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Neuro-Ophthalmology

The Trade-Off Between Luminance and Color Contrast Assessed With Pupil Responses

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Purpose: A scene consisting of a white stimulus on a black background incorporates strong luminance contrast. When both stimulus and background receive different colors, luminance contrast decreases but color contrast increases. Here, we sought to characterize the pattern of stimulus salience across varying trade-offs of color and luminance contrasts by using the pupil light response.

Methods: Three experiments were conducted with 17, 16, and 17 healthy adults. For all experiments, a flickering stimulus (2 Hz; alternating color to black) was presented superimposed on a background with a complementary color to the stimulus (i.e., opponency colors in human color perception: blue and yellow for Experiment 1, red and green for Experiment 2, and equiluminant red and green for Experiment 3). Background luminance varied between 0% and 45% to trade off luminance and color contrast with the stimulus. By comparing the locus of the optimal trade-off between color and luminance across different color axes, we explored the generality of the trade-off.

Results: The strongest pupil responses were found when a substantial amount of color contrast was present (at the expense of luminance contrast). Pupil response amplitudes increased by 15% to 30% after the addition of color contrast. An optimal pupillary responsiveness was reached at a background luminance setting of 20% to 35% color contrast across several color axes.

Conclusions: These findings suggest that a substantial component of pupil light responses incorporates color processing. More sensitive pupil responses and more salient stimulus designs can be achieved by adding subtle levels of color contrast between stimulus and background.

Translational Relevance: More robust pupil responses will enhance tests of the visual field with pupil perimetry.

Introduction

The pupil light response (PLR) is often considered to be a simple subcortical reflex arc. Its neural pathway presumably consists of photoreceptors; bipolar and retinal ganglion cells, with their axons forming the optic nerve; intercalated neurons in the midbrain; the oculomotor nerve; and short ciliary nerves innervating the pupillary sphincter muscle. The activation of this pathway through a bright stimulus onset results in a pupil constriction in response to an increase in retinal illumination.[1–5] However, recent developments suggest that in addition to objective, physical retinal illumination, such pupil responses depend on multiple factors beyond light levels, including the degree to which a stimulus is salient and draws attention.[5] These pupil orienting responses do not seem to be dependent on sensory modality[6–8] and are enhanced by multisensory presentation.[9–11] Moreover, the speed and amplitude of pupil responses scale with stimulus salience.[7,12,13] This novel view explains why subjectively perceived brightness and the degree of awareness for the presented stimulus rather than its physical properties determine pupil response amplitudes.[4,14–17] Furthermore, experience with stimulus content,[18] the degree and locus of attention,[19–25] and visual sensitivity[4,25–29] all shape pupil responses. In summary,

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a pupillary response amplitude reflects how well a stimulus draws attention and is processed. In line with this, the pupil responds not only to brightness but also to other stimulus properties such as luminance contrast,[30] spatial frequency,[31] numerosity,[32] and color hue.[33–36] Two distinct pathways process stimulus color and luminance. Through retinal ganglion cells (RGCs), short-, medium-, and long-wavelength–sensitive photoreceptors provide input to the parvocellular (P) pathway, which is most sensitive to chromatic features, and the more luminance-driven magnocellular (M) pathway.[37–41] Nonetheless, these features must interact somewhere in the hierarchy of visual processing to create a coherent percept. Although these features have been studied in isolation, it remains unclear how they may interact and affect pupil responses together. A pupil response thus likely incorporates a multitude of distinct though additive pupil responses.[6,9,10,42,43] Here, we focus on investigating to what degree pupil size changes incorporate responses to both luminance and color contrast between stimulus and background. How these two stimulus features interact with respect to saliency is not trivial, as each form of contrast may increase separately, though only at the expense of the other.

Effects of the presentation of chromatic stimuli on pupil size have been tested in the context of visual field sensitivity assessment (i.e., pupil perimetry[1,24,44–47]). As the colors blue and yellow and the colors red and green are complementary, opponency colors for the human visual system—as modeled by the CIELAB color space, a three-dimensional color space defined by the International Commission on Illumination that covers the entire gamut of human color perception (see Fig. 1A)—they perfectly lend themselves to investigate the effect of color contrast on the pupil response. The main aim of this study was to investigate whether the addition of color contrast to luminance contrast between a stimulus and the background evokes a stronger pupil response. However, adding color contrast to a scene always comes at the expense of luminance contrast. To explain this more clearly, imagine a white stimulus on a black background. In this case, the stimulus has 100% luminance contrast with its background. By adding color to the stimulus, some degree of color contrast with the background is added but luminance contrast decreases (e.g., a yellow stimulus is not as bright as a white stimulus). Color contrast can be enhanced even more by adding a complementary, opponency color to the background, again at the expense of luminance contrast (e.g., a blue background is not as dark as a black background); for a visualization of the interaction between color and luminance contrasts, see Figures 1B and 1C. The question posed here is where the optimal balance between luminance and color contrast may be found across the color space. This may depend, first, on the shape and curvature of color space representations and, second, to which degree luminance contrast is preferred over color contrast by the visual and

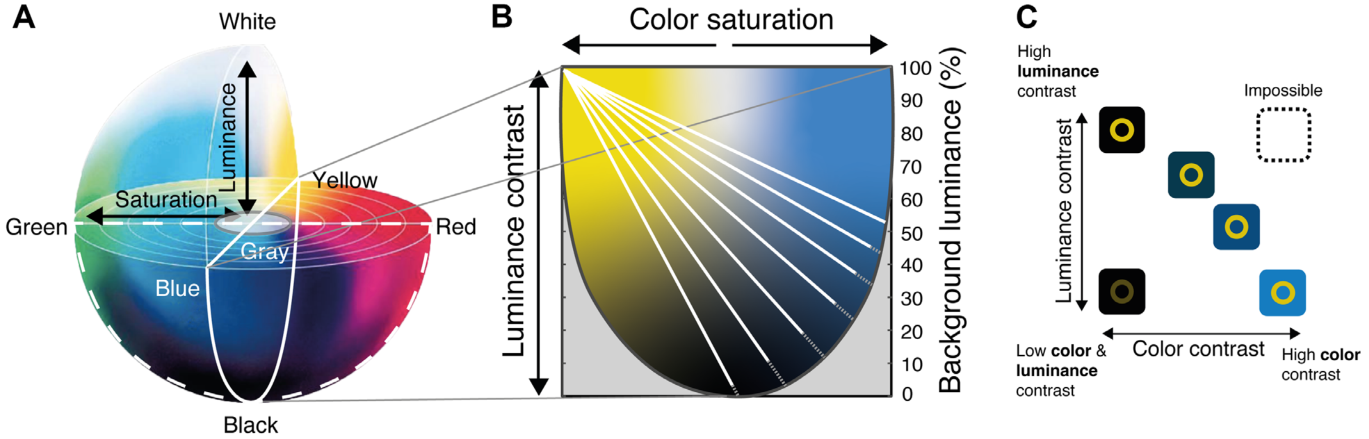

Figure 1. (A) Spherical CIELAB color space modeling color distances to reflect how well colors (and accompanying luminance levels) can be discerned from each other (i.e., color contrasts) in human color perception. Horizontal distance from the center (*gray*) corresponds to color contrast, vertical distance from black to white corresponds to luminance. The *white outline* connecting the color blue with complementary yellow (Experiment 1) and the *white dashed outline* connecting red with complementary red (Experiments 2 and 3) highlight the color spaces targeted in this study. (B) An intersection plane of the sphere shows equal distances (*white lines*) between stimulus (*yellow*) and background (*black* to *blue*) per color contrast level (0%–45%). Depending on the yet unknown dimensions of color space and the pupil's sensitivity to luminance contrast (vertical axis) with respect to color contrast (horizontal axis), the most salient appearance, and thus strongest pupil response (i.e., where the overshoot of *white lines* [*dotted gray*] is longest) may be evoked by using one of several possible background color contrast levels ranging from high luminance contrast to high color contrast. (C) A fully luminant yellow stimulus is offset to variations of the background with the complementary color blue, varying from high luminance contrast when the background has low brightness to high color contrast when the background displays high brightness.

pupillary system. In this study, we aimed to improve pupillary measures of visual field sensitivity by manipulating luminance and color contrast between stimulus and background to find the most optimal pupil response.

In summary, this study aimed to explore to what degree the pupil responds to color contrast with respect to luminance contrast and to investigate whether this response generalizes across directions in color space.

Methods

Participants

The participants of the three experiments consisted of 17 (11 females), 16 (10 females), and 17 (11 females) healthy Dutch students and staff with Caucasian ethnicity (mean age ± SD, 22.8 ± 3.5 years, 22.9 ± 2.6 years, and 23.2 ± 4.2 years, respectively). Subjects were screened for color blindness using the Ishihara test[48] and had normal or corrected-to-normal visual acuity. Furthermore, we verbally inquired about the presence of visual or neurological disorders; none of the subjects reported having any. All experiments were approved by the local ethical committee of Utrecht University (approval number FETC19-006) and conformed to the tenets of the Declaration of Helsinki. Participants gave written informed consent prior to participation. Furthermore, they received financial reimbursement for participation (€8 per hour).

Apparatus and Stimuli

All experiments were conducted in a darkened room without ambient light. Stimuli were generated on a Dell desktop computer (Dell Technologies, Round Rock, TX) with the Windows 7 operating system (Microsoft, Redmond, WA), using MATLAB (MathWorks, Natick, MA) and the Psychtoolbox 3 and EyeLink toolbox extensions.[49–52] We used a 143 × 63-cm LG OLED65B8PLA monitor (LG Electronics, Seoul, South Korea) with a resolution of 1920 × 1080 and a refresh rate of 60 Hz to display stimuli. Pupil size and gaze angle of the right eye were tracked with an EyeLink 1000 eye tracker (SR Research, Ottawa, ON, Canada; 0.5° accuracy of gaze angle) connected to a separate Dell desktop computer with the Windows 7 operating system, which recorded the right eye from above through a hot (infrared-reflecting) mirror (tower mount). We used the EyeLink toolbox extension for the Psychtoolbox[50] on the presentation computer to communicate and synchronize stimulus presentations with the pupil size recordings on the eye-tracking computer. Start and stop triggers and stimulus presentation messages were sent from the presentation computer to the eye-tracking computer by means of an Ethernet cable with negligible latency (for more details, see the SR Research manual). A participant's head and viewing distance were fixed using a forehead and chin rest at a 75-cm distance from the monitor. A schematic of the used apparatus is shown in Figure 2D. The eye-tracker calibration procedure consisted of a five-point grid and took ~1 minute. The EyeLink tracker software outputs pupil size in arbitrary units rather than absolute pupil diameter in millimeters, and we refrained from converting the units as the current study was only concerned with within-subject comparisons.

Experiment 1: Background Color Contrast Yellow/Blue

The stimuli consisted of yellow or blue annuli with 100% luminance (at 141 and 143 cd/m^2, respectively; see Supplementary Table S1), each presented at one of five possible eccentricities (see Fig. 2A). The width of each annulus was increased as a function of eccentricity using a cortical magnification factor (radial width = eccentricity[1,12] in degrees) to activate approximately equal numbers of neurons by both central and peripheral stimuli (e.g., see Ref. 53). Stimuli flickered between colored (i.e., blue or yellow) and black annuli at 2 Hz for 5 seconds per trial, and a red fixation point was placed at the center of the presentation monitor. A flicker paradigm was used, as it is known to produce oscillatory PLRs with amplitudes reflecting the degree to which a stimulus onset is visually processed (i.e., stimulus salience[25,26,28]). The stimuli were superimposed on a complementary colored background (i.e., opposite colors in CIELAB color space) (Fig. 2), which varied in background color contrast depending on the trial condition (0%–45% with intervals of 5% in random order to minimize effects of time; see Supplementary Table S1). Each unique combination of eccentricities (five), colors (two), and color contrast (10) was tested once (100 trials).

Experiments 2 and 3: Background Color Contrast Red/Green

Experiments 2 and 3 were identical to Experiment 1 aside from the different complementary color pairs used: green and red annuli flickered between colored and black (Fig. 2c). The 100% luminant red and green colored annuli used in Experiment 2 differed significantly in brightness (52 and 171 cd/m^2, respectively; see Supplementary Table S1) because of the OLED screen properties and the infrared hot mirror, and Experiment 3 used green annuli with 55% luminance to achieve

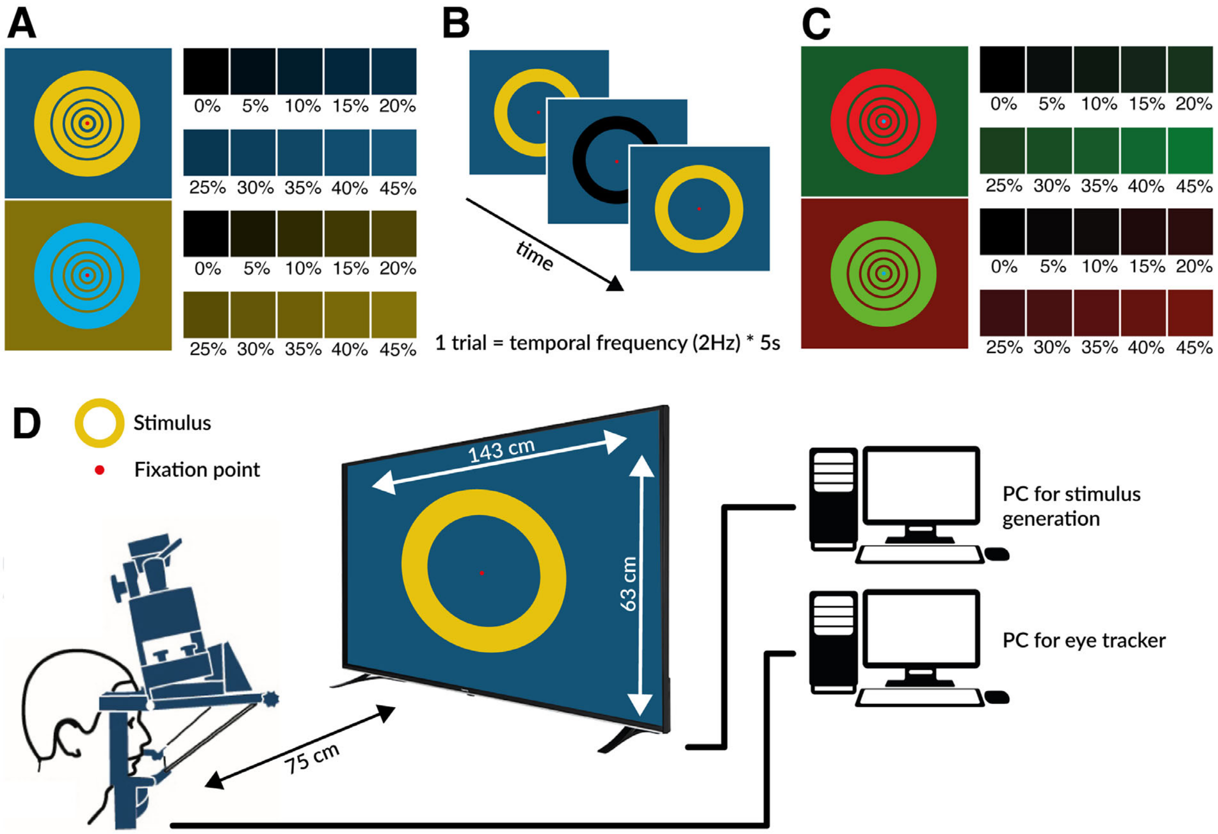

Figure 2. (A) Stimulus color and background color conditions for Experiment 1. The *upper left* panel shows five yellow stimulus locations across different eccentricities (4°, 8°, 12°, 17°, and 23° from the fixation point) superimposed on a blue background. Note that the pictures are cropped for aesthetic reasons; the background actually extended farther to the left and right to fill the entire monitor screen with a 16:9 aspect ratio. The smaller panels adjacent to the *left panel* pertain to the different background color contrast conditions (0%–45%) upon which the stimuli were superimposed. The lower panels show the blue colored stimuli and their complementary yellow background color contrasts. The experiment consisted of 100 trials (5 stimulus rings × 2 stimulus colors × 10 background color contrasts). (B) An example of a trial procedure. One of the five annuli flickered from color to black for 5 seconds at a 2-Hz frequency around a red fixation point. This was repeated for all background color contrast conditions, locations, and stimulus colors in random order. (C) The procedures and stimulus dimensions for Experiments 2 and 3 were equal to those for Experiment 1, but Experiment 3 used red stimuli superimposed on a green background (*upper panel*) and vice versa (*lower panel*). (D) Schematic arrangement used for the pupil measurements in a darkened room. Each participant's head was fixed in a forehead and chin rest under the tower-mounted eye tracker which was positioned in front of the presentation monitor. The presentation monitor and eye tracker were connected to separate computers.

physical equiluminance with the 100% luminant red stimuli. Each unique combination of eccentricities (five), colors (two), and color contrast (10) was tested once (100 trials) for each experiment.

Modeled Weights of Color Contrast in Relation to Luminance Contrast

We modeled the relative degree of luminance and color contrast between stimulus and background per variation of the background color contrasts conditions (see Fig. 3). The weight of color contrast in relation to luminance contrast was varied across models, basically simulating size changes in the horizontal dimension of color contrast in Figure 1B. By varying this weight, the distance in color space between stimulus and background (white lines in Fig. 1B) changed, with an optimal distance at varying background color contrasts. In doing so, the relative contribution (i.e., weight) of color contrast to pupillary responses (relative to luminance contrast) could be determined after the experimental search for the optimum.

Procedure

Participants were instructed to continuously gaze at the red fixation point in the middle of the screen. We additionally instructed participants to covertly attend the flickering stimuli, each presented in a gaze-contingent manner, to evoke strong pupil responses.[22,25] Participants were tested at varying times

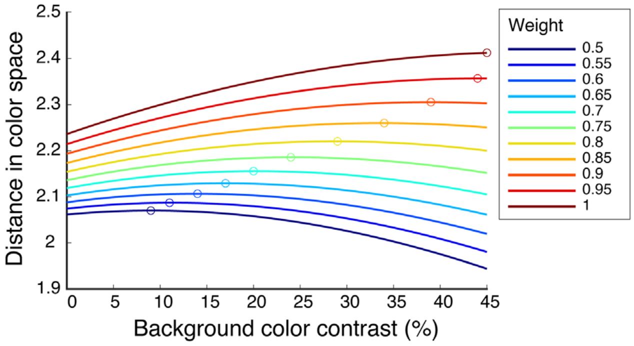

Figure 3. Modeled distance in color space (i.e., *white lines* in Fig. 1B) per background color contrast (0%–45%). Each colored line represents a different weight (range, 0.5–1) assigned to the color contrast space relative to luminance contrast space between background and stimulus. A weight of 0.5 means that the horizontal width of the color space as displayed in Figure 1B, representing visual sensitivities to color contrasts, is equal to the height of the color space, representing visual sensitivities to luminance contrasts. A weight of 1.0 means that the width of the color space in Figure 1b scales by a factor of two, modeling color contrast as sensitive as luminance contrast. This weight determines at what color brightness the background contrasts optimally with the stimulus color (i.e., the largest distance in the modeled color space; see *white/gray lines* in Fig. 1B). *Circles* on the colored traces highlight the optimal background color (and luminance) contrast with the stimulus (i.e., where the *dotted gray line* is longest in Fig. 1B).

of the day. Only the right eye was recorded; the left eye was patched with an adhesive eye patch. Eye-tracker recalibrations were performed whenever participants indicated they wanted to take a break. Each experiment lasted 500 seconds (5 stimulus locations × 5 seconds per location × 10 background color contrast conditions × 2 stimulus colors), excluding (re)calibration and breaks.

Analysis

The continuous pupil recordings were analyzed in an event-related manner with the first stimulus onset per new location as start events. Blinks were detected and filtered using a speed threshold of 4 SD above the mean. The detected blink periods shorter than 600 ms were interpolated with a cubic method (interp1 MATLAB function). Trials with less than 80% pupil data were removed from the analysis. To filter out low-frequency noise, we subtracted pupil traces filtered with a second-order Butterworth filter with a 1-Hz cutoff frequency (i.e., we applied a high-pass filter), which produced baseline corrected traces showing pupillary oscillation patterns around zero. This is a necessity for proper frequency analyses. After this baseline correction, we removed high-frequency noise by filtering the high-pass filtered pupil traces with a fifth-order Butterworth filter with a 15-Hz cutoff frequency. Pupil traces were filtered per event (i.e., per stimulus location) and saved in a matrix with each row presenting a trial and each column representing a time point. Pupil traces were converted to power spectral density estimates in the frequency domain by computing a Lomb–Scargle periodogram using a fast Fourier transform for each trial. This power measurement reflects the amplitude in the pupillary oscillation patterns evoked by a stimulus. For simplification, we refer to this measurement as the pupil response amplitude from now on. These pupil response amplitudes served as the main dependent variable.[25,26,28,29] To determine the statistical significance of differences in pupil amplitudes across background color contrast conditions and stimulus color conditions, we performed a two-way repeated-measures analysis of variance (ANOVA). Paired double-sided *t*-tests were performed to test for differences in pupil amplitudes across conditions as a post hoc test. Experiment data and analysis files are available on https://osf.io/yzavk.

Results

Experiment 1 (Yellow/Blue): Results and Discussion

In Experiment 1, we set out to explore which background color contrast condition produced the strongest pupil responses. First, we inspected whether the pupil properly responded to the 2-Hz flickering. As shown in Figure 4A, this prerequisite was met. The average pupil traces consisted of a 2-Hz oscillatory pattern reflecting the responses to stimulus on and off sets. Next, we investigated whether pupil response amplitudes differed across background color contrast conditions within each chromatic stimulus color variant. The lines in Figure 4B depict the participant group averages of pupil response amplitudes across background color contrast conditions per stimulus variant. A two-way repeated-measures ANOVA revealed a significant main effect of color contrast ($F_{9,19} = 6.15$, $P < 0.001$, partial $\eta^2 = 0.28$), but not for stimulus color ($F_{1,19} = 3.84$, $P = 0.07$, partial $\eta^2 = 0.19$). There was no interaction between the two main effects. This means that the pupil responses did not differ significantly between yellow and blue backgrounds but did differ across background color contrast. The strongest pupil responses were found within the range of 25% to 35% background color contrast (see Supplementary Tables S2 and S3 for post hoc comparisons). Pupil responses were 15% to 20% lower for 0% background color contrast. To

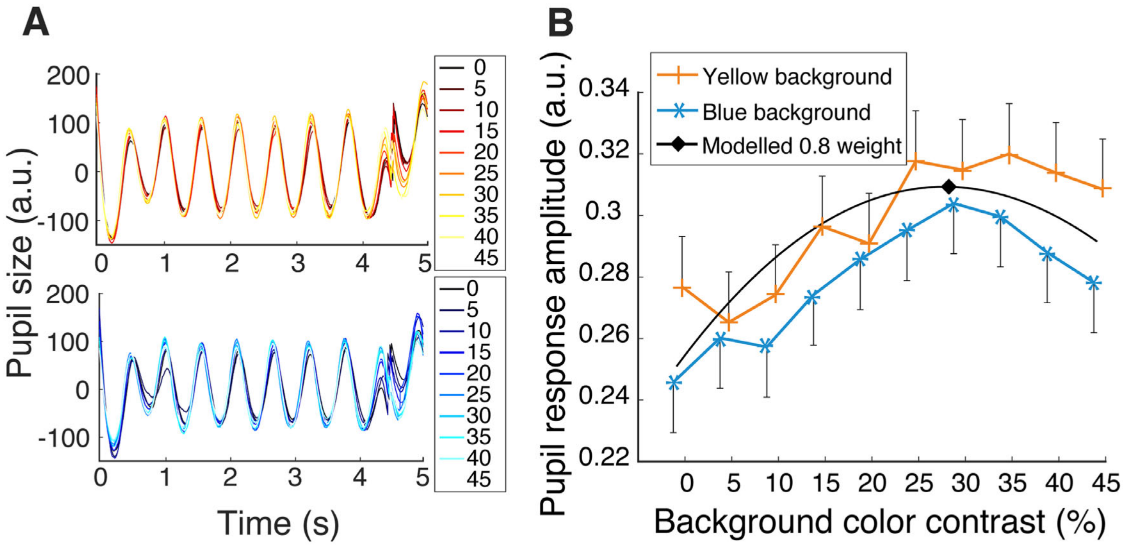

Figure 4. (A) Average pupil traces for yellow (*upper panel*) and blue (*lower panel*) background colors across participants. Each trace represents a different background color contrast (0%–45%). Pupil responses followed roughly the same oscillatory pattern of 2 Hz across experiment conditions. (B) Average pupil response amplitudes per background color contrast with standard errors around the mean. An optimal pupil response for both colors is found between 25% and 35% background color contrast. This optimum corresponded with an 80% relative contribution (i.e., weight) of color contrast to pupillary responses relative to luminance contrast (black trace with optimum ~30% background color contrast). Note that all pupil sizes are outputted in arbitrary units rather than absolute millimeters due to the EyeLink tracker software.

conclude, a background color contrast of approximately 30% evoked the strongest pupil responses for both color background variants. Figure 3 represents the modeled distance in color space between stimulus and background as a function of background color contrast per color contrast weight in relation to luminance contrast. A weight of 0.8 (i.e., 80%) corresponded to an optimal distance (and pupil response amplitude) at 30% background color contrast.

Experiment 2 (Red/Green): Results and Discussion

The goal in Experiments 2 and 3 was to investigate whether the results found in Experiment 1 would generalize across different directions within the color space. First, similar to the previous experiment, the pupil showed appropriate responses to the 2-Hz flickering stimulus on and off sets (Figs. 5A, 5B). Next, differences between pupil response amplitudes across all background color contrasts within the different chromatic stimulus variants were explored. Figures 5A and 5B show the pupil response amplitudes per background color contrast and stimulus color condition averaged across trials and participants. As in Experiment 1, a two-way repeated-measures ANOVA revealed a significant main effect of background color contrast ($F_{9,19} = 6.02$, $P < 0.001$, partial $\eta^2 = 0.29$). Not surprisingly, Experiment 2 also resulted in a significant main effect for stimulus color ($F_{1,19} = 28.59$, $P < 0.001$, partial $\eta^2 = 0.31$) because brightness differed substantially between the red and green flickering stimulus (see Supplementary Table S1 for the CIE color coordinates). There were no interactions between the two main effects. This means that the pupil responses significantly differed across background color contrasts. Similar to Experiment 1, pupil responses were weakest (15%–30% lower compared to optimum) when no color contrast was present (i.e., background was black; see Supplementary Tables S4 and S5 for post hoc comparisons) but got stronger as color contrast increased. For the green background with red stimulus condition, pupil responses significantly weakened when color contrast was increased beyond an optimum of approximately 20%, whereas pupil responses weakened significantly for the red background with green stimulus condition beyond the optimum of 40% (see Supplementary Tables S4 and S5).

Experiment 3 (Equiluminant Red/Green): Results and Discussion

Like the two previous experiments, the two-way repeated-measures ANOVA in Experiment 3 showed a significant main effect of background color contrast ($F_{9,19} = 4.50$, $P < 0.001$, partial $\eta^2 = 0.11$). In contrast

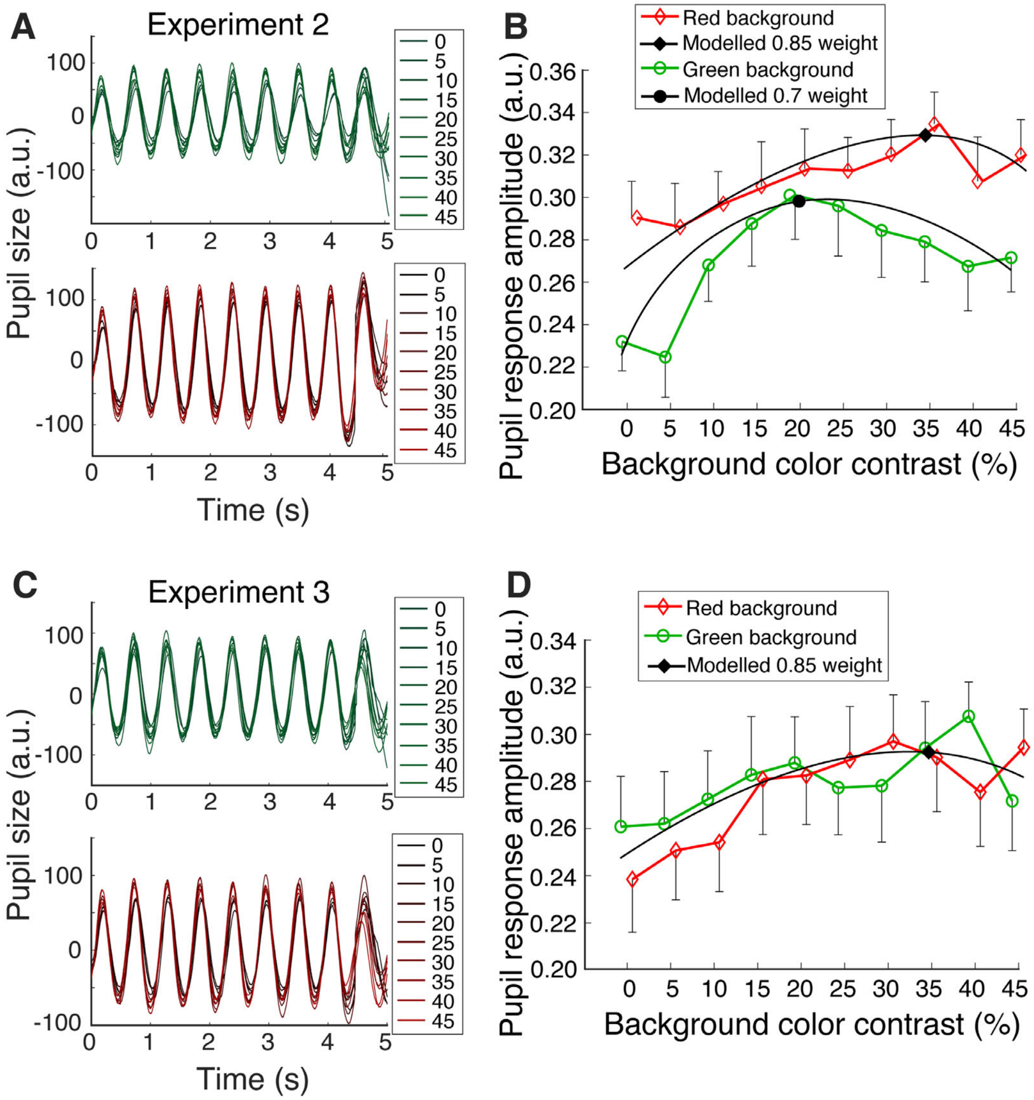

Figure 5. Same as Figure 4 but now for Experiments 2 and 3, where red versus green background and stimulus colors were displayed. Note that the modeled weights of Experiment 2 are different due to a significant main effect of stimulus color (see Results section).

to Experiment 2, in Experiment 3 we succeeded in controlling for previous brightness differences between the red and green stimulus conditions (no significant main effect for stimulus color; $F_{1,19} = 0.28$, $P = 0.61$, partial $\eta^2 = 0.003$). Again, weakest pupil responses were recorded in the absence of color contrast (see Supplementary Tables S6 and S7) and gradually increased ~20% to find an optimum at around 30% to 40% background color contrast. The model portrayed in Figure 3 revealed a relative weight of approximately 0.85 (background color contrast in relation to luminance contrast) to find the optimal distance and pupil response amplitude at 35% background color contrast. Contrary to Experiment 2, no significantly weaker pupil responses were found beyond the optima for both conditions. To conclude, an additive color contrast component to luminance contrast results in stronger pupil responses with an occasional optimal response pattern at an intermediate color contrast level for a selective set of background and stimulus colors.

Discussion

The main objective of this study was to investigate the contribution of color contrast (relative to luminance contrast) to pupillary responses that reflect stimulus salience. We specifically aimed to explore whether adding color contrast between a stimulus and its background (at the expense of luminance contrast) enhanced pupil responses amplitudes to stimulus onsets.

Our findings support the notion that, in the context of enhancing pupil responses to stimuli, an optimal balance exists between luminance and color contrast (between background and stimulus); for all investigated color directions (i.e., yellow, blue, green, and red colors), pupil size changed the strongest when a substantial amount of color contrast was present (e.g., ∼30% background color contrast for blue and yellow). The improvement in such pupil orienting responses acquired through the addition of color contrast could originate from a combined effect of physiological, psychological, and neurological factors.[5,54–58] The pupil responses to chromatic stimuli presumably consist of multiple components. One such component consists of P and M pathways projecting to the lateral geniculate nucleus, whereas the tonic, wavelength-opponent RGCs that project to the P pathway are most sensitive to chromatic modulation and the phasic non-opponent RGCs projecting to the M pathway which supports luminance flicker detection.[37–41] Others stem from an interaction between cortical (frontal–parietal attention network) and subcortical (superior colliculus) processes involved in orienting responses that feed back to the more reflexive pupillary pathway.[5] As such, the degree the pupil constricts in response to a stimulus onset reflects how well the visual system processes features such as color. That the pupil showed utilization in assessing human sensitivity to varying features demonstrates this more clearly.[31,59] An example includes reproducing the contrast sensitivity function. The pupil showed peak responsiveness for gratings with three cycles per visual degree contrast variations.[60,61] This optimal response to spatial frequencies matches the contrast-sensitivity function, indicating that the pupil could be an objective measure of visual sensitivity (and visual acuity). Similar conclusions can be drawn from numerous pupil perimetry studies showing that pupil response amplitudes weaken when stimuli are presented in a scotoma or blind spot.[1,25,26,28,62–66] Our results suggest that the dynamics of human color perception and dimensions of color space representations may also be assessed accurately, objectively, and quickly by inspecting pupil responses to changes in color. The novel findings of this study could also positively impact several ophthalmological practices, such as (1) enhancing pupil perimetry's accuracy, (2) objectively mapping degrees of color blindness, and (3) improving the saliency of traffic lights or presentation slides.

The created models in which we systematically varied the weight of feature dimensions of luminance and color revealed that approximately a 0.7 to 0.85 weight ratio of color contrast with respect to luminance contrast best explains the variations in observed pupil response amplitudes across background color contrasts. This ratio is relatively high and means that, in the context of pupillary color representation of the visual system, the horizontal color dimension in Figure 1B has an approximately 70% to 85% length of the vertical luminance dimension. Similarly, weaker though substantial contributions of chromatic contrast to the pupillary response were found in humans and the rhesus monkey.[34,67] These studies compared pupillary reactions to contrasting chromatic stimuli with spatially equivalent achromatic stimuli and found a ratio of ∼0.5 between pupil response amplitudes of chromatic versus nonchromatic stimuli.

An additive color contrast component to luminance contrast resulted in stronger pupil responses rather than simply luminance contrast (i.e., when background was black) in all experiments and thus across axes of the color space. Previous studies have taught us that the pupil responds to a multitude of contrast modalities, such as changes in luminance,[30] spatial frequency,[31] and color contrast.[31,33–36] Salient changes within these modalities evoke an orienting response consisting of a pupil constriction.[5] Our findings confirm that these pupil responses are enhanced by a multisensory presentation in an additive manner (in this case, color and luminance). For a selective set of color combinations, these responses even showed an optimal response pattern (i.e., the red stimulus with a 20% green background color contrast and green stimulus with 40% red background color contrast of Experiment 2) with significantly weaker responses at both extremes of the background color contrast range. These results confirm that some parts of the color space represented in the human visual system portray a curvature as displayed in Figures 1A and 1B. Although similar, an optimal response pattern was not found for the blue–yellow and equiluminant red–green axes. This could mean that color space is not represented by a sphere (Fig. 1A) with the same curvature for every color axis or that a larger range of background color contrasts (e.g., 0%–60%) is needed to find the optimum response pattern for other color combinations. Future studies may adapt the current pupillometry paradigm to explore a full range of contrasts and colors to test these possibilities.

The novel findings reported here will be of value to visual field testing through pupil perimetry, because the optimal balance between luminance and color contrast leads to stronger and thus likely more robust pupil responses. Another advantage lies in the use of colors to dissociate contributions of distinct pathways to pupil responses,[68,69] such as isolating melanopsin-directed responses.[69,70] Intrinsically photosensitive retinal ganglion cells (ipRGCs) receive input via bipolar cells through short-wavelength cone OFF and medium-wavelength and long-wavelength–sensitive cone and rod ON inputs.[71–74] Additionally, ipRGCs express the photopigment melanopsin (for which the action spectrum peaks at 482 nm and overlaps all three cone types), which renders them directly photosensitive.[75–77] Melanopsin cells mediate the PLR by projecting to the suprachiasmatic nucleus, intergeniculate leaflet, and pretectal olivary nuclei.[76,78,79] As rod/cone photoreceptors and melanopsin differ substantially in their response properties, light stimuli can be designed to preferentially assess their function in patients with retinal diseases.[33,80–84] Unfortunately, the approach used in this study was not feasible to specifically target distinct photoreceptor pathways, but it will be interesting for future research to explore how luminance and color processes in the retina interact and contribute to the optimal pupil sensitivities found here.

Subsequently, the repeated gaze-contingent flickering stimulus presentation reported in this study conveniently lends itself to making optimal use of the newfound optimal color contrast between stimulus and background. In future studies, the addition of not only temporal but also spatial color contrast (e.g., a red, green and yellow checker pattern within the stimulus) to the stimulus ON region might be of value.

This study has some limitations. First, as our study contains only results from healthy adults that did not undergo an ophthalmic examination, our findings cannot be extrapolated to patient populations with complete certainty. Moreover, because it was out of the scope of the current study, we did not compare a 100% luminance contrast (i.e., white vs. black) to the combined color and luminance contrast (i.e., the upper part of the color space in Fig. 1A has not yet been probed). It thus remains unclear whether adding color contrast to stimuli results in greater diagnostic accuracies in detecting visual field defects with the pupil. Future clinical studies might provide more clarity regarding the sensitivity of this.

To conclude, a pupil response to a stimulus contains multiple overlapping components: one component responding to changes in luminance and another additive component responding to changes in color. Stronger pupil responses can be achieved by combining color and luminance contrast between stimulus and background.

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