Functional Damage to Inner and Outer Retinal Cells in Experimental Glaucoma

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PURPOSE. To investigate the cellular sources underlying the functional damage observed by multifocal electroretinography (mFERG) responses of glaucomatous eyes of monkeys.

METHODS. First- and second-order (K1 and K2, respectively) mFERG responses of three normal and three experimentally induced glaucomatous eyes of cynomolgus monkeys were measured at two different levels of luminance. Retinal contributors to the responses were isolated by intravitreal injections of pharmacological agents that suppress specific retinal cells. γ-Aminobutyric acid (GABA) and glycine were administered to block inner retinal function, followed by 2-amino-4-phosphonobutyric acid (APB), to block ON-bipolar cells.

RESULTS. An inner retinal component removed by GABA and glycine was found in both the normal and glaucomatous eyes. However, it was attenuated in the latter, correlating with changes observed in the baseline K1 responses. Delays in the latency of outer retinal components were found in the responses of the glaucomatous eyes. K2 responses were dominated by an inner retinal contribution and were diminished in the responses of glaucomatous eyes. The outer retina responded to increased luminance with a shorter implicit time. A distinct wave part of the inner retinal component responded to increased luminance with increased amplitudes.

CONCLUSIONS. The integration of the retinal sources forming the mFERG response was compared between normal and glaucomatous monkey eyes. Both inner and outer retinal functions were aberrant in the responses of the glaucomatous eyes, with the attenuation of the inner retinal function more conspicuous. Nevertheless, glaucomatous eyes retained certain inner retinal activity, despite the advanced stage of disease. K2 responses were more sensitive to glaucomatous changes than were K1 responses. (Invest Ophthalmol Vis Sci. 2003;44:3675–3684) DOI:10.1167/iovs.02-1236

Glaucoma is a sight-threatening disease of multifactorial etiology affecting more than 65 million people worldwide.1 It is a progressive optic neuropathy that may often go undiagnosed due to the slow decline in vision. It has been estimated that up to 50% of the nerve fibers may be lost before obvious clinical symptoms.2,3 However, because the damage is irreversible, diagnosis and treatment early in the course of the disease are crucial.

Retinal function can be evaluated objectively by electroretinography (ERG). Early detection of pathologic processes and their localization may be facilitated by choosing the stimulation and recording conditions. Evidence of glaucomatous damage encompassing inner and outer retinal layers has been found in both flash and pattern ERG studies.4–10 Latency increases and amplitude decreases in pattern ERG studies were found suitable to distinguish between control and glaucomatous eyes, and also for detecting ocular hypertension.6,9,10 Flash ERG studies have shown reduced amplitudes and delayed responses in glaucoma,11–14 a photopic negative response sensitive to early stages of damage,15 and diminished oscillatory potentials.16 However, these tests are sensitive to widespread retinal damage. The multifocal electroretinography (mFERG) technique17 generates high-resolution maps of discrete responses across the central and near-peripheral retina. It may thus be advantageous in the diagnosis and monitoring of glaucoma, which can affect the retina unevenly.15–17 Recent mFERG studies showed clear evidence of functional loss in glaucoma,18 suggesting damage to inner and outer retinal layers.16–21 These studies showed attenuation of the first- and second-order (K1 and K2, respectively) responses in glaucoma,16–19,21 and the elimination of an optic nerve head component.20 However, injury to specific retinal pathways was not established.

Pharmacological agents that selectively block retinal activity have been used to advance the understanding of the cellular contributions to ERG responses.22–25 The origin of the mFERG response has been studied,20,26–28 but only recently have the underlying sources of the mFERG response been explicitly explored.29,30 Using pharmacological agents that block specific cell types, Hood et al.29 presented a model of the precise retinal sources that constitute the mFERG response in primates. The model suggests that the bipolar cells dominate the mFERG response, with the inner retina and photoreceptors adding smaller contributions to the waveform. The purpose of this study was to investigate the cellular sources that underlie the functional damage observed in mFERG responses of glaucomatous eyes, by comparing the pharmacological dissection of the responses of normal and glaucomatous eyes.

METHODS

Preparation of Experimental Animals

Three normal and three glaucomatous eyes of six adult male cynomolgus monkeys (Macaca fascicularis) were used in this study. Ocular hypertension was induced by photocoagulation of the trabecular meshwork 7 years before the experiment.31,32 An argon laser beam (75–100 μm in diameter, 1100 mW, for 0.5 second) was applied to the entire trabeculum.

Intraocular pressure was measured with a calibrated tonometer (Tono-Pen XL; Mentor Ophthalmics, Inc., Norwell, MA) after intramuscular premedication with ketamine (10 mg/kg). During the mFERG...
recordings monkeys were anesthetized with an inhalation mixture of N₂O and O₂ supplemented with a continuous intravenous (IV) drip of propofol (5 mg/kg per hour). Eye movement was prevented by periodic IV boluses of muscle relaxants (vecuronium 0.1 mg/kg or rocuronium 0.45 mg/kg). Expired CO₂, blood O₂ saturation level, heart rate, respiration rate, core temperature, and blood pressure were monitored continuously and kept within normal ranges. Pupils were dilated with topical drops of tropicamide and atropine.

A noninvasive contact lens electrode (Jet; Metrovision, Peregines, France) was placed on the cornea of the recorded eye, and a similar electrode was set on the contralateral covered eye for reference. Hydroxyethylcellulose gel (1.3%) was applied for corneal protection and for tight adhesion of the electrodes. A gold-cup electrode attached to the skin rostral to the ear served as the ground. Experimental and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and local regulations and ethical considerations for the use of animals.

**mFERG Recordings**

The mFERG stimulus, produced by the Visual Evoked Response Imaging System software (VERIS; Electro-Diagnostic Imaging [EDI], San Mateo, CA), was displayed by a monitor (Landscape UHR 2.1L; Nortech Imaging Technologies, Plymouth, MN) from a distance of 33 cm, stimulating a visual field of approximately 23° radius. The eyes were optically corrected. A series of short recordings (approximately 3.5 minutes) was conducted to center the macula in the stimulated field. The stimulus consisted of 103 unscaled hexagons alternating between black (0 cd/m²) and white (200 cd/m²) at a frame rate of 75 Hz. For stimulation of high luminance the maximum luminance was raised to 600 cd/m². The background was set on average luminance. An experimental run consisted of 2⁵⁹ – 1 elements in the m-sequence, and lasted approximately 7 minutes, recorded as one segment. The stimulation sequence is the same for all hexagons, yet is temporally modulated among them, enabling the extraction of local responses from the overall response derived at the cornea. A detailed description of the mFERG technique is given elsewhere. A band-pass filter of 1 to 300 Hz was used, with no additional notch filter. Signals were amplified 100,000-fold (Model 12; Grass Instrument Co., West Warwick, RI).

**Administration of Pharmacological Agents**

The pharmacological agents were administered by intravitreal injections of 50 µL posterior to the limbus with a 30-gauge needle. Noted are the final vitreal concentrations, assuming vitreal volume of 2 mL. After baseline recordings, a mixture of 2 mM γ-aminobutyric acid (GABA) and 2 mM glycine was administered to suppress inner retinal function. Receptors to GABA and glycine are found mainly on third-order neurons and can also be found in the outer retina. However, the effect of this mixture of neurotransmitters was comparable to the effect of tetrodotoxin (TTX) and N-methyl-D-aspartic acid (NMDA). In two normal and two glaucomatous eyes, 6 mM 2-amino-4-phosphonobutyric acid (APB), an agonist to glutamate metabotropic receptors, was administered sequentially to block transmission from photoreceptors to ON-bipolar cells. The responses obtained after administration of APB reflect the activity of OFF-bipolar cells, horizontal cells, and photoreceptors. The contribution of the horizontal cells to the responses is questionable, owing to their lateral orientation; therefore, we will refer to the component obtained after administration of APB as the OFF-bipolar and photoreceptor component. The time interval between the two injections was 2 to 3 hours. APB was administered only after assuring that the retinal responses were unchanged for a minimum of 30 minutes after the administration of GABA and glycine.

**Data Analysis**

First order (K1) and first slice of the second-order (K2) responses were analyzed with the VERIS as well as extracted for further data processing. The retinal responses were grouped and averaged in five regions of seven signals each (Fig. 1A). Responses were generally consistent across the stimulated retinal field. However, drug effects were not achieved uniformly in all the stimulated retinal field with the same time course. To limit the period of anesthesia, we chose to present the responses of the inferior region where drug effects were observed first and reached steady state indicating complete effect. The responses of the normal eyes in this region were in agreement with those represented before for the normal monkey.
Latency was measured from the time of stimulus onset to the beginning of the response, and implicit time was measured from the time of stimulus onset to the peak of the wave. Amplitudes were measured from the first negative (N) trough to the following positive (P) potential (N1 to P1).

### RESULTS

#### Intraocular Pressure

The IOP of the normal eyes was 15, 13, and 15 mm Hg in monkeys L4, L5, and L8, respectively. A detailed documentation of IOP in the glaucomatous eyes over the concluding 30 months of the total 7-year period since glaucoma induction has been published.\(^\text{16}\) Cumulative IOP, calculated as the integral of IOP over time, was 3.5-, 2.4-, and 3.5-fold higher in the glaucomatous eyes than in the contralateral normal eye of the same animal in monkeys 1331, 468, and DP70, respectively. On the day of the experiment the IOP was 53, 48, and 50 mm Hg in monkeys 1331, 468, and DP70, respectively. The C-D ratio in the glaucomatous eyes was 0.87, 0.82, and 0.66 in monkeys 1331, 468, and DP70 respectively. The C-D ratio in normal eyes in our laboratory is 0.21 \pm 0.13.

#### Baseline Responses

Clear differences between responses of normal and glaucomatous eyes were evident in the baseline records (Fig. 1). Amplitudes were higher in the macula of both eyes than in the periphery, primarily because the stimulus was unscaled. However, the trace array of the normal eye varied in waveshape across the retinal field, whereas that of the glaucomatous eye was generally homogenous, as previously reported.\(^\text{17,20}\) A closer examination of the responses at the inferior region (Figs. 1C, 1D) shows that responses of the normal eyes consisted of a trough, N1, followed by a double positive peak, P1 and P2, and another trough, N2. In the glaucomatous eyes the responses were slightly delayed in comparison to the responses of the normal eyes (Table 1). P2 was diminished in the responses of the glaucomatous eyes, rendering P1 prominent (Fig. 1D). Finally, in the signals of the normal eyes the oscillations persisted longer than in the signals of the glaucomatous eyes which dissipated by 80 msec (Fig. 1D). Sequential administration of pharmacological agents enabled us to study the underlying sources of these differences.

#### Effects of GABA and Glycine

GABA and glycine saturate receptors of inhibitory pathways that are abundant in the inner retina. Thus the evoked mERG response was dominated by outer retinal contributions. In the normal eyes, inhibition of the inner retina removed one or more contributors to the waveform, thereby diminishing P2 and augmenting P1 (Fig. 2). Moreover, it appears that a component masking N1 was eliminated, revealing the full magnitude of N1. The remaining response of the normal eyes after injection of GABA and glycine resembled in general waveform the baseline responses of the glaucomatous eyes, as expected, assuming that glaucoma is primarily a disease of the inner retina. This is clearly evident in Figure 3, where the responses of the normal eyes after injection of GABA and glycine are compared to the baseline responses of the glaucomatous eyes. To eliminate amplitude differences, each response was normalized to the amplitude of N1 to P1.

To assess the contribution of the inner retina to the mERG responses of the glaucomatous eyes, we injected a mixture of GABA and glycine. As shown in Figure 4, inhibition of the inner retina resulted in larger amplitudes of all wave components, N1, P1, and N2, as well as a slower decay of the positive potential. Thus, unlike in the normal eyes, the implicit times of N2 were prolonged in the glaucomatous eyes (by 0.83, 3.32, and 1.66 ms for monkeys 1331, 468, and DP70, respectively). Assuming that the site of action of GABA and glycine is restricted to the inner retina, these observations indicate that the glaucomatous eyes in our study retained some inner retinal function.

As discussed earlier, both normal and glaucomatous eyes exhibited an inner retinal contribution to the mERG response. To compare the outer retinal contribution to the mERG re-

### Table 1. Implicit Time of N1 in Baseline (K1) Responses

<table>
<thead>
<tr>
<th></th>
<th>L(_{\text{max}}) 200 cd/m(^2)</th>
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</tr>
<tr>
<td>L8</td>
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<td>10.79</td>
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<td>1331</td>
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<tr>
<td>468</td>
<td>13.28</td>
<td>13.28</td>
</tr>
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<td>DP70</td>
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**FIGURE 2.** Responses of the three normal eyes before (thin traces) and after (thick traces) administration of GABA and glycine in monkeys (A) L4, (B) L5, and (C) L8.
response, we plotted in Figure 5A the normalized responses that were recorded after administration of GABA and glycine in three normal and three glaucomatous eyes. The outer retinal contribution in normal and glaucomatous eyes was similar in shape, constituting a trough-peak-trough waveform, but differed in implicit time. The leading slope of the first trough and of the positive potential were similar in the two groups, but the trailing slopes were delayed in the responses of the glaucomatous eyes. The implicit times of the first trough were 14.11, 13.28, and 11.62 in the normal eyes, and 15.77, 14.11, and 14.94 in the glaucomatous eyes. This is in correlation with the delayed N1 in the baseline responses of the glaucomatous eyes in relation to the responses of the normal eyes. The difference in implicit time of the positive potential was even greater, measuring 26.56, 24.9, and 24.07 in the normal eyes, and 28.22, 28.22, and 27.39 in the glaucomatous eyes.

By subtracting the signals recorded after administration of GABA and glycine from the baseline responses, we were able to obtain signals representing the inner retinal contribution (Fig. 5B). Inner retinal function had a peak-trough-peak waveform. The major difference between the waveforms of the normal and the glaucomatous eyes lay in the region of 30 to 40 ms (marked by an ellipse). The peak located in this region in the waveforms of the normal eyes was absent in the waveforms of the glaucomatous eyes. This peak corresponds in implicit time to P2 in the baseline responses of the normal eyes.

Isolation of ON-Bipolar Contribution to the mfERG Response

In two monkeys from each group, outer retinal contributions were further dissociated by the administration of APB to block transmission from photoreceptors to ON-bipolar cells. Figure 6A shows the responses that remained after administration of APB, representing the activity of OFF-bipolar cells and photoreceptors, whereas Figure 6B shows the contribution of the ON-bipolar cells that was obtained by subtracting the response...
after APB (Fig. 6A) from the responses after GABA+glycine (Figs. 2, 4).

There was a small delay in the OFF-bipolar and photoreceptor component in glaucomatous eyes compared with that in the normal eyes. The latency of the onset of the leading slope was 4.15 and 6.64 ms for monkeys L4 and L5, respectively, versus 8.30 ms in both glaucomatous eyes. Otherwise, no consistent differences were found between normal and glaucomatous eyes in the responses of the ON and OFF pathways in the outer retina. In all four eyes, the OFF-bipolar and photoreceptor component was oscillatory in character, with reduced oscillations from approximately 80 msec. These oscillations, synthesized with the oscillations contributed by the inner retina, are apparent in K1 responses. The ON-bipolar contribution, in contrast, was restricted to the time window of 10 to 40 ms.

Based on the two-step pharmacological decomposition of the responses, the retinal contributors to the mfERG response were established (Fig. 7). Several observations can be made from this analysis: (1) The ON and OFF pathways of the outer retina dominated the waveform of the mfERG. (2) The inner retina contributed mainly to the region of N1 and P2. (3) The components varied in amplitude among the eyes, and the inner retinal contribution also varied in wave shape. Nevertheless, the relative contribution of each component remained alike. The root mean square, a general amplitude measure, revealed that the ratio between the components was similar among the four eyes, with the OFF-bipolar cells and photoreceptors constituting the major part of the response and the inner retina contributing the smallest portion (Table 2). The relative contribution of the components can be estimated at 20% to 25% for the inner retina, 25% to 35% for the ON-bipolar cells, and 45% to 55% for the OFF-bipolar and photoreceptor cells.
Glaucoma Normal response. function reduced its contribution to approximately 60% of the more, the ratio suggests that the attenuation in inner retinal eyes compared with the responses of the normal eyes. Further-
of the inner retina to the K2 responses of the glaucomatous This indicates a threefold decrease in the relative contribution
retina dominated the remainder of the response. To quantify
this observation, the relative contribution of the retinal sources
was determined by a simple amplitude ratio between the
absolute values of the inner and outer retina at each point
along the waveforms. The amplitudes were measured every
0.83 ms from 0 to 100 ms. In the normal eyes, the ratio was
5.2:1 on average, indicating that the inner retina constitutes
more than 80% of the K2 response in the normal eyes. In the
glaucomatous eyes, both components were diminished, with
an average inner to outer retinal contribution ratio of 1.8:1.
This indicates a threefold decrease in the relative contribu-
tion of the inner retina to the K2 responses of the glaucomatous
eyes compared with the responses of the normal eyes. Furth-
more, the ratio suggests that the attenuation in inner retinal
function reduced its contribution to approximately 60% of the
response.

Responses to Stimulation of Increased Luminance
It has been shown that decreased luminance induces slower
responses. In this study we sought to characterize sepa-
ately the inner and outer retinal contributions to mfERG
responses at different luminance intensities. Furthermore, be-
cause responses of glaucomatous eyes were delayed in compari-
on with responses of normal eyes, we asked whether higher luminance would “compensate” and thus evoke the
glaucomatous eyes to produce responses of implicit time simi-
lar to normal eyes. Measurements of N1, in which significant
implicit time differences between normal and glaucomatous
eyes have been documented (Ofri R, et al. IOVS 2000;41:ARVO
Abstract 535; Ver Hoeve JN, et al. IOVS 2000;41:ARVO Abstract
2772), implied that the glaucomatous eyes did not respond as
well as the normal eyes to the increment in maximum lumi-
nance (Table 1). Figure 9A shows that a stimulus of higher
maximum luminance evoked larger amplitudes of P1 and
shorter implicit times of N1 in the responses of the normal
eyes. The most apparent effects of increased luminance on the
responses of the glaucomatous eyes were an increase in P1
amplitude, and, in two eyes, shorter implicit times of the rising
slope of P1 (Fig. 9B) but the implicit time of N1 did not change
(Table 1). Thus, raising the level of luminance did not com-
penstate for the glaucomatous damage as expressed in the
mfERG, as further emphasized in Figure 9C where the average
responses of the normal and glaucomatous eyes to the two
levels of luminance are compared. The responses were nor-
malized to emphasize the different temporal properties.

The contribution of the outer retina to the mfERG in the
normal eyes, as assessed from the responses that were re-
corded after injection of GABA and glycine, demonstrated
shorter implicit times of the first negative trough and larger
amplitudes. The amplitude of N1 to P1 increased by 11.5, 12.4,
and 12.6 nV/deg² for monkeys L4, L5, and L8, respectively, in
response to 600 cd/m² compared with 200 cd/m² maximum
luminance (Fig. 10A). These observations are in agreement
with the changes observed in K1 (Fig. 9A). Similar effects were
observed in the outer retinal responses of the glaucomatous
eyes (Fig. 10B). The implicit time of the first trough was slightly
decreased, and the N1 to P1 amplitude was increased by 11,
17.6, and 10.5 nV/deg² in monkeys 1331, 468, and DP70,
respectively.

The contribution of the inner retina to the mfERG, assessed
by subtracting the responses recorded after injection of GABA
and glycine from the baseline responses, was also affected by
increasing the maximum luminance of the stimulus as shown
in Figure 11. The inner retinal contribution in the responses of
the normal eyes was slightly increased along the waveforms
(Fig. 11A). In contrast, the increased luminance had a more
dramatic effect on the inner retinal contribution of the glauco-
matous eyes (Fig. 11B). Amplitudes of the second positive
potential (p2 at 40 ms) were increased and resembled those of
the responses of the normal eyes.

**DISCUSSION**

In this study we segregated the mfERGs responses of normal
and glaucomatous eyes into discrete retinal contributors by
pharmacological dissociation to identify the site of attenuated
function in the glaucomatous eyes. A primary finding was that

**TABLE 2. Ratio of RMS Values**

<table>
<thead>
<tr>
<th>Eye</th>
<th>Inner Retina: ON:OFF + Photoreceptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>20:36:44</td>
</tr>
<tr>
<td>L5</td>
<td>26:30:44</td>
</tr>
<tr>
<td>Glaucoma</td>
<td></td>
</tr>
<tr>
<td>1331</td>
<td>19:25:56</td>
</tr>
<tr>
<td>468</td>
<td>25:32:43</td>
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</tbody>
</table>

Data are percentages

**Second-Order Responses**

Second order mfERG responses are used to assess inner retinal
function. Figure 8 shows average K2 responses for the
inferior retina of three normal and three glaucomatous eyes
(Figs. 8A, 8B, respectively). It is clear that the K2 responses of
the glaucomatous eyes were considerably less prominent than
those of the normal eyes, supporting the notion that the pri-
mary glaucomatous damage is to the inner retina. The phar-
macological decomposition of the waveform in the normal eyes
demonstrated that the outer retina contributed mainly at 25 to
45 msec, constituting a trough followed by a peak. The inner
retina dominated the remainder of the response. The am-
plitudes were measured every 0.83 ms from 0 to 100 ms. In the normal eyes, the ratio was
5.2:1 on average, indicating that the inner retina constitutes
more than 80% of the K2 response in the normal eyes. In the
glaucomatous eyes, both components were diminished, with
an average inner to outer retinal contribution ratio of 1.8:1.
This indicates a threefold decrease in the relative contribu-
tion of the inner retina to the K2 responses of the glaucomatous
eyes compared with the responses of the normal eyes. Furth-
more, the ratio suggests that the attenuation in inner retinal
function reduced its contribution to approximately 60% of the
response.

**FIGURE 8.** Baseline K2 responses and their outer and inner retinal contributors in normal (A) and glaucomatous (B) eyes. Each waveform
represents an average response of three eyes.
the glaucomatous eyes, in spite of the prolonged period (7 years) of elevated intraocular pressure (50 ± 12 mm Hg), still retained inner retinal function (Figs. 4, 5B). It has been shown before that, although suppression of the inner retina in normal eyes approximates responses of glaucomatous eyes, the signals are not identical.17 The results of this study suggest that this discrepancy arises mainly from the drugs’ removal of all inner retinal contribution, whereas the inner retina still partakes in the responses of glaucomatous eyes.

Despite small variations from eye to eye, it is possible to construct a single model of mfERG response generators that is consistent with all the data (Fig. 7) and agrees with a previous report.29 The outer retinal contributors are the dominant source of the K1 mfERG response. The descending slope of N1 reflects the hyperpolarization of OFF-bipolar cells and photoreceptors. The ascending slope of N1 and the leading slope of P1 arise from light-induced depolarization of the ON-bipolar cells and from the depolarization of the OFF-bipolar cells as they recover from the light stimulus. Because of the short stimulus, the on phase of the ON-bipolar cells can combine with the off phase of the photoreceptors and OFF-bipolar cells, similar to the flash ERG that is elicited by bright stimulus in the light-adapted state.23,29 The summated contributions of the ON- and OFF-bipolar cells is also expressed in P2 and N2 and the ongoing oscillations. The inner retinal function shapes N1, forming an additional peak (seen clearly in Fig. 7A) which is especially prominent in the nasal region.16,17 Postreceptoral influences at early times of the response have also been shown in cone-driven flash ERG responses (Robson JG. IOVS 2002;43: ARVO E-Abstract 1821). Further, a more conspicuous influence of the inner retina is evident in the segment of 30 to 40 ms in the responses of the normal eyes (Fig. 5B). A peak in the inner retinal contribution in this area yields P2 in the baseline responses. In the inner retinal contribution of the glaucomatous eyes this peak is attenuated, either absent, or delayed and thus integrated with the following signal elements, rendering P2 of the K1 responses diminished. The inner retinal contribution is also apparent in the oscillations after N2 (Fig. 7).

Variations were observed in the inner retinal contribution to the mfERG responses. The general peak-trough-peak form, which was observed in previous studies17,38 can be seen in all the eyes, but varied in shape and in amplitude (Fig. 5B). The simplest explanation to this variability assumes that the drugs did not have an identical effect in all eyes. However, this is less likely because the responses were steady for over 30 minutes, implying maximal effect of the drugs. Alternatively, the mixture of GABA and glycine that was used in the study to suppress inner retinal function probably acted on several cell types. Therefore, it is reasonable to assume that the inner retinal component is actually a superposition of several waveforms, similar to the TTX-sensitive component of the mfERG response.26,29 and the difference between eyes reflects variation in the contributions of the different sources.
In light of the variability in the inner retinal contribution to the mfERG responses, the consistent difference between normal and glaucomatous eyes in the area of 30 to 40 ms, corresponding to P2 in K1, is even more remarkable. Although this study included a small number of eyes, the attenuation of P2 in responses of glaucomatous eyes was consistently documented, and it is reasonable to assume that the underlying cause is the same. Because the ganglion cells are primarily damaged in glaucoma, we suggest that the second positive peak in the inner retinal responses (30–40 ms) reflects ganglion cell function, whereas other parts of this waveform represent activity of other inner retinal cells. This supports conclusions of a recently published study describing mfERG responses in optic neuritis.

It is interesting to note that signals that appear alike may envelop less congruent components, as was the case in the two normal eyes L4 and L5 (Figs. 7A, 7B). The baseline responses of the two normal eyes are almost identical in shape and in amplitude, whereas the individual components differ in amplitude and the inner retinal component also varies in shape. Nevertheless, we found that the amplitude ratio of the components is maintained in the K1 responses (Table 2). This ratio was unexpectedly similar for the glaukomatos eyes as well. The glaucomatous damage that was expected to be most severe in the inner retina was not expressed in a dramatic change of mfERG contributions toward the outer retinal components. It is possible that the long-standing increased IOP did not cause severe damage to the inner retina, or that the damage was severe to both inner and outer retinal layers, thus rendering a similar ratio. Moreover, the contribution of inner retinal function to K1 responses in the normal eyes was estimated at 26% or less (Table 2), and therefore a significant attenuation in inner retinal function would be expressed in only a small change in the ratio of contributing sources.

Direct evidence of glaucomatous damage to outer retinal cells is obtained from the initial part of the waveforms. The trough N1 was delayed in the responses of the glaucomatous eyes (Fig. 1D, Table 1), in agreement with previous reports (Ofri R, et al. IOVS 2000;41:ARVO Abstract 555; Ver Hoeve JN, et al. IOVS 2000;41:ARVO Abstract 2772). This correlates with a similar delay in the outer retinal component (Fig. 5A), indicating that the delay in the K1 responses originates in this component. The usefulness of implicit time of mfERG responses as a diagnostic tool has been reported in other retinal diseases. The pharmacological analysis shows that outer retinal cells are the principal source underlying N1. However, it was difficult to determine which of the outer retinal contributors in the glaucomatous eyes is responsible for the delayed implicit time of N1.

Second-order responses are a measure of retinal adaptation, reflecting the effect of successive flashes on the mfERG responses. The first slice of the second-order kernel (K2) shown in this study represents the effect of an immediately preceding flash on the response. Waveforms of K2 were composed of positive-negative-positive potentials in the normal eyes (Fig. 8). By administration of GABA and glycine, we were able to show that the inner retina was the prominent underlying source of K2 responses, as was suggested before. We estimate that inner retinal mechanisms are responsible for roughly 80% of the adaptation process that is expressed in the K2 responses of normal eyes. Nevertheless, K2 responses include outer retinal contribution as well. Frishman et al. showed previously that suppression of the inner retina eliminated most of the K2 responses, similar to the effect of glaucoma. However, whereas the K2 responses of the glaucomatous eyes were practically eliminated, a negative potential at 25 to 30 ms remained in TTX-NMDA treated eyes. The present study suggests that this negative potential of the K2 response originates in the outer retina.

Because K2 responses are dominated by the inner retinal contribution, they are likely to be a more sensitive measure of inner retinal function. If the inner retina constitutes 80% of the K2 response (compared with only 20% to 25% of the K1 response), impaired inner retinal function would be accentuated in K2 responses. For example, a 50% loss of inner retinal function would result in only a ~10% decrease of the inner retinal contribution in the ratio of the retinal sources that constitute K1. However, the same loss of inner retinal function would result in a decrease of approximately 40% of the inner retinal contribution in the ratio of the retinal sources that constitute K2. In the glaucomatous eyes in this study, both inner and outer retinal contribution to K2 responses were diminished. However, the ratio of inner to outer retinal contribution decreased, suggesting that the inner retina sustained more severe damage than the outer retina (Fig. 8).

Increasing the maximum luminance of the stimulus was expressed in the mfERG responses by increased amplitudes and faster kinetics (Table 1, Fig. 9), in agreement with previous studies. The pharmacological analysis demonstrated that in normal as well as in glaucomatous eyes, outer retinal components participated in the implicit time shift induced by luminance changes (Fig. 10). The pathophysiology underlying the changes in the responses of the glaucomatous eyes could be a decrease in number of cells while the remaining cells function normally, or an attenuated function of cells, or both. The implicit time of N1 in K1 responses suggests that the outer retina in the glaucomatous eyes did not perform as well as that of the normal eyes. The implicit time of N1 under the stimulus of lower luminance in the normal eyes was shorter than that of...
the glaucomatous eyes, even when the glaucomatous eyes were illuminated with stimuli of high luminance (Table 1). This suggests that outer retinal cells in the glaucomatous eyes, whether decreased in number or not, do not perform as cells in the normal retina.

The principal effect of increased luminance on the inner retinal component was the increased amplitude of the second positive peak (30–40 ms) in the responses of the glaucomatous eyes (Fig. 11B). This peak “recovered” to an amplitude comparable to the normal eyes under luminance of 600 cd/m². The difference in effect on this peak relative to the rest of the inner retinal contribution supports the previous assertion that this region reflects one group of cells, whereas other inner retinal cells are manifested in other parts of the waveform. We suggest that the sensitivity of ganglion cells to luminance changes is reflected in the region of 30 to 40 ms of the inner retinal contribution. These changes are not manifested in K1 responses, because they are masked by the outer retinal responses.

In light of the small number of eyes and a degree of inter-subject variability in the responses the analysis presented in this study should be interpreted cautiously. Nevertheless, the findings helped elucidate the cellular contributions to the mfERG responses of normal and glaucomatous eyes. Inner retinal function was clearly portrayed in N1 and in P2 of the first order mfERG responses. It is conceivable that P2 reflects the activity of ganglion cells, whereas other inner retinal cells, possibly amacrine cells, are reflected in the region of N1. Both of these components were attenuated in the responses of the glaucomatous eyes. Outer retinal function was delayed in the responses of glaucomatous eyes. Together, the evidence suggests an attenuated function of inner and outer retinal layers in this glaucoma model.

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References

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