PHOTOPIC ON- AND OFF-PATHWAY ABNORMALITIES IN RETINAL DYSTROPHIES*

BY *Paul A. Sieving,* MD PhD

INTRODUCTION

THIS THESIS INVOLVED AN ELECTROPHYSIOLOGIC ASSESSMENT OF ABNORmalities in the retinal ON- and OFF-pathways of the cone visual system for patients with retinal dystrophies. Cone visual signals in the retina are processed through dual pathways, one involving ON-center bipolar cells (the ON-pathway) and the other through OFF-center bipolar cells (the OFF-pathway).[1-3] Very little currently is known about what differences in visual information are carried by these two cone pathways. Blocking the ON-pathway in monkey, by injecting glutamate neurotransmitter analogs, diminishes responses for stimulus increments and affects red-green color opponency.[4,5]

We used a relatively novel technique of recording the photopic electroretinogram (ERG) using long-duration light flashes to sort out ON- versus OFF-pathway changes in these patients. The premise was that some of these patients might suffer from vision loss owing to abnormal signal processing in the proximal retina, postsynaptic to the cone photoreceptors.

The locus of vision sensitivity loss in human retinal dystrophies is poorly understood.[6] Retinal histopathology of retinitis pigmentosa indicates that the rod and cone photoreceptors are ultimately lost in at least some cases[7] with obvious consequence to vision. The recent molecular genetic finding that rhodopsin mutations are associated with autosomal dominant retinitis pigmentosa (adRP)[8] adds further support to the notion that the primary defect in many retinal dystrophies occurs at the photoreceptor level in cases of rod and rod-cone degenerations.

However, there remain several puzzling aspects to the story. Why, for instance, do some cases of rod-cone dystrophy (traditionally called "retinitis pigmentosa") have ERG b-wave responses reduced disproportionately compared with fairly well-preserved visual fields and only modestly elevated dark-adapted thresholds? The b-wave develops primarily in the proximal

*From the W. K. Kellogg Eye Center, The University of Michigan Medical School, Ann Arbor.
Supported by grant EY06094 from the National Institutes of Health and by the National Retinitis Pigmentosa Foundation, Baltimore.

retina, postsynaptic to the photoreceptors.[9] In tentative answer, Arden[6] has proposed that, although the scotopic visual pathway is functioning, the retinal Müller glial cells are secondarily affected. These Muller cells are responsible for the transretinal potassium currents that ultimately manifest as the ERG b-wave.[10] In other cases, RP patients were found to have considerable rhodopsin visual pigment within the photoreceptors by in vivo fundus reflection densitometry, but they suffered from extensive rod vision loss in dim light.[11] Arden[6] speculated that a sensitivity loss occurs in neurons proximal to photoreceptors. Such cases provide a rationale for analyzing the function of the proximal retina in patients with retinal dystrophy.

A quite strong case for post-photoreceptoral vision loss has been made for the particular retinal dystrophy called congenital stationary night blindness (CSNB). CSNB was studied extensively by Carr[12] and Ripps,[13] and both have written insightful summaries of this work. Their conclusion was that, particularly for Schubert-Bornschein[14] (SB) type of CSNB, the primary defect limiting vision occurred postsynaptic to the photoreceptors. Subsequent work has suggested that neural transmission is defective at the sign-inverting glutamate synapse between the rod photoreceptors and the second-order retinal bipolar cell neurons,[15,16] also called the "rod" depolarizing bipolar cells (DBCs). In the case of SB type CSNB, Carr[12] and Ripps[13] have presented good evidence that the rod photoreceptors remain functional but that the rod signal does not reach the rod bipolar cell. In similar fashion, although not occurring in a category of a retinal dystrophy, disruption of synaptic transmission from photoreceptors to the rod DBCs is thought to be responsible for paraneoplastic night blindness in cutaneous malignant melanoma.[17]

Neither is daylight cone vision exempt from problems occurring in the proximal retina beyond the photoreceptors themselves. Yokoyama and associates[18] reported patients with cone dysfunction who showed sparing of the a-wave from photoreceptors[19] but with diminished ERG b-waves. Grey and co-workers[20] reported cases of bull's eye maculopathy and cone degeneration and puzzled over finding that the b-wave was reduced considerably more than the a-wave. More recently, Young and colleagues[21] reported studying a patient with retinal degeneration for whom the cone b-wave was depressed selectively over the a-wave. Cases such as these emphasize the great diversity of mechanisms by which vision can be lost in patients with cone dystrophies. This sets the stage for considering the possibility that some patients labeled as having cone or even rod-cone dystrophy may suffer from visual loss occurring from cellular dysfunction in the retinal pathway proximal to the cones or rods themselves.

This thesis reports a physiological search for retinal abnormalities occurring in the retinal cone pathway postsynaptic to the photoreceptors, as evaluated by the ERG. The ERG is an appropriate tool for such study. The ERG is a complex response that includes contributions from many different retinal cells. The ERG is particularly useful as a tool for indicating which retinal cells may be dysfunctional. Consider that the ERG a-wave results from activity of the photoreceptors rather directly,[19] whereas the b-wave originates postsynaptic to the photoreceptors.[9,10] Consequently one can judge the involvement of photoreceptors versus cells postsynaptic to the rods or cones by attention to the relative preservation of the a-wave versus the b-wave.[22-24]

Recent studies have demonstrated that recording the photopic ERG with long-duration stimuli provides novel information about the ON- and OFF-pathways of cone vision through the proximal retina under light-adapted conditions.[25,26] New information suggests that, for the light-adapted vertebrate retina, the b- and d-waves reflect activity of the cone ON- and OFF-pathways, respectively.[25,26] Thus, recording the long-flash photopic ERG provides an opportunity to refine the clinical understanding of mechanisms of cone vision loss in the retinal dystrophies. The application of long-flash ERG studies to clinical questions is quite new, and not even a preliminary survey of photopic ERG ON- and OFF-responses in the retinal dystrophies has been published thus far.

This background presents the rationale for this thesis study. First, however, it was necessary to design and construct an ERG stimulator capable of presenting long-stimulus flashes under photopic conditions and with the light delivered in a fashion suitable for recording from untrained subjects. Such stimulators are not yet commercially available. Techniques for recording ERG ON- and OFF-components are quite new, and we first needed to establish appropriate recording conditions and protocols. Control response values were gathered from a normal population, and methods of ERG data analysis were devised for screening a patient population. With this preliminary work completed, a survey of long-flash ERG abnormalities was then performed with patients who had a variety of retinal dystrophies.

ERG RESPONSES TO ONSET AND CESSATION OF LIGHT

The ERG is important for diagnosing and studying human retinal dysfunction. However, as currently recorded in the majority of clinical settings, the ERG is elicited by very brief flashes of light that are produced by a xenon photostrobe tube and that typically last only microseconds. These very brief xenon-flashes give large ERG responses and are suitable for recording the activity of the dark-adapted retina, for which the rods and rod ON-pathway

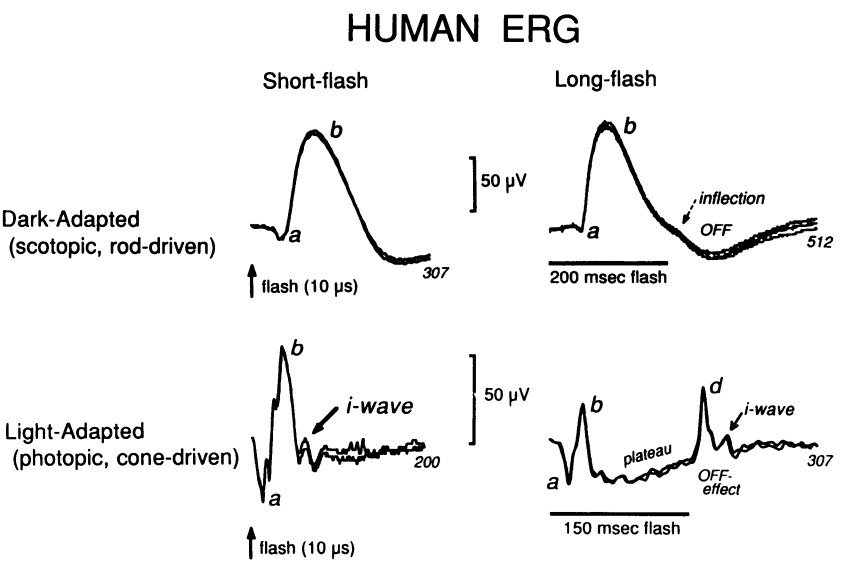

FIGURE 1
Human electroretinogram rod responses recorded dark-adapted with dim flashes and cone responses with bright flashes on a light-adapting background. Stimuli are 10-μsec short-flash or 150- and 200-msec long-flash. Numbers (200, 307, 512) indicate total waveform duration in msec.

mediate vision. However, they are less suitable for dissecting the cellular components of the light-adapted ERG which involves the cone photoreceptors and cone ON- and OFF-pathways.

The main rod ERG components that are measured clinically are the a- and b-waves, shown in Fig 1 for the short flash of 10 μsec. The a-wave provides information about the rod photoreceptors themselves, since this a-wave results from hyperpolarization of rods by the light flash.[19] The b-wave reflects retinal activity postsynaptic to the rods and is believed to result from the depolarizing bipolar cells extruding potassium upon stimulation by light; the Müller cells sequester the potassium and thereby produce the actual b-wave as an epiphenomenon[9,10] to the actual neural signal that subtends vision. Both the rod a- and b-waves are readily elicited by a brief flash, since both are generated primarily by the onset of a light flash and hence are "ON-responses" of the retina.

Although not much used clinically up to the present time, if a long-duration light flash is presented, the vertebrate retina also responds to light cessation and produces an OFF-effect in the ERG, as seen in Fig 1 for the 150-msec flash.[27-29] The OFF-effect of the rod system is small. When long-duration flashes of light are used, the rod OFF-effect is a simple response

that consists primarily of a slow negative-going dip in the ERG wave upon cessation of light (Fig 1). The rod OFF-effect does not contribute significantly to the a- or b-waves as recorded with brief flashes under dark-adapted conditions.

Under light-adapted conditions that reveal cone-driven responses, the ERG response appears quite different. Light adaptation suppress rod activity by the imposition of a steady background light. This is also called the "photopic" condition. The human photopic cone-driven ERG contains the a- and b-wave ON-responses at light onset, but it also shows a separate and large OFF-effect at the termination of the stimulus,[30] as seen in Fig 1 for the 150-msec flash. This photopic ERG OFF-effect is variously called the "OFF-component," "d-wave," or "rapid-off response."[31] By whichever term, these are best reserved to refer exclusively to the photopic cone-driven condition. The terms "d-wave" and "ERG OFF-effect" will be used primarily in this thesis.

The human d-wave OFF-component is a phasic wave of positive polarity at the cornea, like the b-wave ON-component. For very brief flashes the b- and d-waves (ie, the ON- and OFF-responses) occur essentially simultaneously and cannot be observed as separate waves. Further, it is now recognized that when brief flashes are used, the two phasic and positive b- and d-wave responses will summate and merge into a single response that is called the clinical photopic b-wave.[32,33] Thus, stimulating with brief flashes will not capture the complexity of retinal photopic ERG activity. The d-wave can be separated from the photopic b-wave by stimulating with a long-duration pulse of light that lasts upwards of at least 50 msec so that the ON- and OFF-responses do not coincide. Under this condition, the human d-wave is revealed to be nearly as large as the photopic b-wave.

REVIEW OF ERG TERMINOLOGY

A review and clarification of ERG terminology may prove helpful before proceeding. The term "photopic" refers to light-adapted recording conditions for which the ERG reflects the activity of the cone photoreceptors and of those neurons onto which cones synapse in the proximal retina. "Scotopic" refers to the dark-adapted condition for which the ERG is primarily rod-driven, although cones may contribute to a small extent if very bright stimuli are used.[34]

The term "b-wave" is defined as the first positive ERG wave following the initial wave which is the negative a-wave.[35] The true photopic b-wave reflects ON-activity of the retina in response to the onset of a stimulus light. This ON-activity can be observed as separate from the d-wave only by using extended flashes, since for brief flashes the initial positive ERG wave (ie,

nominally but sloppily called the "b-wave" even for instantaneous flashes) is a composite of the true photopic ON-activity plus the d-wave OFF-effect.[32,33] Thus when using the term "photopic b-wave," one must specify whether it is elicited with a short or with a long flash. Consequently, in this manuscript we will refer to the photopic "short-flash (pseudo) b-wave" versus the "long-flash (true) b-wave."

As seen in Fig 1, the a- and b-waves can be elicited for both the scotopic and photopic conditions, and consequently can be driven either by the rod or the cone circuit. The positive and phasic (ie, not sustained) d-wave, however, is primarily cone-driven. Since we are discussing the photopic ERG elicited with extended flashes, we use the term "d-wave" exclusively to refer to the cone-driven OFF-effect.

Some indication of photopic OFF-effect activity can be observed even with a brief flash, in the form of the i-wave.[36,37] As seen in Fig 1, the i-wave is a small and positive wave that follows the photopic short-flash b-wave. However, it was demonstrated recently that ON-responses also contribute to the i-wave,[38] and thus the short-flash i-wave is not a pure measure of retinal OFF-activity.

RETINAL CIRCUITRY OF THE ROD AND CONE PATHWAYS

There are fundamental differences between the rod and cone visual pathways through the retina. The primary requirement of the rod system is to detect an increment of light against a dark background, such as starlight against the blackness of midnight. The rod pathway is optimized around principles that involve processing single quanta visual signals.[1,39] The cone system, by comparison, functions in an environment of surrounding light, and the cone pathway is designed to detect both increments and decrements of light against an illuminated background.

These different visual requirements for rod versus cone vision are reflected in the retinal wiring of the rod and cone pathways, shown by the simplified schematic in Fig 2. The photoreceptors transmit visual information to the retinal bipolar cells, which are second-order neurons. Rods contact only one category of bipolar cells,[40] called the "rod bipolars." The majority of investigators believe that rod bipolar cells depolarize upon being stimulated.[41] In Fig 2 a sign-inverting (−) synapse lies between the rod and DBC. This terminology reflects the fact that when stimulated by light, rods hyperpolarize and their membrane potential becomes more negative. The rod bipolar cell, however, depolarizes when stimulated and the membrane potential becomes more positive. Consequently, the synaptic connection must invert the signal communicated between the hyperpolarizing rod and the depolarizing rod bipolar cell. These DBCs are also called ON-center

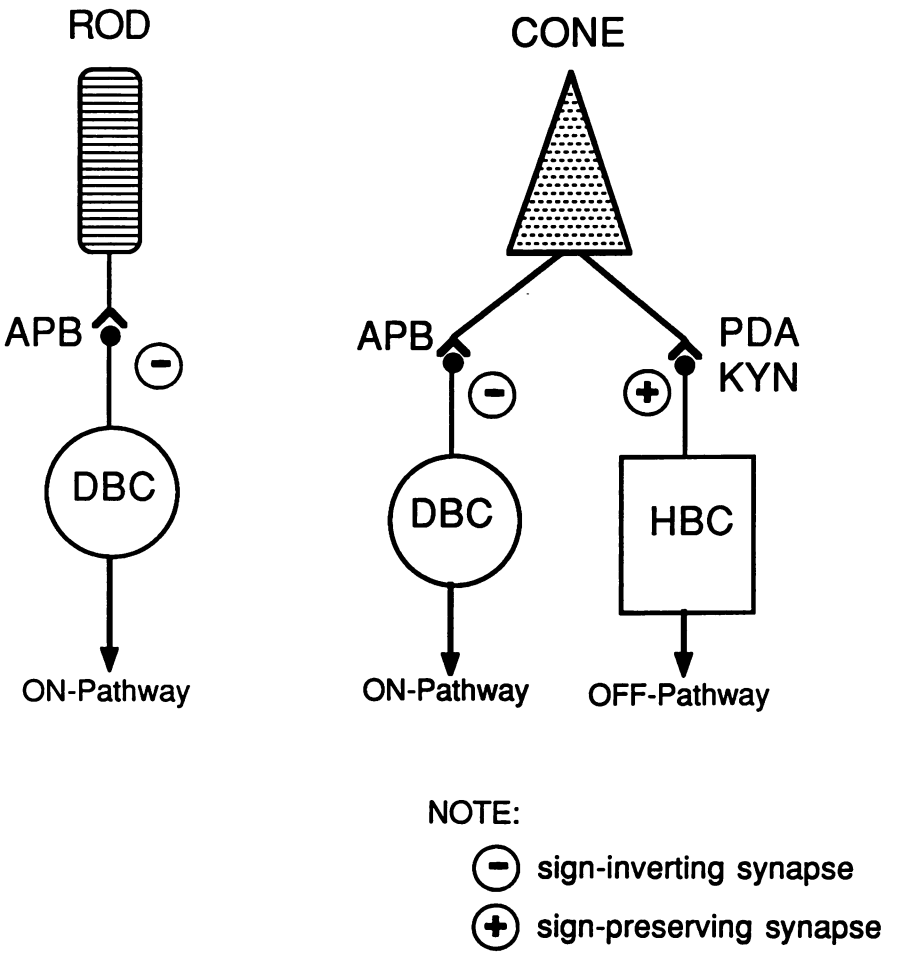

FIGURE 2
Retinal rod and cone pathway wiring. (APE, 2-amino-4-phosphonobutyric acid; KYN, kynurenic acid; PDA, ±cis-2,3-piperidine dicarboxylic acid.)

cells, since they depolarize when a spot of light falls onto the rods that feed them,[41] and the rod pathway is called the "ON-pathway" (Fig 2).

Cones have more extensive postsynaptic connections, and they synapse onto several different types of cone bipolar cells.[41] Some of these are depolarizing cells, and like the rod DBCs, form the cone ON-pathway with a sign-inverting (−) synapse (Fig 2). In addition cones contact hyperpolarizing bipolar cells (HBCs) through a sign-preserving (+) synapse in the cone OFF-pathway.

These two different synapses are each selectively sensitive to different glutamate analogs. The sign-inverting (–) synapse can be blocked by APB (2-amino-4-phosphonobutyric acid).[42] The sign-preserving (+) synapse is not much affected by APB but it can be blocked by either PDA (±cis-2,3-piperidine dicarboxylic acid)[43] or KYN (kynurenic acid).[44] Consequently, these drugs allow for preferential blockade of either the ON- or OFF-pathways in the retina. Further, in connection with these drugs, one can also take advantage of using a steady background light to suppress activity of the rod pathway and thereby isolate either the cone ON- or OFF-pathway quite selectively.

This approach has now led to a preliminary understanding of the contributions of the ON- versus OFF-pathways to the cone ERG in lower vertebrates[25] and even in monkey primate.[26] Although this approach requires the use of long-duration ERG stimuli that currently are not routinely available in clinical laboratories, it is beginning to prove useful for assessing retinal dysfunction in new and more specific ways.[15,17,45,46]

HISTORIC OVERVIEW AND CLINICAL UTILITY OF LONG-FLASH PHOTOPIC ERG STUDIES

Granit[47] described two forms of OFF-effects in the ERG of different animals and recognized that these corresponded to the rod and cone systems. OFF-effects were most prominant in retinas that contained cones. Best and Bohnen[48] demonstrated an ERG OFF-effect for the human eye and found the response more pronounced when light-adapted, consistent with an origin involving the cones. Kawasaki and associates[31] suggested that the human ERG OFF-effect was related rather directly to cone activity. Yonemura and Kawasaki[49,50] described the use of very bright stimuli to elicit a sharply positive potential when the light was turned off. On the basis of application of aspartate in an excised human eye cup, they ascribed this "rapid off-effect" to the termination of Granit's PIII photoreceptor process.[51] Aspartate is a glutamate analog that prevents neural transmission between the photoreceptors and postsynaptic cells and thereby pharmacologically isolates the direct rod and cone contribution to the ERG. Subsequently, a number of studies were published by Yonemura, Kawasaki, and colleagues utilizing the cone rapid OFF-effect to investigate aspects of human cone involvement in patients with color deficiency.[52-57]

The clinical utility of long-flash ERG recordings still remains largely unexplored. The potential benefit of such recordings was demonstrated recently by Miyake and colleagues[45,46] in studies of SB type CSNB. These patients are unable to see at night. The retention of a dark-adapted rod-driven a-wave gives evidence that rod photoreceptors are present and remain functional in SB-CSNB. The scotopic b-wave, however, is severely

diminished, and on this basis the SB-CSNB pathology is ascribed to faulty synaptic transmission between rods and rod bipolar cells.

Although the abnormal rod vision is the striking feature of SB-CSNB, Krill and Martin[58] and Heckenlively and associates[59] showed subtle abnormalities of the photopic a-wave to a brief xenon flash, which suggests involvement of the cone circuit. Proximal cone pathway abnormalities in SB-CSNB had mostly been overlooked, however, because photopic b-wave amplitudes to xenon-flash stimuli could remain normal although with slow implicit times.[58] Lachapelle and colleagues[60] noted that SB-CSNB showed only three rather than the normal four light-adapted oscillatory potentials from the proximal retina.[61]

Miyake and co-workers[45,46] probed the integrity of the cone pathway of SB-CSNB by stimulating with long-duration flashes of 125 msec. Under this condition, they demonstrated that the photopic (long-flash) b-wave was suppressed. This observation enabled Young[15] and Houchin and associates[16] to point out that the suppression of both the rod and cone (true) b-waves could be replicated by applying APB to the monkey retina[62,63] (Fig 2). Since APB blocks synaptic transmission from photoreceptors (both rods and cones) to depolarizing bipolar cells,[42] Young[15] and Houchin and associates[16] suggested that the pathology in SB-CSNB might involve the APB-sensitive glutamate synapse. This hypothesis has yet to be tested on the molecular level and must first await the cloning of the APB-sensitive glutamate receptor, which had not yet been achieved at the time of writing this manuscript. Whether or not Young and Houchin's hypothesis regarding SB-CSNB ultimately is established on the cell biologic level, one can appreciate the potential benefit of understanding and recording the long-flash photopic ERG components in cases of human retinal dystrophies.

"PUSH-PULL MODEL" OF PRIMATE PHOTOPIC ERG

Interpretations of ERG abnormalities, such as those advanced by Young and by Houchin and associates for CSNB, of course depends upon having an appropriate model by which to understand the complexities of the waves. Classic ERG analyses derive largely from Granit's description of several "Processes," termed PI, PII, and PIII, which he elucidated from the cat retina during progressive anoxia.[35,64,65] As recorded at the cornea, the various ERG waves are formed by superposition of Granit's basic Processes. Granit's model is particularly useful for analyzing the dark-adapted rod-driven ERG, from which it was primarily derived. This model, however, serves less well for analyzing the photopic ERG, where, as the data in this paper will show, it does not adequately explain the features of the photopic (true) b- and d-waves in cases of retinal pathology.

In currently existing ERG models, Granit's PIII is a corneal-negative potential, the leading edge of which shapes the a-wave. The a-wave originates from light-evoked hyperpolarization of the rods[19] or cones.[64] In classic analysis, the leading positive edge of the d-wave is shaped by the termination of PIII when the light is turned off.

In the dark-adapted ERG, Granit's PII is positive and phasic, particularly with bright stimuli, and forms the b-wave as the result of local depolarization of Müller (glial) cells consequent to the light-evoked efflux of potassium from depolarizing ON-bipolar cells, which increases extracellular potassium in the distal retina.[9,10,66,67] Synaptic transmission from both rods and cones onto the DBCs can be blocked selectively by APB.[42] APB eliminates the scotopic and photopic b-waves,[62,63] and thereby suggests that the b-wave reflects DBC activity.

However, the imposition of this dark-adapted ERG model to the light-adapted condition is problematic. As previously described (Fig 2), the scotopic retinal pathway of rods is mediated by DBCs alone, whereas in the photopic pathway the cones synapse onto both DBCs (of the cone ON-pathway) and HBCs (of the cone OFF-pathway).[2,39,41] (Of course, both rods and cones synapse onto horizontal cells also.) Thus, whereas it is sufficient to account for DBCs alone when describing the rod-driven ERG, it becomes necessary to consider the HBCs in addition when describing the cone-driven ERG. The drug APB is highly (although perhaps possibly not completely) selective for blocking the input to DBCs. The drugs PDA[43] and KYN[44] are selective for blocking input to HBCs. The ERG photopic OFF-effect d-wave is diminished by drugs that block synaptic transmission to the HBCs in lower vertebrates[45] and pigeon.[68]

We used APB and PDA and/or KYN to elucidate contributions of the depolarizing versus the hyperpolarizing bipolar cells for the monkey ERG (Fig 3). This has led to a more inclusive model for the monkey ERG.[26] A full presentation of this model will not be given here, but a brief overview will provide details pertinent to the analysis to be used in this thesis for ERG recordings of the retinal dystrophy patients.

The central findings are shown in Fig 3, where drugs were given by pars plana intravitreal injections in vivo[69] to cynomolgus and rhesus monkeys. Photopic ERGs were recorded before and after drug application to identify which ERG components were affected by these drugs. Our results demonstrated that the HBCs and horizontal cells exert major control over the photopic b-wave.

Before drug injection the control waveform exhibits the usual photopic components of the a-, b-, and d-waves, with the negative plateau between the b- and d-waves. By blocking DBC activity with an injection of APB, the

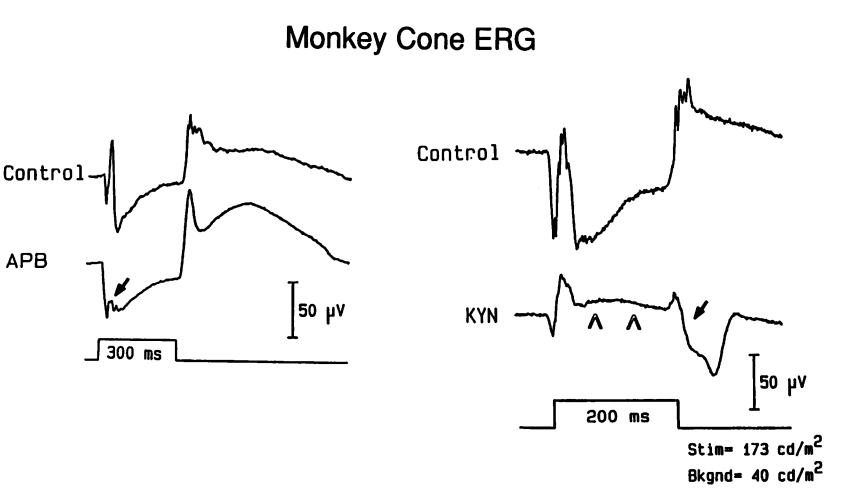

FIGURE 3
Monkey photopic cone-driven ERG. APB blocks ON-pathway and depresses b-wave (*arrow*). KYN blocks OFF-pathway, elevates plateau (*hollow arrowheads*), and causes an unusual negative-going waveform swing (*arrow*) at stimulus termination.

photopic b-wave was suppressed. The normally negative plateau remained negative. A quite different and novel effect was found when HBCs were blocked with KYN: This caused the a-wave to become smaller and the plateau was elevated above baseline such that the b-wave no longer fully turned off. Further, the d-wave amplitude was diminished. Current ERG models derived from Granit's PI, PII, and PII cannot account for this response. Note that these monkey photopic ERG recordings were made using the Ganzfeld stimulator described in the next section.

According to the retinal pathway schema in Fig 2, blocking the DBCs with APB should leave primarily the HBCs active to contribute to the photopic ERG (in addition, of course to the cone photoreceptors themselves). Thus the negative squared-off response after APB can be termed a hyperpolarizing pattern. Similarly, blocking HBCs with KYN gives the overall positive polarity response that can be termed a depolarizing pattern. We are proposing that the "push-pull" of the hyperpolarizing and depolarizing activity are summated in the ERG recorded at the cornea. In our model, photoreceptor negative PIII continues to account for part of the a-wave and for part of the d-wave.[70]

With this Push-Pull Model in hand, we saw a need to begin studying the human ERG OFF-components systematically in clinical patients. Only a few studies have utilized extended flashes to extract information about human retinal processing in human retinal dystrophies. We also sought to

find human responses that might support this new model of the photopic ERG. In addition to concentrating on measuring b- and d-wave response amplitudes, we were particularly interested in patterns of change that would involve both responses in ways that would be predicted by our Push-Pull Model. We have now found such patterns of abnormality from among the many we have studied with retinal dystrophies, and these findings are reported here.

WHAT IS NEEDED

The proposition is raised that devising clinically suitable methods to record the ERG with extended-duration flashes will increase our understanding of retinal pathology in terms of ON- and OFF-activity of the cone retinal pathways. The main impediment to such clinical recordings is the need for a long-flash stimulus itself, since commercial equipment is not currently available to provide other than brief xenon flashes. We have developed equipment and strategies for long-flash ERG studies and have established norms and identified patterns of abnormalities in human retinal dystrophies.

DESIGN AND CONSTRUCTION OF A GANZFELD STIMULATOR FOR EXTENDED FLASHES

DESIGN CONSIDERATION

The main requirement was to develop a stimulator capable of full-field (Ganzfeld) illumination and for which the duration of the light flash could be controlled. Since the ERG component waves vary with light intensity, full-field stimulation is important to ensure that all areas of the retina are stimulated to equal brightness. Otherwise, the ERG from areas stimulated only by dim scattered light will be different from the central areas stimulated directly and more intensely.

The recent ERG standards committee[71] highly recommended use of integrating sphere that is illuminated uniformly inside. The light source is usually positioned on top of the bowl, with light projected onto a diffusing plate that scatters light throughout the inside. The bowl is large enough for the subject to place his or her head into a hole in the front such that the inside surface fills the entire field of view and light reaches the eye from all angles. This method of Ganzfeld stimulation has the advantage of allowing some movement of the subject's head within the front opening while still maintaining uniform retinal illumination and thereby ensuring consistent ERG response amplitudes.

There are a limited number of other ways to accomplish full-field stimulation. Compared with the integrating sphere, most other methods do not

yield truly uniform Ganzfeld stimuli. Light may be delivered directly to the eye through a wide-field lens on an optical bench. This technique is called Maxwellian view[72] and has the advantage of offering precise optical control. Unfortunately, Maxwellian view requires precise positioning of the subject, which is quite cumbersome for use with clinical patients, especially if a dental bite-bar is used to stabilize the head. More problematic is the limited field of view that can be obtained with Maxwellian view, which is constrained by optical principles to:

$$\text{Field diameter} = 2 \tan^{-1}[(0.5 \times \text{lens diameter})/\text{focal-length}].$$

For an F=1 lens (eg, equivalent to the 20-diopter Nikon 2-inch lens used for ophthalmoscopy), the field diameter of direct retinal illumination is only 53.2°. An F=0.7 lens, which is near the theoretical optical maximum for an aspheric lens, offers only 71.1° visual field, which is still less than one third of the total retinal area. Even with an F=0.7 lens, more than two thirds of the retina will be stimulated, only by much dimmer scattered light.

In this context, it can be recalled that although the fovea has the greatest packing density of cones, cones are distributed across the entire retina, and the 1° fovea constitutes only 1% of the cone population. Calculations using photoreceptor counts from Osterberg[73] indicate that the posterior 53° of retina, which encompasses the area within and just beyond the vascular arcades, includes only about 35% of the entire population of cones in the retina. Thus, delivering a light flash by Maxwellian view optics with an F=1 lens will leave 65% of the cones stimulated only by stray light.

Light could also be delivered through a fiber-optic cable positioned near the cornea. However, optical principles of internal reflection in a light pipe limit the field to less than 80° for free viewing, which, like Maxwellian view, leaves nearly two thirds of the retina stimulated indirectly by dim scattered light. Further, fiber-optic delivery again requires cumbersome alignment of the human subject to the optical system.

Other methods use white paper as a diffusing screen illuminated from behind and placed directly in front of the eye to subtend a very wide field of view. A Ping-Pong ball cut in half can also be placed over the eye as a diffuser. However, the uniformity of retinal illumination depends on the spatial distribution of light shined onto the paper from behind. Light scattering follows a mathematical cosine distribution and is scattered best straight ahead and less well at large angles. Unless the diffusing screen is illuminated perpendicularly across its entire surface, light intensity falls off rapidly to the peripheral retina. Unfortunately this is the case when the screen is illuminated by light diverging from a lens on an optical bench or

from a fiber-optic semipoint source. The problem is exacerbated by the large curvature of a Ping-Pong ball diffuser.

Recently, light-emitting diodes (LEDs) have been advocated as ERG light sources.[74] LEDs are placed to cover the inside of a small-diameter bowl that is then placed over both eyes of a subject like goggles. This is a good optical solution but requires a specially designed and expensive linear current supply to drive the LEDs. Furthermore, the small bowl makes it difficult to use speculum electrodes, such as the Burian-Allen type that we prefer for recording from clinical subjects. LED stimulators potentially have the advantage of allowing complex sequences of stimulation, such as "saw-toothed" stimuli in which the light grows increasingly brighter and then turns off abruptly. It is not known whether complex stimuli will have clinical importance, and they are difficult to interpret relative to established paradigms.

Our design solution, described in the following paragraphs, was to construct a Ganzfeld bowl into which light is projected through a mechanical shutter that controls the flash duration. It is difficult to design a simple system in which light is collimated through the small aperture of a shutter and is still sufficiently bright to illuminate the inside of a large Ganzfeld bowl to photopic intensities. The key element in this design was to configure a bright but compact light source close to a mechanical shutter to maximize light capture into the bowl. The final product is inexpensive and is readily constructed in a simple workshop. All parts are available commercially, and the result has proven reliable during seven years of use.

GANZFELD STIMULUS CONSTRUCTION AND CALIBRATION

A schematic of the Ganzfeld stimulator is shown in Fig 4. Fig 5 shows an exploded view of the light housing, shutter, filter wheel and diffusing plate. A light source and shutter are placed on top of the bowl, with light projected inside through neutral density and/or color filters. The bowl is a 20-inch polypropylene streetlight globe (Adjusta-Post Co, Norton, OH). This is sprayed inside with white paint and then coated with magnesium oxide (Eastman Kodak, Rochester, NY) to provide flat spectral reflectance. A 4-inch top hole allows light to be projected in, and a 10-inch hole in the side of the bowl is the viewing port for the subjects. This opening is also large enough to accommodate research animals.[68,75] A red LED light is mounted inside the back of the bowl for subject fixation.

Mechanical shutters impose a trade-off between response time and aperture size. The commercial shutter (Uniblitz, Vincent Associates, Rochester, NY) that was used has a relatively large aperture of 1 inch but also has a fast response for 90% opening and closing times of less than 3 msec. This is

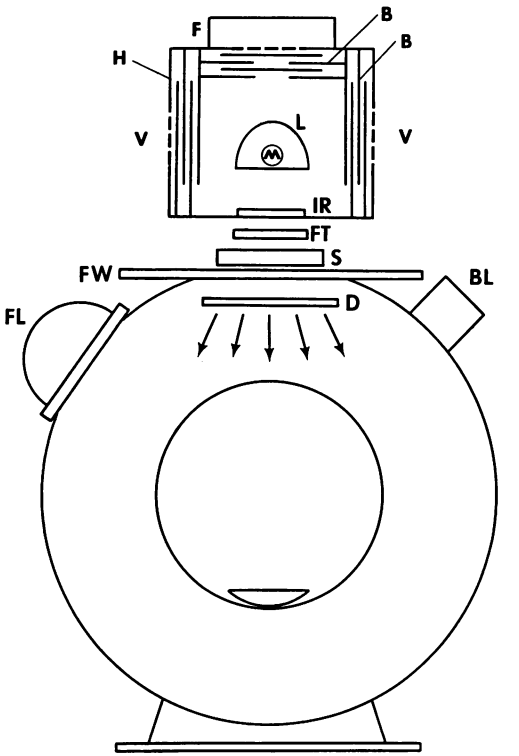

FIGURE 4
Ganzfeld bowl stimulator (see text for description).

quite satisfactory for both scotopic and photopic ERG recordings. This shutter was placed on top of the Ganzfeld bowl, directly in front of the light, and just in front of the filters.

The light source is a 12-V 50-W tungsten-halogen lamp (L7400, Gilway, Woburn, MA) containing a built-in ellipsoidal reflector that captures approximately 70% of the entire light output and focuses this to a ½-inch spot at 1 inch in front of the bulb. The shutter is positioned at this focal point to control the flash duration, while passing essentially all of the focused light into the Ganzfeld bowl. This lamp combines a high color temperature of 2800°K with a long life expectancy of 750 hours, which contributes to the stability and reliability of the source. Power is provided by a 12-V regulated supply (Lambda, Melville, NY).

The lamp is housed in a 7-inch box (H) constructed of ⅜--inch thick black phenolic which withstands heat well and is opaque (Fig 4). All edges are

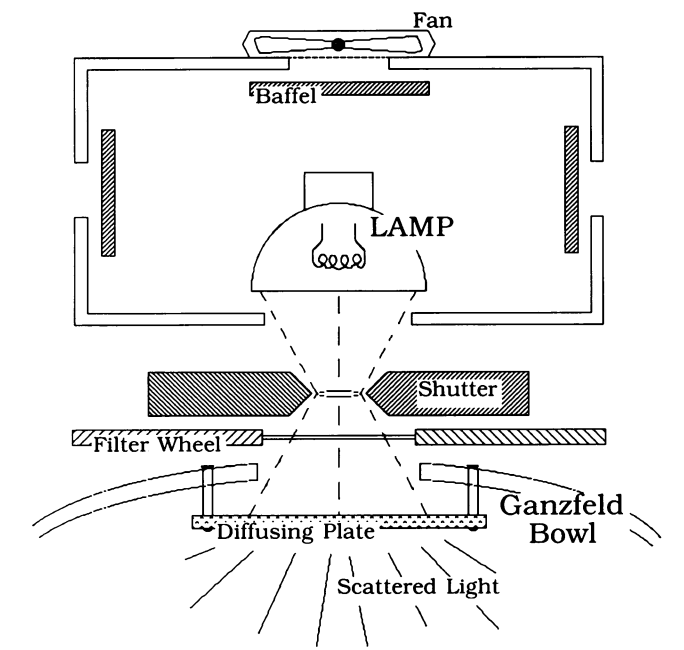

FIGURE 5
Schematic of stimulus lamp housing in exploded view.

machined as step-joints and are set in black epoxy to make the housing completely light-tight. A muffin fan (F) on the top circulates cooling air through vents (V) that allow airflow but are baffled (B) against light leaks. Light exits the box through a hole in the bottom and passes through infrared glass (IR) (Edmond Scientific, Barrington, NJ) to absorb heat that would damage the shutter. Light passes through the shutter and is projected into the Ganzfeld bowl through a hole in the top. Light then scatters from a semitransparent plastic diffusing plate. By direct light measurement the illumination inside the bowl is uniform to within 0.1 log units.

Stimulus intensity is set by the 12 neutral density filters (3×3-inch Wratten filters, Kodak) that are mounted on a large wheel (FW) rotated by a 2-RPM motor. Each filter position is identified by binary-coded holes in the wheel. An electronic circuit matches the filter code to that of a 12-position switch that the operator can set remotely. The wheel is stopped at the desired filter position. The 12 filters cover 4 log unit intensity range that can be extended further to below human or cat rod thresholds[76] by additional filters in a filter tray (FT).

The stimulator is calibrated by measuring the luminance of the inside of the Ganzfeld bowl in ft-L or cd/m^2 using a commercial photometer (United Detector Technologies, Santa Clara, CA). These intensity units are converted to physiologically useful units of the equivalent retinal illuminance, specified in photopic trolands (1 phot Td = [cd/m^2] x [pupil area]).[72] Scotopic trolands are then derived by incorporating the color temperature of light: scot Td = phot Td + 0.18 log unit, for a 2800°K tungsten source.[77] Trolands can be converted to 507-nm equivalent quanta at the human cornea by using the formula 1 scot Td = 5.649 log q(507)/deg^{2}s.[78] Actual values of retinal illuminance must be reduced by light loss to the media, which is approximately –0.233 log units for human.[77]

Constant background illumination inside the bowl from a 12-V bulb (BL) (Sylvania 1152) suppresses rod ERG activity and allows one to record cone responses selectively. A diffuser in front of the bulb is adjusted to give 42 cd/m^2, equivalent to 3.3 log scot Td for pupillary dilation of 9 mm. A 3.3 log Td background effectively saturates the rod photoreceptors[77] and thereby allows recording cone responses.

Maximum stimulus intensity of the Ganzfeld bowl as described here is 180 to 200 cd/m^2, which is about 2.5 log units above rod b-wave saturation for the human dark-adapted ERG.[76] This is sufficiently bright to elicit primate cone-driven a-, b- and d-wave responses against the rod-suppressing background. The stimulus could be made brighter by decreasing the density of the diffusing plate, but with the trade-off of decreasing the uniformity of light in the bowl. Using a 350-W bulb would increase the light by 0.7 log units but would require different focusing optics.

MATERIALS AND METHODS

SUBJECTS AND STUDY PROTOCOLS

Healthy subjects were recruited by newspaper advertisement to obtain unbiased normal ERG control values. Inclusion required no history of retinotoxic drug use, no family history of retinal dystrophy, acuity correctable to 6/6, equivalent spherical refractions of –3 to +3 dipoter, normal ocular tensions, normal visual fields on Goldmann perimetry, normal color vision by Farnsworth D-15 panel, and normal results on retinal examination. None had diabetes or known cardiovascular disease. ERG data were used only from the right eye of each normal subject. Patient subjects with retinal dystrophies were recruited from patients diagnosed by clincial examination and by recording a standard clinical ERG.[79] All subjects were fully informed about the experiments and gave consent.

Pupils were dilated fully with 2.5% phenylephrine hdrochloride and 1% tropicamide. ERGs were recorded at the cornea using Burian-Allen bipolar electrodes (Hansen Ophthalmic Development Labs, Iowa City, IA) with use of topical anesthesia of proparacaine hydrochloride. A cholorided silver reference electrode was placed on the forehead. Full-field stimuli were produced by the Ganzfeld bowl described previously. A continuous rod-suppressing background of 42 cd/m^2 was used. The stimulus was flickered at 3.3 Hz (on 150 msec and off 150 msec, for the long-flash; on 10 msec and off 290 msec, for the short-flash). Responses were recorded for 300 ms epochs, amplified with 3-dB bandwidth of 1 to 1,000 Hz, averaged on a microcomputer, stored, and displayed by digital plotter. These could also be digitally filtered[80] at a later time to examine the oscillatory potentials. The 1-Hz cutoff does not affect amplitudes of the a-, b-, or d-waves, and it alters the shape of the plateau region minimally for flashes of 200 msec or shorter. Of course, DC amplification is prefered, but this requires an exceptionally quiet subject.[81] The b-wave amplitude was measured from the a-wave trough. The d-wave amplitude was measured from the plateau inflection point at the beginning of the initial d-wave rise. Note that the d-wave shows an initial shallow rise followed by a steeper second phase to which an OFF-oscillatory wavelet potential[82,83] may contribute.[38]

CLINICAL XENON-FLASH ERG

Clinical ERGs were recorded with xenon-flash stimuli for diagnostic evaluations according to the new international ERG guidelines.[71] Burian-Allen bipolar corneal electrodes (Hansen Ophthalmic Instruments), were used with topical anesthesia (0.5% proparacaine hydrochloride) after full pupil dilation (10% Neo-Synephrine and 1% Mydriacyl). The light source was a 10-μsec xenon flash from a PS-22 stimulator (Grass Instrument, Quincy, MA) presented in a Ganzfeld bowl. Light intensities were measured with a photometer (40-X Opto-Meter, UDT, Santa Monica, CA). ERG responses were amplified at 0.1 to 1000 Hz (3 dB) and digitized for computer storage and display. Clinical ERG responses are recorded after 1 hour of dark-adaptation. First, an intensity series of dark-adapted rod-predominant ERG responses is recorded using a dim blue flash at 2-sec intervals. Responses are next recorded dark-adapted to intense single-flash, white stimuli to yield roughly 75% rod- and 25% cone-driven responses. Subjects are then light-adapted at 42 cd/m^2 for 5 minutes, after which 30-Hz repetitive stimuli and also single-flash white stimuli are used to elicit cone-predominant responses. Population normal values (Table I) were determined from 40 normal subjects ranging from 5 to 60 years old. Linear regressions were compiled for response amplitudes versus ages and generally followed the data published by Weleber.[84]

TABLE I: POPULATION NORMAL VALUES FOR CLINICAL XENON-FLASH ERG

	DA b-WAVE μV	DA a-WAVE SLOPE μV/ms	ROD b-WAVE μV	LA b-WAVE μV	30 Hz μV
Mean	378	6.61	337	120	103
SD	82.1	2.26	70.6	31.8	30.8
Low value	227	3.12	201	50	61

DA b-wave, dark-adapted, single white flash (4.2 cd-sec/m^2); DA a-wave, dark-adapted, single white flash (4.2 cd-sec/m^2); Rod b-wave, dark-adapted, single dim blue flash (0.42 cd-sec/m^2 with Wratten 47+47A+47B); LA b-wave, light-adapted, single white flash (10.0 cd-sec/m^2); 30 Hz, 30 Hz white flicker (4.2 cd-sec/m^2 per flash).

PSYCHOPHYSICAL METHODS

Rod absolute sensitivity thresholds after 1 hour dark-adaptation are tested on a Goldmann-Weekers Dark Adaptometer (Haag-Streit AG, Berne, Switzerland) with 0.8-sec flashes of a 5.7° target for each eye at fixation and at 20° points in each visual quadrant. Hue discrimination of each eye is tested using the Farnsworth Panel D-15 or 100-Hue test, and standard scoring is used[85] to detect chromatic discrimination loss either along congenital protan, deutan, or tritan axes or as resulting from an acquired basis of cone dysfunction. Visual fields are evaluated by dynamic perimetry on a Goldmann Perimeter and by static testing on a Humphrey Visual Field Analyzer.

CLINICAL DIAGNOSIS OF ROD-CONE VERSUS CONE-ROD DYSTROPHY

The clinical designation was made as either rod-cone or cone-rod dystrophy primarily on the basis of ERG testing. This was not always unambiguous, however. A further important feature was whether or not the psychophysical dark-adapted thresholds reached normal rod levels. These levels were tested at six retinal loci for each eye across the horizontal field. No case considered a rod or rod-cone dystrophy had normal rod threshold. Perhaps more difficult was whether to designate a case as cone or as cone-rod dystrophy. Weleber[86] also has commented on the difficulty that this terminology presents, since a spectrum of relative cone versus rod ERG losses is found in all these conditions. Here again, for us the dark-adapted thresholds were important. In addition to the guidance provided by the ERG testing, we designated cases as having a cone dystrophy only if rod psychophysical thresholds were normal; those with elevated thresholds were called cone-rod dystrophy.

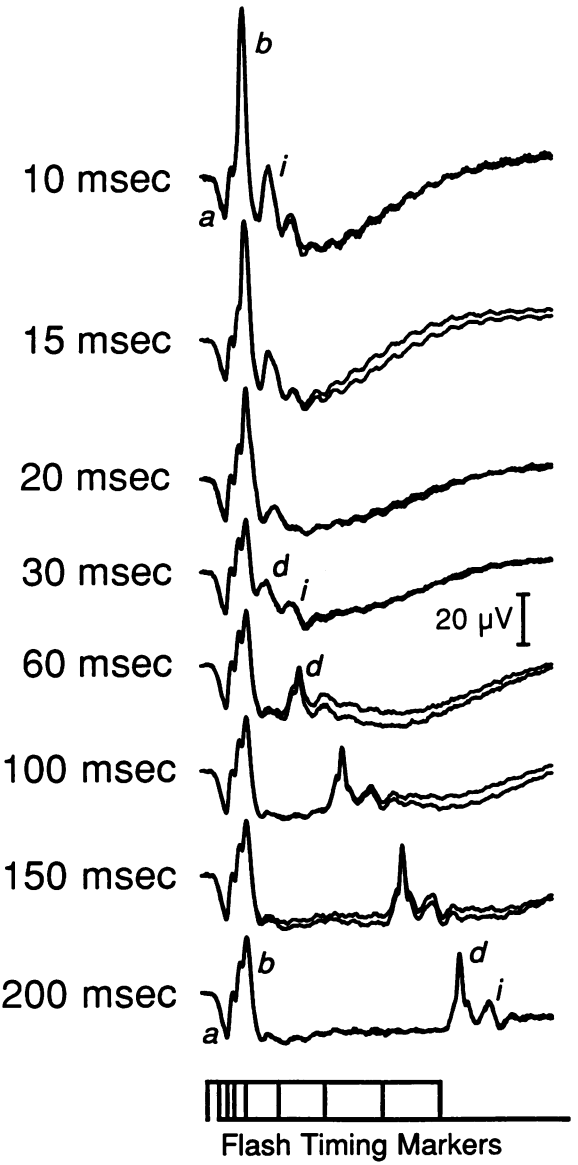

FIGURE 6
Human photopic ERG duration series for 10- to 200-msec flashes (185 cd/m² Ganzfeld stimulus on 43 cd/m² background).

RESULTS: NORMAL SUBJECTS

EVALUATION OF PHOTOPIC LONG-FLASH ERG RECORDING CONDITIONS

Flash-Duration Studies

Before the stimulus conditions were set for recording from clinical patients, the effect of flash duration was evaluated for two normal subjects. Fig 6 shows ERG responses for one of these subjects studied systematically using flash durations from 10 to 200 msec. The b-wave was largest for the 10-msec flash (which was the shortest flash used here), and it progressively became smaller for longer flashes. For flashes of 10 to 20 msec, the b- and d-waves summated into a single large response and could not be distinguished from each other.[32,33] The b-wave amplitude reached a stable asymptote for flash durations of 60 msec and longer, as shown by the graph in Fig 7.

The d-wave was first evident as a separate but small wave with 30-msec flashes, and with 60-msec flashes it was clearly distinguished from the (true) b-wave. The d-wave reached maximum amplitude for 150 msec flashes and thereafter did not change as the flash became longer. The results of duration studies were the same for both subjects, although the absolute response amplitudes were different. Thus, 150-msec flashes were chosen as the standard recording condition.

Repeating the flash at three times per second did not alter the ERG waveform, and we settled on 3.3 Hz stimuli for the standard recording protocol. These stimuli were tolerated well by subjects and allowed for rapid response averaging. Averaging 10 to 15 ERG responses enhanced the clarity of responses and gave clean and reproducible waveforms.

Assessment of Possible Rod Contamination

Evaluation of possible rod contamination of these photopic long-flash ERGs was performed by recording from three human achromats. Achromats have primarily rods and lack essentially all cone photoreceptors, as shown by retinal histology.[87] Recording from an achromat provides an important test of whether the photopic ERG responses elicited using our long-flash Ganzfeld stimulator (with 43 cd/m^2 background) truly derive from cones alone, or whether rod responses intrude, since any ERG response from an achromat would reflect rod contamination. Fig 8 shows photopic long-flash ERG recordings from achromat patient 1, and Fig 9 shows her xenon-flash ERG responses.

Patient 1. This 32-year-old patient had congenital nystagmus and lifelong acuity of 20/200. No color discrimination could be identified by Ishihara plates or by Farnsworth D-15 panel. The fundus appearance was normal with the exception of an abnormally broad foveal reflex. Visual fields were

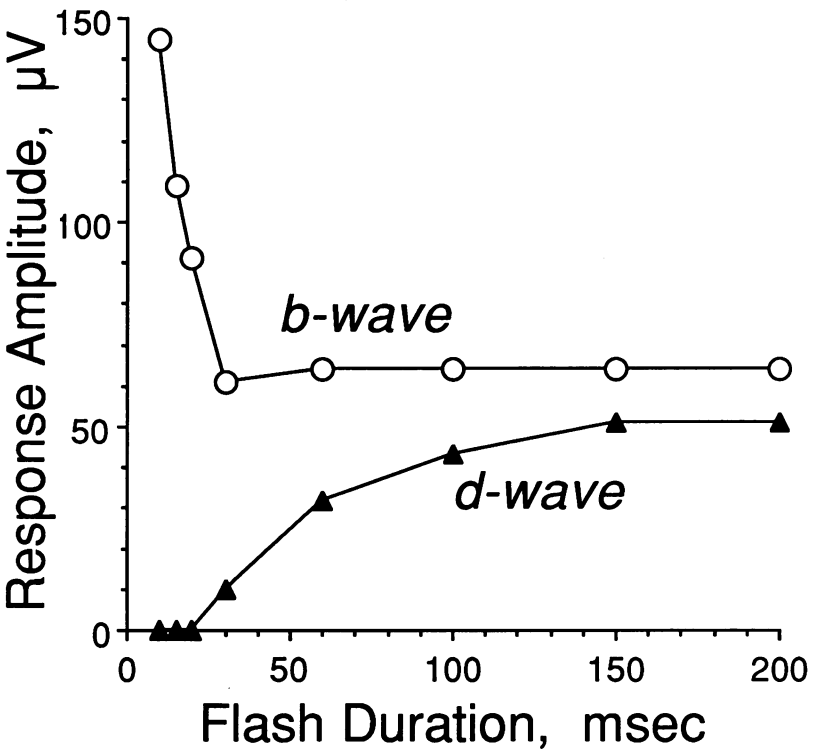

FIGURE 7
Photopic b-wave and d-wave amplitudes for stimuli of 10- to 200-msec duration (data from Fig 6).

full with Goldmann V4e and I4e targets, particularly with the background illumination slightly dimmed to eliminate patient photophobia. Dark-adapted thresholds reached normal rod levels within 30 minutes. The xenon-flash ERG showed normal rod b-wave responses but no responses for 30-Hz or for single-flash photopic (43 cd/m^2 background) stimulation. Clinical diagnosis was achromat.

Recording with 150-msec photopic flashes in the Ganzfeld stimulator produced only tiny responses of less than 0.5 μV above background noise (Fig 8), which is less than 3% of the normal mean b- and d-wave amplitudes (population normal values are given in the "Normal Values" section). Three achromats were tested, and all showed a virtual absence of photopic responses. This confirmed that responses were cone-driven and that rod contamination was negligible under the photopic long-flash stimulus conditions from this Ganzfeld bowl.

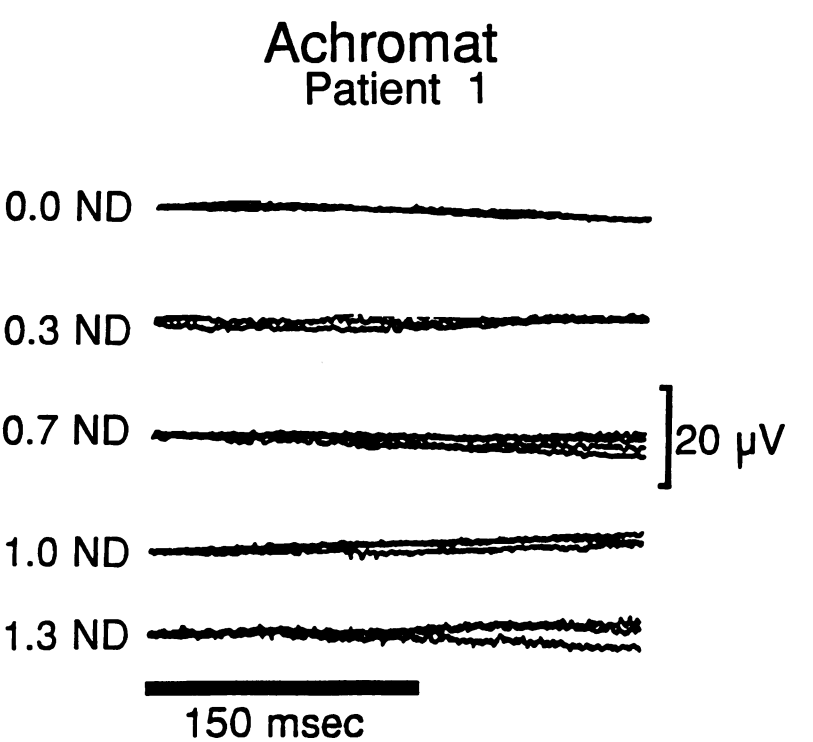

FIGURE 8
Patient 1. Photopic ERG of achromat patient showed no response to 150-msec flashes presented in Ganzfeld bowl stimulator described in text (185 cd/m^2 stimulus on 43 cd/m^2 background).

Assessment of Cone Photoreceptor Contribution

According to Granit's analysis,[35] cones contribute the negative PIII wave to the photopic long-flash ERG. Brown and Watanabe[88] occluded the central retinal artery in a monkey eye to eliminate the proximal retinal activity that depends upon retinal circulation and thereby isolate the cone photoreceptor contribution to the photopic ERG (since photoreceptor metabolism is supported mainly by choroidal circulation). The analogous human "experiment" is to record from a patient with central retinal artery occlusion (CRAO). Fig 10 shows the photopic long-flash ERG recordings from CRAO patient 2, and Fig 9 shows the xenon-flash ERG result.

Patient 2. This 63-year-old man had a recent occlusion of the central retinal artery of the right eye. The left eye was normal on examination. Visual acuities were counting fingers 6 ft OD and 20/20 OS. Ocular media were clear OU. Retina OD showed reperfusion by the time of ERG testing.

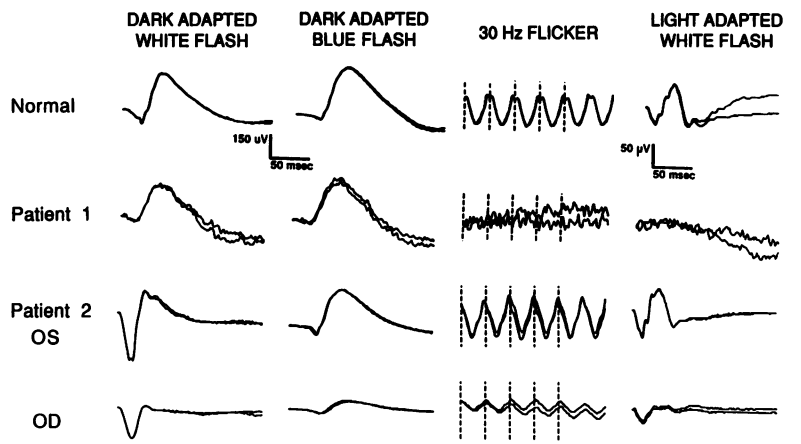

FIGURE 9
Clinical xenon-flash ERG of achromat patient and patient with central retinal vein occlusion (CRVO). Stimulus conditions are given in text. Flashes coincided with onset of trace.

OS revealed no retinal vascular or optic nervehead abnormalities, and macular and peripheral retina appeared normal. Peripheral fields were severely compromised OD with responses to only the Goldmann V4e target; this OD field showed only a temporal crescent between 20° and 40° running superior, temporal, and inferior. Peripheral fields OS were full to V4e and I4e targets. Psychophysical dark-adapted thresholds were elevated 3 log units OD at multiple retinal loci tested but reached normal rod thresholds OS. ERGs were recorded 18 weeks after the artery occlusion. OS showed normal rod and cone responses; OD showed dark-adapted rod b-wave amplitude of 60 µV (30% of normal) and an electronegative a-wave pattern on bright flash. Cone 30-Hz flicker was only 10 µV but retained normal implicit time, while the photopic light-adapted white flash response showed b-wave loss. Clinical diagnosis was CRAO OD.

For this patient the majority of the long-flash photopic b-wave was lost, along with half of the d-wave. The xenon-flash ERG showed a small residual rod b-wave (Fig 9), indicating that the CRAO was extensive but not absolute in eliminating all ERG activity postsynaptic to the photoreceptors. This would account for the residual photopic long-flash b-wave in Fig 10. The photopic long-flash a-wave (Fig 10) was also diminished, indicating either that circulatory support for the photoreceptors was compromised or that part of the photopic a-wave originates postsynaptic to cones, as has been proposed on the basis of pharmacologic studies of monkey.[70] Note that both the (b/a) ratio and the (b/d) ratio were less than 1.0 and consequently

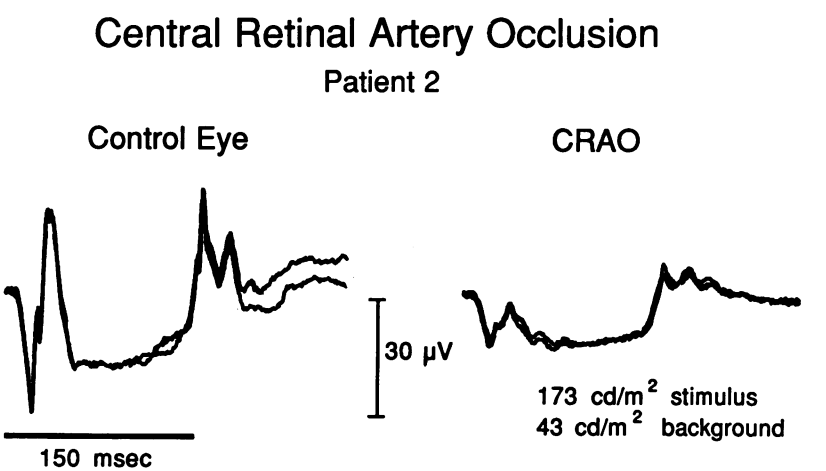

FIGURE 10
Patient 2. Photopic long-flash ERG of patient with unilateral CRVO showed reduction of the a-, b-, and d-waves compared with unaffected fellow eye. (173 cd/m^2 Ganzfeld stimulus on 43 cd/m^2 background).

abnormal (as is demonstrated later in sections that follow).

Although based only on the findings in this one CRAO subject, it appeared that impairment of the cells postsynaptic to the cones (1) revealed a hyperpolarizing response with the plateau remaining negative and below baseline, (2) suppressed the photopic long-flash b- and d-wave amplitudes, (3) altered the (b/a) ratio or (b/d) ratio, and (4) possibly reduced the a-wave amplitude.

NORMAL VALUES FOR THE LONG-FLASH PHOTOPIC ERG

Amplitudes to Maximum Flash Intensity

Photopic ERG responses were recorded with maximum-intensity stimuli from 46 normal subjects using 10- and 150-msec flashes. The bar graph in Fig 11 depicts the b- and d-wave amplitudes for each normal subjects, and the population mean values are given in Table II. Several observations were made:

1. The b-wave for 10-msec flashes was larger than the b-wave for 150-msec flashes for each normal subject, with a mean amplitude ratio of 1.85 (standard deviation [SD] = 0.31; range, 1.36 to 2.68).

2. The short-flash b-wave amplitude was larger than the sum of the long-flash b-wave plus d-wave for the majority (78%) of these normal subjects.

3. For long-flash stimuli, the b-wave was greater than the d-wave in each case, with a mean amplitude ratio of 1.35 (SD = 0.23; range, 1.09 to 1.99).

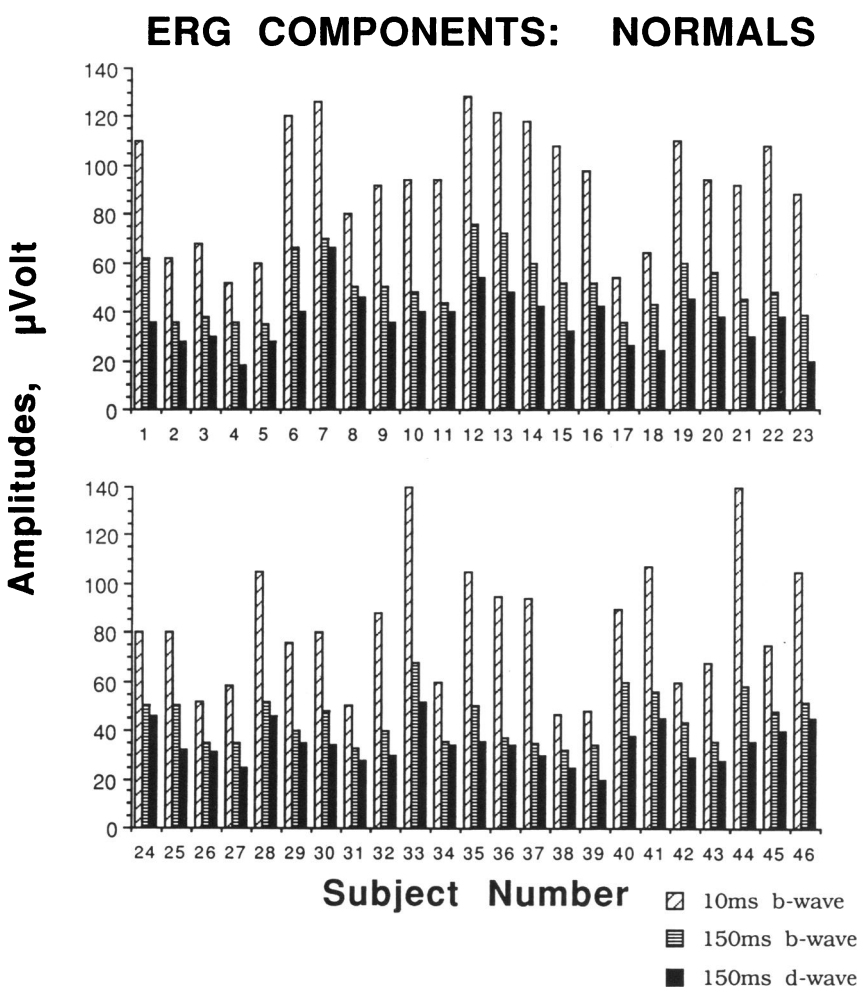

FIGURE 11
Photopic ERG of 46 normal subjects, with b-wave to 10-msec flash and with b- and d-waves to 150-msec flash (185 cd/m² Ganzfeld stimulus on 43 cd/m² background).

TABLE II: PHOTOPIC ERG RESPONSE AMPLITUDES FROM 46 NORMAL CONTROLS (185 cd/m² FLASHES AT 3.3 Hz ON 43 cd/m² BACKGROUND)

RESPONSE, FLASH-LENGTH	MEAN (μV)	SD	RANGE (μV)
b-wave, 10 msec	88.1	25.7	47 - 140
a-wave, 150 msec	27.9	11.6	12 - 66
b-wave, 150 msec	47.6	11.7	32 - 76
d-wave, 150 msec	35.4	9.6	18 - 66

Intensity-Response Characteristics

The effect of stimulus intensity on long-flash ERG responses was evaluated for the normal subjects. As shown for two representative normal subjects in Fig 12, response amplitudes decreased as the stimulus intensity decreased, but the overall waveform shape did not change much. Responses could be tracked down to a threshold near 1.3 neutral density (ND) below the maximum (0.0 ND = 185 cd/m²), but for still dimmer intensities, the responses could not reliably be distinguished from noise. The entire ERG waveform scaled monotonically over the 1.3-log-unit range of flash intensity. The photopic a-, b-, and d-waves each persisted even for dim flashes, unlike the behavior of the dark-adapted human ERG, in which the b-wave, but not the a-wave, is evident for dim flashes.[75] Some variation was noted in the shape of the OFF-effect waveform, but in all cases the d-wave was phasic, and the negative trailing edge was shorter than the leading edge.

The plateau region of the waveform between the b- and d-waves was negative at all intensities between 0 and 1.3 ND. The plateau was most negative for the brightest flashes, consistent with cones contributing PIII to the negative plateau.[89] The reader's attention is directed to the cone plateau, because it was negative for all normal subjects, whereas some patients with cone dystrophies had this plateau shifted to positive polarity. As will be explained, a plateau of positive polarity rather than the normal negative polarity can only be accounted for by postulating a defect in photoreceptor transmission to second-order neurons.

Population values for the 150-msec long-flash photopic a-, b-, and d-waves across a stimulus intensity range were determined for the normal subjects, with results given in Table III and data plots shown in Figs 13 through 15.

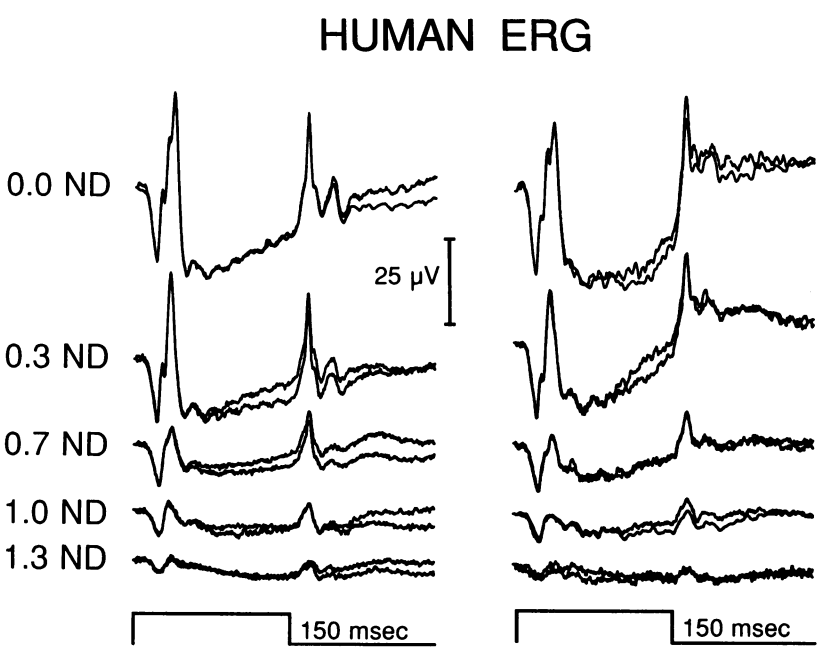

FIGURE 12
Photopic ERG intensity series for two normal subjects (0.0 ND = 185 cd/m² Ganzfeld stimulus of 150 msec on 43 cd/m² background).

TABLE III: NORMAL CONTROLS' AMPLITUDE (μV) VERSUS INTENSITY FOR 150 msec FLASHES

	a-WAVE		b-WAVE		d-WAVE	
INTENSITY	MEAN	SD	MEAN	SD	MEAN	SD
0.0 ND	27.9	11.6	47.6	11.7	35.4	9.6
0.3 ND	18.3	7.0	37.4	10.1	27.8	8.6
0.7 ND	11.1	3.6	18.5	6.1	3.5	5.8
1.0 ND	6.3	1.5	9.9	3.4	6.3	2.9
1.3 ND	2.8	0.9	5.9	1.8	2.7	1.2

0.0 ND = 185 cd/m² flashes at 3.3 Hz on 43 cd/m² background.

The amplitudes at each intensity formed a normal distribution skewed toward high values. As others had previously shown for the dark-adapted ERG b-wave,[90-92] log transformation of the b- and d-wave amplitudes better approximated a normal distribution (Table IV).

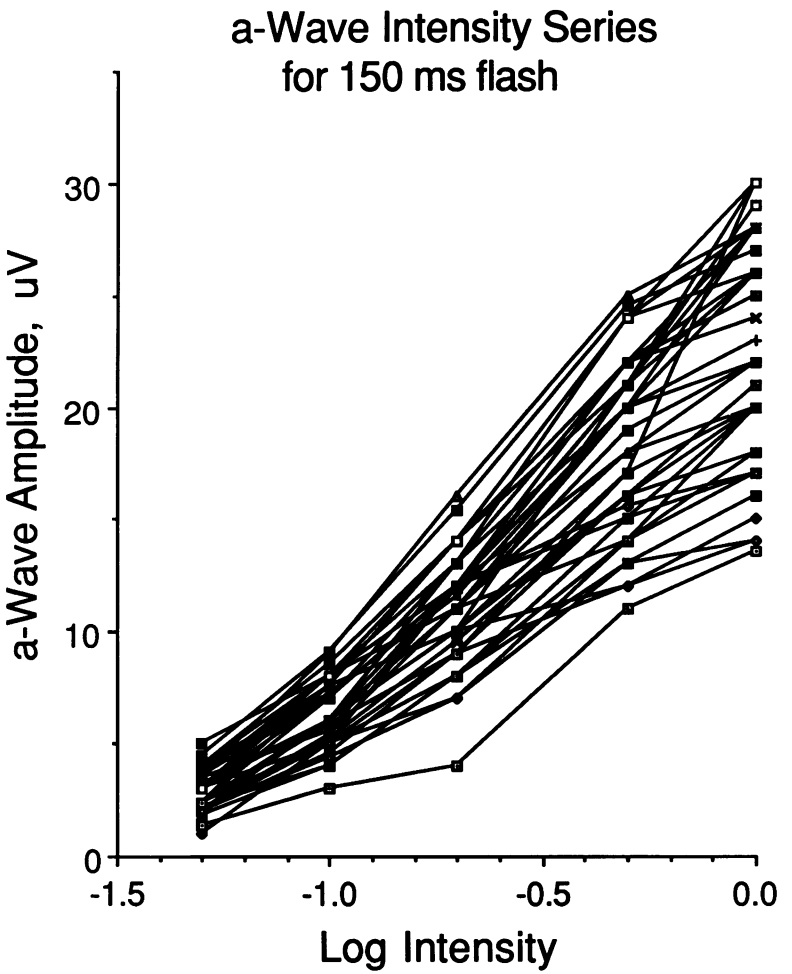

FIGURE 13
Photopic ERG a-wave intensity series of normal subjects (0.0 ND = 185 cd/m^2 Ganzfeld stimulus of 150 msec on 43 cd/m^2 background).

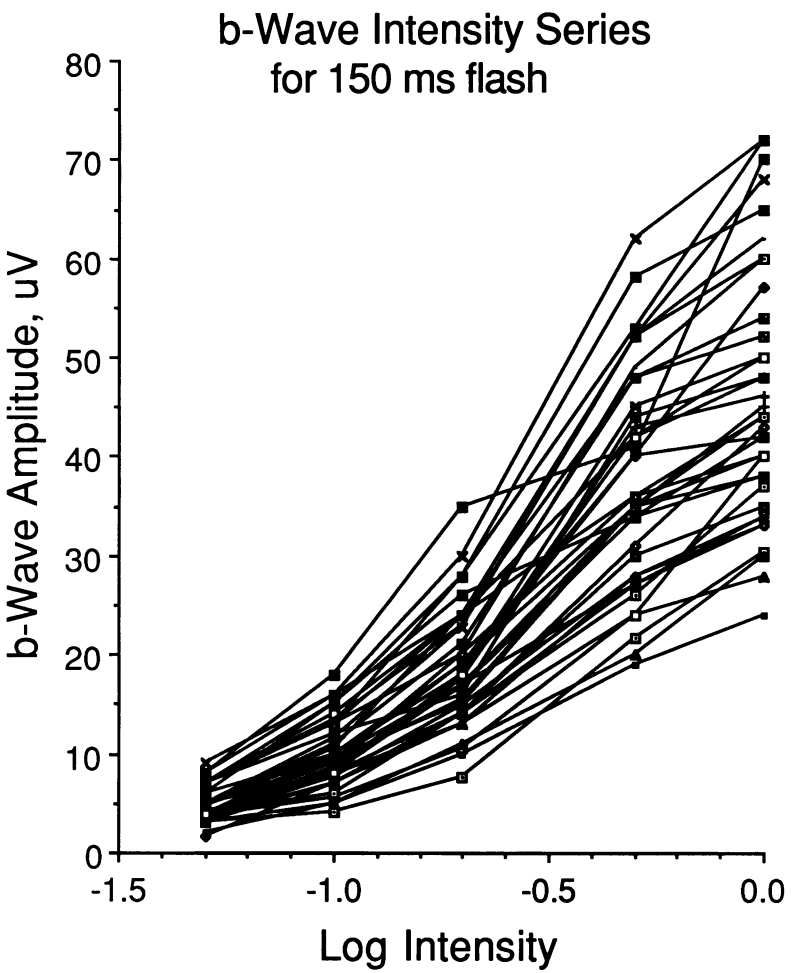

FIGURE 14
Photopic ERG b-wave intensity series of normal subjects (0.0 ND = 185 cd/m² Ganzfeld stimulus of 150 msec on 43 cd/m² background).

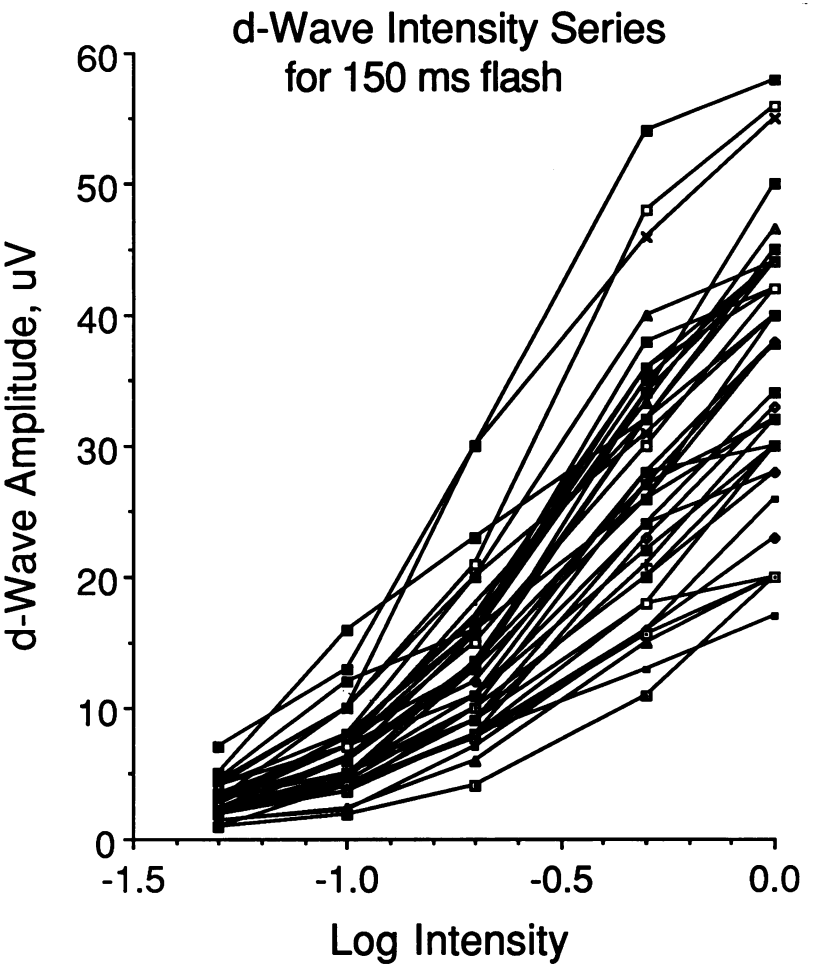

FIGURE 15
Photopic ERG d-wave intensity series of normal subjects (0.0 ND = 185 cd/m^2 Ganzfeld stimulus of 150 msec on 43 cd/m^2 background).

TABLE IV: DISTRIBUTION SKEWNESS OF b- AND d-WAVE AMPLITUDES*

RESPONSE	SKEWNESS	
	RAW DATA	LOG-TRANSFORMED
b-wave	0.616	0.271
d-wave	0.710	–0.116

*150 msec flashes of 185 cd/m² on 43 cd/m² background.

Amplitude Ratios

Response amplitudes showed a considerable range for the normal population, but not dissimilar to the range for the short-flash b-wave to 10-msec flashes (Table II). To narrow the range across subjects, one strategy is to normalize internal to each subject by using ratios of a-, b-, and d-wave amplitudes. This helps to accentuate abnormalities that affect individual components even if the entire waveform has a reduced amplitude.[22,23]

Figs 16 and 17 show scatter plots of b-wave amplitudes versus both a- and d-waves for the normal subjects. Note that for both cases, the line representing (b/a) ratio = 1.0 and (b/d) ratio = 1.0 is a lower discriminator boundary for the normal population values. Further, according to the data in Table III, the b-wave is the largest of the long-flash responses for all intensities tested. All normal subjects had (b/a) and (b/d) ratios > 1.0 for all photopic stimulus intensities tested. We subsequently noted that a number of patients with retinal dystrophies showed amplitude reversed, with ratios < 1.0. on one or both indices.

RESULTS: RETINAL DYSTROPHY PATIENTS

We surveyed long-flash photopic ERG responses recorded from 127 subjects with a variety of retinal dystrophies. Because long-flash recordings have not previously been studied systematically across the spectrum of human retinal dystrophies, we could not anticipate the findings. Consequently, we did not preselect for a specific category of pathology, other than that patients should have indications of disease involving the outer retina. Each patient was first examined clinically and had a xenon-flash ERG recorded.

REDUCED SHORT-FLASH CONE ERG DOES NOT PRECLUDE LONG-FLASH RECORDINGS

Early in these studies it became clear that one could not accurately predict

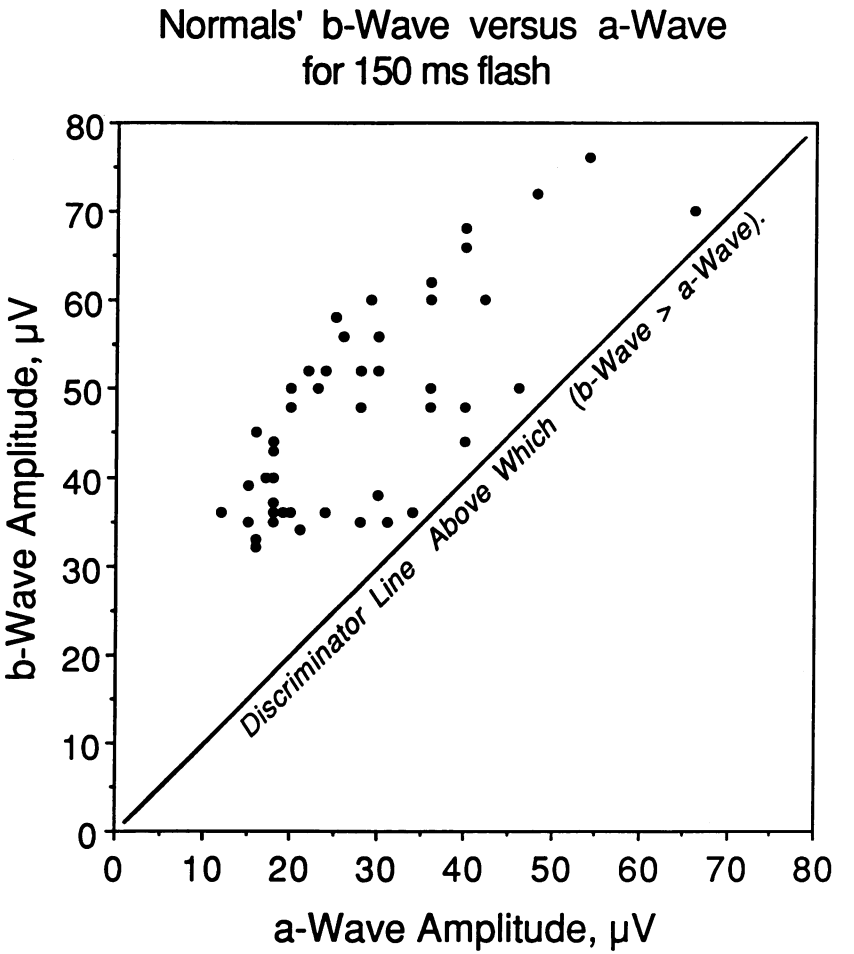

FIGURE 16
Scatter plot of b-wave versus a-wave. Discriminator line defines (b/a) ratio = 1.0 (185 cd/m^2 Ganzfeld stimulus of 150 msec on 43 cd/m^2 background).

whether long-flash photopic ERG responses would be abolished simply by extrapolating from small photopic responses on xenon-flash recordings. Cone-rod dystrophy patient 3 illustrated this by showing considerable ERG activity with long-flash stimuli (Fig 18) despite having markedly reduced cone responses on clinical xenon-flash ERG recordings (Fig 19).

Patient 3. This 37-year-old man had recent difficulty with night driving and progressive photosensitivity to bright daylight. He has no siblings. Visual acuities were 20/30 OU with refraction of +0.25 +0.50 × 26 OD, +0.25

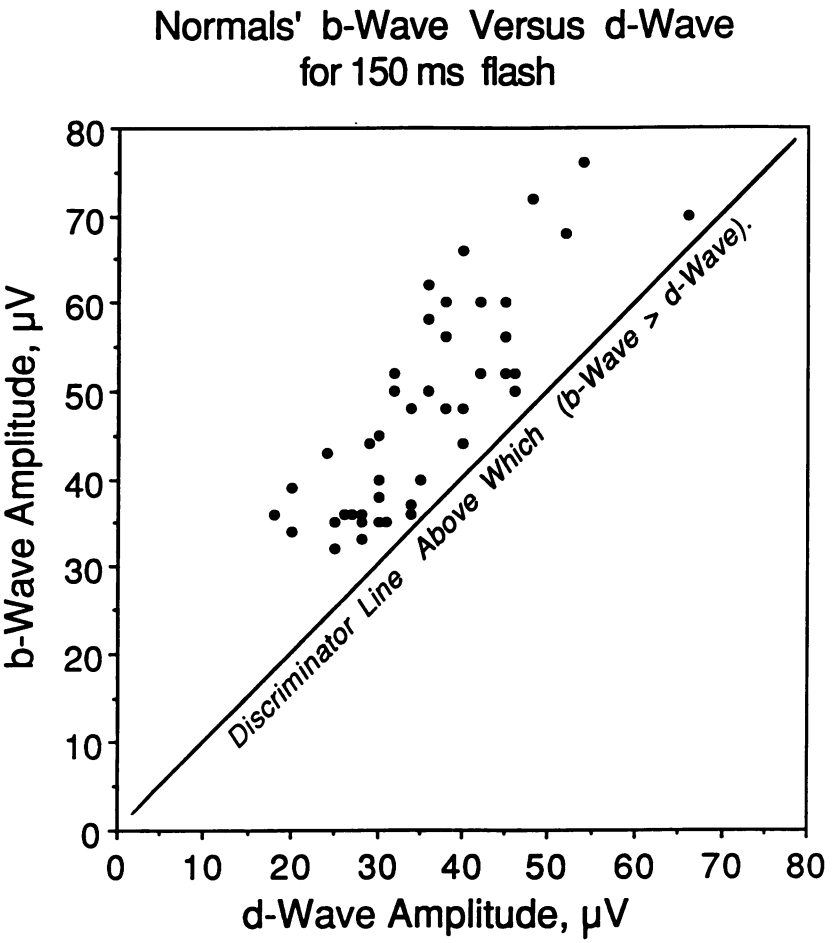

FIGURE 17
Scatter plot of b-wave versus d-wave. Discriminator line defines (b/d) ratio = 1.0 (185 cd/m^2 Ganzfeld stimulus of 150 msec on 43 cd/m^2 background).

+0.50 × 118 OS. Retinal vessel caliber was 50% constricted. Midperipheral fundus showed granular RPE changes and early-stage atrophy but no intraretinal bone spicule pigmentation. OD and OS, respectively, had 3 and 5 tritan errors on Farnsworth D-15. Visual fields were full except for slight superior loss with the V4e target; I4e target revealed complete superior field loss but a preserved inferior crescent between 40° and 60° OU. Dark-adapted thresholds were elevated 2 to 3 log units above normal rod levels OU. Clinical xenon-flash ERG showed rod amplitudes 50% reduced and

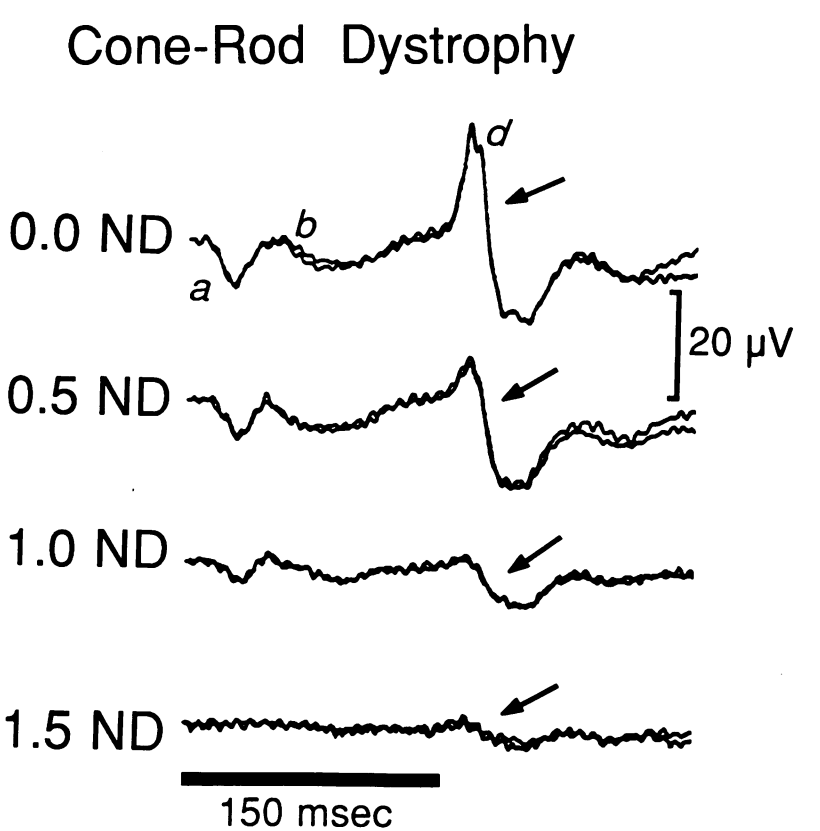

FIGURE 18
Photopic ERG of patient with cone-rod dystrophy. Exaggerated negative swing after d-wave (*arrow*) is similar to effect of KYN on monkey photopic ERG (Fig 3) (185 cd/m² Ganzfeld stimulus of 150 msec on 43 cd/m² background).

cone amplitudes more than 90% reduced. Clinical diagnosis was cone-rod dystrophy.

Patient 3 gave easily recordable a-, b- and d-waves for long-flash photopic stimulation. His clinical xenon-flash 30-Hz cone flicker response was only 10% of normal at 6 μV (Table I: normal mean = 103 μV, with lowest normal 61 μV) and was significantly delayed to 40 msec (normal, 26 to 32 msec); the photopic light-adapted single white flash response was even more reduced at 2 μV (Table I: normal mean = 120 μV, with lowest normal 50 μV). Despite this, although the long-flash a- and b-waves were reduced beyond normal (cf, Table II), the d-wave was 18 μV and still within lowest normal level for our control population (Table II). The (b/a) ratio and the (b/d) ratio both

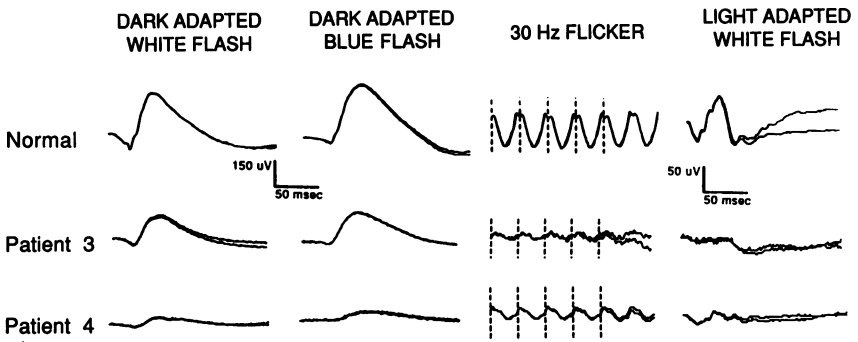

FIGURE 19
Clinical xenon-flash ERG. Stimulus conditions are given in text. Flash coincideds with onset of trace.

were less than 1.0 and consequently were abnormal.

Compared with the normal long-flash ERG waveform (cf, Figs 1, 6, and 12), the morphology of the OFF-response in patient 3 was quite unusual. It contained a sweeping negativity in the OFF-complex following the d-wave (at arrow in Fig 18). This persisted for all intensities down to threshold at 1.5 ND, at which neither a- nor b-waves were evident for this patient. Once this late negativity had been appreciated in the long-flash response, it could even be seen in the xenon-flash ERG (Fig 19).

Several conclusions can be drawn from the findings in this subject:

1. Despite considerably reduced cone ERG to xenon short-flash stimuli, some subjects show substantial responses to long-flash photopic stimuli.

2. Abnormal (b/a) or (b/d) ratios may be sensitive indicators of pathology.

3. From the remarkably altered waveform found for this subject, it is apparent that the analysis of long-flash photopic ERG recordings must go beyond simply measuring a-, b- and d-wave amplitudes and must also include attention to changes in the overall waveform morphology. Indeed, paying attention to the waveform morphology uncovered human photopic ERG responses that mimicked the depolarizing (ON-pathway) or hyperpolarizing (OFF-pathway) responses uncovered in the monkey by pharmacology studies (Fig 3). This observation provided a basis for classifying the human waveforms based on morphology. First, however, the results will be described by attention to amplitudes alone, according to a statistical classification.

STATISTICAL CLASSIFICATION

Amplitude Statistics of a-, b- and d-Waves

Of a total of 127 patients tested prospectively for photopic long-flash ERG changes, the number having normal amplitudes for individual waves or combinations of waves was as follows: a-wave normal = 66 subjects; b-wave normal = 46; d-wave normal = 81; a- and b-waves both normal = 44; and b- and d-waves both normal = 46. Fortunately, after review of all responses for each patient, a simple unifying element emerged from these otherwise bland statistics. Specifically, finding the b-wave amplitude to be reduced was a precondition to finding either the a- or d-waves reduced in these patients. Thus, measuring the long-flash b-wave amplitude was the single most efficient screen for identifying abnormal ERG amplitudes to long-flash photopic stimulation, and this gives the following:

"Normal Photopic Long-flash b-Wave Rule": No patient with a normal long-flash photopic b-wave amplitude had reduced amplitudes for either the a-wave or d-wave.

Amplitude Scatter Plots

To screen the long-flash ERG results of these 127 retinal dystrophy patients quickly, scatter plots were made of b-wave amplitudes versus a- and d-wave amplitudes shown in Figs 20 and 21. In both graphs the diagonal discriminator lines identify the amplitude ratio = 1.0. Data points that lie below these lines are those patients with amplitude ratios of less than 1.0. Such reduced ratios are abnormal by comparison with the normal population values (ie, Figs 16 and 17). Four categories of patients are identified by these scatter plots: (1) patients with normal amplitudes: these points lie in the upper right on each plot, within the normal range outlined by dashed lines; (2) patients with response amplitudes reduced to zero; (3) patients with subnormal amplitudes but with normal (b/a) or (b/d) ratios and consequently lying above the discriminator line = 1.0; and (4) patients with reduced (b/a) or (b/d) ratios < 1.0 and lying below the discriminator line = 1.0.

Normal Amplitude Category—Patients in this category showed normal 1-, b-, and d-wave amplitudes on long-flash photopic ERG testing as well as normal (b/a) and (b/d) ratios. Inclusion of patients subsequently found to have "normal" ERG amplitudes arose because of the exploratory nature of this study, since with the exception of CSNB findings of Miyake and associates,[45,46] one could not predict the outcome of long-flash ERG testing without actually studying a variety of patients. Patients who subsequently showed normal results included genetic carriers of x-linked juvenile retinoschisis, of x-linked ocular albinism, and of autosomal recessive achro-

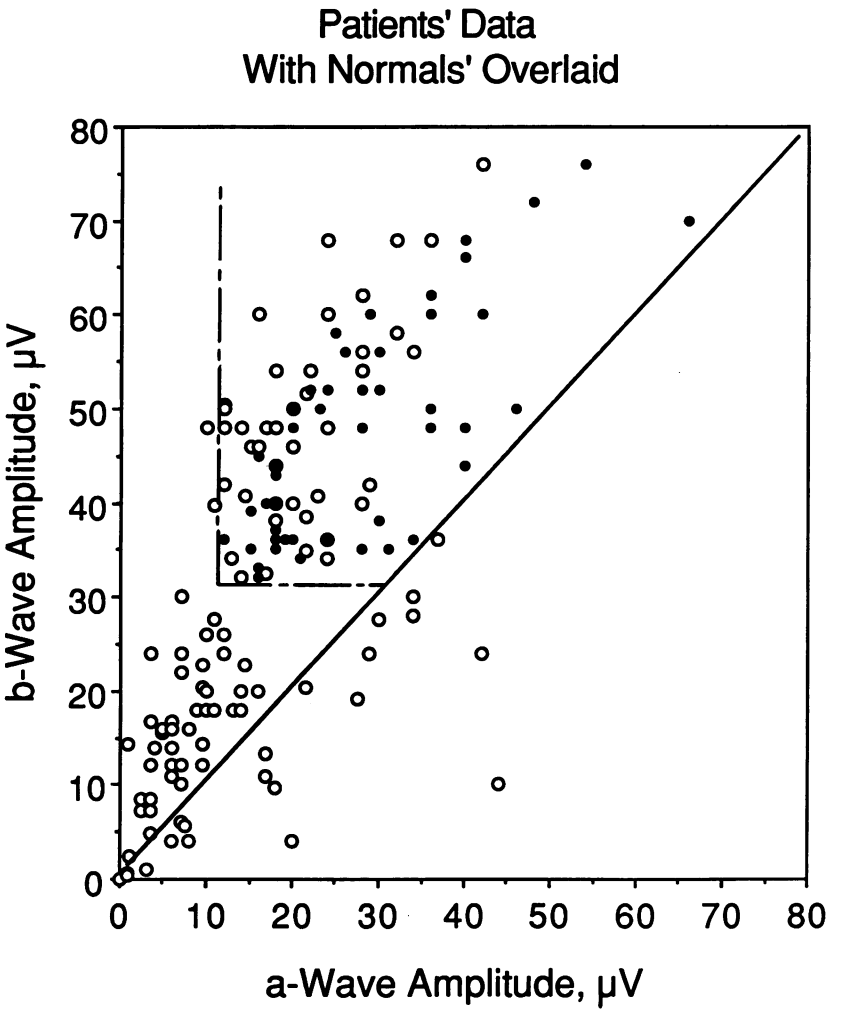

FIGURE 20
Scatter plot of b-wave versus a-wave for 126 retinal dystrophy patients (*open circles*) and 46 normal subjects (*filled circles*). Discriminator line defines (b/a) ratio = 1.0. *Dashed lines* show lowest limit of normal ERG responses (185 cd/m^2 Ganzfeld stimulus of 150 msec on 43 cd/m^2 background).

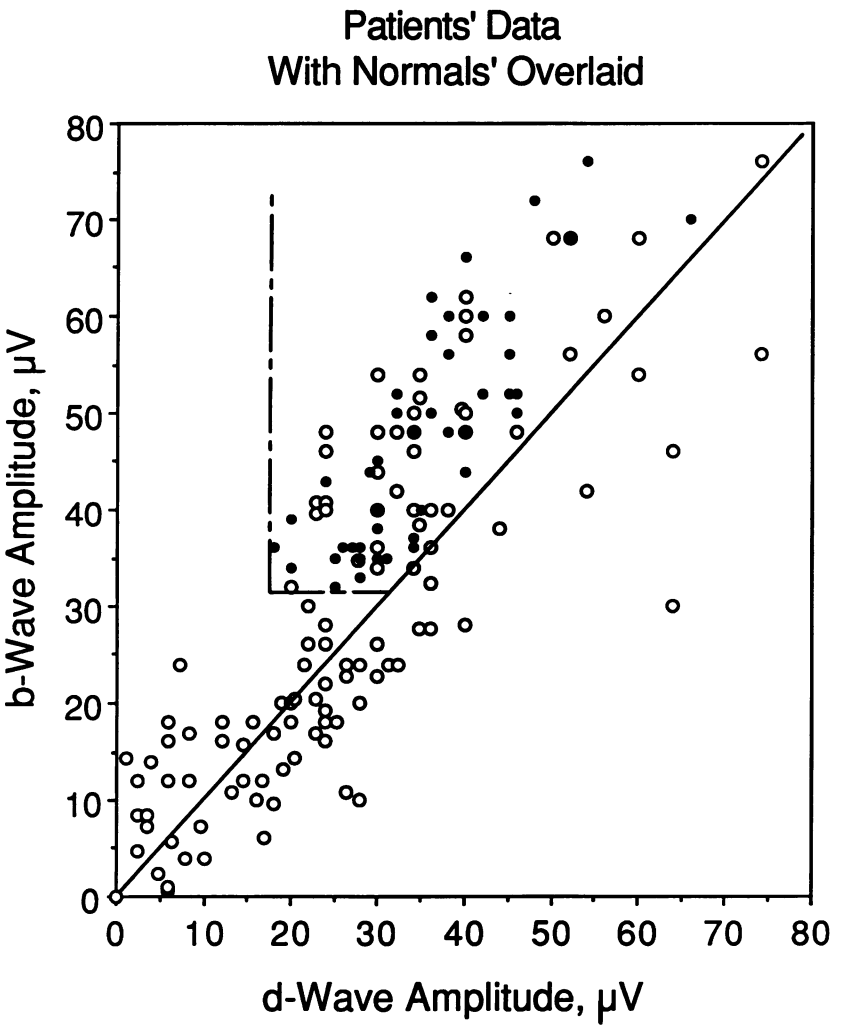

FIGURE 21
Scatter plot of b-wave versus d-wave for 126 retinal dystrophy patients (*open circles*) and 46 normal subjects (*filled circles*). Discriminator line defines (b/d) ratio = 1.0. *Dashed lines* show lowest limit of normal ERG responses (185 cd/m^2 Ganzfeld stimulus of 150 msec on 43 cd/m^2 background).

matopsia. Other subjects with responses subsequently found to be normal included Best's macular dystrophy, pattern dystrophy of the RPE, or early and localized thioridazine hydrochloride (Mellaril) drug toxicity. Again, none of these showed consistent abnormalities on long-flash testing. Some patients in this category had very mild retinitis pigmentosa, with minimally decreased rod ERGs but with good preservation of cone responses by clinical xenon-flash ERG studies; they were studied nevertheless for the possibility of finding unexpected changes on photopic long-flash studies. Eleven patients with Stargardt's macular dystrophy and fundus flavimaculatus (Stargardt's/FF) were tested, and all had normal amplitudes, although three had abnormal (b/d) ratios of less than 1.0.

Zero Amplitude Category—This group included patients who had advanced pathology of the outer retina, as determined by severe reductions of rod and cone ERG responses on xenon-flash testing, markedly constricted visual fields, psychophysical dark-adapted thresholds elevated by three or more log units, and (frequently) poor visual acuity. The great majority of these patients had advanced rod-cone dystrophies. Other subjects in this "zero amplitude" category had achromatopsia (cf, Fig 8, above) or blue cone monochromacy. Surprisingly, few subjects with cone-rod dystrophy were in this category, since those patients who had even barely recordable responses by clinical xenon-flash studies showed at least some response to long-flash photopic stimulation.

Subnormal Amplitudes, But Normal (b/a) or (b/d) Ratios—Patients in this category typically carried a clinical diagnosis of early-stage rod-cone dystrophy. These patients showed proportional a-, b-, and d-wave reduction. In brief, the subnormal response pattern in this category is equivalent to the reduced amplitudes seen for normal subjects upon dimming the stimulus flash (Fig 12). Conceptually, this could result from opaque media or by reducing the overall number of cones in the retina, as from a large atrophic scar. Somewhat more interesting were the cases of known retinal dystrophy that showed a "subnormal amplitude" pattern, such as the case of autosomal dominant retinitis pigmentosa in a family characterized on the molecular level as having a mutation in the rhodopsin gene. (patient 4 described later in connection with Fig 22).

Abnormal (b/a) or (b/d) Ratios—All of the seven patients tested with CSNB were in this category, as were many of the patients with cone or cone-rod dystrophies. When we subsequently looked at the morphology of the individual waveforms of these patients, many showed peculiar changes. For example, cone-rod dystrophy patient 3, described previously (Fig 18), had abnormal (b/a) and (b/d) ratios < 1.0 and a remarkable negative afterswing on the OFF-effect.

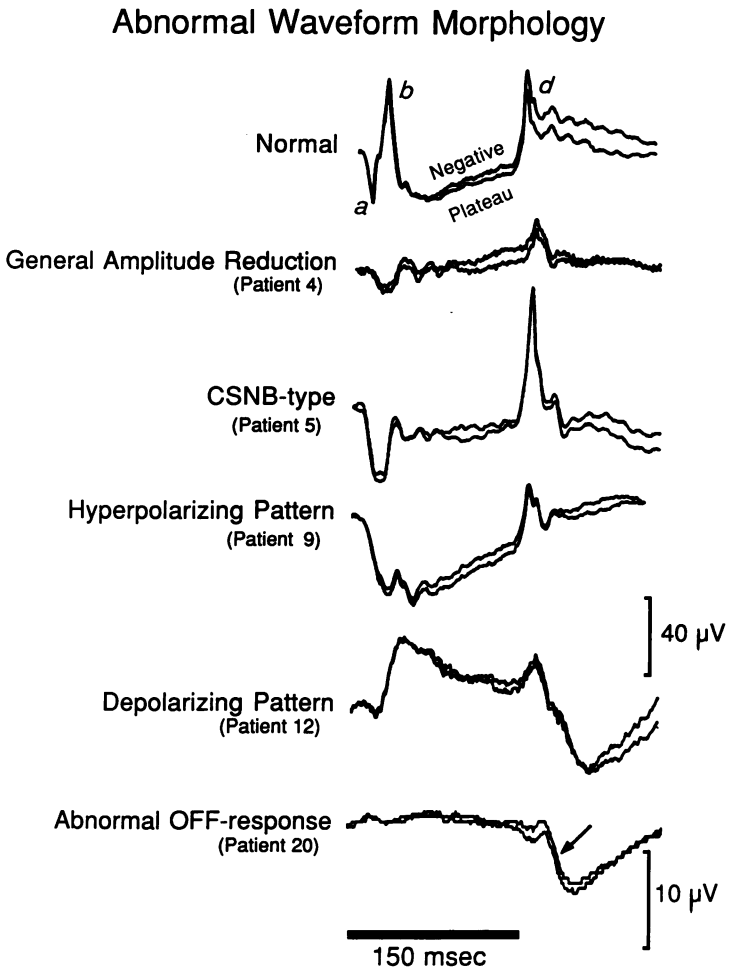

FIGURE 22
Five patterns of abnormal ERG responses to 150-msec flashes (185 cd/m^2 Ganzfeld stimulus on 43 cd/m^2 background). CSNB, congenital stationary night blindness.

A reduced (b/a) ratio < 1.0 proved to be good at discriminating between rod-cone versus cone-rod disease: 11 of 22 (50%) patients with cone and cone-rod dystrophy had an abnormal (b/a) ratio < 1.0, whereas this ratio was normal (greater than 1.0) for all 26 rod-cone subjects tested.

A greater proportion of patients overall were abnormal on the (b/d) ratio than on the (b/a) ratio. For instance, 18 (82%) of 22 patients with cone and cone-rod dystrophy showed (b/d) ratio < 1.0. However, the (b/d) ratio was also abnormal for 9 (35%) of the 26 patients with rod-cone dystrophy.

Consequently, the (b/d) ratio was less helpful in discriminating exclusively cone and cone-rod dystrophy subjects.

Two "(b/a) ratio rules" emerged:

1. Cone and cone-rod dystrophy patients were much more likely to have reduced (b/a) ratios < 1.0 than were rod-cone (RP) subjects.

2. A subject who showed a reduced (b/a) ratio < 1.0 was unlikely to have a rod-cone dystrophy.

Abnormal (b/d) Ratio < 1.0, But Normal b- and d-Wave Amplitudes.—A few patients were noted to have an abnormal (b/d) ratio ≤ 1.0 despite showing normal b-wave amplitudes. Two of these patients had old central retinal vein occlusion (CRVO), one had juvenile retinoschisis, and three had Stargardt's macular dystrophy (two cases) or fundus flavimaculatus (one case). Conceptually, the occurrence of an abnormal (b/d) ratio but with normal b-wave ampltiude could arise either by a selective enhancement of the d-wave or from a small but selective b-wave reduction that nevertheless still lay within the wide normal b-wave amplitude range.

For CRVO one can reasonably hypothesize that the d-wave might be less reduced than the b-wave, since cone PIII (relatively spared in CRVO[24,93]) contributes to the a- and d-waves but not the b-wave. Consistent with this hypothesis, the photopic a-wave amplitude remained normal in these CRVO patients. The analogous result with xenon-flash ERG is well known for using (b/a) ratios in occlusive retinal vascular disease.[24,93]

In the case of juvenile retinoschisis, the pathology is believed to involve primarily the Müller cells and to spare the photoreceptors, at least relatively.[94] Thus, finding an abnormal (b/d) ratio ≤ 1.0 in this patient suggests a reduction of the b-wave but a relative sparing of the d-wave by virtue of its PIII photoreceptor contribution. The a-wave amplitude was normal for this patient with juvenile retinoschisis.

Stargardt's macular dystrophy and fundus flavimaculatus may be different phenotypes of a single retinal dystrophy.[95] Both are thought to affect the retinal pigment epithelium (RPE) and distal retina. Thus the current understanding does not offer a ready explanation for the finding of an abnormal (b/d) ratio in Stargardt's/FF. It is unclear how RPE and/or photoreceptor pathology in Stargardt's could exaggerate the termination of cone PIII and thereby enhance the d-wave amplitude. Not all Stargardt's/FF patients exhibited these changes, and an abnormal (b/d) ratio ≤ 1.0 was found for only these 3 out of 11 Stargardt's/FF patients tested. It remains to be determined whether the range of ERG findings reflects phenotypic variability within Stargardt's/FF or is the result of real diversity that has not yet been incorporated into current clinical subtyping. In a similar vein, recall that the "dark-choroid" on fluorescein angiography is strongly but not

exclusively associated with Stargardt's/FF cases.[96]

CLASSIFICATION BY WAVEFORM MORPHOLOGY

Some retinal dystrophy patients showed ERG waveforms that mimicked the novel monkey ERGs after blocking either the ON- or OFF-pathways by drugs (Fig 3). These monkey ERG changes corresponded to a "depolarizing" or "hyperpolarizing" ERG waveform, dependent upon blocking, respectively, the OFF- or ON-pathway with neurotransmitter glutamate analogs. Quite similar human waveforms were observed in some patients, and this provided a basis for classifying the human long-flash photopic ERG responses by waveform morphology. Five waveform patterns encompassed the majority of the abnormalities observed among these 127 patients. These are listed in Table V, and representative waveforms are shown in Fig 22.

TABLE V: ABNORMAL WAVEFORM MORPHOLOGY CATEGORIES FOR LONG-FLASH PHOTOPIC ERG

General amplitude reduction
CSNB-type
Hyperpolarizing pattern
Depolarizing pattern
Abnormal OFF-response

General Amplitude Reduction

These patients showed a nonselective reduction of all photopic long-flash ERG a-, b-, and d-waves. These changes were the most frequently found among the retinal dystrophy patients. This category corresponds, in Figs 20 and 21, to those patient data points lying above the discriminator line but toward zero, below the normal amplitude boundaries. Frequently, though not exclusively, these patients had rod-cone dystrophy. The waveforms were not very special, and only one example is presented, from retinitis pigmentosa patient 4 in Fig 22. This patient carried a rhodopsin gene mutation. Her xenon-flash ERG is shown in Fig 19.

Patient 4. This 53-year-old woman in an autosomal dominant pedigree has retintis pigmentosa and a cosegregating Thr58Arg rhodopsin gene mutation. (This patient is case III-2 in the family previously reported by Richards and associates.[97]) Acuities were 20/25 OU with +3.25 +0.50 × 144 OD and +3.50 sphere OS. The fundus showed early waxy pallor of the optic

nerveheads, constricted retinal vessel caliber, and intraretinal bone spicule pigmentation in the midperiphery of both eyes. Visual fields showed a midperipheral ring scotoma by Goldmann V4e testing and only central 10° vision with the I4e target. No errors were made on Farnsworth D-15 OU. Psychophysical dark-adapted thresholds were elevated 2 to 3 log units OU at multiple retinal loci. Clinical xenon-flash ERG gave dark-adapted rod b-wave amplitudes of 30 μV (only 15% normal); 30-Hz cone flicker amplitudes were 16 μV (30% normal) and with prolonged implicit time; photopic light-adapted white flash b-wave amplitude was subnormal at 21 μV. Clinical diagnosis was rhodopsin Thr58Arg mutation adRP.

Several mechanisms could account for overall reduced long-flash ERG amplitudes, such as simply fewer cones remaining to drive proximal retinal activity, as from a large atrophic scar involving a major segment of the entire retina. Alternatively, small responses but normal waveform could result from decreased light intensity. The trivial example was seen in Fig 12, where dimmer stimuli caused smaller waves but in similar proportion. Only the plateau, between b- and d-waves, became less negative for dimmer stimuli (which, interestingly, is seen for RP patient 4 in Fig 22).

A biologic equivalent of dimming the light would result from ocular media opaque from hemorrhage.[22] More pertinent for retinal dystrophies would be biologic factors that would decrease the effectiveness of the cones for catching quanta, such as by shortened outer segments that would shorten the optical path length and decrease quantal catch by visual pigment. It is unlikely that photoreceptor misalignment (ie, Stiles-Crawford effect) played much of a role, since these ERGs were recorded with widefield stimulation. Which among all these factors might be pertinent to patient 4 with the Thr58Arg rhodopsin mutation is unknown by ERG technique.

CSNB-Type

Several patients with Shubert-Bornshein type CSNB showed depressed b-waves, with d-wave amplitude relatively spared (Fig 23). As discussed in the "Introduction," blocking the photopic ON-pathway with APB (Fig 3) produces a photopic ERG waveform that mimics these CSNB patients, including enhancing the d-wave in patient 5. Since Miyake and associates[45,46] had previously described the photopic long-flash ERG of SB-CSNB patients, we adhered to this as a separate category, particularly because our SB-CSNB constituted a relatively homogeneous clinical and ERG phenotype. Fig 24 shows the xenon-flash ERG results in these CSNB patients.

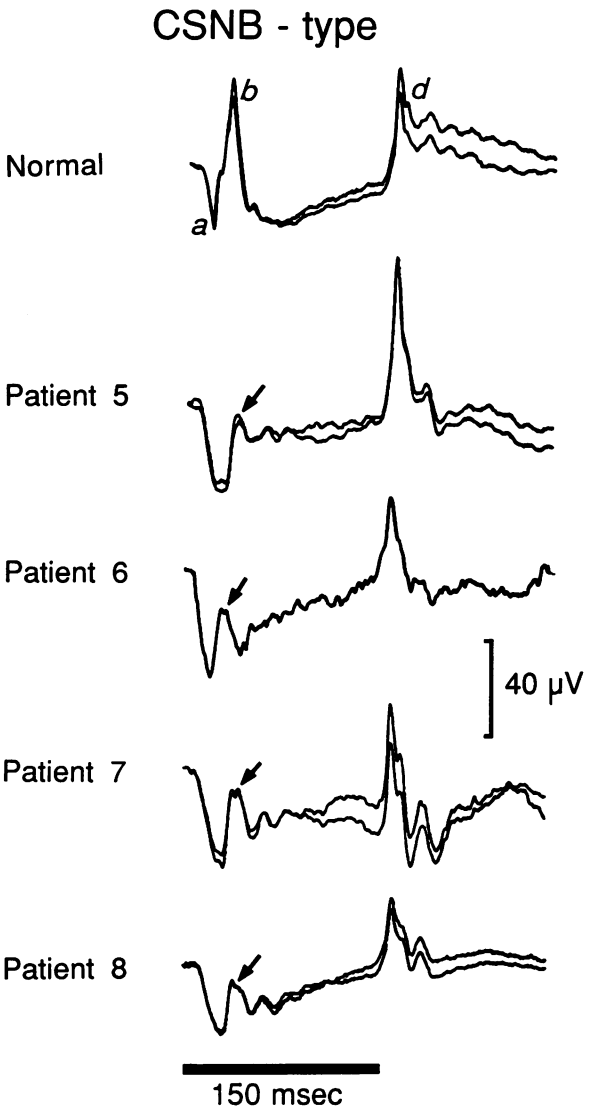

FIGURE 23
CSNB-type photopic ERG to 150 msec flashes shows truncated b-wave (*arrow*) (185 cd/m^2 Ganzfeld stimulus on 43 cd/m^2 background).

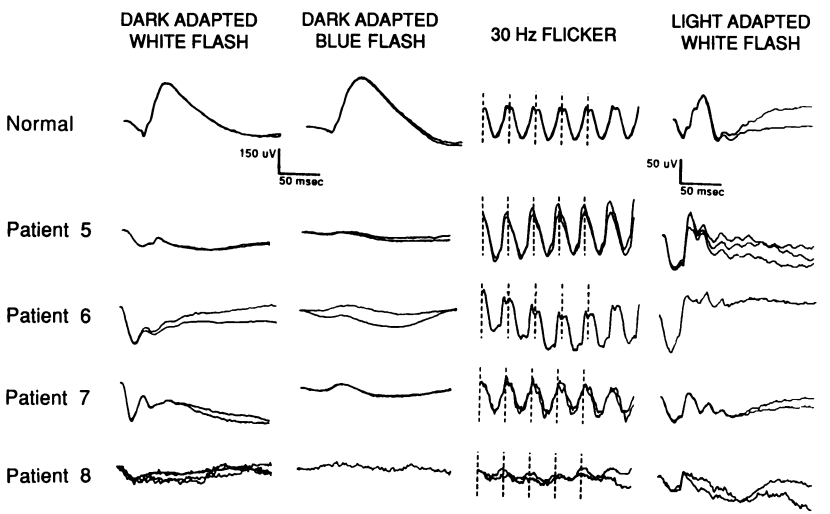

FIGURE 24
Clinical xenon-flash ERG. Stimulus conditions are given in text. Flash coincides with onset of trace.

Patient 5. This 9-year-old boy had had poor acuity and inability to see at night since his youngest years. No other genetic relatives were known to be similarly affected. Acuities were 20/200 OD and 20/70 OS with correction of −1.00 +0.75 × 175 OD and −1.00 +0.50 × 5 OS. The fundus showed mild disc pallor but without a temporal notch or gliosis, normal vessel caliber, normal macular appearance, and good foveal reflexes. Fundus appearance was blond with a prominent choroidal pattern, but no pigment clumping or intraretinal pigmentation was present. Peripheral fields were full with Goldmann V4e and I4e. One minor crossing error for each eye was made on Farnsworth D-15. Dark-adapted psychophysical thresholds were elevated 2 to 3 log units at several retinal loci tested OU. Clinical xenon-flash ERG showed severely diminished rod b-wave of only 10 μV, but the a-wave to dark-adapted bright flash was normal. Cone 30-Hz flicker amplitude and timing were normal, but cone light-adapted white flash response showed a broadened a-wave with loss of 2 oscillatory potentials. Clinical diagnosis was SB type CSNB.

Patient 6. This 5-year-old boy had high myopia and inability to see in the dark. Family history revealed no other similarly affected genetic relatives, but a 1-year-old male sibling was highly myopic. Visual acuities were 20/30 OU with −6.00 +0.75 × 97 OD and −5.00 +0.50 × 167 OS. Fundus examination revealed physiologic color and cupping of the optic nerveheads,

normal vessel caliber, and intact foveal reflexes. The macula and periphery showed a coarse RPE granularity but no pigment clumping or intraretinal pigment aggregation. Visual fields were full with Goldmann V4e and I4e. Four errors were made with each eye on Farnsworth D-15. Dark-adapted thresholds were definitely elevated but of poor reliability due to age. Clinical xenon-flash ERG showed severe depression of rod dark-adapted responses but with a large dark-adapted a-wave to bright flash. Cone 30-Hz flicker had normal timing and amplitude, but cone light-adapted white flash response revealed broad a-wave with selective dropout of the first oscillatory potential. Clinical diagnosis was SB type CSNB with high myopia, possibly X-linked recessive.

Patient 7. This 15-year-old boy had a lifelong history of inability to see at night. No other genetic relatives were similarly affected. Visual acuities were 20/30 with correction of −9.25 +1.75 × 180 OD and −8.25 +1.00 × 179. Fundus showed no retinal or optic nervehead abnormalities. Visual fields were normally full with Goldmann V4e and I4e targets. No errors were made on Farnsworth D-15 panel. Psychophysical dark-adapted thresholds were elevated 3 log units above normal rod threshold at multiple retinal loci tested. Clinical xenon-flash ERG showed the dark-adapted rod b-wave only 10% of normal at 20 μV, but the a-wave reached normal amplitudes with dark-adapted bright flash stimulus. Cone 30-Hz flicker latency and amplitudes were normal, but with light-adapted white flash stimulus the photopic a-wave was broadened and the b-wave amplitude was subnormal, with loss of 2 oscillatory potentials. Clinical diagnosis was SB type CSNB with high myopia.

Patient 8. This 8-year-old boy had lifelong difficulty seeing in dim light. His 10-year-old brother was similarly affected, but no relatives in previous generations were known to be affected. Acuities were 20/400 and 20/40 with −9.25 +1.25 × 9 OD and −9.75 +1.50 × 170 OS. He had known strasbimic amblyopia OD. The discs showed myopic tilt, retinal vessel caliber was normal, and macular reflexes were intact. This was a blond fundus with a prominent choroidal pattern but no intraretinal pigmentary changes or RPE pigment clumping. Visual fields were full with Goldmann V4e and I4e targets. Farnsworth D-15 showed no crossing errors for either eye. Dark-adapted thresholds remained elevated by 1.5 log units at fixation and multiple retinal loci tested OU. Clinical xenon-flash ERG showed loss of dark-adapted rod b-wave; cone 30-Hz flicker had normal amplitude, and the light-adapted white flash response showed a broad a-wave and diminished b-wave amplitude, with loss of 2 oscillatory potentials. Clinical diagnosis was SB type CSNB with high myopia, possibly X-linked recessive.

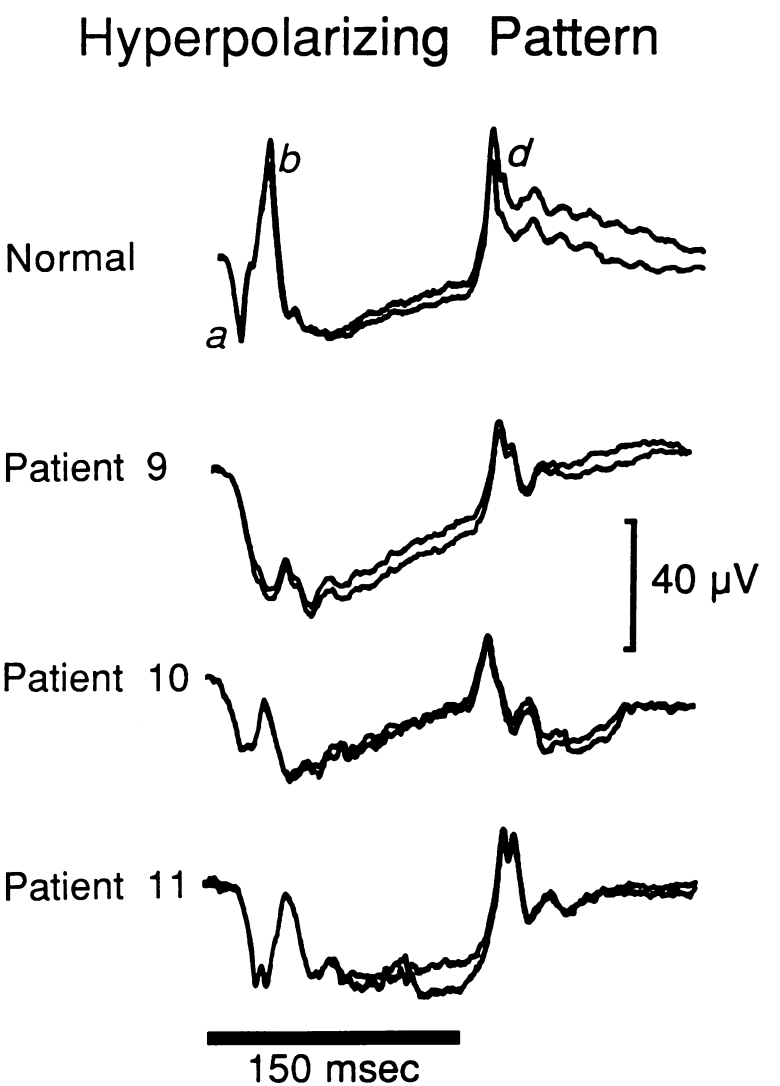

FIGURE 25
Hyperpolarizing pattern of photopic ERG to 150 msec flashes shows depressed negative plateau (185 cd/m² Ganzfeld stimulus on 43 cd/m² background).

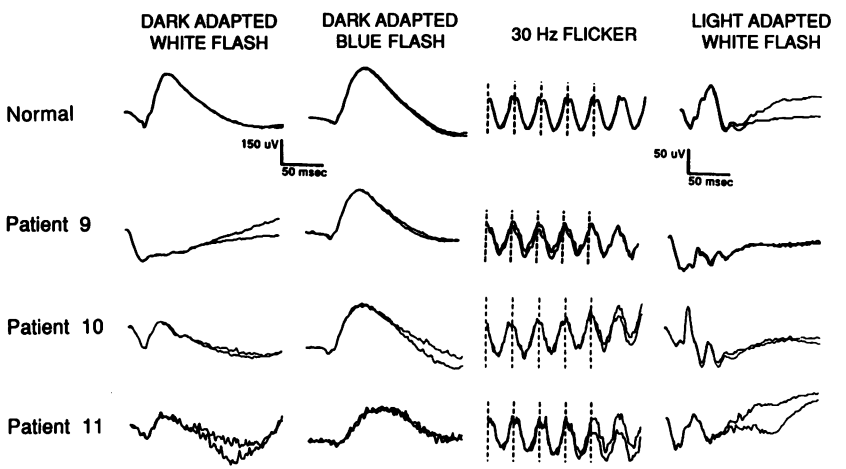

FIGURE 26
Clinical xenon-flash ERG. Stimulus conditions are given in text. Flash coincides with onset of trace.

Hyperpolarizing Pattern

Other patients besides those with CSNB showed predominantly negative responses on photopic long-flash stimulation. Like the CSNB-type, the patients with hyperpolarizing pattern showed a relative suppression of the b-wave which caused both the (b/a) ratio and the (b/d) ratio to be abnormal at < 1.0. Three examples of these photopic long-flash ERG are shown in Fig 25, and the xenon-flash results are shown in Fig 26.

Although the long-flash waveforms were not dissimilar to those of the CSNB-type, these patients constitute a separate category in that they all had normal or near normal rod b-waves on xenon-flash ERG testing. Further, these patients exhibited normal psychophysical dark-adapted rod thresholds, quite unlike those with CSNB.

Patient 11 showed a photopic long-flash ERG response similar to the CSNB-type, but she had bull's-eye macular RPE atrophy quite unlike the clinical appearance of CSNB. Wakabayashi[98] reported patients with bull's-eye maculopathy who had photopic ERG abnormalities similar to those of patient 11 on xenon-flash ERG testing. All patients in the hyperpolarizing pattern group reported that they once had normal visual acuity but suffered progressive loss beginning in their teenage years or later, unlike the congenital onset of nyctalopia in CSNB.

Patient 9. This 55-year-old woman had had poor acuity since her early 20s. She drove a car until age 20. She had no full siblings; 12 half-siblings

were unaffected. Acuities were 20/100 OD and 20/400 OS with +3 equivalent sphere. Retinal vessels showed 50% narrowing. The fovea was normally developed. The entire posterior pole showed RPE granularity but no intraretinal bone spicule pigmentation. Visual fields were full for Goldmann V4e annd I4e targets. Farnsworth D-15 testing showed five minor tritan errors OU. Dark-adapted rod thresholds reached normal levels. Clinical xenon-flash ERG showed normal amplitude on dark-adapted rod and 30-Hz cone flicker testing, but the light-adapted b-wave to single white flash was diminished by one half and the a-wave showed a broadened trough. Clinical diagnosis was cone abnormality in a genetic isolate.

Patient 10. This 31-year-old man had vision not fully correctable since age 13. Color discrimination was thought to be normal in childhood, but the patient claimed it had been poor for 10 years. He was photophobic to bright light and visually more comfortable at night. Acuities were 20/40 –2 OD and 20/50 –1 OS with correction of –0.25 +0.50 × 135 OD and –0.25 +0.50 × 180 OS. Optic nerveheads had physiologic color and cupping. Retinal vessel caliber was normal. Foveal reflexes were broad. The peripheral retina showed no atrophy, RPE pigment clumping or granularity, and no intraretinal pigment. Visual fields were full with Goldmann V4e and I4e targets except for a central relative scotoma of 10° OU with I4e target. There were 2 tritan axis errors OD and 4 OS on Farnsworth D-15. Dark-adapted psychophysical thresholds reached normal rod levels. Clinical xenon-flash ERG had normal dark-adapted rod b-wave amplitudes and waveform, normal cone 30-Hz flicker amplitude and timing, and a low but normal cone light-adapted white flash b-wave, but with a broadened a-wave. Clinical diagnosis was cone dystrophy.

Patient 11. This 44-year-old woman had had poor acuity for several years and was now photoaversive in bright light. No other genetic relatives are known to be similarly affected. Acuities were 20/50 OU with –2.75 OD and –2.00 +0.25 × 113 OS. Optic nerveheads had physiologic color and cupping, and the retinal vessel caliber was normal. The macula showed bull's-eye atrophy OU. No peripheral retinal changes were evident. Visual fields remained normally full on Goldmann V4e and I4e. Six tritan axis errors were made OD on Farnsworth D-15 and two errors OS. Psychophysical dark-adapted thresholds reached normal rod levels between 10°and 60° retinal eccentricity but were elevated by 1 to 2 log units in the central atrophic macular region. Clinical xenon-flash ERG gave normal dark-adapted rod b-wave amplitudes. Cone 30-Hz flicker amplitudes and implicit times were normal; the photopic a-wave was broadened on to light-adapted white flash stimuli, but with normal b-wave amplitude. Clinical diagnosis was bull'e-eye macular degeneration with ERG evidence of cone dystrophy.

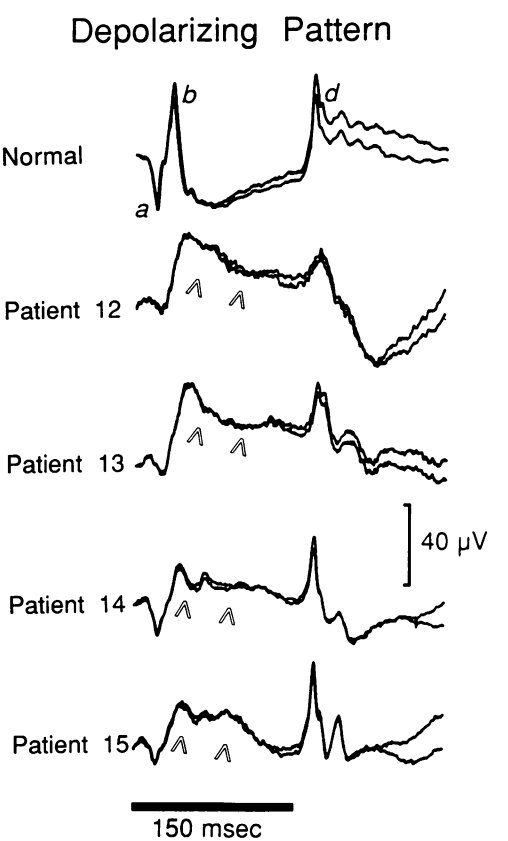

FIGURE 27
Depolarizing pattern of photopic ERG to 150 msec flashes shows plateau elevated above prestimulus base-line (*hollow arrows*) (185 cd/m^2 Ganzfeld stimulus on 43 cd/m^2 background).

Depolarizing Pattern

Patients in this category showed elevation of the plateau to a positive potential above the prestimulus baseline. This is shown for four patients in Fig 27, with xenon-flash ERG results shown in Fig 28. A plateau elevation to this extent was not found for any normal subject at any stimulus intensity (eg, Figs 1, 6, and 12).

These patients showed a normal rise for the long-flash photopic b-wave but lacked the normal falling slope after the b-wave peak. The plateau was elevated above the baseline, and this truncated the normal negative-going termination of the b-wave. Existing paradigms of photopic ERG analysis cannot explain this, since they require that the plateau be negative as a

consequence of the PIII contribution. The "depolarizing type" human ERG could be mimicked quite well in monkey by applying PDA or KYN to block synaptic transfer to the hyperpolarizing bipolar cells of the cone OFF-pathway (Fig 3).

Patient 12. This 14-year-old boy had had limited peripheral and night vision since age 5. No genetic relatives other than his mother were similarly affected. Acuities were 20/30 OU without correction. Visual fields were full using Goldmann V4e but showed tight "tunnel vision" to the central 10° isopter with I4e. No errors were made on Farnsworth D-15 testing. Dark-adapted thresholds were mediated by cones, with rod thresholds elevated above cone threshold when tested at multiple retinal loci. Clinical xenon-flash ERG revealed severely depressed dark-adapted rod responses to noise level. Cone 30-Hz flicker was delayed and reduced to 10% of normal. Cone light-adapted single white flash response of 35 μV was subnormal. Clinical diagnosis was rod-cone dystrophy.

Patient 13. This 66-year-old woman had had difficulty seeing at night for 7 years and recent visual acuity decrement. Her sister, father, and paternal grandmother were diagnosed with retinitis pigmentosa. Acuities were 20/50 OU with correction of −2.00 +1.50 × 143 OD and 1.50 +1.25 × 42 OS. The optic nerveheads showed early gliosis causing "waxy pallor" and reducing the cup-disc ratio to 0.1 OU. Retinal vessel caliber was 50% constricted. The foveal reflexes were broad, and the RPE within the macula was granular. Fine bone spicule pigmentation was present in all quadrants OU. The peripheral RPE showed coarse granularity with early diffuse atrophy that revealed a prominent choroidal pattern. Visual fields showed supratemporal loss on Goldmann V4e testing, and only a 10° central field remained on I4e testing with nasal extension to 30°. Dark-adapted thresholds were elevated 1 to 2.5 log units at multiple retinal loci tested OU. OD showed 4 tritan axis errors and 2 OS on Farnsworth D-15 testing. Clinical xenon-flash ERG revealed dark-adapted rod b-wave amplitudes of 100 μV (50% normal). Cone 30-Hz flicker responses were only 10 μV (20% normal) and delayed, and the photopic light-adapted white flash response was subnormal at 30 μV, with no oscillatory potentials remaining. Clinical diagnosis was rod-cone dystrophy.

Patient 14. This 22-year-old man had had poor visual acuity for several years. One brother of six siblings was reported to be similarly affected. No relatives from previous generations were known to be affected. Acuities were 20/40 OD and 20/100 OS with correction of +1.75 +1.00 × 90 OD and +1.50 +1.00 × 89 OS. The optic nerveheads showed physiologic color and cupping. Retinal vessel caliber was slightly constricted. The foveal reflexes were absent, and the macula showed deep yellowish RPE lesions that were

nearly confluent to the midperiphery. The appearance was dissimilar to, though not exclusive of, fundus flavimaculatus. Fluorescein angiogram showed staining but not window defects. Peripheral fields were intact only with the Goldmann V4e target; only a peripheral crescent between 20°and 40° remained with I4e testing. Six tritan axis errors were made OU on Farnsworth D-15. Dark-adapted thresholds were elevated 3/4 log unit OU at multiple retinal loci. Clinical xenon-flash ERG showed dark-adapted rod b-wave amplitudes only 50% of normal; 30-Hz cone flicker response amplitudes were subnormal and delayed; photopic light-adapted white flash response reached normal amplitudes OU but with a quite abnormal waveform consisting of an initial b-wave rise interrupted by a horizontal transition lasting 15 msec and finally rising to the b-wave peak. No oscillatory potentials remained. Clinical diagnosis was RPE disease with rod and cone involvement.

Patient 15. This 10-year-old girl had poor acuity and color vision abnormality of recent onset. A second cousin was known to be similarly affected. Acuities were 20/60 OU with emmetropic refraction. Optic nerveheads showed physiologic color and cupping. Retinal vessel caliber was slightly constricted. A partially atrophic bull's-eye pattern was present in the macula OU. The peripheral retina showed mild RPE granularity but no pigment clumping or intraretinal pigment. Visual fields were normally full with Goldmann V4e and I4e targets. Seven major deutan axis crossing errors were made OU on Farnsworth D-15 panel. Dark-adapted psychophysical thresholds reached normal rod levels OU at multiple retinal loci tested. Clinical xenon-flash ERG gave dark-adapted rod b-wave amplitudes of 100 μV (50% normal); 30-Hz cone flicker amplitudes were subnormal at 35 μV but with implicit times, and photopic light-adapted white flash responses had normal b-wave amplitude but an abnormal waveform with an initial rise interrupted for 10 msec in the middle before reaching the peak. Clinical diagnosis was cone-rod dystrophy with bull's-eye macular pattern.

Unilateral Depolarizing Pattern—One unique patient showed a depolarizing ERG pattern in only one eye (Fig 29). A photopic long-flash intensity series showed that the pattern held constant down to threshold responses and was not an artifact of brighter flash intensity. The constancy of the depolarizing waveform over the full-intensity range to which normals respond (Fig 12) suggests that the cones were catching and responding to light over a normal range.

Patient 16 presented with unilateral vision complaints involving the right eye. Clinical ERG studies (Fig 30) gave evidence of a cone dystrophy affecting primarily the right eye, although both eyes exhibited an electronegative pattern on dark-adapted bright -flash testing. Few reports of

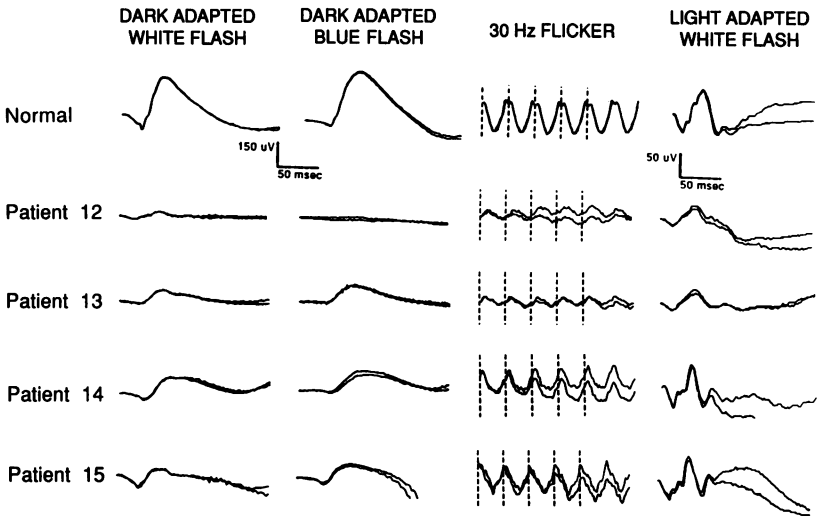

FIGURE 28
Clinical xenon-flash ERG. Stimulus conditions are given in text. Flash coincides with onset of trace.

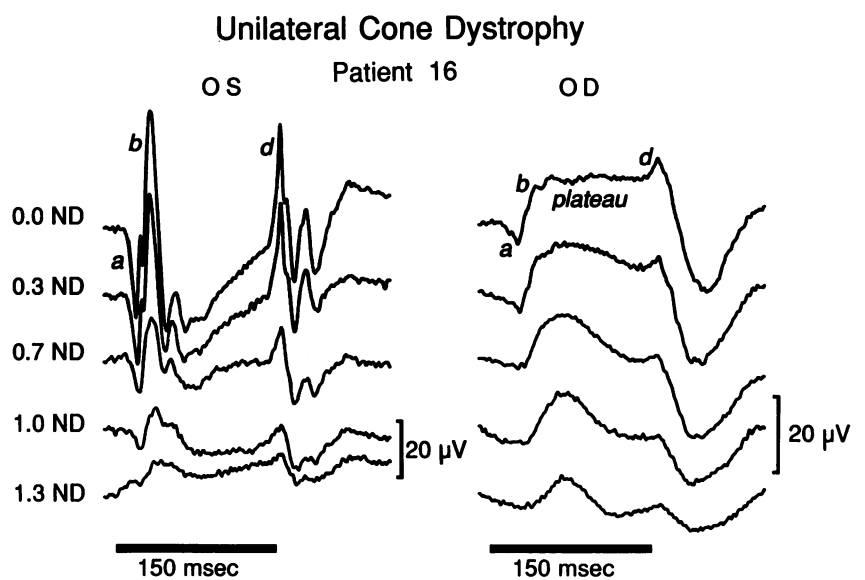

FIGURE 29
Photopic ERG intensity series in case of unilateral cone dystrophy for 150 msec flashes shows depolarizing pattern of involved eye, with plateau elevated above prestimulus baseline (185 cd/m^2 Ganzfeld stimulus on 43 cd/m^2 background).

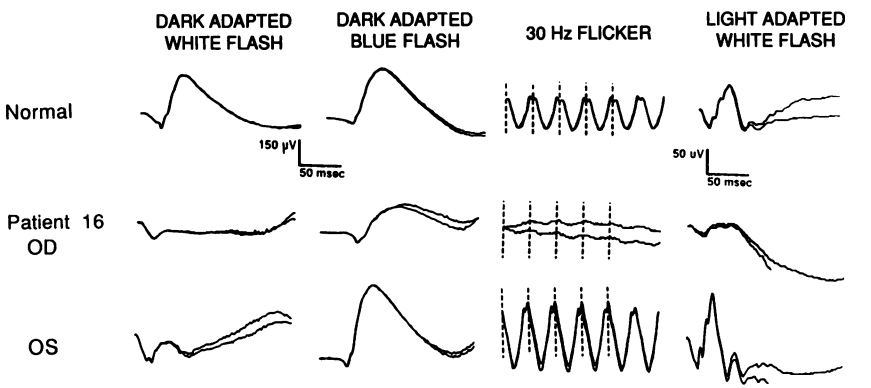

FIGURE 30
Clinical xenon-flash ERG. Stimulus conditions are given in text. Flash coincides with onset of trace.

unilateral cone dystrophy exist,[99,100] and none for which photopic long-flash ERG recordings are available. In the monkey, blocking of the OFF-pathway with KYN (Fig 3) resulted in a depolarizing pattern. Currently nearly nothing is known for any species about the consequences to vision of blocking the OFF-pathway. The patient's complaint of gray peripheral vision in daylight suggested that the HBC OFF-pathway might be important for color processing. However, thus far in primate, color vision has only been studied in conjunction with blocking the ON-pathway DBCs using APB,[3-5] and the consequences to color vision of eliminating the OFF-pathway are not known.

Patient 16. This 63-year-old woman had a 2-year history of poor acuity and color desaturation OD but not OS. She had received regular ophthalmic examinations in prior years and reported a definite time of onset of the vision change 2 years previously. She reported best vision for the right eye directly ahead and claimed "fuzzy vision and greyness" outside of a central 5° zone. No genetic relatives were similarly affected. Acuities were 20/40 OD and 20/20 OS with +2.25 +0.50 × 1 OD and +2.50 +0.50 × 166 OS. Pupil responses were both normal and with no afferent defect. Optic nerveheads both showed physiologic color and cupping of 0.25 OD and 0.35 OS. Retinal vessel caliber was normal and symmetric between the eyes. The foveal reflexes were intact but broad OU. The macula showed no atrophy, fundus flecks, or pigmentary granularity. The peripheral retina appeared normal. Visual fields OU were normally full to Goldmann V4e target, but with the I4e they reached to only 60° temporal OD versus 70° OS. Five tritan axis errors were made OD on Farnsworth D-15 panel, but no errors

were made OS. Dark-adapted psychophysical thresholds reached normal rod levels OS but were 1/2 log unit elevated OD at multiple retinal loci. Clinical xenon-flash ERG showed fully normal for rod and cone responses OS. OD rod dark-adapted b-wave was normal (but mildly depressed relative to OS), and an electronegative pattern was seen on dark-adapted bright flash testing. OD cone flicker was less than 5 μV and cone light-adapted white flash response was 10 μV, both severely reduced from normal. Electrooculogram Arden ratios were 2.18 OD and 2.75 OS. Clinical diagnosis was unilateral cone dystrophy.

As clarification, this diagnosis was given on the basis of nearly normal scotopic visual function testing by psychophysics and xenon-flash ERG, and the complaint of daylight vision disturbance, with an absence of color vision outside the central 5°.

Abnormal OFF-Responses

These patients were clinically less homogeneous than those in the previous categories. Those with late-negativity in the ERG OFF-complex with photopic long-flash stimuli (Fig 31) mimicked the pronounced negative swing of the OFF-effect in the monkey photopic ERG after application of KYN, which blocks the OFF-pathway (Fig 3). Clinical xenon-flash ERG testing (Fig 32) indicated that these patients had variants of cone-rod degeneration. Patients 18 and 20 showed patterns of cone dystrophy, and patients 17 and 19 had bull's-eye maculopathy.

Patient 17. This 9-year-old boy had a 3-year history of poor visual acuity. No relatives were known to be similarly affected. Acuities were 20/200 OU with correction of −1.50 +1.25 × 91 OD and −2.50 +2.25 × 90 OS. The optic nerveheads showed physiologic color and cupping. Retinal vessel caliber was normal. Atrophic bull's-eye macular lesions measured 3 disc diopters in both eyes. The peripheral retina appeared normal. Visual fields were full on Goldmann V4e and I4e testing, but with 10° central relative scotomata OU. Four nonspecific crossing errors were made OD and 3 OS on Farnsworth D-15. Psychophysical dark-adapted thresholds were elevated 3/4 log unit OU at multiple retinal loci. Clinical xenon-flash ERG showed dark-adapted rod b-wave amplitudes of 60 μV (30% normal); no 30-Hz cone flicker responses remained, and photopic light-adapted white flash responses had electronegative patterns with (b/a) ratio less than 1.0. Clinical diagnosis was bull's-eye maculopathy in a cone-rod dystrophy.

Patient 18. This 32-year-old man complained of color confusion, limited night vision, and decreased acuity. No relatives were known to be similarly affected. Acuities were 20/25 OU with emmetropic refraction. Optic nerveheads showed physiologic color and 0.2 cupping OU. Retinal vessel caliber

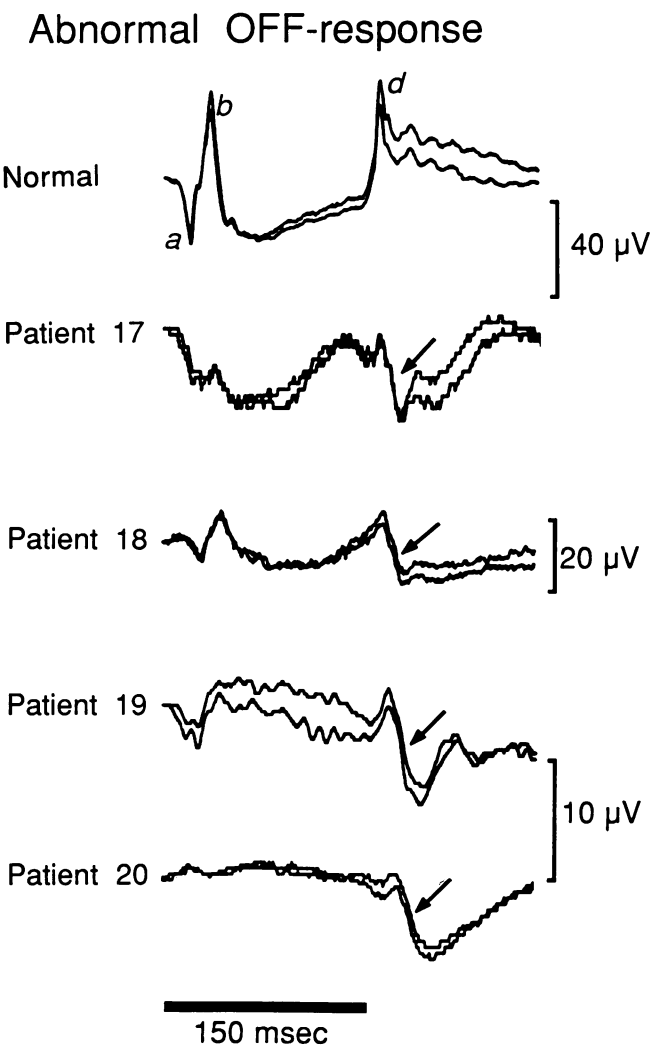

FIGURE 31
Abnormal OFF-response pattern to photopic ERG with 150-msec flashes shows exaggerated negative waveform swing (*arrows*) after d-wave (185 cd/m² Ganzfeld stimulus on 43 cd/m² background).

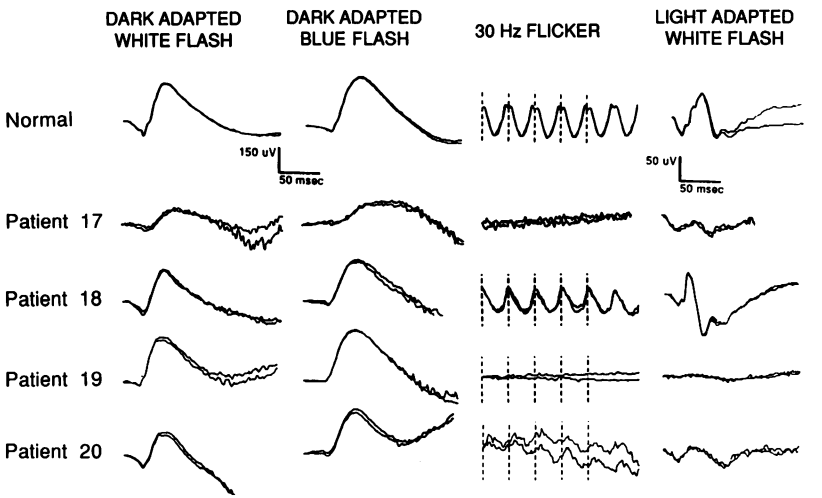

FIGURE 32
Clinical xenon-flash ERG. Stimulus conditions are given in text. Flash coincides with onset of trace.

was normal. The foveal reflexes were intact. The peripheral retina appeared normal, although with a prominent choroidal pattern. Visual fields were full on Goldmann V4e and I4e testing. Four major tritan axis errors were made on Farnsworth D-15 panel OU. Psychophysical dark-adapted thresholds reached normal rod levels after 35 minutes OU at multiple retinal loci tested. Clinical xenon-flash ERG gave subnormal dark-adapted rod b-wave amplitudes of 150 μV (80% normal). Cone 3-Hz flicker responses had normal implicit times but subnormal amplitudes of 30 μV (50% normal). Photopic light-adapted white flash responses had subnormal b-wave amplitudes and an abnormal waveform with a sweeping negativity after the b-wave. Only two rather than four, oscillatory potentials were present. Clinical diagnosis was cone dystrophy.

Patient 19. This 29-year-old man had poor acuity and progressive neurologic dysfunction from spinopontocerebellar degeneration. No other relatives were affected. Acuities were 20/400 OU with slight myopic correction by retinoscopy. The fundus showed physiologic cupping of the optic nerveheads, slightly constricted retinal arteriolar caliber, RPE granularity throughout the macula and periphery, and a central atrophic macular bull's-eye lesion measuring 1 disc diameter. Visual fields were full OU with Goldmann V4e but showed temporal constriction to 50° with I4e. Ten crossing errors were made OU along no specific color axis on Farnsworth D-15. Psychophysical dark-adapted thresholds reached normal rod levels

OU at multiple retinal loci tested. Clinical xenon-flash ERG gave low but normal dark-adapted rod b-wave amplitudes, but cone 30-Hz flicker and photopic light-adapted white flash responses were reduced to nearly the level of noise, at less than 5 μV. Clinical diagnosis was bull's eye maculopathy and cone dystrophy associated with spinopontocerebellar degeneration.

Patient 20. This 36-year-old man complained of a decline in acuity over several years OS and progressive sensitivity to bright daylight OU. The right eye was amblyopic. He was known to be "red-green color blind" since childhood. Three full siblings were unaffected. Acuities were 20/400 OD and 20/40 OS with refraction of −3.00 +2.25 × 172 OD, +1.25 +1.50 × 27 OS. Retinal vessel caliber was normal. Disc cupping was 0.5 and showed a temporal notch. Foveal reflexes were broad. RPE granularity was seen throughout the macula and midperiphery but without intraretinal pigment clumping or bone spicule pigment forms. Major protan axis errors for each eye occured on Farnsworth D-15. Visual fields were full with Goldmann V4e and I4e targets. Dark-adapted thresholds reached normal rod levels on Goldmann-Weekers testing. Clinical xenon-flash ERG gave normal dark-adapted rod amplitudes, but cone responses were delayed and reduced 70% on 30-Hz flicker and the photopic response was 80% reduced to single white flash. Clinical diagnosis was cone dystrophy.

DISCUSSION

This study explored the use of photopic long-flash ERG recordings to evaluate ON- and OFF-pathway abnormalities of the cone system for patients with retinal dystrophies. The cone photopic ERG OFF-effect has rarely been studied in retinal dystrophy patients to this time, and then only for individual cases or small groups of patients.[17,21,45,46,101] This investigation accomplished the following:

- demonstrated the feasibility of recording photopic long-flash ERGs routinely from patients in a clinical setting;
- described ERG stimulus parameters appropriate for studying retinal activity of the cone ON- and OFF-pathways;
- developed statistical criteria for analyzing photopic long-flash ERGs to segregate patients with cone (cone-rod) dystrophy from those with rod-cone dystrophy;
- identified a new category of patients with a depolarizing ERG pattern, putatively resulting from physiologic dysfunction of the cone OFF-pathway;
- identified hyperpolarizing ERG pattern patients with putative ON-pathway dysfunction of the cone but not rod system (and thereby unlike CSNB);

• suggested that vision loss in some cases of cone and cone-rod dystrophy results from pathology in the retinal pathways proximal to the photoreceptors.

STATISTICAL VERSUS MORPHOLOGIC ANALYSIS

The statistical and waveform morphologic analyses represent two quite different approaches to utilize the information from photopic long-flash ERG recordings. Consideration of the group in statistical aggregate provided several new ERG descriptors that may have diagnostic value for distinguishing cone-rod from rod-cone dystrophies. The statistical outcomes of this study were discussed in the "Results" section. In brief, classifying the response amplitudes by (b/a) and (b/d) ratios identified patient subpopulations that were enriched for recognizable categories of clinical pathology. In particular, the (b/a) ratio of photopic long-flash ERG responses identified many of the patients with cone and cone-rod dystrophy while excluding all of the cases with rod-cone dystrophy. Further exploration of this (b/a) ratio is warranted to understand its potential as a diagnostic parameter for the retinal dystrophies, as well as to provide new insights into physiologic distinctions between the cone-rod and rod-cone groups.

In addition to providing statistical analysis of the entire group, however, this study pointed out the benefit of paying attention to waveform configurations of each individual case. This approach identified waveforms that conformed to expectations for ON- versus OFF-pathway losses as indicated by monkey ERG changes after selectively blocking these mechanisms pharmacologically.

Morphologic analysis of the response waveforms allowed for sorting patients' ERGs into subcategories along physiologic grounds. This approach is potentially quite powerful, in that it identified an entirely new category of ERG abnormality called the "depolarizing pattern" (Figs 22 and 33). This unique ERG waveform had not previously been described in human patients, but it corresponded quite well to the monkey ERG that resulted from blocking the photopic retinal hyperpolarizing OFF-pathway with KYN (Fig 3). Morphologic waveform analysis also identified patients who had putative photopic ON-pathway abnormalities that mimicked CSNB but who had normal scotopic b-waves and dark-adapted thresholds that distinguished them from CSNB; such patients have previously been reported only rarely, by Young and associates[21] and by U. Kellner and M. H. Foerster (unpublished data). These patients fit into a category called hyperpolarizing ERG pattern (Figs 22 and 33) that is newly described in this thesis.

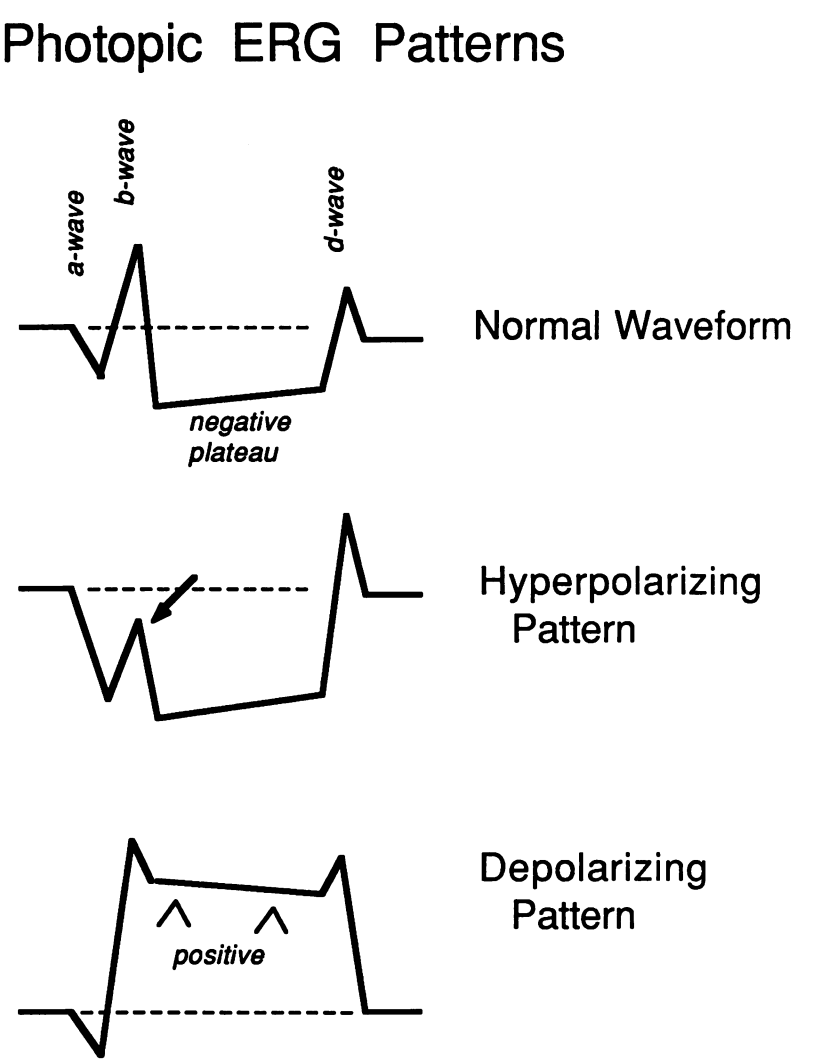

FIGURE 33
Cartoon of "depolarizing" and "hyperpolarizing" patterns found in retinal dystrophy patients for photopic long-flash ERG stimulation.

RELATIONSHIP BETWEEN THE PHOTOPIC "HYPERPOLARIZING ERG PATTERN" AND THE SCOTOPIC "ELECTRONEGATIVE" RESPONSE

Scotopic ERGs that have a-waves larger than b-waves are said to have a "negative ERG waveform" and are labeled "electronegative ERGs."[105] Electronegative scotopic ERGs are characteristically found for CSNB,[14,44,45,106] juvenile X-linked retinoschisis[94,107] and retinal vascular disease.[105] Each occurs by a different mechanism: CSNB is believed to be due to dysfunction of the APB-sensitive DBC pathway[15,16]; X-linked juvenile retinoschisis is believed to result from Müller cell pathology that affects its potassium processing underlying b-wave production[108]; negative scotopic ERG in retinal vascular disease is thought to be due to selective anoxia of the proximal retinal b-wave but with sparing of the distal retinal photoreceptor a-wave.[24]

Patient 9, with a photopic "hyperpolarizing ERG pattern" (Fig 25), showed an electronegative scotopic rod ERG on clinical xenon bright-flash recording but had a normal rod b-wave amplitude for the dimmer blue flash (Fig 26). Kellner and Foerster reported several cases similar to our patient 9, although they did not perform long-flash stimulation to evaluate the photopic system in their patients (written communication, 1993). Patient 9 showed bull's-eye maculopathy and had neither CSNB, retinoschisis (the patient was not male), nor retinal vascular disease; none of the mechanisms described previously explain her severely electronegative scotopic response.

An alternate mechanism for cases such as patient 9 would invoke the cone hyperpolarizing OFF-pathway. Clinical bright-flash ERG testing with a photostrobe definitely stimulates cones as well as rods.[34] Thus, although recorded under dark-adapted conditions, this bright flash ERG will activate the cone hyperpolarizing OFF-pathway in addition to the rod DBC pathway. Since the cone depolarizing ON-pathway is suppressed in these patients (as evidenced by the suppressed photopic b-wave), the cone hyperpolarizing OFF-pathway may depress even the scotopic ERG waveform for high-intensity flashes. However, since the dimmer blue flashes evoke activity of the rod system nearly exclusively, the rod b-wave will remain present and be positive.

DEPOLARIZING ERG PATTERN

Patients showing a photopic depolarizing ERG pattern, with the plateau elevated to a positive potential, are new. This ERG pattern was mimicked by blocking the cone OFF-pathway with application of KYN (Fig 3), but this result cannot be accounted for by current models of ERG analysis. The positive plateau cannot be the result of drug action on the cone photoreceptors, since light-evoked hyperpolarization of cones contributes the negative PIII response to the ERG for all photopic stimulus conditions.[51,88,89]

DIAGNOSTIC VALUE OF ON- AND OFF-PATHWAY ERG INDICES

Distinguishing cone and cone-rod dystrophy from rod-cone dystrophy still remains a clinical art.[86,109] This distinction carries considerable importance for the patients in question, because of the quite different prognoses for future vision. Cone and cone-rod dystrophy patients as a group show lower cone ERG amplitudes on photopic xenon-flash testing and have worse visual acuity in the short term than do patients with rod-cone dystrophy.[109]

Part of our quest in studying retinal dystrophy patients was to search for human photopic ERG responses that had a depolarizing pattern which would support our ERG theory developed in monkey.[26] Finding human patients with a depolarizing pattern of response (Fig 27) provided welcome support for our idea that the drug results in monkey truly reflect actual retinal physiology.

CSNB-TYPE VERSUS HYPERPOLARIZING ERG PATTERN

The photopic ERGs of CSNB-type and of hyperpolarizing-type patients (Fig 22) are both electronegative, with the b-wave suppressed to varying extent. Both patterns of response were mimicked by blocking the ON-pathway in monkey with APB (Fig 3). However, these two categories differed fundamentally in that the scotopic rod-driven b-wave was markedly depressed for CSNB patients (Fig 24) whereas it was nearly normal for hyperpolarizing-type patients (Fig 26). This indicates that dysfunction need not affect the sign-inverting (–) synapses of both the rod and the cone ON-pathways simultaneously. The ERG of hyperpolarizing-type patients is consistent with dysfunction involving only the photopic ON-pathway rather than simultaneously involving the scotopic ON-pathway as was suggested might be the basis for CSNB by Young[15] and Houchin and associates.[16]

The existence of both CSNB-type and hyperpolarizing-type pathology in human patients suggests that the two sign-inverting (–) synapses of the rod and cone ON-pathways (Fig 2) are not identical, since the scotopic rod b-wave appears affected to remarkably different degrees in CSNB versus hyperpolarizing ERG pattern patients. Physiologic single-cell studies of animal retina have not yet identified pharmacologic differences that distinguish between these two APB-sensitive glutamate synapses of the rod and cone ON-pathways (Fig 2). However, with the recent advances in identifying molecular diversity of the multiple glutamate-sensitive synapses,[102-104] it is reasonable to propose what is suggested by the long-flash ERG recordings from human dystrophy patients, namely that unique differences will ultimately be found between the rod- and cone-DBC synapses. Of course, this is speculation that must await future work.

However, they have a somewhat better long-term prognosis for retaining useful vision, albeit peripheral and mediated by rods, than do patients with rod-cone loss, many of whom eventually reach functional blindness. The proliferation of terms that have been applied to these patients attest to the continuing struggle to refine the clinical description and to subclassify cone-rod dystrophies. These terms include cone dystrophy,[110] cone degeneration,[111] cone dysfunction syndrome,[112,113] cone-rod dystrophy,[114] and ideopathic photoreceptor dysfunction of later adulthood.[79]

Our present finding from photopic long-flash ERG testing indicates that the group of cone-rod dystrophy patients is far from homogeneous and suggests that further classification along physiologic lines may be possible. Undoubtedly some of these patients suffer vision loss from retinal cone ON-pathway dysfunction. In some cases, including the CSNB patients, it is congenital whereas in others it is degenerative, such as in patients 9 through 11 with hyperpolarizing ERG pattern. Regarding future therapeutics, one can postulate that it might be more difficult to intervene in cases of acuity loss from cone photoreceptor death than in cases that potentially involve synaptic/neurotransmitter dysfunction.

If one can dare to speculate on basis of these few patients, we noted that a hyperpolarizing ERG pattern, putatively from ON-pathway abnormalities, predicted worse visual acuity than the depolarizing ERG pattern, which, on basis of the monkey drug studies, involved the OFF-pathway. This difference could be seen most clearly in patient 9, with the pronounced hyperpolarizing ERG pattern (Fig 25) and marked acuity loss, versus patient 16, with a pronounced depolarizing pattern (Fig 29) but relatively good acuity. Such cases suggest that the human cone DBC ON-pathway may be more critical than the OFF-pathway for processing of retinal visual acuity signals.

The abnormal OFF-waveform presents a further category with possible clinical value. Those patients who showed OFF-response late-negativity (Fig 31) all had clinical evidence of cone or cone-rod dystrophy. Similarily, the OFF-response late-negativity in Fig 18 occurred for patient 3 with cone-rod dystrophy. This pattern did not occur with patients having rod-cone dystrophies. Since Granit's PIII from the photoreceptors goes positive at the termination of a light stimulus,[35] this late-negativity must arise postsynaptic to the cones, possibly by a defect in the cone OFF-pathway, as mimicked by applying KYN to the monkey eye (Fig 3). This is a recognizable waveform category that may have diagnostic value. Further, this category suggests again that synaptic transmission abnormalities cause the vision limitation in some types of cone and cone-rod dystrophies.

SPECULATIONS AND FUTURE WORK

A New Model of Electronegative Photopic b-Wave

SB-type CSNB is thought to result from dysfunction of the sign-inverting (–) synapses of both the rod and cone DBC ON-pathway.[15,16] Schiller and co-workers[3,62,115] blocked these synapses in monkey with APB and reported the suppression of the photopic b-wave and a dramatic loss of sensitivity to light incremental steps but not to decremental steps. However, R. L. Purple, PhD (personal communication) reports that extensive and careful testing of a human (male) SB-type CSNB patient failed to find evidence for a selective ON-pathway visual deficit. This raises questions regarding the suggestion that the block of the sign-inverting (–) DBC synapse by APB truly mimics the pathology of CSNB.

If, however,as suggested by the findings in this present study, both DBCs and HBCs contribute to the photopic long-flash ERG, then an alternate possibility could explain the suppression of the photopic b-wave without an accompanying significant loss of photopic visual information. We have suggested that the b-wave results from subtractive interference between the DBC and HBC contributions.[26] As shown by the reconstructed monkey ERG waveforms in Fig 34, delaying the latency of DBC light response, but without eliminating synaptic transfer, creates an electronegative response as found for CSNB-type patients (Fig 23). The underlying waveforms to reconstruct the responses shown in Fig 34 were obtained from monkey ERGs by use of the drugs APB and KYN plus PDA to isolate the DBC and HBC components. Once isolated, these component responses were stored digitally and then added together with the required time delays imposed on the DBC waveform. This computer manipulation simulated a slowing but not blocking of ON-pathway signals reaching the DBCs from cones through the APB-sensitive synapse (Fig 2). Note that retarding the DBC signal by 2.5 to 5 msec in Fig 34 mimicked the photopic long-flash ERG findings in CSNB patients (Fig 23).

This model simulates photopic ERG effects caused by delayed rather than blocked transmission through the cone sign-inverting (–) ON-pathway synapse. The rationale for adding a time delay into the depolarizing cone pathway signal derived from noting that CSNB patients exhibited a clear delay of b-wave latency, which caused the broadened a-wave trough (Fig 23). Since the DBCs are thought to contribute the initial positive rise of the b-wave,[10,25,26,42,68] the mechanism of CSNB might be presumed to involve a slowing rather than a complete block of the APB-sensitive cone DBC synapse.

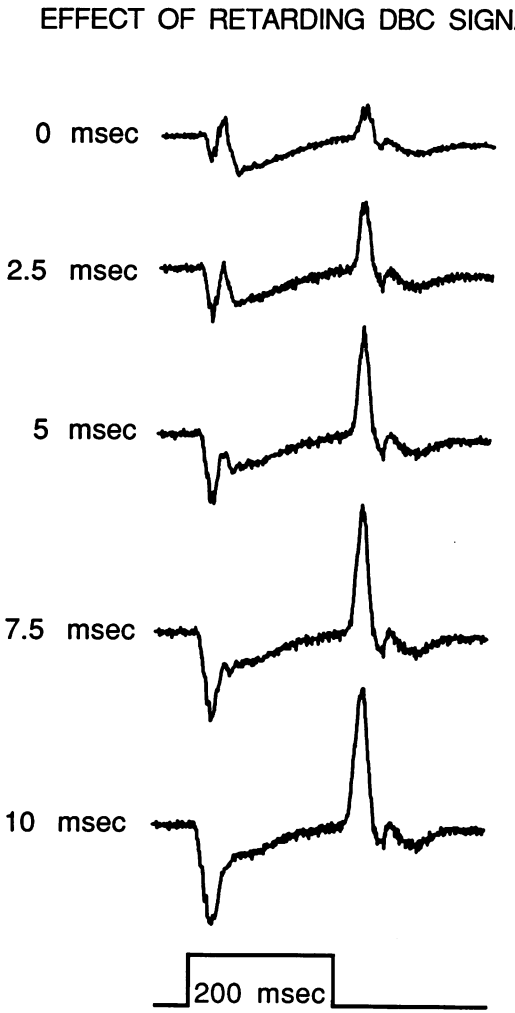

FIGURE 34

Reconstruction of monkey photopic ERG from components obtained pharmacologically shows that slight delay of depolarizing component produces a "hyperpolarizing pattern" as seen in several of the retinal dystrophy patients. "0 msec" waveform was reconstructed with no delay and has normal (b/a) and (b/d) ratios > 1.0. However, delays even as small as 2.5 msec cause these ratios to become < 1.0. The 5 msec delay causes double bump on a-wave trough that mimics result found for patient 11 with a "hyperpolarizing pattern." Enhanced d-wave in these reconstructed monkey ERG responses particularly mimic CSNB patient 5.

Stimulus Parameters

A cautionary note must by added regarding the conditions used in this paper to record the photopic ERG with long-flash stimuli. Future investigations of cone ON- and OFF-pathway activity by similar ERG recordings must contend with replicating appropriate recording conditions. Whereas the rod ERG is normally recorded under the single condition of full dark-adaptation, photopic ERG recordings have additional parameters with which to contend. Background brightness interacts with response amplitudes and timing characteristics of the photopic ERG.[110] Whereas the rod ERG can be studied with very brief flashes, stimulus duration has different effects on the various photopic ERG components.[116,117] Stimulus intensity is also important, since the relative contribution of the cone photoreceptors increases with brighter stimuli.[70] Dimmer photopic stimuli near photopic ERG threshold favor contributions from the second-order neurons and consequently allow for probing the ON-and OFF-pathways, as described for this paper. Stimuli much brighter than used in this study may be desirable for observing cones directly, utilizing the rapid-off effect.[31] Clearly, further work is required to complete our understanding of the cone ERG to long-flash stimuli under the broad range of stimulus conditions that are possible.

Further Questions Regarding Degeneration Retinae

One unresolved dilemma is whether these ERG waveform changes attributed to cone pathway dysfunction might be due to degeneration of the cones themselves. Basic information from physiologic single-cell studies is not yet available on this point.

Cellular electrophysiologic studies have begun to characterize synaptic input-output transfer properties between normal photoreceptors and their postsynaptic cells.[118-120] However, essentially nothing is known at this point about how degeneration of these cones would affect synaptic output.

Cones contact multiple second-order neurons of both the hyperpolarizing and depolarizing type through their respective sign-conserving (+) and sign-inverting (−) synapses (Fig 20).[1-3] One might speculate as to what could happen if these two synapse types were differentially impaired during cone degeneration. The sign-inverting (−) synapse onto DBCs has higher gain,[121] due to a G-protein coupled mechanism,[122] than does the sign-preserving (+) synapse onto HBCs.

This could mean that the ON-pathway synapses of degenerating cones, with their higher signal gain (which is localized in the postsynaptic G-protein mechanism and not in the presynaptic cone pedicle) might be degraded less by noise than the OFF-pathway signal, and consequently be less susceptible to loss of signal transfer causing a loss of visual sensitivity.[3-5]

Immediately, we must reiterate that this is entirely speculation in the absence of applicable physiologic single-cell studies, but it would appear to be important information for understanding vision loss in human retinal degenerative conditions. These ideas present hypotheses testable by single-cell physiologists.

CONCLUSION

In this study we were particularly interested in attempting to elucidate mechanisms of vision loss by attention to the cone ON- and OFF-pathways of the proximal retina. A central finding was that the photopic long-flash ERG waveforms from retinal dystrophy patients could be organized into several physiologically distinct categories. The five categories of waveform morphology suggested that unique physiologic differences are found among the retinal dystrophy patients. Some categories strongly suggested that their vision loss was attributable to pathology within the ON- or OFF-pathways and was not necessarily due to loss of cone photoreceptors themselves. These ideas may provide conceptually new approaches to subtyping the human retinal dystrophies on functional-physiologic principles, beyond current clinical-structural considerations such as "bull's-eye patterns." While much remains to be learned about recording the photopic long-flash ERG in a clinical setting, this study has demonstrated its feasibility on a large patient population.

REFERENCES

1. Falk G: Retinal physiology, in JR Heckenlively, GB Arden (eds): *Principles and Practice of Clinical Electrophysiology of Vision*. St Louis, CV Mosby, 1991, Chap 7, pp 69-84.
2. Famiglietti EV Jr, Kolb H: Structural basis for ON- and OFF-center responses in retinal ganglion cells. *Science* 1976; 194:193-195.
3. Schiller PH: The On and Off channels of the visual system, in B Cohen, I Bodis-Wollner (eds): *Vision and the Brain*. New York, Raven Press, 1990, pp 35-41.
4. Smith EL III, Harwerth RS, Crawford MLJ, et al: Contribution of the retinal ON channels to scotopic and photopic spectral sensitivity. *Vis Neurosci* 1989; 3:225-239.
5. Sperling HG, Mills SL: Red-green interactions in the spectral sensitivity of primates as derived from ERG and behavioral data. *Vis Neurosci* 1991; 7:75-86.
6. Arden GB: The electroretinogram in dominantly inherited retinitis pigmentosa, in R Clayton, J Haywood, HW Reading, et al (eds): *Problems of Normal and Genetically Abnormal Retinas*. New York, Academic Press, 1982, pp 349-351.
7. Szamier RB, Berson EL: Retinal ultrastructure in advanced retinitis pigmentosa. *Invest Ophthalmol* 1977; 16:947-962.
8. Dryja TP, McGee TL, Reichel E, et al: A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* 1990; 343:364-366.
9. Faber DS. Analysis of the slow transretinal potential in response to light. Buffalo, NY, State University of New York at Buffalo, 1969 [Dissertation].
10. Dick E, Miller RF: Light-evoked potassium activity in mudpuppy retina: Its relationship to the b-wave of the electroretinogram. *Brain Res* 1978; 154:388-394.

11. Ripps H, Brin KP, Weale RA: Rhodopsin and visual threshold in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1978; 17:735-745.
12. Carr RE: Congenital stationary nightblindness. *Trans Am Ophthalmol Soc* 1974; 72:448-486.
13. Ripps H: Night blindness revisited: From man to molecules. Proctor Lecture. *Invest Ophthalmol Vis Sci* 1982; 23:588-609.
14. Schubert G von, Bornschein H: Beitrag zur Analyse des menschlichen Elektroretinograms. *Ophthalmologica* 1952; 123:396-413.
15. Young RSL: Low-frequency component of the photopic ERG in patients with X-linked congenital stationary night blindness. *Clin Vis Sci* 1991; 4:309-315.
16. Houchin KW, Purple RL, Wirtschafter JD: X-linked congenital stationary night blindness and depolarizing bipolar system dysfunction. *Invest Ophthalmol Vis Sci* (ARVO abstract) 1991; 32:1229.
17. Alexander KR, Fishman GA, Peachey NS, et al: "On" response defect in paraneoplastic night blindness with cutaneous malignant melanoma. *Invest Ophthalmol Vis Sci* 1992; 33:477-483.
18. Yokoyama M, Ui K, Yoshida T: The spectral response in the retina with progressive cone dystrophy. *Jpn J Clin Ophthalmol* 1974; 28:805-813.
19. Penn RD, Hagins WA: Signal transmission along retinal rods and the origin of the electroretinogram a-wave. *Nature* 1969; 223:201-204.
20. Grey RHB, Blach RK, Barnard WM: Bull's eye maculopathy with early cone degeneration. *Br J Ophthalmol* 1977; 61:702-718.
21. Young R, Price J, Gorham N, et al: Selective abnormality of the cone b-wave in a patient with retinal degeneration. *Doc Ophthalmol* 1985; 211-218.
22. Perlman I: Relationship between the amplitudes of the b wave and the a wave as a useful index for evaluating the electroretinogram. *Br J Ophthalmol* 1983; 67:443-448.
23. Asi H, Perlman I: Relationships between the electroretinogram a-wave, b-wave and oscillatory potentials and their application to clinical diagnosis. *Doc Ophthalmol* 1992; 79:125-139.
24. Johnson MA, McPhee TJ: Electroretinographic findings in iris neovascularization due to acute central retinal vein occlusion. *Arch Ophthalmol* 1988; 106:348-352.
25. Stockton RA, Slaughter MM: B-wave of the electroretinogram: A reflection of ON bipolar cell activity. *J Gen Physiol* 1989; 93:101-122.
26. Sieving PA, Murayama K, Naarendorp F: Monkey cone ERG b-wave results from interaction of depolarizing and hyperpolarizing pathways. *Invest Ophthalmol Vis Sci* (ARVO abstract) 1991; 32:927.
27. Brucke ET, Garten S: Zur vergleichender Physiologie der Netzhautstrome. *Pflugers Arch Ges Physiol Menschen Tiere* 1907; 120:290-348.
28. Piper H: Ueber die Netzhautstrome. *Arch Anat Physiol Lpz* 1911; pp 85-132.
29. Chaffee EL, Sutcliffe E: The differences in electrical response of the retina of the frog and horned toad according to the position of the electrodes. *Am J Physiol* 1930; 95:250-261.
30. Heck J: The flicker electroretinogram of the human eye. *Acta Physiol Scand* 1957; 39:158-166.
31. Kawasaki K, Tsuchida Y, Jacobson JH: Positive and negative deflections in the OFF response of the electroretinogram in man. *Am J Ophthalmol* 1971; 72:367-365.
32. Tamsley K, Copenhaver RM, Gunkel RD: Some observations on the off-effect of the mammalian cone electroretinogram. *J Opt Soc Am* 1961; 51:207-213.
33. Howarth CI: On-Off interaction in the human electroretinogram. *J Opt Soc Am* 1961; 51:345-352.
34. Berson EL: Electrical phenomena in the retina, in WJ Hart Jr (ed): *Adler's Physiology of the Eye: Clinical Application*. Nineth edition. St Louis, CV Mosby, 1992, pp 641-707.
35. Granit G: *Sensory Mechanisms of the Retina*. London, Oxford University Press, 1947.
36. Nagata M: Studies on the photopic ERG of the human retina. *Jpn J Ophthalmol* 1963; 7:96-124.

37. Peachey NS, Alexander KR, Fishman GA, et al: Properties of the human cone system electroretinogram during light adaptation. *Appl Optics* 1989; 28:1145-1150.
38. Murayama K, Sieving PA: Different rates of growth of human and monkey photopic ERG suggests two sites of light adaptation. *Clin Vis Sci* 1992; 7:385-392.
39. Sterling P, Freed M, Smith RG: Microcircuitry and functional architecture of the cat retina. *Trends Neurosci* 1986; 9:186-192.
40. Ramon y Cajal S: *Recollections of My Life*. Philadelphia, American Philosophical Society, 1937.
41. Wassle H, Boycott BB: Functional architecture of the mammalian retina. *Physiol Rev* 1991; 71:447-480.
42. Slaughter MM, Miller RF: 2-amino-4-phosphonobutyric acid: A new pharmacological tool for retina research. *Science* 1981; 211:182-185.
43. Slaughter MM, Miller RF: Bipolar cells in the mudpuppy retina use an excitatory amino acid neurotransmitter. *Nature* 1983; 303:537-538.
44. Slaughter MM, Miller RF: An excitatory amino acid antagonist blocks cone input to sign-conserving second-order retinal neurons. *Science* 1983; 219:1230-1232.
45. Miyake Y, Yagasaki K, Horiguchi M, et al: On- and Off-responses in photopic electroretinogram in complete and incomplete types of congenital stationary night blindness. *Jpn J Ophthalmol* 1987; 31:81-87.
46. Miyake Y, Yagasaki K, Horiguchi M, et al: Congenital stationary night blindness with negative electroretinogram: A new classification. *Arch Ophthalmol* 1986; 104:1013-1020.
47. Granit R: Two types of retinas and their electrical responses to intermittent stimuli in light and dark adaptation. *J Physiol* 1935; 85:421-438.
48. Best W, Bohnen K: Untersuchungen uber das Elektroretinogramm des Menschen bei Verwendung farbiger Lichtreize, in H Sautter, W Straub (eds): *Electroretinographie, Hamburger Symposium 1956; Bibliotheca Ophthalmologica*. New York, S Karger, 1957, pp 77-86.
49. Yonemura D, Kawasaki K: Electrophysiological study on activities of neuronal and non-neuronal retinal elements in man with reference to its clinical application. *Jpn J Ophthalmol* 1978; 22:195-213.
50. Yonemura D, Kawasaki K: New approaches to ophthalmic electrodiagnosis by retinal oscillatory potential, drug-induced responses from retinal pigment epithelium and cone potentials. *Doc Ophthalmol* 1979; 48:163-222.
51. Yonemura D, Kawasaki K, Shibata N, et al: The electroretinographic PIII component of the human excised retina. *Jpn J Ophthalmol* 1974; 18:322-333.
52. Nakazato H, Kawasaki K, Yonemura D, et al: Objective examination of genetic carrier of congenital red-green color blindness by electroretinography. *Acta Soc Ophthalmol Jpn* 1985; 89:548-555.
53. Nakazato H, Hanazaki H, Kawasaki K, et al: Electroretinographic OFF-response in congenital red-green color deficiency and its genetic carrier. *Doc Ophthalmol* 1986; 63:179-186.
54. Hanazaki H, Nakazato H, Tanabe H, et al: The ERG rapid OFF-response in different grades of abnormality in congenital red-green color deficiency. *Acta Soc Ophthalmol Jpn* 1987; 91:860-864.
55. Hanazaki H, Tanabe J, Kawasaki K, et al: Electrophysiological study of the pigmentfarbenamblyopie. *Doc Ophthalmol* 1987; 66:227-232.
56. Kawasaki K, Nakazato H, Hanazaki H, et al: Objective diagnosis of congenital red-green color deficiency on the basis of the electroretinographic rapid OFF-response. *Folia Ophthalmol Jpn* 1988; 39:1501-1511.
57. Hanazaki H, Saitoh Y, Nakazato H, et al: The ERG rapid OFF-response in cases of congenital red-green color deficiency erroneously classified with pseudoisochromatic plates. *Acta Soc Ophthalmol Jpn* 1988, 92:658-662.
58. Krill AE, Martin D: Photopic abnormalities in congenital stationary nightblindness. *Invest Ophthalmol* 1971; 10:625-636.

59. Heckenlively J, Martin D, Rosenbaum AL: Loss of electroretinographic oscillatory potentials, optic atrophy, and dysplasia in congenital stationary night blindness. *Am J Ophthalmol* 1983; 96:526-534.
60. Lachapelle P, Little J, Polomeno R: The photopic electroretinogram in congenital stationary night blindness with myopia. *Invest Ophthalmol Vis Sci* 1983; 24:442-450.
61. Heynen H, Wachtmeister L, van Norren DF: Origin of the oscillatory potentials in the primate retina. *Vision Res* 1985; 10:1365-1373.
62. Knapp AG, Schiller PH: The contribution of ON- bipolar cells to the electroretinogram of rabbits and monkeys: A study using 2-amino-4-phosphonobutyric (APB). *Vision Res* 1984; 24:1841-1846.
63. Evers HU, Gouras P: Three cone mechanisms in the primate electroretinogram: Two with, one without OFF-center bipolar responses. *Vision Res* 1986; 26:245-254.
64. Granit R: The components of the retinal action potential and their relation to the discharge in the optic nerve. *J Physiol* 1933; 77:207-240.
65. Brown KT, Watanabe K: Isolation and identification of a receptor potential from the pure cone fovea on the monkey retina. *J Physiol* 1962; 158:257-280.
66. Newman EA, Odette LL: Model of electroretinogram b-wave generation. *J Neurophysiol* 1984; 51:164-182.
67. Miller RF, Dowling JE: Intracellular responses of the Müller (glial) cells of mudpuppy retina: Their relation to b-wave of the electroretinogram. *J Neurophysiol* 1970; 33:323-341.
68. Porciatti V, Bagnoli P, Alesci R: ON and OFF activity in the retinal and tectal response to focal stimulation with uniformed or patterned stimuli. *Clin Vis Sci* 1987; 2:93-102.
69. Naarendorp F, Sieving PA: The scotopic threshold response of the cat ERG is suppressed selectively by GABA and glycine. *Vision Res* 1991; 31:1-15.
70. Bush R, Sieving PA: Do photoreceptors alone contribute to the primate photopic ERG a-wave? *Invest Ophthalmol Vis Sci* (ARVO abstract) 1992; 33:836.
71. Marmor MF, Arden GB, Nilsson SEG, et al: The International Standardization Committee: Standards for clinical electroretinography. *Arch Ophthalmol* 1989; 107:816-819.
72. Westheimer G: The Maxwellian view. *Vision Res* 1966; 6:669-682.
73. Osterberg G: Topography of the layers of rods and cones in the vertebrate retina. *Acta Ophthalmol* (Suppl) 1935; 13:6.
74. Hogg C: The use of light-emitting diodes in electrophysiology and psychophysics, in JR Heckenlively, GB Arden (eds): *Principles and Practice of Clinical Electrophysiology of Vision*. St Louis, CV Mosby, 1991, Chap 28, pp 221-227.
75. Wakabayashi K, Gieser J, Sieving PA: Aspartate separation of the Scotopic Threshold Response (STR) from the photoreceptor a-wave of the cat and monkey ERG. *Invest Ophthalmol Vis Sci* 1988; 29:1615-1622.
76. Sieving PA, Nino C: Scotopic threshold response (STR) of the human electroretinogram. *Invest Ophthalmol Vis Sci* 1988; 29:1608-1614.
77. Wyszecki G, Stiles WS: *Color Science*. New York, John Wiley & Sons, Inc, 1967.
78. Aguilar M, Stiles WS: Saturation of the rod mechanism of the retina at high levels of stimulation. *Optica Acta* 1954; 1:59-65.
79. Rowe, SE, Trobe JD, Sieving PA: Idiopathic photoreceptor dysfunction causes unexplained visual acuity loss in later adulthood. *Ophthalmology* 1990; 97:1632-1637.
80. Khani-Oskouee K, Sieving PA: A digital band-pass filter for electrophysiology recording systems, in JR Heckenlively, GB Arden (eds): *Principles and Practice of Clinical Electrophysiology of Vision*. St Louis, CV Mosby, 1991, Chap 26, pp 205-210.
81. Taumer R, Rohde N, Wichmann W, et al: A method for DC-ERG recording of alert humans. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1976; 198:45-55.
82. Kojima M, Zrenner E: Off-components in response to brief light flashes in the oscillatory potential of the human electroretinogram. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1978; 206:107-120.

83. Lachapelle P: Analysis of the photopic electroretinogram recorded before and after dark adaptation. *Can J Ophthalmol* 1987; 22:354-361.
84. Weleber RG: The effect of age on human cone and rod Ganzfeld electroretinograms. *Invest Ophthalmol Vis Sci* 1981; 20:392-399.
85. Farnsworth D: *The Farnsworth Dichromatous Test for Color Blindness: Panel D-15.* New York, Psychological Corporation, 1947.
86. Weleber RG: Retinitis pigmentosa and allied disorders, in SJ Ryan (ed): *Retina,* vol 1. St Louis, CV Mosby, 1989, Chap 20, pp 299-420.
87. Falls HF, Wolter JR, Alpern M: Typical total monochromacy. *Arch Ophthalmol* 1965; 74:610-616.
88. Brown KT, Watanabe K: Isolation and identification of a receptor potential from the pure cone fovea of the monkey retina. *Nature* 1964; 193:958-960.
89. Baron WS, Boynton RM: The primate foveal local electroretinogram: An indicator of photoreceptor activity. *Vision Res* 1974; 14:495-501.
90. Birch DG: Clinical electrophysiology. *Ophthalmol Clin North Am* 1989; 2:469-497.
91. Fishman GA: Basic principles of clinical electrophysiology. *Retina* 1985; 5:123-126.
92. Iijima H, Martin DA, Heckenlively JR: Autosomal dominant retinitis pigmentosa: A log quotient analysis of the photopic and scotopic b-wave amplitude. *Br J Ophthalmol* 1989; 73:337-341.
93. Johnson MA: Use of electroretinographic ratios in assessment of vascular occlusion and ischemia, in JR Heckenlively, GB Arden (eds): *Principles and Practice of Clinical Electrophysiology of Vision.* St Louis, CV Mosby, 1991, Chap 80, pp 613-618
94. Hirose T, Wolf E, Hara A: Electrophysiological and psychophysical studies in congenital retinoschisis of X-linked recessive inheritance, in T Lawwill (ed): *ERG, VER and Psychophysics.* (14th ISCERG Symposium, Louisville, KY, 1976.) The Hague, Dr W Junk, 1977, pp 173-184. (Doc Ophthalmol Proc Ser; 13).
95. Fishman GA: Fundus flavimaculatus: A clinical classification. *Arch Ophthalmol* 1976; 94:2061-2067.
96. Fish G, Grey R, Sehmi KS, et al: The dark choroid in posterior retinal dystrophies. *Br J Ophthalmol* 1981; 65:359-363.
97. Richards JE, Kuo C-Y, Boehnke M, et al: Rhodopsin Thr58Arg mutation in a family with autosomal dominant retinitis pigmentosa. *Ophthalmology* 1991; 98:1797-1805.
98. Wakabayashi K: Electrodiagnosis of primary macular dystrophies. *J Juzen Med Soc* [Kanazawa Daigaku Igakka Zasshi] 1986; 96:399-439.
99. Brigell M, Celesia GG: Electrophysiological evaluation of the neuro-ophthalmology patient: An algorithm for clinical use. *Semin Ophthalmol* 1992; 7:65-78.
100. Zervas JP, Smith JL: Neuro-ophthalmic presentation of cone dysfunction syndromes in the adult. *J Clin Neuro-Ophthalmol* 1987; 7:202-218.
101. Foerster MH: The significance of the DC-ERG in hereditary retinal disease, in T Lawwill (ed): *ERG, VER and Psychophysics.* (14th ISCERG Symposium, Louisville, KY, 1976.) The Hague, Dr W Junk, 1977, pp 185-193 (Doc Ophthalmol Proc Ser; 13).
102. Meguro H, Mori H, Araki K: Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature* 1992; 357:70-74.
103. Kutsuwada T, Kashiwabuchi N, Mori H: Molecular diversity of the NMDA receptor channel. *Nature* 1992; 358:36-41.
104. Gasic GP, Hollmann M: Molecular neurobiology of glutamate receptors. Molecular neurobiology of glutamate receptors. *Nature* 1992; 54:507-536.
105. Karpe G: The basis of clinical electroretinography. *Acta Ophthalmol* (Suppl) 1945; 24:1-118.
106. Young RSL: Low-frequency component of the photopic ERG in patients with X-linked congenital stationary night blindness. *Clin Vis Sci* 1991; 6:309-315.
107. Kellner U, Brummer S, Foerster MH, et al: X-linked congenital retinoschisis. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1990; 228:432-439.
108. Murayama K, Kuo C-Y, Sieving PA: Abnormal threshold ERG response in X-linked juvenile retinoschisis: Evidence for a proximal retinal origin for the human STR. *Clin Vis Sci* 1991; 6:317-322.

109. Heckenlively JR: RP cone-rod degeneration. *Trans Am Ophthalmol Soc* 1987; 85:438-470.
110. Francois J, DeRouck A, DeLaey JJ: Progressive cone dystrophies. *Ophthalmologica* 1976; 173:81-101.
111. Krill AE, Deutman AF, Fishman M: The cone degeneration. *Doc Ophthalmol* 1973; 35:1-80.
112. Francois J, DeRouck A, DeLaey JJ, et al: Progressive generalized cone dysfunction. *Ophthalmologica* 1974; 169:255-284.
113. Goodman G, Ripps H, Siegel IM: Cone dysfunction syndromes. *Arch Ophthalmol* 1963; 70:214-231.
114. Rabb MF, Tso MOM, Fishman GA: Cone-rod dystrophy: A clinical and histopathologic report. *Ophthalmology* 1986; 93:1443-1451.
115. Dolan RP, Schiller PH: Evidence for only depolarizing rod bipolar cells in the primate retina. *Vis Neurosci* 1989; 2:421-424.
116. Heck J: Der Off-effect im menschlichen Elektroretinogramm. *Acta Physiol Scand* 1957; 40:113-120.
117. Armington JC: *The Electroretinogram.* New York, Academic Press 1974, p 262.
118. Baylor DA and Fettiplace R: Transmission from photoreceptors to ganglion cells in turtle retina. *J Physiol (Lond)* 1977; 271:425-448.
119. Nawy S, Copenhagen DR: Multiple classes of glutamate receptor on depolarizing bipolar cells in retina. *Nature* 1987; 325:56-58.
120. Jardon B, Yucel H, Bonaventure N: Glutamatergic separation of ON and OFF retinal channels: Possible modulation by glycine and acetylcholine. *Eur J Pharmacol* 1989; 162:215-224.
121. Copenhagen DR, Ashmore JF, Schnapf JK: Kinetics of synaptic transmission from photoreceptors to horizontal and bipolar cells in turtle retina. *Vision Res* 1983; 23:363-369.
122. Shiells RA, Falk G: Glutamate receptors of rod bipolar cells are linked to a cyclic GMP cascade via a G-protein. *Proc R Soc Lond* [Biol] 1990; 242:91-94.