Retinal Pathway Origins of the Pattern Electroretinogram (PERG)

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PURPOSE. To determine retinal pathway origins of pattern electroretinogram (PERG) in macaque monkeys using pharmacologic dissections, uniform-field flashes, and PERG simulations.

METHODS. Transient (2 Hz, 4 reversals/s) and steady state (8.3 Hz, 16.6 reversals/s) PERGs and uniform-field ERGs were recorded before and after intravitreal injections of L-AP4 (not APB) (2-amino-4-phosphonobutyric acid, 1.6–2.0 mM), to prevent ON pathway responses; PDA (*cis*-2,3-piperidinedicarboxylic acid, 3.3–3.8 mM), to block activity of hyperpolarizing second- and all third-order retinal neurons; and TTX (tetrodotoxin, 6 μ M), to block Na⁺-dependent spiking. PERGs were also recorded from macaques with advanced unilateral experimental glaucoma, and were simulated by averaging ON and OFF responses to uniform-field flashes.

RESULTS. For 2-Hz stimulation, L-AP4 reduced both negative- and positive-going (N₉₅ and P₅₀) amplitudes in transient PERGs, and their counterparts, N₂ and P₁ in simulations, to half-amplitude. PDA eliminated N₉₅ and N₂, but increased P₅₀ and P₁ amplitudes, in that it enhanced b-waves. As previously reported, severe experimental glaucoma or TTX eliminated photopic negative responses, N₉₅, and N₂; glaucoma eliminated P₅₀ and reduced P₁ amplitude; TTX reduced P₅₀ and hardly altered P₁. For 8.3-Hz stimulation, L-AP4 eliminated the steady state PERG and reduced simulated PERG amplitude, whereas PDA enhanced both responses. TTX reduced PERG amplitude to less than half; simulations were less reduced. Blockade of all postreceptoral activity eliminated transient and steady state PERGs, but left small residual P₁ in simulations.

CONCLUSIONS. Transient PERG receives nearly equal amplitude contributions from ON and OFF pathways. N₉₅ reflects spiking activity of ganglion cells; P₅₀ reflects nonspiking activity as well. Steady state PERG, in contrast, reflects mainly spike-related ON pathway activity. (*Invest Ophthalmol Vis Sci.* 2011; 52:8571–8584) DOI:10.1167/iows.11-8376

The pattern electroretinogram (PERG) is commonly recorded noninvasively from the cornea under light-adapted conditions using a checkerboard or grating pattern with equal numbers of bright and dark elements. The pattern stimulus covers the central retina and commonly reverses in contrast at full-cycle temporal frequencies between 1 and 8 Hz (2 and 16 reversals per second [r/s]). The lower frequencies (e.g., 1 to 2

Hz, 2 to 4 r/s) elicit transient PERGs with discrete positive- and negative-going (P₅₀ and N₉₅) responses to each stimulus contrast reversal, peaking approximately at the time in milliseconds past the reversal noted in the subscripts, whereas the higher frequencies (e.g., 8 Hz, 16 r/s) produce steady state second-harmonic responses. It is assumed that since the overall luminance of the pattern stimulus remains constant, the linear responses for all frequencies of stimulation that contribute to standard waves (e.g., a-, b-, and d-waves) of the full-field flash ERG will cancel, leaving only nonlinear responses in the signal. Previous studies have shown that the PERG can be substantially reduced or eliminated after optic nerve crush/section or severe glaucoma in humans, or experimental glaucoma in rodents and monkeys.^{1–10} The PERG in macaque, whose retina is very similar to that of humans, can also be substantially reduced, but not completely eliminated, by blocking Na⁺-dependent spiking with intravitreal tetrodotoxin (TTX).^{10,11} N₉₅ of the transient PERG was found to be affected to a greater degree by TTX than P₅₀ was, similar to findings in several optic nerve disorders in humans.^{4,10,11} Together these findings indicate a primary role for retinal ganglion cells (RGCs) and their spiking activity, in generating the PERG. However, nonspiking portions of P₅₀ may, in some pathologic situations, reflect activity of more distal neurons.

The PERG has been used clinically for many years to assess RGC function in diseases that affect their integrity (e.g., glaucoma).^{1,4} The PERG is sensitive to early ganglion cell dysfunction caused by ocular hypertension and early glaucoma when visual field defects are minimal.^{12–19} PERG amplitude reduction in early glaucoma also exceeds that predicted from ganglion cell axon loss, suggesting the presence of a group of viable but dysfunctional ganglion cells (or glial cells whose currents are likely involved in generation of the PERG) at this stage.¹⁹

Although it is established that generation of the PERG is dependent on functional ganglion cells, the exact contributions from spiking versus nonspiking activity of retinal ON and OFF pathways to the primate PERG remain unclear. Indications with respect to these issues from other studies have not been entirely consistent. A recent study of the mouse transient PERG using TTX and pharmacologic blockade of ON or OFF pathways indicates that P₁ in the mouse PERG (the counterpart of P₅₀ in primate transient PERG) is dominated by spiking activity from the ON pathway, whereas N₂ (the counterpart of N₉₅) depends on spiking activity and nonspiking activity from the OFF pathway.⁸

The finding that X-linked congenital stationary night blindness (CSNB), which eliminates ON pathway responses, practically eliminates the human transient PERG,⁴ raising the possibility of ON pathway dominance for the human PERG. The predominance of a particular pathway in formation of PERG waveforms is somewhat surprising. Viswanathan et al.²⁰ demonstrated in macaques that the PERG could be simulated by averaging ON and OFF responses to long-duration uniform-field flashes, both of which contained negative-going photopic neg-

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Supported in part by National Eye Institute Grants R01-EY06671 (LJF) and P30-EY07751 (University of Houston College of Optometry).

Submitted for publication August 8, 2011; revised September 12, 2011; accepted September 14, 2011.

Disclosure: X. Luo, None; L.J. Frishman, None

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ative responses (PhNRs) after the b- and d-waves. It was proposed that N_{95} of the transient PERG reflects an average of the PhNR in the ON and OFF responses.¹⁰ PhNRs, like the PERGs, are believed to have origins from ganglion cells, particularly the TTX-sensitive activity of those neurons.²⁰

In the present study, we used both uniform-field flash ERGs and pharmacologic agents that targeted specific retinal receptors and channels to further investigate ON versus OFF pathway origins (both spiking and nonspiking) of the PERG in macaque monkeys. The results should provide useful information for interpretation of the PERG in human patients, with pathologies that affect pathway elements distal to the ganglion cells, as well as the ganglion cells themselves. Some of the findings were reported previously in abstract form (Luo X, et al. *IOVS* 2009;50:ARVO E-Abstract 2177).

METHODS

Subjects

Ten adult monkeys (*Macaca mulatta*; 7 to 10 years of age) were studied. All experimental and animal care procedures adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Houston.

Animal Preparation

Animals were anesthetized intramuscularly with ketamine (15–25 mg·kg⁻¹·h⁻¹) and xylazine (0.7–0.9 mg·kg⁻¹·h⁻¹) and were treated with atropine sulfate (0.04 mg·kg⁻¹, injected subcutaneously), as previously described.^{5,10,21} The depth of anesthesia was monitored and kept at a level sufficient to suppress eye movements. Pupils were fully dilated to approximately 8.5 mm in diameter with 1% topical tropicamide, 1% atropine sulfate, and 2.5% phenylephrine hydrochloride. Body temperature was maintained between 36.5 and 38°C with a thermostatically controlled heating blanket (TC1000 temperature controller; CWE Inc., Ardmore, PA). Heart rate and blood oxygen were monitored with a pulse oximeter (model 9847V; Nonin Medical, Inc., Plymouth, MN).

Pharmacologic Interventions

The pharmacologic interventions that the macaque eyes ($n = 11$) received are summarized in Table 1. Acute blockade of retinal pathways was achieved by intravitreal injection of L-AP4 (hitherto, called APB in this paper) (2-amino-4-phosphonobutyric acid, 1.6–2.0 mM estimated vitreal concentration based on a 2 mL vitreal volume) to mimic the effect of glutamate at the mGluR6 (metabotropic glutamate) receptors expressed in ON cone bipolar cells, thereby blocking ON bipolar cell light responses and activity in the ON pathway thereafter.^{22,23} PDA (*cis*-2,3-piperidine dicarboxylic acid, 3.3–3.8 mM) to block AMPA/KA (α -amino-3-hydroxy-5-methylisoxazole-4-propionate/kainite) receptors found on hyperpolarizing second-order (OFF cone bipolar cells and horizontal cells), and all third-order (both ON and OFF amacrine and ganglion cells) neuron responses.^{22,24,25} Intravitreal injection of TTX (6 μ M) was used to eliminate spiking mediated by voltage-gated sodium channels (Na_v)²⁶ expressed in third-order neurons that contribute to PERG generation (mainly RGCs).

Experimental Glaucoma

ERGs were recorded from three macaques with unilateral experimental glaucoma that were being used in other studies, as well, to compare with results from a previous study of the PERG from the laboratory using a slightly different setup,¹⁰ and with the results of the other interventions used in the present study. The experimental glaucoma was the result of laser-induced ocular hypertension.^{27,28} Development of experimental glaucoma was confirmed by retinal nerve fiber layer (RNFL) thickness loss revealed with optical coherence tomography

(Stratus OCT, v. 4.0.4; Carl Zeiss Meditec, Dublin, CA), PhNR loss in full-field ERG, and, in one animal, visual sensitivity (VS) loss measured by perimetry (Humphrey Field Analyzer [HFA], model 630; Carl Zeiss Meditec).

ERG Recording

All full-field flash ERGs, uniform-field flash ERGs, and PERGs were recorded differentially between the two eyes using DTL (Dawson, Trick, Litzkow) fiber electrodes²⁹ placed horizontally across the center of each cornea, moistened with saline and 1% carboxy-methyl cellulose sodium solution, and covered with gas-permeable contact lenses with correction for the viewing distance. One eye was stimulated and the other was occluded. Stimulation and recording were done using a multifocal ERG system software (Espion system, first generation with updated software package; Diagnosys, Lowell, MA) with filter settings of DC–300 Hz. Long-duration flash full-field ERGs were recorded to monitor and confirm effects of pharmacologic agents on retinal pathways, and to compare to uniform-field flash ERG results. The full-field stimuli were 200-ms red flashes ($\lambda_{\text{max}} = 640$ nm, 2.5–560 photopic [phot] cd/m²) on a rod-saturating blue background ($\lambda_{\text{max}} = 462$ nm, 100 scotopic [scot] cd/m², or 10 phot cd/m²), which have been reported to be optimal in generating the PhNR,³⁰ and 200-ms white flashes (2.5–280 phot cd/m²) on a white background (100 scot cd/m², 40 phot cd/m²), which was more similar in wavelength to the black-and-white uniform-field ON–OFF and pattern ERG stimuli. In this study, we report mainly ERG results for intense white-on-white (W/W) long-duration stimuli.

After injections of pharmacologic agents, PERG and uniform-field flash ERGs were recorded only after characteristic waveform changes in full-field flash ERG responses^{10,20,22,30,31} had stabilized, which usually occurred within 1 hour after injection. Uniform-field flash and pattern stimuli were presented on the same display monitor (model HL7955SKF; Mitsubishi Electric Corp., Nagasaki, Japan), frame rate of 100 Hz, with a contrast of 84%, mean luminance of 55 cd/m², and square-wave modulation frequencies of 1, 2, 3.1, and 8.3 Hz (i.e., 2, 4, 6.3, and 16.6 r/s) (Figs. 1A, 1B). The viewing distance was 46 cm and the screen subtended 44° of visual angle horizontally and 34° vertically. The PERG stimuli were contrast-reversing checkerboards with a check size of 1°, which is a good stimulus for macaques^{1,5} and close to the ISCEV (International Society for Clinical Electrophysiology of Vision) standard range for humans.³² The check size provides a PERG that is affected in both early and late glaucoma, whereas the PERG response to much larger or smaller checks can be relatively normal early in the disease.^{1,5} To minimize stray light effects, the display was fitted with a white cardboard surround that extended approximately 18 cm beyond the screen, and was illuminated by the room lights in the ceiling (55 cd/m²). Both uniform-field flash and pattern ERGs were recorded after the fovea was aligned to the center of the display using an adapted monocular indirect ophthalmoscope.³³

More than 1000 complete cycles of uniform-field flash ERG and PERG were recorded. Each trial was approximately 6 seconds in length and contained multiple cycles that could be segmented for averaging of single cycles, or illustrating waveforms of multiple cycles. The uniform-field flash ERG protocols were designed so that all odd-numbered trial recordings started with a light-onset frame, whereas all even-numbered trial recordings started with a light-offset frame. Data collection commenced approximately 12 seconds after the protocol started to allow for stabilization, and half a cycle between each trial thereafter was discarded to obtain the appropriate 180° phase shift. As shown in Figure 1C, averaging the odd-numbered trials yielded an ON–OFF response, whereas averaging the even-numbered trials yielded an OFF–ON response, and the average of all the trials provided a simulation of the PERG. Both the PERG and the simulated responses were measured at reversal frequencies, which were twice the stimulus frequencies. As illustrated in Figure 1D, P_1 in the simulation came from the average of b- and d-waves, whereas N_2 was from the average of PhNR_{on} and PhNR_{off} in transient uniform-field responses.

TABLE 1. Number of Eyes, Interventions and Their Effects, and ERG Protocols Used in the Present Study

Intervention (and Order of Intervention)				Number of Eyes	Light Responses Reduced or Eliminated	Remaining Light Responses	Number of Eyes for Each Protocol			
1	2	3	W/W				Full Field	2-Hz Uniform	2-Hz PERG	8.3-Hz Uniform
A. One Intervention										
Glaucoma*			3	RGCs	All cells distal to RGCs Nonspiking retinal responses		3			
TTX*			2	Spiking due to Na _v s in inner retinal neurons			1	1 (OHT-53)	3	1 (OHT-59)
B. Serial Interventions										
TTX			1	Spiking due to Na _v s in inner retinal neurons	Nonspiking retinal activity		1	1	1	1
APB	APB		3	Nonspiking activity in ON pathway	Nonspiking activity in OFF pathway					
	TTX			ON pathway responses	OFF pathway responses		3	3	3	3
		PDA		Spiking activity in OFF pathway	OFF pathway, nonspiking activity					
			2	Remaining post-receptoral responses	Isolated photoreceptor response					
PDPA				OFF pathway, horizontal cells, and inner retinal responses	Photoreceptors and ON bipolar cells		2	2	2	2
	TTX			Remaining spiking activity in ON pathway	Photoreceptors and ON bipolar cells, nonspiking activity					
		APB		Remaining post-receptoral responses	Isolated photoreceptor response					

*The present study is a replication of a similar previous study in the same laboratory.¹⁰

FIGURE 1. Uniform-field ON-OFF flash and pattern ERG techniques used in this study. (A) Uniform-field flash stimulus. (B) PERG stimulus. (C) Generation of second-harmonic responses from transient ON-OFF uniform-field flash responses in a simulated 2-Hz PERG, compared with a PERG recorded using a pattern stimulus (beneath). Calibration: 100 ms, 5 μ V. (D) Uniform-field ON-OFF responses on an expanded time axis to show how the ON and OFF responses shape the second-harmonic responses in the simulated PERG. Calibration: 25 ms, 5 μ V.

Data Analysis

Full-field flash ERG, uniform-field flash ERG, and PERG data (exported from Espion) were processed using custom programs (MATLAB, v. 7.1; The MathWorks, Inc., Natick, MA).

Although four temporal frequencies were tested, only 2-Hz (transient PERG response to 4 r/s) and 8.3-Hz (steady state PERG response to 16.6 r/s) data were fully described (Fig. 2). The duration of ON or OFF portions of the 2-Hz uniform field protocol was 250 ms, which

FIGURE 2. Full-field flash ERGs, uniform-field flash ERG, and PERGs in nine control eyes and nomenclature used in the present study. For (A–H), the *black traces* are averages of individual responses that are shown by the *gray traces*. (A, B) White and red full-field long-flash ERGs (280 phot cd/m² for all full-field flash stimuli used for this and subsequent figures). (C) 2-Hz uniform-field ON-OFF response. (D) 2-Hz simulation. (E) 2-Hz PERG. (F) 8.3-Hz uniform-field ON-OFF response. (G) 8.3-Hz simulation. (H) 8.3-Hz PERG. (I) Uniform-field ON responses recorded using stimuli with four temporal frequencies (1, 2, 3.1, and 8.3 Hz). (J) Uniform-field OFF responses recorded with the same stimuli. (K) Simulations derived from responses in (I) and (J). (L) PERGs recorded using pattern stimuli with the same temporal frequencies.

was close to the duration of long flashes used for full-field flash ERG (200 ms, Fig. 2A) and was long enough for both PhNR in uniform-field flash ERG and N_{95} in PERG to return to the baseline, which was not quite the case for 3.1-Hz stimulation, for which the N_{95} amplitude was small (Figs. 2I–L). Results using 1 Hz were very similar to those using 2 Hz.

Amplitudes and implicit times of 2-Hz and 8.3-Hz PERG and uniform-field flash ERGs and simulations were measured after low-pass filtering (DC-50 Hz with an off-line digital filter) the averaged signals. In general, for 2-Hz stimulation, amplitudes were measured from baseline, and implicit times of OFF response components were measured from the time of reversal of the uniform field from ON to OFF. For 8.3-Hz stimulation, second-harmonic responses were measured. The details of how waveform components were measured under particular conditions are presented in corresponding sections of the Results.

RESULTS

Flash ERG and PERG Responses in Control Eyes

Figure 2 shows the full-field long-flash ERG, uniform-field flash ERG, simulated PERG, and actual PERG recordings from control eyes. The thick black solid curves in Figures 2A–H are the averages of the individual responses (thin gray curves) from all the macaque eyes that were studied. The averages are useful

for appreciating the major attributes of the waveforms. However, the reported average implicit times and amplitudes in figures and tables (see, e.g., Table 2) were all based on measurements of individual responses. The numerical sign of the PhNR (negative-going) peak amplitude was reversed so that an elevation of the record due to an intervention at the implicit time of the PhNR in control eyes would manifest as a reduction in its amplitude. This transformation was also used for negative waves N_2 and N_{95} .

Figures 2A and 2B show ERG responses to a white flash on white background (W/W) and red on blue (R/B) long full-field flashes, with the various waves at light onset and offset labeled for comparison with the uniform field responses below. The full-field flash responses illustrated here were generated by stimuli stronger than the PERG/uniform-field display, and were selected for the figure primarily because the major ERG waves were easily identified with this intensity. As described previously, PhNRs are better isolated by R/B than by W/W flashes for full-field stimulation, which elicit greater influence from second-order hyperpolarizing neurons in the responses.^{22,30} As indicated by the labeling, the uniform-field ON–OFF responses (Fig. 2C) contained all the waves seen in the flash ERGs. The general shape and timing of the PhNR_{on} and PhNR_{off} troughs looked more similar to the response to the R/B flash, even

TABLE 2. Control ERG and PERG Data

A. ERG Wave Amplitudes

Wave Variables	Full-Field ($n = 10$)		2-Hz Uniform Field ($n = 7$)	
	Mean	SD	Mean	SD
a-wave				
Implicit time, ms	15.0	1.0	18.0	1.2
Amplitude, μV	−35.4	7.9	−7.1	2.6
b-wave				
Implicit time, ms	29.0	2.2	34.0	1.1
Amplitude, μV	34.1	27.1	11.8	4.8
PhNR_{on}				
Implicit time, ms	74.0	13.7	115.0	9.6
Amplitude, μV	61.3	24.9	13.5	5.8
d-wave				
Implicit time, ms	31.0	5.9	44.0	4.1
Amplitude, μV	21.0	17.8	3.0	1.1
PhNR_{off}				
Implicit time, ms	79.0	14.7	118.0	18.0
Amplitude, μV	−4.2	15.0	6.9	1.9

B. Simulation and PERG Amplitudes

Variables	2-Hz Simulation ($n = 7$)		2-Hz PERG ($n = 10$)		t	P
	Mean	SD	Mean	SD		
N_1 – N_{35}						
Implicit time, ms	17.0	1.6	33.0	1.0	−25.5	<0.001
Amplitude, μV	−2.0	1.1	−0.2	0.3	−5.2	<0.001
P_1 – P_{50}						
Implicit time, ms	34.0	1.3	50.0	0.8	−32.3	<0.001
Amplitude, μV	8.8	3.9	3.6	1.3	3.8	0.002
N_2 – N_{95}						
Implicit time, ms	118.0	10.1	114.0	8.8	1.0	0.324
Amplitude, μV	7.6	2.2	6.1	2.4	1.3	0.218

Peak amplitudes were measured from the baseline for 2-Hz data. Implicit times of d-wave and PhNR_{off} were measured from the time of reversal of the uniform field from ON to OFF.

though the wavelength spectrum of the visual display was broader.

Figure 2D shows the simulated 2-Hz PERG. The nomenclature for the waves P_1 and N_2 distinguishes them from their counterparts (P_{50} and N_{95}) in the transient PERG (Fig. 2E). Measurements of implicit times in the uniform-field ON-OFF responses and the simulated response (Table 2) show that the implicit time of P_1 (~ 34 ms) was determined mainly by the implicit time of the b-wave (rather than the d-wave) in the uniform-field ON response. The difference in implicit time between the b-wave in the uniform-field ON response and P_1 in the simulation (-0.6 ± 0.5 ms, mean $\pm$ SD) was significantly smaller ($t = -6.36$, $P = 0.0007$) than the difference between P_1 and the d-wave in the OFF response (9.7 ± 4.3 ms).

P_1 in the 2-Hz simulation was larger and peaked significantly earlier (~ 15 ms) than its counterpart, P_{50} in the PERG (Table 2B and Fig. 2E), which had an implicit time of 50 ms, the same as the nominal time in humans. In contrast, N_2 and N_{95} were similar in their implicit times and amplitudes, which indicated that they might be more closely related to each other than P_1 and P_{50} were.¹⁰ Although N_1 and N_{95} were similar in timing, they were both slightly delayed compared with the nominal 95 ms in humans. The reasons for this difference, stimulus versus species, were not investigated in this study. The earlier peak time of P_1 than that of P_{50} may be due, in part, to the large effective "check" size of the uniform field stimulus that occupied the entire screen.³⁴

In contrast to what was observed in 2-Hz responses, second-harmonic amplitudes for 8.3-Hz simulation and PERG (not reported in Table 2, Figs. 2F–H) were quite similar: 5.5 ± 0.9 and 4.3 ± 1.5 μ V, respectively. The difference in the amplitudes between the two was not statistically significant ($t = 1.39$, $P = 0.2224$, $n = 5$; paired two-tail t -test).

Figures 2I–L show half-cycle average responses for 2- and 8.3-Hz stimulations, as well as for the other two low temporal frequencies tested, 1 and 3.1 Hz, for which full results are not described here. For the lower frequencies, the first 120 ms is shown. For 8.3 Hz only 60 ms (a half cycle, i.e., one reversal) is shown. Temporal frequency had little impact on the timing of major waveform components of the PERG (N_{35} , P_{50} , and N_{95}) and simulations (N_1 , P_1 , N_2), up to 120 ms postreversal for the three low frequencies. The 8.3-Hz responses, however, differed from those transient responses in the following respects. First, in the half-cycle uniform-field ON response (Fig. 2I), although 8.3-Hz a- and b-waves overlapped responses to other frequencies, the half-cycle of an 8.3-Hz uniform-field was too short for the PhNR_{on} to be present. Second, in the half-cycle uniform-field OFF response (Fig. 2J), neither the d-wave nor the PhNR_{off} was readily identifiable in the 8.3-Hz response. Third, for the simulations (Fig. 2K) and the actual PERG (Fig. 2L), the 8.3-Hz stimuli were too short for identifiable N_2 and N_{95} to appear. Although initial negative and positive waves of the 8.3-Hz simulation (Fig. 2K) and the PERG overlapped their counterparts in transient responses, these waves were the result of interactions between responses to current and previous stimulus reversals.

Implicit times of waves in control full-field and 2-Hz ERG recordings documented in Table 2 were used in the sections that follow to measure amplitude changes at these fixed times caused by the interventions used in this study. For instance, the average b-wave implicit time in the control 2-Hz ON-OFF uniform-field response was 34 ms, and this time was used to measure these b-waves in all phases of the study. Effects of interventions on N_1 and N_{35} were not reported because they were relatively small waves compared with those that were measured. Because fixed times derived from controls were used, measurements could be made even when the wave of

interest was no longer distinguishable in the record after an intervention.

Effects of Experimental Glaucoma or TTX on Flash ERGs and PERGs

Long-duration flash responses, uniform-field ON-OFF responses, and PERGs from three macaques with advanced unilateral experimental glaucoma and in three with intravitreal TTX injection in the absence of any other intervention were included in this study, both to confirm results of similar previous experiments in the laboratory¹⁰ and for use in comparison with results of other interventions in this study. For experimental glaucoma, as shown in Table 3A, RNFL thickness and brief flash PhNRs were greatly reduced in experimental eyes, as was the visual sensitivity in the one animal that was examined with perimetry. The ERG and PERG results from one animal, OHT-53, shown in Figure 3, are typical of the three animals. As reported previously,^{20,30,35} advanced experimental glaucoma led to a profound loss of PhNR_{on} in the full-field long-flash ERG (Fig. 3, top). The flattened negative wave remaining in the record of the experimental eye is related to hyperpolarizing activity of second-order retinal neurons, rather than ganglion cells (also see the subsection on use of PDA later in the Results section).²⁹ The PhNR_{off} was hardly visible even in the control eye for this experiment.

Waveform changes for the glaucomatous eye in the 2-Hz uniform-field ON-OFF response were similar to those in the full-field ERG (Fig. 3A, second row), although effects on PhNR_{off} were easier to see. Both PhNR_{on} and PhNR_{off} lost substantial amplitude. These changes in uniform-field responses led to changes in the simulation (Fig. 3A, third row). N_2 amplitude decreased dramatically, but P_1 only slightly. The prominence of the residual P_1 marked a major difference between the simulation and the 2-Hz PERG shown just below (Fig. 3A, bottom). In the PERG, both P_{50} peak amplitude and N_{95} were greatly reduced by experimental glaucoma (see Table 3B).

In the present study, the 8.3-Hz PERG was recorded from both eyes of OHT-59. The steady state PERG response was almost eliminated in the eye with experimental glaucoma (Fig. 4A, bottom row inset), which was consistent with previous results.¹⁰ The 8.3-Hz uniform-field ERG was not recorded, but in the previous work¹⁰ from the laboratory the second-harmonic amplitudes of 8-Hz simulations were reduced.

Effects of TTX on flash ERGs, 2-Hz uniform field responses, and PERGs (Fig. 3B and Table 3B) were also similar to those observed in experimental glaucoma.¹⁰ PhNR amplitudes in the flash ERGs and 2-Hz uniform field responses were substantially reduced. In the 2-Hz simulation, TTX practically eliminated N_2 in both animals for which simulations were done (Table 3B), and it eliminated N_{95} of the 2-Hz PERG in all three animals. P_1 peak amplitude was not attenuated after TTX, but its implicit time advanced by 6 ms in Figure 3B (third row), and by 2 ms in the other animal. TTX also advanced the implicit time of the PERG P_{50} wave (by 8 ms on average), and reduced P_{50} peak amplitude by 54.6% on average.

Effects of TTX on 8.3-Hz responses for the same animal are illustrated in Figure 3C. TTX reduced trough-to-peak amplitude in the uniform-field ON response, the simulation, and most dramatically in the PERG itself, as previously observed (see Table 3B).¹⁰ Implicit times measured for the positive peak of the wave, advanced by 2 ms in the simulation and by 8 ms in the PERG in the records illustrated.

Effects of APB on Flash ERGs and PERGs

Contributions from the retinal ON pathway to ERGs can be assessed using intravitreal injections of APB in normal eyes (see Table 1 for retinal locations of effects of pharmacologic

TABLE 3. Effects of Experimental Glaucoma on Intravitreal TTX on ERGs and 2-Hz PERGs

A. Functional and/or Structural Test Results for the Three Monkeys with Experimental Glaucoma									
Variable	OHT-53			OHT-59			OHT-61		
	Control	Exp	Difference	Control	Exp	Difference	Control	Exp	Difference
RNFL thickness, μm	98.2	58.6	39.6	103.7	57.2	46.6	95.3	71.6	23.7
Mean deviation, dB	0.8	-9.9	10.7						
PhNR @ 65 ms, μV	13.3	-24.2	-37.5	16.7	-29.9	-46.6	31.4	2.9	-28.5
B. ERG and PERG Results									
Variables	Experimental Glaucoma		Intravitreal TTX						
	Control	Exp	Control	Exp					
Full field									
b-wave peak	18.2 (78.2)	16.9 (63.2)	56.0 (21.5)	36.6 (21.1)					
PhNR _{on}	79.5 (31.2)	32.3 (3.1)	40.6 (84.3)	12.9 (35.7)					
d-wave	86.9 (48.4)	83.3 (49.1)	44.1 (41.5)	47.3 (51.1)					
PhNR _{off}	14.9 (12.8)	12.2 (2.1)	31.2 (19.3)	2.8 (16.9)					
Uniform field									
b-wave	18.1	12.8	6.6 (16.0)	6.0 (16.0)					
PhNR _{on}	24.4	11.1	11.6 (11.9)	3.2 (4.3)					
d-wave	15.2	11.8	3.1 (5.1)	1.8 (2.7)					
PhNR _{off}	13.3	5.2	8.8 (10.2)	0.2 (0.7)					
Simulation									
P ₁ peak	16.9	11.9	5.1 (9.4)	4.9 (11.1)					
N ₂	10.5	2.1	8.2 (8.2)	0.1 (0.9)					
PERG									
P ₅₀ peak	5.2 (3.3)	0.3 (1.0)	3.4 (1.9, 5.1)	1.5 (1.0, 1.9)					
N ₉₅	6.8 (5.6)	1.4 (0.4)	5.3 (3.0, 6.9)	0.2 (0.0, 0.5)					

d-wave: measured from the level of the recording at light offset (200 ms for full-field; 250 ms for uniform field) to the peak of the d-wave; PhNR_{off}: measured as the amplitude difference between d-wave peak and a measurement at the time of peak PhNR_{off} in control full-field response (average of 79 ms) and control uniform-field response (average of 188 ms).

agents). Results for one animal are shown in Figure 4, and results for all three animals are listed in Table 4. APB eliminated the b-wave and PhNR_{on} in both full-field and uniform-field flash responses. However, amplitudes at the peak time of control d-waves, measured from the level of the response at stimulus offset, and PhNR_{off}, measured from the d-wave peak, increased by >100% in both full-field and uniform-field ERGs after APB injection. In the uniform-field response, a positive-going potential during the ON phase of the stimulus (Fig. 4B, column 2) remained after APB, as noted previously by Kondo et al.³⁶ in a similar study in macaque. This positive potential was revealed, in the present study, to be the recovery of the PhNR_{off} because it was removed by TTX injection (Fig. 4B, column 3, described later in this section).

The net effect on the 2-Hz simulations of the changes in b- and d-wave amplitudes in uniform-field responses after APB injection was a 43.4% average loss in P₁ peak amplitude, and the net effect of changes in PhNR_{on} and PhNR_{off} amplitudes was a 41.3% average loss in N₂ amplitude. Losses of about half of the positive and half of the negative waves were also observed in PERG results. APB attenuated P₅₀ peak amplitude by 53.0% and N₉₅ by 57.7% on average. These APB results serve as strong evidence that ON and OFF pathways are of roughly equal importance shaping P₅₀ and N₉₅ in the 2-Hz PERG. This is a major difference from what was found in mice, where ON pathway activity dominated P₁ and OFF pathway activity dominated N₂.⁸

Effects of APB on the 8.3-Hz uniform-field ON/OFF responses (i.e., loss of b-wave and enhancement of the response to the light offset) were similar to those observed in 2-Hz responses (Fig. 4B). In the simulation, the second-harmonic

amplitude decreased by 58% on average. In contrast to the transient PERG results, however, the 8.3-Hz PERG (Fig. 4B) was practically eliminated after APB, which suggested an almost exclusively ON-pathway origin for the steady state response.

Effects of TTX after APB

Next we injected TTX into the eyes that had already received APB injections to evaluate the contribution of spiking activity to the remaining response, which would have come only from the OFF pathway (Table 1). The major effects of TTX after APB conditioning for 2-Hz stimulation (Fig. 4A) were the elimination of the enhanced PhNR_{off} wave in full-field and uniform-field ERGs that was present after APB alone, elimination of N₂ in the simulation, and N₉₅ in the PERG. In the simulated PERG, P₁ duration was more prolonged. Changes in P₅₀ were minimal.

Waveform changes in the 8.3-Hz uniform-field OFF response after TTX injection in eyes conditioned with APB were less obvious than those observed for 2 Hz (Fig. 4B). There was just a slight smoothing of the trough of the response. As a consequence of the minimal changes in uniform-field responses, simulated waveforms were scarcely altered. The average second-harmonic amplitudes for the simulation before and after TTX injection were similar, which suggested that the spiking activity from the OFF pathway had little impact on the simulated PERG. The small residual 8.3-Hz PERG response itself became a little larger and more sinusoidal (i.e., less distorted by higher harmonics after TTX injection).

The TTX, APB, and APB + TTX results together indicate that the ON pathway response (spiking activity in particular) dominates the second-harmonic response in the steady state PERG.

FIGURE 3. Effects of experimental glaucoma and TTX on ERG and PERG. Con, control (gray lines); Exp, experimental glaucoma (black lines). (A) For one animal with advanced experimental glaucoma. Rows: top, full field-flash ERG; second, 2-Hz Uniform ON-OFF ERG; third, 2-Hz Uniform simulation; bottom, 2-Hz PERG, with an inset showing 8.3-Hz responses. (B) For one macaque eye before and after injection of TTX. Con, control (gray lines); TTX (black lines). Rows: top, full field-flash ERG; second, 2-Hz uniform ON-OFF ERG; third, 2-Hz uniform simulation; bottom, 2-Hz PERG. (C) Effect of TTX. Rows: second, 8.3-Hz uniform ON-OFF ERG; third, 8.3-Hz uniform simulation; bottom, 8.3-Hz PERG.

Effects of PDA after APB and TTX

After APB and TTX injections, the remaining response would be expected to originate from nonspiking activity in the OFF pathway, which could be further blocked by PDA. With complete blockade, combined injections of APB, TTX, and PDA should result in isolated photoreceptor responses. Addition of PDA slowed the leading edge of the sustained negative responses to light onset in both full-field and 2-Hz uniform-field ERGs (Fig. 4A). This change is consistent with a loss of post-receptoral OFF pathway responses that add to the photoreceptor response.^{22,37} PDA also attenuated the d-wave amplitude, as previously reported.^{22,37} Although a positive wave at light offset was present in the uniform-field ON-OFF response, this did not always occur when APB/TTX/PDA were injected. Although some residual P_1 (approximately 19% of the original peak amplitude) remained in the simulation, elimination of P_{50} in the PERG was nearly complete.

Observation of the effects of PDA on 8.3-Hz responses (Fig. 4B) was possible for one of the three eyes that received APB and TTX in prior injections. The amplitude of the ON-OFF response and the simulation was reduced by more than half, and the PERG was hardly visible after PDA injection.

Effects of PDA on Flash ERGs and PERGs

Unlike APB, PDA cannot completely separate ERG responses from ON and OFF pathways. Injection of PDA, through its action on ionotropic glutamate receptors, eliminates OFF, rather than ON, bipolar cell responses, but also blocks horizontal cell responses (and inhibitory influence on bipolar cells), and responses of amacrine and ganglion cells in both ON and OFF pathways. Therefore, post-PDA responses will be dominated by ON bipolar cell and photoreceptor contributions. After PDA injections in two normal eyes, one of which is illustrated in Figure 5A, b-wave amplitude in full-field ERG increased dramatically (also see Table 5), as previously reported.²² The b-wave peak time was delayed by 11 ms in Figure

5 (and by 16 ms in the other eye) after PDA. Consistent with blockade of the inner retina, PDA eliminated PhNR_{on} and, consistent with blockade of the entire OFF pathway, PDA eliminated the d-wave and PhNR_{off} .

Waveform changes in 2-Hz uniform-field ON-OFF response after PDA were similar to those observed in the full-field response, except that the remaining large b-wave was more transient than that in the full-field ERG. The b-wave peak time was delayed by 16 ms in Figure 5A, and by 10 ms in the other eye. In the 2-Hz PERG stimulation, the amplitude of P_1 in the 2-Hz simulation also increased after PDA, and the P_1 peak time was delayed in both animals (14 and 11 ms). Waveform changes in the 2-Hz PERG were similar to those in the simulation. P_{50} amplitude increased substantially and peak time was delayed by 9 and 11 ms. Both N_2 and N_{95} were practically eliminated in both animals.

Effects of PDA on 8.3-Hz responses are illustrated in Figure 5B and listed in Table 5. PDA enhanced and prolonged the b-wave in the uniform-field responses. Changes in the amplitudes of the second-harmonic responses, simulated PERG, and actual PERG differed for the two animals; the responses were larger in one animal, as illustrated in Figure 5B, but smaller in the other animal (see Table 5).

Effects of TTX after PDA

An injection of TTX after PDA conditioning blocked spiking activity not already eliminated by PDA blockade of inner retinal activity (Table 1). Effects were less dramatic than those seen in Figure 4 after APB. Small effects on b-wave amplitudes in full-field flash and 2-Hz uniform flash ERGs were mixed (Table 5), with one slightly reduced (Fig. 5A) and the other elevated. However, the entire waveform past the peak of the b-wave was elevated in both cases, and both P_1 of the simulation and P_{50} of the PERG were reduced in amplitude by TTX.

In the 8.3-Hz response (Fig. 5B), TTX after PDA reduced small oscillations present on and after the b-wave. The 8.3-Hz

FIGURE 4. ERG and PERG after serial injections of APB, TTX, and PDA. **(A)** Transient 2-Hz ERG and PERG waveform changes in one eye after serial injections. *Columns 1:* APB, *2:* TTX, and *3:* PDA. *Rows: top,* full field-flash ERG; *second:* 2-Hz uniform ON-OFF ERG; *third,* 2-Hz uniform simulation; *bottom,* 2-Hz PERG. **(B)** 8.3-Hz steady state ERG waveform changes after serial injections in the same eye whose responses are in **(A)**; *columns 1:* APB; *2:* TTX; and *3:* PDA. *Rows: top,* 8.3-Hz uniform ON-OFF ERG; *middle,* 8.3-Hz uniform simulation; *bottom,* 8.3-Hz PERG. Uniform ON-OFF ERG, uniform simulation, and PERG waveforms are plotted with the same scales.

simulation and 8.3-Hz PERG were also smoother, and they were reduced in amplitude compared with the response before TTX injection. The post-PDA and TTX steady state PERG amplitude was larger (roughly the same size as that in the control) than its counterpart in post-TTX only response in Figure 3C, where it was roughly one quarter the size in the control. The 8.3-Hz PERG waveforms in Figure 5B were large even after PDA and TTX (although not for another animal; Table 5). The large 8.3-Hz “PERG” may have occurred because it was dominated by ON bipolar cell responses that, due to the PDA, were no longer subject to inhibitory horizontal cell feedback, or cancellation by OFF bipolar cell responses, as would normally occur in the PERG. Unlike a normal PERG, the large ON bipolar cell-dominated response, which remained after PDA injection, was not greatly affected by TTX.

Effects of APB after PDA and TTX

After PDA and TTX injections, only nonspiking activity from photoreceptors and ON bipolar cells should have remained in the responses. APB was then injected to isolate photoreceptor responses (Fig. 5A, rightmost column), as had been done after APB + TTX. In both full-field ERGs and 2-Hz uniform-field flash responses, the enhanced ON b-wave responses were completely removed, leaving the negative-going photoreceptor response after all three blockers were injected. Only small resid-

ual P_1 responses were still evident in the simulation and the 2-Hz PERG was nearly abolished, supporting the earlier finding for the same mixture of pharmacologic agents that isolated photoreceptor responses will not generate a transient PERG.

Effects of APB on 8.3-Hz responses were similar to those observed in 2-Hz responses (Fig. 5B, rightmost column), and to the responses seen after the same drug combination in Figure 5. The ON response in the uniform-field ERG was removed by APB, leaving a negative-going photoreceptor response. The second harmonic, distorted by a higher harmonic, was still present in the simulated response, but PERG was hardly visible.

Relation between Positive and Negative Waves in Simulations and PERGs

Simulation of N_{95} (by N_2 in the present study) using uniform-field ON-OFF responses and averaging the PhNR_{on} and PhNR_{off} in those responses was previously described by Viswanathan et al.¹⁰ for macaques and by Simpson and Viswanathan³⁸ for humans. A similar approach was also used by Holden and Vaegan³⁹ to compare PERG and the sum of ON and OFF ERGs. In the present study, simulations were compared with transient PERGs for a series of additional interventions to separate ON and OFF pathway contributions to the PERG. We observed that changes in N_{95} of the 2-Hz transient PERG were

TABLE 4. Effects of Sequential Injections of APB, TTX, and PDA on ERG and PERG Waveform Amplitudes

ERG Type/Measure	Average (min, max) Amplitude (in μV ; $n = 3$)			
	Control	APB	TTX	PDA
Full-field				
b-wave	36.1 (14.1, 71.1)	Eliminated		
PhNR _{on}	52.7 (29.5, 86.8)	Eliminated		
d-wave	39.1 (37.3, 42.0)	81.2 (62.6, 107.9)	102.5 (98.6, 106.4)	Eliminated
PhNR _{off}	15.7 (4.8, 26.9)	56.8 (33.8, 71.2)	16.9 (0.9, 33.6)	Eliminated
2-Hz				
Uniform				
b-wave	11.6 (8.0, 15.6)	Eliminated		
PhNR _{on}	10.1 (7.2, 13.9)	Eliminated		
d-wave	5.2 (4.8, 5.9)	13.3 (7.1, 19.3)	16.5 (14.6, 19.6)	Eliminated
PhNR _{off}	8.0 (7.2, 9.5)	16.8 (15.9, 17.9)	3.2 (1.1, 6.2)	Eliminated
Simulation				
P ₁ peak	8.0 (6.6, 9.7)	4.5 (3.1, 5.4)	2.8 (2.1, 3.7)	1.5 (0.7, 2.0)
N ₂	6.2 (4.5, 9.1)	3.7 (1.6, 5.2)	1.1 (0.8, 1.3)	0.2 (0.0, 0.4)
PERG				
P ₅₀ peak	3.9 (2.2, 4.8)	1.7 (1.4, 2.0)	1.9 (0.7, 3.3)	0.6 (0.0, 1.2)
N ₉₅	6.0 (2.1, 9.9)	2.3 (1.3, 3.7)	3.2 (0.2, 0.7)	-0.1 (-0.6, 0.5)
8-Hz				
Simulation				
Second harmonic	6.1 (5.7, 6.9)	3.2 (1.2, 5.9)	2.9 (2.4, 3.3)	0.6 ($n = 1$)
PERG				
Second harmonic	4.2 (1.8, 5.5)	0.4 (0.3, 0.7)	0.8 (0.2, 1.3)	0.3 ($n = 1$)

d-wave: measured the same way as in Table 3; PhNR_{off}: measured the same way as in Table 3.

generally consistent with changes predicted in stimulations (N₂) regardless of the intervention(s). Data pooled from control recordings and recordings after each of the interventions were well fit by a linear function. The slope of the function was approximately 0.8, showing N₉₅ amplitude to be just slightly smaller than that predicted by the N₂; the correlation was strong ($r > 0.9$) and statistically significant ($P < 0.001$; Fig. 6A). This is consistent with a strong relationship between the two measures, likely through the same mechanisms of generation.

The same analysis was also applied to study the relationship between P₁ and P₅₀ peak amplitudes for the transient PERG (Fig. 6B), and between second-harmonic amplitudes in simulations and steady state PERGs (Fig. 6C). In both cases, the slopes were shallower than those for the negative-going responses, indicating that both P₅₀ and 8.3-Hz PERG amplitudes were smaller than simulated amplitudes, and the correlations between these paired measures were not as strong as those between N₂ and N₉₅, although both of them were statistically significant.

DISCUSSION

A major objective of the present study was to determine the relative contributions from ON and OFF pathways to the PERG of the macaque monkey, a good model for studying origins of the ERG in humans. Using APB, an analog of glutamate that suppresses ON pathway activity, we found that approximately half of each of the major waves of the transient (2 Hz, or 4 r/s) PERG was eliminated, indicating that ON and OFF pathways have nearly equally weighted contributions to the transient PERG, with contributions from the inner retina in responses at the timing of b- and d-waves contributing to P₅₀, and the PhNR in ON and OFF responses summing and dominating N₉₅. In contrast to this combined pathway response, the steady state (8.3 Hz, or 16.6 r/s) PERG was found to represent ON pathway activity almost exclusively. This occurred despite the presence

of a large negative signal in the uniform-field ON-OFF responses from photoreceptor and postreceptor OFF pathway after APB injection, indicating the relatively linear nature of that remaining ON-OFF response. One possible reason for the predominance of the ON pathway, as mentioned in the Results section in reference to Figure 2, is that the 8.3-Hz stimuli reversed too frequently for the OFF responses to develop as a separate wave.

A second goal of the study was to better define the role of spiking versus nonspiking activity (and that of ganglion cells) in generation of the PERG in the two pathways. Our findings confirmed previous reports that the N₉₅ of the transient PERG and much of the steady state PERG originate from spiking activity (of ganglion cells) in macaques. We also found indications that more distally generated, nonspiking activity can contribute to the PERG when specific pathways are compromised and this is addressed later in the discussion.

Similarities and Differences in Retinal Pathway Origins of PERG in Macaques and Mice

A similar study of transient PERG origins recently done in mice⁸ allows us to identify interspecies similarities and differences in pathway origins of the PERG between macaque and mouse (C57BL/6, which is a commonly studied mouse strain). The transient PERGs of the two species were similar in the sense that RGC lesions (experimental glaucoma with laser-induced ocular hypertension in macaques, and optic nerve crush and subsequent ganglion cell loss in mice) practically eliminated PERG, as does glaucoma (experimentally induced in monkey, genetically induced in DBA/2J mice).⁴⁰ These similarities confirm results from numerous other studies that indicate that PERGs in both species rely on functional integrity of RGCs.^{2,5,7-9}

Although the normal PERG originates from ganglion cells in both species, the relative contributions of Na_vs in the two

FIGURE 5. ERG and PERG after serial injections of PDA, TTX, and APB. **(A)** Transient ERG waveform changes after serial injections in one eye. *Columns 1:* PDA; *2:* TTX; and *3:* APB. *Rows:* *top*, full field-flash ERG; *second*, 2-Hz uniform ON-OFF ERG; *third*, 2-Hz uniform simulation; *bottom*, 2-Hz PERG. **(B)** 8.3-Hz steady state ERG waveform changes after serial injections in the same eye whose responses are in **(A)**. *Columns 1:* PDA; *2:* TTX; and *3:* APB. *Rows:* *top*, 8.3-Hz uniform ON-OFF ERG; *second*, 8.3-Hz uniform simulation; *bottom*, 8.3-Hz PERG. Uniform simulation and PERG waveforms are plotted with the same scales.

species were different. Blockade of Na_v s with TTX greatly reduced P_{50} ($>50\%$) in the macaque, but reduced P_1 in mouse (counterpart of P_{50} in human/macaque PERG) even more, to approximately 25% of control. For the negative-going waves, N_{95} in macaque was dominated by spiking activity; TTX practically eliminated it from the PERG, whereas in the mouse PERG, a significant amount of the N_2 (counterpart of N_{95}) remained after TTX injection.

The significance of the ON and OFF pathways in shaping the PERG was also different between the two species. In blockade of ON pathway light responses, APB removed about half of P_{50} and half of N_{95} in monkey PERG, indicating that both ON and OFF pathways contributed to generation of the entire transient PERG. In contrast, in mouse, APB completely eliminated P_1 but had little effect on N_2 . Thus in the mouse PERG, P_1 comes exclusively from the ON pathway and N_2 originates from the OFF pathway.

A TTX injection after APB conditioning removed the remaining N_{95} and even raised it over the baseline in monkey PERG. In contrast, although a cocktail of APB and TTX removed two thirds of N_2 in mouse PERG, a negative PERG was still present. This confirms that N_{95} in monkeys originates from spiking activity of both ON and OFF pathways, whereas N_2 in mice comes from both spiking and nonspiking activities mainly of the OFF pathway.^{8,10} In both species, the late time to peak amplitude (trough) and prolonged duration of the negative response suggest that glial currents are involved in generating

the response. There is support in rats⁴¹ and humans⁴² for glial involvement in generation of the PhNR of inner retinal origin.

Effects of PDA on the PERG were also quite different in the two species. Blockade of second-order hyperpolarizing neurons and third-order neurons with PDA practically eliminated N_{95} in macaque, but increased P_{50} . In contrast, in mice PDA reduced both N_2 and P_1 by $>60\%$. The differences in PDA effects between the two species are consistent with PDA causing much greater b-wave enhancement in macaques than that in mice for which ON bipolar cell contributions to the normal ERG are less opposed or inhibited by second-order hyperpolarizing neurons.⁴³ The failure of interventions that eliminate ganglion cell activity in only one or the other of the ON and OFF pathways to eliminate P_{50} in macaques, and both P_1 and N_1 in mice, would indicate that nonlinear activity created by the use of PDA prevented cancellation of responses from distal neurons that normally occurs in the transient PERG.

Utility of Uniform-Field Flash ERG Technique

The uniform-field protocol differed from typical flash protocols in that it used periodic stimulation, rather than having long intervals between flashes that allow slow responses, such as the PhNR, to fully recover before the next stimulus. PhNRs recorded with black-and-white uniform-field periodic stimuli of the same temporal frequency as the PERG stimulus had implicit times very close to those of N_{95} in PERG, in this and previous

TABLE 5. Effects of Sequential Injections of PDA, TTX, and APB on ERG and PERG Waveform Amplitudes in Two Eyes

ERG Type/Measure	Amplitude (μV) of the Responses Illustrated (Not Illustrated)			
	Control	PDA	TTX	APB
Full-field				
b-wave	1.2 (51.7)	114.4 (92.7)	92.5 (94.4)	Eliminated
PhNR _{on}	73.1 (38.4)	Eliminated		
d-wave	50.6 (43.2)	Eliminated		
PhNR _{off}	7.8 (24.2)	Eliminated		
2-Hz				
Uniform				
b-wave	6.8 (10.9)	32.0 (18.8)	28.5 (22.8)	Eliminated
PhNR _{on}	11.5 (11.1)	Eliminated		
d-wave	7.7 (5.7)	Eliminated		
PhNR _{off}	8.6 (10.3)	Eliminated		
Simulation				
P ₁ peak	6.4 (7.7)	19.6 (11.3)	11.1 (7.5)	5.4 (3.2)
N ₂	6.2 (8.0)	-0.5 (0.8)	-3.8 (-3.2)	-0.7 (0.0)
PERG				
P ₅₀ peak	2.5 (5.1)	11.5 (6.4)	5.6 (3.9)	0.4 (0.2)
N ₉₅	6.3 (7.8)	-1.2 (0.1)	-2.2 (-1.7)	0.0 (0.0)
8-Hz				
Simulation				
Second harmonic	5.0 (5.2)	8.8 (3.4)	3.2 (1.9)	2.2 (2.1)
PERG				
Second harmonic	3.3 (5.1)	5.5 (1.4)	3.7 (0.5)	0.5 (0.3)

d-wave: measured the same way as in Table 3; PhNR_{off}: measured the same way as in Table 3.

studies,^{10,44} most likely due to the similar periodic nature of both stimuli, as well as to other shared stimulus characteristics.

The waveform changes observed in the simulation generated by averaging ON and OFF responses of uniform-field flash ERG agree with those observed in PERG, particularly well for the negative-going N₂ and N₉₅ responses. The similarities between N₂ and N₉₅ in implicit times and amplitudes (Table 2) and the strong correlation between these two components (Fig. 6A) all indicate that N₂ from uniform-field simulation can serve as a good estimation of N₉₅ or even replace it as a tool to assess inner retinal function when a reliable PERG recording is hard to obtain. With appropriate stimulus conditions, the uniform-field flash response is larger than that of the PERG, does not require refractive correction, and is less affected by opacities in the ocular media and strict foveal fixation during recordings than those of the PERG.

Another benefit of the stimulus paradigm, not quite as well accomplished with use of intermittent flashed stimuli with

longer off times, is that the status of both ON and OFF cone circuits distal to the ganglion cells can be observed in the same recordings that are used to assess ganglion cell function by the PERG. Reversals of each element in pattern stimuli generate local ON and OFF responses, which are then averaged in the retinal circuits and recorded from the cornea as PERG. In contrast, the uniform-field flash ERG technique can record retinal ON-OFF responses right from the cornea, and the averaging process to generate a simulation of PERG occurs outside the retina. These differences could allow insights on mechanisms underlying PERG waveform changes in various disease and experimental conditions.

The simulations contained a larger P₁ signal than that of PERG P₅₀ in many cases (Fig. 6), such as in the example just cited. Part of the reason for this discrepancy is that the uniform field, although reversed at the same temporal frequency and having the same full luminance excursion as that of the PERG stimulus, was flashed ON and OFF, creating a global change of

FIGURE 6. Relations between waveform components in simulations and PERGs. (A) Relation between N₉₅ and N₂ amplitude. (B) Relation between P₅₀ and P₂ amplitude. (C) Relation between PERG second harmonic and Simulation second-harmonic response. The formula for linear regression is: PERG component amplitude = simulation component amplitude $\times$ ($a + b$). The open symbols represent results from normal control eyes; the filled symbols are from eyes with experimental glaucoma, or after injection of pharmacologic agent. The plots are square, with equal absolute ranges (24 μV) on both x - and y -axes.

mean luminance that filled the entire screen. Theoretically, modulation of the uniform field up and then down from a presentation of the mean luminance level would have made the luminance changes more similar to those introduced by the PERG stimulus. Although the size of the positive signals was larger in the simulation, insights about contributions to PERGs, such as those reported herein, were still possible.

Distal Retinal Contributions to the PERG When Retinal Function Is Perturbed

Sources distal to ganglion cells for P_{50} generation have been suggested to account for findings in patients with diseases other than glaucoma that affect the ganglion cells and optic nerve, such as optic neuritis after the acute stage, dominant optic atrophy,⁴⁵ and optic nerve resection,³ in which P_{50} is less affected than N_{95} in the transient PERG.⁴ In the present study on macaques, both severe experimental glaucoma and TTX removed N_{95} of the transient PERG, but P_{50} was eliminated only in severe glaucomatous eyes, but not in eyes injected with TTX (Fig. 4D vs. Fig. 5D).¹⁰ Effects in the same direction occur in the simulations (Fig. 4C vs. Fig. 5C) with some loss of P_1 in glaucoma, but none in the TTX-treated eyes. The TTX-insensitive contribution to P_1/P_{50} may reflect nonspiking RGC activity, which is lost in severe experimental glaucoma. However, it could also reflect activity from more distal retinal cells, if TTX altered their responses sufficiently that they would contribute nonlinearly, as occurs after PDA injections (described earlier), to averaged responses of opposite polarity that normally cancel in the PERG.

More specifically, TTX injections would have altered responses of neurons distal to ganglion cells that produce sodium spikes. For example, Na_v s are known to be present in some amacrine cells in macaques.⁴⁶ However, recent work in several species indicates the presence of TTX-sensitive voltage-gated sodium channels in bipolar cells as well, and in rodents, specifically in cone bipolar cells.^{47–54} Furthermore, preliminary ERG studies provide some evidence for the presence of Na_v s in cone bipolar cells in the macaque retina (Luo and Frishman, unpublished observations, 2010). Our finding that ON-OFF and PERG responses were affected by injection of TTX after PDA conditioning (Fig. 6) could be explained by the presence of Na_v s in ON cone bipolar cells, since PDA should have eliminated all OFF bipolar cells as horizontal, amacrine as well as ganglion cell responses.

APB results in the present study indicate that both ON and OFF pathways are of equal importance for the generation of 2-Hz PERG in monkeys. We expect that this will be the case for human PERG as well. This result is in apparent conflict with findings by Holder⁴ that retinal diseases that compromise ON pathway activity such as melanoma-associated retinopathy and X-linked CSNB eliminate 2-Hz PERG. It would be interesting to examine these patients with the uniform-field flash ERG technique to see if OFF pathway function is also affected. Abnormalities in ganglion cell activity of the OFF pathway have been documented in the NOB1 mouse, a mouse model for complete CSNB in which the gene for nyctalopin, a protein associated with the signal transduction cascade in ON bipolar cells, is mutated.⁵⁵

CONCLUSIONS

Uniform-field flash ERG and simulations were useful for investigating the retinal origins of PERG waveforms. The present study showed that ON and OFF pathways are of equal importance in shaping the transient PERG waveform in macaques. P_{50} in the 2-Hz (4 r/s) transient PERG originates from both spiking and nonspiking activity of ON and OFF pathways,

whereas N_{95} originates solely from the spiking activity of the two pathways. In contrast, the 8.3-Hz (16 r/s) steady state PERG originates mainly from spiking activity in the ON pathway. This study also provided insights about the generation of residual P_{50} in the transient PERG in cases where ganglion cell activity that normally dominates the PERG may not be present, and the linear cancellation of distal retinal elements to the PERG is perturbed.

Acknowledgments

The authors thank Bijoy Nair for his help with experiments and Ronald S. Harwerth and Nimesh Patel for allowing us to record from their macaque monkeys with unilateral experimental glaucoma.

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