Declines in Wavelength Discrimination and Shifts in Unique Hue with Hypoxia

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INTRODUCTION: Hypoxia can be a problem for warfighters, compromising visual and cognitive performance. One area of study has been hypoxia-induced decrements in color vision.

METHODS: The present study examined how hypoxia affected the perception of wavelengths associated with unique green and with unique yellow as well as discriminability by the blue vs. yellow (b-y) and the red vs. green (r-g) spectrally opponent color channels while breathing O_2 levels found at sea level and at 5500 m. Measurements of wavelengths producing unique green (minimizing response by the b-y channel) and unique yellow (minimizing response by the r-g channel) preceded measurements of wavelength discriminability near those unique hues.

RESULTS: Relative to sea level, unique yellow shifted to shorter wavelengths (0.54 nm) and unique green shifted to longer wavelengths (2.3 nm) under hypoxia. In terms of an equal psychophysical scale, both unique hues shifted by similar magnitudes. Wavelength discriminability of both color channels was compromised by statistically reliable amounts of 16–17% under hypoxia.

DISCUSSION: These results were consistent with previous studies and the inference that postreceptor, M-cone neurons were differentially compromised by hypoxia. However, these measurable changes in color vision due to hypoxia were not perceived by the subjects.

KEYWORDS: hypoxia, color vision, wavelength discriminability, unique hue.

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Hypoxia is the diminished availability of oxygen (O_2) to body tissues; its causes are many and varied, including a deficiency of oxygen in the atmosphere at high altitudes. A substantial proportion of warfighters can be subjected to hypoxia which can affect cognitive performance. Diminished or altered personality, mood, or cognitive functions are usually the first symptoms of hypoxia.[11] Warfighters are often not aware of impairments to their own cognitive performance when subject to hypoxia, so it is desirable that warfighters be provided with rapid, accurate, and reliable feedback on the degree of hypoxia they might be experiencing.

Together with a decline in cognitive functions, visual functions are also diminished under hypoxia.[22,25] These deleterious effects are more prominent under dim light conditions[2,8,21] where the retina has greater metabolic demand.[23] Chronic exposures to low concentrations of O_2 are more evident than acute exposures. For example, Ernest and Krill[8] and Phillips et al.[24] showed no effect of hypoxia in the first few minutes of exposure. In contrast, mountain climbers exposed to low O_2 concentrations at high altitudes for days can show robust and reliable decrements in performance.[26,32,34]

One area of interest in studies of hypoxia has been compromises to color vision (e.g., Connolly et al.), particularly tests associated with short wavelength sensitivity.[3] Although these phenomena have been called S-cone deficits, it is unlikely that the S-cone photoreceptors are affected alone by hypoxia. One of the first studies by Smith et al.[31] acknowledged that hypoxia is likely to affect the postreceptor neurons in the blue vs. yellow (b-y) spectrally opponent color channel, where the blue (b) response of this neural channel originates from the S-cone and

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the yellow (y) response is formed from the sum of the L-cone and the M-cone inputs. Subsequent authors have come to the same conclusion; e.g., Vingrys and Garner.[33] More directly, Schatz and colleagues,[29] using electroretinographic methods, have inferred that hypoxia affects amacrine and ganglion neurons in the inner plexiform layer rather than photoreceptors. They also argue that hypoxia affects S-cone, ON-bipolar neurons[28] as well as the rod bipolar neurons[27] in the outer plexiform layer. A possible explanation for the deficits associated with short wavelengths relative to long wavelengths has been offered by Hood and colleagues.[10,13] They show the b-y channel has a limited response range relative to the red vs. green (r-g) channel, where the red (r) response originates from the L-cones and green (g) response from M-cones, and the achromatic, luminance channel, which is formed from the sum of the L- and M-cone inputs. This explanation would be consistent with the reported preferential loss of sensitivity along the tritan axis of the Farnsworth-Munsell 100 Hue test (FM100)[9] relative to the protan and deutan axes reported by a number of authors.[31,32,34] It should be noted, however, that the relative loss of the b-y pathway can be small and indeed some authors report losses[14,26] or even larger losses[33] of sensitivity in the r-g pathway. Further, Davies et al.[6] showed no effects and Leid and Campagne[20] showed marginal reductions in color vision, although as the authors state, the test they employed was perhaps less sensitive than those used by other researchers; e.g., Willmann et al.[34] Where deficits in the b-y and r-g color opponent channels have been tested together, there does seem to be a greater, but not exclusive loss in the b-y channel[34] although, again, this is not always the case.[33]

To better elucidate the mechanism and magnitude of the effects of hypoxia on color vision, a sensitive laboratory experiment was designed and conducted to measure changes in the spectral sensitivity of the two opponent color channels, b-y and r-g, at sea level and at altitude. In an attempt to isolate the effects of hypoxia on each of the two color channels, unique, or "pure," hues were used as stimuli. Unique green (or unique red) is perceived when the cone inputs to the b-y channel are balanced (i.e., b-y = 0). Thus, the S-cone and the sum of the L-cone and M-cone inputs to the b-y channel are the same, leaving the r-g color channel to signal green (or red). Similarly, unique yellow (or unique blue) is perceived when the L-cone and M-cone inputs to the r-g are balanced (i.e., r-g = 0), leaving the b-y color channel to signal yellow (or blue). In addition to measuring changes in unique green and unique yellow from sea level to altitude, it is also possible to measure changes in wavelength discriminability centered on these unique hues. This approach effectively isolates changes in wavelength discriminability for a single, spectrally opponent color channel. Thus, the effects of hypoxia on the two color channel responses were examined by looking for two possible changes: 1) the shift in the wavelength associated with pure yellow or pure green (i.e., the wavelengths associated with no response from the r-g and the b-y channels, respectively); and 2) the wavelength discriminability associated with a unique hue. With regard to the second type of observation, relative to sea level, hypoxia should make *more* wavelengths appear unique, thereby decreasing wavelength discriminability.

METHODS

The study protocol was approved in advance by the Rensselaer Institutional Review Board. Each subject provided written informed consent before participating.

A 2 × 2, within-subjects, repeated measurements experiment was conducted to assess the effects of altitude at sea level and 18,000 ft (~ 5500 m) on monochromatic wavelength settings and wavelength discriminability for two unique hues (green and yellow). The unique hue and wavelength discriminability tasks were administered in phases for both sessions: initial, altitude 1, and altitude 2 (**Table I**). The unique hue used in a session was estimated during the initial phase and became the center wavelength for the unique hue and wavelength discrimination measurements during that session for both altitudes. Subjects wore a mask where the air composition was controlled for both the sea level and the simulated altitude conditions. After each phase, the subject's breathing air would be slowly altered to the next altitude.

Subjects

At least 1 d before subjects were presented with the experimental conditions the nurse conducted an initial medical screening session. The nurse recruited healthy subjects, aged 18–29 yr, with normal acuity and color vision. Subjects with any significant medical history including neurological disease, cognitive dysfunction, pulmonary disease, or cardiovascular disease, history of anxiety, panic attacks or claustrophobia, prescription medication use (except oral contraceptives), smoking and pregnancy, were excluded. To be included in the study, subjects had to have normal color vision as assessed using plates 1–25 of the Ishihara test for color blindness[17] and visual acuity equivalent to 20/20 as assessed with a Landolt-ring eye chart viewed at 2 m.[1] During the initial medical screening session, every subject also performed a practice trial to become familiar with the tasks they would complete. For the practice trial, each subject wore the mask that was open to room air so they could get accustomed to how it felt and to make sure they could find a comfortable fit. There were 27 people who met the screening criteria for participation. One subject dropped out before completing the experimental protocol resulting in complete data sets for 26 subjects. As explained in the Results section, the entire data sets for two subjects were excluded from the analysis because their responses for the discriminability task (below) on two occasions suggested noncompliance. The mean age of the final 24 subjects was 20 yr, (SD 1.7); 6 subjects were female. Subjects were asked to abstain from consuming alcohol or caffeine for 24 h prior, and abstain from exercise for 8 h prior to the experiment.

Equipment

Two monochromators (**Fig. 1**) were used to control the lighting conditions shown to the subjects (Spex model 1681B for the

Table I. Experimental Order for the Unique Green and Unique Yellow Conditions for the Two Sessions.

	SESSION 1		SESSION 2	
TEMPORAL ORDER	**ALTITUDE**	**RATE (DURATION)**	**ALTITUDE**	**RATE (DURATION)**
Start*	0 m	—	0 m	—
Transition	—	0 m · min^{-1} (10 min)	—	762 m · min^{-1} (4.8 min), 0 m · min^{-1} (1 min), 610 m · min^{-1} (3 min), 0 m · min^{-1} (2 min)
Altitude 1†	0 m	—	5500 m	—
Transition	—	762 m · min^{-1} (4.8 min), 0 m · min^{-1} (1 min), 610 m · min^{-1} (3 min), 0 m · min^{-1} (2 min)	—	152 m · min^{-1} (3.6 min), 0 m · min^{-1} (6.4 min)
Altitude 2†	5500 m	—	0 m	—
Transition	—	152 m · min^{-1} (3.6 min)	—	0 m · min^{-1} (3.6 min)
End‡	0 m	—	0 m	—

Notes: * = Initial unique hue determination (green or yellow); † = Data collection for unique hue (green or yellow) and wavelength discrimination; ‡ = Recovery and vitals check (blood pressure, heart rate, and Spo_2 measurements).

reference stimulus, Spec Industries, Edison, NJ; and 2 x Acton, model SP-2300i configured as a double monochromator for the test stimulus, Princeton Instruments, Trenton, NJ). The monochromators were configured for 4 nm full-width-half-maximum (FWHM) bandpasses and calibrated for a centroid wavelength accuracy of ± 0.1 nm over a 510 to 590 nm range using a spectroradiometer (Photoresearch, model PR740, 0.25° spot size, Photoresearch, Topanga Canyon Place, CA). The monochromator outputs were coupled to single core, plastic (PMMA) fiber-optic cables that terminated at a viewing fixture that held the cable ends one degree apart. The cable ends each provided a one-degree diameter, circular stimulus field. An optical diffuser (Luminit, light shaping diffuser, 10° circular, Luminit, Torrance, CA) was placed directly in front of the fiber ends to ensure spatially uniform stimuli. Light at the entrance of each monochromator was provided by a phosphor-converted lime green LED (Luxeon, LXML-PM02, Lethbridge, Alberta, Canada), mounted on a heat sink (Endo star, 1-up) and powered by a programmable constant-current power supply (Agilent, model E3632A, Santa Clara, CA). The LED currents were automatically adjusted during the experiment to maintain constant stimulus luminance of 20 ± 0.40 cd · m^{-2} for all wavelengths presented. Ambient room lighting was from ceiling mounted fluorescent lamps (Philips model F32T8\TL841) providing roughly 120 lux horizontal illuminance and 50 lux vertical. The ambient lighting added less than 3% to the stimuli luminance. The stimulus surround was a matte white board illuminated by the ambient lighting to 14 cd · m^{-2}. Other surface finishes visible to the subjects were neutral colors (white, gray and black).

A Reduced Oxygen Breathing Device (Environics® Model 6202, Reduced Oxygen Breathing, ROBD2, Tolland, CT) was used to control oxygen concentrations for simulating high altitude. The ROBD2 controlled the proportions of air, nitrogen (N_2), and O_2 delivered to a breathing mask worn by the subject. The sea level condition provided 21% O_2 and the 5500 m altitude condition provided 9.5% O_2. Air mixtures flowed continuously to the mask which was fitted with a one-way expiratory valve to vent the excess gas not breathed by the subject. Note that published tables list 10.5% O_2 at 5500 m, so the simulated altitude indicated by the ROBD2 was probably greater than 5500 m.

Procedure

A nurse was present during every experimental session. Prior to starting a session, the nurse would take the subject's blood pressure, heart rate, and Spo_2 measurement to verify that they were healthy enough that day to complete the experiment.

The presentation orders of the four experimental conditions (two unique hues and two altitudes) were counterbalanced across subjects by changing the presentation order (Table I). Of the 24 possible ordering permutations of 4 conditions, there were 8 presentation orders meeting a procedural requirement that the same unique hue needed to be presented at both altitudes during an experimental session; yellow and green stimuli were presented to subjects during separate sessions on different days. Each of the 8 presentation orders was presented to 3 subjects in the course of running the study ($N = 24$).

Two measurement tasks were conducted for each color; unique hue wavelength determination and wavelength discriminability. The first task was designed to find the subject's unique green and unique yellow wavelengths. The individual's unique hues were used as the reference stimuli for the subsequent wavelength discriminability tasks within the session.

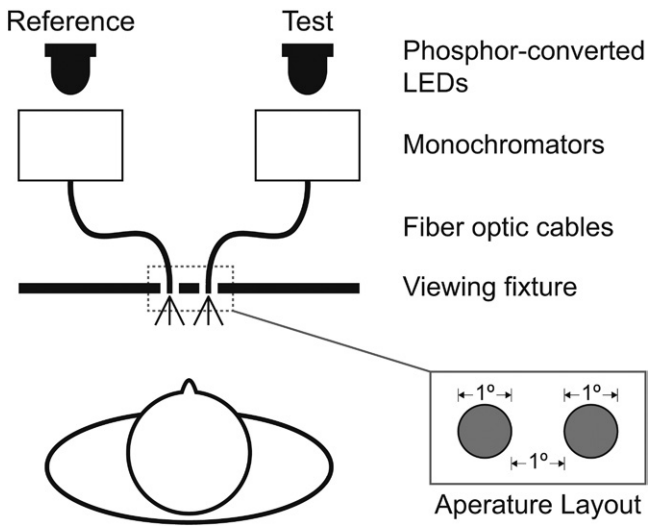

Fig. 1. Schematic diagram of the apparatus providing visual color stimuli for unique hue and wavelength discriminability measurements.

A double random staircase method was used to determine each subject's unique green and unique yellow.[5] For each unique hue, two staircases were generated from responses by the subject. For unique green, the staircases started at 505 and 540 nm, while for unique yellow they started at 570 and 585 nm. The subject would be randomly shown one of the staircase conditions for 2 s, after which they would give a forced-choice response as to whether or not they thought the light source was bluish or yellowish for unique green, or greenish or reddish for unique yellow. The center wavelength of the next stimulus, presented after a 3-s interval, depended on the subject's previous response; for unique green, if the response was bluish, then the next stimulus presented for that staircase had a center wavelength 3 nm longer, otherwise, the next stimulus was 3 nm shorter. For unique yellow, the procedure was the same except the wavelength would shift in steps of 0.5 nm. Pilot tests revealed that these step sizes corresponded to roughly the same range of subject responses where the two wavelengths farthest from the center had a high probability of being judged different than the matching stimulus. Trials continued until there were 10 reversals in the direction of wavelength setting. Unique hue was calculated by averaging the reversals, starting at the third reversal, that led to stimuli that were farther from the starting wavelength, and then averaging the two randomly interleaved staircase results.[7] Unique green and unique yellow were each measured three times during each session, initially at sea level to determine the center wavelengths for the wavelength discriminability tests, and then again at sea level and at 5500 m. The reference stimulus was switched off for the entire unique hue test (Fig. 1).

Wavelength discriminability measurements followed the unique hue task. The test stimulus was set to one of five wavelengths centered about the subject's own unique hue wavelength (Fig. 1) in steps of, again, 3 nm for unique green, and 0.5 nm for unique yellow. The reference and test stimuli were switched on together for 2 s, after which the subject responded whether the two stimuli were the same or different. The next trial was presented 3 s later. Each subject made 150 wavelength comparisons at sea level (30 repetitions for each of the 5 test wavelengths) and 150 comparisons at simulated altitude.

Subjects were asked the following three questions every 30 trials during the wavelength discriminability test:

1. On a scale of 1 (low) to 5 (high) rate your desire to stop the experiment.
2. On a scale of 1 (low) to 5 (high) rate your confidence in answering same or different.
3. Describe any symptoms you are feeling, if any.

The attending nurse would monitor any problems with the breathing mask and would record subject responses, taking these into account to decide whether adjustments had to be made and if it was safe for the subject to continue. If at any time the subject would rate question 1 at a level 5, or if the nurse felt that continuing might harm the subject, the experiment would be stopped, and the subject would be given 100% O_2 until their vital signs came back to normal; three subjects required O_2 administration. Testing was suspended during hypoxic conditions for two subjects, one because the nurse deemed him to be displaying unsafe symptoms and the other upon the subject's request. Supplemental O_2 (100%) was administered through the breathing mask and the subjects quickly recovered. The subjects completed the session the following day. Another subject requested and received supplemental O_2 upon completing a session and also quickly recovered. Prior to leaving the facility, the nurse would collect another set of blood pressure, heart rate, and Spo_2 measurements to make sure that the subject was fully recovered. The nurse reported that no subject hyperventilated at any time during the study.

Statistical analyses

For each dependent variable, unique hue wavelength and discriminability, a repeated-measures ANOVA was performed with factors of color (green and yellow), altitude (sea level and 5500 m); subject was a random variable. Post hoc paired 2-sample Student's *t*-tests were performed to further investigate statistically significant ($P < 0.05$) interaction effects revealed by the ANOVAs. One-sample *t*-tests on the wavelength differences between the initial subsequent unique hue determinations at sea level were also performed for green and yellow unique hues.

The predictability of Spo_2 and pulse rate by wavelength discriminability, σ, were analyzed by linear regression. Subject self-reports were analyzed by repeated-measures ANOVAs following the same methods as done for unique hue wavelength and discriminability. A binomial proportion test aided in comparing the frequencies of reported symptoms between sea level and 5500 m.

RESULTS

The initial determinations of unique hues for each experimental session performed at sea level were not significantly different ($P > 0.05$) than the corresponding unique hue determinations at sea level in the experiment when subjects were wearing the breathing mask.

From the ANOVA on unique hue determinations color was highly significant [$F(1,23) = 1.2 \times 10^3$; $P < 0.0001$] because the unique hue wavelengths for yellow and green stimuli are widely separated. There was no main effect of altitude, but the interaction between altitude and color was statistically significant [$F(1,23) = 6.21$; $P = 0.020$].

Table II shows that the mean and median wavelengths associated with each unique hue were quite similar, indicating a normal distribution for the wavelength settings for both unique yellow and unique green. The standard deviations for unique green (the b-y channel) were approximately 4.5 times larger than that for unique yellow (the r-g channel) indicating much higher sensitivity to wavelength differences for unique yellow. Regarding the significant interaction between altitude and color in the ANOVA, the relative shifts in unique hue from sea level to 5500 m were in opposite directions for the b-y and r-g channels; unique green shifts to longer wavelengths while unique

Table II. Unique Hue Summary Statistics and *t*-Tests.

UNIQUE HUE	CHANNEL	ALTITUDE	MEDIAN	MEAN	SD	DELTA MEAN HUE		*t*-TEST
		m	nm	nm	nm	nm	1σ discriminability steps	Two-tail *P* value (d.f. = 23)
Green	b-y	0	524.5	525.2	7.2	2.3	0.55	0.068
		5500	526.2	527.5	8.5			
Yellow	r-g	0	578.4	578.4	1.7	-0.54	-1.06	0.016
		5500	578.1	577.9	1.6			

yellow shifts to shorter wavelengths. Student's *t*-tests revealed a nearly significant [$t(23) = 1.92$; two tail $P = 0.068$] shift to longer wavelengths at 5500 m for unique green (2.3 nm) and a significant shift to shorter wavelengths for unique yellow [$t(23) = -2.61$; two tail $P = 0.016$] despite a smaller wavelength shift (0.54 nm).

Wavelength discriminability was determined from the rate at which subjects reported the test stimulus as "different" from the reference stimulus. The "different" response rates ($r_{different}$) were complemented (i.e., changed to "no difference" rates) and normalized to unity producing values of y by Eq. 1. These transformed values were then fitted with a Gaussian distribution having two free parameters, the mean (peak wavelength, in nm) and width of the standard deviation (σ, in nm).

$$y = \frac{1 - r_{different}}{\max(1 - r_{different})} \qquad \text{(Eq. 1)}$$

The two-parameter curve fits for 81% of subjects had R^2 values greater than 0.9 and 93% had R^2 greater than 0.8. Two subjects were excluded from the final subject pool ($N = 24$) because their responses for one of the four conditions did not have a single peaked response that could be unambiguously represented with a Gaussian curve-fit. In these cases the R^2 values were less than 0.7 and the fitted Gaussian was highly sensitive to slight variations in the data, in one case giving nonsensical results ($\sigma = 13$ nm) and for the other case the response data had two widely separated, equal height peaks. The fitted peak wavelength estimates were submitted to a within-subjects, repeated measures ANOVA revealing one statistically significant effect, color [$F(1,23) = 6.5 \times 10^4$; $P < 0.0001$]; peak wavelengths for green and yellow were obviously very different at both sea level and at altitude. Since neither altitude nor its interaction with color was significant, peak wavelength was not used in any further analysis. The fitted σ values were used as a dependent variable in a separate within-subjects, repeated measures ANOVA. As with the peak wavelength, color was highly significant [$F(1,23) = 170.0$; $P < 0.0001$]. The value of σ was approximately 7.5 times greater for green than for yellow. Altitude was significant [$F(1,23) = 11.2$; $P = 0.0028$] as well as the interaction between color and altitude [$F(1,23) = 7.43$; $P = 0.012$]. Post hoc paired 2-sample Student's *t*-tests comparing σ at the two altitudes revealed a significant difference for both the b-y channel (green) and the r-g channel (yellow). **Table III** provides descriptive statistics for the discriminability task and the results of the Student's *t*-test. Exemplar curves for green and yellow test stimuli are shown for one subject (#23) in **Fig. 2**.

The systematic difference of σ for green (1.45 to 1.66) and for yellow (0.52 to 0.61) shown in Table III affects the statistical power of the unique hue ANOVA. To better evaluate the interaction between color and altitude, these systematic differences in variance were minimized by converting wavelengths associated with unique hue from physical units, λ in nm, to a unitless psychophysical scale λ_p. The λ values associated with the green stimulus were divided by the average discriminability for unique green (4.23 nm) and the unique yellow hue measurements were divided by the average discriminability for unique yellow (0.56 nm). These λ_p values replaced λ as the dependent variable in another two-color-by-two-altitude, within-subjects, repeated measures ANOVA.

This ANOVA again revealed a highly significant difference between color [$F(1,23) = 2.1 \times 10^6$; $P < 0.0001$]. There was also a highly significant interaction between color and altitude [$F(1,23) = 16.39$; $P = 0.0005$]; in fact, a much higher statistical significance than using λ as the dependent variable**. Fig. 3** compares the findings from the unique hue determinations when using physical wavelength, λ, as the dependent variable and psychophysical discriminability, λ_p, as the dependent variable. In terms of perceptual steps in color differences, unique yellow showed a greater shift than unique green and in the opposite direction.

Spo_2 had a negative correlation with wavelength discriminability with *P*-values of 0.10 for green and 0.010 for yellow stimuli for a linear relationship (**Fig. 4**). Removal of the outlier for yellow wavelength discriminability near 1.4 nm maintained

Table III. Wavelength Discriminability Grouped by Color and Altitude.

COLOR	COLOR CHANNEL	ALTITUDE [m]	MEDIAN WAVELENGTH DISCRIMINABILITY (λ)	MEAN WAVELENGTH DISCRIMINABILITY (λ)	SD (σ)	DELTA WAVELENGTH DISCRIMINABILITY (altitude – sea level)		*t*-TEST
			nm	nm	nm	nm	%	One-tail *P* value (d.f. = 23)
Green	b-y	0	3.74	3.89	1.45	0.68	16	0.0027
		5500	4.41	4.57	1.66			
Yellow	r-g	0	0.46	0.52	0.14	0.10	17	0.0022
		5500	0.55	0.61	0.20			

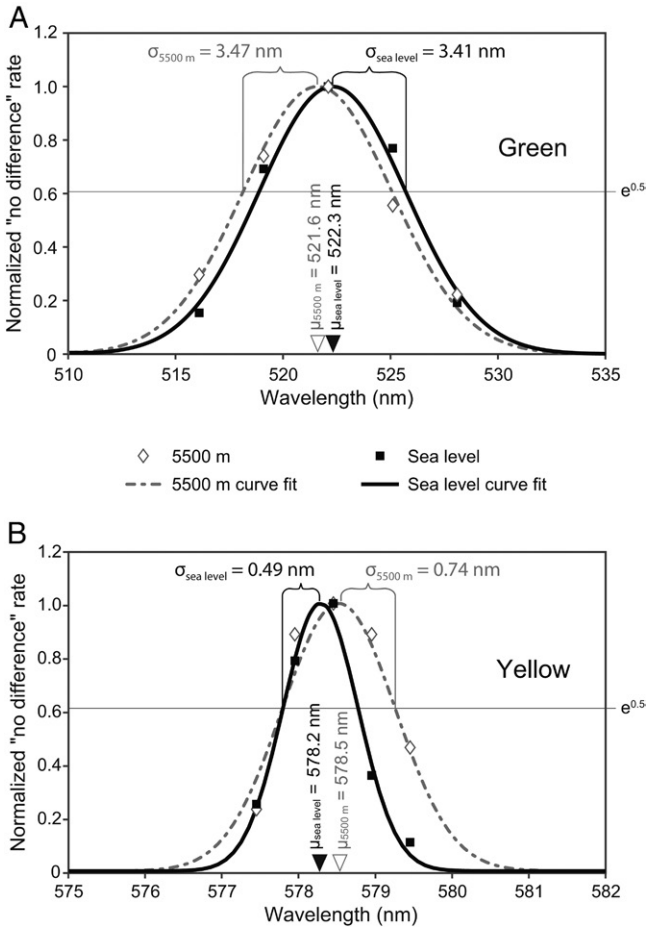

Fig. 2. Exemplar, two-parameter (mean and σ) Gaussian curve fits for subject number 23. Discriminability was operationally defined as the wavelength width (in nanometers) of 1 SD (σ) of the fitted distribution as indicated on the plots.

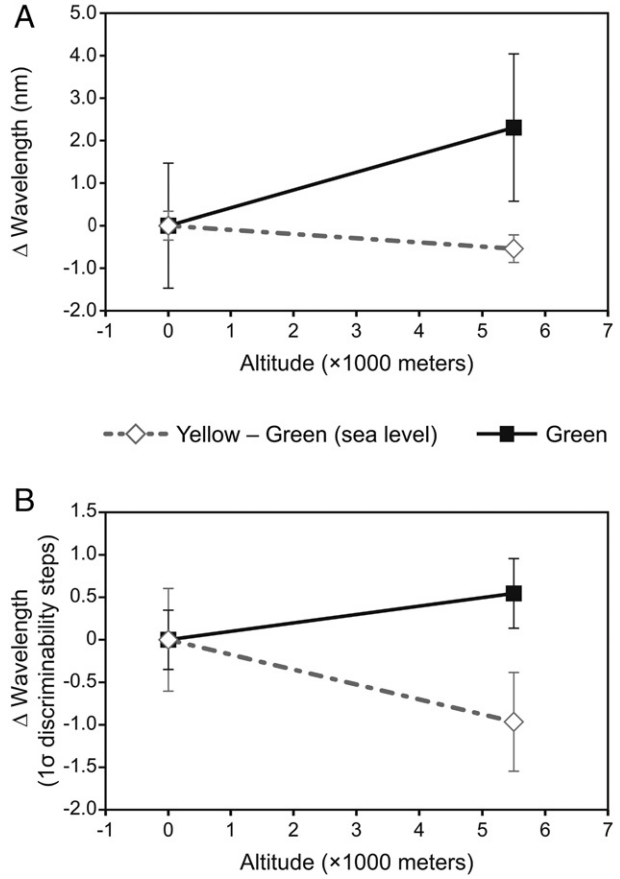

Fig. 3. Unique hue change for yellow and green stimuli plotted in physical wavelength units (λ, top) and psychophysical discriminability units (λ_p, bottom) at sea level and 5500 m. Error bars show SEM.

a significant linear relationship (P-value = 0.027). Pulse rate measurements have a positive correlation, but without a significant slope.

Self-reported desire to stop the experiment was low for all conditions, with slightly higher values at altitude [$t(23) = -2.73$; $P = 0.012$]. On average, subjects responded 1.21 out of 5 for sea level, and 1.38 out of 5 for 5500 m.

Average subject confidence in performing the discriminability task decreased at altitude [$t(23) = 3.61$; $P = 0.0015$] with average rating of 3.79 out of 5 for sea level, and 3.40 out of 5 for 5500 m. Confidence levels also decreased as the subject completed more trials in a session, going from 3.86 at trial 30 to 3.36 at trial 150 across both altitudes [$F(4,92) = 11.22$; $P < 0.0001$].

Table IV shows the symptoms and number of subjects reporting them during sea level and 5500 m experimental conditions. The number of subjects reporting no symptoms was significantly less at sea level then at altitude ($P < 0.0001$ for the binomial proportion test where $p_{sea\ level} = 32/52$ and $p_{altitude} = 6/52$).

DISCUSSION

Restricting the level of oxygen in respiratory air induces several interrelated physiological consequences. For example, capnic status, not monitored in this experiment, was likely lower at altitude than at sea level due to some degree of compensatory hyperventilation. Therefore, restricted O_2 in respiratory air, as employed in this experiment, may not isolate the effects of hypoxia alone on color vision. Color vision is also affected by restricted oxygen in respired air, which was the topic of interest in the present study.

Among the changes to color vision, reduced respiratory oxygen impairs one's ability to perceive hue differences; however, there have been mixed findings regarding differential effects of hypoxia on the r-g and b-y spectral opponent channels. The Farnsworth-Munsell 100-Hue (FM100) test[9] is primarily used to assess congenital or acquired losses in the two color channels, but it has also been used to assess the effects of hypoxia on hue discriminability.[18,31,32] All of these studies have shown a loss in hue discriminability in the b-y channel (tritan axis) under hypoxic conditions ranging in altitude from 3658 to 5486 m. However, Vingrys and Garner[33] found more general losses at a simulated altitude of 3658 m and argued that those studies showing a decrement in color difference sensitivity due to hypoxia have required subjects to perform color vision tests at low light levels which have been shown many times to affect overall visual sensitivity.[2,4,21] Since the FM100 is designed to test hue discriminability at high levels of daylight-simulating illumination, low light levels may differentially affect the

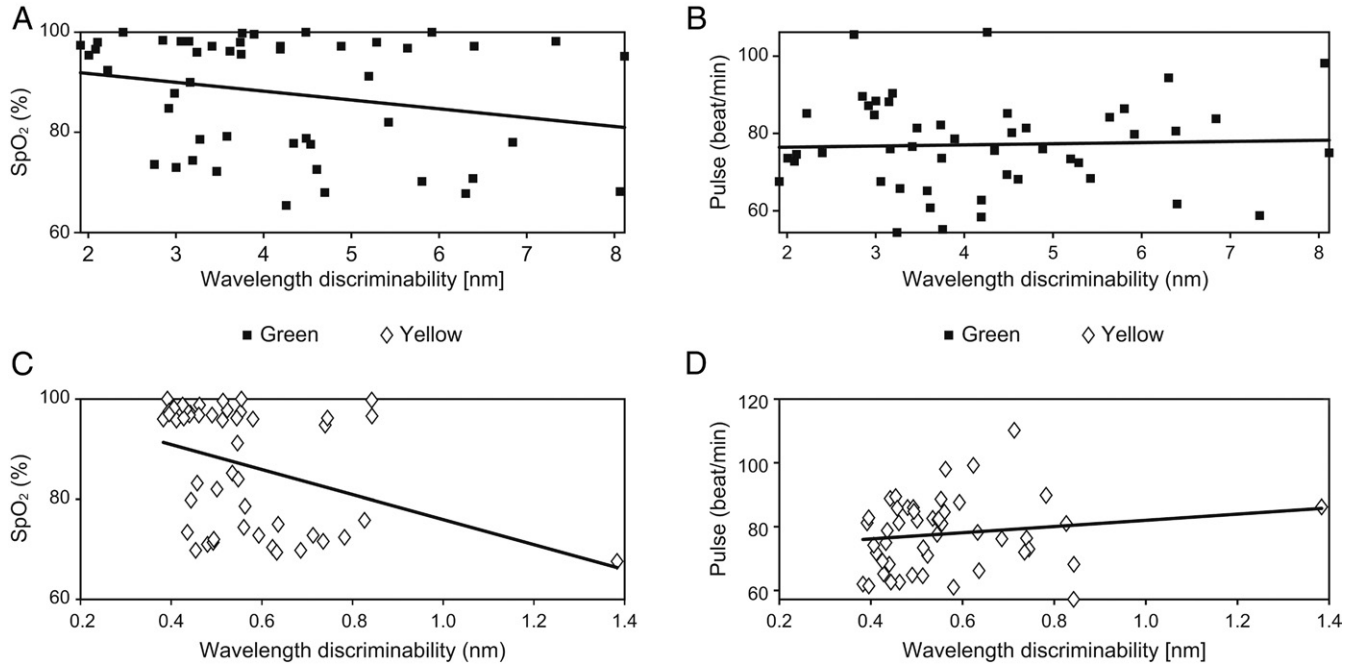

Fig. 4. Regressions of Spo_2 (A, C) and pulse rate (B, D) as linear functions of wavelength discriminability for the green and the yellow stimuli. Discriminability was measured in terms of σ.

discriminability of color discs used to assess tritan losses relative to those used to assess protan and deutan losses.[33] In fact, Knoblauch et al.[19] showed this to be true, namely, that lowering the light level differentially affected hue errors of color normal observers on the tritan axis. It should be noted, however, that at least two studies have shown losses on the tritan axis under bright, daylight conditions at an altitude of 3000 m[18] and at 5486 m.[32] Both of these studies were conducted under conditions of chronic exposure to hypoxia, however. Thus, even though hypoxia may differentially affect the b-y channel relative to the r-g channel, those differences may be exaggerated at low light levels or under chronic exposures to hypoxia when using the FM100.

Like the FM100, congenital deficiency in trichromacy can be assessed by an anomaloscope.[16] Matches between a yellow reference field and a test field comprised of two, red and green, primaries are presented to an observer and the ratio of the red and green radiances indicates relative photon absorption by the L- and M-cone, respectively. An observer with an anomalous photopigment will exhibit a different ratio than color normal observers. For example, a deutan with an anomalous M-cone photopigment will require more green primary radiance than a color normal to make an anomaloscope match. At altitude (1000 to 5000 m) one study showed the relative contribution of the green primary in anomaloscope matches needed to be greater relative to sea level (e.g., Richalet et al.[26]). These findings would suggest that the probability of photon absorption by the M-cone photopigment was compromised, analogous to matches made by a deuteranomalous trichromat.

On the surface, the findings from Richalet et al.[26] appear to be consistent with other studies of hypoxia using red and green primaries to make flicker matches. In these studies, a red and a green primary are rapidly alternated, and the radiance of one primary is carefully modulated until the alternating field no longer appears to flicker. If M-cone photopigment absorption was compromised relative to the L-cone photopigment under hypoxia, more green primary would be needed in the flicker match. In fact, Hovis[15] showed that under hypoxia more green primary was needed at altitude than at sea level to make a flicker match. Moreover, Schellart et al.[30] showed that the green primary relative to the red primary varies systematically with the available O_2 during flicker matches, ranging from 100 to 10% normobaric O_2; again, under hypoxia there was a relative loss in M-cone sensitivity. It should be made clear, however, that flicker photometry results, unlike the anomaloscope results, do not necessarily mean that hypoxia affects the M-cone photopigment. Flicker matches require neural interactions in the retina and brain and it seems clear from the electroretinographic (ERG) studies like those performed by Schatz and

Table IV. The Types of Symptoms and the Number of Subjects Reporting Them During Sea Level and 5500 m Experimental Conditions.

SYMPTOM	SEA LEVEL	5500 m
Drowsiness	18	26
Lightheadedness	3	19
Difficulty focusing	5	11
Fidgetiness	0	7
Nausea	1	5
Headache	1	5
No symptoms*	32	6

* Statistically different ($P < 0.0001$).
Note: The same 26 subjects, including the 2 subjects excluded from the ANOVA analyses, reported for each of the 4 experimental conditions yielding 52 total opportunities to report symptoms for each altitude.

colleagues[27–29] that hypoxia affects neural activity in the retina more than photoreceptor photopigments.

To rationalize the findings from Richalet et al.,[26] and those from Hovis,[15] Shellart et al.,[30] and Schatz et al.[27–29] one must look to potential artifacts in the anomaloscope measurements by Richalet and colleagues. They measured the red-green ratio at sea level and at altitude using a portable device containing red and green primaries produced by two types of LEDs. The luminous output and spectral distribution (peak wavelength and FWHM) of LEDs varies with temperature. Red LEDs in particular are affected by temperature, shifting to shorter wavelengths at lower temperatures. At altitude the ambient temperatures during anomaloscope measurements in the Richalet et al.[26] experiment were almost certainly lower than they were at sea level. If Richalet and colleagues did not take steps to ensure constant stimulus conditions at both sea level and altitude, more of the green primary would have been required to make the anomaloscope matches because the red LEDs would have shifted to shorter wavelengths at altitude. Absent calibration, the increased amount of green primary would be interpreted as a change in M-cone photopigment under hypoxia.

Compromised M-cone neural activity by hypoxia would not only affect flicker photometry and ERG recordings, hypoxia should also, as shown in the present study, cause a shift to shorter wavelengths associated with unique yellow. A reduction in M-cone input relative to the L-cone input to the r-g spectral opponent channel would shift the wavelength at which the red and green signals are equal, that is, the channel cross point associated with unique yellow. Similarly, a reduction in M-cone neural activity by hypoxia would reduce the magnitude of the yellow signal in the b-y spectral opponent channel, where the yellow signal represents the sum of the L-cone and M-cone responses. This reduction in M-cone neural activity would reduce the yellow signal from the b-y channel and would, consistent with present results, shift the cross point associated with unique green to longer wavelengths. Thus, the present results are consistent with the conclusions by Vingrys and Garner[33] that both the r-g and b-y color channels are affected by hypoxia.

Compromised neural activity in the retina from hypoxia not only causes systematic changes in color vision, but it causes greater variability in psychophysical measurements as well. Indeed, the systematic loss in hue discriminability with the FM100 is, by definition, a reflection of greater psychophysical variability. Greater psychophysical variability is also associated with anomaloscope measurements. Vingrys and Garner[33] showed, for example, an increased variability at altitude when adjusting the red-green radiance ratio to match a yellow reference light. The present results also show loss in discriminability with altitude (Table III). The change in discriminability, σ, associated with unique green and with unique yellow were both about the same (16% and 17%, respectively), suggesting a general loss in neural homology with color stimuli.

It has been commonly observed[12,14,25] that the biomarkers Spo_2 and heart rate are poor indicators of changes in an individual's neural activity due to hypoxia. The present study reinforces these observations (Fig. 4), namely that these two biomarkers cannot be exclusively used to assess hypoxic-induced changes in color vision.

It has also been observed that self-reported symptomology associated with hypoxia varies among individuals. Some people feel light headed while others feel fatigued, for example. Support for this conclusion was offered through the results of the present study. There is, however, a clear difference between altitude and sea level in terms of the reporting of *no* symptoms. Despite idiosyncratic types of symptoms, the absence of symptoms was clearly a differentiator between altitude and sea level. As noted by Harding and Mills,[11] self-reports are often the earliest indicators of hypoxia.

Hypoxic-induced changes in color vision are small and difficult to measure.[3] In the present study, assessments of unique hue and hue discriminability were more difficult to acquire than self-reports even though there were clear, statistically reliable differences in unique hues and hue discriminability at sea level and altitude. The small color changes were consistent with other published studies (e.g., Vingrys and Garner[33]) and support the inference that the M-cone neurons are differentially compromised by hypoxia.[26,30] Despite the reliability of these measurements and the added insight into the underlying neural mechanisms compromised by hypoxia, these measurements are time consuming and, importantly, observers are completely unaware of these hypoxic-induced changes in color perception. With the goal of developing a reliable, rapid and practical test for assessing hypoxia in warfighters, measurements of changes in color vision are probably not particularly useful. Rather, to meet that goal, systematic investigations into the measurement of warfighter symptomology under hypoxia may be the most useful approach for the future. This suggestion is consistent with the recommendation by Phillips and colleagues[24] as an approach to reexamine the Time of Useful Consciousness (TUC) table used by the military as a guide for estimating the "effective performance time at altitude."

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REFERENCES

1. Bailey IL, Lovie-Kitchin JE. Visual acuity testing. From the laboratory to the clinic. Vision Res. 2013; 90:2–9.

2. Connolly DM, Barbur JL. Low contrast acuity at photopic and mesopic luminance under mild hypoxia, normoxia, and hyperoxia. Aviat Space Environ Med. 2009; 80(11):933–940.
3. Connolly DM, Barbur JL, Hosking SL, Moorhead IR. Mild hypoxia impairs chromatic sensitivity in the mesopic range. Invest Ophthalmol Vis Sci. 2008; 49(2):820–827.
4. Connolly DM, Hosking SL. Oxygenation state and mesopic sensitivity to dynamic contrast stimuli. Optom Vis Sci. 2009; 86(12):1368–1375.
5. Cornsweet TN. The staircase method in psychophysics. Am J Psychol. 1962; 75(3):485–491.
6. Davies AJ, Morris D, Kalson N, Wright A, Imray C, Hogg C. Changes to colour vision on exposure to high altitude. J R Army Med Corps. 2011; 157(1):107–109.
7. Dixon WJ, Massey FJ, Jr. Introduction to statistical analysis. New York (NY): McGraw-Hill Book Company, Inc; 1951.
8. Ernest JT, Krill A. The effect of hypoxia on visual function: Psychophysical studies. Invest Ophthalmol. 1971; 10(5):323–328.
9. Farnsworth D. The Farnsworth-Munsell 100-hue and dichotomous tests for color vision. JOSA. 1943; 33(10):568–578.
10. Greenstein VC, Hood DC, Ritch R, Steinberger D, Carr RES. (Blue) cone pathway vulnerability in retinitis pigmentosa, diabetes and glaucoma. Invest Ophthalmol Vis Sci. 1989; 30(8):1732–1737.
11. Harding RM, Mills FJ. Aviation medicine. Problems of altitude I: hypoxia and hyperventilation. Br Med J (Clin Res Ed). 1983; 286(6375): 1408–1410.
12. Harshman SW, Geier BA, Fan M, Rinehardt S, Watts BS, et al. The identification of hypoxia biomarkers from exhaled breath under normobaric conditions. J Breath Res. 2015; 9(4):047103.
13. Hood DC, Benimoff NI, Greenstein VC. The response range of the blue-cone pathways: a source of vulnerability to disease. Invest Ophthalmol Vis Sci. 1984; 25(7):864–867.
14. Hovis JK, Milburn NJ, Nesthus TE. Hypoxia, color vision deficiencies, and blood oxygen saturation. J Opt Soc Am A Opt Image Sci Vis. 2012; 29(2):A268–A274.
15. Hovis JK, Milburn NJ, Nesthus TE. Trichromatic and dichromatic relative sensitivity to green light in a mild hypoxic environment. Aviat Space Environ Med. 2013; 84(11):1125–1130.
16. Hsia Y, Graham C. Color blindness. Vision and visual perception. New York: Wiley New York; 1965: 395–413.
17. Ishihara S. Tests for colour-blindness. 15th ed. London: H. K. Lewis; 1960.
18. Karakucuk S, Oner A, Goktas S, Siki E, Kose O. Color vision changes in young subjects acutely exposed to 3,000 m altitude. Aviat Space Environ Med. 2004; 75(4):364–366.
19. Knoblauch K, Saunders F, Kusuda M, Hynes R, Podgor M, et al. Age and illuminance effects in the Farnsworth-Munsell 100-hue test. Appl Opt. 1987; 26(8):1441–1448.
20. Leid J, Campagne JM. Colour vision at very high altitude. Color Res Appl. 2001; 26(Suppl. 1):S281–S283.
21. McFarland AR, Halperin M. The relation between foveal visual acuity and illumination under reduced oxygen tension. J Gen Physiol. 1940; 23(5):613–630.
22. McFarland RA, Evans JN. Alterations in dark adaptation under reduced oxygen tensions. Am J Physiol. 1939; 127(1):37–50.
23. Petrassi FA, Hodkinson PD, Walters PL, Gaydos SJ. Hypoxic hypoxia at moderate altitudes: review of the state of the science. Aviat Space Environ Med. 2012; 83(10):975–984.
24. Phillips JB, Drummond LA, Robinson F, Funke ME. Hypoxia: exposure time until significant performance effects. Dayton (OH): Naval Medical Research Unit Dayton; 2016. Report No.: 16-19.
25. Phillips JB, Hørning D, Funke ME. Cognitive and perceptual deficits of normobaric hypoxia and the time course to performance recovery. Aerosp Med Hum Perform. 2015; 86(4):357–365.
26. Richalet J-P, Duval-Arnould G, Darnaud B, Keromes A, Rutgers V. Modification of colour vision in the green/red axis in acute and chronic hypoxia explored with a portable anomaloscope. Aviat Space Environ Med. 1988; 59(7):620–623.
27. Schatz A, Breithaupt M, Hudemann J, Niess A, Messias A, et al. Electro-retinographic assessment of retinal function during acute exposure to normobaric hypoxia. Graefes Arch Clin Exp Ophthalmol. 2014; 252(1): 43–50.
28. Schatz A, Fischer MD, Schommer K, Zrenner E, Bartz-Schmidt K-U, et al. Attenuation of S-cone function at high altitude assessed by electro-retinography. Vision Res. 2014; 97:59–64.
29. Schatz A, Willmann G, Fischer MD, Schommer K, Messias A, et al. Electroretinographic assessment of retinal function at high altitude. J Appl Physiol. 2013; 115(3):365–372.
30. Schellart NA, Pollen M. Effect of dysoxia and moderate air-hyperbarism on red-green color sensitivity. Undersea Hyperb Med. 1997; 24(1):7–13.
31. Smith VC, Ernest JT, Pokorny J. Effect of hypoxia on FM 100-hue test performance. Mod Probl Ophthalmol. 1976; 17(1):248–256.
32. Tekavčič-Pompe M, Tekavčič I. Color vision in the tritan axis is predominantly affected at high altitude. High Alt Med Biol. 2008; 9(1):38–42.
33. Vingrys AJ, Garner LF. The effect of a moderate level of hypoxia on human color vision. Doc Ophthalmol. 1987; 66(2):171–185.
34. Willmann G, Ivanov IV, Fischer MD, Lahiri S, Pokharel RK, et al. Effects on colour discrimination during long term exposure to high altitudes on Mt Everest. Br J Ophthalmol. 2010; 94(10):1393–1397.