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# Color Perception Is Mediated by a Plastic Neural Mechanism that Is Adjustable in Adults

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## Summary

**An intensely debated issue concerning visual-experience-dependent neural plasticity is whether experience is required only to maintain function or whether information from experience is used actively, relieving the necessity to hard-wire all connections and allowing adaptive adjustments. Here, an active role for experience is demonstrated in circuits for color vision. Chromatic experience was altered using colored filters. Over days there was a shift in color perception, as measured by the wavelength of unique yellow, which persisted 1–2 weeks after the filters were discontinued. Moreover, color-deficient adults were shown to have altered weightings of inputs to chromatic channels, demonstrating a large neural adjustment to their inherited photopigment defect. Thus, a neural normalization mechanism for color perception, determined by visual experience, operates to compensate for large genetic differences in retinal architecture and for changes in chromatic environment.**

## Introduction

Visual-experience-dependent neural plasticity is a recognized property of the developing mammalian cortex (Wiesel and Hubel, 1963). Early on, it was assumed that this malleability was restricted to the preadult; however, evidence for some degree of neural plasticity that remains throughout adulthood has accumulated (Baseler et al., 1999; Buonomano and Merzenich, 1998; Chino et al., 1992; Gilbert and Wiesel, 1992; Kaas et al., 1990). A fundamental question, which has been under intense debate, concerns the purpose of the ability of sensory processes to remodel their function in response to changes in experience (Hubel, 1988). An engaging hypothesis is that plasticity allows sensory experience to act as a guide, relieving the necessity to hard-wire all connections during development according to genetic instructions and, in adult cortex, allowing remodeling of neural connections to make adaptive adjustments, compensate for minor genetic abnormalities, and recover from damage (Albright et al., 2000; Andrews, 1964; Kaas et al., 1990). However, detractors argue that while activity is necessary for maintenance of proper neural function, convincing experimental evidence is lacking for a guiding role in which the information gained from visual experience is actively used by the nervous system in the formation of the proper circuits and in adjusting their characteristics (Crowley and Katz, 1999, 2000; Hubel, 1988; Yuste and Sur, 1999). Recent developments suggest that color vision may provide the first example where it is possible to demonstrate that information from experience is actively used to guide the neural function underlying a basic sensory capacity.

It is becoming appreciated that there is amazing variation in the ratio of L to M cones in the retina (Carroll et al., 2000; Hagstrom et al., 1998; Kremers et al., 2000; Roorda and Williams, 1999; Wesner et al., 1991), yet individuals with very different ratios do not seem to have correspondingly different color vision (Brainard et al., 2000; Pokorny et al., 1991). Considering that visual experience is similar for most people, one possible explanation is that, perhaps, there is a plastic neural normalization mechanism that allows the visual system to use information from experience in order to compensate for individual differences in the cone ratio and make perception uniform (Mollon, 1982; Pokorny and Smith, 1977).

In the series of experiments presented here, we tested the hypothesis that there is a plastic normalization mechanism for color perception. We demonstrate that a cortical mechanism uses information acquired from visual experience to adjust the human red/green chromatic circuits. This compensates both for large idiosyncratic genetic differences in retinal architecture and for changes in the chromatic environment. First, L:M cone ratios were estimated for 32 normal individuals using a newly developed procedure that combines the techniques of electroretinogram (ERG) flicker photometry and examination of photopigment gene sequences (Carroll et al., 2000). We found an enormous range in estimated cone ratio, but no corresponding variation in color perception. This is consistent with the presence of a plastic mechanism that compensates for differences in genetic makeup by normalizing to a shared environment. That hypothesis predicts that a person raised in an altered chromatic environment should have altered color perception. Historically, except for short-term contrast effects and contingent form-color aftereffects (e.g., the McCollough effect [Stromeyer, 1978]), vision scientists have not usually considered color vision to be modifiable by experience. However—amazingly—we found that prolonged exposure to chromatic alteration in adults induced long-term changes in color perception. The effects of monocular chromatic alteration transferred to the unexposed eye, as predicted if the neural substrate for the normalization mechanism resides in the cortex. A fourth set of experiments provided an independent test for the presence of a neural mechanism capable of compensating for altered chromatic input. We used a combination of genetic analysis and color vision psy-

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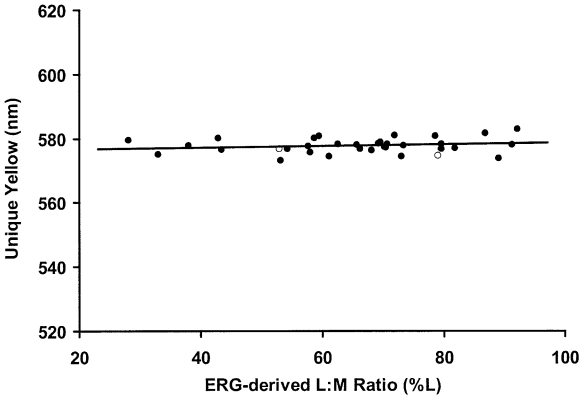


Figure 1. Unique Yellow as a Function of L:M Cone Ratio

ERG spectral sensitivity functions were obtained from 32 subjects with normal color vision. An average of three to six unique yellow measurements, obtained using the two-alternative forced-choice method, is shown for each subject (closed circles). Error bars represent ±1 SEM, and are smaller than the size of the data points. Solid line is a best-fitting linear regression, with $r^2 = 0.0321$, $p = 0.32$. Two additional subjects (open circles) are shown from a previous study in which L:M ratio was determined using both the ERG method and an adaptive optics technique (Brainard et al., 2000; Roorda and Williams, 1999), and unique yellow was measured using the method of adjustment technique.

chophysics to determine the characteristics of inputs to the red/green chromatic channel in deuteranomalous trichromats and found that the inherited difference in the spectral sensitivity of their photopigments is associated with a large shift in the neural weighting of the inputs to their red/green chromatic mechanism. This indicates that the nervous system has made a large compensatory adjustment in response to the abnormal input that results from having anomalous photopigments. Results from these experiments demonstrate that information gathered from experience can be used to actively optimize the neural circuits for this fundamental perceptual ability and that the plasticity persists in adults.

## Results

### Color Vision Is Uniform Despite Enormous Variability in L:M Cone Proportions

The wavelength that appears uniquely yellow (neither reddish nor greenish) was determined for 32 subjects with normal color vision. This is a standard measure of color appearance that can be determined easily and with great precision. In the same subjects, estimates of the ratio of L to M cones were obtained using ERG flicker photometry. Previously, L:M cone ratio estimates from this ERG technique were shown to be reliable within ± 2.5% L (Carroll et al., 2000). Corrections were made from an analysis of each subject's photopigment genes to eliminate errors in the estimates of cone ratio that would derive from normal variations in cone spectral sensitivities (Carroll et al., 2000). Dramatic individual differences in L:M ratio were observed (from about 10:1 to 0.4:1; a 25-fold range). However, we found no associated differences in the wavelength of unique yellow (Figure 1).

In earlier experiments, it was reported that two subjects who had different cone ratios, as measured by direct imaging of the cone mosaic using adaptive optics (Roorda and Williams, 1999), did not show a corresponding difference in unique yellow (Brainard et al. 2000). Also, in previous experiments that used psychophysical flicker photometry to estimate cone ratio, differences in unique yellow did not correlate with large differences in estimated cone ratio. This was true both in heterozygous carriers of color vision defects, who are known to have highly skewed cone ratios (Jordan and Mollon, 1997; Miyahara et al., 1998), and in color normal individuals (Pokorny et al., 1991). The method used here eliminates errors inherent in earlier psychophysical measurements, and it is highly efficient; testing a large sample of normal men allowed a statistical evaluation of the hypothesis that there is a correlation between unique yellow and cone ratio. There is no significant correlation ($r^2 = 0.0321$; $p = 0.32$) (Figure 1).

One might expect, in a very simple model (Cicerone, 1987; Otake and Cicerone, 2000), that someone with a preponderance of L cones would have a sensation of redness for wavelengths others see as yellow and thus choose a much shorter wavelength for unique yellow; conversely, people with relatively high numbers of M cones would choose longer wavelengths for unique yellow. The observed lack of association between variation in cone ratio and unique yellow could be accounted for if the relative weighting of the inputs to the red/green chromatic channel is adjustable. The nervous system could use information gathered from experience to make adjustments in the red/green chromatic mechanism, perhaps normalizing to the "average white" of the surroundings (Mollon, 1982; Pokorny and Smith, 1977, 1987) to compensate for the individual differences in the retinal mosaic and thus make perception uniform. This hypothesis is tested in the following experiments.

### Long-Term Changes in Color Perception in Response to Chromatic Alteration of Visual Experience

The familiar transient aftereffects in color perception induced by short periods of chromatic adaptation have been studied extensively (Jameson et al., 1979). There has also been a report that prolonged wearing of colored filters can induce changes in spectral sensitivity lasting for hours (Eisner and Enoch, 1982). However, the normalization model, introduced above, predicts that by restricting the wavelength content of the input to the visual system, it should be possible to induce changes in the red/green chromatic mechanism that are more enduring. To test this, we subjected three males (CL, YY, and JC) and one female (EM) to chromatically altered conditions for a series of days. Each subject's visual input was restricted by wearing colored contact lenses, by wearing goggles fitted with colored filters, or by spending time in a room where all light sources had been covered with colored filters. Each day, immediately prior to the introduction of the chromatically altered experience, unique yellow was measured. Following the chromatic alteration period (4, 8, or 12 hr), each subject spent the remainder of the day under illumination that was not altered from his normal routine. For example, in the 4 hr condition, the subject spent a total of 20

hr under normal illumination before beginning the next day's alteration period. Thus, the daily unique yellow measurements were not influenced by traditional color after effects, which last for only minutes (or hours at most).

As predicted by the normalization model, the result was that, over time, each subject's unique yellow shifted progressively further away from his or her baseline (Figure 2). Initially, the size of the shift was small; however, after many days of exposure, for example, to the red alteration conditions, the shift had grown so large that wavelengths previously called red came to be consistently called green in appearance. The absolute size of the shift varied between the subjects, presumably due to a combination of different filtering devices, different amounts of time spent filtered, and the activities performed while subjected to the altered conditions.

That it takes a long time to induce the change in perception, and that it persists for weeks in the absence of exposure to chromatic alteration, demonstrates an adaptive change in the nervous system. The persistence of the change in perception observed here is reminiscent of contingent form-color after effects (e.g., the McCollough effect, a color aftereffect produced when viewing black and white gratings after adaptation to colored bars of the same orientation [Stromeyer, 1978]), and at the cellular level, the neural mechanisms underlying these two phenomena may be related. However, the change in color perception described here is unique in that it is omnipresent and not contingent. The global normalization described here apparently compensates for the huge genetic variation in the ratio of L to M cones.

### A Cortical Locus for the Normalization Mechanism

One feature of the McCollough effect is that it is largely monocular (Stromeyer, 1978), indicating that it depends on mechanisms at a neural locus early in visual processing (e.g., the retina) prior to the point where information from the two eyes is combined. To determine the neural locus of the normalization mechanism observed here, we did two additional experiments. First, we used the ERG to record relative spectral sensitivities from subject JC. Figure 3A shows average spectral sensitivity functions from before/after and during the chromatic alteration period. The relative L and M cone contributions to spectral sensitivity measurements made before or after the 10 day alteration period did not differ from those taken within the alteration period ($p = 0.24$). The variability of these readings was not significantly different than that observed for multiple readings taken from a control subject who did not undergo chromatic alteration ($p = 0.25$; data not shown). These results indicate that the normalization effects are mediated by a mechanism beyond the outer retina.

In an additional experiment, one subject (YY) underwent monocular chromatic alteration, while the other eye was occluded (4 hr per day). Unique yellow was recorded from the occluded eye each day prior to the chromatic alteration period. As shown in Figure 3B, unique yellow shifted in the same direction that was observed with binocularly altered chromatic experience, though perhaps, to a lesser extent. This interocular transfer suggests that the normalization mechanism ex-

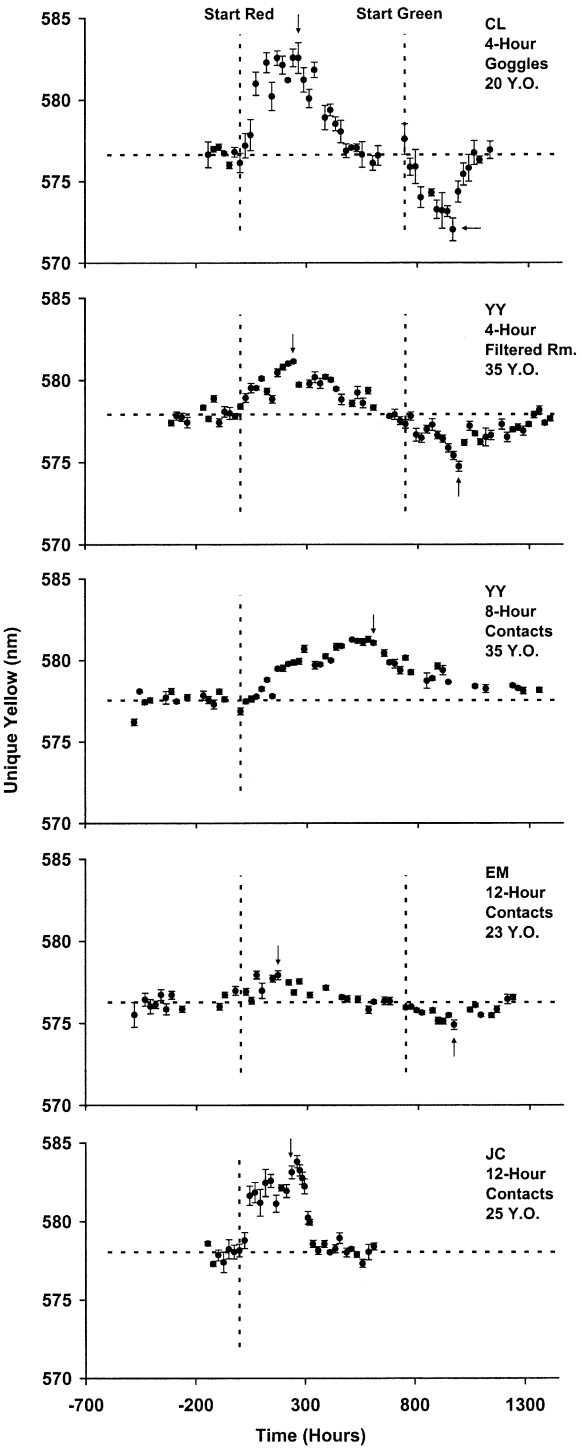


Figure 2. Long-Term Effects of Exposure to a Chromatically Altered Environment

Unique yellow settings from four subjects (five different data sets) are aligned relative to the start of their respective red (0 hr) and green (744 hr) alteration periods (vertical dashed lines). Horizontal dashed lines give the baseline unique yellow value for each subject. Arrows indicate last day of exposure to the altered environments, with subsequent data points illustrating the recovery of unique yellow to baseline. Error bars represent $\pm 1$ SEM, with five measurements taken during each recording session.

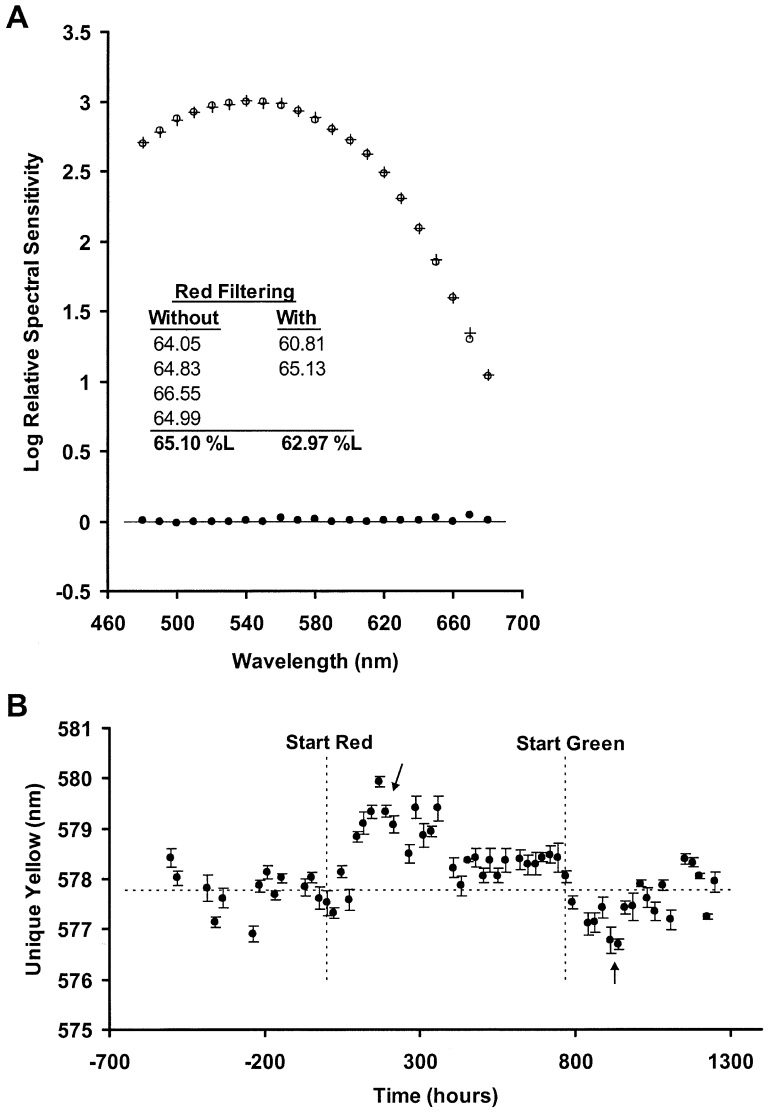


Figure 3. Experiments to Localize the Normalization Mechanism

(A) Subject JC's visual environment was filtered using red contact lenses for 12 hr a day. ERG spectral sensitivity functions were obtained before (two times), during (two times), and after (two times) the 10 day alteration period. The average ERG spectral sensitivity functions are shown (without filtering, crosses; with filtering, open circles). During the alteration period, the recordings were obtained in the morning, before the 12 hr chromatic alteration began. In addition, recordings were obtained after 5 days of the filtering regimen, after the shift in unique yellow had reached a maximum. Relative L and M cone contributions to spectral sensitivity measurements are given as percent L values (inset). The closed circles plot the absolute difference in sensitivity for the two conditions. A change in the percent L value induced by chromatic alteration would predict a systematic difference between the two conditions, which was not found. Thus it is unlikely that the change in perception as measured with unique yellow is mediated by a receptoral mechanism.

(B) Monocular chromatic alteration was used to investigate interocular transfer of the shift in unique yellow. Subject YY wore goggles containing a red filter over the left eye, while his right eye was occluded for 4 hr a day, for a period of 8 days. Unique yellow measurements were obtained from the occluded eye using the method of adjustment technique (closed circles; error bars ± 1 SEM; measurements per session = 5). Vertical dashed lines indicate the start of the red and green chromatic alteration periods, and arrows indicate the last day of exposure to the altered environments, with subsequent data points illustrating the recovery of unique yellow to baseline (horizontal dashed line, 577.72 nm).

ists, at least in part, in central visual pathways at a locus postsynaptic to where chromatic information from both eyes has been integrated.

### Cone Inputs to Chromatic Channels Have Adjustable Weighting Factors

The chromatic alteration experiments (above) demonstrate that long-term changes in chromatic experience induce a change in the weighting of the L and M cone inputs to the chromatically opponent red/green channel. This weighting value can be determined from knowing the spectral sensitivities of the cone pigments and the spectral location of unique yellow. As illustrated in Figure 4 (top panel), the average normal M and L pigment sensitivity curves (which peak near 530 and 560 nm, respectively) have equal quantal catches at a point intermediate between the peaks, about 545 nm. Theoretically, unique yellow is the point at which the L and M signals are balanced, thus if the weighting were equal to 1.00, unique yellow would be near 545 nm. However, the average value of unique yellow is 578 nm (Miyahara et al., 1998), indicating that the input of the L cone mechanism to the red/green chromatic channel has been adjusted, reducing its sensitivity by a gain factor of 0.64 (Figure 4, middle panel). This reduced gain of the L cone input is predicted from the idea that the nervous system adjusts itself by normalizing to the average spectral profile experienced at the level of the photoreceptors. Because of a combination of factors, including that short-to-middle wavelengths are preferentially absorbed by the lens and macular pigment and that the L pigment is slightly broader in its absorption spectrum, the M cones absorb fewer photons, on average, than the L cones. Knowing the precise ratio of L to M photopigment absorptions averaged over time requires exact information about the spectral characteristics of the average light reaching the eye. It is a combination of direct illumination and reflected light for which exact information is not available. However, all reasonable estimates of the average spectral distribution of light reaching the eye yield the result that M cones absorb fewer photons than do L cones. For example, the assumption that the char-

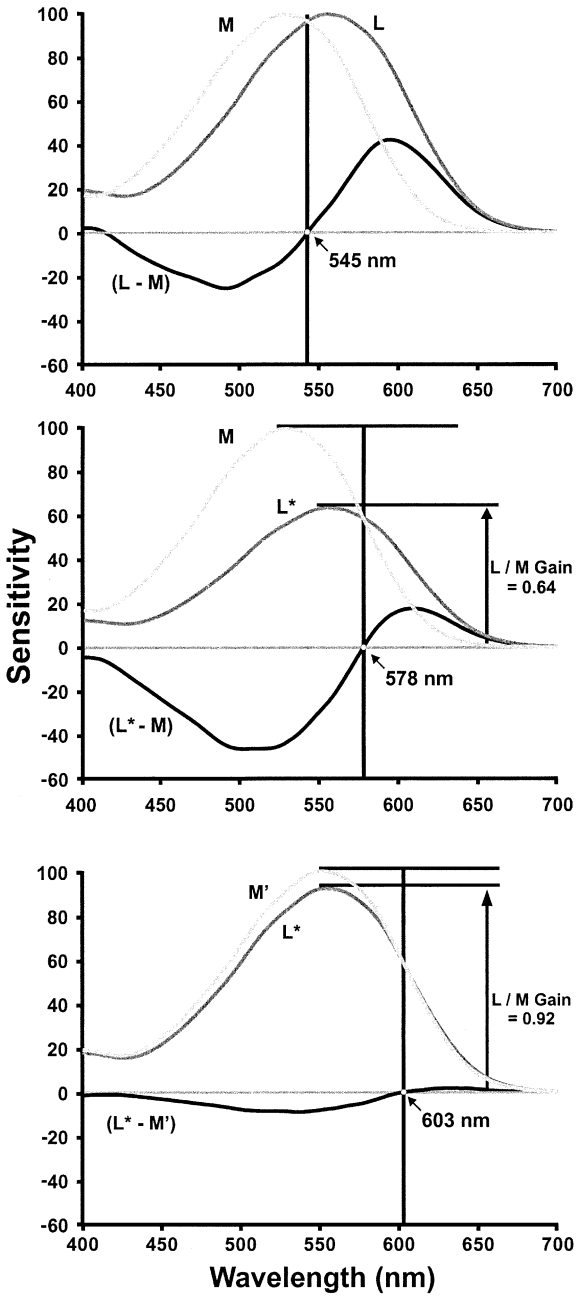


Figure 4. L and M Photopigment Sensitivity and Chromatic Opponent (L-M) Curves

Shown in the top panel are the "normal" L- and M-photopigment spectra, with $\lambda_{max}$ of 557.23 and 530 nm, respectively. The L-M opponent is calculated by subtracting the L and M sensitivity values. The L-M opponent curve crosses 0 at 545 nm. The middle panel illustrates that in order to obtain a cross point of 578 nm (the average value for unique yellow in the normal population), the L pigment curve needs to be adjusted downward by a gain factor of 0.64. This weighted L pigment curve is labeled L*. The L-M opponent (L*-M) in this case crosses at 578 nm. The bottom panel shows the L/M gain factor for a pair of deuteranomalous pigments (555.5 and 552.5 nm). The additional L pigment of the deutan is denoted as M', while the weighted L pigment curve is labeled as L*. The gain was adjusted until the cross point of the L-M opponent (L*-M') equaled subject JG's unique yellow of 603 nm.

acteristics of the light reaching the eye are similar to average daylight (Wyszecki and Stiles, 1982) predicts that, on average, the M cones absorb only about six to seven photons for every ten absorbed by the L cones. Thus, the hypothesis of a normalization mechanism in which the relative gain of the L and M cones is adjusted to be in equilibrium for the average illuminant would predict a compensating reduction in the relative weighting for the L cone input to the red/green chromatic mechanism on the order of 0.60–0.70, just as is observed here (Figure 4, middle panel).

The long-term chromatic alteration conditions, used above, produced changes in unique yellow that correspond to changes in gain of about ±10% after a 10 day exposure (Figure 2). This demonstrates that a plastic neural mechanism controls the gain; however, we would like to know how adjustable the gain is. The change in unique yellow induced by wearing colored lenses is largest during the first few days, and it appears to change more slowly at the end of a 2 week chromatic alteration period, as if it were approaching an asymptote (Figure 2). What would happen if the chromatic alteration was continued for a year, or if someone lived with abnormal chromatic input for his or her whole life? This can be investigated because people who live with abnormal chromatic input exist as a common occurrence. These are people with congenital color vision deficiencies. The most frequent color vision deficiency is deuteranomaly, in which normal M cone pigment is missing, but a reduced form of red/green color vision is based on two closely separated pigments with absorption peaks in the long-wavelength region of the spectrum (Figure 4, bottom panel).

By measuring unique yellow in deuteranomalous men whose inputs to the red/green chromatic mechanism are abnormal because of their congenitally aberrant photopigment, we were able to explore the degree to which the gain can be changed by a lifetime of abnormal input. Since the normal M pigment is replaced by a pigment with a more long-wavelength-sensitive absorption spectrum, there is much less of a difference in the average quantal catches of the cones responsible for red-green color vision in deuteranomalous men compared to normals (Figure 4, bottom panel). In normals, according to our theory, the reduced gain (0.64) of the L cone input is the result of a compensatory adjustment to the difference in average quantal catch rate that occurs because of the difference in the absorption spectra of the L and M cones. It has recently become possible, using molecular biological techniques, to determine the spectral locations of the pigments underlying color vision in individual deuteranomalous subjects (Carroll, 2002; Neitz et al., 1996; Shevell et al., 1998). Thus, the relative gain of the cone inputs can be calculated from the unique yellow settings (Figure 4). The spectral separation of the X-encoded photopigments in deuteranomals can vary from a maximum near 10 nm to a minimum of 1–2 nm. Thus, if the gain is fully adjustable by experience and can completely compensate for the photopigment difference, then for deuteranomalous individuals with pigments that have smaller spectral differences than normal, the gain should approach 1.00 as the absorption spectra approach being identical. This is a very robust prediction of the normalization hypothesis, be-

Table 1. Unique Yellow Values and L/M Gains for Three Groups of Deuteranomalous Men

| Subject | $\lambda_{max}$ (nm) L | $\lambda_{max}$ (nm) M′ | Estimated Difference | Unique Yellow (nm ± SEM) | Gain | | Predicted Unique Yellow w/ No Gain Change (nm) |
|---|---|---|---|---|---|---|---|
| JG | 555.5 | 552.5 | 3.0 | 602.6 ± 1.5 | 0.92 | | >700 |
| JW | 555.5 | 548.5 | 7.0 | 607.0 ± 3.4 | 0.80 | | 645 |
| GA | 559 | 548.5 | 10.5 | 605.4 ± 2.2 | 0.74 | | |
| MA | 559 | 548.5 | 10.5 | 613.4 ± 1.6 | 0.70 | 0.72 | |
| SL | 559 | 548.5 | 10.5 | 610.8 ± 0.8 | 0.71 | | 624 |

cause the value of 1.00 projected as the limit does not depend on any assumptions about the average spectral distribution of light reaching the eye.

Molecular genetics was used to determine the sequence and arrangement of the X-encoded visual pigments in five deuteranomalous men. Shown in Table 1 are estimates of the spectral separation of their pigments and their respective unique yellow settings. The results show that deuteranomalous individuals have very different weighting factors than normal, and as predicted, the gain values monotonically increase toward a value of 1.00 as the spectral separation between the two pigments decreases. For the three types of deuteranomalous men examined here, we calculated weighting factors of 0.72, 0.80, and 0.92. Figure 4 (bottom panel) illustrates results for the subject who was most different from normal; his unique yellow of 603 nm indicates that his L pigment is weighted by a factor of 0.92, near 1.00 as predicted above, and it is 44% higher than normal. We conclude from the fact that the gain adjustment is so near the theoretical limit and so large that it is possible that the gain mechanism is fully adjustable and could be set to any value dictated by the environment.

If the gain factor for the deuteranomalous subject illustrated in Figure 4 (bottom panel) was not altered from the average normal person (0.64), his unique yellow would occur at a wavelength well beyond 700 nm, a wavelength outside of the visible range. This person would be able to extract virtually no useful color vision from his two X chromosome photopigments if the gain (and hence unique yellow) were not adjustable to a wavelength that was optimized to his aberrant photopigments. Shown in Table 1 are the predicted unique yellows for all the deuteranomalous men, calculated using a constant gain factor of 0.64. In all three cases, the unique yellow predicted by a fixed gain is extremely different from the actual values observed for deuteranomals. For comparison, we looked at values of unique yellow for deuteranomalous individuals from previous studies (Hurvich and Jameson, 1964; Nelson, 1938; Rubin, 1961). Without genetic results, like those obtained here, it is not possible to determine the gain values or predicted unique yellow values that correspond to the previously published results. However, we have obtained an estimate of the average spectral separation for pigments in deuteranomalous individuals (~5 nm) (Carroll, 2002). This average separation, together with the average deutan unique yellow from previously reported measurements, provides a comparison which shows that previous values are in reasonable agreement with the present results.

The unique yellow values for deuteranomalous individuals found here demonstrate that the gain values of the inputs to the red/green chromatic channel are different from normal in association with differences in the average relative quantal catches of the pigments underlying red/green color vision. The fact that deuteranomals have different values for unique yellow than normal is also significant. The experiments reported here were stimulated by the observed lack of variation in unique yellow in the normal population despite large individual differences in cone ratio. One explanation that might have been offered for the close agreement between individuals could have been cultural rather than neural. For example, one might argue that through shared language, it is possible that people with significantly different color perception might come to agree on a consistent set of color terms. However, in the previous experiments, we showed that altered experience, through the use of colored filters, changes the wavelength called unique yellow, and in this set of experiments, we show (in agreement with previous reports [c.f., Hurvich and Jameson, 1964; Nelson, 1938; Rubin, 1961]) that anomalous observers give different values for unique yellow compared to color normals, even though they are subject to the same cultural influences.

## Discussion

### Advantages of Using Information Gained from Experience

Here, it has been demonstrated that the nervous system actively uses information gathered from visual experience to adjust the gain of the inputs to the red/green chromatic channel. The idea of a plastic nervous system that is able to actively use information from the surroundings in the self-organization and adjustment of circuits responsible for basic sensory capacities has long been attractive; however, convincing demonstrations have been lacking. For instance, patching one eye during the critical period causes the loss of sensory capacity (amblyopia), but that does not eliminate the possibility that all the information for making the proper connections and fine tuning them is not genetically programmed. It could be that the effect of the eye patch is to simply interrupt the normal genetic program (Hubel, 1988). It has been difficult to demonstrate an active role for neural plasticity either during development or in adults because experimental manipulations in many of the systems studied have been limited to deprivation and ablation (though, see Baseler et al., 2002). However, here, in studying the color vision system, we have introduced large shifts in chromaticity rather than completely removing an input, and it has been possible to take

advantage of widespread genetic differences that produce large variability at the input end of the system. In these cases, the ability of the color vision system to actively use information from the altered input is demonstrated by the fact that the perceptual changes are those predicted from how the input was changed; for example, wearing the red lenses for a prolonged period makes the world without them appear more greenish, while the aftereffect of the green lenses is a redder visual world.

Here, the plasticity of color vision illustrates the advantages, both in economy and flexibility, of having a nervous system that can actively use information drawn from the environment. The red/green color vision system is relieved of needing a genetically encoded value for the gain. The flexibility is demonstrated by the ability of the visual system to adjust to different chromatic environments, to correct for large differences in cone ratio, and to maximize color vision in anomalous trichromats. This same plasticity may operate to maintain color perception during aging (Schefrin and Werner, 1990), adjusting the gain as the spectral properties of the lens change, and perhaps compensating for neural changes (i.e., damage) that may occur with disease or age (Schefrin et al., 1991).

### Purpose of the Plasticity

Unique yellow is the wavelength that produces the same ratio of absorption in the L and M cones, as does the average illuminant of our environment (Mollon, 1982, 1991). When we see unique yellow, stimulation on both sides of the red/green spectrally opponent system is equal, its output is zero, and it is said to be in equilibrium. By adjusting itself to be in equilibrium for lights that produce the same ratio of quantal catches as the mean chromaticity experienced in everyday life, the red/green color vision system is able to perform its job of signaling departures from that equilibrium point. It is well known that the visual system has an ability to adapt very quickly in response to routine changes in illumination. This familiar adaptation is very different, both mechanistically and functionally, than the plastic normalization mechanism illustrated here. Adaptation is a fast acting, largely retinal, mechanism in which the visual system adjusts to the ambient illumination level. When the spectral distribution of the ambient illumination changes, chromatic adaptation works in the direction of giving us color constancy (Kraft and Brainard, 1999). For example, a piece of paper that appears white under sunlight continues to appear white under incandescent lights.

In contrast, the plastic normalization mechanism revealed here works at a more central level with a different purpose. This mechanism determines the color appearance of objects in the world. The familiar transient chromatic adaptation mechanisms operate in a direction to preserve appearance under transiently changing illuminations. As an illustration, imagine that you grew up in a "green-dominant" spectral environment and your centrally determined gain was set to a different value than everyone else. In our world, a paper that looks white to everyone else would look pinkish to you. On the other hand, your fast-acting chromatic adaptation mechanisms would work in the direction of maintaining that same pink color appearance under the daily changes in our normal illumination.

Because of the plastic normalization mechanism, the red/green color vision system is set to be in equilibrium for the average illuminant. That is, the average illuminant produces no response from spectrally opponent color vision mechanisms. Our color vision provides information about the spectral reflectance of objects, allowing us to detect them, to discriminate them from their surroundings, and to determine their internal properties. By being normalized to the average illuminant, the color vision system is optimized to perform these functions. Nearly all natural surfaces reflect some wavelengths from the illuminant more than others, and the reflectances are well distributed around neutral; thus, because of the normalization, the red/green chromatic channel is matched to the input by having the equilibrium response at the average value of the input distribution.

The ability of the color vision system to normalize itself to the average illuminant means that color perceptions will be very much the same for everyone who shares the same environment. This stability allows color coding as a form of communication, which has tremendous importance in our modern lives. It would not be possible to assign different meanings to relatively small differences in wavelength content, like the difference between a yellow caution light and a red stop light, unless there was very good agreement in color perception across individuals. During our evolution, the utility of having color perception standardized would have offered a selective advantage long before there was man-made color coding. For example, fruits have evolved (or coevolved with primates) to change color when going from an inedible to an edible state (Mollon, 2000; Polyak, 1957; Regan et al., 2001; Sumner and Mollon, 2000). Having the change be one in which the color crosses an unvarying hue boundary (e.g., from green to yellow like a banana [Snodderly, 1979]), simplifies signaling between the fruit and the primate that eats it.

### A Broader Purpose

Recent experiments on the genetic basis of color vision (Balding et al., 1998; Wang et al., 1999) predict that the L and M cones are not intrinsically different from one another in any way except the pigment they contain. This leaves open the question of how the circuits for color vision become correctly wired in the absence of distinguishing labels that would allow the L and M cones to be recognized by second-order neurons. As a solution that would relieve the necessity to guide each connection, it has been suggested that at the first stage of color processing, the center-surround red/green opponent ganglion cells might be formed by chance connections (Lennie, 2000; Lennie et al., 1991; Paulus and Kroger-Paulus, 1983; though see Martin et al., 2001). Midget ganglion cells in the fovea draw their center input from a single cone. Thus, for example, when the central cone is M, then according to chance it will not be unusual for the neighbors that form the surround to be all (or mostly) L cones, naturally creating a spectrally opponent receptive field. This would explain the first stage, but the process must be maintained and extended at higher levels of visual processing in the cortex. For this, participation of "Hebbian mechanisms" could be hypothesized (DeValois and DeValois, 1993; Mollon, 1999), in which

synapses on to a particular cell are strengthened or lost through visual experience according to how well its inputs are correlated (Hebb, 1949). Such a capacity during development would allow experience to mold the higher cortical circuits for red/green color vision. Until now, there has not been direct evidence for the existence of plastic chromatic mechanisms at the level of the cortex. However, the malleability of the color vision system we have demonstrated here makes it plausible that neural plasticity might not only adjust the gain but also might have a role in initially establishing the cortical connections for color vision.

## Experimental Procedures

### Subjects

All research on human subjects adhered to the tenets of the Declaration of Helsinki and was approved by the IRB at the University of Rochester and the Medical College of Wisconsin. Informed consent was obtained after explanation of the nature and possible consequences of the study. 32 color normal subjects (14 females, 18 males) were used to obtain the results in Figure 1. Three of these males (YY and JC, authors and CL, naïve observer) and one additional female subject, who did not participate in the experiments summarized in Figure 1 (EM, naïve observer), performed the chromatic adaptation experiments summarized in Figure 2. In addition, unique yellow and ERG spectral sensitivities were obtained from five deuteranomalous males. Each subject's color vision was classified based on results from a variety of color vision tests including the Ishihara plates, The Neitz Test of Color Vision (Neitz and Neitz, 2001), AO-HRR plates, the Farnsworth-Munsell 100-Hue Test, and color matching performance on an anomaloscope.

### Genetic Analysis

Whole blood was obtained from each subject, and DNA was extracted using the ABI 341 automated nucleic acid purification system. Genomic DNA was used in the polymerase chain reaction (PCR) to selectively amplify the L pigment genes, and exons 2–5 of the L genes were sequenced. The PCR and sequencing conditions and primers have been reported previously (Carroll et al., 2000; Neitz et al., 1995). For deuteranomalous subjects, the L gene that occurs at the 5′ end of the X chromosome visual pigment gene array was amplified separately from the other L genes in the array according to a previously published protocol (Neitz et al., 1996), and they were sequenced separately.

### ERG Flicker Photometry

The details of the flicker photometric ERG have been described previously (see Carroll et al., 2000; Jacobs et al., 1996). Briefly, the subject's right pupil was dilated (Tropicamide 0.5%), and the ERG was differentially recorded using fiber from the DTL Plus electrode as an active corneal electrode. Two beams (identified here as a reference and a test beam) of a three-channel Maxwellian-view optical system were superimposed to illuminate a circular portion of the retina subtending approximately 70°. High-speed electromagnetic shutters were used to alternately present the reference and test lights at 31.25 Hz, with a neutral density wedge used to control the intensity of the test light. The wavelength of the test light was controlled by a Varispec liquid-crystal electronically tuneable filter (Cambridge Research & Instrumentation, Boston, MA). Spectral sensitivity was recorded by measuring the null point, where the ERG signals produced by the test and reference light were equal. The null point was found by adjusting a neutral density wedge within the test path, while the intensity of the reference light remained fixed. The average of two complete runs through a range of wavelengths between 480 and 680 nm was used to derive a spectral sensitivity function. Final spectral sensitivity values are reported as quantal intensities, corrected for lens absorption and age-dependent lens correction (Pokorny et al., 1987).

We estimated the L:M cone ratio for the color normals by determining the weighted sum of an L and an M cone spectral sensitivity function required to best fit their spectral sensitivity data. To account for differences in the underlying cone pigments between subjects, L cone spectra were derived for each subject by comparing the amino acid sequences deduced from their L pigment genes to those from deuteranopes for whom an ERG-derived $\lambda_{max}$ estimate was also obtained (Carroll et al., 2000). An average protanope spectral sensitivity function was used as the M cone fundamental for each subject. Spectral sensitivity functions were also obtained from each of the five deuteranomalous subjects. Their data was best fit to a wavelength-shiftable visual pigment template function (Carroll et al., 2000, 2001). The spectral sensitivity function for the deuteranomalous subjects is derived from contributions from both of their L cones. Thus the single $\lambda_{max}$ value that results represents some weighted contribution of the values for the underlying photopigments (Carroll et al., 2001). All five deuteranomalous $\lambda_{max}$ values fell in-between those of their respective photopigment pairs, indicating that both photopigments were contributing to the ERG, and in turn, to color vision.

### Unique Yellow Measurement

A monochromatic light, presented in Maxwellian view, was used to obtain estimates of unique yellow. For subjects YY and EM, the stimulus subtended 0.5°, and the retinal illuminance was approximately 50 td. For the other two color normal subjects (CL and JC), the visual angle subtended by the stimulus was 70°, with retinal illuminance of 1000 td. Before starting a recording session, the subject was dark adapted for 5–10 min. Then the subject was asked to respond either with the method of adjustment (YY, EM) or the 2-alternative forced choice (2AFC) method (CL, JC). Subjects who used the method of adjustment set the wavelength so that it appeared neither reddish nor greenish. This was done five times per session; each time a new starting wavelength was randomly chosen. The mean of these setting was taken as the wavelength corresponding to unique yellow for that session.

For the 2AFC method, unique yellow was determined using two randomly interleaved two-alternative forced-choice staircases. Each staircase began at one hue extreme (clearly red or clearly green, chosen randomly) and moved in varying step sizes toward the opposing hue extreme. The start points were chosen to be equidistant from the known average unique yellow value for color normals (580 nm). The direction the staircase moved was determined by the observer's response to the previous stimulus in that staircase. If a stimulus appeared to be "too green," the next stimulus from that staircase (not necessarily the next stimulus seen, as the two staircases are randomly interleaved) moved to a longer wavelength. Likewise, if a stimulus appeared "too red," the next stimulus from that staircase moved toward a shorter wavelength. The step size for each staircase began at 10 nm and was decremented to 5 nm after the first reversal on that particular staircase. After the second reversal on a given staircase, the step size for that staircase was decremented to 2 nm. A third reversal dropped the step size to the final value of 1 nm. After the first reversal with 1 nm steps, the reversals were counted, and the experiment continued until ten reversals had been reached. The wavelength of unique yellow was taken as the average of the 1 nm reversals on each staircase. Deuteranomalous unique yellows were obtained with the same procedure, though the starting wavelength for each staircase was shifted relative to their expected unique yellow of about 600 nm. Because of the way in which the experiment was performed, the subject (author or nonauthor) never knew the wavelength of the light he or she was judging. This restricted the possible effects of bias to criterion shifts, which even at their theoretical extremes are many times smaller than the observed shifts in unique yellow.

### Chromatically Altered Environments

Subjects were exposed to an altered chromatic environment for 4, 8, or 12 hr each day. In the 4 hr experiment, the alteration was achieved with either color-filtered goggles or with a room in which the light sources were covered completely with filters similar to Kodak Wratten filters 29 and 58. In the 8 and 12 hr alteration conditions, contact lenses were used that were either dyed professionally (Adventures in Color, Inc., Golden, CO) or with a commercially avail-

able dye (Softchrome, Inc., San Ramon, CA) to mimic the transmittance of the Wratten filters as closely as possible.

A baseline unique yellow value was obtained for approximately 1 week prior to the start of the experiment for each of the color normals. Each subject's visual input was then restricted by filtering the incoming light. Each day, immediately prior to the introduction of the chromatically altered experience, unique yellow was measured. Following the chromatic alteration period (of 4, 8, or 12 hr), each subject spent the remainder of the day under illumination that was not altered from his normal routine. The type, period, and duration of alteration were: 4 hr, red and green, 10 days (YY and CL); 8 hr, red, 24 days (YY); 8 hr, green, 21 days (YY); 12 hr, red and green, 10 days (EM); and 12 hr, red, 10 days (JC). At the end of the experiment (for between 7 and 24 days), the recovery of unique yellow was monitored by taking daily measurements for each subject until their unique yellow setting returned to the baseline value obtained at the start of the experiment.

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