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## Pupillary response to chromatic flicker

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**Abstract** There is significant evidence for higher-level cortical control of pupillary responses to visual stimuli, suggesting that factors other than luminance changes may induce a pupillary response. In the present study, the pupillary responses to equiluminant flickering stimuli in a range of 3–13 Hz were examined. Flicker stimuli included color-black (luminance-modulated) and color-color (hue-modulated) flicker. Equiluminance was determined both by objective luminance measures as well as by subjective, perceptual equiluminance for each subject. For both objectively and subjectively equiluminant flicker, significant, sustained pupillary constrictions were recorded. The magnitude of these responses was sensitive to both color and frequency parameters; red-blue color-paired flicker consistently produced the strongest constrictions. These responses occurred even when the flicker was of a lower luminance, both physically and perceptually, than a preceding nonflickering color, indicating that chromatic rather than luminance-sensitive mechanisms are involved in this response. Interestingly, the color- and frequency-sensitivity of constriction parallels those of flickers which maximally stimulate photosensitive epileptic patients, raising the possibility that chromatic response may be a factor in photosensitivity.

**Keywords** Pupil · Flicker · Photosensitive · Chromatic

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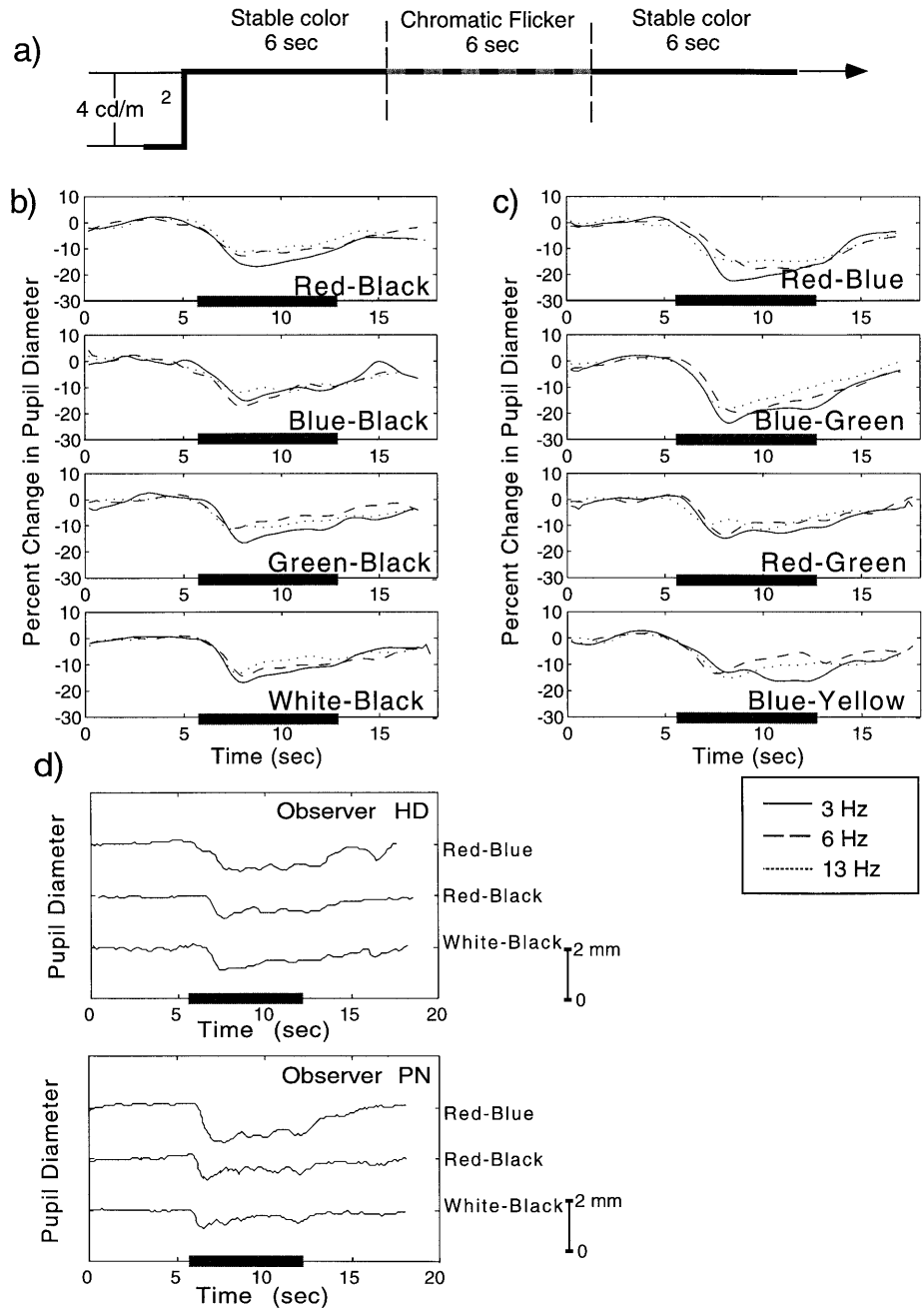
### Introduction

The pupil serves as a gain-control device for the visual system. It responds to luminance changes in the environment, and this response is largely governed by a well-characterized subcortical projection through the pretectum to the accessory oculomotor nucleus (Lowenfeld 1993). However, in addition to this luminance sensitivity, pupillary responses have been found for a number of other stimuli, including single changes in color and pattern, as well as onset of coherent motion (Kohn and Clynes 1969; Young and Alpern 1980; Barbur et al. 1992). Change in pupil size has also been implicated in such higher-level behaviors as attention (Hess and Polt 1960, 1964) and social signaling (Niedenthal and Cantor 1986). Additionally, it was found that a single flash of color on a white background produced a pupillary-constriction response made of several distinct components, including transients related to flash onset and offset as well as a sustained component (Kimura and Young 1995).

The role of the pupil as a gain control may have some clinical and social significance. Unusual luminance-contrast gain control has been linked to photosensitivity, in which afflicted individuals are abnormally sensitive to particular forms of photic stimulation and which can result in seizure attacks (Doose and Waltz 1993; Porciatti et al. 2000). A particularly notorious incident involving photosensitivity happened in 1997, when over 700 children in Japan were hospitalized after exposure to patterned red-blue flicker on a children's television cartoon show (Ishida et al. 1998). Particular color and frequency parameters have been shown to be favorable in inducing seizure-like symptoms in clinical settings (Takahashi and Tsukahara 1976).

The role of the pupillary response in regard to non-luminance-related changes such as transient chromatic exchanges is not clear. Kimura and Young (1995, 1996, 1999) draw parallels between the pupil action spectra to single flashes on a colored background and the spectral sensitivity curve of the psychophysical chromatic

**Fig. 1a–d** Pupillary responses for experiment 1. **a** Schematic diagram of experiment 1. **b**, **c** Average waveforms of pupil diameter for different color and frequency flickers in experiment 1, expressed as mean percent change (from control-period baseline) in diameter. *Solid line* Response at 3 Hz, *dashed line* at 6 Hz, *dotted line* at 13 Hz. *Gray bar* at bottom indicates flicker stimulus. **b** shows responses to luminance-modulated (color-black) flicker, **c** shows responses to hue-modulated (color-color) flicker. **d** Representative waveforms from different observers for 3-Hz flicker. The example waveforms for responses to red-black and white-black flicker have been displaced 2 mm successively downwards for clarity



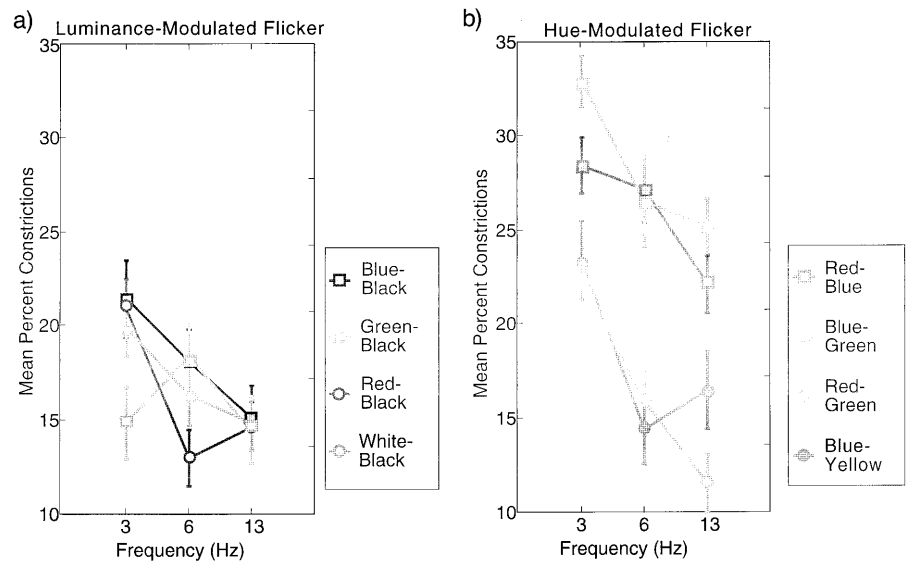
channel. These parallels imply a close relationship between pupillary response and color perception. Previously, Troelstra (1968) used the pupillary response as a measure of brightness enhancement, examining the pupil's response to a temporally modulated light stimulus.

A further exploration of the role of these responses is clearly valuable. For instance, if the pupil responds transiently to individual changes, yet most natural visual scenes feature myriad individual changes of this nature, how does the pupil respond to these sequences of changes? How does a non-luminance-driven pupillary response extend to dynamic, constantly changing stimuli? The present study attempts to address these questions by investigating flickering stimuli that are temporally

modulated over relatively long periods of time (6 s) and that have been shown to have unusual effects on some people – notably, the dangerous excitatory effects on photosensitive patients.

Preliminary studies performed in our laboratory indicated a sustained pupillary constriction in response to a flickering stimulus. The amplitude and duration of this response appeared to be sensitive to color and temporal frequency parameters, with some overlap between those parameters producing the strongest constrictions and those most likely to produce seizure activity in photosensitive patients. These preliminary findings were based on flicker in which the color components of flicker were perceptually equiluminant. The presence of

**Fig. 2a, b** Dependence of constriction size (expressed as percent change from control-period baseline) on color and frequency parameters for experiment 1. **a** Luminance-modulated flicker, **b** hue-modulated flicker. Error bars represent standard error of the mean



constrictions raised the question of whether this response was due to small, physical luminance changes between perceptually isoluminant colors in the flicker. In order to establish whether this response was due to the action of luminance channels, we performed two experiments: one in which the stimulus had the same physical luminance throughout, and one in which the flickering components were perceptually equated, yet of a lower mean luminance than a preceding solid color.

## Materials and methods

### Subjects

Subjects were six healthy volunteers, three male and three female, between the ages of 18 and 28 years. Subjects were screened for photosensitivity and personal or familial history of epilepsy. The study was approved by the Human Subjects Committee; all subjects gave their informed consent prior to their inclusion in the study.

### Stimulus

The stimulus was presented on a 16" NEC RGB monitor operating at a resolution of 1152×870 pixels and a refresh rate of 76 Hz. The monitor was placed 19 cm in front of the subjects' eyes, for a visual angle of 58×53°. For a given color/frequency combination, the subject was presented with a stimulus loop composed of five trials; each trial followed the sequence of 6 s of stable control color, followed by 6 s of full-screen flicker, followed by 6 s of control color. The control color for a given condition was a fusion between the color components of flicker: for instance, for red-blue flicker, the control color was purple. For luminance-modulated flicker such as red-black flicker, the control color was the same as the flickering color, although lower in luminance so that the time-averaged luminance of flickering and non-flickering stimuli were both 3.6 cd/m<sup>2</sup>. All stimuli were generated in MATLAB (MathWorks, Natick, Mass., USA), using the extensions provided by the high-level Psychophysics Toolbox (Brainard 1997) and low-level VideoToolbox (Pelli 1997).

Flicker stimuli were presented at three frequencies: 3.16, 6.33, and 12.6 Hz, corresponding to a color change every 12, 6, and 3 frames, respectively. At each frequency, eight different color combinations were used, grouped into two categories: hue-modulated

(color-color) flicker, which included red-blue, blue-green, red-green, and blue-yellow flicker; and luminance-modulated (color-black) flicker, which included red-black, blue-black, green-black, and white-black flicker.

Two experiments are reported here. In the first experiment, the flicker was of equal physical luminance to the preceding solid color: both stimuli had a time-averaged luminance of 3.6 cd/m<sup>2</sup>. For hue-modulated flicker (e.g., red-blue flicker), this meant that both components of flicker (e.g., red and blue) as well as the stable fusion color (e.g., purple) were at 3.6 cd/m<sup>2</sup>. For luminance-modulated flicker, the stable fusion color was at 3.6 cd/m<sup>2</sup>, while the flicker components alternated between <0.1 cd/m<sup>2</sup> for black, and ~7.5 cd/m<sup>2</sup> for the color. Thus, control colors and flicker had the same time-averaged luminance.

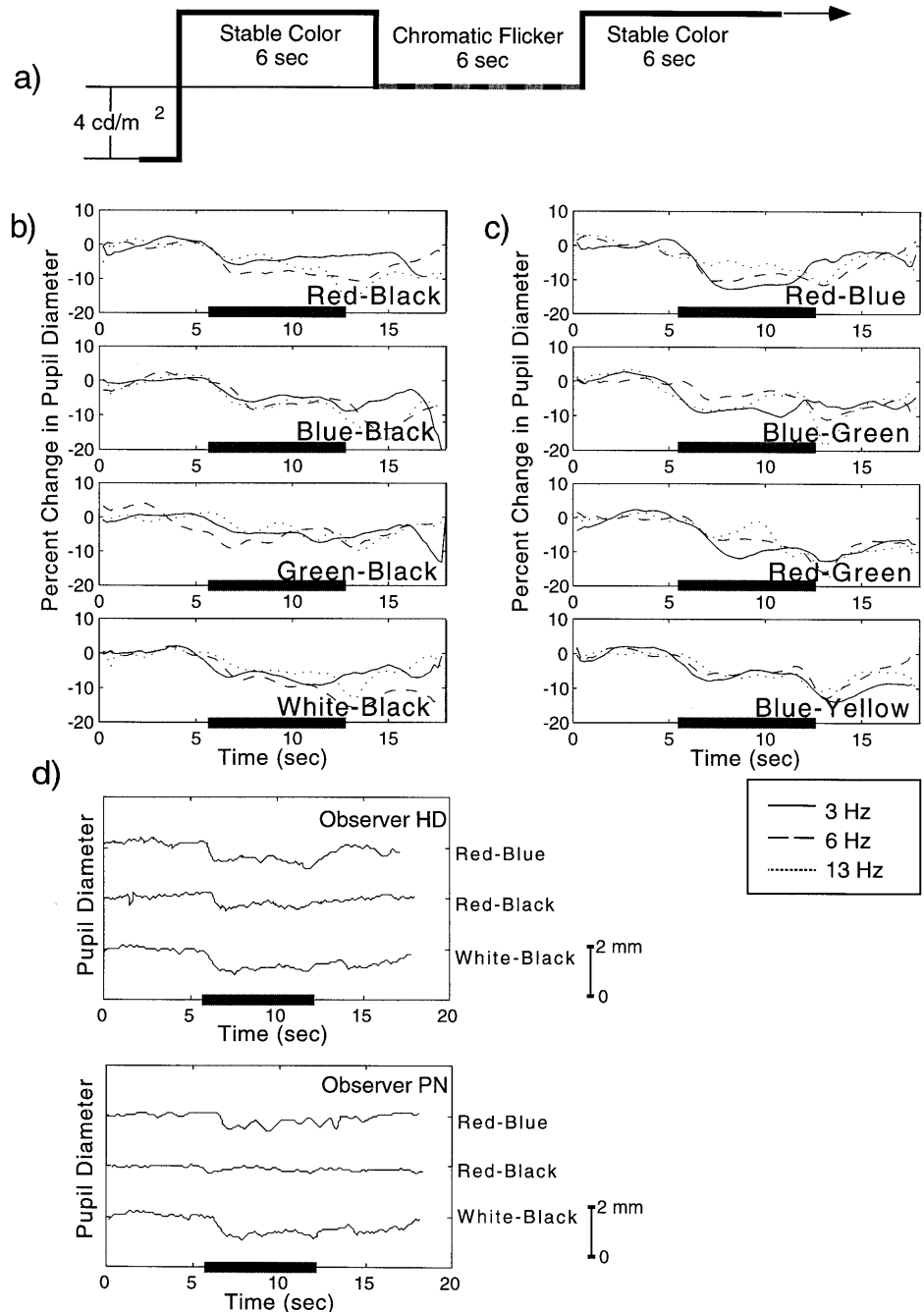
In the second experiment, the flicker components were perceptually equiluminant as determined by a minimum-motion criterion (Antsis and Cavanaugh 1983), relative to a red [(x, y, Y)=(0.58, 0.35, 3.6)]. In order to address possible concerns that small luminance differences between colors might be a possible factor mediating pupillary response, the flicker was also of a lower physical and perceptual luminance than the stable control color, which both preceded and followed it. Specifically, the stable color was the combination of the RGB values in a software color table of the flicker components: for instance, for red-blue flicker, red [(x, y, Y)=(0.58, 0.35, 3.6)] and blue [for one subject, (x, y, Y)=(0.15, 0.08, 3.7)] combined to generate a purple [(x, y, Y)=(0.24, 0.13, 7.3)] approximately twice the luminance of either the red or blue. For luminance-modulated flicker, the stable color was identical to the non-black color of the flicker.

### Procedure

Pupil sizes were recorded by video pupillometry techniques. The display was controlled by a Power Macintosh 7300/200, and the subjects were instructed to observe it passively. Head movements were constrained during experiments by the use of a chinrest. During experiments, the left eye was illuminated by the monitor, while the right eye was illuminated through the use of an infrared (IR) light-emitting diode (LED) and shielded from the display by a mirror. This mirror was oriented 45° to the monitor, and used to reflect the image of the eye into an IR CCD camera, which input into a digital video recorder, which also received timing information about stimulus onset and offset. During rest periods between tests, room lights were turned on in order to prevent rod dominance due to dark adaptation.

The diameter of the right-eye video was sampled off-line using the ViewPoint eye-tracking software (Arrington Research, Mesa,

**Fig. 3a–d** Pupillary responses for experiment 2. **a** Schematic diagram of experiment 2. **b**, **c** Average waveforms of pupil diameter for different color and frequency flickers in experiment 2, expressed as mean percent change (from control-period baseline) in diameter. *Solid line* Response at 3 Hz, *dashed line* at 6 Hz, *dotted line* at 13 Hz. *Gray bar* at bottom indicates flicker stimulus. **b** shows responses to luminance-modulated (color-black) flicker, **c** shows responses to hue-modulated (color-color) flicker. **d** Representative waveforms from different observers for 3-Hz flicker. The example waveforms for responses to red-black and white-black flicker have been displaced 2 mm successively downwards for clarity



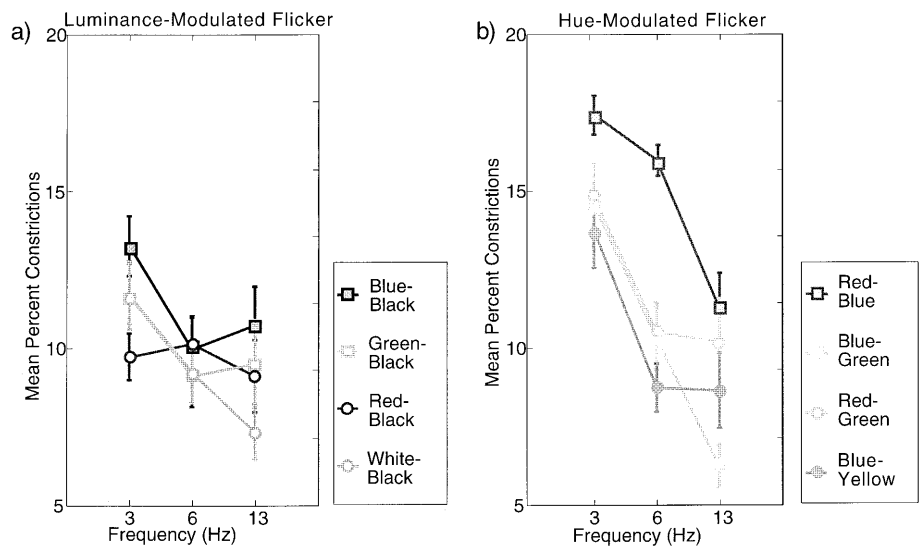
Az., USA) at a rate of 20 Hz. Size of constriction was defined as the difference between the minimum pupil diameter during the flicker period and the mean pupil diameter during the preceding control period, for each trial.

## Results

Experiment 1 investigated pupillary responses to flicker whose components were equal in physical luminance to each other as well as a preceding stable color. For all conditions, significant pupillary constrictions were found during flicker. Waveforms of the pupillary responses

obtained for experiment 1 are presented in Fig. 1. As can be seen by the average traces for each condition, the size of constriction depended on both color and frequency of the stimulus. The dependence of amplitude of constriction in relation to these parameters is plotted in Fig. 2. In general, size of constriction decreased with increasing frequency and also for hue-modulated as opposed to luminance-modulated flicker. Red-blue and blue-green flickers produced the strongest constrictions, with decreases in diameter of up to 38.3% and 52.8% and mean constrictions of 24.0% and 24.7%, respectively. A two-way ANOVA on size of constrictions revealed main

**Fig. 4a, b** Dependence of constriction size (expressed as percent change from control-period baseline) on color and frequency parameters for experiment 2. **a** Luminance-modulated flicker, **b** hue-modulated flicker. Error bars represent standard error of the mean



effects of color [ $F(7,35)=50.1$ ,  $P<0.01$ ] and frequency [ $F(2,10)=54.75$ ,  $P<0.01$ ], as well as an interaction between color and frequency [ $F(14,70)=4.81$ ,  $P<0.01$ ].

In order to test the possible effect of small luminance effects in the observed response, experiment 2 was performed, in which time-averaged luminance decreased with flicker onset. As with experiment 1, significant constriction responses were found for all conditions. Figure 3 shows sample waveforms obtained in this experiment, while Fig. 4 plots the color- and frequency-dependence of the strength of constriction. In this experiment, constrictions were generally smaller in amplitude than those in experiment 1, indicating that constrictions were weakened by a luminance-driven dilation response in experiment 2. As a notable exception, some trials at 13 Hz yielded dilation in response to flicker onset, which would be expected if luminance-driven channels dominated this response, since luminance is decreasing by nearly a factor of 2 with flicker onset. Most notably, the difference between red-blue and other colors in efficacy at generating responses became striking. Constrictions by red-blue were significantly larger than any other color flicker (one-tailed  $t$ -test:  $P<0.02$ ) at 3 and 6 Hz, though not at 13 Hz. A two-way ANOVA on size of constrictions again revealed main effects of color [ $F(7,35)=17.9$ ,  $P<0.01$ ] and frequency [ $F(2,10)=64.0$ ,  $P<0.01$ ], as well as an interaction between the two [ $F(14,70)=4.67$ ,  $P<0.01$ ]<sup>1</sup>.

Additional analysis of the duration of constriction and likelihood in a given trial of a significant constriction showed the same trends as those for amplitude for both experiments. Duration increased with increasing size of constriction, as did the likelihood of a significant constriction [defined as the pupil diameter during the

flicker period being significantly smaller than during the preceding control period by a one-tailed  $t$ -test ( $P<0.05$ )] on a given trial (data not shown).

## Discussion

This study found a pupillary constriction in response to chromatic flickering stimuli. This response existed whether the components of the flicker were perceptually equiluminant or of an equal physical luminance, and the response persisted even when the flicker was of a lower luminance than a preceding solid color. The amplitude of pupillary response depended on both color and temporal frequency parameters. For the color dependence, hue-modulated (color-color) flicker, particularly red-blue and blue-green flicker, consistently produced stronger constrictions than luminance-modulated (color-black) flicker. For frequency dependence, lower frequency flicker generally produced more powerful constrictions, although the color-dependence of flicker was most visible between 3 and 6 Hz.

These results are significant in at least two respects. First, the pupillary constriction appears to be due specifically to the dynamic change in chromaticity of the display, rather than the luminance. The color-dependence strongly suggests an interaction between color and temporal dynamics in producing a response, supporting the notion of more complex control of pupillary responses. Second, the results may have some implication to the condition of photosensitivity, due to overlap between parameters that cause stronger constrictions in normal observers and those which preferentially induce photoparoxysmal response in photosensitive patients.

### Interaction between color and temporal dynamics

In regards to the first point mentioned above, luminance does not seem to be an active factor in the constriction

<sup>1</sup> Worth noting is the presence of relatively large constrictions at the end of flicker periods; this likely represents transients corresponding to the luminance increment at the end of flicker periods, but may also represent a potential OFF transient to the response (Kimura and Young 1995). Further analysis is necessary to determine if this is the case.

responses reported here, for several reasons. In the first experiment, when the stimulus was always at the same physical luminance throughout, luminance can be excluded as a cause of constriction, since it never varied for hue-modulated flicker and the mean temporal luminance does not change for luminance-modulated flickers. Further, it can be argued that small perceptual differences between physically equiluminant colors were also not responsible for this constriction. Consider that, if small differences in the perceived brightness of the color components of flicker caused a response like the one seen, then color-black flicker, which has much larger alternations in perceived brightness during flicker than color-color flicker, would be expected to be a much more powerful stimulus. Instead, color-black flicker produced constrictions of a significantly smaller amplitude than color-color flicker.

Moreover, pupil constrictions were found in the second experiment, when the luminance of the flickering stimulus was always less than that of the nonflickering stimulus. If luminance was governing this response, dilation would be expected; and in fact dilation was found for some conditions at 13 Hz. However, for some trials in all conditions, and especially at lower temporal frequencies, constrictions and not dilations were found.

It is interesting to note, however, that the responses to both color-black and color-color flickers were much reduced in the second experiment, most likely resulting from an additional luminance-driven response tending towards dilation. This suggests that both of these flickers are at least partially mediated by the same mechanism and suggests that the dynamic nature of the display is a critical factor. However, the temporal modulation is not the only critical factor to constriction, since color-color flickers produce such stronger responses than color-black flickers. The interaction between changes in chromaticity and temporal modulation seems to be necessary for the strongest responses.

It is this unique interaction that is not suggested by previous investigations on the pupillary response. The pupillary response to single changes in chromaticity of a stimulus has been well-documented by Barbur and others (1992, 1998), and the pupillary response to single flashes has been examined as a function of wavelength (Kimura and Young 1995). It might be expected that the mechanisms responsible for these constrictions could cause transient constrictions for single changes (essentially at very low frequencies) while producing sustained responses at higher frequencies. Indeed, the inverse relationship between a flicker's temporal frequency and its power in producing constrictions may be consistent with this. However, this would not account for the fact that juxtaposed colors can produce stronger responses than color-black flickers of the same time-averaged luminance.

#### Relation to photosensitivity

The unusual color-dependence of the responses reported here suggests a possible link between these responses

and the condition of photosensitivity. Photosensitivity is defined as the occurrence (on an EEG) of spikes or spikes and waves in response to intermittent light stimulation (Doose and Waltz 1993). In most photosensitive subjects, both frequency and color play an important role in the likelihood of seizures. Takahashi and Tsukahara (1976) found that red-black flicker between 10–20 Hz, particularly at 15 Hz, is the most common color-frequency combination to which photosensitive patients are sensitive, other colors having much weaker effects. Others have reported that frequencies up to 25, 50, and even 84 Hz are effective for some patients (Wilkins et al. 1978; Jeavons and Harding 1975).

Until recently, however, hue-modulated flicker has not been intensively investigated as a seizure-inducing stimulus. This is partially because the equipment used in clinical settings to generate intermittent photic stimulation cannot easily be made to generate color-color flicker. Yet the most notorious case of photosensitivity in recent memory was generated by red-blue flicker, when in 1997 over 700 children in Japan were hospitalized after experiencing seizures while viewing an episode of the cartoon "Pocket Monsters" (Ishida et al. 1998). The stimulus which provoked this incident was a red-blue flicker of about 15 Hz with a jittering pattern and was generally viewed on televisions which had a further interlaced pattern and a 60-Hz refresh rate.

It has been known for some time that flickering stimuli can induce rhythmic activity in the brain (Walter and Walter 1949; Ranger and Singer 1998). The differential pupillary response to different color and frequency flickers may reflect differential ability at inducing rhythmic activity for these stimuli. If this is the case, then the pupil response may reflect the action of a cortical gain-control mechanism. Recently, Porciatti et al. (2000) examined visual-evoked potential (VEP) responses to temporally modulated luminance gratings in photosensitive and non-photosensitive subjects. The responses showed a number of differences between groups, including an apparent absence of amplitude saturation with increasing contrast and overall higher VEP contrast in photosensitive subjects. This evidence strongly indicates a contrast gain-control mechanism in non-photosensitive subjects, the lack of which may underlie photosensitivity. Although differences in VEP response to chromatic red-green gratings were not found to be significant, the study did not examine flickers including blue and another color, which may be more important, since red-blue and blue-green flickers were found to be most powerful at producing pupil constrictions, and the former was the causal stimulus in the 1997 incident.

The pupillary response to a dynamic stimulus such as flicker may be a defensive response. Since we know these stimuli can generate rhythmic activity in the brain to the point of seizure generation, there is a known risk to this stimulus; the pupil's constriction may reduce exposure to that risky stimulus in non-photosensitive observers. It has been shown that brighter flickers have a greater power at inducing seizures (Takahashi and

Tsukahara 1976, 1998), and presumably greater retinal illuminance from a flicker only serves to make the stimulus more dangerous. Constrictions reported here can range up to 50% of the pupil diameter, which reduces the overall illuminance to 25% of its original value; this may well be enough to keep a stimulus out of the range where it is strong enough to induce seizure, and it may be some abnormality of this mechanism in the patients that feedforwards to seizure generation.

Our results also have a more practical implication. In some studies, the pupils of photosensitive patients were pharmacologically dilated before EEG recordings were made during a visual stimuli. If the pupillary mechanism is involved in this condition, findings that are made with the pupil-dilation procedure may not be applicable to seizures occurring in a non-laboratory setting, such as ordinary viewing of a television. Further examination of the pupillary response, as well as other measures of activity (EEG, MEG, fMRI) in photosensitive patients could reveal the exact mechanism and relation between flicker-induced pupillary constriction and photosensitive seizures.

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