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Habituation of Retinal Ganglion Cell Activity in Response to Steady State Pattern Visual Stimuli in Normal Subjects

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Abstract

Purpose—To evaluate autoregulatory changes of retinal ganglion cell (RGC) activity, as measured by the pattern electro-retinogram (PERG), when the eye is exposed to a steady state presentation of stimuli that maximize PERG amplitude and blood flow.

Methods—The PERG was recorded from both eyes of 14 normal subjects in response to steady state presentation (4 minutes) of contrast-reversing (16.28/s) gratings (1.6 cyc/deg) with different contrast (12%–99%) and mean luminance (40–1.3 cd/m²). One temporal period of the stimulus (122.8 ms) was sampled and averaged in packets of 50 sweeps (~15 seconds each). PERG amplitude and phase were evaluated by Discrete Fourier Transform and displayed as a function of time. Data were fitted with an exponential decay function to evaluate PERG changes with time.

Results—For patterns of 99% contrast, the PERG amplitude progressively decreased with time until reaching a plateau approximately 30% lower than the initial amplitude after approximately 2 minutes (habituation). The ratio between initial and plateau amplitude did not change by reducing the stimulus luminance by 1 log unit. However, reducing contrast decreased amplitude habituation. The habituation was abolished at 25% contrast.

Conclusions—Decrease of PERG amplitude with time is consistent with a slow adaptive change of RGC activity in response to high-contrast, steady state stimuli. The authors propose that the initial amplitude represents an index of RGC activity, and the plateau amplitude represents a dynamic equilibrium between RGC activity and the available energy supply. These results are relevant for a better understanding of glaucomatous optic neuropathy.

Neural activity has a high metabolic cost,^{1,2} reflected by the increase in blood flow and in oxygen^{3,4} and glucose consumption^{5–7} in the brain and in retinal regions where neural activity is highest. The precise relationship between electrical activity/metabolic demand of neurons (energy sink) and the associated vascular response (energy source), which provides the basis for functional imaging, is still a matter for debate.^{8,9} In addition, little is known about the constraints imposed by the available supply on physiological neural activity. Since the brain is separated from the general circulation by the blood–brain barrier and has a low energy-storage capacity,¹⁰ available supply depends almost entirely on the cerebral blood flow. Temporary reductions of cerebral blood flow may cause reversible losses of brain function. Chronic deficits in blood flow are believed to cause premature neuronal death.¹¹ This may be

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the case in some forms of glaucoma,^{12,13} because an adequate blood flow is necessary to sustain a high metabolic demand of retinal ganglion cells and their unmyelinated axons.^{14,15}

A neural ensemble must have the ability to maintain its activity within a range of conditions (referred to as *dynamic equilibrium*). The maintenance of dynamic equilibrium requires that a series of self-correcting mechanisms (*autoregulation*) of both neural activity and blood supply be active. The dynamic equilibrium depends on several factors including the metabolic demand of activated neurons, the available supply provided by the vascular system, the metabolic pathways involved,^{16,17} and the time constant of the source–sink connection. An extreme situation is when a neuronal ensemble has to cope with a metabolically challenging task of responding to a steady state stimulus that maximizes neural activity. Under these conditions, the metabolic demand of neurons may be larger than the available supply. Therefore, the neurons must reduce their activity to maintain a dynamic equilibrium compatible with the *energy budget* (e.g., Ref. 18).

Reduction of neural activity also occurs under conditions that do not necessarily depend on the energy budget. For example, the fast reduction in gain of visual neurons in response to high-contrast stimuli (*contrast gain control*) reflects rapid adaptive changes of the transfer function of visual neurons themselves at retinal^{19–24} and cortical^{25–28} levels. In this study, we describe a much slower reduction of retinal ganglion cell activity with time, as measured by the pattern electroretinogram (PERG). The spatiotemporal characteristics of patterned stimuli were chosen to maximize PERG amplitude and blood flow. The properties of this slow dynamic change of PERG amplitude differ from those of fast contrast gain control and seem to depend on the balance between energy demand and available energy supply.

Methods

Subjects

Subjects of this study were 14 normal individuals of both sexes and different age (mean, 32.4 ± 15 years). Ten of them participated in the study on the effect of contrast, and 10 participated in the study on the effect of luminance. Six of them participated in both studies. Subjects were free of systemic or ocular diseases as assessed by routine ophthalmic examination and had best corrected Snellen visual acuity of 20/20 or better. Subjects had refractive errors smaller than ± 3.0 spherical diopters and ± 1.5 cylindrical diopters. The methods applied in the study adhered to the tenets of the Declaration of Helsinki for the use of human subjects in biomedical research. Institutional Review Board/Ethics Committee approval was obtained for the study, and informed consent was obtained from each subject before recording.

Evaluation of Retinal Ganglion Cell Function by Means of the Pattern Electroretinogram

Retinal ganglion cell (RGC) function can be objectively evaluated by means of the pattern electroretinogram (PERG). The PERG is a special kind of ERG in response to patterned fields modulated in contrast, rather than to uniform flashes of light. RGCs are believed to be the main generators of the PERG, since selective RGC death alters the PERG waveform. Several studies in different experimental mammals with optic nerve lesions causing retrograde RGC degeneration^{29–32} and several case reports of human patients with corresponding clinical conditions^{33–35} have demonstrated a strong correlation between the PERG losses and RGC losses. A strong correlation between the PERG loss and RGC loss has also been reported in monkeys with experimental glaucoma.^{36,37} An important characteristic of the PERG is that it requires physiological integrity of viable RGCs. The PERG amplitude is markedly reduced during a transient blockade of RGC spiking activity induced by intravitreal injections of tetrodotoxin in cats³⁸ and monkeys.³⁹ The PERG amplitude is reversibly reduced in human

subjects by a transient increase of the IOP to 30 mm Hg or greater with a suction cup that causes a reduction of the vascular perfusion pressure.^{40–42}

Technique of PERG Recording

Traditionally, the PERG is recorded from metallic or carbon fiber electrodes in contact with the cornea or inserted in the conjunctival sac.⁴³ In this study, the PERG is recorded simultaneously from both eyes by means of small (10 mm diameter) skin electrodes taped on the lower eyelids (references ipsilateral temples, common ground central forehead) as recently reported.⁴⁴ Because it is known that the PERG is a response of small amplitude that needs robust averaging to be isolated from the background noise, it may seem counterproductive to use skin electrodes instead of corneal electrodes (thereby reducing the signal by a factor of about two).⁴⁵ However, the use of skin electrodes allows exceptional stability, which is necessary to evaluate slow temporal changes. Electrode instability may result in nonspecific changes of PERG amplitude and increased variability with time. As detailed herein, the signal-to-noise ratio of the steady state PERG recorded with skin electrodes and frequency analysis was high enough to characterize dynamic changes under a wide variety of stimulus conditions in subjects of different ages.

The spatiotemporal characteristics of the stimulus have been optimized to yield the highest amplitude in control subjects. On average, the peak spatial frequency was between 1 and 2 cyc/deg, and the peak temporal frequency was ~8 Hz, in agreement with previous data.^{46,47} The same optimal stimulus conditions elicit maximum vascular response from capillaries overlying the optic nerve head as measured by Laser Doppler Flowmetry (Logeant E, et al. *IOVS* 2002;43:ARVO E-Abstract 3314). The pattern stimulus consisted typically of horizontal gratings (1.7 cyc/deg, 25° diameter circular field, 99% contrast, 40 cd/m² mean luminance), reversed in counterphase at 8.14 Hz (16.28 reversals/s) and displayed on a TV monitor. The effect of contrast was studied by setting the contrast at different values (99%, 50%, 25%, or 12%) at a constant mean luminance of 40 cd/m². The effect of luminance was studied by adding neutral filters of increasing density (0.5, 1, and 1.5 log units) to each eye, whereas the visual stimulus on the display had 99% contrast and 40 cd/m² mean luminance. Signals were band-pass filtered (1–30 Hz), amplified (100,000-fold), and averaged in successive packets of 50 sums each (~15-second bins). Subjects fixated on a target at the center of the stimulus with the appropriate correction for a viewing distance of 30 cm. Subjects did not receive dilating drops and were allowed to blink freely. Typically, subjects underwent several recordings, each approximately 5 minutes long, during one session. The minimum intervals between successive presentations were 15 minutes, during which subjects were free to roam indoors while keeping the surface electrodes in place. None of the subjects reported visual strain or problems in maintaining fixation. Sweeps contaminated by eye blinks or gross eye movements were automatically rejected over a threshold voltage of 25 μ V. Typically, a couple of rejections per packet occurred. Because the PERG was recorded in response to relatively fast alternating gratings, the response waveforms were sinusoidal-like with a frequency corresponding to the reversal rate. Packets were automatically evaluated in the frequency domain by Discrete Fourier Transform (DFT) to isolate the component at the reversal frequency (16.28 Hz), whose amplitude in microvolts and phase in π rad were displayed as a function of time. Phase values are bound within $\pm 1 \pi$ rad. To avoid inherent discontinuity of phase data around 0 and $\pm 1 \pi$ rad, phase readings were automatically unwrapped by subtracting actual readings from the value modulo of 2π rad (2 minus actual reading). Phase values are thus bound between 1 and 3π rad without discontinuities. At the reversal rate of 16.28 Hz, the value modulo of 2π rad corresponds to 61.4 ms. Phase advances (shorter latencies) are associated with increasing values, and phase delays (longer latencies) are associated with decreasing values.

Temporal Dynamics of PERG in Response to Steady State Stimuli

An example of PERGs (packets of 50 sums or ~15-second bins) recorded simultaneously in both eyes of a representative subject is shown in Figure 1.

The pattern presentation (approximately 4 minutes) was preceded by an unmodulated uniform field (approximately 1 minute) of the same mean luminance (blank), which was used to evaluate the background noise level. In the control experiment shown above, the cycle blank-pattern was repeated three times to show reproducibility. To minimize transients at the onset of pattern presentation, the first five sweeps of the first packet were discarded. During the pattern presentation, the PERG amplitude was much larger than the noise and had a stable phase. During blank presentation, the phase assumed random values within the modulo. The interpacket variability of PERG amplitude was of the same order as the noise variability, indicating that the noise was the major source of amplitude variance with time. Even with a limited averaging of 50 sweeps, however, the signal-to-noise ratio and variance were adequate to evaluate dynamic changes of the PERG amplitude, in particular after interpolating data with a suitable function. The major feature of Figure 1 is that, during pattern presentation, the PERG amplitude slowly decreased with time and tended to level off after eight packets (~2 minutes). We defined this response decline with time as *habituation*, in accordance with previous reports of pattern visually evoked potential (VEP) amplitude diminishment with repetitive stimulation.^{48,49} To have an objective evaluation of the dynamic changes of PERG amplitude, data were fitted with an exponential decay function, $y = p + d \cdot e^{(-n/\tau)}$, where y is the PERG amplitude at any given packet, p is the plateau amplitude, d is the Δ between the peak amplitude and the plateau amplitude, e is the exponential symbol, n is the packet number, and τ is the time constant of the decay function. Using a double exponential function did not improve the fitting of the data. To normalize data among subjects, dynamic changes of PERG amplitude were expressed as the ratio r between the peak amplitude and plateau amplitude [$r = (p + d)/p$]. As shown in Figure 1, pattern-evoked dynamic changes of PERG amplitude were repeatable and did not show apparent signs of fatigue when an unmodulated blank field was interleaved between pattern presentations for approximately 1 minute (five packets).

Results

PERG Dynamics as a Function of Contrast

Figure 2 summarizes the average changes of PERG amplitude and phase with time as a function of decreasing contrast. For each individual, raw data of both eyes were averaged and used as a single entry for the group average.

At 99% and 50% contrast, the PERG amplitude displayed a clear habituation with time (Fig. 2A, 2B) that occurred at constant phase (Figs. 2E, 2F). The effect is statistically significant for 99% and 50% contrast (repeated-measures ANOVA, $P < 0.001$). When the contrast was progressively reduced, the PERG amplitude decreased, and the amount of habituation tended to decrease. Fitting data with an exponential function yielded the following habituation ratios and time constants: 99%: $r = 1.34$, $\tau = 6.8$ packets or ~103 seconds; 50%: $r = 1.32$, $\tau = 4.06$ packets or ~61 seconds. At 25% contrast, r is close to 1, and τ was not measurable. At 12% contrast, the PERG amplitude was close to the noise range, also indicated by the large variability of phase. The phase increased (advanced) with decreasing contrast from 99% to 25% by approximately 0.2π rad, corresponding to ~6 ms.

Exponential decay functions were also calculated for individual subjects (average of two eyes), and the parameters are displayed in Figure 3 as average $\pm$ SEM.

Note in Figure 3A that both the peak and the plateau amplitude increased linearly with increasing contrast, whereas the phase progressively lagged (Fig. 3C). An approximately linear

relationship between PERG amplitude and contrast has been reported.^{46,50} For fast reversal rates, the PERG amplitude may display accelerating contrast characteristics.⁵¹ As shown in Figure 3A, the peak amplitude was significantly larger than the plateau amplitude at 99% (paired *t*-test, $P < 0.001$) and at 50% (paired *t*-test, $P < 0.05$) contrast. At 25% contrast, the peak amplitude virtually coincides with the plateau amplitude. Figures 3B and 3D show the average ratios and time constants for 95% and 50% contrast, at which curve fitting was reliable. Ratios and time constant were significantly (paired *t*-test, $P < 0.05$) larger at 99% ($r = 1.3$, $\tau = 7.5$ packets, approximately 113 seconds) than at 50% ($r = 1.15$, $\tau = 4.19$ packets, approximately 62 seconds) contrast. Ratios and time constants displayed in Figure 3, obtained by fitting responses of individual subjects, are in very good agreement with those obtained by fitting average curves. Overall, the data presented in Figures 2 and 3 indicate a strong dependence of PERG amplitude habituation on stimulus contrast: the higher the contrast, the larger the habituation, the longer the time needed to get the plateau amplitude, and the longer the response phase.

PERG Dynamics as a Function of Mean Luminance

Figure 4 summarizes the average changes of PERG amplitude and phase with time in response to 99% contrast stimuli of decreasing mean luminance. Reducing mean luminance has no effect on stimulus contrast, typically defined as $(\text{Lum}_{\text{Max}} - \text{Lum}_{\text{Min}})/(\text{Lum}_{\text{Max}} + \text{Lum}_{\text{Min}})$. PERG changes are expected to reflect changes of activity primarily occurring in the photoreceptors, rather than ganglion cells. For each individual, raw data of both eyes were averaged and used as a single entry for the group average.

The PERG amplitude progressively decreased with decreasing luminance. At all luminances, the initial amplitude was higher than the plateau amplitude, indicating habituation. The effect is statistically significant (repeated-measures ANOVA) up to 1 log unit of luminance attenuation ($P < 0.001$; Figs. 4A–C) and borderline significant ($P = 0.07$) for 1.5 log units of luminance attenuation (Fig. 4D). At this low luminance level, however, the PERG amplitude was close to the noise range, also indicated by the large variability of phase (Fig. 4H). The phase decreased (lagged) by approximately $0.2 \pi \text{rad}$ (approximately 6 ms) when stimulus luminance was reduced by 1 log unit (Fig. 4E–G). Fitting average data with a single exponential function yielded the following habituation ratios and time constants: 0 log units: $r = 1.38$, $\tau = 6.6$ packets, or ~99 seconds; 0.5 log unit: $r = 1.37$, $\tau = 5.26$ packets, or 79 seconds; 1.0 log unit: $r = 1.46$, $\tau = 3.13$ packets, or ~47 seconds.

Exponential decay functions were also calculated for individual subjects (average of two eyes), and the parameters are displayed in Figure 5 as the average $\pm$ SEM.

Figure 5A shows that both the peak and the plateau amplitude decreased with decreasing luminance, as did the phase (Fig. 5C). The peak amplitude was significantly higher than the plateau amplitude at 0, 0.5, and 1.0 log unit of luminance attenuation (paired *t*-test, $P < 0.01$). Figures 5B and 5D show the corresponding average ratios and time constants. The ratios and time constants displayed in Figure 5, obtained by fitting responses of individual subjects, are in good agreement with those obtained by fitting average curves displayed in Figure 4. Ratios and time constants are not significantly different (paired *t*-test) at different luminance values.

Overall, data presented in Figures 4 and 5 indicate that PERG amplitude habituation was virtually independent of stimulus luminance over at least 1 log unit. In contrast, as shown in Figures 2 and 3, reducing the stimulus contrast by a factor of four abolished PERG habituation. This provides further support to the notion that the PERG is subserved by the activity of contrast-sensitive, rather than luminance-sensitive, generators.^{29,52} For stimuli of high contrast, the sustained activity of RGCs and their unmyelinated axons seems to have a

metabolic cost higher than the available energy budget and must settle to a lower level compatible with it.

Discussion

This study shows that the PERG in response to a steady state presentation of a high-contrast pattern stimulus of optimal spatial and temporal frequency displayed a slow decline of amplitude at constant phase, until reaching a dynamic equilibrium after approximately 2 minutes at a level approximately 30% lower than the initial amplitude. Amplitude habituation was not seen under ordinary conditions of PERG recording, due to the long averaging necessary to increase the signal-to-noise ratio. With the limited averaging we used, the signal-to-noise ratio of the PERG was lower, yet adequate to show dynamic changes with a time resolution of 15 seconds. That the signal-to-noise ratio is not a limiting factor to the detection and measurement of dynamic PERG changes is also demonstrated by the fact that, by reducing stimulus luminance, the signal-to-noise ratio progressively decreased, whereas the amount of habituation remained unchanged.

It seems unlikely that PERG habituation reflects passive changes of retinal electrical resistivity⁵³ resulting from stimulus-induced vasodilation^{54,55} and concurrent retinal stretching. First, the increase in vessel diameter (approximately 5%) is expected to cause minimal increase of retinal thickness. Second, the time constant of stimulus-induced vasodilation⁵⁶ is about one order of magnitude shorter than that of PERG changes (~10 seconds vs. >100 seconds). It seems also unlikely that PERG habituation is affected by unspecific physiological factors, such as declining accommodation, fixation accuracy, or level of vigilance. Indeed, the effect was specific for high-contrast stimuli. Habituation was no longer present with stimuli of 25% contrast or lower.

Habituation of PERG amplitude appears to be a process closely related to the activity of retinal neurons responding to contrast rather than luminance changes. Reducing the stimulus contrast abolished amplitude habituation, whereas reducing stimulus luminance had no significant effect on habituation. Neurons best responding to contrast changes are found in the inner retina, at the level of RGCs. Indeed, RGCs are believed to be the main generators of the PERG.^{29,52}

Initial rapid adaptive changes of RGCs to stimulus onset attributable to contrast gain control also cannot be excluded. Our paradigm, however, precluded their analysis, since the first second of acquisition was rejected to avoid transients at stimulus onset. In addition, the time resolution was necessarily limited to 15 seconds to achieve a suitable signal-to-noise ratio. However, the characteristics of PERG habituation are not consistent with the properties of rapid contrast gain control, or adaptation, described for retinal ganglion cells^{19–22} and cortical neurons.^{25–27} Contrast gain control is believed to represent a fast adaptive change of neurons themselves that scale the input contrast by the average local contrast and speed their kinetics. Accordingly, single units and VEPs show response saturation and phase *advance* with increasing contrast. In the present study, the PERG amplitude did not saturate with increasing contrast, and the phase *lagged*. In addition, the time constant of contrast gain control spanned from milliseconds to seconds,²⁸ whereas that of PERG habituation was longer than 1 minute.

VEPs also show habituation in normal subjects.^{48,49} The time course of VEP habituation spans from seconds to >10 minutes. Slow habituation of VEPs is believed to represent an adaptive mechanism to minimize elevated lactate levels in the visual cortex.^{48,57} VEP habituation, as well as habituation deficits in subjects with migraine, has implications for neurovascular coupling.⁴⁸

Overall, the contrast-dependent increase in PERG amplitude habituation and the corresponding increase in time constant and phase lag, together with the known increase in blood flow, appear

to be consistent with a slow adaptive change to the high metabolic demand of RGCs and their unmyelinated axons. Because of the limited energy-storage capacity of RGCs, the energy supply for neuronal demand must originate from external sources (i.e., vascular supply) and be transported and metabolized to the active energy sink.⁵⁸ This may explain the long-time constant of the process.

There is good evidence indicating that the increase in retinal and optic nerve blood flow after flicker or pattern reversal stimulation is a direct consequence of neural retinal activity. That is, the larger the flicker- or the pattern-ERG amplitude, the larger the blood flow (Logean E, et al. *IOVS* 2002;43:ARVO E-Abstract 3314).^{55,59,60} There is also evidence that the vascular-neural connection may represent a limiting factor for neural activity. The blood flow of the optic nerve head^{61,62} and the ocular perfusion pressure may be transiently decreased by applying a suction cup to the eye or by body inversion. Under these conditions, the steady state PERG amplitude in response to high-contrast stimuli is reversibly reduced in amplitude in a dose-dependent manner.⁴⁰⁻⁴² In these studies, however, the PERG was recorded with standard methods of long averaging, which did not allow determining the temporal dynamics of PERG changes.

In our study, habituation of PERG amplitude occurs under *physiological* conditions. This may suggest that a limited vascular reserve is normally available to match the metabolic demand of active RGCs. At low contrast, the metabolic demand of RGCs is easily matched by the available supply. By increasing the stimulus contrast, however, the metabolic demand of RGCs may be larger than the available supply. Therefore, RGC activity must settle to a lower level compatible with the energy budget. According to this model, the peak amplitude represents a specific index of RGC activity, and the plateau amplitude represents a dynamic equilibrium between RGC activity, metabolic demand, and available energy supply. Neural activity and hemodynamic changes may not overlap spatially in the brain.⁶³ In the primate retina, the capillary network is denser in proximity to the optic nerve head, where the optic nerve fiber layer is thicker.¹⁴ This implies a substantial metabolic demand from unmyelinated RGC axons for ion pumping, in keeping with the observation that the number of mitochondria⁶⁴ and oxidative enzyme levels⁶⁵ drop abruptly when the nerve fibers become myelinated proximally to the lamina cribrosa. The vascular system also presents different blood-brain barrier properties anterior and posterior to the lamina, possibly reflecting the different metabolic needs of the unmyelinated and myelinated fibers.¹⁵ In glaucoma the steady state (16 rev/s) PERG is reported to be substantially more reduced in amplitude than the transient (2 rev/s) PERG (e.g., Ref. 66). This difference between steady state and transient PERG in glaucoma may be understood assuming a higher energy demand under steady state conditions, compared with transient, which is not met in metabolically compromised glaucomatous retinas. This would result in a greater habituation (decreased averaged amplitude).

The energy budget model has a potential interest for a better understanding of glaucomatous optic neuropathy. In glaucoma, both mechanical and vascular factors have been thought to cause damage to the optic nerve head.^{12,67} In this context, determining PERG habituation, in combination with imaging and functional techniques to evaluate the diameter and flow in retinal vessels,⁶⁸ as well as their autoregulatory capacity in response to increased retinal activity,^{69,70} may represent an important tool to establish the relative role of neural and vascular factors.

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Figure 1.

PERG amplitude (*top*) and phase (*bottom*) during sequential presentation of either a blank field or a patterned field of high contrast (*bars over the x-axis, top*). During the pattern presentation, the PERG amplitude decreased, whereas the phase remained constant. During the blank presentation, the amplitude represents the noise level, and the phase assumes random values. Packets on the x-axis correspond to sequential averages of 50 sums (~15 seconds).

Figure 2.

PERG dynamics as a function of contrast. (A–D) The PERG amplitude decreased with decreasing contrast, together with the ratio between the initial and the plateau amplitude. (E–H) The PERG phase advanced with decreasing contrast. (A, *dashed line*) average noise level. Symbols and error bars represent the average and the SEM, respectively.

Figure 3.

(A) Average (±SEM) initial and plateau amplitudes evaluated from exponential decay fitting of individual eyes. Both the initial and plateau amplitude increased linearly with increasing contrast, however with a different slope. (B) The ratio between initial and plateau amplitude increased with increasing contrast. (C) The average (±SEM) phase lagged with increasing contrast. (D) The average (±SEM) time constant evaluated from exponential decay fitting of individual eyes increased with increasing contrast. (A, dashed line) Average noise level.

Figure 4.

PERG habituation for stimuli of 99% contrast at different levels of luminance attenuation with neutral filters of increasing density (0–1.5 log units [l.u.]). (A–D) The PERG amplitude decreased with decreasing mean luminance, whereas the ratio between the initial and the plateau amplitude tended to be constant. (E–H) The PERG phase lagged with decreasing mean luminance. (A, dashed line) Average noise level. Symbols and error bars represent the average and the SEM, respectively.

Figure 5.

(A) Average ($\pm$ SEM) initial and plateau amplitudes evaluated from exponential decay fitting of individual eyes. Both the initial and plateau amplitude increase with increasing *mean luminance* with an approximately similar slope. (B) The ratio between initial and plateau amplitude was not significantly different at different luminances. (C) The average ($\pm$ SEM) phase advanced with increasing mean luminance. (D) The average ($\pm$ SEM) time constant evaluated from exponential decay fitting of individual eyes was not significantly different at different luminances. (A, *dashed line*) Average noise level.