Retinal Responses to Simulated Optical Blur Using a Novel Dead Leaves ERG Stimulus

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Purpose. The purpose of this study was to evaluate retinal responses to different types and magnitudes of simulated optical blur presented at specific retinal eccentricities using naturalistic images.

Methods. Electroretinograms (ERGs) were recorded from 27 adults using 30-degree *dead leaves* naturalistic images, digitally blurred with one of three types of optical blur (defocus, astigmatism, and spherical aberrations), and one of three magnitudes (0.1, 0.3, or 0.5 µm) of blur. Digitally computed blur was applied to the entire image, or on an area outside the central 6 degrees or 12 degrees of retinal eccentricity.

Results. ERGs were significantly affected by blur type, magnitude, and retinal eccentricity. ERGs were differentially affected by defocus and spherical aberrations; however, astigmatism had no effect on the ERGs. When blur was applied only beyond the central 12 degrees eccentricity, the ERGs were unaffected. However, when blur was applied outside the central 6 degrees, the ERG responses were significantly reduced and were no different from the ERGs recorded with entirely blurred images.

Conclusions. Blur type, magnitude, and location all affect the retinal responses. Our data indicate that the retinal area between 6 and 12 degrees eccentricity has the largest effect on the retinal responses to blur. In addition, certain optical blur types appear to have a more detrimental effect on the ERGs than others. These results cannot be solely explained by changes to image contrast and spatial frequency content, suggesting that retinal neurons might be sensitive to spatial cues in order to differentiate between different blur types.

Keywords: blur, retina, defocus, spherical aberrations, astigmatism, myopia, pattern ERG, peripheral retina

Visual information processing begins at the retina as a response to a visual stimulus that is transformed and projected to the retina through the eye's optical system. The excitation of the photoreceptors initiates the photochemical cascade generating an electrical impulse that is then transmitted through neurons in the inner nuclear layer to different ganglion cell pathways toward subcortical and cortical areas. The retinal response to a specific stimulus can be measured using noninvasive electroretinography (ERG). A flash of light is sufficient to elicit a response from distal first- and second-order neurons. However, responses of ganglion cells recorded with the pattern ERG (pERG) depend largely on contrast variations across the retina,[1–4] due to center-surround receptive fields that respond to contrast and preferred spatial frequencies.[5]

The contrast and spatial frequency information of the retinal image depends on the quality of the optics of the eye.[6] Lower and higher-order aberrations alter the image content, resulting in blurry retinal images, and therefore reducing the retinal responses. Berninger and Arden,[7] showed that the pERG amplitude decreases as a function of dioptric defocus, with zero defocus (emmetropia) producing the highest response. Attenuated pERGs to defocus (with plus lenses) and diffuse blur (Bangerter filters) have also been found in chicks.[8] Chin et al.[9] have recently studied retinal responses to defocus blur as a function of the retinal region using global flash pattern multifocal ERGs (mfERG). They found a sign-dependent response to defocus in the pattern mfERGs, with the effect being stronger in near peripheral regions between approximately 9 degrees and 19 degrees eccentricity (mfERG rings 3 to 6). Their results, and other relevant results of accommodation responses in this retinal area,[10,11] suggest that the human retina has a decoding system for defocus located in the near retinal periphery. However, specifics on the retinal location that responds to blur, and research regarding how the retina responds to other types of optical blur at different retinal eccentricities are lacking.

Increasing evidence indicates that the quality of the retinal image plays an important role in the emmetropization process.[12–23] A blurred retinal image at specific retinal eccen-

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tricities might be the culprit for abnormal eye growth. Differences in flash ERG responses have been found between lens-induced and deprivation myopia in animal models,[24] suggesting two different mechanisms are involved in causing myopia. Flicker ERG studies in chicks also indicate that retinal responses to hyperopic and myopic defocus may be different.[25] A small study ($n = 8$) in humans found no significant effect of refractive blur (with plus and minus lenses) correction on mfERGs.[26]

Previous studies have measured retinal responses to blur using positive or negative ophthalmic lenses.[9,27–30] The use of these lenses primarily induces retinal defocus, but they also change the level of spherical aberration, as they affect accommodation, and have other optical effects, such as minification or magnification of the retinal image. When using ophthalmic lenses, blur is applied uniformly across the retina, hence making it impossible to disentangle the effect of different types of blur on different retinal locations.

In the present study, we digitally blurred stimuli by modifying specific Zernike coefficients to different areas of a digital image, in order to differentiate the effect of each specific blur type on specific retinal areas. It is worth noting that this method applies an ideal modification to the image, with assumed pupil diameter, and ignores additional aberrations introduced by the optics of the subjects' eyes, who were all optimally corrected for their low-level aberrations with ophthalmic lenses. We measured retinal responses to different types of simulated optical blur (defocus, spherical aberrations, and astigmatism) at a number of retinal eccentricities. The aims of this study are two-fold: first, by collecting retinal responses to different types of optical blur, we aim to identify those blur types to which the retina might be more sensitive. Second, by simulating blur at different retinal eccentricities, we will identify which retinal areas or zones might be more sensitive to blur. These results may have applications in future studies on understanding how blur affects abnormal eye growth and the development of myopia.

Methods

Subjects

Young adult subjects were recruited ($n = 27$, ages between 23 and 32 years, mean ± SD = 25.5 ± 2.5 years) for the main study. Five additional subjects were recruited for the steady-state ERG recordings (ages between 23 and 32 years, mean ± SD = 28 ± 4.6 years). All subjects were recruited from the New England College of Optometry (NECO) population. Subjects had normal healthy eyes based on a thorough ocular history and slit-lamp examination. Amblyopia, binocular vision or accommodative disorders, or eye diseases were all exclusion criteria. Subjects had best-corrected visual acuity of logMAR 0.00 or better in each eye. The study procedures followed the tenets of the Declaration of Helsinki and were approved by the NECO institutional review board (IRB). After the experiments and procedures were explained and any questions had been answered, the informed consent form was signed by the subjects.

Subjects had refractive error between +0.50 and −8.00 D (spherical equivalent for each eye) as measured with standard objective and subjective refraction. All subjects had less than 1.00 D of anisometropia (comparing the SE for each eye) and less than 1.75 D of astigmatism in each eye. Subjects with refractive error wore soft contact lenses with their optical correction (including astigmatism if present) appropriate for the viewing distance of the experimental testing. Sixteen subjects had myopia (-3.26 ± 2.25 D, mean ± SD) and 15 emmetropia (−0.16 ± 0.35 D, mean ± SD).

ERG Recording Protocol

For all ERG recordings, subjects wore their full refractive correction, as indicated above, and their pupils were not dilated. No anesthetic or cycloplegic agents were used that could alter the quality of the retinal image. The ERGs were recorded with a corneal DTL electrode placed along the lower eyelid margin of each eye. A 9 mm gold-cup skin electrode placed on the subject's forehead served as ground, while two ear clip electrodes placed on the subject's ear lobes served as reference. Electrode placement was in accordance with International Society for Clinical Electrophysiology of Vision (ISCEV) standards.[31,32] Subjects were under normal room lighting conditions (~600 lux) for at least 10 minutes for electrode preparation and explanation of procedures prior to testing.

The cone-driven retinal responses of both eyes were recorded simultaneously under dim photopic lighting conditions (~30 lux),[33] using an acquisition system (Diagnosys, LLC, Lowell, MA, USA) that was triggered externally by MATLAB. The amplifier bandwidth was set between 1 and 100 Hz, with an 8 times signal amplification and 1000 Hz sampling frequency. The 256 sweeps of 250 ms epoch each were averaged for each of the stimuli. The testing lasted approximately 2 hours, and subjects were given ample opportunities for breaks between each of the testing conditions.

Dead Leaves ERGs

When used for clinical applications, a pERG stimulus consists of a high contrast checkerboard with squares of varying size (usually 0.5 degrees or 1 degree visual angle).[32] For this study, we used a dead leaves stimulus (DLS).[34] These stimuli have the advantages that: (1) they contain a wide range of spatial frequencies throughout the entire image, and (2) the contrast between each individual image component can be varied independently. Therefore, these stimuli better resemble natural images than the traditional checkerboards.[35] Additionally, computational methods can be used to alter the type and magnitude of blur on the DLS. Although the DLS has previously been used for psychophysical tasks,[36,37] their complexity and computational manipulation have not been leveraged to study retinal function to date. Similar to a checkerboard, the DLS can be used to elicit a retinal response by either reversing its contrast or appearing in an on-off fashion.

The DLS used consisted of 2000 ovals of random orientation and sizes randomly drawn from a uniform distribution with a minimum of 0.1 degrees and a maximum of 3 degrees visual angle. The grayscale value of each ellipse was randomly drawn to a grayscale value between 0 and 255, giving rise to local variation in image contrast. After applying blur (see Supplementary Material S1), the luminance histogram of the image was rescaled to the range of 0 to 255 so as the contrast range always varied between 0 and 100%. The DLS was displayed on a CRT monitor (GDM-520 SONY Trinitron, refresh rate 120 Hz) controlled by MATLAB (MathWorks Inc., USA) and Psychtoolbox,[38] and subtended 30 degrees × 30 degrees of visual angle at a viewing distance

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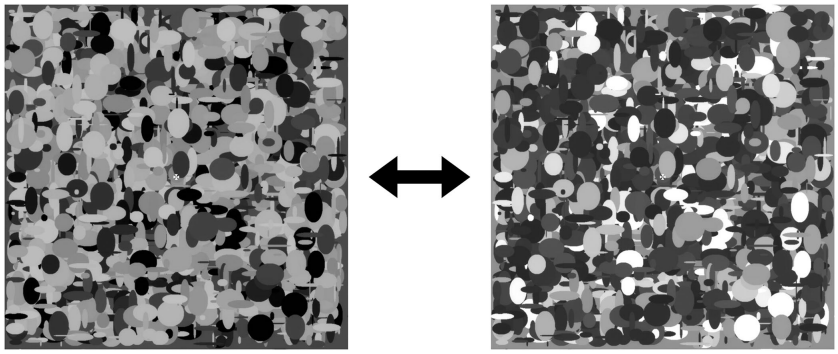

Figure 1. Example of contrast reversal for the DLS. Each DLS is shown for 250 ms before the other DLS is shown on the screen. The ovals change luminance in a decrement/increment fashion symmetrically from the average grayscale value of 127, resulting in a change in contrast polarity.

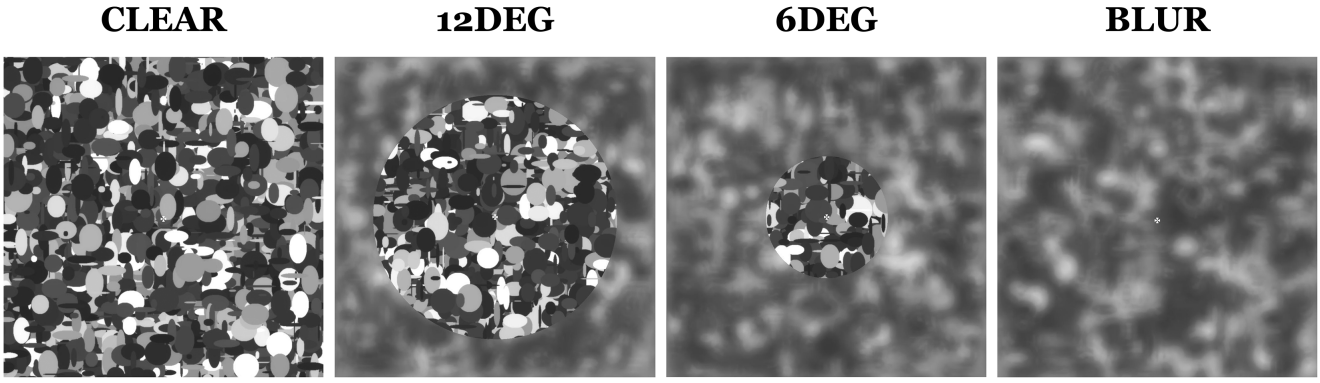

Figure 2. Illustrations of the four different blur spatial configurations of the DLS. The CLEAR image illustrates an unmodified image that is in focus at all eccentricities. The 12 DEG and 6 DEG images illustrate a stimulus in which the central 12 degrees or 6 degrees eccentricity (respectively) are in focus while the rest of the image is blurred. The BLUR image illustrates a stimulus that is uniformly blurred. The blur type of the three illustrated blurred images is DEF with 0.5 µm magnitude. A white 0.6 degree Maltese cross is placed at the center of the stimulus to facilitate fixation.

of 40 cm. The DLS was contrast-reversed at 2 Hz and had a mean luminance of 52.5 cd/m^2 (see Fig. 1). It is evident from Figure S6 that the grayscale distribution of the DLS is symmetrical around the average grayscale luminance, resulting in a steady mean luminance between reversals while contrast changes polarity.

The DLS were computationally convolved, using MATLAB, with three different types of optical blur: defocus (DEF), spherical aberration (SA), and oblique astigmatism (AST) with meridian at 45 degrees. The DLS were convolved in the Fourier domain, a Fourier transform of the pupil function was used to obtain the point spread function (PSF) that was then multiplied by the Fourier Transformed Image (see Supplementary Section S1 for a detailed description).[39] The aberrated DLS were obtained by performing an inverse Fourier Transform to create a spatial domain image. The level of aberration was quantified by the root mean square (RMS) wavefront error, the square of the coefficient of each Zernike polynomial function.[40] The resultant mean RMS levels calculated for a 5 mm pupil ranged from 0.1 to 0.5 µm. Based on Optical Society of America (OSA) guidelines,[40] it would be misleading to quantify blur in diopters because aberrations other than defocus are asymmetrical, depend on the pupil size, and are proportional to the focal distance from the retina.[39,41] For example, for a 5 mm pupil, 0.5 µm of defocus blur would be equivalent to approximately 0.17 D, for other pupil sizes this value would be significantly different (e.g. for a 2 mm pupil 0.5 µm defocus blur would correspond to approximately 1.09 D).

The specific types of optical blur used in this study are found naturally in all individuals as they view everyday scenes.[42] DEF is important for accommodation and depth scale estimation.[43–45] SA is also important for accommodation, and its effect in depth of focus is applied to intraocular lenses to improve presbyopia.[46] AST also affects the eye's depth of focus.[47,48] DEF, SA, and AST are all thought to play a vital role in eye growth regulation and refractive error development.[49]

Four different spatial blur/no-blur stimulus configurations were used, as illustrated in Figure 2. Each DLS subtended 30 degrees × 30 degrees and were, (1) clear with no blur (CLEAR), (2) blurred in its entirety (BLUR), blurred only beyond 12 degrees eccentricity (12 DEG), or blurred beyond 6 degrees eccentricity (6 DEG).

Three magnitudes of RMS blur (0.1, 0.3, or 0.5 µm) were used for each of the BLUR, 12 DEG, and 6 DEG conditions and each type of blur (DEF, AST, and SA). Therefore, subjects viewed 9 images for each type of blur and one clear condition for a total of 28 conditions presented in random order.

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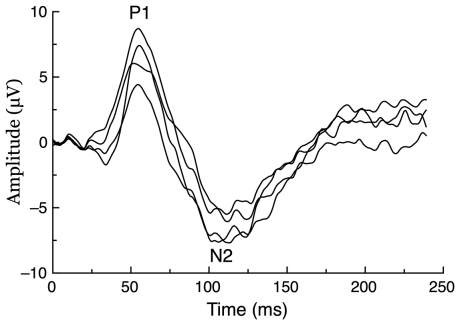

Figure 3. Four representative ERG traces recorded with the CLEAR DLS from four different subjects. The P1 indicates the first positive peak of the waveform, and N2 the second negative trough.

Each image had a 0.6 degree Maltese cross in the center to facilitate fixation (see Fig. 2)

Steady-State Dead Leaves ERGs

In traditional pERGs, the P50 and N95 originate from the activity of the retinal ganglion cells in both the ON and OFF pathways with some contribution of more distal neurons (namely cone bipolar cells) on the formation of P50.[2] Although the transient responses shown in Figure 3 are similar to pERGs recorded with a standard checkerboard stimulus, this does not itself guarantee that the origins of the responses we recorded are post-receptoral like in a pERG. To test whether our DLS indeed generates responses originating from the inner retina, we ran two additional experiments on five subjects.

A steady-state checkerboard stimulus used for pERGs elicits nonlinear retinal responses resulting from contrast change while there is no overall luminance change on the retina.[1,50] When there is a luminance imbalance on the steady-state checkerboard stimulus, then the retinal responses differ from the contrast-only responses; in essence, they are linear.

In the first additional experiment, we presented CLEAR DLS, identical to the images used in the main experiment described above (i.e. only contrast change and no luminance change), reversed 15 times per second (i.e. temporal frequency of 7.5 Hz). In the second experiment, we introduced a luminance imbalance on the CLEAR DLS by not forcing an average luminance on the stimulus. This stimulus still had contrast changes between reversals but also had a luminance change. The temporal characteristics of this stimulus were again 15 reversals per second (i.e. 7.5 Hz). One hundred sweeps, each 1000 ms long, were recorded and averaged for both these 2 additional experiments.

Data Analysis

Kolmogorov-Smirnov normality tests showed that both the P1 ($P > 0.059$, $t < 0.171$) and N2 ($P > 0.063$, $t < 0.169$) amplitudes across all conditions were normally distributed. A paired-sample *t*-test showed no significant differences between the responses of the two eyes for both P1 ($P = 0.071$, $t = -1.807$) and N2 ($P = 0.099$, $t = -1.654$), and therefore, we averaged the P1 and N2 amplitudes of the two eyes for each subject. The P1 and N2 amplitudes were then compared using a univariate general linear model ANOVA. The ERG amplitude was the dependent variable, and the refractive group (myopes/emmetropes), blur type, blur magnitude, and the retinal area being stimulated were fixed factors. Bonferroni corrections for multiple comparisons were applied for any pairwise testing. For the steady-state ERGs, a Fast Fourier Transform was performed.

Results

Dead Leaves ERGs

Figure 3 shows examples of ERGs recorded using DLS. Similar to checkerboard stimuli, our DLS generates a peak at around 50 ms (P1) and a trough around 100 ms (N2). For data analysis purposes, we used the amplitude of the P1 that was measured from the baseline and the amplitude of N2 that was measured from the P1.

The P1 and N2 amplitudes were extracted from all 28 conditions (Fig. 4) and for all 27 subjects. The statistical analysis showed that the P1 amplitude was affected by the type of blur ($F = 7.939$, $P < 0.001$), blur magnitude ($F = 13.025$, $P < 0.001$), and blur eccentricity ($F = 4.117$, $P = 0.017$). Similarly, blur type ($F = 19.248$, $P < 0.001$), blur magnitude ($F = 29.465$, $P < 0.001$), and blur eccentricity ($F = 11.614$, $P < 0.001$) affected the N2 amplitude. Refractive error had no effect on the amplitude of P1 ($F = 2.329$, $P = 0.127$) or N2 ($F = 3.125$, $P = 0.078$).

Figures 5A and 5B, and Supplemental Material S2, S3, S4, and S5 depict the ERG data collected (P1 and N2) with SA, DEF, and AST, respectively, for all the subjects. Figure 6 shows the average data for all the different conditions tested.

Retinal Sensitivity to Different Amounts of Simulated Blur

Pairwise comparisons between the ERG responses to the CLEAR and the BLUR image for each type and amount of blur for all subjects showed a decreased amplitude when the images were convoluted with 0.5 μm DEF for N2 responses only, and with 0.3 μm or 0.5 μm SA for P1 and N2 responses. There were no statistical differences between the CLEAR image and BLUR image for any of the AST conditions. Tables 1 and 2 summarize these findings.

Retinal Responses to Simulated Blur for the 12 DEG and 6 DEG Stimuli

We compared the effect of the retinal area stimulated for these five conditions in which we found a statistical difference between the CLEAR and BLUR stimuli (see Fig. 6, Table 3). When the simulated blur was applied only in the periphery, outside the central 12 degrees of an otherwise clear stimulus, no changes in the ERGs occurred compared to the CLEAR image: no statistically significant differences were found between the N2 responses of the CLEAR and 12 DEG stimuli for either DEF 0.5 μm, and SA 0.3 μm or 0.5 μm. In addition, no statistically significant differences were found between the P1 responses of the CLEAR and 12 DEG stimuli for SA 0.3 μm and SA 0.5 μm. Figure 6 and Table 3 summarize these results.

When the simulated blur was applied to the area outside the central 6 degrees, and the central 6 degrees eccentricity remained clear, a significant effect on the ERG responses was noted compared to the CLEAR image. N2 amplitudes for the CLEAR images were significantly larger than the responses to the 6 DEG stimuli for the three conditions: DEF 0.5 μm,

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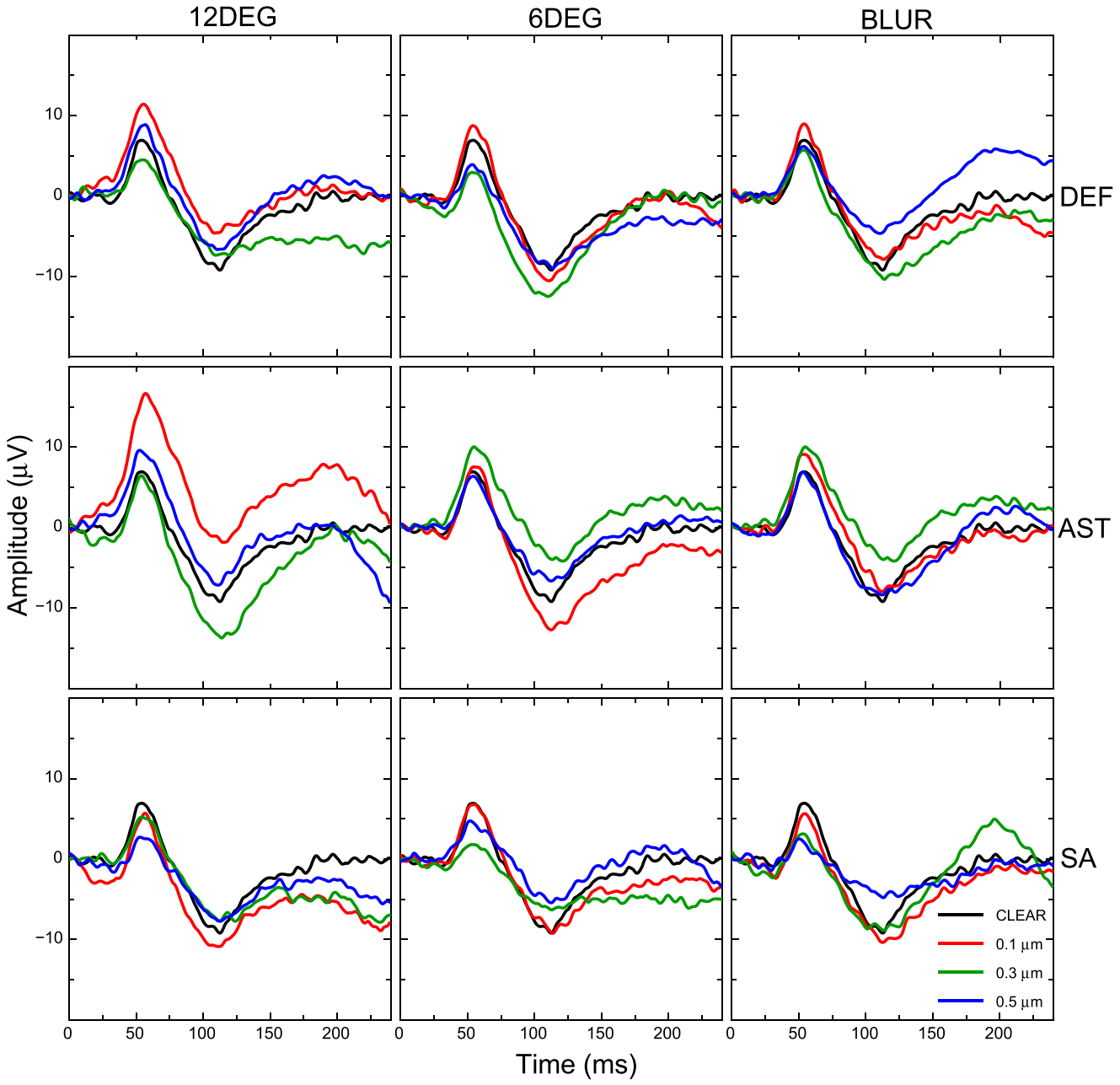

Figure 4. Representative averaged traces for all 28 conditions recorded from one eye of one subject. The top row shows the results of the DEF condition, the middle row of AST, and the bottom row of SA. The left column depicts the results of the 12 DEG spatial configuration, the middle column of the 6 DEG, and the right column of the BLUR. The colored traces correspond to different blur magnitudes (*red* is for 0.1 μm, *green* for 0.3 μm, and *blue* for 0.5 μm). The results of the clear condition (*black trace*) are shown on all nine graphs for comparison.

SA 0.3 μm and 0.5 μm; and the P1 amplitudes for the CLEAR images were larger for the 6 DEG stimuli, but only for the condition SA 0.5 μm.

The N2 responses for the 12 DEG were significantly higher than the BLUR conditions for SA 0.3 μm and 0.5 μm, however, no statistically significant difference was found between 12 DEG and BLUR for DEF 0.5 μm. The P1 responses differed between the 12 DEG and the BLUR stimuli for SA 0.3 μm but no difference was found for the SA 0.5 μm. No statistically significant differences were found between the 6 DEG and BLUR images for any condition, for either the N2 or the P1 data. Further comparisons between the ERG responses of different stimuli showed no statistically significant differences between the 12 DEG and 6 DEG stimuli for any of the five conditions for both the N2 and P1 data.

Retinal Responses to Different Types of Blur

Figure 7 depicts the P1 and N2 amplitudes for the BLUR images for DEF and SA and the three blur magnitude conditions. P1 and N2 amplitudes decrease as a function of blur

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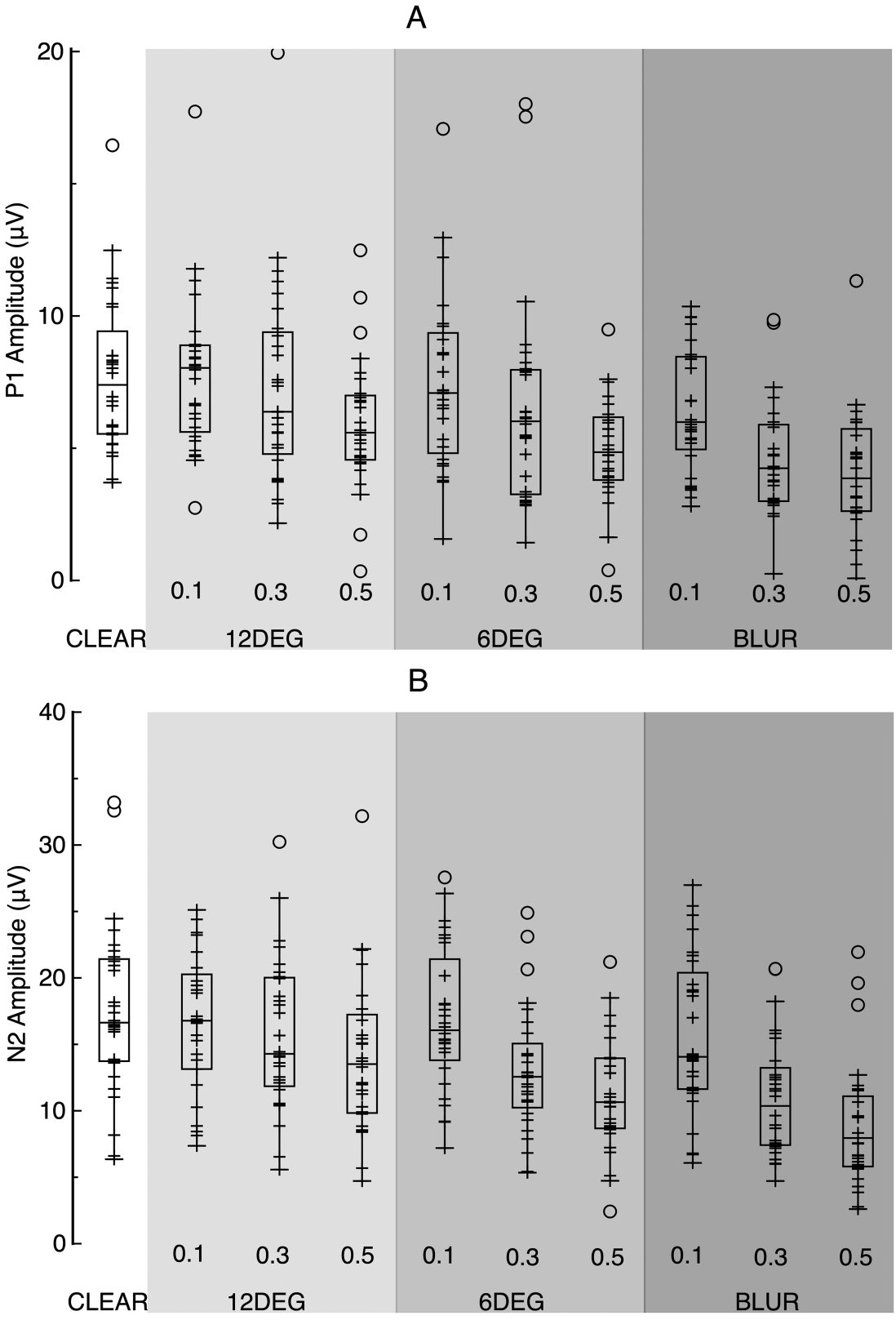

Figure 5. Box plots depicting the P1 (**A**) and N2 (**B**) amplitude for the SA conditions. The individual data points are shown with a cross. The open circles depict data points that are M ± 1.5*IQD, where M is the median, and IQD is the interquantile distance. For comparison, the CLEAR data are shown in this figure.

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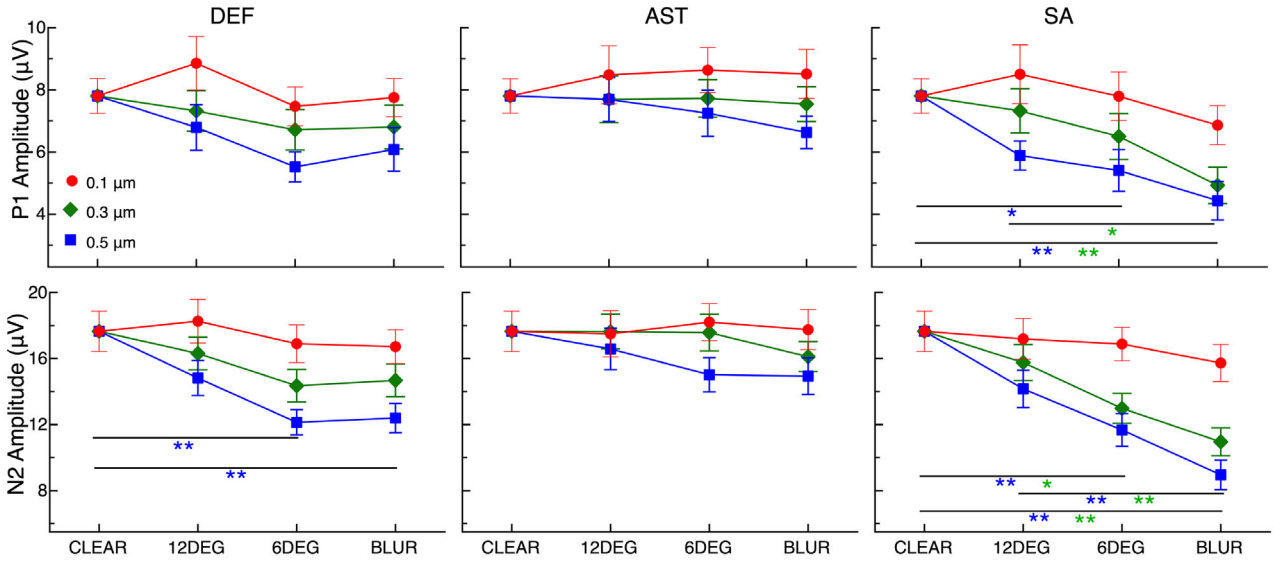

Figure 6. Average ERG P1 (top row) and N2 (bottom row) amplitudes for DEF (left column), AST (middle column), and SA (right column). *Red*, *green*, and *blue* data points correspond to 0.1, 0.3, and 0.5 μm blur magnitude, respectively. Asterisks indicate the statistical significance level (** for $P < 0.01$, and * for $P < 0.05$), and they are color-coded according to the blur magnitudes.

Table 1. Comparison Between CLEAR and BLUR Stimuli for Each Blur Type and Amplitude for P1

	DEF			AST			SA		
Blur Type Magnitude (μm)	**0.1**	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**
t value	0.279	1.052	1.625	0.623	0.340	1.332	1.026	**3.262**	**3.562**
P value	0.990	0.654	0.297	0.900	0.981	0.467	0.671	**0.006**	**0.003**

The bold font indicates a statistically significant difference.

Table 2. Comparison Between CLEAR and BLUR Stimuli for Each Blur Type and Amplitude for N2

	DEF			AST			SA		
Blur Type Magnitude (μm)	**0.1**	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**
t value	0.584	1.89	**2.466**	0.058	1.01	1.649	1.161	**4.515**	**5.744**
P value	0.793	0.112	**<0.001**	0.999	0.681	0.283	0.579	**<0.001**	**<0.001**

Table 3. Comparison Between Different Stimuli for Each Blur Type and Magnitude for P1 and N2

CLEAR versus	12 DEG	P1	N2
	DEF 0.5 μm		t = 1.756, $P = 0.233$
	SA 0.3 μm	t = 0.452, $P = 0.958$	t = 1.155, $P = 0.583$
	SA 0.5 μm	t = 2.386, $P = 0.062$	t = 2.095, $P = 0.118$
CLEAR versus	6 DEG		
	DEF 0.5 μm		**t = 3.833, $P < 0.001$**
	SA 0.3 μm	t = 1.876, $P = 0.256$	**t = 3.073, $P = 0.010$**
	SA 0.5 μm	**t = 2.428, $P = 0.038$**	**t = 3.792, $P = 0.001$**
12 DEG versus	BLUR		
	DEF 0.5 μm		t = 1.756, $P = 0.233$
	SA 0.3 μm	**t = 2.488, $P = 0.048$**	**t = 3.476, $P = 0.003$**
	SA 0.5 μm	t = 1.574, $P = 0.323$	**t = 3.604, $P = 0.002$**
6 DEG versus	BLUR		
	DEF 0.5 μm		t = 0.223, $P = 0.995$
	SA 0.3 μm	t = 1.507, $P = 0.361$	**t = 1.641, $P = 0.287$**
	SA 0.5 μm	t = 0.948, $P = 0.722$	t = 2.03, $P = 0.135$
12 DEG versus	6 DEG		
	DEF 0.5 μm		t = 2.059, $P = 0.127$
	SA 0.3 μm	t = 0.828, $P = 0.796$	t = 1.954, $P = 0.158$
	SA 0.5 μm	t = 0.442, $P = 0.961$	t = 1.647, $P = 0.284$

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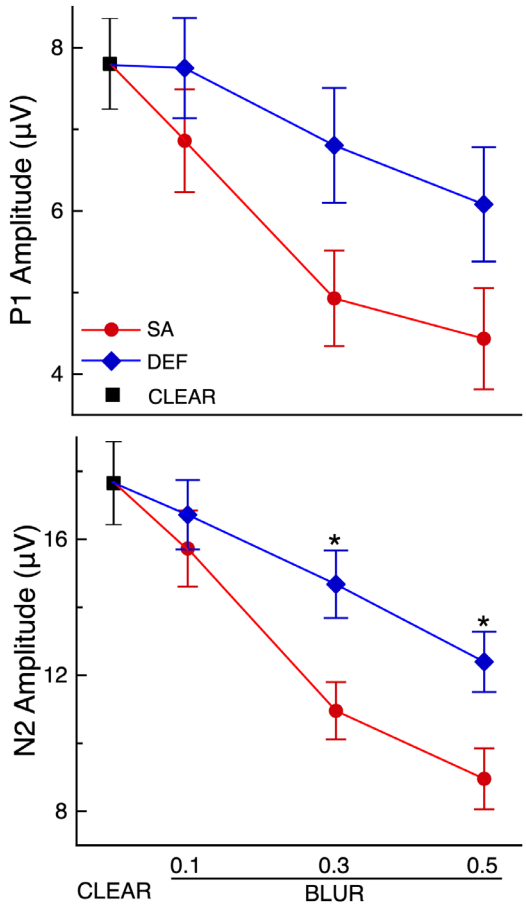

Figure 7. P1 (top) and N2 (bottom) amplitudes for CLEAR and BLUR images with DEF and SA. The *black square* corresponds to the ERG amplitude of the CLEAR image, the *blue diamonds* depict the amplitude of the DEF condition, and the *red spots* the amplitude of the SA condition. The error bars correspond to ±1 SE. The asterisks indicate the statistically significant differences between the SA and DEF conditions.

magnitude for both DEF and SA. With the same level of blur, the amplitude of the N2 responses for the BLUR images was higher for DEF than SA (0.3 μm: $t = 2.857$, $P = 0.018$; 0.5 μm: $t = 2.73$, $P = 0.025$; see Fig. 7). No differences were found in the P1 responses between the BLUR images of DEF and SA for 0.3 μm ($t = 1.892$, $P = 0.181$) or 0.5 μm ($t = 1.661$, $P = 0.279$).

Steady-State ERGs Using DLS

Figure 8 shows the ERG responses of one subject using the steady-state stimulation described in the Methods section. The top-left graph shows the steady-state response to a CLEAR DLS stimulus similar to the ones we used in the main study. The bottom left graph shows the steady-state response to a CLEAR DLS where we introduced a luminance imbalance. The graphs on the right column depict the Fast Fourier Transforms of the raw data. When stimulated with only a change in contrast between reversals (top line), the recorded signal had a temporal frequency of 15 Hz. In contrast, when we introduced luminance change between reversals, the recorded signal had a temporal frequency of 7.5 Hz. The results of the other four subjects are qualitatively identical to the results shown in Figure 8.

Discussion

In this study, we recorded retinal responses to DLS with different types and magnitudes of simulated optical blur presented at specific retinal eccentricities. The results show that the amplitude of post-receptoral responses decreases, in general, for images blurred with DEF and SA as a function of the blur magnitude. On the other hand, AST blur had no significant effect on the ERGs, with up to 0.5 μm of astigmatic blur applied to the stimuli in this study. Importantly, blurring the stimulus (with DEF or SA) only outside the central 12 degrees eccentricity did not alter the ERG amplitude. However, when blur was applied to the retinal area between 12 degrees and 6 degrees eccentricity, the ERGs amplitudes were significantly reduced and no different from the responses to images that were entirely blurred. These notable results suggest that the retinal area between 6 degrees and 12 degrees may be differentially sensitive to optical blur, compared to more central and more peripheral retinal areas. This finding might have implications for the emmetropization process, as discussed below.

The stimulus used in this study has not been used for recording ERGs before. Our stimulus has similar characteristics to the standard pERG stimulus, and the ERGs we recorded resemble the pERGs recorded with checkerboard stimulus. With our steady-state experiment, we showed that the retinal responses to our DLS are similar to the nonlinear responses recorded with a checkerboard stimulus, reinforcing our view that the ERGs we recorded are indeed post-receptoral in nature.[1,50] We should, however, emphasize here that the similarity of the responses we recorded with the standard pERGs and the nonlinearities we demonstrated with the steady-state ERGs are not substitutes for pharmacological studies to unequivocally pinpoint the origins of our signal.

As expected, we found that the amplitude of the ERGs decreases when blur is applied to the entire image (BLUR versus CLEAR), and this reduction is more pronounced with increasing blur magnitude.[27–30] Blur alters the spatial frequency and contrast content of the retinal image, and image analysis (see Supplementary Material S6) shows that for both DEF and SA, as the blur magnitude increases, the power of intermediate and higher spatial frequencies reduces, while the low spatial frequency content increases. Image analysis shows that for all three blur types, the grayscale value distribution differs significantly between the CLEAR and BLUR images of any blur type, which would result in a different frequency of contrast values across the images. It is known that the amplitude of post-receptoral responses decreases as luminance contrast decreases and as the spatial frequency content of the image shifts from intermediate spatial frequencies toward the lower end of the visible spatial frequency range.[4,7] Therefore, the reduction we observe in the ERG amplitudes could be due to the changes in the contrast levels and spatial frequency range of our blurred images. Whereas applying DEF and SA to the viewed images resulted in a significant reduction of the retinal responses, AST had no significant impact on the amplitude of ERGs. The high and intermediate spatial frequency component of the images' changes with AST, but there is little change for lower spatial frequencies. The distribution of the grayscale values of the AST images changes similarly to DEF and SA; hence the contrast content of AST images should change in a similar way. However, according to our results, the contrast content change in the AST conditions

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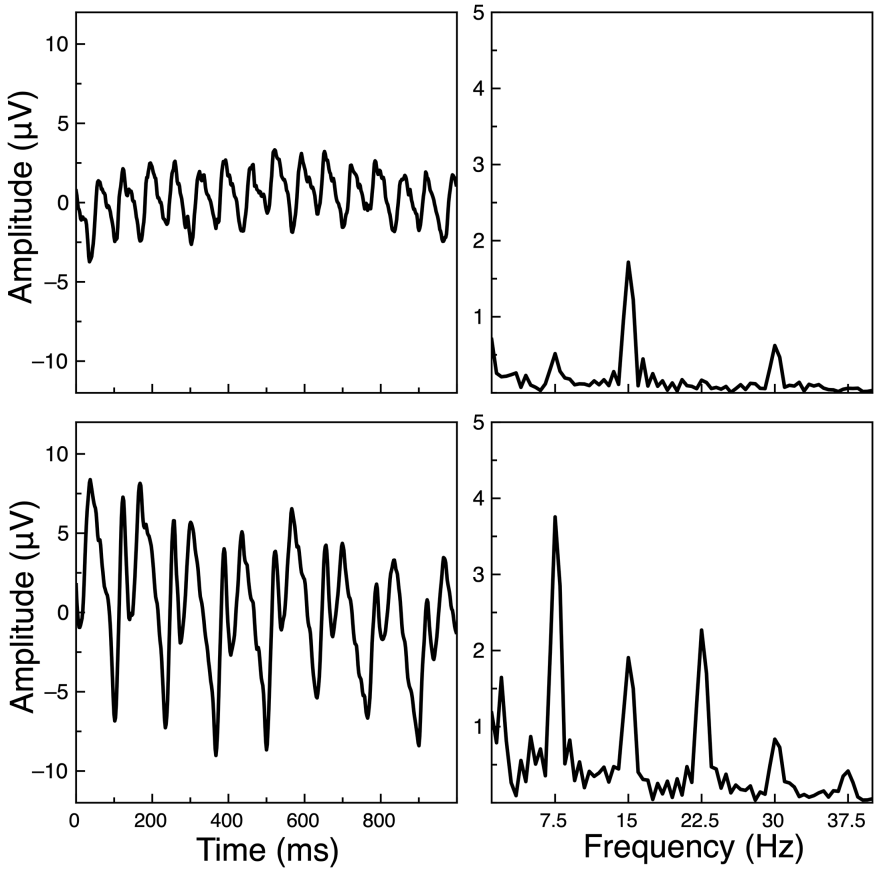

Figure 8. The left column shows the average data for the CLEAR DLS stimulus (top) and the CLEAR DLS stimulus with luminance imbalance (bottom). The corresponding Fast Fourier Transforms are shown on the right column.

is insufficient to reduce the ERG responses, even though orientation-sensitive retinal ganglion cells have been identified in the primate retina.[51] We speculate that the amount of astigmatic blur used in this study was insufficient to produce a reduction in the amplitude of the ERGs, and a higher AST magnitude is needed for a change in the retinal responses.

With our novel paradigm, we were able to selectively stimulate different retinal eccentricities with image blur. No differences in ERG amplitudes were found between the CLEAR and 12 DEG stimuli either for DEF or SA. These results indicate that DEF or SA, within the magnitudes used in this study, have no significant impact on the retinal responses when applied to retinal areas beyond 12 degrees eccentricity from the fovea. It is important to note that the amount of blur we used is perceivable, so the presence of blur should be encoded in the retinal response. However, when blur is applied closer to the fovea (beyond 6 degrees eccentricity), the retinal responses were significantly lower than the CLEAR image. Of most interest in our results is that there is no difference between the 6 DEG and BLUR conditions, suggesting that blurring the area between 6 degrees and the fovea has no additional detrimental effect on the retinal responses.

Given that no differences are found between (a) the CLEAR and 12 DEG conditions and (b) the 6 DEG and BLUR conditions, our findings indicate that the retinal area that significantly contributes to ERG amplitude reduction is between 12 degrees and 6 degrees eccentricity. Even though one would expect that when blur is applied to the entire image (BLUR), covering the central retina, there would be a larger reduction in the ERG responses compared to blur applied only up to 6 degrees eccentricity (6 DEG), this is not the case. In agreement with this finding, Ho et al.[29] found that using global flash multifocal ERGs and ophthalmic lenses to induce blur, the central retina is less sensitive to blur than the paracentral retinal area (6.5 degrees to 11.7 degrees eccentricity in their study). Similarly, a number of studies have found a significant effect of near peripheral blur (around 8 degrees eccentricity) in the accommodation response, even when the fovea is stimulated by a clear retinal image.[10,11] Our findings build on the previous studies and suggest that the retinal area within 6 degrees and 12 degrees eccentricity is most responsive to blur, and therefore might play a significant role in blur decoding for a normal emmetropization process. One might correctly observe that the change in the area that is blurred between the 12 DEG and 6 DEG conditions is much greater compared to the change in the area that is blurred between the 6 DEG and BLUR conditions. Therefore, the lack of statistically significant difference between the 6 DEG and BLUR conditions might be due to the lower number of ganglion cells that are stimulated between the two conditions, as a result of a lower signal-to-noise ratio. However, the ganglion cell retinal distribution and density in the human retina[52] negate the difference in area size, suggesting that our results are due to the applied blur and not due to the difference in the area size being blurred. We should note, however, that ERGs recorded with a patterned stimulus, are the results of retinal

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nonlinearities, and might not be directly related to ganglion cell density or count.

The retinal responses to SA for the BLUR condition are significantly lower than DEF for 0.3, and 0.5 µm blur magnitude (see Fig. 7), implying that the retina is more sensitive to SA than DEF. Image statistics show no significant differences in grayscale values between DEF and SA, and therefore we do not expect significant differences in the contrast content of the images. Hence, contrast alone could not possibly account for the ERG differences observed between stimuli blurred with DEF or SA. The spatial frequency content differs between DEF and SA in a systematic way. The difference in the high spatial frequency content between these two blur types could cause a difference in the retinal responses when blurring the image with SA (BLUR) because the central part of the retina that exhibits high spatial resolution would be stimulated optimally with SA but not DEF.

We tested adult observers with either emmetropia or myopia and found no differences in the retinal responses between the two groups. This is in disagreement with previous psychophysical studies that showed that myopes are less sensitive to peripheral blur,[10,16,37] which could indicate that the peripheral ERG responses of myopes might also differ from those of emmetropes. Of course, a direct comparison between retinal physiological responses and psychophysical responses is not possible, as many compensatory or adaptation/habituation mechanisms might be in play. The lack of difference between refractive groups in our study may be a consequence of testing adult subjects with stable refractive error. A recent study in children showed that when blur sensitivity was measured as depth-of-focus, children with progressing myopia had lower sensitivity compared with emmetropes.[17] A longitudinal study of sensitivity to blur and retinal responses to blur on children before they become myopic would be able to answer these questions and elucidate the discrepancies discussed above.

In conclusion, we demonstrated that retinal responses to different types of digitally blurred images can be decoupled and studied in isolation. DLS blurred with DEF, AST, and SA at varying blur magnitudes can be used to elicit retinal responses. DEF and SA significantly decrease the amplitude of adult observers' retinal responses; however, simulated AST does not affect ERGs, at least for the levels of blur used in this study. Our results indicate a retinal area between 6 degrees and 12 degrees eccentricity of increased sensitivity to blur for DEF and SA, whereas SA affects the retinal responses to a greater extent than DEF. To our knowledge, this is the first study to show greater retinal sensitivity for SA than DEF. SA might play a more important role in guiding the emmetropization process than DEF. Testing young children on the path for developing myopia with our novel paradigm would shed light on a peripheral retinal mechanism that is differentially sensitive to one type of blur than another and may lead to an abnormal emmetropization process.

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