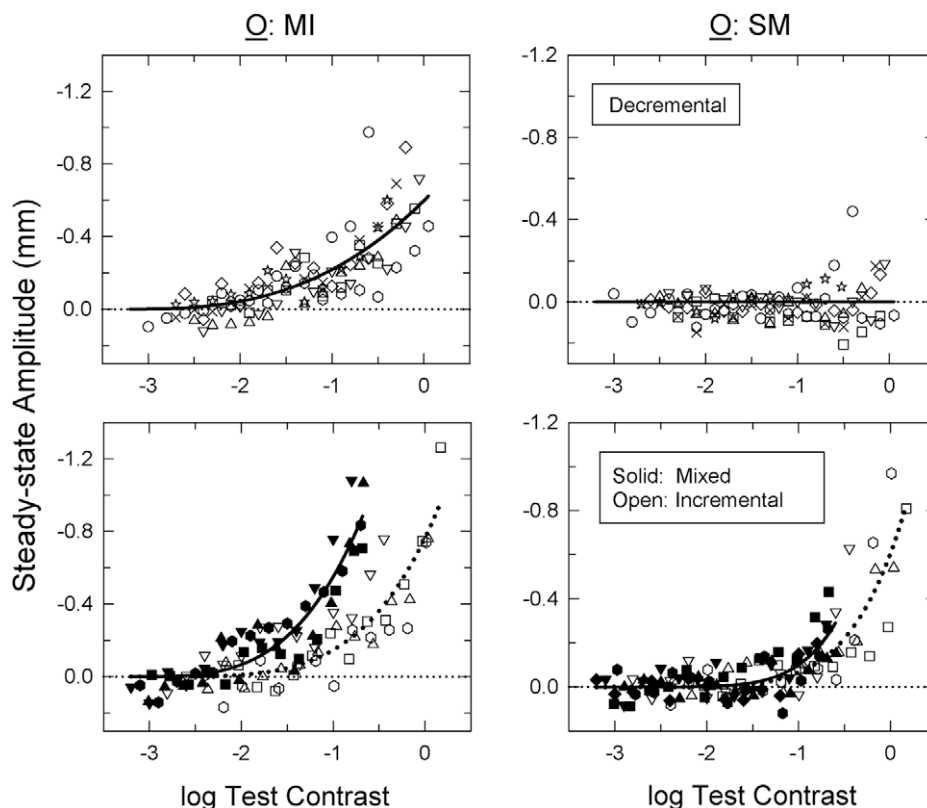
Fig. 3. Sustained constriction amplitudes in different experimental conditions. Negative millimeters of amplitudes represent pupillary constrictions. **Top**: Amplitudes for stimuli that produced a decremental contrast change in all photoreceptors (i.e., −**L**−**M**−**Rd**−**Mel** condition). If sustained responses are only produced by an irradiance-coding process, we expect that the pupil would dilate as the decremental contrast increases. Different symbols represent the repeated measures, i.e., the results for different test directions shown as a function of test contrast. **Bottom**: Comparison of the sustained amplitudes in the mixed contrast condition (i.e., −**L**+**M**+**Rd**+**Mel** or +**L**−**M**−**Rd**−**Mel** condition) and those in the incremental contrast condition (i.e., +**L**+**M**+**Rd**+**Mel** condition). If sustained constrictions are only produced by an irradiance-coding process, we expect that the amplitudes in the mixed contrast condition would be smaller than those in the incremental contrast condition. Solid and dotted lines are Naka–Rushton functions (Boynton, Demb, Glover, & Heeger, 1999) fitted to each set of the data. Different symbols represent the results for different test directions.

tained pupillary constriction as the endpoint (e.g., Kimura & Young, 1995). As the sustained amplitude varied monotonically with test contrast (Fig. 3), the criterion contrast could be interpolated from the contrast-amplitude data.

The results showed that the pupillometric and psychophysically derived functions were similar. In both cases, the functions were linear in shape with a positive slope (Fig. 4A and B). Specifically, the contributions of L- and M-cones were described by the subtractive interaction, $\Delta L/L - \Delta M/M$ = constant. As judged by the distance separating the two lines, the pupillary chromatic sensitivity, however, is lower than that for perception. For observer MI, the pupillary threshold function could be derived using still higher amplitude criteria (Fig. 4A, right panel). Her results suggest that the subtractive interaction is not specific to near-threshold responses.

If rods and/or melanopsin photoreceptors also contributed to the function, their contributions would, in effect, decrease the apparent contrast sensitivity of M-cones ($\Delta M/M$) in cone contrast space and, thus, would have changed the slope and/or shape of the function. But we found little, if any, evidence to support this possibility (Fig. 4).

For the sake of completeness, we also analyzed the photoreceptor interactions underlying the ON and OFF transient aspects of the pupillary responses. The results showed that the threshold functions could also be described by two parallel lines with unity slope. However, because these results are confirmatory of previous findings (Kimura & Young, 1995, 1996), we only wish to mention this finding here.

4. Discussion

The steady-state pupil diameter of human eyes varies systematically with the level of retinal irradiance and, to a lesser extent, the spatial frequency of an image. This has led previous investigators to speculate that the sustained aspect of the pupillary light response is associated with an achromatic afferent channel (e.g., Young & Kennish, 1993). The objective of the present study was to test the hypothesis that the sustained pupillary responses are mediated solely by irradiance-coding against the alternative that the responses were also mediated by wavelength-coding. The hypotheses are discernable because the pupil is expected to constrict and dilate with incremental and decremental changes in irradiance, respectively, but only constrict in response to purely chromatic changes (e.g., Young & Alpern, 1980). In other words, each hypothesis predicts a different pattern of responses (Table 1).

4.1. Irradiance-coding

If the pupillary sustained responses are mediated solely by irradiance-coding, we expect that the direction of responses, i.e., constriction, dilation, or no response, would depend on the net direction of the signal change summated across L- and M-cones, i.e., a purely incremental change (stimulus quadrant 1, Fig. 1), a purely decremental change (quadrant 3), or minimal if any change (quadrants 2 and 4), respectively. The change in stimulus chromaticity is expected to have little, if any, bearing on the predicted directions of response. Our results, however, were inconsistent
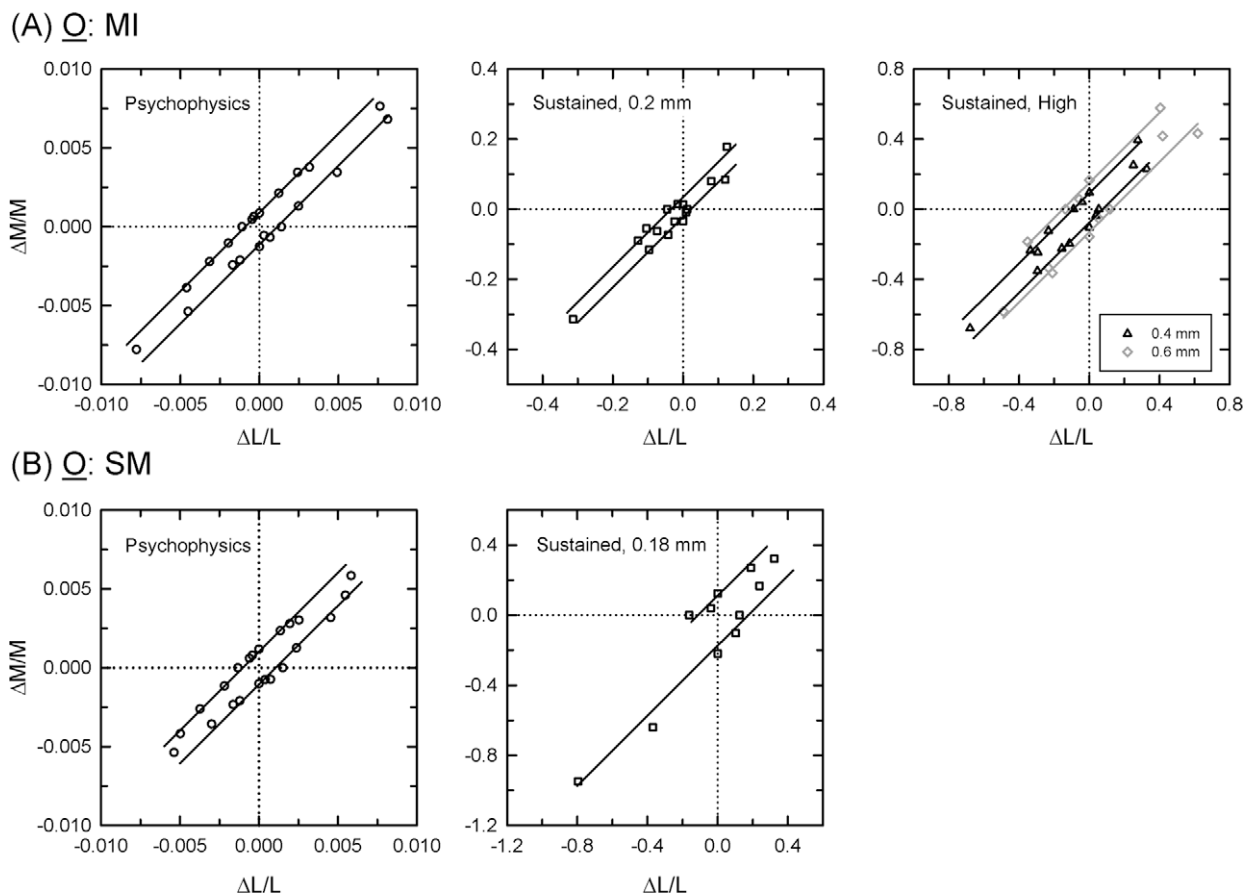
Fig. 4. Comparison of the M- and L-cone interdependencies in the psychophysical detection (left) and sustained pupillary threshold (middle and right) analyses. The psychophysical determinations confirm the expectation that the detection thresholds under the present condition are fitted by the relationship that $\Delta L/L - \Delta M/M$ = constant (solid lines). The new finding is that pupillary thresholds derived from various criterion amplitudes are also fitted by the similar relationship. (A) The results for observer MI. (B) The results for observer SM.

with the expectations. Instead of the expected pupillary dilation in quadrant 3, the results revealed either distinct (observer MI) or smaller (observer SM) sustained constrictions (Figs. 2 and 3 top). To be sure, we examined additional directions besides those shown in Fig. 2 but were still unable to find evidence of the expected pupillary dilation. Specifically, in observer MI, we examined the responses to stimuli in the directions of 207.9° 215°, 220°, 230°, 235°, and 240°. In observer SM, we examined the stimulus directions of 200°, 215°, 220°, 230°, 235°, and 240° (results not shown for brevity). The irradiance-coding hypothesis would also predict that the stimuli producing an incremental contrast in every photoreceptor (i.e., **+L + M + Rd + Mel**) should produce constrictions with amplitudes that are larger than those producing a mixed contrast (i.e., **+L−M−Rd−Mel** or **−L + M + Rd + Mel**). But the results for observer MI showed that the sustained amplitudes are larger in the mixed contrast in comparison to the incremental contrast conditions (Fig. 3, bottom). The results of observer SM are consistent with those of observer MI.

However, when the above results are considered with respect to the alternative hypothesis – that the responses are mediated jointly by wavelength- as well as irradiance-coding – we find a close match between the expected and observed patterns in the responses. Specifically, a sustained pupillary constriction is observed in quadrants 1, 2, and 4. Constrictions in quadrants 2 and 4 are expected from the stimulus chromaticity change, but not expected from irradiance-coding because the incremental signal from L- (or M-) cones would be reduced by the decremental signal from the fellow cones. A response with reduced amplitude in quadrant 3 is consistent with the prediction of the alternative hypothesis that the pupil should dilate in response to the decrement in irradiance and constrict to the change in chromaticity. As the two response tendencies oppose each other, the net response would be expected to be small. The difference found between observers might be explained in terms of variations in the relative strength of the two tendencies.

In summary, all of our evidence failed to support the irradiance-coding hypothesis. But the results were explicable in terms of the alternative hypothesis that the sustained pupillary response is mediated by wavelength-as well as irradiance-coding processes. We found no evidence suggesting that the intrusion of rod and melanopsin signals would change our conclusions (Fig. 3).

4.2. Wavelength-coding

To ascertain whether chromatic stimulus changes contributed to the sustained pupillary constrictions, we examined the underlying photoreceptor interactions. In particular, we investigated the interdependency of the L- and M-cone signals using a paradigm developed by psychophysicists for differentiating the chromatic and luminance effects (e.g., Eskew et al., 1999). If the sustained constrictions are associated with the stimulus chromaticity, the L- and M-cone thresholds should deviate from a purely additive (irradiance-coding) interaction. What we found was that the pupillary and psychophysical functions were similar (Fig 4, middle and right columns), implying that not only are the sustained constrictions mediated by a wavelength-coding process but also that the wavelength-coding involves a subtractive L- and M-cone interaction that is similar to the L- and M-cone opponent interaction observed psychophysically.

4.3. Visual significance of the chromaticity-evoked constrictions

The pupil is the aperture stop of the eye. Changes in its diameter affect the quality and intensity of the retinal image and, thus, impact vision. Currently, the significance of bidirectional sustained pupillary responses is better understood than that of the unidirec-

tional sustained response reported here. In a duplex retina, the widening of the pupil under low illumination and the narrowing under high illumination provide complementary benefits to vision. Whereas the widening in darkness increases the retinal irradiance and thus facilitates the visibility of dim objects, the narrowing of the pupil in daylight illumination contributes to a reduction in the amount of achromatic (e.g., spherical) aberration by blocking passage of the marginal light rays from degrading the retinal image (Young & Freedman, 2007).

Although there is still more to be learnt about unidirectional sustained responses, it seems likely that the sustained constrictions would primarily benefit photopic vision. One benefit might be to reduce the chromatic aberration present in a retinal image. Similar to the change in chromatic contrast from white in the present study, the aberrant dispersion of an achromatic contour into a rainbow of wavelengths might also evoke a sustained constriction. A constriction reflex would be beneficial because it would reduce the magnitude of the aberration and, in turn, serve to restore image clarity. A second benefit might be to minimize image defocus following saccadic eye movements. As saccadic eye movements often produce large changes in the chromaticity of the foveal image, we predict a subsequent narrowing of the pupil following each fixation. The benefit of such pupillary response would be to increase the depth of focus and insure image clarity even when an observer refixates slightly different distances. If the pupil dilated in either of these two situations, the clarity of retinal images would worsen. Thus, a unidirectional response, i.e., always a constriction, would be ideal.

Acknowledgments

Appendix. Bootstrap statistical test for the significance of the sustained pupillary constrictions

To investigate whether the sustained constrictions are significant, we tested the hypothesis that the sustained amplitudes, y_i, are all identical against the alternative possibility that the y values grow as a function of test contrast. The statistic ϕ used in the test is $\phi = \Sigma w_i y_i$, where y_i are the sustained amplitudes corresponding to the different stimulus conditions i for each test direction and w_i are weighting coefficients that are derived from the mean-center of the log test contrast values. For example, if the log contrast values were $c = \{-2.7, -2.5, -2.3, -2.1, -1.9, -1.7, -1.5, -1.3\}$, the mean-centered values would be $w = \{-0.7, -0.5, -0.3, -0.1, 0.1, 0.3, 0.5, 0.7\}$. The important property of this statistic is that when all y values are identical, the statistic ϕ becomes zero. When the values of y increase as a function of test contrast, then the value of ϕ becomes large. The present test is similar to the dose–response test discussed in Westfall and Young (1993, p. 99).

The probability that the null hypothesis is true is evaluated by comparing the magnitude of ϕ against the statistic derived when all values of y are identical, aside from experimental errors. The algorithm for deriving the probability p for a one-tailed test is as follows:

Step 1: Create a bootstrap sample of y^* values from the actual sample Y for each test direction. For sample $Y = \{y_1, y_2, y_3, y_4, y_5, y_6, y_7, y_8\}$, a bootstrap sample y^* might look like $\{y_2, y_7, y_2, y_1, y_4, y_8, y_7, y_6\}$. Bootstrap simulates the operation of selecting random, independent observations from a population.

Step 2: Derive the statistic ϕ^* from the y^* values, i.e., $\phi^* = \Sigma w_i y_i^*$.

Step 3: Repeat Steps 1–2 a large number of times, say, $n\text{Rep} = 10{,}000$ to provide a distribution of ϕ^* values.

Step 4: Compute the probability for different test directions k from $p_k = \{\#\text{ of times } \phi \geq \phi^*\}/n\text{Rep}$.

Step 5: Finally, draw inference from the p-values derived from k-number of test directions using a meta-analysis approach. In specific, derive the probability that the p-values obtained could have been acquired by chance using the *Fisher's combined probability test* which provides a chi-square statistic, $\chi^2 = -2\Sigma\log(p_k)$ with degrees of freedom equal to twice the number of test directions (Wolf, 1986, pp. 18–19).

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