An Electrophysiological Comparison of Contrast Response Functions in Younger and Older Adults, and Those With Glaucoma

Jia Jia Lek, Bao N. Nguyen, Allison M. McKendrick, and Algis J. Vingrys

Department of Optometry & Vision Sciences, The University of Melbourne, Parkville, Victoria, Australia

Correspondence: Jia Jia Lek, Department of Optometry & Vision Sciences, The University of Melbourne, Level 4 Alice Hoy Building, Monash Road, Parkville, VIC 3010, Australia; jjlek@unimelb.edu.au.

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Purpose. Aging and glaucoma both result in contrast processing deficits. However, it is unclear the extent to which these functional deficits arise from retinal or post-retinal neuronal changes. This study aims to disentangle the effects of healthy human aging and glaucoma on retinal and post-retinal contrast processing using visual electrophysiology.

Methods. Steady-state pattern electroretinograms (PERG) and pattern visual evoked potentials (PVEP) were simultaneously recorded across a range of contrasts (0%, 4%, 9%, 18%, 39%, 73%, 97%; 0.8° diameter checks, 31° diameter checkerboard) in 13 glaucoma patients (67 ± 6 years), 15 older (63 ± 8 years) and 14 younger adults (27 ± 3 years). PERG and PVEP contrast response functions were fit with a linear and saturating hyperbolic model, respectively. PERG and PVEP magnitude, timing (phase), and model fit parameters (slope, semi-saturation constant) were compared between groups.

Results. PERG responses were reduced and delayed in older adults relative to younger adults, and further reduced and delayed in glaucoma patients across all contrasts. PVEP signals were also reduced and delayed in glaucoma patients, relative to age-similar (older) controls. However, despite having reduced PERG magnitudes, older adults did not demonstrate reduced PVEP magnitudes.

Conclusions. Older adults with healthy vision demonstrate reduced magnitude and delayed timing in the PERG that is not reflected in the PVEP. In contrast, glaucoma produces functional deficits in both PERG and PVEP contrast response functions. Our results suggest that glaucomatous effects on contrast processing are not a simple extension of those that arise as part of the aging process.

Keywords: contrast response function, glaucoma, aging, electroretinography, visual evoked potentials

Visual cortical neurons predominantly respond to contrast information rather than absolute luminance within the prevailing visual environment. In primary visual cortex (V1), single neurons typically—but not all—show a saturating response to increasing contrast.[1,2] That is, as stimulus contrast increases, the response produced by a given amount of contrast increases monotonically until it reaches a plateau. Varying response magnitudes to different contrast levels is a feature found in both cortical (V1) and pre-cortical (retinal and lateral geniculate nucleus, LGN) cells of the visual system.[3–6]

Although primarily a retinal ganglion cell disease,[7,8] the neurodegenerative changes in glaucoma extend to post-retinal structures including the LGN[9,10] and visual cortex.[11–13] Accordingly, functional visual processing deficits in glaucoma may arise from dysfunction of retinal and/or post-retinal structures. While it is well established that contrast sensitivity is reduced in people with glaucoma (most typically measured across the visual field using perimetry), perceptual measures of contrast gain signatures are also altered by glaucoma.[14–16] Although these contrast processing deficits have been measured using behavioral methods in patients with glaucoma, such perceptual exploration is very limited in the ability to disentangle whether such deficits arise from retinal and/or post-retinal sources.

In this study, we used pattern electrophysiology to investigate retinal and post-retinal contrast processing deficits in early glaucoma. Pattern electrophysiology provides an indirect measure of the neuronal response to contrast, with the pattern electroretinogram (PERG) providing a measure of retinal ganglion cell function[17] and the pattern visual evoked potential (PVEP) providing a representation of pooled responses from visual cortical neurons, including at the primary visual cortex, V1.[18] Previous studies in glaucoma patients report reduced PERG and PVEP responses to high contrast stimuli (>70%).[19–21] Given that natural vision requires interpretation of environments with a wide range of contrasts, we simultaneously measured PERG and PVEP responses from low to high contrast instead of measuring responses to a single contrast level. Previous electrophysiological research measuring responses elicited by a range of stimulus contrasts reports flatter PVEP contrast response functions in those with glaucoma,[22] although concurrently measured PERG data is lacking.

A secondary aim of our study was to compare PERG and PVEP contrast response functions between older and younger adults with healthy vision. Aging is an established risk factor for

Table. Characteristics of Participants With Glaucoma: ID Number, Age, Diagnosis (POAG: Primary Open Angle Glaucoma, NTG: Normal Tension Glaucoma), Moorfields Regression Analysis (MRA), Glaucoma Probability Score (GPS) Tool of the Heidelberg Retinal Tomograph II, and Average Defect (AD), Pattern Defect (PD) on the Central Test of the Medmont Perimeter

ID	Age	Diagnosis	MRA	GPS	AD	PD
G1	73	POAG	**	**	−0.84	11.9***
G2	77	POAG	**	**	−3.96*	18.31***
G3	63	POAG	**	*	−0.46	16.42***
G4	64	POAG	**	**	−0.96	20.61***
G5	73	POAG	**	**	−1.72	12.13***
G6	71	POAG	Normal	Normal	−1.7	10.46**
G7	62	POAG	**	**	−6.08**	0.65
G8	63	POAG	**	**	−3.58*	17.43***
G9	71	POAG	**	**	−2.39	9.03**
G10	61	NTG	**	*	−0.98	5.62*
G11	69	NTG	**	**	−0.65	4.58
G12	61	POAG	*	*	−0.39	0
G13	58	POAG	*	Normal	−3.17*	1.07

MRA and GPS: * Borderline, $P \leq 0.05$. ** Outside normal limits, $P \leq 0.01$.
AD and PD: * $P \leq 0.05$. ** $P \leq 0.01$. *** $P \leq 0.001$.

glaucoma.[23] Studies in older adults have shown that aging can result in neurological alterations at the retina including the loss of retinal ganglion cells,[24,25] as well as at the LGN[26] and visual cortex,[27] the same sites that undergo neurodegeneration in glaucoma. Healthy aging alters a range of perceptual contrast effects, including contrast detection,[28,29] contrast discrimination,[28] spatial contrast suppression[30,31] and contrast gain signatures.[32] Such perceptual effects are consistent with changes to both structural and neurochemical changes in the senescent visual system (for review, see McKendrick et al.[33]) In many ophthalmic conditions, the effects of aging and the effects of disease appear along a continuum. For example, the retinal nerve fiber layer thins somewhat with aging but is pathological with glaucoma; lenticular changes arise in most older adults but are pathological with cataracts. Such observations may lead to the expectation that any observed alterations to contrast response functions with age and those with glaucoma should exist on a continuum of change from those of younger adults. However, given the complexity of cortical visual neural circuitry involving substantive feedforward, feedback, and lateral connectivity (for reviews, see for example[34,35]) and the fact that aging appears to alter the cortical neurochemical environment that influences visual perception (rather than just structural change),[36] it is not necessarily clear that the effects of aging on the contrast response are similar in form to those of glaucoma. Our results demonstrate that the characteristics of altered retinal and post-retinal contrast response functions that arise from aging can be distinguished from those of glaucoma, revealing that glaucomatous post-retinal deficits are not simply an extension of aging changes.

Methods

Participants

All participants provided written informed consent in accordance with the University of Melbourne Human Research Ethics Committee approved protocol and all procedures complied with the tenets of the Declaration of Helsinki.

No previous studies have investigated the effects of aging and glaucoma on PERG and PVEP contrast response functions. Consequently, our a priori power analysis was based on previous literature comparing electrophysiological responses in those with glaucoma to age-matched controls. Specifically, we utilized simultaneous PERG and PVEP data from Parisi's study[19] at high contrast (80%) in glaucoma patients with mild visual field loss (Humphrey visual field analyzer mean deviation: −1.50 to −6.00 dB) and age-similar controls. Five participants in each group provided a power of 80% for detecting a significant reduction in PERG magnitude, while 9 participants in each group provided the same power for detecting a significant reduction in PVEP magnitude (alpha = 0.05). Hence, for the current study, we recruited at least 12 participants in each group to achieve a minimum power of 90% for establishing a difference at higher contrast levels between groups.

Participants were recruited via written advertisements placed in community newspapers and electronic newsletters associated with the University of Melbourne. In addition, people with glaucoma were recruited via Glaucoma Australia, Royal Victorian Eye and Ear Hospital, or the Melbourne Optometry Clinic (Australian College of Optometry). Fourteen young adults aged 22 to 31 years (mean ± standard deviation: 27 ± 3 years), 15 older adults aged 49 to 76 years (63 ± 8 years), and 13 people with early glaucoma (11 with primary open-angle glaucoma and 2 with normal-tension glaucoma) aged 58 to 77 (67 ± 6 years) participated. Details of the glaucoma participants are provided in the Table.

All participants underwent a comprehensive eye examination, including slit-lamp biomicroscopy, applanation tonometry, ophthalmoscopy, optic nerve head imaging with the Heidelberg Retinal Tomograph II (HRT; Heidelberg Engineering, Heidelberg, Germany) and visual field testing with the Medmont perimeter (Medmont Pty. Ltd., Camberwell, Vic, Australia). Inclusion criteria were: visual acuity of 6/7.5 (20/25) or better, and distance refractive errors of no more than ±6.00 D sphere and/or −2.00 D cylinder in the tested eye, no systemic or ophthalmological disease (other than glaucoma for glaucoma participants), and no medications known to affect vision. Non-glaucoma participants were required to have (1) optic nerve head imaging parameters within the one-tailed 95% range of the normative database of the Moorfields Regression Analysis (MRA) or Glaucoma Probability Score (GPS) tool, and (2) normal visual field results (Medmont Central test) as indicated by the average defect (AD) and pattern defect (PD) indices (within the one-tailed 95% range of the perimeter's normative database).

Participants with glaucoma had an ophthalmologic diagnosis consistent with glaucoma and were flagged as failing the visual field test (AD or PD $\leq$ 5% probability of falling within the normative database) or optic nerve head imaging assessment (MRA or GPS $\leq$ 5% of falling within the normative database), as shown in the Table. Glaucoma patients were using their current medications as prescribed by their ophthalmologist. Exclusion criteria were: a history of migraine, intra-ocular pressures of >21 mm Hg (for non-glaucoma participants), significant age-related lens changes (>NC 1.5 as classified using the LOCS III[37]), and unreliable visual field results (reliability indices $\geq$20%).

Electrophysiology Recordings

The PERG and PVEP were recorded simultaneously while participants viewed a black-and-white square-wave checkerboard generated on a gamma-corrected 21-inch CRT monitor (frame rate: 100 Hz; resolution: 1024 × 768 pixels; Trinitron G520; Sony Corp., Tokyo, Japan) by an Espion Electrodiagnostics system (Version V5; Diagnosys LLC, Cambridge, UK). Observers fixated centrally on a 0.5° diameter red square, wearing an appropriate refractive correction for the working distance (50 cm). A translucent occluder was used to cover the non-tested eye to minimize the effects of luminance adaptation during testing.[38] Observers were allowed to blink freely during the session and take breaks as required. Ocular lubricants were used for comfort when required.

The checkerboard comprised 0.8° checks, subtending 31° × 31° in total. The checkerboard reversed every 60 msecs (16.7 reversals/sec) with a square wave profile and one complete cycle (change from light to dark) occurring every 120 msecs (8.3 Hz, steady-state). The chosen check size[39,40] and reversal rate[41,42] are within the optimal range for detecting retinal ganglion cell dysfunction in glaucoma. To minimize adaptation effects, the six contrast levels (Michelson contrast: 4%, 9%, 18%, 39%, 73%, 97%) were presented from low to high contrast, and a spatially homogenous gray stimulus (0% contrast, mean luminance: 52 cd/m^2) preceded each change in contrast for approximately 1 minute (4 batches of 25 sweeps), which was used to define noise.[43] Each contrast level was sampled over a 480 msecs epoch (4 cycles) with signals collected in 8 batches of 25 sweeps (200 trials in total).

The PERG was recorded with corneal DTL carbon-fiber electrodes (Roland Consult, Brandenburg, Germany) placed near the lower limbus and referenced to an inactive silver-silver chloride electrode (Viasys Healthcare, Madison, WI, USA) at the ipsilateral canthus. Gold cup electrodes (Grass Technologies, West Warwick, RI, USA) were used for PVEP recordings. Three were placed on the head at O_z (10% of the nasion-inion distance above the inion), and at 5% nasion-inion distance above and below O_z, since previous studies have shown that the location of the calcarine fissure relative to the inion can vary between individuals.[44,45] Therefore, to take into account possible inter-individual variability due to anatomical variability, the largest response returned from the three locations was taken as the PVEP signal. The reference electrode was located at F_z (30% nasion-inion distance above the nasion) and the ground at C_z (halfway between the nasion and inion). Electrode impedance was kept below 5kΩ. Signals were amplified (100 times), bandpass-filtered (1.25 to 100 Hz) and digitized (1000 Hz) to 16-bit resolution. Blink artefacts (± 50 μV) were rejected post-hoc.

Data Analysis

Analysis of the contrast signal for all contrast levels in the second harmonic of the recordings (2F = 16.7 Hz, i.e., twice the stimulation frequency) was achieved offline using Microsoft Excel (Microsoft, Redmond, WA, USA). The time series data (480 ms) was resampled to yield 512 data points prior to a Discrete Fourier Transform.[46] The 2F PERG and PVEP magnitude in the Fourier domain was returned. The 2F phase was adjusted with an additive constant of 2π radians and bound within ±1π radians to avoid continuity. A decrease in phase of 0.1π radian corresponds to a 3 msecs signal delay in the time domain.

Figure 1 shows examples of PERG and PVEP waveforms obtained at 97% contrast for a younger and older healthy observer, and an observer with glaucoma (G12). Noise was returned as the upper 95% confidence interval of the 0% contrast stimulus (blank screen) that preceded signal collection for each group. As PERG magnitudes for low contrast stimuli often fell within the 95% confidence interval of noise, we conservatively excluded PERG data for 4%, 9%, and 18% from statistical analysis to ensure that noise signals were not mistakenly being interpreted as containing meaningful signal. All contrast levels for PVEP were outside the 95% confidence interval of noise.

Statistical analysis was carried out using SPSS (ver. 20; SPSS, Inc., Chicago, IL, USA). Data were tested for normality using the Kolmogrov-Smirnov test ($P < 0.05$). Mann–Whitney U tests were used as appropriate. When using independent t-tests, Levene's test was applied to check for equality of variances. To investigate the effects of aging, the healthy younger and older groups were compared, whereas the older and glaucoma age-similar observers were compared to evaluate the effect of glaucoma. Groups were compared by mixed repeated-measures ANOVA (mixed RM-ANOVA), where the between-subjects factor was group (Aging: young, older; Glaucoma: older, glaucoma) and the within-subjects factor was contrast (PERG: 39%, 73%, 97%; PVEP: 4%, 9%. 18%, 39%, 73%, 97%). Where data violated the sphericity assumption of repeated measures (Mauchly's test of sphericity), a Greenhouse-Geisser correction was applied. Results were considered statistically significant if $P < 0.05$.

Contrast Response Function Modeling

Individual data were modeled in Microsoft Excel to characterize the slope of the contrast response functions using a linear function (Equation 1) for PERG data:[47,48]

$$y(c) = mc + a \quad (1)$$

where $y(c)$ is PERG magnitude at a specific contrast, m represents the steepness of the function (slope), c is the contrast level, and a is the noise value of y when $c = 0$. Parameters m, and a were allowed to float during the optimization process.

PVEP data were fit with a monotonic saturating hyperbolic (Equation 2[49]), which best fits the non-linear contrast response functions of neurons in primary visual cortex, V1:[1,50]

$$R(c) = R_{max} \times \frac{C^n}{C^n + C_{50}^n} + R_0 \quad (2)$$

where $R(c)$ is the PVEP magnitude at a specific contrast, R_0 is the noise, R_{max} is the maximum saturated response, C_{50} is the contrast at which the response reaches halfway between baseline and maximum, n represents the exponent that determines the slope, and C is the contrast of the stimulus. Only n and C_{50} were allowed to float during the optimization process. R_0 was fixed to the noise magnitude at 0% contrast, and R_{max} was fixed to the average magnitude of 73% and 97% contrasts.[43,51]

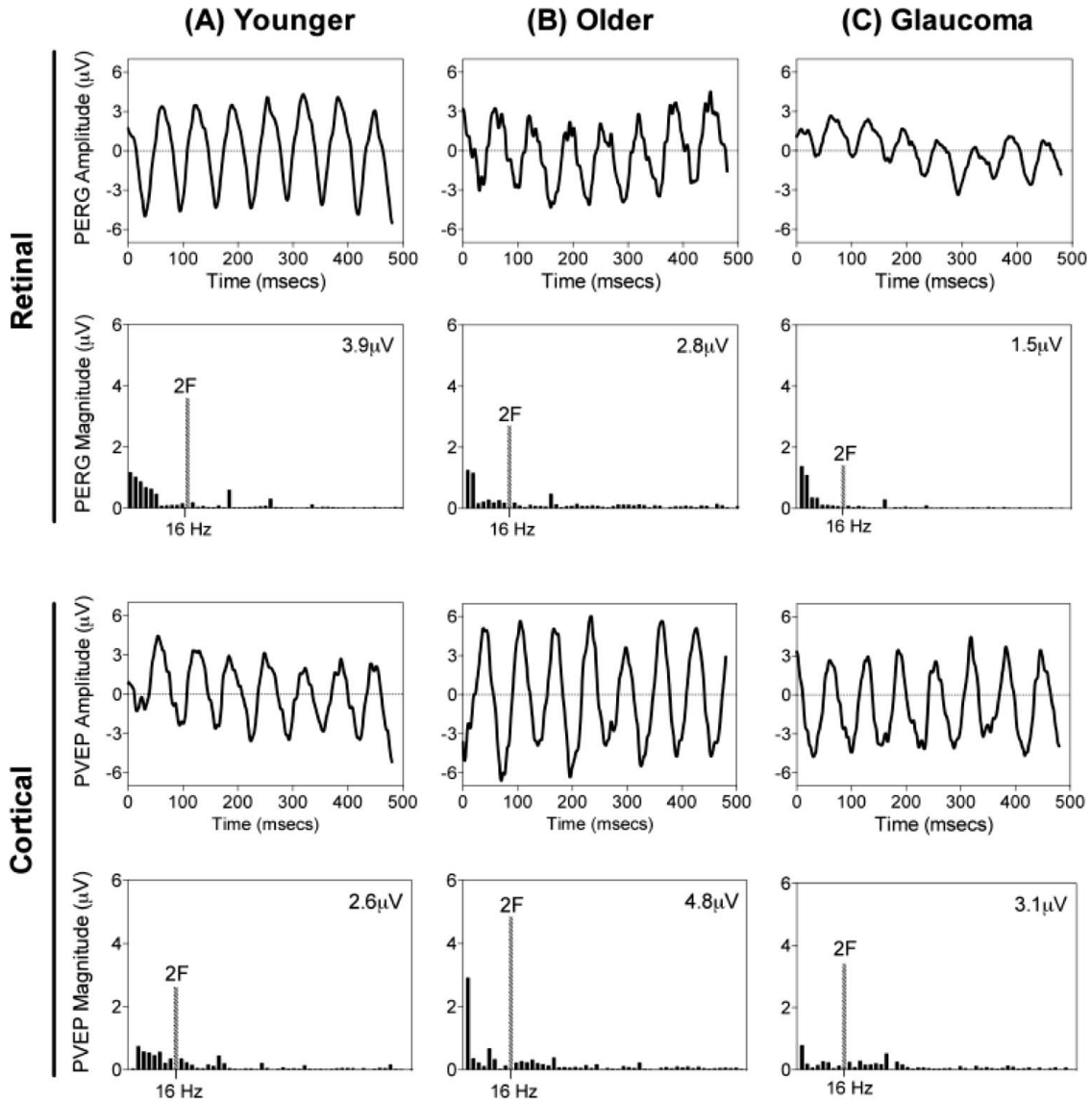

FIGURE 1. Representative PERG and PVEP steady-state waveforms in response to a 97% contrast stimulus recorded from (**A**) a healthy younger observer (31 years old), (**B**) a healthy older observer (57 years old), and (**C**) an observer with glaucoma (G12, Table, 61 years old). The magnitude of the 2F (16 Hz) component is shown in the *top right hand corner* of each Fourier domain panel.

RESULTS

PERG and PVEP Contrast Response Functions With Age

Noise levels at 0% contrast did not differ between age groups for both the PERG ($t(27) = -0.68$, $P = 0.50$) and PVEP ($t(27) = -0.80$, $P = 0.43$). Figure 2A illustrates the group mean PERG data for the six contrast levels tested. Using Equation 1, all participants showed the expected linear increase in PERG magnitude with increasing contrast, with the largest response observed at 97% contrast for both groups. Overall, raw PERG magnitudes were significantly reduced in older adults relative to younger adults (RM-ANOVA main effect of group: $P = 0.001$). A significant interaction ($P = 0.02$) between group and contrast was found, indicating that PERG slope differed with age. The slope of the older group was significantly flatter ($t(27) = 2.51$, $P = 0.02$) relative to the younger group (Fig. 3A). Figure 2E shows that when the raw PERG data of the older adults were normalized to the average PERG of the young group ([individual magnitude − younger group mean magnitude] / younger group mean magnitude), aging resulted in a significant reduction in PERG magnitudes (mean ± 95% CI = −29.48 ± 13.09%) (RM-ANOVA main effect of group; $P < 0.001$).

Figure 2B shows the PVEP contrast response functions and group mean data, indicating that, on average, PVEP magnitudes showed a monotonic saturating response in both younger and older adults. A significant interaction between group and contrast ($F_{1.79,48.43} = 4.22$, $P = 0.02$) was found, where the younger and older groups had similar magnitudes at the first two contrast levels (4%, 9%) followed by a trend for elevated magnitudes in the older group at higher (18%, 39%, 73%, 97%) contrasts. The individual model fits to Equation 2 (which assumes response saturation at high contrast) did not converge for one younger and one older participant, both showing lower PVEP magnitudes at high contrast levels (39%, 73%, 97%—also called "supersaturation," a relatively uncommon but normal finding in a small proportion of healthy observers[43]). Consequently, for these individuals, it was not possible to obtain a sensible estimate of PVEP contrast response slope (n)

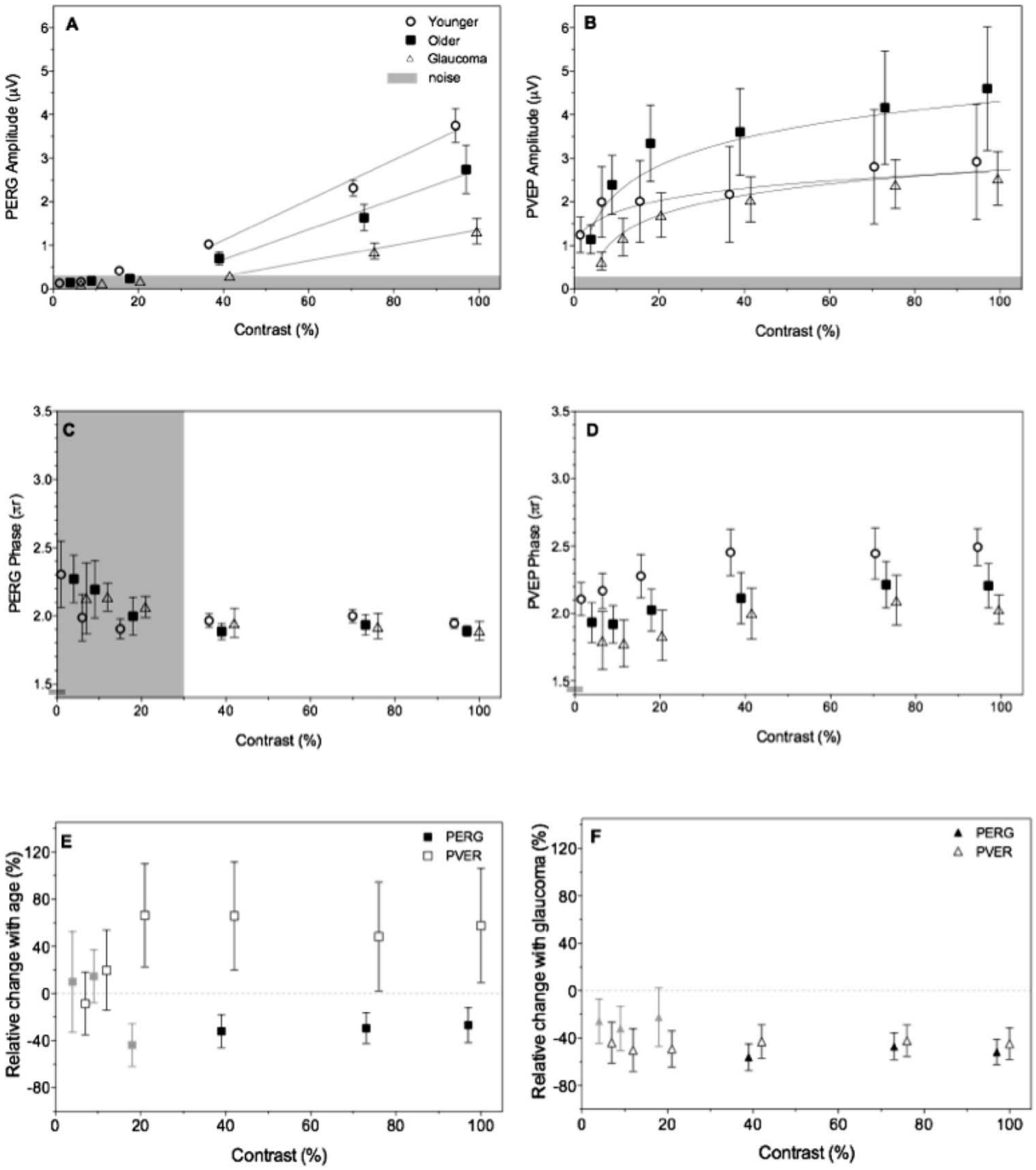

FIGURE 2. Group average retinal (**A**), (**C**) and cortical (**B**), (**D**) responses with aging and with glaucoma. Group mean (±95% confidence interval of the mean) PERG and PVEP magnitude (**A**), (**B**), phase (**C**), (**D**) as a function of contrast in younger (*circles*), older (*squares*) and glaucoma (*triangles*) observers. *Shaded regions* in (**A**) and (**B**) represent the upper 95% limits of noise levels as measured with 0% contrast, and in (**C**), data that have been excluded from statistical analysis due to lying in the region of noise. Relative change (mean ± 95% confidence interval) in PERG (*filled symbols*) and PVEP (*unfilled symbols*) with (**E**) aging and (**F**) with glaucoma. *Gray symbols* represent PERG signals that were in noisy regions. Data have been shifted horizontally for clarity.

and PVEP semi-saturation constant (C_{50}). For the remainder of the participants, the model fit well and the slope (n) and semi-saturation constant (C_{50}) of the PVEP contrast response functions were not significantly different between the younger and older groups (slope: Fig. 3B; $P = 0.59$; semi-saturation constant: Fig. 3C; $P = 0.79$).

Although younger and older groups returned a similar PVEP slope, older adults showed a trend for larger PVEP magnitudes

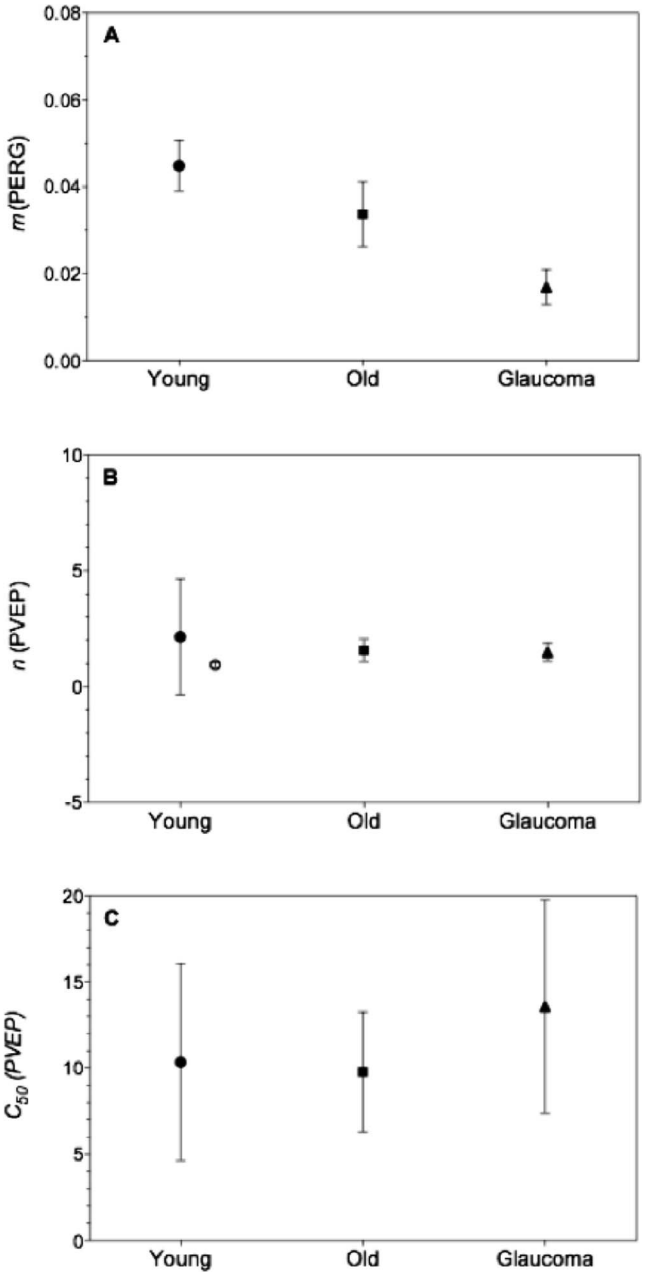

FIGURE 3. Group average for (**A**) PERG slope, and (**B**) PVEP slope, and (**C**) semi saturation constant (C_{50}) of younger (*circles*), older (*squares*), and glaucoma (*triangles*) groups. Error bars are 95% confidence intervals of the mean. Note that in Figure 3B, the error bars are largest in the young group (*filled circle*) due to a single observer's data returning a slope (n) of 17.2 (median of young group = 0.99). The *unfilled symbol* next to the young group data shows the mean ± 95% confidence interval of the mean without this observer included in the group.

(Fig. 2B). This trend did not reach statistical significance (no main effect of group: $F_{1,27} = 2.94$, $P = 0.10$). Figure 2E shows the same data but with the older adult data normalized to the average of the younger group. Aging resulted in minimal relative change at low contrast levels (4%, 9%) but substantial elevation in PVEP magnitudes at higher contrasts (18%, 39%, 73%, 97%). This is confirmed by a statistically significant interaction between group (younger, older) and contrast ($P = 0.004$).

In both young and older adults, PERG responses were similar in timing across mid to high contrast levels as shown in Figure 2C, while PVEP responses initially become faster with increasing contrast to reach a constant after approximately 20% contrast (Fig. 2D). Differences in signal phase between the younger and older groups with PERG (Fig. 2C) and PVEP (Fig. 2D) were consistent across contrast levels. This was confirmed by the absence of a significant interaction between group (younger, older) and contrast for both PERG ($F_{1.6,41.9} = 0.30$, $P = 0.68$) and PVEP ($F_{2.34,63.06} = 0.79$, $P = 0.48$) phase. The age related phase shift ($F_{1,27} = 4.76$, $P = 0.04$) translates to a significant delay (2.1 msecs) in the PERG response, and a further delay (10.8 msecs) of PVEP response ($F_{1,27} = 8.56$, $P < 0.01$).

PERG and PVEP Contrast Response Functions With Glaucoma

Relative to age-similar healthy controls, glaucoma patients did not show a significant difference in noise of the PERG ($t(26) = 1.33$, $P = 0.2$) and PVEP ($t(26) = 0.47$, $P = 0.65$). Overall, PERG magnitudes were reduced in individuals with glaucoma (Fig. 2A; $F_{1,26} = 23.31$, $P < 0.001$), with differences between groups increasing with contrast as indicated by the significant interaction (Fig. 2F; $F_{1.13,29.47} = 16.05$, $P < 0.001$) between group and contrast. Retinal contrast response function slopes (m) were significantly flatter in patients with glaucoma relative to age-similar controls (Fig. 3A; $t(26) = 4.06$, $P < 0.001$).

PVEP magnitudes of observers with glaucoma were also significantly reduced relative to controls as shown in Figure 2B ($F_{1,26} = 9.24$, $P = 0.005$). The interaction between group and contrast did not reach statistical significance ($F_{1.71,44.40} = 3.22$, $P = 0.06$), indicating that the reduction in PVEP magnitudes with glaucoma was similar across contrast levels. The cortical contrast response function slope (n) was similar between patients with early glaucoma and controls (Fig. 3B; $t(25) = 0.30$, $P = 0.77$). Semi-saturation (C_{50}) was not statistically significantly different between patients with glaucoma and older adults as shown in Figure 3C ($t(25) = -1.18$, $P = 0.25$).

Figure 2C shows no statistical difference in PERG phase between the older and glaucoma groups, indicating that glaucoma did not result in a change in timing ($F_{1,26} = 0.21$, $P = 0.65$). Figure 2D shows that although the glaucoma group demonstrated a trend toward slower PVEP responses relative to the older group, this difference was not statistically significant ($F_{1,26} = 1.50$, $P = 0.23$). There was no interaction between group and contrast for either PERG ($F_{1.6,42.9} = 1.30$, $P = 0.27$) or PVEP ($F_{2.65,68.93} = 0.34$, $P = 0.77$) phase responses.

Figure 2F shows that when the glaucoma group was normalized to age-similar controls ([individual magnitude – older group mean magnitude] / older group mean magnitude), there was a significant relative reduction in PERG (mean ± 95% CI = –51.67 ± 10.27) ($P < 0.001$) and PVEP magnitudes (mean ± 95% CI = –45.54 ± 19.94) ($P < 0.01$) that was not contrast dependent (no significant interaction with contrast: PERG: $P = 0.38$; PVEP: $P = 0.85$).

DISCUSSION

This study used simultaneously recorded PERG and PVEP for stimuli presented from low to high contrast to explore the nature of the contrast response function in younger and older adults, in addition to those with glaucoma. Specifically, we were interested in exploring whether the effects of glaucoma on the contrast response are similar but exaggerated versions of the impact of simple aging on contrast functions, at both the retinal and cortical levels. A simple prediction is that aging

might result in a reduction of amplitude of response for both retinal and cortical recordings and that glaucoma would exacerbate the same. However, relative to younger adults, older adults showed reduced (~29%) PERG magnitudes but elevated (~57%) PVEP magnitudes across mid to high contrast levels. Glaucoma resulted in a reduction in both in PERG magnitudes (~52%) and in PVEP magnitudes (~43%). Both PERG and PVEP deficits with glaucoma were not contrast dependent.

A reduced PERG response per unit contrast resulted in a flatter retinal contrast response function in older adults. This finding is consistent with reduced contrast gain signatures previously reported using perceptual methods.[32] At high contrasts, the reduction in PERG magnitude (~29%) with age found in this study is consistent with previous studies using high contrast stimuli.[52,53] Age-related reductions in PERG magnitude have been attributed to retinal ganglion cell loss with age,[24,54] although it is noted that the PERG can also be affected by optical degradation from senile miosis and age-related lens opacities.[55] We measured PERG and PVEP responses according to International Society for Clinical Electrophysiology of Vision (ISCEV)[56,57] standards where pupils are undilated. As we did not record the pupil size, we are unable to evaluate the influence of pupil size on these responses. However, we consider it unlikely that optical changes can completely account for the PERG magnitude reductions observed, given that the minimum age of the healthy "older" adults included in this study as age-similar controls for the glaucoma cohort was 49 years (i.e., not elderly). Moreover, all participants had normal visual acuity and no significant lens opacity, as determined during the screening eye examination.

Despite significantly reduced relative PERG magnitudes, there was no evidence for older adults in this study to demonstrate reduced PVEP magnitudes (Fig. 2B). We acknowledge that previous reports find PVEP responses are reduced with aging (see, for example, a review by Tobimatsu[58]) and that PVEP responses can be highly variable between individuals, as evidenced in our data. In an attempt to reduce the inter-individual variability in PVEP magnitudes, we used the largest signal captured by one of three electrodes. We also investigated whether any systematic differences could be due to gender[59]; however, we did not find any differences between males and females (data not shown). Nevertheless, when normalized to the younger adult PVEP, there was a trend for the older adult PVEP magnitudes to be elevated for mid to high contrast levels (Fig. 2E). These results are relevant to the specific stimulus parameters we used (0.8° check size or 0.625 cyc/deg spatial frequency, 8Hz temporal frequency) and may not be not directly comparable to previous literature.

Another difference worth noting between our study and previous electrophysiology literature is that the majority of previous simultaneous PVEP and PERG studies measure only a single contrast (typically high contrast >90% to evoke a strong retinal response), whereas we have measured responses to sequential patterns of increasing contrast. As noted by Nguyen et al.,[43] this method may have produced differential contrast adaptation effects that could influence the recorded PVEP magnitude, despite our attempts to minimize adaptation by presenting stimuli from low to high contrast and introducing a gray (0% contrast) homogenous background in between each contrast level. Nevertheless, to offer a perspective of how our results compare to other accounts of aging effects on PVEP magnitude, at lower temporal frequencies (4–6 Hz), older adults demonstrate lower PVEP magnitudes but a trend for higher PVEP magnitudes with increasing temporal frequency (above 8–10 Hz)[52,60] relative to younger adults. This trend for a "cross-over" effect is also seen when different spatial frequencies are tested, where aging produces a relative increase in PVEP magnitude for 1 cyc/deg and 3 cyc/deg spatial frequencies but not at 4 cyc/deg.[61,62] Thus, our results add to the general finding that age-dependent effects on steady-state PVEP are highly stimulus dependent.[58]

The precise mechanisms underpinning our finding of elevated PVEP magnitudes at mid to high contrast levels in older adults is unclear, and why there is an apparent compensation for some retinal reduction in signal amplitude cannot be ascertained by our electrophysiological methods. In interpreting changes to PVEP signals, it is important to keep in mind that PVEP depends on the integrity of the visual pathway from retina to cortex,[57] other visual cortical areas,[18] and feedback from extrastriate cortical areas to V1.[63] Still, a prevailing theory in the past decade regarding the effects of aging on visual processing is that there is less inhibition in older adult visual cortices. A simple model of reduced inhibition could possibly be proposed to explain the increased magnitude of cortical responses to the large high contrast stimuli used in our experiments. This theory has arisen from neurophysiological studies where some V1 neurons of older primates and cats show less orientation selectivity and direction selectivity relative to those of younger animals.[64,65] Furthermore, the orientation and direction selectivity of aged primate V1 neurons can, at least partially, be restored with local administration of inhibitory neurotransmitter agonists.[66] However, there is no direct evidence in humans for this proposal. Indeed, a recent study has shown elevated, rather than reduced, levels of the inhibitory neurotransmitter GABA in the visual cortex of older people.[36] Previous studies in patients with epilepsy[51] and migraine[43] have proposed that altered contrast response functions could be a result of an imbalance of excitatory/inhibitory processes, rather than a simple reduction in cortical inhibition. Additionally, as previously noted, care needs to be taken in interpreting PVEP signals as its contributions are not limited to V1.[18,57,63]

In this study, glaucoma participants demonstrated flatter retinal contrast response functions, a finding consistent with elevated contrast discrimination thresholds[14,28,67] and reduced contrast gain signatures found in perceptual studies.[14–16] The PERG magnitude loss (~52%) in our early glaucoma cohort was of a similar extent as compared to previous studies,[41,42,68,69] and is likely due to retinal ganglion cell loss[7] or dysfunction[70,71] in glaucoma. Interestingly, due to elevated PVEP magnitudes with age, PVEP magnitudes in participants with glaucoma were similar to those of younger adults. This finding demonstrates that care needs to be taken not to infer perceptual performance from PVEP amplitude alone and that it is important to consider possible aging effects when interpreting glaucoma data, a primary motivation for our study.

A novel aspect of this study was the simultaneous recording of PERG and PVEP across a range of contrast conditions. This method enabled us to compare the characteristics of the relative loss to PERG and PVEP signals in glaucoma across these two electrophysiological methods in the same patients to the same stimulus set. We were interested in examining whether there might be evidence for neuroplasticity in post-retinal structures in glaucoma, as suggested by animal models following localized or absolute loss of retinal outputs[72,73] and in animal models of glaucoma.[74,75] Our data show similar differences in PERG and PVEP relative to age-matched controls (Fig. 2F). Assuming that the visual pathway processes signals in a serial manner, the cortical deficit found in our early glaucoma observers might be a simple reflection of an upstream retinal deficit. As the current study only included observers with early glaucoma, the extent of cortical deficits relative to retinal deficits in patients with moderate to advanced glaucoma is unclear. To further improve our understanding of the disease

glaucoma, future studies should consider inclusion of participants with different stages of glaucoma and participants with monocular glaucoma to allow for comparison of responses between eyes.

Through the use of simultaneous PERG and PVEP recordings, we demonstrate that retinal signals and contrast response function slopes were reduced in older adults and further reduced in those with glaucoma. The effects of aging manifest very differently for cortical signals, with a trend of elevation in response magnitude in the older cohort for the specific spatial and temporal frequency tested (0.8° or 0.625 cyc/deg and 8 Hz). Glaucoma decreased responses relative to age-matched controls, resulting in a net effect of the PVEP magnitude for high contrast stimuli being similar between those with glaucoma and younger healthy adults with normal vision. These findings provide insight into the nature of contrast processing in aging and glaucoma and clearly demonstrate the importance of age-matched norms for the interpretation of visual electrophysiological results in glaucoma.

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