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Pupil responses associated with coloured afterimages are mediated by the magno-cellular pathway

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Abstract

Sustained fixation of a bright coloured stimulus will, on extinction of the stimulus and continued steady fixation, induce an afterimage whose colour is complementary to that of the initial stimulus; an effect thought to be caused by fatigue of cones and/or of cone-opponent processes to different colours. However, to date, very little is known about the specific pathway that causes the coloured afterimage. Using isoluminant coloured stimuli recent studies have shown that pupil constriction is induced by onset and offset of the stimulus, the latter being attributed specifically to the subsequent emergence of the coloured afterimage. The aim of the study was to investigate how the offset pupillary constriction is generated in terms of input signals from discrete functional elements of the magno- and/or parvo-cellular pathways, which are known principally to convey, respectively, luminance and colour signals. Changes in pupil size were monitored continuously by digital analysis of an infra-red image of the pupil while observers viewed isoluminant green pulsed, ramped or luminance masked stimuli presented on a computer monitor. It was found that the amplitude of the offset pupillary constriction decreases when a pulsed stimulus is replaced by a temporally ramped stimulus and is eliminated by a luminance mask. These findings indicate for the first time that pupillary constriction associated with a coloured afterimage is mediated by the magno-cellular pathway.

1. Introduction

Sustained fixation of a bright light stimulus will, on extinction of the stimulus and continued steady fixation, induce an afterimage whose colour is complementary to that of the initial stimulus. Sustained fixation of a bright green spot will, for example, induce on offset, an afterimage of complementary colour (i.e. orange) at the same location. The effect can be explained in part by fatigue of cones and/or by cone-opponent processes to different colours. However, to date, very little is known about the specific pathway that causes the colour afterimage (Burbeck, 1986; Kelly & Martinez-Uriegas, 1993; Schiller & Dolan, 1994).

Barbur, Weiskrantz, and Harlow (1999) reported two types of pupil constriction when the pupillary response was measured for an isoluminant green stimulus: the first is evoked following onset of the stimulus; the second is evoked by the offset of the stimulus and corresponds to the emergence of the coloured afterimage. It was also shown that pupillary responses associated with coloured afterimages could be demonstrated in the absence of both a primary visual cortex and conscious awareness. These second constrictions have also been known as an OFF response for chromatic stimuli (e.g. Kimura & Young, 1995; Kohn & Clynes, 1969).

The aim of the present study is to investigate whether the second pupillary constriction is associated with input signals from discrete functional elements of the magno- and/or parvo-cellular pathway. It is widely accepted that pupillary responses are driven by luminance signals (i.e. the magno-cellular pathway) (e.g. Alpern & Campbell, 1962; Kohn & Clynes, 1969) and recently it has been shown that signals in the linear $|L - M|$ cone-opponent mechanism (i.e. the parvo-cellular pathway) also contribute to a pupillary control mechanism (Tsujimura, Wolffsohn, & Gilmartin, 2001). Since both pathways contribute to the pupillary responses, it is feasible that the second pupillary constriction could be generated by either or both mechanisms.

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Kimura and Young (1995) used a principle component analysis to investigate whether the pupillary responses could be attributed to magno-/parvo-cellular pathways. They partitioned the pupillary response into transient and sustained components, to match those that might, respectively, correspond to signals in magno- and parvo-cellular pathways. They showed that the intensity–amplitude function derived for the off-portion (i.e. the second pupillary constriction) of the pupillary response is similar to that for the transient component (i.e. the magno-cellular pathway), whereas the action spectra as a function of wavelength from the OFF response was similar to that for the sustained component (i.e. the parvo-cellular pathway).

The present study investigates the second offset pupillary constriction (corresponding to the emergence of the coloured afterimage) in terms of its potential association with discrete functional elements occurring within the magno-cellular and parvo-cellular pathways. It is likely that there is a link between offset stimuli and the magno-cellular pathway as offset stimuli have high energy in high temporal frequency regions that are known to stimulate preferentially the magno-cellular pathway. The data confirm an association between the second offset pupillary constriction and the magno-cellular pathway as removal of high-frequency energy from the stimulus, by substituting the pulse stimulus with a ramped stimulus, is shown to decrease the amplitude of the offset constriction. Further confirmation that the magno-cellular pathway contributes directly to the offset constriction is provided by a luminance-masking paradigm to suppress selectively the magno-cellular pathway.

2. Methods

2.1. Stimulus generation

The stimulus was generated by a video controller (Cambridge Research Systems VSG2/3) and displayed on a colour monitor (EIZO, T68). The resolution of the monitor was 640×480 pixels and the frame rate was 150 Hz. A 12-bit digital-to-analogue converter drove each phosphor. Cone excitation was calculated according to the spectral radiation of each phosphor, measured by a Photo Research PR-650 instrument, using three cone fundamentals obtained by Smith and Pokorny (1975).

2.2. Background stimulus

A yellow background with CIE coordinates $(0.40, 0.47)$ and luminance 34.5 cd/m^2 was used throughout. The retinal illuminance of the background was $\approx$650 photopic trolands with an average pupil size of 4.9 mm, which minimized the involvement of rods (Aguilar & Stiles, 1954; Lee, Smith, Pokorny, & Kremers, 1997). In a previous paper (Tsujimura et al., 2001), the pupillary iso-response contour in L, M cone contrast space was well described ($r^2 = 0.99$) by a straight line with a positive slope under similar stimulus conditions. The results indicated that the pupillary response was determined solely by an $|L - M|$ linear cone-opponent, suggesting very little rod intrusion. Lee et al. (1997) showed that the parvo-cellular ganglion cells (i.e. $|L - M|$ cells) demonstrate only weaker rod input than the magno-cellular cells. They concluded that rod intrusion for the parvo-cellular pathway is minimal above two trolands. Here we will also show (Fig. 2) that the iso-response contours in this experiment form a straight line with a positive slope, thus indicating the minimal rod intrusion.

A yellow background was chosen in order to avoid the effect of short-wavelength cone excitation on the pupillary response. The fraction of the background luminance for L cone contrast (i.e., $\mathbf{r} = \mathrm{LBg}/(\mathrm{LBg} + \mathrm{MBg})$ in the cone excitation diagram proposed by MacLeod & Boynton, 1979) was 0.67 and the ratio of the L- and M-cone excitation was 2.0, being identical to that for 570 nm isochromatic light. The fraction used minimized the apparent variation in the background colour when either the mean luminance level, temporal frequency or spatial frequency of the test stimulus was changed. For a 570 nm yellow background field, Pokorny, Jin, and Smith (1993) showed that the red–green ratio in flicker detection was approximately constant with respect to changes in the mean luminance. Stromeyer, Chaparro, Tolias, and Kronauer (1997) also showed that the ratio of L- and M-cone inputs to the luminance mechanism were constant with respect to the change in spatial and temporal frequencies.

2.3. Test stimulus

The uniform test stimulus was displayed in a circular region of 10° diameter at the centre of the screen for 2 s. Despite being relatively large, and hence susceptible to contamination from the rod system and mango-cellular pathway, the stimulus is shown later (Fig. 2) to be influenced minimally by either of these sources. The pupillary response was recorded for 6 s comprising the 2 s test presentation and 2 s pre- and post-task presentation. Photometrically isoluminant green stimuli were used as the isoluminant stimuli and bright yellow stimuli as the luminance stimuli. The contrast for isoluminant and luminance stimuli was the same and was defined in cone-contrast space. The two test stimuli had different temporal envelopes: a pulsed stimulus and a ramped stimulus (i.e. a smooth temporal envelope, ramped on for 200 ms with a raised cosine, held constant for 1600 ms, then ramped off). A contrast of 0.11 was used for the pulsed stimulus and 0.12 for the ramped stimulus so that

the total energy of each stimulus was the same. These contrasts were approximately 15× greater than the psychophysical contrast threshold initially measured.

2.4. Masking stimulus

In the second experiment, in order to suppress selectively the magno-cellular pathway, a luminance-masking paradigm was used. The spatially uniform masking stimulus overlapped the same circular area as the test stimuli and was presented 2 s before and after the test stimulus. A sinusoidal luminance function with contrast of 0.04 defined in cone-contrast space was used to determine the amplitude of the masking stimulus. Every 7 ms, the phase of the sinusoidal function was randomly selected and the amplitude at that phase was selected as the amplitude of the masking stimulus. This temporally jittering mask can suppress selectively the magno-cellular pathway since it is modulated only in luminance and has a broadband temporal spectrum.

2.5. Measurement of pupil size

The pupil of the right eye was imaged using a video camera (Pulnix TM6) located 1 m from the observer and 15° temporal to the visual axis. To facilitate measurement of pupil size by image analysis, an infra-red light source was mounted on the video camera. The video image was fed into an IMAQ PCI-1407 image acquisition board (National Instruments) and analysed using LabView and IMAQ Vision software (National Instruments) at a frequency of 50 Hz. The pupil was located using thresholding and edge detection techniques, allowing the pupil diameter to be analysed at a resolution of <0.01 mm.

2.6. Procedure

Six observers including one of authors (JW) each with normal colour vision and ametropia corrected with ultra-thin hydrophilic contact lenses (*Bausch and Lomb*, 55% water content) participated in the experiment. Observers were seated 66 cm in front of the display monitor and fixated binocularly a black Maltese Cross (90% contrast) which subtended 0.8° and was always present in the centre of the screen. The cross acted as an accommodative lock, providing a strong closed-loop stimulus to maintain accommodation at a constant level. A previous study has shown that these experimental conditions ensure that pupil responses do not receive any input from the accommodative system (Tsujimura et al., 2001). Pupil responses were recorded continuously from the observer's right eye and experimental trials followed an initial adaptation period of 3 min. Each of the test stimuli presentations were repeated and summed so that each trace represented an average of more than 30 recordings.

2.7. Isolation of the mechanism

We confirmed that isoluminant stimuli used in the experiments described below were solely determined by the parvo-cellular pathway (i.e. $|L-M|$ mechanism). The iso-response contours in cone-contrast space were determined (Tsujimura et al., 2001) to examine whether the parvo-cellular pathway was solely responsible for the pupillary responses evoked by the isoluminant stimulus. If the $|L-M|$ chromatic mechanism drives the pupillary response, the iso-response contour should form a straight line with a positive slope in cone-contrast space (Tsujimura et al., 2001; see also Chaparro, Stromeyer, Chen, & Kronauer, 1995). The characteristics of the iso-response contour change when other mechanism(s) (e.g. $|L+M|$ mechanism and/or rod system) determine the response. For example, if the $|L+M|$ luminance mechanism drives the pupillary response, the contour should form a straight line with a negative rather than positive slope (Stromeyer et al., 1997; Tsujimura, Shioiri, Hirai, & Yaguchi, 1999, 2000). Furthermore, the iso-response contours should form a parallelogram when both mechanisms determine the response. When the rod system determines the response, the point(s) of the iso-response contour around the M-cone axis do not fall on a straight line. Rod effectivity pertaining to the M-cone axis is stronger than that for the L-cone axis as the spectrum of rod activity is more similar to the M-cone than to the L-cone.

Fig. 1 shows hypothetical iso-response contours in cone-contrast space when both $|L-M|$ and $|L+M|$ mechanisms determine the pupillary response. The form of the iso-response contour varies depending on the difference in sensitivity between $|L-M|$ and $|L+M|$ mechanisms. The upper panel represents the iso-response contour when the sensitivity of the $|L-M|$ mechanism is greater than that of the $|L+M|$ mechanism and the lower panel represents the contour when the sensitivity of the $|L+M|$ mechanism is greater than that of the $|L-M|$ mechanism. The sensitivity of each mechanism is defined as the distance between the origin and each respective line. The circles in the second quadrant represent contrasts of stimuli that induce the same amplitude of the pupillary response in each condition. These stimuli are several colours including the isoluminant stimulus.

In the upper panel, the iso-response contour in the second quadrant represents a positive slope, indicating that the $|L-M|$ mechanism solely determines the thresholds in this region. Conversely, in the lower panel the iso-response contour in the second quadrant no longer forms a straight line since the three iso-responses without an isoluminant stimulus are determined by the

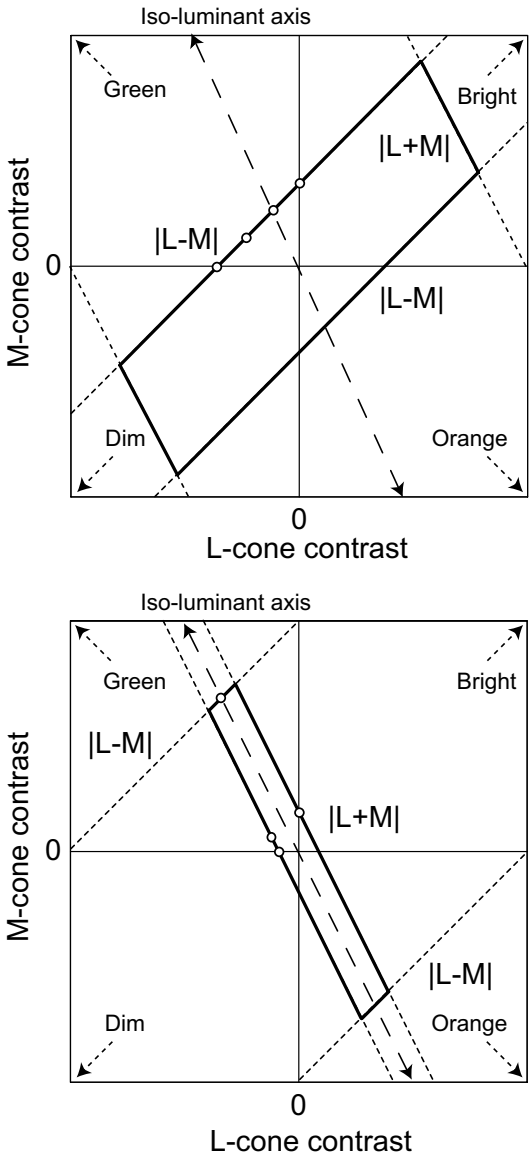

Fig. 1. The hypothetical iso-response contours in cone-contrast space when both $|L - M|$ and $|L + M|$ mechanisms determined the pupillary response. The form of the iso-response contour varies depending on the difference in sensitivity between $|L - M|$ and $|L + M|$ mechanisms. The upper panel represents the iso-response contour when the sensitivity of the $|L - M|$ mechanism is greater than that of the $|L + M|$ mechanism and the lower panel represents the contour when the sensitivity of the $|L + M|$ mechanism is greater than that of the $|L - M|$ mechanism.

$|L + M|$ mechanism. Therefore, the shape of the iso-response contour in cone-contrast space indicates the isolation of each mechanism.

The pupillary responses to the isoluminant uniform stimuli along four vector directions (90°, 117°, 153° and 180°) were measured to obtain the iso-response contour. The response amplitude was recorded as the difference between the initial pupil diameter and the peak constriction. The iso-response contrast (using a criterion of a 0.2 mm change in pupil size) for each vector direction was calculated from the pupillary response evoked by a test stimulus with a contrast of 0.12 since a linear relationship between the test contrast and the amplitude of the pupillary response was found previously for a similar condition (Tsujimura et al., 2001).

The pupillary iso-response contour in cone-contrast space for subjects JW and RC are shown in Fig. 2. White circles represent the iso-response contrasts for the 90°, 117°, 153°, and 180° vector directions. It was found to be linear in the second quadrant, indicating that the sensitivity of the $|L - M|$ mechanism is greater than that of the $|L + M|$ mechanism. Therefore, the isoluminant stimuli (117°) vector direction used in the following experiments can isolate the $|L - M|$ mechanism alone. In other words, the responses along the isoluminant axis were determined solely by the parvo-cellular pathway.

The d-isoluminant stimuli have been used to isolate the $|L - M|$ mechanism since they may not respond to either the rod system or the $|L + M|$ mechanism (Young

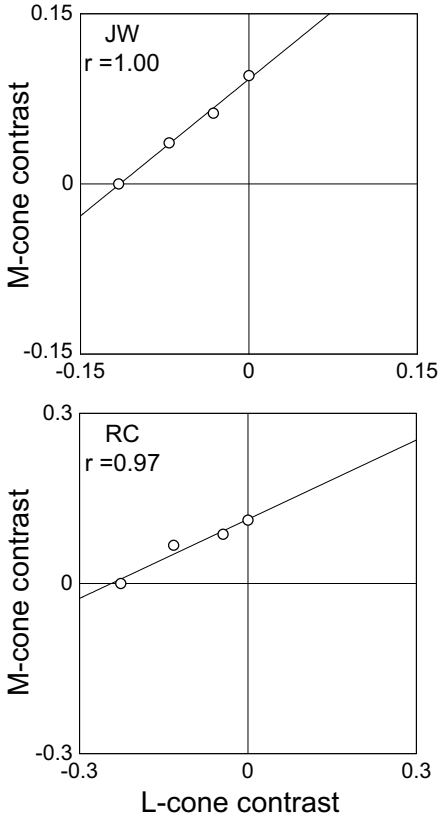

Fig. 2. The pupillary iso-response contour (for a change in amplitude of 0.2 mm) evoked by various colours of stimuli modulated in cone-contrast space. White circles represent the iso-response contrasts for the 90°, 117°, 153°, and 180° vector directions. It was found to be linear in the second quadrant, indicating that the sensitivity of the $|L - M|$ mechanism is greater than that of the $|L + M|$ mechanism. Therefore, the responses evoked by the isoluminant stimuli were determined solely by the parvo-cellular pathway.

& Teller, 1991). A preliminary test was carried out to confirm the isolation by plotting the iso-response contour in cone-contrast space (Fig. 2) rather than using the d-isoluminant stimuli since plotting the former is one of the techniques to confirm the isolation of the $|L-M|$ mechanism. D-isoluminant stimuli are, however, not always able to isolate the $|L-M|$ mechanism. For example, when the weight ratio of L- and M-cone signals to the $|L+M|$ mechanism is different from that of the CIE standard observer, the stimuli are no longer isoluminant. It is technically difficult to obtain the weight ratio since it varies substantially, depending on spatial and temporal frequency and background colour (Stromeyer et al., 1997). Furthermore, when there is a phase shift between L- and M-cone signals in the magno-cellular pathway (Smith, Lee, Pokorny, Martin, & Valberg, 1992), the iso-response contour will form an ellipse rather than parallel lines (Stromeyer et al., 1997; Tsujimura et al., 2000). In this case, the responses evoked by the stimuli along the isoluminant axis are determined by the $|L+M|$ mechanism instead of the $|L-M|$ mechanism. In addition, as indicated in Fig. 1, the isolation of the mechanism is also dependent on the relative sensitivity of the mechanisms. Therefore, some preliminary test is required to confirm the isolation. The isolation was confirmed in this study by plotting the iso-response contour in cone-contrast space.

3. Results

3.1. Pupil responses evoked by colour and luminance stimuli

The pupillary responses evoked by isoluminant green stimuli for all six subjects are shown in Fig. 3. All test stimuli produced relatively large pupillary responses (on average 0.40 ± 0.12 mm). All observers reported observing a strong afterimage following the offset of each green stimulus. The afterimage evoked a large transient second constriction whose trough was reached approximately 700–1000 ms following the offset of the test stimulus. This finding supports data from previous studies and has been attributed to afterimage cortical mechanisms (Barbur et al., 1999; Barbur, Wolf, & Lennie, 1998) and to OFF responses (Kimura & Young, 1995).

We observed the second constrictions evoked by the isoluminant green stimuli. However, it is possible that these were produced by small changes in luminance stimuli since observers' isoluminant points could be different from the isoluminant point defined in CIE. To investigate further which mechanism evokes the second constriction, a luminance stimulus was used in the next experiment. If the second constriction is evoked by the change in luminance component of the stimulus, the amplitude of the constriction evoked by the luminance stimulus is likely to be greater than that evoked by isoluminant stimulus.

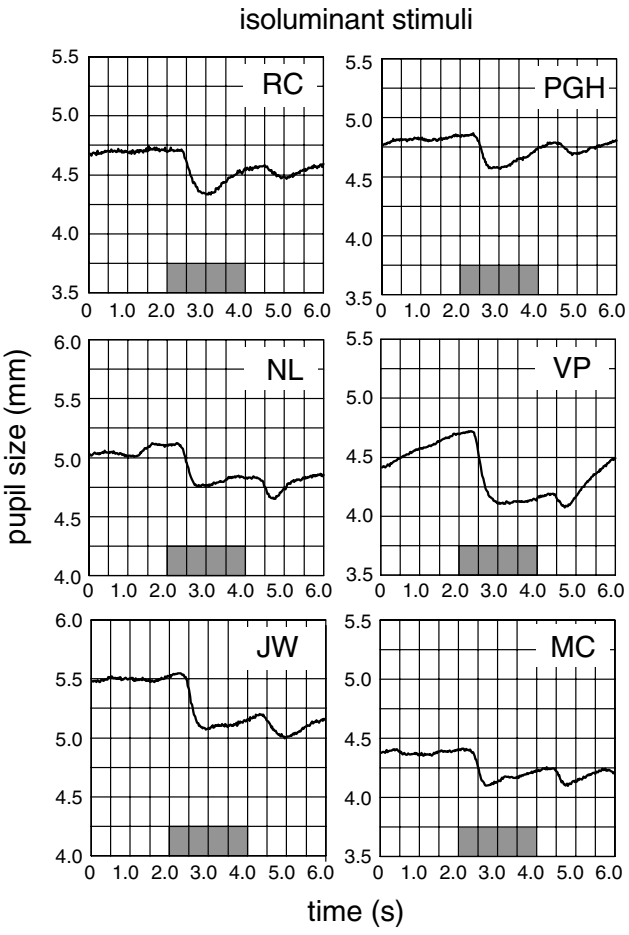

Fig. 3. Pupil responses evoked by the isoluminant green stimulus for six observers. The green stimuli were photometrically isoluminant to the yellow background field. Each trace represents the average of more than 30 recordings. The onset and offset of the test stimuli is shown by the grey box on the time scale axis. For all observers the second pupil constriction was observed following the offset of the stimulus.

Fig. 4 shows the pupillary responses evoked by luminance stimuli. All test stimuli produced relatively large pupillary responses similar to the responses for isoluminant stimuli (on average 0.28 ± 0.11 mm). In contrast, the large second constriction observed with the isoluminant stimulus disappeared. These results indicate that the second constriction evoked by the isoluminant green stimulus was not evoked by the change in luminance component of the stimulus.

The difference in pupillary response between isoluminant green and luminance stimuli suggests that a different mechanism drives the pupillary response. To investigate whether the different mechanism involves the pupillary response, the latency of the pupillary response was analysed. Latency of the pupillary response to the test stimuli was calculated using the normalization

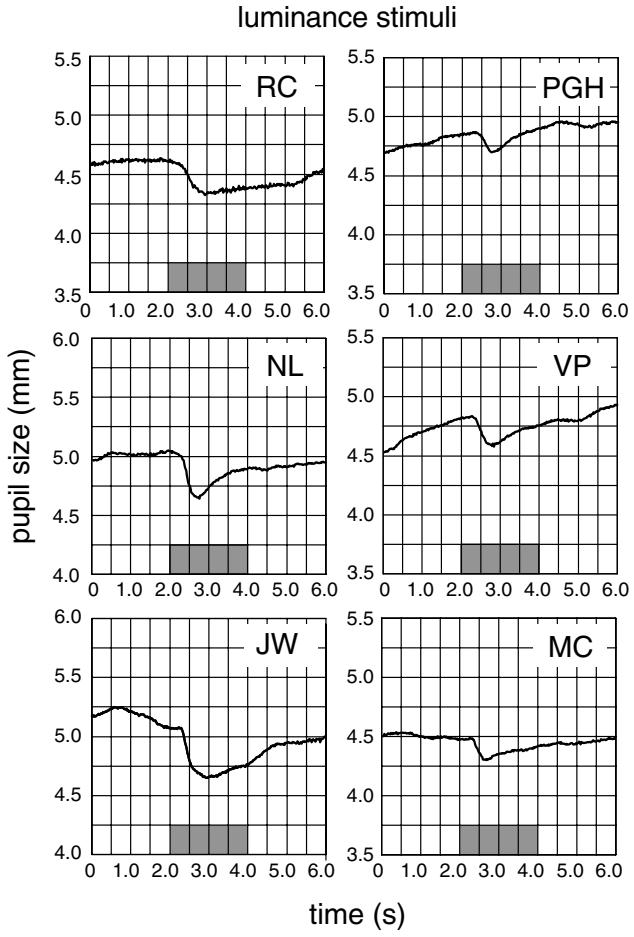

Fig. 4. Pupil responses evoked by the luminance stimulus for six observers. The bright yellow test stimulus was presented on the yellow background field. All other details are the same as for Fig. 3.

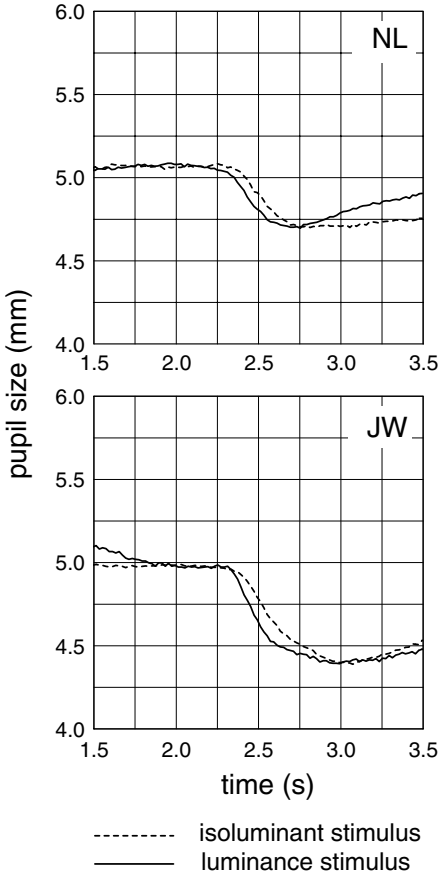

Fig. 5. Normalised pupillary response amplitudes for subjects NL and JW to isoluminant green (dashed line) and luminance (solid line) stimuli, allowing response latencies to be calculated. The amplitudes of the pupillary response to isoluminant green and luminance stimuli were normalised with respect to the average amplitude and the traces overlaid such that differences in latency could be detected. The latency in pupil response ranged from 70 to 370 ms for all observers. The pupillary response evoked by the isoluminant green stimulus is delayed by approximately 75 ms compared to that evoked by the luminance stimulus.

technique developed by Barbur et al. (1998). The time courses for the earlier phase of the response (i.e. the time to reach maximum first pupil constriction from baseline) were shown to have two characteristics: first, time courses were independent of contrast level after normalisation; secondly, time courses were equivalent for within-class variations in stimulus level (e.g. contrast) but different for between-class variation in stimulus level (e.g. colour and luminance stimuli). The amplitude of the pupillary response to isoluminant and luminance stimuli were normalised with respect to the average amplitude and the traces overlaid such that differences in latency could be detected.

Fig. 5 shows onset latencies for observers NL and JW. The latency in pupil response ranged from 70 to 370 ms for six observers, similar to that found by Barbur et al. (1998). The latencies for the isoluminant stimulus were significantly longer (the differences for each of the subjects were 53, 88, 102, −24, 72 and 59 ms $P < 0.05$), on average by 75 ms, than those found for the luminance stimuli and were again similar to those reported by Barbur et al. (1998). In a previous paper (Tsujimura et al., 2001), very similar latencies (30–100 ms: average 62 ms) were obtained for the isoluminant grating and the uniform luminance stimulus. Since the parameters of the isoluminant green stimulus used in this experiment were the same, except for the spatial frequency, as used in the previous experiment (i.e., for contrast, field size and temporal frequency), the similarity between latencies suggests that the pupillary response evoked by the isoluminant green stimuli was driven by the L − M cone-opponent mechanism.

The second constriction following the stimulus offset suggested a relationship between the constriction and the temporal properties of the stimulus since the stimulus offset had high energy in high temporal frequency regions. Consequently, the effect of manipulating the temporal envelope of the test stimuli was investigated in

the third experiment for three observers (RC, NL and JW) using ramped stimuli.

The three panels in the left column of Fig. 6 show the pupillary responses evoked by the ramped stimuli. The amplitudes of the second constriction were smaller than those obtained by pulsed stimuli (Fig. 3, left column). Differences in amplitudes of the second constrictions induced by the ramped stimuli were compared using the normalization technique similar to that used in the latency analysis. Hence response amplitudes to pulsed and ramped test stimuli were normalised with respect to the average amplitude and the traces overlaid such that differences in amplitude of the secondary constriction could be detected.

The three panels in right column of Fig. 6 show normalised pupillary responses evoked by ramped and pulsed stimuli. The pupillary response evoked by the ramped stimulus had a more sustained temporal property, and the relative amplitude of the second constriction evoked by a ramped stimulus was significantly smaller than that evoked by a pulsed stimulus ($P = 0.000$ for JW and NL, and 0.015 for RC). The relative amplitude was measured as the difference in amplitude between the beginning of the second constriction and the peak constriction of the averaged response. The relative amplitudes (mm) for ramped stimulus are 0.026 for RC, 0.014 for NL, 0.044 for JW; and 0.087, 0.215 and 0.249 respectively for pulsed stimulus. The average standard deviation for relative amplitude was 0.19.

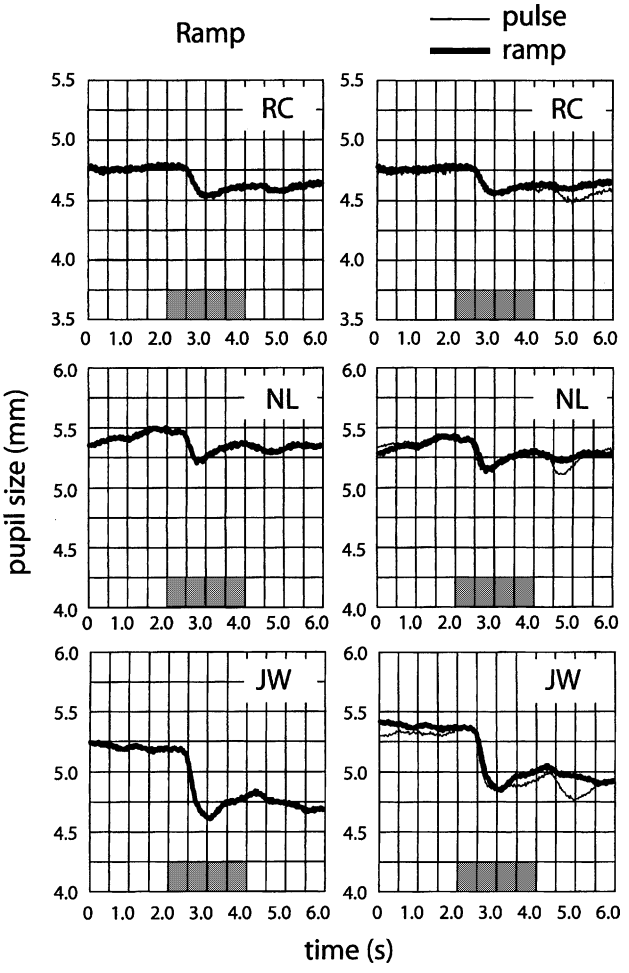

Fig. 6. Pupil responses evoked by the ramped stimuli (the left column) and normalised pupillary responses to ramped (thick line) and pulsed (thin line) stimuli (the right column) for three observers. The amplitudes of the pupillary response to these stimuli were normalised in the same way as in Fig. 3. The pupillary response evoked by the ramped stimulus had a more sustained temporal property, and the relative amplitude of the second constriction was substantially smaller than that evoked by a pulsed stimulus.

It appears, therefore, that the second pupillary constriction is a consequence of the high temporal frequency properties of the initial stimulus which suggests the involvement of magno-cellular rather than the parvo-cellular pathways as the former pathway is known to exhibit greater sensitivity to higher temporal frequencies than the latter (e.g. Merigan, Katz, & Maunsell, 1991).

To substantiate further whether the second pupillary constriction can be attributed to the magno-cellular pathway, a luminance-masking paradigm was used, as it is known to suppress selectively the pathway.

The six panels in Fig. 7 show pupillary responses for pulsed stimuli with and without the luminance mask; the panels in right column show the normalised pupillary responses. The results demonstrate that the pupillary response evoked by the masked stimulus had a more sustained temporal property, and that the form of the pupil response characterising the initial pupillary constriction is relatively unaffected by the presence or absence of the luminance mask. In contrast, the amplitude of the second pupil constriction was significantly smaller for all observers with the luminance mask than without the mask ($P < 0.01$). The amplitudes of masked stimuli (mm) were −0.027 for RC, 0.046 for NL, 0.051 for JW; and 0.077, 0.210 and 0.286 respectively without the mask. The average standard deviation for relative amplitude was 0.31. Before the test stimulus presentation some ripples were observed for observers NL and JW. Since the masking stimulus was presented 2 s before the test stimulus presentation, the ripples have been attributed to a response associated with the initial presentation of the masking stimulus. As the temporal loci of the first pupillary constriction in the masking condition were very similar for each observer the effect of the ripples on the main results has been discounted.

4. Discussion

The results of this study demonstrate that pupillary constriction associated with a coloured afterimage is

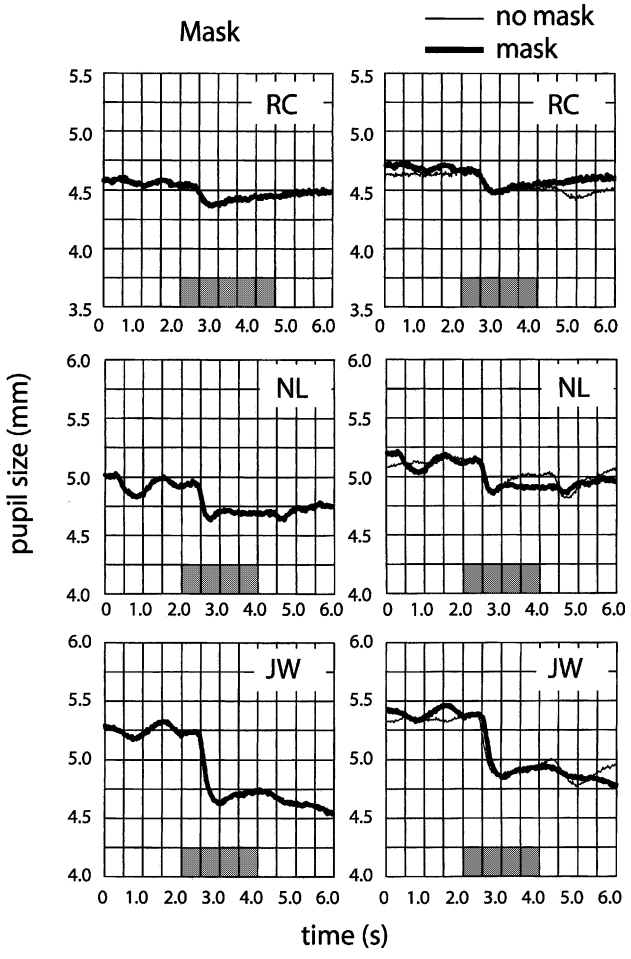

Fig. 7. Pupil responses evoked by the masked stimuli (the left column) and normalised pupillary responses to masked (thick line) and no-mask (thin line) stimuli (the right column) for three observers. The amplitudes of the pupillary response to these stimuli were normalised in the same way as in Fig. 3. The pupillary response evoked by the masked stimulus had a more sustained temporal property and the form of the pupil response characterising the initial pupillary constriction is relatively unaffected by the presence or absence of the luminance mask.

mediated by the magno-cellular pathway. Using similar experimental conditions Tsujimura et al. (2001) observed a second pupil constriction in response to an isoluminant uniform green stimulus and showed that it disappeared at a relatively high spatial frequency (1.6 c/deg), suggesting that, at this frequency, the magno-cellular pathway contributes less to the response. It would appear then that, as suggested by Kimura and Young (1995), the pupillary responses evoked by coloured light flashes could consist of functionally separable components comprising a combination of magno-cellular and parvo-cellular pathways. Using principle component analysis Kimura and Young (1995) demonstrated that the intensity–amplitude function derived for the off-portion (i.e. the second pupillary constriction) of the pupillary response is similar to that for the transient component of the response.

We propose that the magno-cellular pathway is responsible for the generation of the second constriction associated with the coloured afterimage and, once formed, the parvo-cellular pathway is responsible for its transmission to higher visual processing centres. A number of studies have reported that the magno-cellular pathway receives $|L - M|$ chromatic signals as well as luminance signals (Smith et al., 1992; Stromeyer et al., 1997; Tsujimura et al., 1999). Smith et al. (1992) measured phase shift between L- and M-cone signals in the magno-cellular pathway and suggested that the receptive field surround of retinal ganglion cells in the magno-cellular pathway is colour-opponent (see also Stromeyer et al., 1997). Tsujimura et al. (1999) also showed that the $|L - M|$ chromatic mechanisms, using similar stimuli to those in this study, could selectively suppress L- and M-cone signals in the luminance pathway. If this is the case, since the magno-cellular pathway is able to receive chromatic signals, its output could affect the pupillary response. Here we show that whereas the second pupil constriction was evident for an isoluminant green stimulus, it disappeared for a luminance stimulus, suggesting that the second constriction is formed in the parvo-cellular pathway which conveys principally colour signals. Kelly and Martinez-Uriegas (1993) also showed that chromatic or luminance afterimages elevate only the thresholds for chromatic patterns, suggesting further that the afterimage is formed in the parvo-cellular pathway. Barbur et al. (1999) proposed a model for coloured afterimages based on cone-specific adaptation. However, their model does not explain the finding in this study that amplitude of the second constriction is decreased by the ramped and the masking stimuli.

The question that remains is whether the second constriction is induced by the chromatic afterimage, rather than by the stimulus onset and offset. Suppose that stimuli activate two temporally separate systems in the pupillary pathway: one that responds rapidly to the stimulus, the other more slowly, as is the case with cone and rod systems. In this dual system, the second constriction could be driven by the stimulus onset, i.e., the first constriction being driven by the fast system and the second constriction being driven by the slow system. However, a dual system having these characteristics was not evident in the present experiment. We compared the amplitude of the second constriction following the pulsed and the ramped stimuli. A slow system is more sensitive to a ramped stimulus rather than to a pulsed stimulus since the slow system is more sensitive at lower temporal frequencies. Our ramped-stimulus experiment showed that the amplitude of the second constriction was significantly smaller than that of the pulsed stimuli. Hence the second constriction is not induced by the onset of the stimulus. Furthermore, it is likely that the second constriction is induced by the chromatic afterimage rather than by stimulus offset. The second pup-

illary constriction is not observed when the duration of stimulus presentation is short and where correspondingly there is little or no afterimage. Barbur et al. (1999) varied the presentation times for their chromatic stimuli and found that the second constriction disappeared when the duration was short (i.e. 300 ms). Our preliminary data show similar results. The second constriction to isoluminant stimuli disappear when the duration is 100 ms (data not shown) which suggests that the second constriction is induced by the chromatic afterimage.

The results of this study indicate that the second pupil constriction associated with the coloured afterimage is mediated by the magno-cellular pathway. A number of researchers (e.g. Silverman, Trick, & Hart, 1990) have reported that primary open angle glaucoma might selectively damage the magno-cellular pathway and consequently conventional psychophysical screening tests for glaucoma are designed to measure contrast thresholds for flicker in high temporal frequency regions which are known to stimulate preferentially the magno-cellular pathway. However, psychophysical measurement is often intricate with, for example, elderly patients or patients with systemic or ocular disease, owing to the increased variability in data compared with normal observers. As the variability in these measures results principally from the subjective and judgmental nature of psychophysical measurements, the objective characteristics of pupil response measurement has special clinical utility in general and, in the context of this study, specific utility where a deficit of the magno-cellular pathway is likely to be a distinct feature of the clinical condition. However, the clinical use of pupil responses will, in order to optimise signal-to-noise ratio, require high contrast stimuli such as those used in this study. In addition, the observation that the second pupil constriction can be demonstrated even in observers with damage to, or loss of, area V1 (Barbur et al., 1999) demonstrates further the potential clinical utility of pupil measurements where standard psychophysical procedures are not feasible.

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