The short wavelength sensitive (S) cones represent a minority of the cone photoreceptors in the human retina, about 10% of the 7–8 million cones. There is a long history indicating that S-cones are more vulnerable to damage by certain retinal diseases than L and M-cones, which gives S-cone function unique clinical relevance. The traditional methods used to assess S-cone function have been subjective, either by the use of pseudoisochromatic color plates, color matching, or increment threshold techniques. There have been several reports that the human electroretinogram (ERG) of the S-cones can be detected at the cornea, but this S-cone ERG has never been standardized for age and interindividual variability or methodology. We have previously reported a method to detect this response that can be used with some modifications in most clinical ERG systems. We now report the range of amplitudes and implicit times of the S-cone ERG using this method in a group of normal subjects of different ages. To clarify the ERG components that are relevant to the S-cone and not L and M cones, we have also examined the ERG of an S-cone achromat.

MATERIALS AND METHODS

The method used to record the human ERG has been described previously. The ERG is recorded from both eyes simultaneously as the subject sits with his head on a chin rest and gazes at a point of fixation on the inner surface of a matte white Ganzfeld. The subject's pupils are dilated, usually to a diameter of 8 mm. Pupil size is checked before and after the test to express retinal illumination in trolands. The ERG is produced by a strobe that has been placed into a metal box mounted on the Ganzfeld above the head of the subject and diffused homogeneously over the interior of the Ganzfeld. The spectral characteristics of this flash can be changed by interposing 7.5 × 7.5 cm filters (Wratten, Rochester, NY) 36 (410 nm), 98 (450 nm), 48 (471 nm), 75 (488 nm), 61 (534 nm), 21 (593 nm), and 29 (633 nm). The energy in the flash can be changed by neutral density filters, usually in 0.1 density units. The
flashes are presented on a steady white adapting field of 17,000 photopic trolands.

The ERGs were recorded with a Nicolet (Madison, WI) CA 1000 signal averager at a sensitivity of 10–25 μV/cm and with an artifact rejection window. The frequency response was 5–1500 Hz, but this did not completely eliminate large responses of slightly lower frequency from being detected. About 500–1000 responses were averaged; it usually took about 3–4 minutes for a particular response. The eyes of 84 subjects were studied. Each patient had a complete ophthalmologic examination before the ERG test, to establish that their eye(s) were normal. This research followed the tenets of the Declaration of Helsinki. Informed consent was obtained after the nature and possible consequences of the study were explained, and the research was approved by our institutional review board on human investigation.

In general, the data from both eyes were averaged to obtain results for a particular subject. In some patients, one eye was aphakic and the other was not; in these patients, the normal eye was treated separately. The S-cone achromat, from a 25-year-old man, has been described by us in previous publications. To determine the difference in relative light intensity required to match the L and M cone b-wave produced by the blue (450 nm) flash to that produced by the red (633 nm) flash, we determined the relationship between L–M cone b-wave amplitude and light intensity for the red flash and then graphically determined what relative light intensity produced an equal amplitude L–M cone b-wave for the blue (450 nm) flash. At this amplitude match, the L–M cone b-wave implicit times invariably matched. For action spectra based on a similar constant response criterion, which was only done on seven subjects, intensity amplitude function was determined for all spectral stimuli. For illustrative purposes, we obtained permanent recordings from our subjects using the maximum light intensities available at 410 and 450 nm and dimming the other stimuli by an estimated amount, because all longer wavelength stimuli had more effective energy. Sometimes our estimates were less accurate at one or two wavelengths, producing an L–M cone ERG mismatch, but this had no influence on the quantitative measurements.

RESULTS

The S-cone ERG, elicited by short (410, 450, and 471 nm) wavelength flashes, appears as a separate b-wave riding on an earlier b-wave of the L and M cone ERG (Fig. 1). Light adaptation exposes this S-cone response by saturating rod responses and light-adapting the L and M cone responses. Light-adaptation reduces the amplitude and speeds up the L and M-cone response more than the S-cone response. We measured the S-cone b-wave response from its initial appearance, after the peak of the L and M cone b-wave, to its own peak for the 450 nm stimulus, as illustrated in Figure 1.

We determined the action spectrum based on equal response criteria for both the early (L and M-cone) and later (S-cone) b-waves in six normal subjects.
and in an S-cone achromat (Fig. 2). The earlier b-wave (filled circles) has its peak sensitivity to the 550 nm flash, which is consistent with an L and M cone origin. The sensitivity of this L and M cone response is relatively low at the short wavelength region of the spectrum. The second b-wave (open circles) includes the S-cone response and a small oscillation (labeled 2 in Fig. 1, bottom trace), which is produced by longer wavelength stimuli. Some patients have not one, but two, late oscillations (2 and 3, Fig. 1, bottom trace). The action spectrum of these later positive waves (Fig. 2) shows that the short wavelength-sensitive one has its peak sensitivity at 450 nm and the longer wavelength-sensitive one (the oscillation) is identical to the spectral sensitivity of the early L and M cone b-wave. The S-cone achromat (open squares) has a peak sensitivity at 450 as well. There are other properties that distinguish the S-cone b-wave from this L and M cone b-wave oscillation. The S-cone b-wave is followed by a negative wave, which is absent at longer wavelengths that generate the L and M cone b-wave oscillations (top 3 traces, Figs. 1).

The S-cone ERG of the S-cone achromat (Fig. 3) demonstrates that all responses elicited by wavelengths at 534 nm or longer must be from L and M cones because they are absent from this ERG even though the longer wavelength stimuli used are about 10 times stronger than those used in normal subjects (Figs. 1). The cone ERG of the S-cone achromat is virtually identical in waveform (Fig. 3) and action spectrum (Fig. 2) to the S-cone ERG of normal subjects. Both have S-cone b-waves of similar amplitude and implicit time, and both show a negativity after the b-wave response.

Because L and M-cones do not contribute to the ERG of the S-cone achromat, it is possible to examine the S-cone ERG in such a subject over a larger dynamic range using white instead of the less energetic light.
short wavelength flashes required for normal subjects. Figure 4 illustrates the S-cone ERG of the S-cone achromat when tested with high-intensity white light. This reveals that even larger S-cone ERGs can be obtained with stronger stimuli, although there is a suggestion of saturation to the strongest flash (Fig. 4, upper trace). Even with the strongest stimuli, the implicit time of the S-cone b-wave remains later than that of the L and M cone b-wave in normals. With the stronger stimuli, an initial a-wave (Fig. 4, arrow) can be seen in the S-cone ERG that is not detectable with the weaker chromatic stimuli. In addition to the late negativity, a large positive wave appears with an implicit time of about 100 msec. This is a repeatable response in the S-cone achromat’s ERG; a suggestion of a late positive wave after the late negativity is also seen in a normal subject’s S-cone ERG (Fig. 1).

The amplitude of the S-cone b-wave decreases progressively with age (Fig. 5), and this is accompanied by an increase in implicit time (Table 1). Some of this change with age may be caused by the properties of the diopteric media because the ratio of long to short wavelength filtering required to produce identical L–M cone b-waves increases progressively with age, implying a progressive loss in short wavelength transmission with age. This is supported by the fact that average amplitude and implicit time of the S-cone ERGs of aphakic subjects are less affected by age (Table 1).

Table 1 breaks down these changes into three different age groups of normal subjects and the aphakic subjects, showing averages and standard deviations of the mean. The S-cone ERG amplitudes of the young (11–29 yrs) and middle-aged (30–59 yrs) subjects are significantly different from each other and from the older subjects (60–83 yrs) at the 1% ($P = 0.01$) level using the Student t-test comparison. The amplitudes of the S-cone ERG of aphakics are almost significantly different than those of older subjects at the 5% ($P = 0.05$) level.

**DISCUSSION**

The major reason this unique ERG response to blue light is considered to be caused by S-cones is its action spectrum, which has a relatively narrow peak sensitivity in the blue region of the spectrum. A second reason is that an S-cone achromat has exactly the same response under the same conditions of light adaptation that saturate the rod ERG. Another reason is the absence of this response in a well-defined tritanope. We have not had the opportunity of examining such a subject, but two other groups have. One group, using methods that are exactly the same as ours, has exam-
TABLE 1. S-Cone Electroretinogram B-Wave

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of Subjects</th>
<th>Amplitude (µV)</th>
<th>Implicit Time (ms)</th>
<th>LM Cone Balance (Density Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>11–29</td>
<td>19</td>
<td>2.15</td>
<td>0.99</td>
<td>38.0</td>
</tr>
<tr>
<td>30–59</td>
<td>40</td>
<td>1.42</td>
<td>0.78</td>
<td>39.6</td>
</tr>
<tr>
<td>60–83</td>
<td>20</td>
<td>0.61</td>
<td>0.45</td>
<td>44.8</td>
</tr>
<tr>
<td>Aphakic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58–79</td>
<td>5</td>
<td>1.17</td>
<td>0.55</td>
<td>40.7</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>1.38</td>
<td>0.91</td>
<td>40.5</td>
</tr>
</tbody>
</table>

SD, standard deviation.

ined two subjects heterozygous for a defect in the S-cone opsin gene on chromosome 7. For both, this S-cone ERG was reduced but not absent. A second group using a similar method examined two subjects homozygous for such a gene defect and found absent S-cone ERG. Therefore, the evidence is substantial that this blue-sensitive ERG response obtained in the presence of a strong adapting field is the result of activity of the S-cone system. Because there is significant interindividual variability in these responses, it is difficult to rule out some L–M cone contribution to the particular S-cone b-wave we have measured.

In normal subjects, the optimal blue light stimulus for producing an S-cone ERG also produces an early small ERG from the L and M cone system. Recently, the pattern ERG (PERG) of the S-cone system has also been identified using selective chromatic (yellow) adaptation and blue pattern stimuli. Even in the PERG, a contribution from the L and M cones was present under optimum conditions for isolating the S-cones. It is not surprising that a blue light flash elicits a response from all three cone systems because all cones absorb light in this region of the spectrum. There is an advantage in having both L–M and S-cone ERGs identifiable and measurable on the same trace because it allows distinguishing abnormalities that affect certain or all cone mechanisms at the same time.

Our S-cone b-wave implicit times, ranging from 38–45 msec, are shorter than those reported by some other investigators. In particular, Sawusch et al. reported S-cone b-wave implicit times of 60–70 msec. They used a silent substitution method to detect the S-cone ERG that may integrate slower components of the S-cone system response, as revealed in the ERG to white light of the S-cone achromat (Fig. 4).

An important factor in standardizing the human S-cone ERG is the age of the patient because there is a progressive increase in short-wave absorption by the aging lens. This is a much greater factor for the S than the L–M cone ERG. The latter is less influenced by short wave light because the maximum absorption of the L and M cones occurs in the yellow region of the spectrum. Our results suggest a means to distinguish whether a reduction in an S-cone ERG results from a spectral change in the media, in particular lens senescence, or to a change in the S-cone response.

The S-cone system in the primate retina is unusual because it transmits its signals to a unique set of retinal ganglion cells, undoubtedly through a subset of S-cone bipolar cells. S-cones do not appear to influence the more numerous bipolars and ganglion cells that subserve L and M cones because most primate retinal ganglion cells have no S-cone input. L and M cones invariably antagonize the S-cone signals that reach S-cone-subsuming ganglion cells. This antagonism must occur at the external or the internal plexiform layer, or both. All the antagonism at the outer and some antagonism at the inner plexiform layer must influence these S-cone bipolars and, consequently, the b-wave. Nevertheless, the S-cone b-wave appears to be merely added to the L and M cone b-wave and not obviously influenced (suppressed) by the earlier L and M cone responses. Perhaps the flash is too brief to influence strongly the antagonistic channels of the horizontal and amacrine cells that mediate this antagonism from L and M cones to the S-cone pathway in the retina, or perhaps some of the antagonism is exerted directly on the ganglion cells, which make little or no contribution to the flash ERG.

This point is also relevant to a syndrome that has been described in subjects who have cone and rod dysfunction but generate a large ERG response to short wavelength stimuli, which could be a supernormal response of S-cones. The fact that an S-cone achromat does not have a supernormal S-cone ERG indicates that reduction of L and M cone function does not lead to a supernormal S-cone ERG.

There are diseases, such as diabetic retinopathy and glaucoma, in which defects in blue-yellow color vision are common and may indeed be early indicators of these diseases. Part of this S-cone abnormality may be from inner retinal disease, which may not influ-
ence the flash ERG but may influence the PERG. Never-
theless, an objective test of S-cone function that
monitors the outer layers may be a useful adjunct to
the examination of these diseases, although one must
be cognizant of considerable intersubject variability.

Key Words
S-cones, electroretinogram, S-cone achromat

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References
1. Padmos P, van Norren D, Jaspers Faijer JW. Blue
cone function in a family with an inherited tritan de-
cfect tested with electroretinography and psychophys-
2. Sawusch M, Pokorny J, Smith VC. Clinical electroreti-
nography for short wavelength sensitive cones. Invest
3. Miyake Y, Yagasaki K, Ichikawa H. Differential diag-
nosis of congenital tritanopia and dominantly in-
4. Gouras P, MacKay CJ. Electroretinographic re-
sponses of the short-wavelength-sensitive cones. Invest
5. Gouras P, MacKay CJ, Lewis AL. The blue cone elec-
 troretinogram isolated in a sex-linked monochromat.
In: Drum B, Verriest G, eds. Colour Vision Deficien-
cies. Dordrecht, Netherlands: Kluwer Academic Pub-
6. Bailey JE, Montag E. Short wavelength sensitive cone
system function in hereditary tritanopia. Invest Oph-
7. Korth M, Nguyen NX, Rix R, Sembritzki O. R-G and
B-mechanisms in PERG and their spatial selectivities.
8. Marmor MF, Jacobson SG, Foerster MH, Kellner U,
Weteber RG. Diagnostic clinical findings of a new syn-
drome with night blindness maculopathy and en-
124–134.
sensitivity and color discrimination changes in glau-
coma and glaucoma-suspected patients. Invest Ophthal-
10. Greenstein VC, Hood GC, Ritch R, Steinberger D, Carr RE. S (blue) cone pathway vulnerability in retini-
tis pigmentosa diabetes and glaucoma. Invest Ophthal-