Mild Hypoxia Impairs Chromatic Sensitivity in the Mesopic Range

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Purpose. The effect of mild hypoxia on chromatic sensitivity in the mesopic range is poorly documented. This study was conducted to examine the effects of mild hypoxia and hyperoxia on red-green (R-G) and yellow-blue (Y-B) chromatic sensitivity thresholds at low photopic (22.3 cd · m^{-2}), borderline upper mesopic (1.67 cd · m^{-2}) and mid-mesopic (0.21 cd · m^{-2}) luminance.

Methods. The Color Assessment and Diagnosis (CAD) test was used to measure binocular and monocular R-G and Y-B chromatic sensitivity by using dynamic luminance contrast noise to isolate the use of color signals. Mild hypoxia was imposed by breathing 14.1% oxygen and was investigated relative to control exposures breathing air (normoxia) at each light level. Subsequently, hyperoxia, breathing 100% oxygen, was assessed relative to hypoxia under the mesopic conditions. A balanced, repeated-measures design allowed assessment of main effects and interactions of light level, viewing condition, gender, breathing gas, and exposure order by using multivariate analysis of variance (MANOVA), with post hoc analysis employing ANOVA and paired *t*-tests.

Results. Light level, number of viewing eyes, and oxygenation state were significant determinants of chromatic sensitivity. One man and one woman introduced orthogonal sources of gender bias. The CAD test revealed minimal deuteranomaly (R-G deficiency) in the man and loss of Y-B sensitivity in the only woman using hormonal contraception.

Conclusions. In the mesopic range, mild hypoxia impairs chromatic sensitivity progressively with reducing luminance. Binocular summation of chromatic signals is consistent and independent of the luminance channel. The CAD test is highly sensitive to mild congenital and acquired color vision deficiencies. (*Invest Ophthalmol Vis Sci.* 2008;49:820–827) DOI:10.1167/iovs.07-1004

In aviation, minimum color vision requirements are essential for distinguishing colors critical for air navigation, precision approach, warning, and emergency purposes.[1,2] However, the increasing use of color is placing ever greater demands on aircrew color vision, not least with regard to in-flight use of maps, manuals, and contemporary electronic flight displays. This trend is likely to continue, as color increases target conspicuity and enhances visual performance in coding or grouping and segmentation tasks.[3] Thus, the use of color coding enhances visual performance and can reduce operator error.[4,5]

Atmospheric air comprises 20.95% oxygen by volume, whereas barometric pressure falls almost exponentially with increasing altitude, and so hypobaric (low pressure) hypoxia (oxygen deficiency) is an inevitable consequence of breathing air at ambient pressure during ascent. Investigators in various studies have examined color vision during hypoxia, to deduce the effects of acute altitude exposure, employing a variety of visual stimuli and methodologies.[6–11] The results have not always been consistent, but it is generally accepted that moderate hypoxia, at equivalent altitudes above 3,048 m (10,000 ft), can produce impairment of color discrimination that varies with the level of light adaptation and may be more pronounced in the visual and retinal periphery, where rod photoreceptor density is high.

Retinal phototransduction processes require considerable metabolic energy, for rhodopsin phosphorylation, synthesis of cyclic guanosine monophosphate, chromophore transport, and support of enzyme activity.[12] In the dark, rod photoreceptor oxygen uptake increases dramatically to support the ion pumps that maintain the "dark current," consuming more oxygen than any function of any other cell.[13,14] In consequence, the retinal partial pressure of oxygen (Po_2) falls to remarkably low levels near photoreceptor inner segments and may even compromise oxidative phosphorylation in inner segment mitochondria.[15–19] Furthermore, breathing 100% oxygen (hyperoxia), rather than air, hastens the onset of scotopic sensitivity during dark adaptation, implying that rods may even be functionally hypoxic in the dark in normal respiratory and barometric conditions.[20]

During night flights, aircraft cockpits and flight decks are illuminated typically at mesopic levels, and so normal color vision is degraded.[21,22] In dim light, rod-driven retinal hypoxia may be anticipated to compromise cone oxygenation and function and to render threshold color sensitivity vulnerable to further exogenous hypoxia—specifically, the hypobaric hypoxia that results from altitude exposure. Progressive reduction in luminance would be expected to exaggerate the impairment. We report the results of two studies designed to investigate the effects of respiratory disturbance on mesopic color sensitivity. In study 1, we compared the effects of mild hypoxia (breathing 14.1% oxygen in nitrogen) with those of normal air (normoxia control) in subjects at low photopic, upper mesopic, and mid-mesopic luminances. In study 2, responses to hyperoxia (breathing 100% oxygen) were compared with those to hypoxia (14.1% oxygen control) at upper and mid-mesopic luminances. For convenience, physiological gas pressures are reported as measured (in millimeters of mercury), with SI units (in kilopascals) in parenthesis in the text.

Methods

Subjects

The work adhered to the principles of the Declaration of Helsinki, the study protocol was approved in advance by an independent Local Research Ethics Committee, and all subjects provided written informed consent before participating. Twelve healthy volunteers (six men and

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six women) completed each experimental condition. However, one female subject withdrew from the study due to relocation and was replaced. The mean age of the seven women was 26.1 years (range, 22–34) and of the six men was 31.7 years (range, 24–39).

Comprehensive medical screening ensured fitness to participate and included a medical questionnaire, detailed physical examination, electrocardiogram, and urinalysis to exclude glycosuria. Ophthalmic screening included a detailed ophthalmic history, external and funduscopic examination, and assessments of near and distant visual acuity, accommodation, convergence, visual fields, ocular movements and alignment, and pupillary reactions. All subjects were assessed as having normal trichromatic color vision by an experienced aviation medical examiner using the first 17 Ishihara pseudoisochromatic plates and by a postdoctoral vision scientist using a Nagel type I anomaloscope. Neither examiner was involved in the subsequent study. The Snellen acuity of all test eyes was 6/6 or better, using untinted corrective spectacles (two women) or contact lenses (one man), as necessary. The ophthalmic prescriptions for these subjects were unchanged throughout the study.

Subjects were nonsmokers who were asked to avoid alcohol for 24 hours before and caffeine on the day of an experiment. Subjects were masked to the presentation order of the breathing gases, and these were randomized such that three men and three women undertook each possible exposure order for each luminance condition. All subjects were familiar with breathing from pressure-demand oxygen regulators through aircrew oxygen masks. The women took a urine test to exclude pregnancy before each experiment.

Respiratory and Monitoring Equipment

Each subject was fitted with a modified U.K. Royal Air Force Aircrew Respirator Mk 5 (AR5), comprising an appropriately sized oxygen mask (over mouth and nose) with an attached polycarbonate visor and butyl rubber cowl, covering the head, neck, and shoulders. The neck seal was removed for comfort, and the helmet suspension harness was not needed. The mask antisuffocation valve was sealed to exclude light, but the microphone was retained for the same reason. The bulk of the visor was cut away to leave a residual scaffold on which matching polycarbonate scaffolds could be mounted. These supported neutral density (ND) gelatin filters (Wratten; Eastman Kodak, Rochester, NY) of either 1 or 2 optical density units. A port to the mask cavity allowed breathing gas composition to be analyzed continuously with a mass spectrometer (MGA 2000; Airspec, Kent, UK). The AR5 allowed ambient air to be blown gently and independently of the breathing gas supply, across the facial aspect of the ND filters, ensuring that they remained free from misting. Hence, normal corneal oxygenation was preserved, regardless of respiratory condition, and subjects could wear normal corrective spectacles and lenses, as required. Thus, the AR5 enabled total, contemporaneous control of visual and respiratory adaptation state.

Each of the three breathing gases (air, oxygen, and the hypoxic mixture) was supplied to its own dedicated, pressure-demand, breathing gas regulator at a nominal supply pressure. The regulators had identical pressure–flow characteristics and imposed minimal breathing resistance, making them indistinguishable to the user, and their breathing gases were delivered, via a selection tap, to a common mask hose. Mask valves prevented rebreathing of expired gas, and the mass spectrometer traces were monitored to ensure that good face–oxygen mask seals were maintained. Mass spectrometer calibrations were conducted immediately before and after each respiratory condition by using various gas mixtures of known composition, giving a measurement error of less than 1% for physiological partial pressures of oxygen and carbon dioxide. Noninvasive monitoring of blood pressure, heart rate, and oxygen saturation were undertaken with a blood pressure monitor (Finapres 2300; Ohmeda, Englewood, NJ) and a pulse oximeter (7840; Kontron Instruments, Ltd.; Watford, UK) with finger probe. Analog outputs from both devices were calibrated and recorded, together with the mass spectrometer data, using a PC-based data recording and analysis system with software (Powerlab/Chart software; AD-Instruments, Castle Hill, NSW, Australia).

Vision Test Equipment and Stimuli

Vision testing was conducted binocularly and then monocularly (dominant eye) using the Color Assessment and Diagnosis (CAD) test.[23,24] Randomly interleaved staircases were used with variable step sizes and a four-alternative, forced-choice procedure, to measure threshold chromatic sensitivity along eight color directions in the CIE 1931 (x, y) chromaticity chart. The uniform background field was approximately equivalent to daylight at a color temperature of 6500 K, with x, y chromaticity of 0.305, 0.323, respectively. The directions of chromatic displacement away from background chromaticity were chosen to maximize the output of either the red-green (R-G) or yellow-blue (Y-B) color channels. Thus, the cone-contrast signal generated by the test stimuli in S-cones is approximately zero along the R-G axis whereas the M- and L-cone contrast signals are approximately zero along the Y-B axis.

The CAD test stimulus is generated in the center of a large, uniform background field (30° × 24°) and comprises a square array of 15 × 15 achromatic checkers subtending a horizontal visual angle of ~3.3°. The uniform background provides a steady state of light adaptation while the luminance of each checker scintillates rapidly, independently, and randomly above and below background, to generate achromatic, dynamic, luminance contrast noise. The function of the noise is to mask the detection of residual luminance contrast signals in the "isoluminant" colored stimulus, a condition that does not affect significantly the threshold for detection of the color signal.[25] The color-defined stimulus comprises 5 × 5 checkers (~1.1°) moving diagonally across the checkerboard, along one of four possible directions. The subject's task is to indicate the direction of stimulus movement by pressing one of four buttons arrayed in a square, with each button corresponding to a destination corner on the checkerboard. The chance probability of a correct response is reduced to 1 in 16 by requiring the subject to report the correct direction of stimulus motion twice before the strength of the color signal is reduced according to the interleaved staircase procedure.

The stimulus for the CAD test was generated on a CRT display (Trinitron Multiscan G520 monitor; Sony, Tokyo, Japan) calibrated for both luminance and chromaticity with a luminance photometer (LMT, model 1009; Lichtmesstechnik, Berlin, Germany) and a telespectroradiometer (model CS1000; Minolta, Osaka, Japan). The luminance calibration was repeated automatically at regular intervals by using the internal calibration program. Subjects positioned a notch in the expiratory port of the oxygen mask against a chin rest, viewing the display screen along a matt-black viewing tunnel from a distance of 70 cm. A central fixation point was provided, and the subjects were familiarized with the test before starting the study.

Respiratory and Viewing Conditions

At 1 atmosphere, breathing 14.1% oxygen with a balance of nitrogen simulates mild hypobaric hypoxia by imposing an alveolar partial pressure of oxygen (P_AO_2) of ~60 mm Hg (8 kPa). This approximates the P_AO_2 achieved when breathing air at an altitude of 3,048 m (10,000 ft) and lowers arterial hemoglobin oxygen saturation (S_AO_2) to ~90%. The use of supplementary oxygen by aircrew is generally regarded as unnecessary below this altitude. It is therefore pertinent to establish the possible effects that this "acceptable" level of hypoxia may have on visual performance challenges relevant to aviation. In study 1, the effect of hypoxia on chromatic sensitivity was established relative to normoxia, viewing the display directly (low photopic, 22.3 cd · m^{-2}), and, on subsequent visits, through ND 1.0 (borderline upper mesopic, 1.67 cd · m^{-2}) and ND 2.0 (mid-mesopic, 0.21 cd · m^{-2}) filters. Study 2 repeated the mesopic experiments to investigate the potential benefits on chromatic sensitivity of breathing supplementary 100% oxygen, relative to performance during mild hypoxia. Each respiratory

condition was imposed for 15 minutes, to achieve a respiratory steady state before commencing vision testing.

Experimental Procedure

The subject was seated at rest and adapted to the low ambient light level (1–2 lux). The AR5 was fitted and the subject breathed room air through the mask hose. A halter was worn, supporting the breathing gas supply hose and the blower for the de-mist air supply. The subject was prepared for physiological monitoring, and the mass spectrometer sampling line was connected to the mask. Concurrent visual and respiratory adaptation were then undertaken with the purpose of establishing unambiguous, stable and procedurally reproducible adaptation states, within and between subjects. The process was based on a dark-adaptation regimen that measured time to scotopic sensitivity under analogous conditions of respiratory disturbance, reported previously.[20] Five minutes of light adaptation were undertaken by viewing directly, with natural pupils, a light box with a peak mean luminance of 735 cd · m^{-2}. Subsequently, 15 minutes of dark adaptation commenced with application of the appropriate polycarbonate visor frame and filter, together with an opaque material cover, and gentle blown air was supplied from ambient air to prevent filter misting. After 15 minutes the material cover was removed to allow 5 minutes of light adaptation while viewing the test display either directly or through the relevant ND filter. Five minutes into dark adaptation, the breathing gas supply hose was connected to the mask hose to begin 15 minutes of respiratory adaptation, so that vision testing began on completing 25 minutes of visual adaptation and 15 minutes of concurrent respiratory adaptation. Tests of achromatic spatial contrast sensitivity, in matching background viewing conditions, preceded the CAD tests. The CAD tests commenced after approximately 30 minutes of respiratory exposure, to within a minute or so and occupied a total of ~20 minutes. After binocular testing, an opaque occluder was placed in front of the nondominant eye and the CAD test was repeated monocularly. Further unrelated vision testing occupied another 10 minutes or so, after which the subject rested for 15 minutes before repeating the entire procedure in the alternate respiratory condition.

Analysis

Cardiovascular and respiratory parameters were recorded continuously throughout all exposures. The significance of differences in physiological parameters between respiratory conditions was assessed using paired *t*-tests on within-subject data. Chromatic signals for threshold detection of color-defined motion were measured along each of the eight color directions. For both studies, these thresholds were treated as eight related, dependent variables in multivariate analysis of variance (MANOVA), assessing main effects and interactions of light level, viewing condition (binocular or monocular), gender, breathing gas and respiratory exposure order ($\alpha = 0.05$). Post hoc analysis was conducted by using a variety of ANOVA techniques and paired *t*-tests. All analyses were conducted on computer (Minitab 14 software; Minitab, State College, PA). Mean R-G and Y-B axes were calculated for each respiratory condition in relation to "standard normal" CAD units.[24,26] Mean threshold data are represented in graphs of CIE 1931 (*x*, *y*) color space using group mean (*x*, *y*) coordinates for each color direction. Increases in individual thresholds may appear small when represented in this way. However, it should be considered that the consequent and disproportionate increases in area of the color ellipses defined by increases in R-G and Y-B axis lengths may represent substantial losses of net color sensitivity.

Cardiorespiratory Parameters

The inspired P_{O_2} (Pi_{O_2}), end tidal P_{O_2} ($P_{ET}O_2$), end tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), peripheral S_AO_2, heart rate, and systolic, diastolic, and mean blood pressures were measured during all exposures and remained stable during vision testing. Mean (±SE) respiratory responses between subjects are shown for both studies in Table 1.

Table 1. Respiratory Parameters Imposed during Studies 1 and 2

	Normoxia	Hypoxia
Study 1: Comparing Hypoxia (14.1% Oxygen) with Normoxia Control		
Direct viewing (22.3 cd · m^{-2})		
Gas tension (mmHg)		
Pi_{O_2}	156 (0.7)	108 (0.5)
$P_{ET}O_2$	107 (1.2)	60 (0.9)
$P_{ET}CO_2$	36.7 (0.5)	37.5 (0.5)
S_AO_2	97.7 (0.3)	90.6 (0.4)
ND 1.0 filter (1.67 cd · m^{-2})		
Gas tension (mm Hg)		
Pi_{O_2}	157 (0.6)	107 (0.5)
$P_{ET}O_2$	109 (1.1)	60 (1.1)
$P_{ET}CO_2$	38.0 (0.7)	38.9 (0.6)
S_AO_2 (%)	97.9 (0.3)	91.3 (0.4)
ND 2.0 filter (0.21 cd · m^{-2})		
Gas tension (mm Hg)		
Pi_{O_2}	155 (0.7)	105 (0.6)
$P_{ET}O_2$	108 (1.2)	58 (1.0)
$P_{ET}CO_2$	38.2 (1.1)	38.2 (1.1)
S_AO_2 (%)	98.3 (0.3)	91.0 (0.6)
Study 2: Comparing Hyperoxia (100% Oxygen) with Hypoxia Control (14.1% Oxygen)		
ND 1.0 (1.67 cd · m^{-2})		
Gas tension (mm Hg)		
Pi_{O_2}	744 (4.8)	105 (0.6)
$P_{ET}O_2$	681 (4.9)	58 (1.5)
$P_{ET}CO_2$	35.0 (0.8)	39.1 (1.1)
S_AO_2 (%)	99.7 (0.2)	91.5 (0.6)
ND 2.0 filter (0.21 cd · m^{-2})		
Gas tension (mmHg)		
Pi_{O_2}	740 (5.4)	105 (0.7)
$P_{ET}O_2$	681 (5.1)	59 (1.4)
$P_{ET}CO_2$	33.2 (0.9)	38.0 (1.2)
S_AO_2 (%)	99.8 (0.1)	91.8 (0.7)

Data are expressed as the mean ± SE (1 kPa = 7.501 mm Hg). The imposed changes in gas tension and resultant S_AO_2 were closely controlled and stable throughout. The hypoxia exposures were highly reproducible.

The respired gas tensions and S_AO_2 data show that the intended respiratory conditions were achieved and were closely controlled. There was no effect of mild hypoxia to induce secondary hyperventilation. However, hyperoxia was accompanied by a slight but unequivocal, consistent, and highly statistically significant ($P < 0.001$) reduction in $P_{ET}CO_2$ by approximately 5 mm Hg (~0.7 kPa). This appears paradoxical but is attributable to the Haldane effect, whereby the enhanced oxygenation of hemoglobin in venous blood reduces carriage of carbon dioxide from the tissues in the carbamino form.[27,28] This has not confounded interpretation of the effects of altered oxygen tension on threshold color sensitivity.

The group mean cardiovascular responses, including derived pulse pressure (systolic minus diastolic), are shown for both studies in Table 2. The results suggest a slight tendency for blood pressures to increase under mild hypoxia, but no increase achieved statistical significance. However, a statistically significant increase in heart rate with hypoxia was consistent with expectations.[20]

Results

Derived mean (±SE) Y-B and R-G axis lengths are shown in Table 3 with reference to the standard normal CAD observer (CAD units). However, the data for one man and one woman

TABLE 2. Cardiovascular Parameters Recorded during Studies 1 and 2

	Normoxia	Hypoxia
Study 1: Comparing Responses to Hypoxia (14.1% Oxygen) with Normoxia Control		
Direct viewing (22.3 cd · m^{-2})		
Blood pressures (mm Hg)		
Systolic	120 (3.9)	128 (7.1)
Diastolic	68 (3.7)	70 (3.8)
Mean	85 (3.6)	89 (4.7)
Pulse	52 (2.9)	58 (4.6)
Heart rate (min^{-1})	65 (1.8)	69 (2.1)
ND 1.0 (1.67 cd · m^{-2})		
Blood pressures (mm Hg)		
Systolic	117 (2.9)	121 (2.9)
Diastolic	69 (2.6)	70 (2.5)
Mean	85 (2.5)	87 (2.3)
Pulse	48 (2.5)	51 (2.4)
Heart rate (min^{-1})	72 (2.9)	75 (3.2)
ND 2.0 (0.21 cd · m^{-2})		
Blood pressures (mm Hg)		
Systolic	117 (4.0)	120 (4.5)
Diastolic	66 (3.5)	65 (3.4)
Mean	83 (3.5)	84 (3.6)
Pulse	51 (2.5)	55 (2.7)
Heart rate (min^{-1})	65 (2.8)	68 (2.9)
Study 2: Comparing Responses to Hyperoxia (100% Oxygen) with Hypoxia Control (14.1% Oxygen)		
ND 1.0 (1.67 cd · m^{-2})		
Blood pressures (mm Hg)		
Systolic	116 (3.8)	118 (2.7)
Diastolic	68 (3.0)	67 (2.6)
Mean	84 (3.1)	84 (2.4)
Pulse	47 (2.0)	51 (2.2)
Heart rate (min^{-1})	63 (3.3)	72 (3.8)
ND 2.0 (0.21 cd · m^{-2})		
Blood pressures (mm Hg)		
Systolic	123 (4.4)	132 (2.4)
Diastolic	69 (4.5)	71 (2.8)
Mean	87 (4.3)	91 (2.4)
Pulse	54 (2.1)	61 (2.5)
Heart rate (min^{-1})	63 (2.7)	72 (3.2)

Date are expressed as the mean ± SE. Cardiovascular status remained stable during all exposures. The slight increase in heart rate during hypoxia was statistically significant for direct viewing in study 1 ($P < 0.01$) and for both exposures in study 2 ($P < 0.001$). The slight tendency for systolic and pulse pressures to increase with hypoxia was not statistically significant. Cardiovascular status remained essentially stable between respiratory conditions.

are presented separately from those of the remaining 10 normal trichromats as these two subjects appear to contribute to gender bias, discussed later. The binocular, photopic CAD data of the 10 normal trichromats, breathing air, are consistent with those of the standard normal CAD observer.[24,26] The mean (±SE) Y-B and R-G thresholds in study 1 are shown in Figure 1, and those in study 2 are in Figure 2. Unless indicated otherwise, the following probabilities are from the initial balanced, repeated-measures MANOVA conducted on the data from all 12 subjects.

A main effect of light level is unambiguous for both study 1 ($P < 0.001$) and study 2 ($P < 0.001$) and is self-evident in Figures 1 and 2 for all viewing and respiratory conditions.

The number of viewing eyes was statistically significant in both study 1 ($P < 0.001$) and study 2 ($P = 0.011$), such that monocular viewing impaired chromatic sensitivity along both the Y-B and R-G axes. The effect is illustrated in CIE 1931 (x, y) color space in Figure 3, using just the normoxia data from study 1. A statistically significant interaction between light level and viewing condition was seen ($P < 0.05$), such that the monocular loss of color sensitivity increases in absolute value with decreasing luminance.

A statistically significant main effect of breathing gas was achieved in both study 1 ($P = 0.001$) and study 2 ($P = 0.002$). Considering study 1 (Fig. 1), hypoxia clearly impairs chromatic sensitivity at the lowest light level, viewing through the ND 2.0 filter. Y-B and R-G axis lengths are compromised progressively by mild hypoxia alone, by monocular viewing alone, and by viewing monocularly when hypoxic. In contrast, there was no unambiguous effect of breathing gas at photopic luminance. At the intermediate, upper mesopic luminance the data for some color directions suggest a tendency in favor of hypoxic impairment. A statistically significant interaction between light level and breathing gas ($P < 0.01$) supports a progressive effect of hypoxia with reducing luminance, reinforced by post hoc paired *t*-tests on the data obtained at each of the three light levels.

In study 2, a consistent benefit of breathing 100% oxygen over hypoxia on R-G and Y-B sensitivity was apparent at both light levels when viewing binocularly and monocularly (Fig. 4). Although not always achieving statistical significance at $\alpha = 0.05$, post hoc analysis on study 2 data subsets, disaggregated by light level and/or number of viewing eyes, indicate a consistent trend for impairment under hypoxia.

A statistically significant main effect of respiratory exposure order was seen in study 1 ($P = 0.01$) but not study 2 ($P = 0.717$). The respiratory exposures were controlled closely (Table 1) and test–retest variability on the CAD test is small, so neither explains the order effect. The color direction data were examined by exposure order and light level, revealing a slight but consistent tendency for overall chromatic sensitivity to be worse when hypoxia preceded normoxia than vice versa. This suggests that preceding hypoxia may have compounded possible fatigue or inattention in subsequent normoxic exposures, whereas the alerting effect of supplementary oxygen would mitigate such an effect of exposure order in study 2.

A main effect of gender was seen in study 1 ($P = 0.016$) but not in study 2 ($P = 0.1$). Two consistent trends were apparent when comparing thresholds in the male and female participants for all color directions at each luminance, with the data sorted by gender, viewing eyes, and breathing gas: the R-G axes tended to be longer in the men, whereas the Y-B axes were longer in the women. Two systematic and orthogonal sources of gender bias were apparent when considering individual responses, introduced by one male and one female subject. The man had marginally but consistently poorer R-G sensitivity than his peers, whereas the woman had poorer Y-B sensitivity than hers. Post hoc ANOVA conducted on each threshold axis in both studies 1 and 2 revealed consistent effects of gender only along the 60° to 240° Y-B axis. The results for both subjects were removed from the data for study 1, and a further MANOVA was performed. There was no longer a statistically significant effect of gender ($P = 0.291$), but main effects of light level ($P < 0.001$), viewing condition ($P < 0.001$), and breathing gas ($P = 0.004$) remained, together with the interactions identified previously.

DISCUSSION

Both R-G and Y-B thresholds are impaired significantly at low light levels, with Y-B thresholds affected the most. Consistent with previous findings,[21,26] an asymmetry in Y-B thresholds is apparent at the lowest light level, with yellow thresholds,

TABLE 3. R-G and Y-B Chromatic Sensitivity Expressed in CAD Units

Light Level (cd · m^{-2})	Number of Viewing Eyes	Respiratory Condition	R-G Thresholds (CAD Units)			Y-B Thresholds (CAD units)		
			10 Normal Trichromats Mean (SD)	Man with Minimal Deuteranomaly	Woman Using Hormonal Contraception	10 Normal Trichromats Mean (SD)	Man with Minimal Deuteranomaly	Woman Using Hormonal Contraception
Direct viewing (22.3)	Binocular	Normoxia	1.14 (0.19)	1.79	1.01	0.99 (0.24)	0.77	1.12
		Hypoxia	1.06 (0.18)	1.42	0.95	0.95 (0.20)	0.87	1.02
	Monocular	Normoxia	1.36 (0.30)	1.78	0.90	1.24 (0.27)	0.79	1.30
		Hypoxia	1.37 (0.33)	1.80	1.39	1.25 (0.33)	0.98	1.66
ND 1.0 (1.67)	Binocular	Normoxia	1.99 (0.47)	2.67	1.64	2.64 (0.85)	1.64	3.06
		Hypoxia	2.00 (0.60)	2.94	1.56	2.68 (0.69)	2.09	3.37
	Monocular	Normoxia	2.29 (0.80)	2.42	2.03	3.37 (0.87)	2.19	4.40
		Hypoxia	2.52 (0.99)	2.82	1.95	3.29 (0.83)	2.20	3.49
ND 2.0 (0.21)	Binocular	Normoxia	3.78 (1.20)	6.03	4.68	7.74 (2.11)	9.42	12.82
		Hypoxia	4.32 (1.58)	7.67	4.42	9.21 (3.03)	9.99	11.92
	Monocular	Normoxia	5.07 (1.92)	6.83	4.99	10.45 (2.77)	10.17	10.98
		Hypoxia	6.15 (2.44)	8.64	8.26	10.55 (2.97)	11.41	15.77
ND 1.0 (1.67)	Binocular	100% Oxygen	2.51 (2.13)	2.50	1.75	2.85 (0.90)	1.98	2.86
		Hypoxia	3.03 (1.85)	2.81	3.26	3.54 (1.39)	2.08	5.80
	Monocular	100% Oxygen	2.17 (0.54)	2.45	1.62	3.30 (1.01)	2.10	3.62
		Hypoxia	3.93 (2.89)	2.97	5.37	4.60 (1.63)	3.02	6.21
ND 2.0 (0.21)	Binocular	100% Oxygen	3.48 (1.04)	5.79	3.42	7.77 (2.03)	7.51	8.16
		Hypoxia	4.09 (1.30)	6.43	7.42	9.07 (2.81)	8.68	12.60
	Monocular	100% Oxygen	4.96 (2.25)	5.71	4.34	10.06 (2.77)	8.09	10.20
		Hypoxia	5.58 (1.61)	8.10	10.33	11.35 (3.07)	14.36	10.45

One CAD unit is the mean threshold chromatic sensitivity for the "standard normal CAD observer," viewing binocularly at photopic luminance under normoxic conditions, established by testing a large number of normal trichromatic subjects.[24,26] Note the substantial standard deviations of the normal trichromats, which reflect considerable within- and between-subject variability in response in the different conditions. Where coefficients of variation exceed 35% the outlying (poor performance) data points were contributed inconsistently by half of the 10 trichromatic subjects. Comparison of the trichromats' mean responses with those of the mildly deuteranomalous male and the female with tritan deficiency reveals the consistently orthogonal nature of their confounding influences under almost all conditions, explaining the apparent gender effect seen with initial balanced MANOVA.

corresponding to S-cone decrements, compromised more than the complementary blue thresholds that correspond to S-cone increments (Fig. 5). This suggests an asymmetry for increments and decrements in S-cone signals at low light levels, supporting a greater metabolic demand of the S-cone system and a possible role of hypoxia in the etiology of disease-induced tritan defects.[29,30]

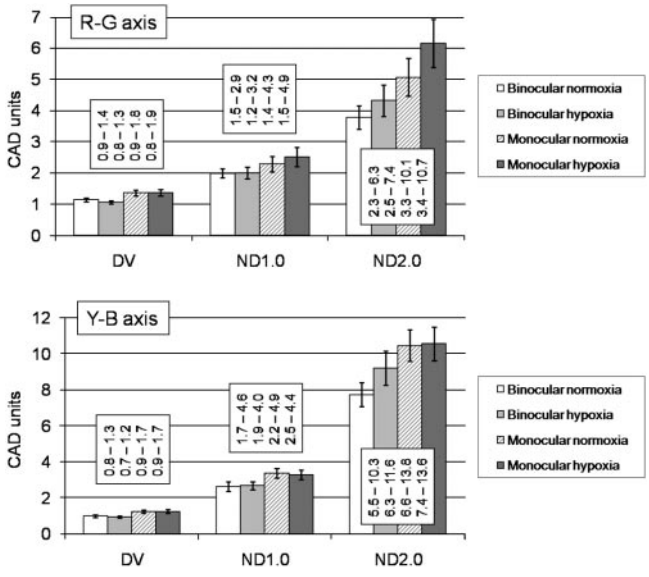

FIGURE 1. Mean ± SE Y-B and R-G thresholds of 10 normal trichromats in study 1, viewing directly (DV; 22.3 cd · m^{-2}) and through ND 1.0 (1.67 cd · m^{-2}) and 2.0 (0.21 cd · m^{-2}) filters. The range for each data set is shown, illustrating the considerable overlap between conditions.

Thresholds for color detection were consistently lower in binocular than in monocular viewing. As the chromatic stimuli were presented against luminance contrast noise, this benefit of binocular viewing relates specifically to improved discrimination of the color signal. This finding conflicts with one recent report[31] but is in accord with others.[32–35] For normal trichromats, R-G thresholds in Table 1 approximate the semiminor axes of corresponding color ellipses and the Y-B thresholds approximate the semimajor axes. Thus, while monocular axes may not appear much longer than binocular axes, they may represent substantial increases in ellipse area and considerable net loss of color sensitivity. Although the difference does not appear significant in Figure 3, the calculated ellipse area of the normal trichromats when viewing monocularly rather than binocularly increased by 67% at 22.3 cd · m^{-2}.

The man with deuteranomaly passed the screening tests for the study but, unfortunately, his Nagel anomaloscope matching range data were not recorded. However, records from an unpublished previous study indicate that he made repeated errors on Ishihara testing some years previously. His baseline R-G threshold of 1.79 CAD units was just within the range consistent with minimal deuteranomaly.[24,26] At photopic and upper mesopic luminance his R-G sensitivity was marginally worse than that of normal trichromats, but his Y-B sensitivity was somewhat better. However, at the lowest light level, his sensitivity to yellow deteriorated dramatically (Fig. 6). Despite

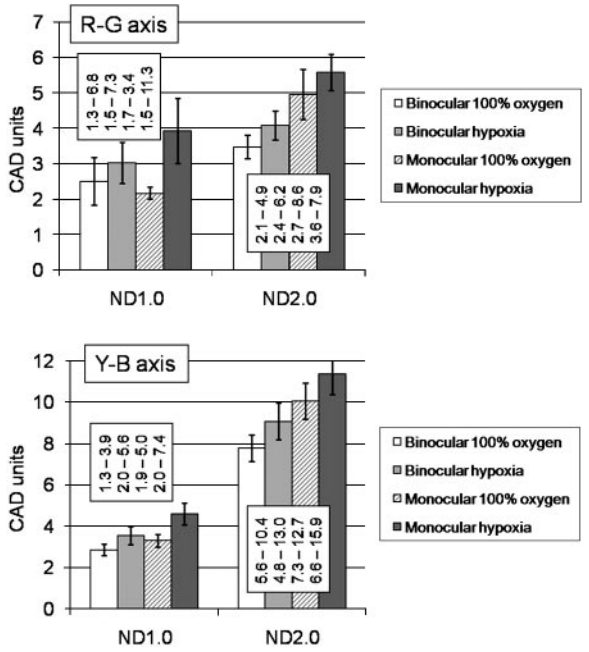

FIGURE 2. Mean ± SE Y-B and R-G thresholds of 10 normal trichromats in study 2, viewing through ND 1.0 (1.67 cd · m^{-2}) and ND 2.0 filters (0.21 cd · m^{-2}). The range for each data set is shown. Performance breathing 100% oxygen is comparable to that breathing air in study 1. Performance under hypoxia varies from that in study 1 and is markedly worse for the ND 1.0 condition.

near-normal photopic and upper mesopic color sensitivity, he manifests a specific loss of sensitivity to yellow at mid-mesopic luminance. Such responses call into question the suitability of conventional color tests for determining the functional acceptability of color vision at reduced luminance.

The woman with tritan deficiency exhibited the most severe Y-B loss at mid-mesopic luminance, particularly to blue. She was the only woman using hormonal contraception, known to influence color vision.[2] Figure 6 illustrates how the

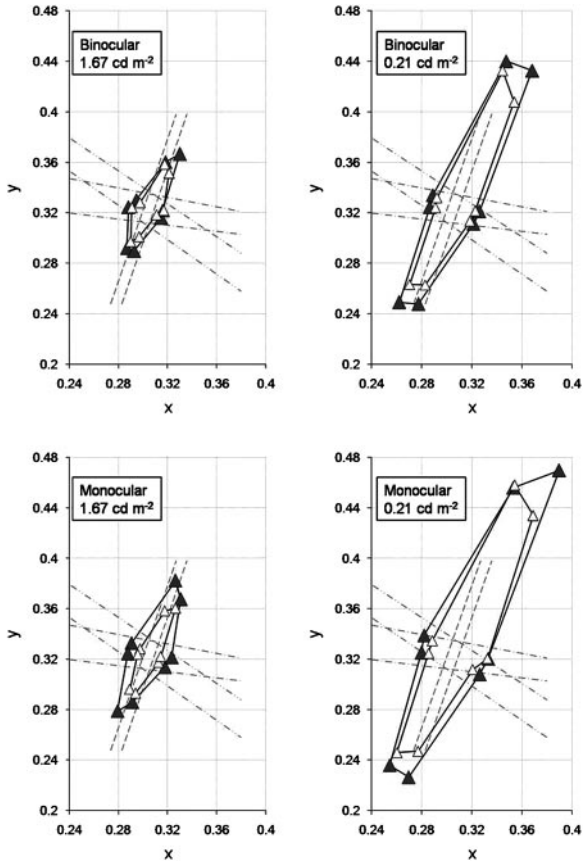

FIGURE 4. Effect of 100% oxygen (*white symbols*) consistently to optimize chromatic sensitivity along all eight color directions in CIE 1931 *x*, *y* color space, relative to mild hypoxia (*gray symbols*), at both upper and mid-mesopic luminance ($n = 12$). Use of the same *x*, *y* scales allows direct comparison of the data by light level. Small increases in R-G and Y-B axis lengths under hypoxia may represent substantial increases in the corresponding color ellipse area.

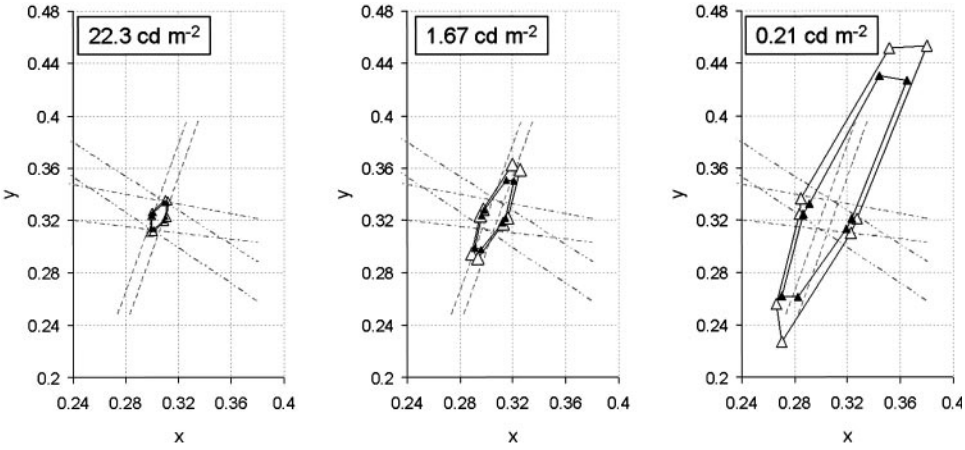

FIGURE 3. Effect of viewing monocularly (*white symbols*) rather than binocularly (*black symbols*) in study 1 to compromise normoxic chromatic sensitivity in CIE 1931 (*x*, *y*) color space at three light levels ($n = 12$). Mean thresholds are shown, measured along eight color directions from $x = 0.305$, $y = 0.323$. The effect of monocular viewing to compromise thresholds in all eight color directions is consistent and independent of the respiratory component of the study. Use of the same *x*, *y* scales allows comparison of the data by light level but tends to mask the discrete R-G threshold data between viewing conditions. Loss of color sense is reflected by the increasing area of the corresponding color ellipses. Monocular viewing increases calculated color ellipse area by 67% at 22.3 cd · m^{-2}.

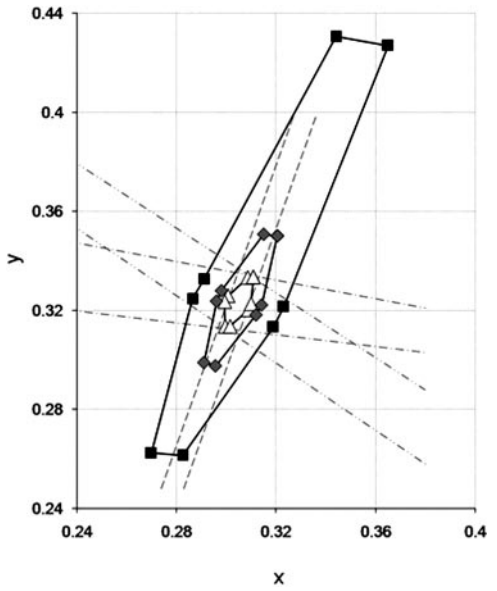

FIGURE 5. Effect of light level on binocular, normoxic mean chromatic thresholds in CIE 1931 (*x*, *y*) color space at 22.3 cd · m^{-2} (*triangles*), 1.67 cd · m^{-2} (*diamonds*) and 0.21 cd · m^{-2} (*squares*). The normal mesopic tritanopia is apparent, but there is far greater loss of sensitivity to yellow than to blue at the lowest light level ($n = 12$).

combined, orthogonal influences of the tritan woman and deuteranomalous man would be likely to introduce a gender bias in relation to the mean performance of the normal trichromats.

The effect of hypoxia on mesopic color sensitivity is well established at an equivalent altitude of 3,048 m (10,000 ft), and so lesser impairment may be anticipated at lower altitudes. However, chromatic sensitivity was preserved at photopic luminance, contrary to previous reports of threshold elevation[36] and loss of color discrimination[37] with this level of hypoxia.

Optimal chromatic sensitivity breathing 100% oxygen in study 2 was similar to that breathing air in study 1 and showed that supplementary oxygen conferred no benefit over normoxia. Hypoxia was associated with far greater loss of chromatic sensitivity in study 2 than in study 1, particularly at 1.67 cd · m^{-2} (Fig. 4), yet the hypoxia exposures are highly reproducible and virtually identical between studies (Table 1). However, familiarity with the challenging study procedure and reduced levels of psychological arousal may have increased susceptibility to cognitive or attentional effects of hypoxia during later experiments. Responses to hypoxia are notoriously variable, within and between subjects, and this may be compounded in dim light if attention wanders. In contrast, 100% oxygen is arousing and appears to maintain normal chromatic sensitivity.

In general, the magnitude of the effect of hypoxia is less than but broadly comparable to that of viewing monocularly. As with viewing monocularly, small increases in hypoxic Y-B and R-G thresholds may result in substantial increases in the area of the corresponding color ellipses. However, the asymmetry in S-cone responses, in conjunction with a progressive effect of hypoxia with decreasing mesopic luminance, and the suggestion of a tilt in the major axis of mesopic color ellipses,[21] suggest that it is inappropriate to extend comparison of ellipse area into the mesopic range, when using the data available herein. Nonetheless, progressive hypoxic loss of color sensitivity may be anticipated with decreasing mesopic luminance at 3,048 m (10,000 ft).

As the effect of hypoxia is progressive with falling light it is therefore more likely due to an ocular rather than central mechanism. A drop in P_AO_2 to ~60 mm Hg (~8 kPa) will reduce ciliary artery Po_2 to ~50 to 55 mm Hg, reducing the choroidal oxygen "pressure head" by ~50%. As the available light decreases, the compound influences of progressive rod-driven retinal hypoxia and the reduced choroidal Po_2 may compromise cone oxygenation and function. An effect of mild hypoxia on cone-mediated vision commences at upper mesopic luminance and is well-established at mid-mesopic luminance (0.21 cd · m^{-2}), supporting an increased retinal vulnerability to hypoxia in dim light. Chromatic sensitivity may be more vulnerable to hypoxia with distance from the fovea, as rod density increases and cone density decreases.

Hypoxia's compromising the acquisition of color-coded information in the mesopic cockpit has implications for aircrew operating in unpressurized cabins at or above 3,048 m (10,000 ft). Besides reduced conspicuity of colored targets, color deficiency is associated with extended search times and higher error rates.[38,39] However, the consequences of reduced color sensitivity go beyond impaired extraction of chromatic information. At mesopic levels, the chromatic signal contributes to "effective" luminance contrast and reaction time,[40,41] so hypoxia may compromise wider aspects of aircrew performance.

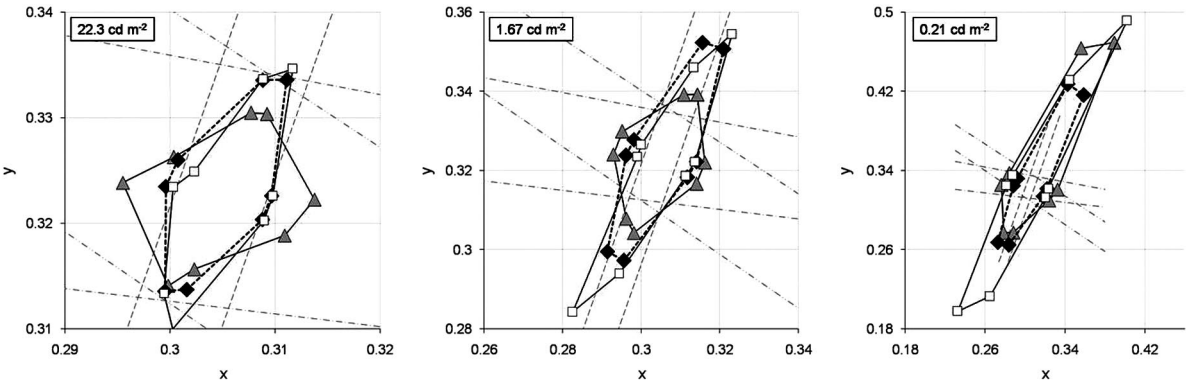

FIGURE 6. Comparison of the chromatic thresholds of the man with minimal deuteranomaly (*gray triangles*) and the woman using hormonal contraception (*white squares*) with the means of the 10 normal trichromats (*black diamonds*) at each light level (binocular, normoxic). The different (*x*, *y*) scales are necessary to resolve the detail of the different patterns of response at the different light levels. The protan, deutan, and tritan color confusion axes shown (*dashed lines*) are the same plots in each diagram. Note the mildly but clearly elevated deutan thresholds of the man with minimal deuteranomaly at 22.3 cd · m^{-2} and the consistent loss of Y-B sensitivity of the woman using hormonal contraception.

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