AMERICAN ACADEMY OF OPHTHALMOLOGY®

Restoring Color Perception to the Blind

An Electrical Stimulation Strategy of Retina in Patients with End-stage Retinitis Pigmentosa

Lan Yue, PhD,[1,2] *Johnny Castillo, BA,*[2] *Alejandra Calle Gonzalez, PhD,*[1,2] *Jay Neitz, PhD,*[3] *Mark S. Humayun, PhD*[1,2]

Purpose: Bioelectronic retinal prostheses that stimulate the remaining inner retinal neurons, bypassing degenerated photoreceptors, have been demonstrated to restore some vision in patients blinded by retinitis pigmentosa (RP). These implants encode luminance of the visual scene into electrical stimulation, however, leaving out chromatic information. Yet color plays an important role in visual processing when it comes to recognizing objects and orienting to the environment, especially at low spatial resolution as generated by current retinal prostheses. In this study, we tested the feasibility of partially restoring color perception in blind RP patients, with the aim to provide chromatic information as an extra visual cue.

Design: Case series.

Participants: Seven subjects blinded by advanced RP and monocularly fitted with an epiretinal prosthesis.

Methods: Frequency-modulated electrical stimulation of retina was tested. Phosphene brightness was controlled by amplitude tuning, and color perception was acquired using the Red, Yellow, Green, and Blue (RYGB) hue and saturation scaling model.

Main Outcome Measures: Brightness and color of the electrically elicited visual perception reported by the subjects.

Results: Within the tested parameter space, 5 of 7 subjects perceived chromatic colors along or nearby the blue-yellow axis in color space. Aggregate data obtained from 20 electrodes of the 5 subjects show that an increase of the stimulation frequency from 6 to 120 Hz shifted color perception toward blue/purple despite a significant inter-subject variation in the transition frequency. The correlation between frequency and blue-yellow perception exhibited a good level of consistency over time and spatially matched multi-color perception was possible with simultaneous stimulation of paired electrodes. No obvious correlation was found between blue sensations and array placement or status of visual impairment.

Conclusions: These findings present a strategy for the generation and control of color perception along the blue-yellow axis in blind patients with RP by electrically stimulating the retina. It could transform the current prosthetic vision landscape by leading in a new direction beyond the efforts to improve the visual acuity. This study also offers new insights into the response of our visual system to electrical stimuli in the photoreceptor-less retina that warrant further mechanistic investigation. *Ophthalmology 2020;■:1–10 © 2020 by the American Academy of Ophthalmology*

As a critical component of human vision, color plays an important role on different levels of visual processing.[1] At lower levels, color helps segment the visual scene and enhances the saliency of the visual inputs; at higher levels, objects seen in their characteristic colors are associated with color knowledge stored in memory.[2-4] In a healthy eye, color vision originates in S, M, and L cone photoreceptors that are respectively sensitive to short, medium, and long wavelength of light. It is widely agreed that early in the visual pathway the encoding of color vision is transformed from these 3 types of cones to 3 different opponent systems: red (R), green (G), blue (B), yellow (Y), and black-white. Output of these color opponent systems is believed to form the computational basis of color interpretation in higher visual centers in the brain.[5,6]

Retinal degeneration is a leading cause of blindness, affecting 30 to 50 million people worldwide and approximately 3 million in the United States alone.[7,8] In end-stage outer retinal degeneration, photoreceptors are nearly entirely absent,[9,10] depriving patients of the ability to perceive light and color. Our group and others have shown that bioelectronic retinal implants that stimulate the remaining inner retinal neurons can restore some vision, but at a much reduced spatial resolution of 16 to 1600 electrodes—typically an individually stimulated electrode represents 1 pixel in the stimulation pattern.[11-13] As such,

https://doi.org/10.1016/j.ophtha.2020.08.019
ISSN 0161-6420/20

any means of providing more visual information for object recognition and orientation in an environment is valuable. We describe a methodology that could be implemented in retinal prostheses to provide limited color sensation as an extra dimension of vision.

Independent studies of 2 epiretinal implants, Argus II (Second Sight Medical Products, Sylmar, CA) and Intelligent Medical Implant (Zug, Switzerland; later acquired by Pixium Vision, Paris, France), both surveyed electrically elicited color perception in blind patients with retinitis pigmentosa (RP).[14-16] Notably, studies by Stanga et al[14,16] reported up to 9 different colors perceived by 14 patients with Argus II implants and suggested an association between the percentage of blue percepts and the delivery frequency of stimuli. Yet from the brief conference reports of the studies, the reliability and stability of the electrically elicited color perception remain unclear, as does the interplay between frequency and amplitude tuning. Compelled by these unanswered questions, we investigated color perception in the blind patients with Argus II implants under the dual control of frequency and amplitude—we adjusted current amplitude to maintain phosphene brightness during frequency modulation. The color sensation generated along the blue-yellow axis exhibited a strong correlation with the stimulation frequency, with a good level of consistency over time. Spatially matched multi-color perception was possible with the simultaneous stimulation of paired electrodes. Our findings potentially offer a stimulation strategy in the inner retina, bypassing the severely damaged cone photoreceptor system, to partially restore color vision to the blind. Such a strategy will involve the development of an algorithm to encode color information extracted from the visual scene into frequency-modulated electrical stimuli. In a patient, the algorithm will be calibrated for individual electrodes based on the frequency-color correspondence and the amplitude-brightness function of the electrode. Long-term stability of the color sensations and the possibility of color interaction between the phosphenes generated by neighboring electrodes will also be considered in the algorithm development.

Color vision, even at low spatial and chromatic resolution, could enable the patients to search the outstanding color(s) for guidance, facilitating quicker visual field scanning and more accurate visual field segmentation. As illustrated in Figure 1, color facilitates edge detection of the blue flowers in the green and brown background in the normal vision (Fig 1A), while it practically becomes the only visual cue of the flowers in the pixelized vision that lowpass filters the spatial information (Fig 1B and C). Likewise, color is helpful, though not required, for a healthy eye to distinguish an apple from an orange (Fig 1D), but it is crucial in pixelized vision where the subtle distinguishing features in shapes and texture are lost (Fig 1E and F). Increased pixelization leads to exacerbated loss of spatial information, further highlighting the importance of having color as a visual cue (compare Fig 1B, C, E, and F). Additional studies are required to elucidate how color could add to the visual experience of the blind in daily life.

Methods

Argus II Retinal Prosthesis and Test Subjects

The Argus II Retinal Prosthesis System (Second Sight Medical Products, Inc, Sylmar, CA) is an epiretinal implant that converts visual scenes into electrical stimuli that are delivered via an array of 6×10 electrodes to the retina. The array is surgically attached to the inner surface of retina, nearby the ganglion cell layer. It is indicated for patients with end-stage RP in both eyes. Details on the device have been provided by Yue et al.[13] A total of 7 subjects (5 female and 2 male) monocularly fitted with the Argus II were tested in the study. Subjects 1 and 2 received surgical implantation in 2007 and 2012, respectively. Other subjects received implants between 2015 and 2018. Subjects provided informed consent to participate in the postmarket study with approval from the Institutional Review Board of the University of Southern California. The research adhered to the tenets of the Declaration of Helsinki. No serious adverse event has been filed on the Argus II implant in the tested subjects before or after our testing. All subjects claimed having normal color vision in childhood and most subjects (except for subject 7) in early adulthood until being visually deprived by RP. Despite being blind for decades, all subjects stated remembering the appearance of colors.

Threshold Detection and Choice of Test Electrodes

Threshold detection was performed with the proprietary Argus II Swift Programming Assistant, which provides a rapid mechanism to estimate the current levels necessary to elicit phosphenes from each electrode or a group of electrodes. Electrical stimuli were delivered at 6 Hz with the pulse width (PW) of 0.46 ms per phase. Subjects were required to respond "yes" or "no" based on whether they perceived phosphene or not upon delivery of electrical stimulus. Current level of the stimulus was automatically adjusted in predetermined steps according to the subjects' response. Perceptual threshold is defined as the minimum current level at which the subject responds "yes" to at least 2 of 3 trials. Electrodes were tested individually and automatically grouped in pairs or quads with the neighboring electrodes by the program if the minimum current of the electrode exceeds 168 μA. Catch trials (zero stimulation) were used to eliminate the false-positive rate of the responses. Only electrodes with low individual threshold (current level <168 μA) were chosen for the testing of color percepts to minimize risks of tissue damage at higher stimulation frequencies.

Direct Stimulation of Electrodes

Frequency modulation was tested when the implant was in the direct stimulation mode; the stimulation chip was under direct control of an external computer, bypassing the image acquisition of the prosthetic system. Stimulation parameters were set on the proprietary software. In this program, electrodes can be selected individually or in a group. With the current software, when multiple electrodes were selected and stimulated in a group, identical stimuli were delivered to the entire group. This limited the choice of electrodes in the psychophysical testing to the ones with similar perceptual threshold to be compared at similar brightness levels.

Electrical Stimulation and Psychophysical Tests

Electrical stimulus consists of charge-balanced biphasic pulses with a cathodic leading phase and an anodic trailing phase. Under

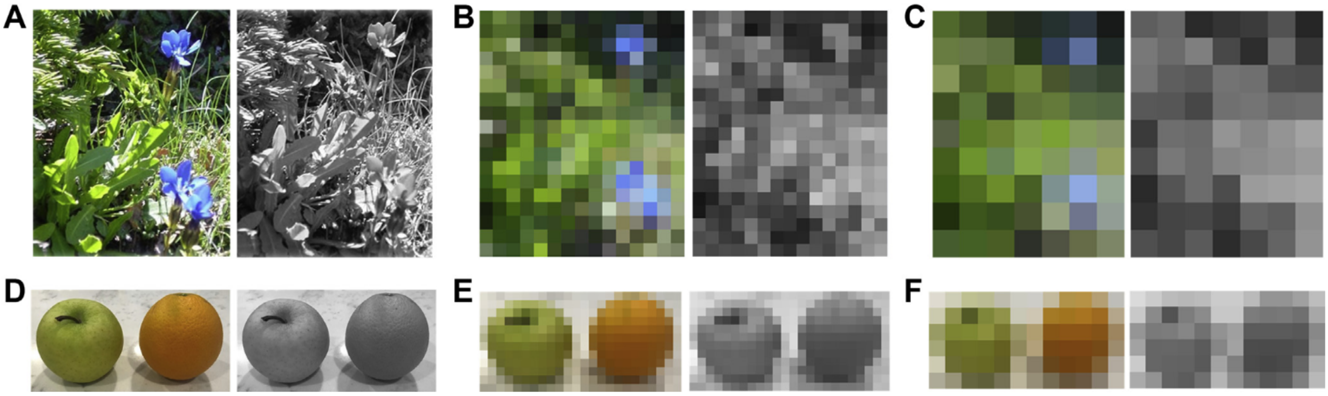


Figure 1. Demonstration of the importance of color in simulated prosthetic vision. **A,** Colored and black-and-white presentations of a visual scene (371×456 pixels; adapted from http://webvision.med.utah.edu). **B** and **C,** images in **A** but downsampled to 14×18 pixels **(B)** and 7×9 pixels **(C)**, respectively. **D,** Colored and black-and-white presentations of an apple and an orange side-by-side (542×273 pixels). **E** and **F,** images in **D** but downsampled to 24×12 pixels **(E)** and 12×6 pixels **(F)**, respectively.

the direct stimulation mode, variations in the delivery frequency (6−200 Hz) and PW (0.2−2 ms) were tested. In the early testing, we noticed that the subjects typically became more hesitant and less certain about the colors they perceived of very bright or dim phosphenes. To reduce interference of phosphene brightness on color sensation, we took the approach of adjusting stimulation amplitude (I) to maintain phosphene perception at medium brightness when other stimulation parameters, such as frequency and PW, were altered. Upon delivery of an electrical stimulus, the subjects were requested to choose the perception brightness from 6 options: not detected, dim, dim to medium, medium, medium to bright, and bright. The amplitude was tuned on the basis of the subjective response. Along with the brightness, the subjects were also requested to describe the shape, size, and color of the phosphene they perceived. The subjects were instructed to report colors by name (e.g., red, green, pink, brown) as well as by quantitative descriptions in RYGB hue and saturation scaling, a color categorizing model extensively used in psychophysical studies. In this model, all colors can be named using various amounts of fundamental hue sensations in red (R), yellow (Y), green (G), and blue (B). Following the method described by Gordon et al,[17] the subjects were asked to report the percentages of his/her sensation of R, Y, G, B hues for a total of 100%, or zero if no hue, as well as the apparent saturation, that is, percentage of the entire sensation that is chromatic. The "washed jeans" analogy was often used to explain the meaning of saturation to the test subjects and seemed well received. Most often, the subjects reported hues such as blue or yellow with no contribution from other RYGB components. When subjects were uncertain about the percentage of the RYGB contribution in the hue they perceived, they were asked to describe the hue the best they can to the detail or in the natural hue of something they are familiar with, for example, royal purple and lavender purple. All hues reported, whether quantitatively in the RYGB model or qualitatively in a specific name of a hue, along with saturation, were converted to the Hue, Saturation, Value model for further quantitative analysis and for color presentation in the figures. Images showing color perception of the subjects were plotted with MATLAB (MathWorks, Natick, MA) scripts.

Blue Scores and Statistical Analysis

A blue score of each color sensation was calculated by the following scaling system: 0 = no blue or purple perception; 1 = blue or purple sensation reported, but the color is highly unsaturated (saturation ≤ 0.2); 2 = more significant blue or purple sensation reported ($0.2 <$ saturation ≤ 0.5); 3 = strong blue or purple sensation reported (saturation > 0.5). No attempt to differentiate blue and purple in calculating the blue score was made because the reported purple hues are strongly blue biased and the focus of the present study is the yellow-blue color pathway. Statistical significance of data was determined with paired sample *t* tests. All statistical analysis was performed in R with a significance level of 0.05. When fitting curves, fits were weighted with the inverse of variance and were optimized.

Results

Electrically Elicited Color Sensation Under Controlled Phosphene Brightness

Details of the psychophysical tests of the electrically elicited color perceptions are described in the "Methods" section. Waveforms of the biphasic pulses and the reduction in the current amplitude that were needed to maintain medium phosphene brightness under increased stimulation frequency are shown in Figure 2A and B. Within the parameter space examined (combinations of variations of frequency between 6 and 120 Hz and variations of PW between 0.2 and 2 ms), 11 colors were named by 7 subjects from the stimulation of a total of 29 electrodes, including 3 achromatic colors (white, gray, and black) and 8 chromatic colors (yellow, blue, purple, gold, green, pink, black, orange, and brown). Figure 2C shows the number of electrodes that generated the leading colors ($\geq$5 electrodes). When plotted in the red-green versus blue-yellow color space, it became obvious that the dominant chromatic colors fell along or nearby the blue-yellow axis (Fig 2D). Among the 7 subjects tested, 5 were able to perceive blue (3 subjects) or purple (2 subjects) under the test condition. The purples reported were royal purple and/or lavender purple, both strongly blue biased. As such, the following quantitative analysis of blue sensation does not attempt to differentiate blue from purple. Of the 2 subjects who did not describe seeing blue or any blue-tinted colors, one reported seeing yellow and white, and the other reported seeing nonwhite colors but was unable to identify them. Changes in the phosphene brightness by variations in the stimulation amplitude did not elicit blue percepts in these subjects either.

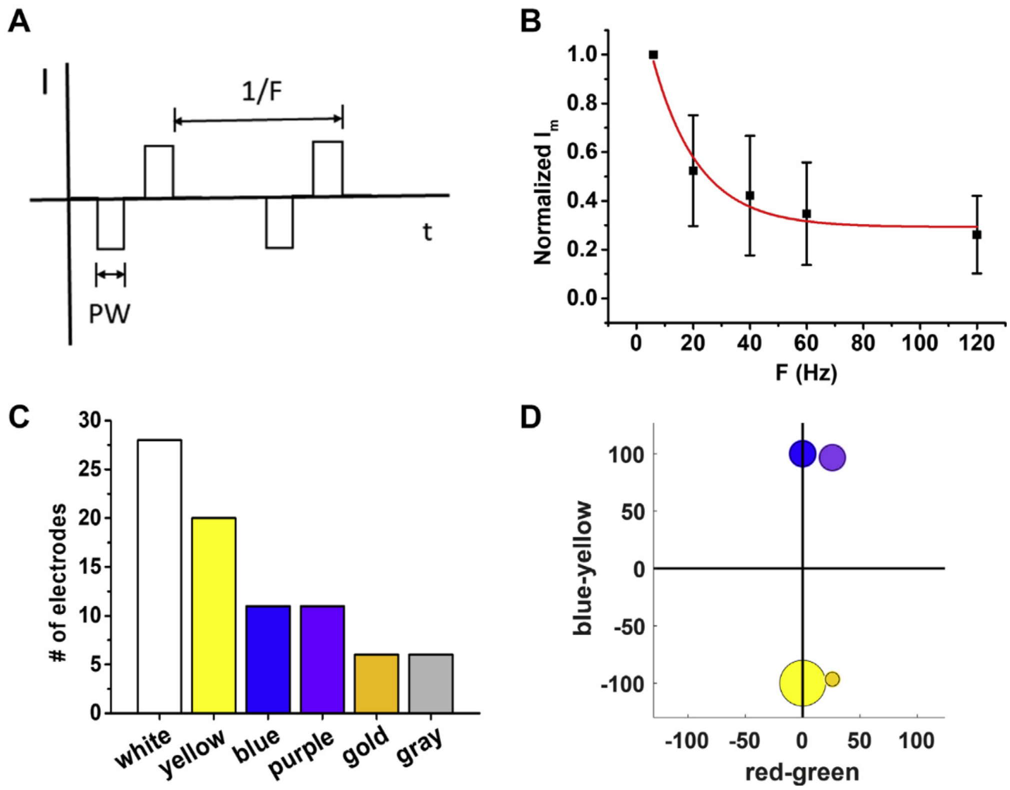


Figure 2. Color perceptions reported by test subjects under brightness control. **A,** Stimulation waveform showing current amplitude (I) stimulation at medium brightness under frequency modulation. **B,** Current amplitude needed to maintain medium brightness (I_m) with the increase of the frequency. Aggregate results obtained from 19 electrodes in 7 subjects. Fitting of the data with an exponential decay function shown in red, $R^2 = 0.92$. **C,** Dominant colors reported and the number of electrodes that have generated each color (colors generated from <5 electrodes not shown). Data obtained from 29 electrodes across 7 subjects. **D,** Plot of the dominant chromatic colors in the color space formed by blue-yellow and red-green axis. Color and size of the circles represent the hue perceived and its frequency of occurrence, respectively.

Shift of Color Sensation with Stimulation Frequency

A representative frequency dependence of color sensation is illustrated in Figure 3A. Subject 2 perceived mostly yellow and white under lower frequencies, and the color shifted toward blue when the frequency was increased to 120 Hz and above. Variations of PW were tested for 2 frequencies (20 and 120 Hz) that respectively elicited yellow and blue perceptions at the default PW of 0.45 ms. Within the tested range of 0.2 to 1.5 ms, PW did not significantly affect the colors perceived. For ease of illustration, multiple colors perceived in 1 phosphene, for example, a blue center surrounded by a white outline or a blue top with a white bottom, are presented in concentric rings, each portraying the hue and saturation of 1 color. Color sensation elicited in subject 4, who perceived purple instead of blue at higher frequencies, is illustrated in Figure 3B. Phosphenes were perceived in yellow/white at 6 Hz and changed to purple at 20 Hz and above. Variations in PW did not have an obvious impact. Both subjects described similar trends of color percept transition with frequency, despite an inter-subject variation in the transition frequency (20 vs 120 Hz). In subject 4, the frequency response obtained from 8 electrodes at different locations in the array confirmed that most electrodes (except electrode 7) elicited steady purple sensation between 20 and 60 Hz, whereas colors elicited at 6 Hz had much less purple component (Fig 3C). To quantitatively evaluate the blue/purple components in a phosphene, we assigned blue scores to subjects' color sensation ("Methods"). At the default PW, aggregate data from 20 electrodes across 5 blue/purple sensing subjects (Fig 3D) show a clear trend of enhanced blue sensation with the increase of frequency ($P = 0.002$ between 6 and 20 Hz, $P = 0.02$ between 20 and 60 Hz; paired-sample t test). In contrast, no significant change in the blue score with variations in PW, either at low or high frequency, was found (Fig 3E).

Long-term Stability of Electrically Elicited Color Sensation

Long-term stability of the electrically elicited color sensation is crucial for establishing color as a reliable dimension of visual cues in prosthetic vision. Thus, we compared color perception generated over 6 months apart and presented the representative results obtained from a blue-sensing and a purple-sensing subject. At 10 and 19 months postimplantation, subject 3 consistently perceived yellow at 6 Hz and blue at 60 and 120 Hz stimulation, although the saturation level of the colors fluctuated. This fluctuation is not surprising given the highly subjective and perhaps slightly time-variant understanding of the term "saturation" by the long blind subject. Variations in hue perception existed in the middle frequency range (20 and 40 Hz); the pink and green sensations sporadically reported were not replicated between the 2 time points. Nonetheless, the general trend of frequency-dependent shift between yellow and blue persisted (Fig 4B). Similar results were obtained with subject 4 (who sensed purple instead of blue) at 7

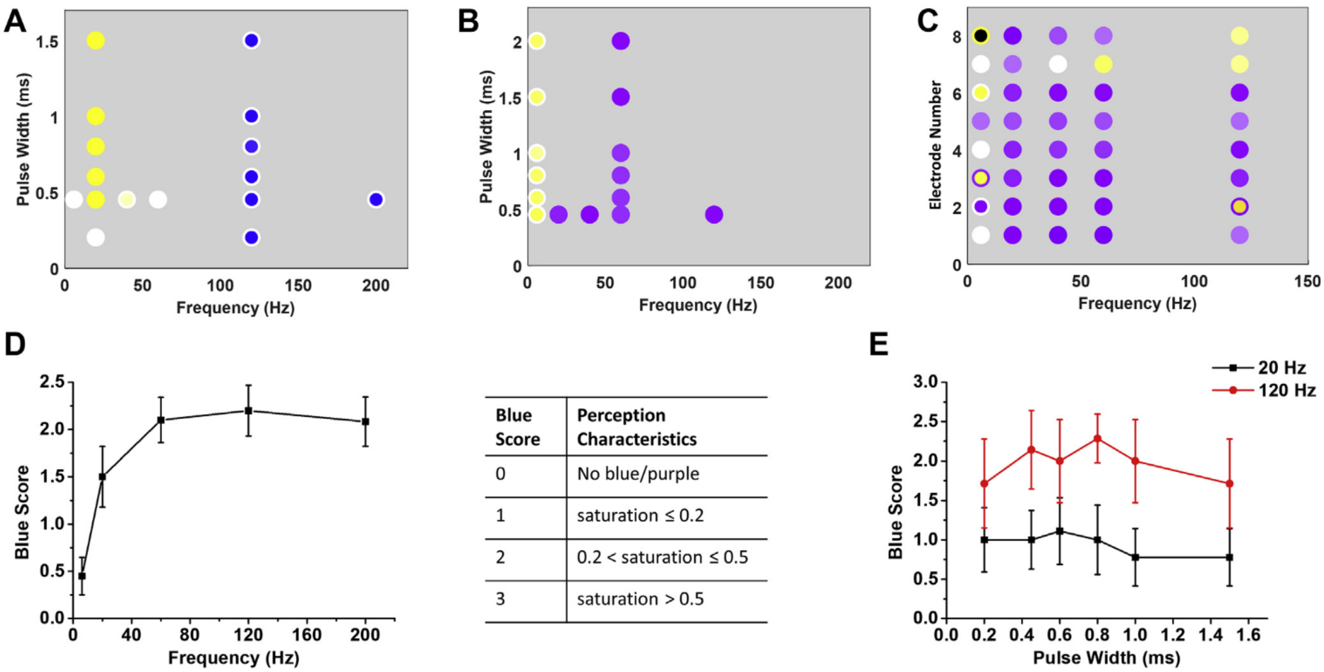


Figure 3. Changes of color perception with stimulation frequency and pulse width (PW). **A,** Representative colors perceived by subject 2 under different combinations of frequency (6, 20, 40, 60, 120, and 200 Hz) and PW (0.2, 0.45, 0.6, 0.8, 1.0, and 1.5 ms). As the frequency increased, the subject's perception changed from yellow (6–60 Hz) to blue (120–200 Hz) dominated colors. For the ease of illustration, if more than 1 color was perceived in 1 phosphene, they are presented in concentric rings, each portraying the hue and saturation of 1 color reported. These rings do not depict the actual spatial relations of each color. **B,** Representative colors perceived by another subject (subject 4) under different combinations of frequency (6, 20, 40, 60, and 120 Hz) and PW (0.45, 0.6, 0.8, 1.0, and 2.0 ms). As the frequency increased, the subject's color perception shifted from yellow and white (6 Hz) to purple (20–120 Hz). **C,** Color perception obtained from 8 electrodes in different retinal locations of subject 4 under variations of frequency (PW = 0.45 ms). **D, Left:** aggregate data of blue scores under variations of frequency; **right:** the conversion scale from subjective descriptions of color perception to the blue scores. Data obtained from 20 electrodes in 5 subjects. **E,** Aggregate data of blue scores under variations of PW at 20 Hz (black) and 120 Hz (red). Data obtained from 6 electrodes in 3 subjects for 20 Hz and 7 electrodes in 4 subjects for 120 Hz. Data presented in mean ± standard error.

and 14 months postimplantation (Fig 4C and D), except for a little more variation at 120 Hz. Overall, we found a good level of long-term stability of color perception along the yellow-blue axis. It is possible that some frequencies produced better stability than others, but the range of such frequency exhibited inter-subject variability, likely to be correlated with the different transition frequencies for different subjects.

Simultaneous Stimulation of Two Electrodes

Patterned color sensation, the ability to discriminate different colors in different areas of the visual field, is required for integrating color information with the spatial information. We examined the ability of the subjects to simultaneously perceive 2 colors with stimulation of paired electrodes. Figure 5 shows the data obtained in a blue-sensing and a purple-sensing subject. In blue-sensing subject 1, electrode 1 was tested in pairs with a cluster of electrodes (electrodes 2–4) on its right in the mapping of the visual field (Fig 5A). When stimulated at both lower and higher frequencies, these electrode pairs produced simultaneous perception of 2 phosphenes: one on the left and the other on the right. The left phosphene exhibited the same color as the one elicited by stimulating electrode 1 alone, and the right phosphenes were of the same colors elicited by individually stimulating electrode 2, 3, or 4.

In purple-sensing subject 4, electrode 1 was paired with the fellow electrodes in multiple directions in the visual field (Fig 5B). When stimulated individually at 20 Hz, only electrode 1 generated yellow perception and all the other electrodes generated purple perception. Paired electrodes produced colored phosphenes that spatially matched the sites of stimulation in the visual field in all directions. Separated by approximately 1400 µm on the array from edge to edge (calculated from the 225 µm electrode diameter and the 575 µm pitch size), Electrodes 1 and 3 form the closest pair tested. The fact that the subject was able to discern different phosphene colors generated by this pair suggests the feasibility of creating color-encoded stimulation patterns at this level of spatial resolution. Refined spatial sampling of the array will be investigated in the future when multi-electrodes can be set at different stimulation parameters so that the testing is no longer limited to electrodes sharing similar perceptual thresholds.

Blue Sensation, Electrode-Retina Interface, and Visual Impairment

As aforementioned, in contrast with the majority of the test subjects (5/7), 2 subjects did not report blue-tinted sensation within the tested parameter space. To find out what could cause this difference in blue sensations, we compared the electrode-retina interface and the status of visual impairment in these 2 groups of subjects. First, we examined the electrode-retina interface by fundus imaging (for array location) and perceptual threshold (for electrical contact). All arrays were implanted in the macula straddling the fovea, with

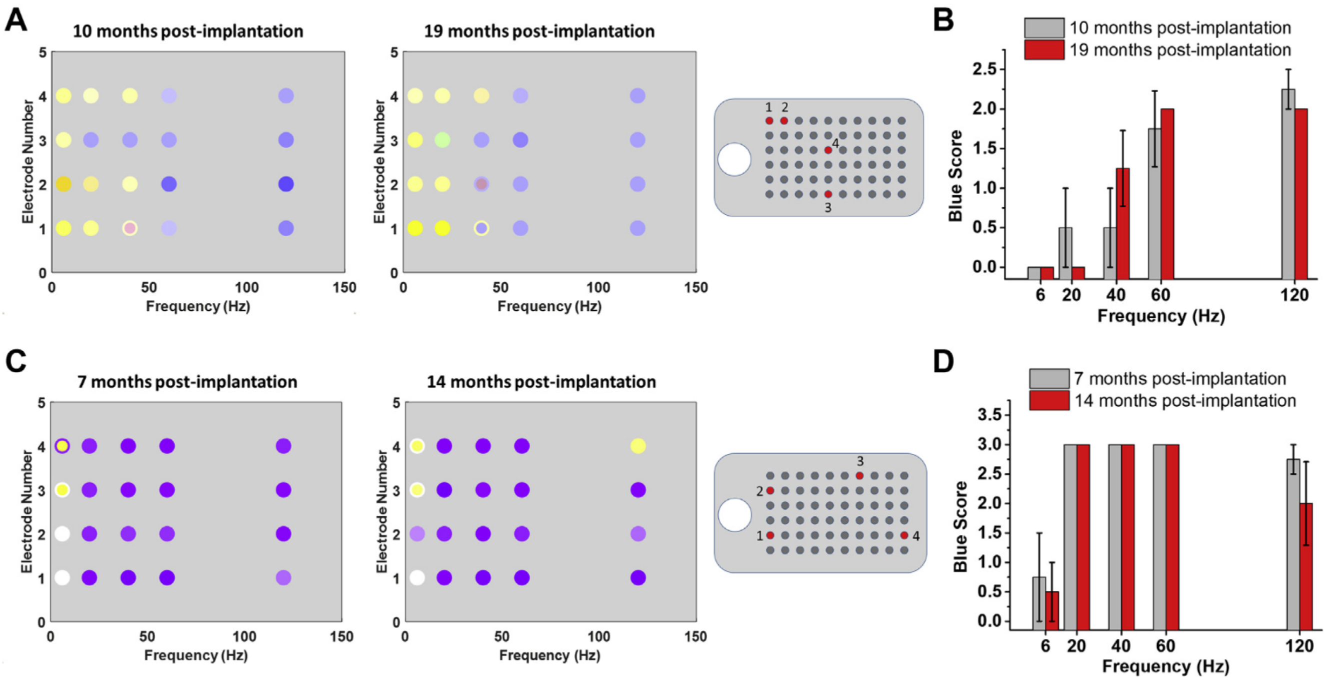


Figure 4. Stability of frequency-shifted yellow-blue perception over time. **A,** Color perception under different frequencies in subject 2. Data obtained from 4 electrodes at 10 months (left) and 19 months (middle) postimplantation. **Right:** locations of the electrodes in the array. **B,** Average blue scores of the 4 electrodes at the 2 postimplantation time points. **C,** Color perception under different frequencies in subject 4. Data obtained from 4 electrodes at 7 months (left) and 14 months (middle) postimplantation. **Right:** Locations of the electrodes in the array. **D,** Average blue scores of the 4 electrodes at the 2 postimplantation time points. Data presented in mean ± standard error.

most electrodes in similar parafoveal locations superior temporal to the optic disc (Fig 6A–E). There is no indication of a significant difference in the implantation site of the array in blue-sensing versus non–blue-sensing subjects. Perceptual threshold was analyzed as a gauge of electrical contact between retina and electrode; low threshold typically represents good retina-electrode contact. In both blue-sensing and non–blue-sensing subjects, perceptual threshold of color-tested electrodes exhibited similar data range, without any sign of worse retina-electrode contact in the non–blue-sensing subjects. In contrary, the very low perceptual threshold (<40 μA) was contributed mostly by the non–blue-sensing Subject 6.

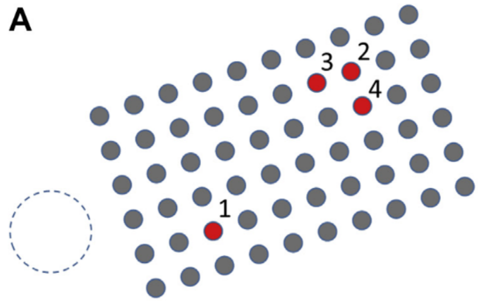
Second, we compared the visual impairment status of the subjects (Table 1). All have had severe vision loss for decades as a result of advanced nonsyndromatic RP or Usher syndrome; the average self-reported time of being completely blind is 21.2 years for blue-sensing and 23.5 years for non–blue-sensing subjects. All subjects have no light perception or bare light perception in the implant eye, and whether it was no or bare light perception did not seem to carry much weight in blue sensation as 4 of 5 blue-sensing subjects had no light perception. Self-reported family history of RP suggests the likelihood of an autosomal dominant inheritance pattern in subject 2 and an autosomal recessive, maternally acquired, or X-linked inheritance pattern in other subjects, without an obvious correlation between the inheritance pattern and blue sensation. All 5 blue-sensing subjects are female and both non–blue-sensing subjects are male, but it is unknown whether gender is a delineating factor given the very small sample size. Genetic testing confirmed Usher syndrome II in 1 blue-sensing subject and 1 non–blue-sensing subject. We found no indication for a difference in blue sensation between patients with Usher syndrome II and those with nonsyndromic RP.

Discussion

Implications for a Color-Encoded Stimulation Strategy

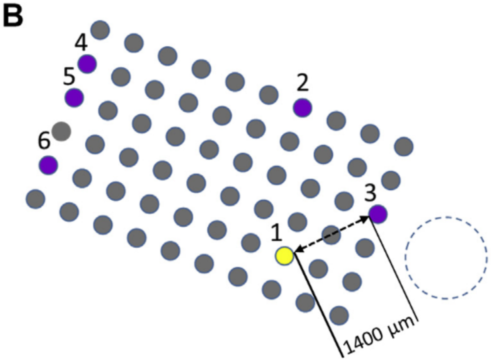
Present retinal prostheses typically elicit black, white, and limited gray scale visual perceptions in blind patients with RP who, without the retinal prostheses, only have bare or no light perception and no color vision due to photoreceptor loss. In this study, we tested the feasibility of restoring color sensation by delivering frequency-modulated stimuli under amplitude control in the blind patients with RP. Our testing was performed in the Argus II subjects, but the overall strategy of encoding color information in electrical stimulation that can be decoded by inner retinal neurons could find applications in other retinal prostheses. Despite our methodology being currently limited to color sensations along the blue-yellow axis, it is an important first step toward bioelectronic restoration of color vision in photoreceptor-less retina. In the ultimate clinical application, specific color-encoding algorithm will be determined for each electrode based on its frequency-color correspondence, amplitude-brightness correlation, and the frequency range that exhibits better long-term stability. What also needs to be considered when integrating color information with spatial information is the possible interaction between phosphenes of different colors when the stimulation sites are close enough; such interaction may be shaped by both current distribution in the retina and color processing in the higher visual centers. Given that frequency modulation

A



Electrodes	F (Hz)	Left	Right
1 & 2	20	blue	white
	60	blue	blue
1 & 3	20	blue	white
	60	blue	blue
1 & 4	20	blue	yellow
	120	blue	blue

B



Electrodes	F (Hz)	yellow	purple
1 & 2	20	bottom	top
1 & 3	20	lower left	upper right
1 & 4	20	lower right	upper left
1 & 5	20	lower right	upper left
1 & 6	20	right	left

Figure 5. Color perception with simultaneous stimulation of paired electrodes. **A,** color perception of subject 1 when electrode 1 was separately paired with electrodes 2, 3, and 4. Data obtained at 2 frequencies (20 and 60 Hz for pairs 1 and 2 and pairs 1 and 3; 20 and 120 Hz for pairs 1 and 4). **Left:** locations of the electrodes in the visual field (labeled in red); **right:** Color percepts produced by activation of different electrode pairs and the relative locations of each color. **B,** Color perception of subject 4 when electrode 1 was separately paired with electrodes 2−6. Data obtained at 20 Hz. **Left:** Locations of the electrodes in the visual field. Difference in the orientation of the visual field between **A** and **B** reflect the array position in the right versus left eye. At 20 Hz, electrode 1 consistently generated yellow percepts, and other electrodes generated purple percepts (electrodes labeled by the corresponding colors). Edge-to-edge separation between electrodes 1 and 3 is labeled by the **dashed line** with **arrows**. **Right:** Relative locations of the yellow and purple percepts under activation of different electrode pairs.

within the tested parameter space generated blue perception in 5 of 7 subjects, the possibility remains that such a color encoding strategy may not fit a subset of the RP population. A larger patient cohort is required to identify key determinants in blue sensation.

Possible Origins of the Frequency-Based Color Response

The origin of the frequency-shifted blue-yellow color sensation is yet unclear. Earlier studies of signal transmission from S-cones to ON pathways in primates suggested that the blue-ON signal is carried by the small bistratified retinal ganglion cells (RGCs), although the retinal origin of the blue-OFF signal remained elusive.[6,18] In recent years, compelling evidence that mGluR6-deficient patients who have disrupted S-cone input to the small bistratified RGCs exhibit normal central blue vision gave rise to a new school of thought arguing that all conscious hue perception, including blue, is mediated by the midget RGCs and that the small bistratified RGCs, despite receiving S-cone inputs, only facilitate the unconscious sensing of the spectral changes of the light.[19,20] In this theory, S-cone input feeds the receptive field surrounds of the L/M opponent midget RGCs in 4 different ways, providing the physiologic basis of 4 distinct color channels by summing with either M input for the blue-yellow axis or summing with L for the red-green axis. Given these 2 competing theories, unveiling the underlying mechanism of the electrically generated blue-yellow perception may demand a systematic study of the frequency response of the small bistratified and the midget RGCs in the retinal network. An interesting aspect of our results with respect to the midget RGC theory is that according to that theory, yellow, white, and green are mediated by ON-center midget RGCs, and red and blue are mediated by OFF-center midget RGCs. Purple is the simultaneous sensation of blue and red; thus, it is possible that the higher frequencies are more effective at stimulating the OFF-center RGCs, giving rise to blue and purple percepts, while lower stimulus frequencies preferentially stimulate the ON-center RGCs, giving rise to sensations of white and yellow. It awaits to be determined how morphology, stratification, and ion channel distribution could potentially contribute to multi-pulse integration of these and other cell types and whether it is possible to further separate out sensations of red and green as well as yellow and blue.

It is noteworthy that retina is only the first step in the generation of color sensation. In normal color vision, signals from the blue-mediating RGCs (i.e., small bistratified

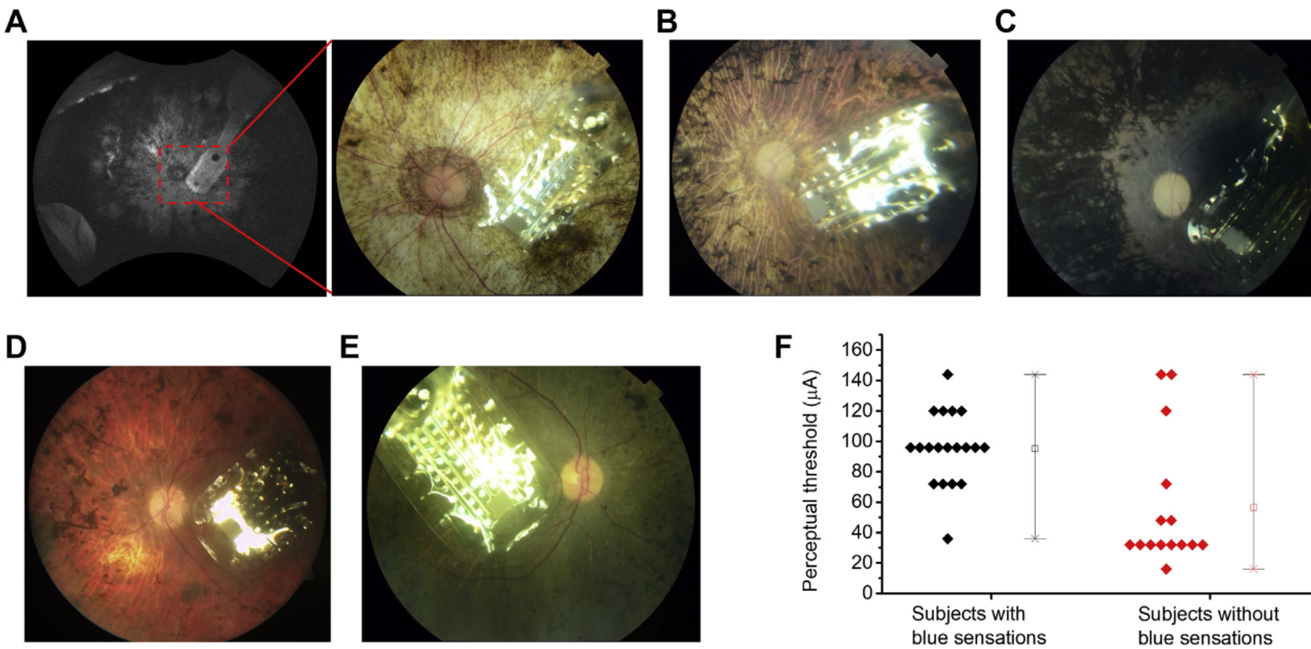


Figure 6. Fundus images and perceptual threshold of blue-sensing and non−blue-sensing subjects. **A,** Fundus images showing overall placement of the intraocular implant in subject 3 **(left)** and the blown-up view of the retinal area nearby the electrode array **(right)**. **B** and **C,** Fundus images obtained from the implant eye of subjects 4 (**B**; left eye) and 5 (**C**; left eye). All 3 subjects reported blue/purple sensation at higher stimulation frequencies. **D** and **E,** fundus images obtained from the implant eye of subjects 6 (**D**; left eye) and 7 (**E**; right eye). Neither subject reported blue/purple sensation at higher stimulation frequencies. **F,** Perceptual threshold of 19 electrodes in the blue-sensing subjects versus that of 15 electrodes in the non−blue-sensing subjects.

RGCs, midget RGCs, or other subpopulations) are decoded by higher visual centers in the context of other visual information such as contrast and brightness. Likewise, in electrically elicited prosthetic vision, interpretation of color encoded signals is likely to involve neural computation of the output from various spatially and temporally relevant cells, as demonstrated by our observation of skewed color perception of very bright or dim phosphene. Thus, electrical activation of other visual pathways may interact with the color pathway, affecting color interpretation in the higher visual centers. The extent of this influence and its contribution to frequency-modulated color perception under the roughly controlled phosphene brightness warrant further evaluation.

Frequency Response and Retinal Ganglion Cell Desensitization

Decrease in RGC response to repeated delivery of electrical stimuli at high frequency was noted in vitro in multiple animal models.[21-25] On the basis of the findings that profound RGC desensitization occurred after approximately 200 ms of stimulation, Cai et al[26] predicted altered phosphene properties midway through a stimulus longer than 200 ms. Pulse train durations of 250 ms and 1 second were both explored in our initial study, and the subjects reported longer-lasting phosphene sensation for 1-second stimulus without consistently sensing a sharp change in brightness or color of the phosphenes midway through the

Table 1. Subjects' Blue Sensations and Retinitis Pigmentosa Conditions

Subject	Blue Sensation	Age	Gender	Diagnosis	Age of Diagnosis*	Years Being Blind*	Family History of RP*	Vision Status in the Implant Eye
1	Y	68	F	RP	23	30	1 brother	OD, NLP
2	Y	63	F	RP	18	18	Paternal grandmother, father, and 1 sister but neither of the 2 brothers	OS, NLP
3	Y	71	F	RP	45	15	None known	OS, NLP
4	Y	75	F	USH2	22	23	1 sister	OS, BLP
5	Y	61	F	RP	~25	20	1 sister	OS, NLP
6	N	71	M	RP	37	26	None known	OS, NLP
7	N	48	M	USH2	15	21	1 aunt	OD, NLP

BLP = bare light perception; NLP = no light perception; OD = right eye; OS = left eye; RP = retinitis pigmentosa; USH2 = Usher syndrome 2.
*Subject report.

stimulus. At the default PW of the Argus II, RGC firing is evoked by both direct and network-mediated indirect activation. Previous studies have shown that although indirect activation undergoes pronounced desensitization with repeated stimulation, the RGC response to direct activation remains stable, and lengthening of PW increasingly favors desensitization-prone indirect activation of RGCs.[23,27] Yet no obvious impact on the subjects' color perception was observed under variations of PW in a range that was demonstrated to cause a significant shift between direct and indirect activation of RGCs in vitro (Fig 3E). These observations, along with the finding that current amplitude needs to be markedly lowered rather than raised to maintain phosphene brightness under increased frequency, suggest a frequency response more complex than RGC desensitization the way we currently understand it. One possibility could be resistance of blue-mediating RGCs to desensitization under high-frequency stimulation or alternatively a multi-pulse integration mechanism that selectively enhances activity of blue-mediating RGC subtypes or suppresses the antagonistic input to blue signaling.

Safety of High-frequency Stimulation in Retina

A limitation of the present study is the uncertainty of the risks of tissue damage for stimuli delivered at high frequencies over long periods of time. An earlier study of the sciatic nerve in cats suggested increased risks of axon damage associated with prolonged high rate stimulation, that is, 8 hours of continuous stimulation at 50 and 100 Hz.[28] Later, Cohen et al[29] found increased reflectance of the inner plexiform layer and edema with the pulse trains $\geq$ 442 $\mu C/cm^2$ per phase delivered continuously at 50 Hz for 5 minutes. It remains to be determined whether a clinical stimulation paradigm that uses intermittent pulse trains each lasting subseconds long would impose similar accumulative damage to the tissues, but both studies suggested that an increase in the stimulation frequency lowered the maximum current amplitude that the target tissue can be safely exposed to. For Argus II electrodes of 225 μm in diameter, given the default PW of 0.45 ms, a charge density limit of 442 $\mu C/cm^2$ per phase yields a maximum current amplitude of approximately 300 μA, which is above the amplitudes we have tested so far for frequencies $>$ 20 Hz. Our finding of the need to lower the current amplitude at higher frequencies to maintain phosphene brightness may further reduce the chance of tissue damage. Nonetheless, caution needs to be taken in the calibration of the current strength of high-frequency stimuli.

In conclusion, our findings offer a strategy to encode blue-yellow color information in the delivery frequency of the electrical stimuli for retinal prostheses as an extra visual cue. Color sensation, even at lower spatial resolution, could add enormously to the visual ability of the blind, enabling them to search the outstanding color(s) for guidance, potentially facilitating quicker and more accurate object recognition, target localization, and visually guided orientation. Further studies are required to verify the benefits of clinical utility of these color clues in daily living and reveal the underlying mechanisms of the frequency-modulated bioelectronic color perception.

Acknowledgments

The authors thank Dr. Jessy Dorn at Second Sight Medical Products for sharing earlier clinical findings.

References

1. Bramão I, Faísca L, Petersson KM, Reis A. The Contribution of Color to Object Recognition. In: Kypraios I, ed. *Advances in object recognition systems*. Rijeka, Croatia: InTech; 2012: 73−88. Available at: http://www.intechopen.com/books/advances-in-object-recognition-systems/the-contribution-of-color-in-object-recognition.
2. Tanaka J, Weiskopf D, Williams P. The role of color in high-level vision. *Trends Cogn Sci*. 2001;5:211−215.
3. Rossion B, Pourtois G. Revisiting Snodgrass and Vanderwart's object pictorial set: the role of surface detail in basic-level object recognition. *Perception*. 2004;33:217−236.
4. Uttl B, Graf P, Santacruz P. Object color affects identification and repetition priming. *Scand J Psychol*. 2006;47:313−325.
5. Nathans J. The evolution and physiology of human color vision: insights from molecular genetic studies of visual pigments. *Neuron*. 1999;24:299−312.
6. Dacey DM. Circuitry for color coding in the primate retina. *Proc Natl Acad Sci*. 1996;93:582−588.
7. Bressler NM. Age-related macular degeneration is the leading cause of blindness. *JAMA*. 2004;291:1900−1901.
8. den Hollander AI, ten Brink JB, de Kok YJ, et al. Mutations in a human homologue of Drosophila crumbs cause retinitis pigmentosa (RP12). *Nat Genet*. 1999;23:217−221.
9. Jackson GR, Owsley C, Curcio CA. Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. *Ageing Res Rev*. 2002;1:381−396.
10. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet*. 2006;368:1795−1809.
11. Stanga P, Jalil A, Tsamis E, et al. *Argus II® Electronic Epiretinal Prosthesis in Advanced Dry AMD: Safety and Feasibility Study and Preliminary Functional Results*. Seattle, WA: ARVO; 2016.
12. Stingl K, Bartz-Schmidt KU, Besch D, et al. Subretinal visual implant alpha IMS−clinical trial interim report. *Vision Res*. 2015;111:149−160.
13. Yue L, Weiland JD, Roska B, Humayun MS. Retinal stimulation strategies to restore vision: Fundamentals and systems. *Prog Retin Eye Res*. 2016;53:21−47.
14. Stanga P, Hafezi F, Sahel J, et al. Patients blinded by outer retinal dystrophies are able to perceive color using the Argus II Retinal Prosthesis System. *ARVO*. 2011;2011:1−5.
15. Hornig R, Zehnder T, Velikay-Parel M, et al. *The IMI retinal implant system. Artificial Sight*. New York: Springer; 2007: 111−128.
16. Stanga PE, Sahel Jr JA, Hafezi F, et al. Patients blinded by outer retinal dystrophies are able to perceive simultaneous colors using the Argus® II retinal prosthesis system. *Invest Ophthalmol Vis Sci*. 2012;53, 6952-6952.
17. Gordon J, Abramov I, Chan H. Describing color appearance: hue and saturation scaling. *Percept Psychophys*. 1994;56:27−41.
18. Dacey DM. Physiology, morphology and spatial densities of identified ganglion cell. Paper presented at: Symposium on Higher-order processing in the visual system, held at the Ciba Foundation, London, 19-21, October 1993.

19. Neitz J, Neitz M. Evolution of the circuitry for conscious color vision in primates. *Eye*. 2017;31:286.
20. Neitz J, Neitz M. The genetics of normal and defective color vision. *Vision Res*. 2011;51:633−651.
21. Jensen RJ, Rizzo III JF. Responses of ganglion cells to repetitive electrical stimulation of the retina. *J Neural Eng*. 2007;4:S1.
22. Freeman DK, Fried SI. Multiple components of ganglion cell desensitization in response to prosthetic stimulation. *J Neural Eng*. 2011;8:016008.
23. Ahuja AK, Behrend MR, Kuroda M, et al. An in vitro model of a retinal prosthesis. *IEEE Trans Biomed Eng*. 2008;55:1744−1753.
24. Stronks HC, Dagnelie G. The functional performance of the Argus II retinal prosthesis. *Expert Rev Med Devices*. 2014;11:23−30.
25. Hadjinicolaou A, Savage C, Apollo N, et al. Optimizing the electrical stimulation of retinal ganglion cells. *IEEE Trans Neural Syst Rehabil Eng*. 2014;23:169−178.
26. Cai C, Ren Q, Desai NJ, et al. Response variability to high rates of electric stimulation in retinal ganglion cells. *J Neurophysiol*. 2011;106:153−162.
27. Fried SI, Hsueh H-A, Werblin FS. A method for generating precise temporal patterns of retinal spiking using prosthetic stimulation. *J Neurophysiol*. 2006;95:970−978.
28. McCreery D. Tissue reaction to electrodes: The problem of safe and effective stimulation of neural tissue. In: *Neuroprosthetics: Theory and Practice*. Singapore: World Scientific; 2004:592−611.
29. Cohen E, Agrawal A, Connors M, et al. Optical coherence tomography imaging of retinal damage in real time under a stimulus electrode. *J Neural Eng*. 2011;8:056017.

Footnotes and Financial Disclosures

Originally received: June 5, 2020.
Final revision: August 5, 2020.
Accepted: August 10, 2020.
Available online: ■■■. Manuscript no. D-20-01563.

[1] Roski Eye Institute, University of Southern California, Los Angeles, California.

[2] Ginsburg Institute for Biomedical Therapeutics, University of Southern California, Los Angeles, California.

[3] Department of Ophthalmology, University of Washington, Seattle, Washington.

Financial Disclosure(s):
The author(s) have made the following disclosure(s): M.S.H.: financial interest − Second Sight Medical Products Inc.

Supported by National Science Foundation EAGER #1833288; National Science Foundation Biophotonics #1805210; Dean's Pilot Funding Program, USC (Los Angeles, CA); funding from Ginsburg Institute for Biomedical Therapeutics, USC (Los Angeles, CA); Juliette RP Vision Foundation; National Institutes of Health RO1EY027859; Unrestricted Grant to the Department of Ophthalmology from Research to Prevent Blindness (New York, NY). The sponsors or funding organizations had no role in the design or conduct of this research.

HUMAN SUBJECTS: Human subjects were included in this study. The human ethics committees at the University of Southern California approved the study. All research adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Yue, Neitz, Humayun

Data collection: Yue, Gonzalez

Analysis and interpretation: Yue, Castillo

Obtained funding: Yue, Neitz, Humayun

Overall responsibility: Yue, Humayun

Abbreviations and Acronyms:
F = frequency; **PW** = pulse width; **RGC** = retinal ganglion cell; **RP** = retinitis pigmentosa; **RYGB** = Red, Yellow, Green, and Blue.

Keywords:
Color vision, Retinal degeneration, Retinal stimulation, Visual prosthesis, Vision restoration.

Correspondence:
Lan Yue, PhD, 1450 San Pablo St, HC4 Room 6526, Los Angeles, CA 90033. E-mail: lyue@usc.edu.