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Electrophysiological measures of dysfunction in early-stage diabetic retinopathy: No correlation between cone phototransduction and oscillatory potential abnormalities

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Abstract

Purpose: To define the relationship between abnormalities in the activation phase of cone phototransduction and the oscillatory potentials (OPs) of the light-adapted electroretinogram in diabetics who have mild or no retinopathy.

Methods: Subjects included 20 non-diabetic controls and 40 type-2 diabetics (20 had no clinically-apparent diabetic retinopathy [NDR] and 20 had mild nonproliferative DR). Single flash responses for a series of retinal illuminances were measured under light-adapted conditions using conventional techniques. The a-waves of the responses were fit with a delayed Gaussian model to derive R_{mp3} (maximum amplitude of the massed photoreceptor response) and S (phototransduction sensitivity). OPs were extracted from the responses by conventional bandpass filtering.

Results: Analysis of variance (ANVOA) indicated that both diabetic groups had significant OP amplitude and S reductions compared to the controls, whereas R_{mp3} did not differ significantly among the groups. Although log OP amplitude and log R_{mp3} were significantly correlated for the control subjects for each flash retinal illuminance (all $r > 0.49$, $p < 0.03$), log OP amplitude and log R_{mp3} were not correlated for either diabetic group for any flash retinal illuminance (all $r \leq 0.36$, $p \geq 0.13$). Log OP amplitude and log S were generally not correlated significantly for the control or diabetic groups.

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Informed consent: Informed consent was obtained from all individual participants included in the study.

Conclusion: OP amplitude losses do not appear to be related to reduced cone sensitivity in early-stage diabetic retinopathy. This suggests that diabetes may separately affect cone function, as evidenced by cone phototransduction sensitivity losses, and inner-retina function, as evidenced by OP amplitude losses.

Keywords

electroretinogram; a-wave; oscillatory potentials; diabetic retinopathy

INTRODUCTION

Although diabetic retinopathy (DR) is classified clinically according to retinal vascular abnormalities, there has been growing interest in the effects of diabetes on the function of the neural retina (Adams and Bearse [1] and Lynch and Abramoff [2] provide recent reviews). Studies of neural abnormalities in DR have largely focused on responses of the inner-retina, with particular attention to the oscillatory potentials (OPs) of the single flash electroretinogram (ERG). The OPs are a series of high frequency wavelets that are superimposed on the ascending limb of the b-wave recorded under both light- and dark-adapted conditions [3–7]. The neural generators of the OPs are incompletely understood at present, but interactions among inner-retina neurons are thought to play a key role [8,9]. Previous work has shown that the OPs are sensitive to disturbances in retinal circulation [10] and these responses have been reported to be abnormal in DR, even at the earliest stages of the disease (see Tzekov and Arden [10] for a review).

In addition to abnormalities of the inner retina, previous electrophysiological studies have provided evidence for outer-retina dysfunction in early-stage DR [3,11–17]. In particular, there is evidence that the activation phase of phototransduction can be abnormal in these individuals [12,15]. For example, Holopigian et al. [12] measured dark- and light-adapted ERGs for a series of flash retinal illuminances. From these recordings, they derived measures of the activation phase of rod and cone phototransduction from the leading edge of the a-wave, based on the delayed Gaussian model of Hood and Birch [18,19]. This model provides two important parameters that are related to phototransduction: R_{mp3} , the maximum amplitude of the massed photoreceptor response, and S , which is related to the sensitivity of the activation phase of phototransduction. Holopigian et al. [12] found S reductions in 9 of their 12 diabetic subjects who had a wide range of retinopathy severity; only two of their diabetic subjects had reduced R_{mp3} .

Abnormalities at the photoreceptor level can potentially complicate the interpretation of the OPs. That is, if the photoreceptor input into the generators of the OPs is abnormal, then reduction in OP amplitude and/or alterations in timing may be expected. Much of the classic literature on OP abnormalities in DR does not comment on the characteristics of the a- and b-waves [20–23]. In OP studies that do comment on these characteristics, the a- and b-waves are typically reported to be normal in early-stage DR [24,25]. Holopigian et al. [12] addressed the relationship between OP implicit time and cone sensitivity ($\log S$) directly. In a sample of twelve subjects who had various stages of DR, nine subjects were shown to have $\log S$ and OP1 implicit time abnormalities. Of these nine subjects, the OP delay could be

accounted for by the reduced cone a-wave log S in six subjects, suggesting that the OP abnormalities were secondary to photoreceptor dysfunction. For the other three subjects, the OP delays were longer than those predicted based on reduced cone sensitivity, and additional post-receptor cone pathway losses were inferred. Thus, there appears to be variability among individual subjects in the extent to which diabetes selectively affects photoreceptor function versus both photoreceptor and inner-retina function. A number of unresolved questions arise from this previous work. For example, it is unclear how abnormalities in log S and R_{mp3} might affect OP amplitude, as previous work focused on OP timing [12]. It is also unknown whether the previously reported differences among DR subjects are due to disease stage and if a similar distribution of outer- and inner-retina dysfunction would be observed in larger sample of subjects.

The primary goal of the present study was to evaluate the relationship between the activation phase of cone phototransduction and light-adapted OPs in diabetics who have mild or no nonproliferative diabetic retinopathy (NPDR). We sought to determine whether abnormalities at the level of the cone photoreceptors (log S and log R_{mp3}) might affect inner-retina function in these individuals. The results of this study are intended to help clarify the nature and extent of abnormalities in electrophysiological function of the inner- and outer-retina in diabetics who have mild or no NPDR.

METHODS

Subjects

The study followed the tenets of the Declaration of Helsinki and was approved by a University of Illinois at Chicago institutional review board. All subjects provided written informed consent prior to participating. The ERG data that form the basis for the present report were obtained from a previous ERG study in diabetic individuals [15]. The sample consisted of 40 subjects (13 male and 27 female) diagnosed with Type-2 diabetes mellitus (DM) and 20 visually-normal, non-diabetic control subjects (8 male and 12 female). The mean ($\pm$ SD) age of the control group, diabetic with no DR group, and diabetic with mild NPDR group was 51.9 ± 12.2 , 52.0 ± 8.2 , and 53.7 ± 8.6 years, respectively; there was no significant difference in age among the subject groups ($F = 0.21$, $p = 0.81$). The subject characteristics are detailed in the original report [15]. In brief, histories were obtained from medical records and each subject received a comprehensive examination by a retina specialist. The stage of NPDR was graded and the subjects were clinically classified as diabetic with no clinically-apparent DR ($N = 20$) or diabetic with mild NPDR ($N = 20$) according to the early treatment of diabetic retinopathy study (ETDRS) scale [26]. Subjects classified as mild NPDR had retinal vascular abnormalities including: microaneurysms, hard exudates, cotton-wool spots and/or mild retinal hemorrhage (equivalent to ETDRS level 35 or less [26]). Other than diabetes, no subject had systemic disease known to affect retinal function, other ocular disease, or significant cataract. No subject had a history of panretinal photocoagulation, but three of the mild NPDR subjects had a history of anti-VEGF injection.

Procedure apparatus and stimuli

The procedure, apparatus, and stimuli are described in detail in the original report of these data [15] and are briefly reviewed here. ERG measurements from all subjects were performed monocularly, with the fellow eye patched. The pupil of the tested eye was dilated with 2.5% phenylephrine hydrochloride and 1% tropicamide drops. Single flash stimuli were generated by and presented in a ColorDome desktop ganzfeld system (Diagnosys LLC, Lowell, MA) [16,17]. The stimuli were full-field, brief (<1 ms) achromatic flashes of 2.8, 3.2, 3.6, and 4.0 log Td-s (assuming a dilated pupil diameter of 8 mm) that were generated by a xenon strobe. The xenon flashes were presented against an LED-generated achromatic adapting field of 3.3 log Td. ERGs were recorded with DTL electrodes, and gold-cup electrodes were used as reference (ear) and ground (forehead). A minimum of 5 responses for each flash retinal illuminance were obtained and averaged for analysis. Amplifier bandpass settings were 0.30 to 500 Hz and the sampling frequency was 2 kHz.

Analysis

The leading edges of the a-waves elicited by the four flash retinal illuminances were ensemble-fit using the delayed Gaussian model of Hood and Birch [19]. The Hood and Birch model describes the massed photoreceptor response of the cone system as a function of time (t) in response to a brief flash of light that has a retinal illuminance (I) as:

$$P3(I, t) = R_{mp3} \{1 - \exp[-IS(t - t_d)^2]\} \otimes \exp[-(t/\tau)], \quad (1)$$

where R_{mp3} is the maximum amplitude of $P3$ response, S represents the sensitivity of phototransduction, t_d defines the delay before the onset of the response, $\otimes$ represents convolution, and τ defines the time constant of a low-pass exponential filter due to cone outer segment capacitance. The value of t_d was fixed at 2 ms and τ was 1.8, for consistency with previous work [27].

The OPs were isolated from the responses to each flash retinal illuminance with a conventional finite impulse response bandpass filter (70–300 Hz pass band) that is described in detail elsewhere [28]. Four OPs were apparent in the response at each retinal illuminance. The individual trough-to-peak amplitude of each of the four OPs was measured and these amplitudes were averaged to provide a single mean OP (MOP) amplitude for each stimulus retinal illuminance. The peak time of each OP was also measured and these peak times were summed to provide a single measure of OP timing at each stimulus retinal illuminance. This previously used approach assumes approximately similar alterations in OP amplitude and timing in diabetes, which is consistent with findings of previous work [3].

RESULTS

Figure 1 shows the mean single flash responses for the control subjects (black), NDR subjects (green), and mild NPDR subjects (red) for the four stimulus retinal illuminances. From the mean waveforms, it is apparent that the control subjects tended to have larger a-waves than either diabetic group for all four stimuli. The b-wave was somewhat larger for the controls and had a slightly faster implicit time compared to both diabetic groups for the

2.8 log Td-s flash; OPs are clearly present on the ascending limb of the b-wave for all three subject groups for this stimulus. For higher flash retinal illuminances, the b-wave amplitudes are similar for the three groups (measured from the trough of the a-wave to the peak of the b-wave), but are slightly delayed for the diabetic groups compared to the controls. OPs are also apparent for each group at the higher flash retinal illuminances, but are somewhat less obvious for the diabetic groups for the 3.6 and 4.0 log Td-s flashes (e.g. the waveforms for the diabetic groups appear smoother).

Figure 2 shows the mean OPs extracted from the waveforms in Fig. 1 by band-pass filtering (color coding conventions are as in Fig. 1). The peaks of the four OPs obtained at each retinal illuminance are numbered. In general, the OP amplitude tended to be reduced for each of the OPs for the diabetic groups, as compared to the controls. Slight delays in OP peak time for the diabetic groups, compared to the controls, are also apparent in many of the responses. Overall, the later OPs (OP3 and OP4) were somewhat more affected in the diabetic groups, relative to the two earlier OPs.

Figure 3 provides a quantitative assessment of the mean waveforms shown in Figs. 1 and 2. Log a-wave amplitude (top left), log b-wave amplitude (middle left), and log MOP amplitude (bottom left) are plotted as a function of log stimulus retinal illuminance. The a-wave amplitude increased as the stimulus retinal illuminance increased from 2.8 to 3.6 log Td-s for all three subject groups; there were minimal differences in the a-wave amplitude elicited by the 3.6 and 4.0 log Td-s stimuli. In general, the controls had slightly larger a-wave amplitudes than the diabetic groups for all flash retinal illuminance, but the differences were statistically significant only at 2.8 and 3.2 log Td-s. Specifically, a two-way repeated measures ANOVA was performed to compare the a-wave amplitudes among the groups (subject group and flash retinal illuminance were included as main effects). The ANOVA indicated a significant difference among the subject groups ($F = 3.30$, $p = 0.04$). Holm-Sidak multiple comparisons indicated that the control a-wave amplitude was significantly larger than the NDR a-wave amplitude at 2.8 and 3.2 log Td-s (both $t > 2.55$, $p < 0.03$), but not at 3.6 or 4.0 log Td-s (both $t < 1.84$, $p > 0.13$). Holm-Sidak multiple comparisons also indicated that the control a-wave amplitude was significantly larger than the mild NPDR a-wave amplitude at 3.2 log Td-s ($t = 2.23$, $p = 0.03$), but not at 2.8, 3.6 or 4.0 log Td-s (all $t < 1.44$, $p > 0.15$). Thus, there were small a-wave amplitude losses for the diabetic groups that reached statistical significance for some, but not all, flash retinal illuminances. The open black circles show the effect of shifting the control data rightward by 0.4 log units, which aligned the control and diabetic amplitudes. This finding indicates that the diabetic a-wave amplitude can be simulated in the control subjects by reducing the flash retinal illuminance by 0.4 log units (a factor of 2.5). As discussed further below, this is consistent with a cone sensitivity loss in the diabetic subjects.

The b-wave amplitude decreased as the stimulus retinal illuminance increased from 2.8 to 4.0 log Td-s for all three subject groups. This is opposite of the pattern observed for the a-wave, but is expected as the flash retinal illuminances are in the “photopic hill” range [29]. In general, the controls had slightly larger b-wave amplitude, compared to the diabetic groups for the 2.8 log Td-s flash. The b-waves for the three groups were highly similar for the higher flash retinal illuminances. A two-way repeated measures ANOVA indicated no

significant difference among the subject groups in b-wave amplitude ($F = 0.79$, $p = 0.46$). Given the highly similar b-wave amplitudes among the groups, shifting the control data rightward by 0.4 log units to simulate a sensitivity loss results in poor alignment of the control and diabetic data.

The OP amplitude decreased sharply as the stimulus retinal illuminance increased from 2.8 to 3.2 log Td-s for all three subject groups, and essentially plateaued or decreased slightly thereafter. In general, the controls had larger OP amplitudes than the diabetic groups for all flash retinal illuminances, but the differences among the groups were small at 3.6 log Td-s. A two-way repeated measures ANOVA indicated a significant difference in OP amplitude among the subject groups ($F = 4.92$, $p = 0.01$). Holm-Sidak multiple comparisons indicated that the control OP amplitude was significantly larger than the NDR OP amplitude at 2.8 and 3.2 log Td-s (both $t > 2.41$, $p < 0.02$), but not at 3.6 or 4.0 log Td-s (both $t < 1.85$, $p > 0.06$). Holm-Sidak multiple comparisons indicated that the control OP amplitude was significantly larger than the mild NPDR OP amplitude at 2.8, 3.2, and 4.0 log Td-s (all $t > 2.41$, $p < 0.04$), but not at 3.6 log Td-s ($t = 1.53$, $p = 0.24$). Thus, there were OP amplitude losses for the diabetic groups that reached statistical significance for some, but not all, flash retinal illuminances. The open black circles show the effect of shifting the control data rightward by 0.4 log units, which resulted in poor alignment of the control and diabetic amplitudes. This finding indicates that the diabetic OP amplitudes cannot be accounted for by reducing the flash retinal illuminance.

The right column of Fig. 3 replots the control and diabetic flash ERG waveforms from Fig. 1. However, the control response in each panel was obtained at a flash retinal illuminance that was 0.4 log units lower than that for the diabetic groups. These waveforms further emphasize that the a-waves can be matched for the control and diabetic groups by reducing the flash retinal illuminance for the controls by approximately 0.4 log units. Although this nearly equates the a-waves in the top two panels, the b-waves and OPs remain poorly matched for the control and diabetic groups. For the highest flash retinal illuminances tested (lower right), reducing the flash retinal illuminance for the controls by approximately 0.4 log units provided a reasonable approximation to the diabetic groups for both the a- and b-waves. Despite approximating the b-wave amplitude, the shape of the b-wave differed for the control and DM groups. Specifically, the b-wave for the DM groups was broader in shape than that of the controls, with a reduction and delay of the slow negative potential that follows the b-wave. This negative potential is likely related to the photopic negative response (PhNR), which has been shown to be reduced in DM [17,30,31]. The PhNR, however, is typically recorded using chromatic stimuli at substantially lower flash retinal illuminances [32].

The data shown in Figs. 1 to 3 are based on the mean responses of the three subject groups. These figures are intended to provide examples of the general patterns of data obtained at each stimulus retinal illuminance. Data and analyses based on individual subjects will be presented in Figs. 4 to 6. Figure 4 shows the log OP amplitude for each subject for each of the four flash retinal illuminances (top four panels). Control subjects are shown in black (circles), NDR subjects are shown in green (squares), and mild NPDR subjects are shown in red (triangles). The horizontal lines mark the mean for each subject group. It is clear that the

OP amplitude is reduced for the NDR and mild NPDR subjects compared to the controls for the 2.8 and 3.2 log Td-s flashes. The differences between the control and diabetic groups are statistically significant (as discussed above). In contrast, the group distributions for the 3.6 log Td-s flash largely overlap and there are no significant difference among the groups (as discussed above). For the 4.0 log Td-s flash, the OP amplitude was reduced significantly for the mild NPDR group, but not the NDR group.

ANOVA was also performed to compare the timing of the OPs among the subject groups for the four flash retinal illuminances. ANOVA indicated no significant differences among the subject groups in the sum of OP peak times ($F = 0.23$, $p = 0.80$). We note that the timing of the OPs tended to be variable for the DR subjects, with some subjects having OPs that were advanced in time and other subjects having OP delays.

Figure 4 also shows the log R_{mp3} and log S values for individual control and diabetic subjects, as derived from the delayed Gaussian model (Eq. 1). The R_{mp3} values were similar among the subject groups and the ranges largely overlapped. A one-way ANOVA indicated a non-significant trend for differences in log R_{mp3} among the subject groups ($F = 2.85$, $p = 0.07$). In contrast, the log S parameter differed significantly among the three groups ($F = 9.49$, $p < 0.001$). Holm-Sidak pairwise comparisons indicated a statistically significant reduction in log S for the NDR ($t = 3.61$, $p = 0.001$) and mild NPDR ($t = 3.92$, $p < 0.001$) groups compared to the control group. (These log R_{mp3} and log S data have been reported previously [15].)

An advantage of analyzing the mean OP amplitude (as in Fig. 4) is that variability due to the small, noisy nature of the OPs is reduced by averaging. Nevertheless, Fig. 2 suggests that the later OPs (OP3 and OP4) may be more affected in diabetes than the earlier OPs. To evaluate this possibility, the amplitude and time-to-peak for each OP was quantified, with the results presented in Supplementary Tables 1 and 2. In brief, this analysis confirmed that the amplitude of the later OPs tended to be more reduced than the earlier OPs, but there were no significant differences in OP timing among the groups for any OP.

Figure 5 shows the relationship between log OP amplitude for each flash retinal illuminance and log R_{mp3} (left column) and log S (right column) for the control subjects. The solid lines in each panel (left column) have unit slope and are fit to the grand mean of the control data. The Pearson correlation values and the corresponding p-value are also provided in each panel (and in Table 1). For the control subjects, there were significant correlations between log OP amplitude and log R_{mp3} for each retinal illuminance. That is, control subjects who had high R_{mp3} (large a-waves for the high retinal illuminance flash) tended to have larger OP amplitudes as well. By contrast, there was no significant correlation between log OP amplitude and log S for the control subjects (right column).

Figure 6 shows the relationship between log OP amplitude and log R_{mp3} (left column) and between log OP amplitude and log S (right column) for the NDR (green squares) and mild NPDR (red triangles) subjects. Data are shown in separate panels for the different flash retinal illuminances. The vertical dashed lines in each panel mark the lower limit of the control log R_{mp3} (left column) or log S (right column). The horizontal dashed lines mark the

lower limit of the control log OP amplitude. In contrast to the results obtained for the controls, there was generally no correlation between log OP amplitude and log R_{mp3} or between log OP amplitude and log S for either diabetic group (Table 1), with two exceptions: 1) log OP amplitude for the 2.8 log Td-s stimulus was weakly, but significantly, correlated with log R_{mp3} for the mild NPDR group; 2) log OP amplitude for the 2.8 log Td-s stimulus was weakly, but significantly, correlated with log S for the NDR group. Note, however, if a correction for multiple comparisons is applied, then neither of these correlations would achieve statistical significance.

The plots of log OP amplitude versus log R_{mp3} (Fig. 6, left column) show that most subjects fall in either the upper right quadrant (normal OP amplitude and normal R_{mp3}) or the lower right quadrant (reduced OP amplitude and normal R_{mp3}). Subjects falling in the lower right quadrant are likely to have selective inner-retina dysfunction. In comparison, almost no subjects fall in the lower left quadrant, which would indicate abnormalities in both OP amplitude and R_{mp3} . Subjects falling in the upper left quadrant have reduced R_{mp3} and normal OP amplitude. There are relatively few of these subjects, and all of the subjects that fall in this quadrant cluster near the vertical dashed line, indicating that their R_{mp3} is only slightly outside of the normal range.

The plots of log OP amplitude versus log S (Fig. 6, right column) show that many subjects fall in the upper left quadrant (normal OP amplitude and reduced S). For these subjects, there is an apparent abnormality in cone phototransduction (evidenced by reduced S), but there is no significant correlation between log OP amplitude and log S . A smaller number of subjects fall in the lower left quadrant (reduced OP amplitude and reduced S). Combining data from the upper and lower left quadrants indicates that over half of the DM subjects had a log S value that was outside of the control range. Consequently, relatively few patients fall in the lower right quadrant (reduced OP amplitude and normal S).

DISCUSSION

The purpose of this study was to evaluate the relationship between the activation phase of cone phototransduction and light-adapted OPs in diabetics who have mild or no NPDR. Based on previous work [12], it is known that both cone phototransduction sensitivity and OP timing can be abnormal in these individuals. We sought to determine how abnormalities at the level of the cone photoreceptors might affect electrophysiological responses of the inner-retina. Overall, there was no strong relationship between the activation phase of cone phototransduction and OP amplitude in the diabetic subjects. For the control subjects, however, R_{mp3} was significantly associated with OP amplitude. These findings are consistent with the predicted effects of R_{mp3} and S loss on the ERG response to flashes of high retinal illuminance. That is, cone sensitivity loss (reduced log S) is not expected to markedly reduce the amplitude of the a- and b-waves elicited by flashes of high retinal illuminances [33]. Rather, reduced photoreceptor sensitivity is expected to reduce log S and the slope of the leading edge of the a-wave, as well as delay the implicit time of the b-wave. Although subtle, these changes can be seen in the mean waveforms of Fig. 1. Reduced R_{mp3} , by contrast, is expected to scale the response waveform, reducing the a-wave, b-wave, and OP amplitudes. The diabetic subjects of the present sample generally did not have marked

R_{mp3} losses, which accounts for the minimal a-wave, b-wave, and OP amplitude losses and the lack of a significant correlation between R_{mp3} and OP amplitude.

The effect of reduced log S and its effect on the a-wave, b-wave, and OP amplitude and timing can also be seen in Fig. 3. For moderate retinal illuminances, the mean a-wave of the diabetic groups was highly similar to the normal control mean a-wave that was elicited by a weaker flash (i.e. 0.4 log units lower in retinal illuminance). The mean b-wave and OP amplitudes of the diabetic groups, however, could not be accounted for by a 0.4 log unit retinal illuminance reduction (i.e. a 0.4 log unit loss in cone sensitivity). For the highest flash strength (4.0 log Td-s), the mean waveform (a-wave, b-wave, OPs) of the diabetic groups could be reasonably well approximated by the control waveform that was elicited by a retinal illuminance that was 0.4 log units lower. Thus, the diabetic response is consistent with a loss of cone photoreceptor sensitivity, which does not appear to have substantial effects on OP amplitude. The reduced OP amplitude observed in the present sample of diabetic subjects is more likely related to direct effects of diabetes on inner-retina function (i.e. the OP generators), than abnormalities in cone phototransduction.

The present results are consistent with those of Holopigian et al. [12] who reported reduced log S and OP abnormalities in their sample of diabetic subjects who had a broad range of disease severity. However, the nature of the OP abnormality observed by Holopigian et al. and in the present study differ somewhat: Holopigian et al. reported OP delays in 9 of their 12 subjects, whereas OP delays were not consistently observed in our sample of mild to no NPDR subjects. Of note, small OP delays for individual subjects were observed in the present sample, particularly for the later OPs at the lowest flash retinal illuminance. As a group, however, the delays were not statistically significant. Despite the non-significant OP delays, there was a significant correlation between OP implicit time and log S for the diabetic subjects for the 2.8 log Td-s flash retinal illuminance ($r = -0.40$, $p = 0.01$). No significant correlations between log S and log OP timing were observed for the higher flash retinal illuminances. Thus, DM subjects with reduced log S tended to have longer OP implicit time, at least for the lowest retinal illuminance, which is consistent with previous work [12].

One consideration is that all recordings in the present study were performed under light adapted conditions to limit rod pathway contributions to the responses. OPs are most commonly measured under dark-adapted conditions in response to moderate to high luminance flashes (e.g. ISCEV DA 3.0 response [34]). Under these conditions, both rods and cones likely contribute to OP generation (i.e. a mixed response) [4,5,35], which can complicate the interpretation of which pathway may be contributing to the abnormality [3]. The use of a rod suppressing adapting field in the present study helps to simplify the interpretation, and more directly link the cone phototransduction parameters to the cone pathway OP responses. Relatively few studies in NPDR have examined the OPs under light adapted conditions. Of the available reports, one showed that both light- and dark-adapted OPs can be reduced in early-stage NPDR, but the abnormalities were found to be larger and statistically reduced relative to non-diabetic controls only under dark-adapted conditions [3]. A second report showed reductions in OP amplitude under both light- and dark-adapted conditions for subjects who had clinically-apparent DR [36]. Thus, future work could focus

on the relationship between dark-adapted OP responses and rod photoreceptor activation, as the neural abnormalities may be greater within the rod pathway.

The present study focused on responses to stimuli of high retinal illuminance (e.g. higher than the ISCEV standard 3.0 flash stimulus), as stimuli of high retinal illuminance are used to model the a-wave. It is possible that the correlation between the a-wave parameters and OP amplitude would be stronger if OP measurements were performed with stimuli of lower retinal illuminance. An advantage of the present approach is that the a-wave and OP parameters were derived using identical stimuli, but a disadvantage is that analyses of these relationships are limited to a relatively narrow range of stimulus conditions.

An additional consideration is that the a-wave, particularly the later portion of the response, likely has contributions from OFF bipolar cells [37–39]. It is difficult to determine the amount of post-receptor intrusion into the a-waves of the present study, as the extent of post-receptor contributions may depend on the specific conditions used to elicit the responses. In the context of the present study, post-receptor contributions may complicate the interpretation of the relationship between the model fit parameters and the OP responses. That is, what we have considered a cone sensitivity loss could, in some part, also reflect an OFF bipolar cell abnormality. Nevertheless, the primary finding holds: parameters derived from the leading edge of the a-wave do not appear to predict OP amplitude losses in early-stage diabetic retinopathy. This finding suggests that diabetes may separately affect cone, and possibly OFF bipolar cell, function and inner-retina function (e.g. amacrine and RGC).

In summary, approximately half of the diabetic subjects in the present study had abnormalities in the activation phase of cone phototransduction (reduced log S) and approximately half had reduced OP amplitude at one or more flash retinal illuminance level. However, there was no significant correlation between these measures. Indeed, individual diabetic subjects had reduced log S with normal OP amplitude ($N = 9$) and vice versa: reduced OP amplitude with normal log S ($N = 5$). For the thirteen subjects who had reduced log S and reduced OP amplitude, there was no significant correlation in the magnitude of the abnormality. These findings suggest that in the early stages of DR, diabetes may independently affect photoreceptor and inner-retina function, as inferred from reduced log S and reduced OP amplitude, respectively. Although DR is typically considered a disease of the retinal vasculature, the present results emphasize that diabetes can have multiple effects on neural function at early disease stages.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Mean waveforms obtained for flash retinal illuminance of 2.8 log Td-s (upper left), 3.2 log Td-s (upper right), 3.6 log Td-s (lower left), and 4.0 log Td-s (lower right). Each trace represents the mean response from the control subjects (black), NDR subjects (green), and mild NPDR subjects (red).

Figure 2:
Oscillatory potentials extracted from the waveforms shown in Fig. 1. Conventions are as in Fig. 1.

Figure 3: Mean (±SEM) a-wave (upper left), b-wave (middle left), and OP (lower right) log amplitude as a function of stimulus log retinal illuminance. Data are shown for the control subjects (black circles), NDR subjects (green squares), and mild NPDR subjects (red triangles). Green and red asterisks indicate significant differences from the control for the NDR and mild NPDR subjects, respectively. The open black circles are the control data displaced to the right by 0.4 log units. The left panels replot the waveforms for the control and diabetic groups from Fig. 1. However, the control data in these panels were obtained at a lower stimulus retinal illuminance, as compared to the diabetic groups. As described in the text, this permits evaluating the effect of reduced flash retinal illuminance on the shape and amplitude of the waveforms.

Figure 4:

Log amplitude for individual subjects for each stimulus retinal illuminance (top four panels). The bottom row shows log R_{mp3} (left) and log S (right) for each subject. The horizontal bars represent the group means and asterisks indicate significant differences from the control.

Figure 5:

Log OP amplitude is plotted as a function of log R_{mp3} (left column) and log S (right column) for the control subjects. Each row shows data for a different stimulus retinal illuminance. The solid lines have unit slope and are fit to the grand mean of the data. The Pearson correlation values and associated p-values are given in each panel.

Figure 6:

Log OP amplitude is plotted as a function of $\log R_{mp3}$ (left column) and $\log S$ (right column) for the NDR subjects (green) and the mild NPDR subjects (red). All other conventions are as in Fig. 5.

Table 1:

Association between OP amplitude and phototransduction parameters

OP		Log R_{mp3}	Log S
2.8 log Td-s	Control	$r = 0.63, p = 0.003$	$r = 0.24, p = 0.30$
	NDR	$r = 0.21, p = 0.38$	$r = 0.51, p = 0.02$
	Mild NPDR	$r = 0.47, p = 0.04$	$r = 0.12, p = 0.61$
3.2 log Td-s	Control	$r = 0.72, p < 0.001$	$r = 0.17, p = 0.47$
	NDR	$r = 0.14, p = 0.55$	$r = 0.09, p = 0.69$
	Mild NPDR	$r = 0.25, p = 0.29$	$r = 0.11, p = 0.63$
3.6 log Td-s	Control	$r = 0.49, p = 0.03$	$r = 0.14, p = 0.56$
	NDR	$r = 0.22, p = 0.35$	$r = 0.21, p = 0.38$
	Mild NPDR	$r = 0.36, p = 0.13$	$r = 0.08, p = 0.75$
4.0 log Td-s	Control	$r = 0.74, p < 0.001$	$r = -0.04, p = 0.88$
	NDR	$r = 0.20, p = 0.40$	$r = 0.03, p = 0.90$
	Mild NPDR	$r = 0.18, p = 0.45$	$r = -0.07, p = 0.75$