

# A Comparison of Photopic and Scotopic Electroretinographic Changes in Early Diabetic Retinopathy

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**Previous studies of early diabetic retinopathy have shown that oscillatory potential (OP) amplitudes are reduced in many diabetic patients. OP amplitude is believed to be a more sensitive indicator of the development of future retinopathy than b-wave amplitude of the scotopic electroretinogram (ERG). Because OPs measured to a bright white flash reflect both rod and cone system activity, it is important to compare OP amplitudes to photopic ERG measures as well as scotopic measures in early diabetic retinopathy. In this study, OPs and ERG responses were measured under photopic and scotopic conditions in a group of diabetic patients. Although OPs were reduced in amplitude in the diabetic group, several other parameters of the scotopic and photopic b-waves were impaired. The results indicate that b-wave activity may indicate retinal changes in early diabetic retinopathy in the same manner as the OPs. Invest Ophthalmol Vis Sci 33:2773-2780, 1992**

Diabetic retinopathy is one of the ocular complications associated with diabetes mellitus. For some diabetic patients, the retinopathy will progress to a proliferative stage (including neovascularization and retinal hemorrhages). Because these patients require laser photocoagulation to slow the progression of retinopathy, early identification is needed to ensure adequate follow up.

Some reports indicate that the oscillatory potentials (OPs) of the flash electroretinogram (ERG) are reduced in amplitude in many diabetic patients and that this reduction in OP amplitude may predict the development of proliferative retinopathy.<sup>1-5</sup> Yonemura et al<sup>1</sup> were the first to note the relationship between OP amplitude and the severity of diabetic retinopathy. They found that patients with advanced diabetic retinopathy had extremely small or extinguished OPs, even when the a- and b-waves of the ERG were normal in amplitude. In addition, they found that the OP amplitude was reduced in more than 50% of diabetic patients with no ophthalmologic changes. Later studies on patients with retinopathies ranging from

moderate nonproliferative to proliferative supported the conclusion that the OPs are selectively impaired in diabetes, relative to the amplitude of the scotopically measured b-wave.<sup>3-5</sup>

OP amplitude also has been shown to predict the continued progression of diabetic retinopathy. Simonsen<sup>2</sup> studied the predictive value of OP amplitude in a longitudinal study of diabetes patients whose retinopathies ranged from no detectable retinopathy to proliferative retinopathy. He found that OP amplitude at the initial visit was a reliable predictor of proliferative retinopathy development.

One implication of this literature is that the OPs are better indicators of diabetic retinopathy than other components of the ERG, such as b-wave amplitude. That is, the OPs are specifically affected early in this disease. This conclusion, however, never has been adequately tested. Previous studies have two major problems. First, in the studies where the OPs were compared to b-wave amplitudes, patients with intermediate or advanced stages of retinopathy typically were examined.<sup>3-6</sup> However, diabetic patients with little or no diabetic retinopathy must be tested to assess the prognostic value of OP amplitude reductions.

The second problem is the appropriate ERG comparisons. Typically, the amplitude of the OPs has been compared to the amplitude of the scotopically measured a- and b-waves.<sup>1-2,6,7</sup> However, the OPs measured under standard clinical conditions are composed of both scotopic and photopic components.<sup>8-10</sup> Some investigators have argued that the OPs may be specific to the photopic system.<sup>11</sup> As a result, compari-

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sons of OP amplitude to scotopic ERG components may not be complete.

The present study differed from existing studies in two important ways. First, patients with early diabetic retinopathy were tested (Table 1). Second, OP amplitudes were compared to a range of ERG parameters. Amplitudes and implicit times of scotopic and photopic parameters of the standard ERG were recorded. In addition, OPs were measured under recording conditions designed to maximize photopic or scotopic contributions. This protocol allowed us to determine whether the OPs are selectively impaired in early diabetic retinopathy and to assess the relative effects of early diabetic retinopathy on scotopic versus photopic ERG parameters.

## Materials and Methods

### Observers

Fourteen patients with diabetes mellitus and 14 age-similar controls were subjects in this experiment. All were recruited from the Ophthalmology Clinic in Bellevue Hospital (New York), after a complete ocular exam. The subjects gave informed consent to participate after a full explanation of the procedure was given.

The diabetic patients had visual acuities better than 20/50, a history of diabetes ranging from 4–21 yr (median 12 yr), no history of cataracts, and no other ocular or health problems. Four of the diabetic patients were classified as Type I and the remaining 10 were classified as Type II. The mean age ( $\pm$ standard deviation) of the diabetics was  $49.1 \pm 12.8$  yr. All diabetic patients received a fluorescein angiogram and fundus

photographs. The level of retinopathy and degree of macular edema were independently classified by two retinal specialists, according to a modified Airlie House classification scheme, and the scores were averaged. The clinical characteristics of these patients are given in Table 1.

The control observers had normal visual acuity and no history of ocular or health problems. The mean age of the controls was  $45.5 \pm 11.6$  yr.

### Apparatus and Procedure

The eye with the better visual acuity was tested after pupil dilation (1% tropicamide and 2.5% phenylephrine). All patients had pupil diameters greater than 6 mm. The patients were patched and dark adapted for 40 min, and full-field ERGs were measured with a Grass (Boston, MA; PS22) flash with a ganzfeld surround. ERGs were recorded with a monopolar Burian-Allen (Hansen Ophthalmic, Iowa City, IA) electrode. The forehead served as reference and the ipsilateral ear served as ground. For the a- and b-wave recordings, the signal was amplified (1 K; Grass preamplifier P511) and filtered (1–300 Hz). For the OP recordings, the amplification was increased to 5 K and the signals were filtered between 100 and 1000 Hz. All ERG recordings were digitized (512 Hz), signal averaged ( $n = 5$ ), and analyzed on a computer.

Under scotopic conditions, ERGs were recorded to a low intensity blue flash (Grass S1 and a Wratten [Kodak, Rochester, NY] 47b filter) and to white flashes of increasing intensity (Grass S16) for a voltage versus intensity function (from  $-4.1$  to  $1.9$  log td). Scotopic OPs were measured to a low intensity (Grass

**Table 1.** Clinical characteristics—diabetics

Obs	Eye	Sex	Age	Acuity	Type	Dur.	Ret. level* (grader)		Edema level* (grader)	
							1	2	1	2
CB	OD	F	51	20/25 <sup>+1</sup>	II	20 yr	2	2	0	0
CC	OD	M	48	20/20	II	15 yr	3	3	1	0
CF	OD	F	61	20/30 <sup>-2</sup>	II	5 yr	1	1	0	0
DH	OD	F	33	20/25 <sup>-2</sup>	I	21 yr	2	2	0	0
HM	OD	M	25	20/25 <sup>+2</sup>	I	4 yr	1	1	0	0
RO	OS	F	51	20/20 <sup>-3</sup>	I	15 yr	3	2	0	0
AO	OD	F	59	20/20 <sup>-1</sup>	II	Unknown	2	2	0	0
SP	OS	F	68	20/40 <sup>-2</sup>	II	8 yr	2	2	0	0
DS	OS	F	52	20/30 <sup>-3</sup>	II	12 yr	1	1	0	0
ST	OD	M	58	20/20 <sup>-1</sup>	II	12 yr	1	1	0	0
DL	OD	F	26	20/20	I	18 yr	2	2	1	1
MC	OD	F	49	20/20	II	12 yr	2	2	0	0
JA	OD	M	50	20/20	II	10 yr	2	2	0	0
LR	OD	F	57	20/25 <sup>-1</sup>	II	9 yr	2	2	0	1

Mean age = 49.1, SD = 12.8.

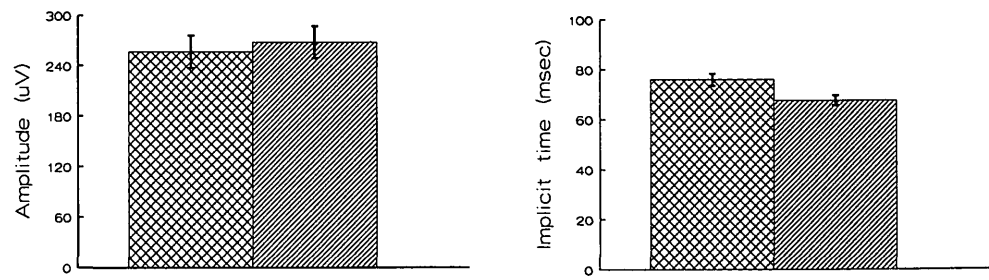
\* The level of retinopathy and the degree of macular edema were assessed from fluorescein angiograms by two independent graders using the following modification of the Airlie House classification scheme:

Level of retinopathy: Level 1—normal fundus; Level 2—one or more mi-

croaneurysms only; Level 3—microaneurysms with one or more other non-proliferative lesions present of mild to moderate degree.

Degree of macular edema: Grade 0—none; Grade 1—questionable macular edema.

**Fig. 1.** Averaged scotopic b-wave responses to the blue (Wratten 47a) flashes. The left side of the figure shows the averaged b-wave amplitudes and the right side shows the averaged implicit times. Each pair of histograms shows the results for the diabetics (hatched) and the controls (diagonal stripes). Error bars are  $\pm 1$  standard error.



S8) blue flash (Wratten 47b; rod dominated response) and to a bright (Grass S16) white flash (mixed rod and cone response). After 10 min of light adaptation, a photopic flash response, a 30 Hz flicker response, and a photopic flash OP response (cone dominated) were measured to a Grass S16 white stimulus. For all OP recordings, a conditioning flash was used.<sup>12,13,10</sup>

In addition, 12 of these diabetic patients also had the course of dark adaptation at one retinal locus measured and had measures of dark-adapted thresholds in four retinal quadrants. These results are presented elsewhere.<sup>14,15</sup> After all testing was completed, fluorescein angiograms and retinal photographs were obtained on the diabetic patients.

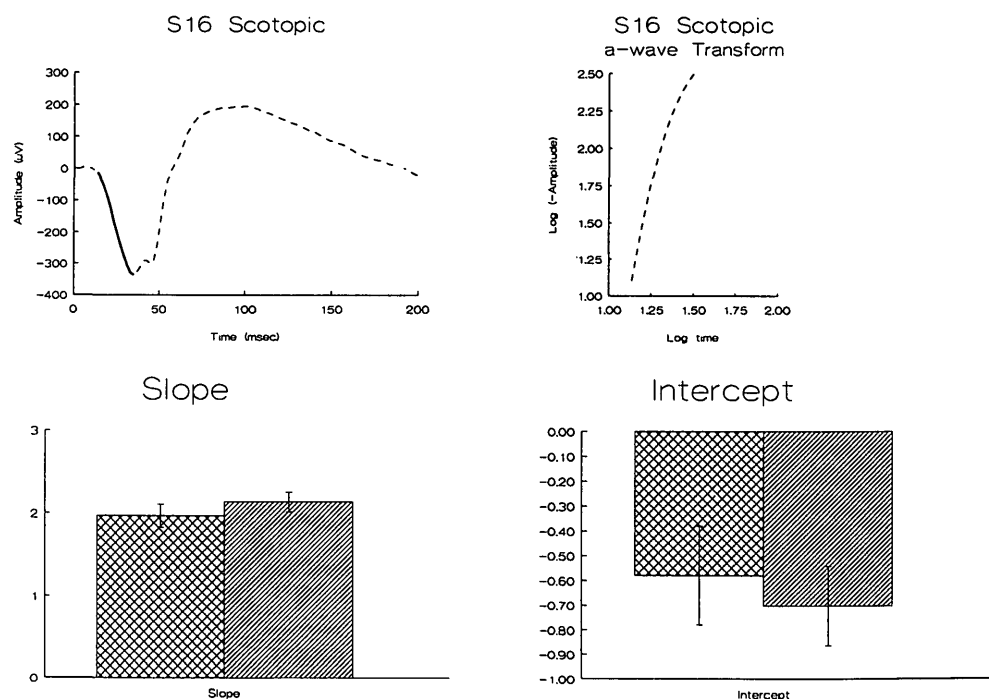
**Results**

The data obtained to the low intensity blue flashes (S1 blue) are presented in Figure 1. The left side of the

figure shows the averaged b-wave amplitudes for the diabetics (hatched) and the controls (diagonal stripes). There were no significant differences in amplitude between the two groups, ( $t(26) = 0.42; P > 0.05$ ). The right side of the figure shows the averaged implicit times. The implicit times for the diabetic patients were significantly delayed, relative to the controls ( $t(26) = 2.64; P < 0.05$ ).

The a-wave findings are presented in Figure 2. Because the response of the outer retina reflected in the a-wave cannot be measured independently of the b-wave, we transformed a-wave responses using a method proposed by Hood and Birch.<sup>16-17</sup> For each subject, the a-wave response to the highest intensity flash was truncated before intrusion of the b-wave. These data then were plotted as log (-amplitude) versus log time. From these functions, the slope and intercepts were calculated and averaged for the control

**Fig. 2.** Transformation of scotopic a-wave amplitude data.<sup>16,17</sup> The portion of the ERG (solid line) used in this analysis is shown in the top left and the a-wave amplitude function is shown at the top right. The bottom portion of the figure shows the averaged slopes and intercepts for the diabetics (hatched) and controls (diagonal stripes). Error bars are  $\pm 1$  standard error.



and diabetic groups. Shown in the top left of Figure 2 is a graphic demonstration of the portion of the ERG (solid line) used in this analysis. Shown in the top right is the a-wave amplitude function plotted on log amplitude versus log time axes. The bottom portion of Figure 2 shows the averaged slopes and intercepts for the diabetic (hatched) and control groups (diagonal stripes). There were no significant differences between the groups for either measure (slope:  $t(20) = 0.21, P > 0.05$ ; intercept:  $t(20) = .08, P > 0.05$ ).

The averaged b-wave amplitudes (measured from the trough of the a-wave to the peak of the b-wave) as a function of flash intensity are shown in Figure 3. The results for the diabetics are shown as open squares and the results for the controls as filled circles. For both groups, b-wave amplitude increased with flash intensity. The repeated measure design analysis of variance (ANOVA) indicated there were no significant differences in amplitude between the diabetic and control groups, nor was there a significant interaction between group and flash intensity (see Table 2 for ANOVA values). In addition, for each observer, the Naka-Rushton equation was best fit to the amplitude versus intensity data. The estimates of Rmax and k (semi-saturation constant) were averaged for each group. There were no significant differences in Rmax or k for the two groups (Rmax:  $t(20) = 1.35, P > 0.05$ ; k:  $t(20) = 1.80, P > 0.05$ ).

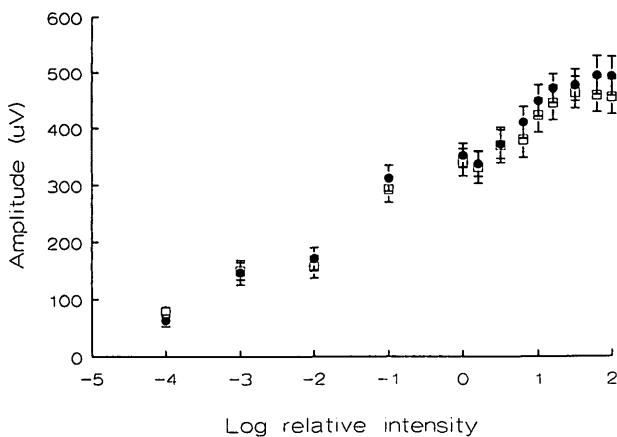
The averaged b-wave implicit times as a function of flash intensity are presented in Figure 4. The implicit times for the diabetic patients (open squares) were delayed at all flash intensities relative to the control observers (filled circles). Significant differences in implicit times between the two groups were found, as was a significant interaction between group and flash intensity (Table 2).

**Table 2.** Repeated measures analysis of variance

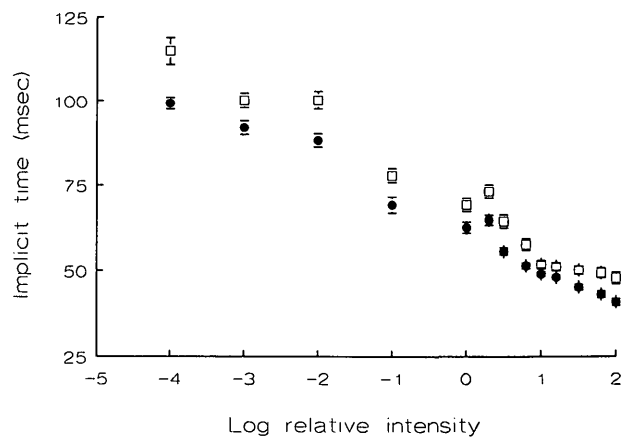
	<i>F</i> value	<i>df</i>	<i>P</i> value
1. Scotopic b-wave amplitude			
Group	0.3	1	>0.05
Filter strength	121.3	12	<0.001
Interaction	1.2	12	>0.05
2. Scotopic b-wave implicit time			
Group	12.8	1	<0.005
Filter strength	486.0	12	<0.001
Interaction	3.5	12	<0.001
3. Scotopic blue OP amplitude			
Group	10.5	1	<0.005
OP (1-4)	44.8	3	<0.001
Interaction	3.7	3	<0.05
4. Scotopic blue OP implicit time			
Group	4.2	1	>0.05
OP (1-4)	1300.1	3	<0.001
Interaction	2.0	3	>0.05
5. Scotopic white OP amplitude			
Group	10.3	1	<0.005
OP (1-4)	33.1	3	<0.001
Interaction	0.3	3	>0.05
6. Scotopic white OP implicit time			
Group	1.7	1	>0.05
OP (1-4)	14.0	3	<0.001
Interaction	1.3	3	>0.05
7. Photopic white OP amplitude			
Group	3.2	1	>0.05
OP (1-3)	18.0	2	<0.001
Interaction	0.2	2	>0.05
8. Photopic white OP implicit time			
Group	2.2	1	>0.05
OP (1-3)	893.9	2	<0.001
Interaction	2.2	2	>0.005

OP, oscillatory potential.

The results for the photopic components of the ERG are shown in Figures 5 and 6. The averaged amplitudes (left) and implicit times (right) of the photopic b-wave for the diabetics (hatched) and controls (diagonal lines) are shown in Figure 5. There were no significant differences in either measure for the two

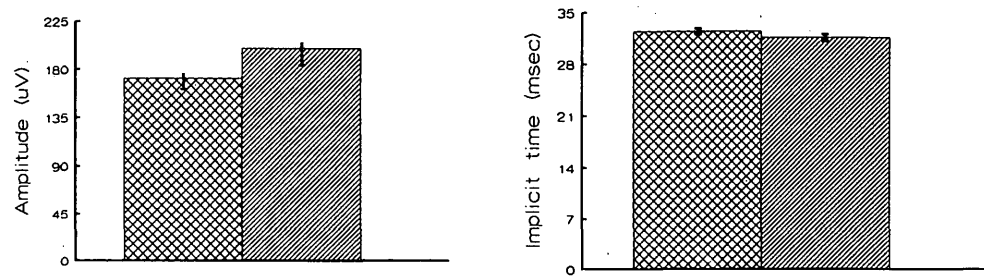


**Fig. 3.** Averaged scotopic b-wave amplitude data as a function of flash intensity for the diabetics (squares) and controls (circles). Error bars are  $\pm 1$  standard error.



**Fig. 4.** Averaged scotopic b-wave implicit time data as a function of flash intensity for the diabetics (squares) and controls (circles). Error bars are  $\pm 1$  standard error.

**Fig. 5.** Averaged photopic b-wave responses to the photopic white flashes. The left side of the figure shows the averaged b-wave amplitudes and the right side shows the averaged implicit times. Each pair of histograms shows the results for the diabetics (hatched) and the controls (diagonal stripes). Error bars are  $\pm 1$  standard error.

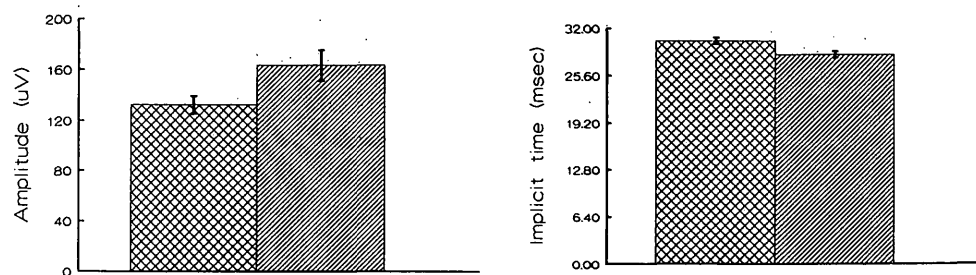


groups (amplitude:  $t(26) = 1.49, P > 0.05$ ; implicit time  $t(26) = 1.44, P > 0.05$ ). The averaged amplitudes and implicit times of the photopic 30 Hz flicker are presented in Figure 6. For the diabetic group, amplitude was significantly reduced ( $t(25) = 2.19; P < 0.05$ ) and implicit time was significantly delayed ( $t(25) = 2.88; P < 0.01$ ).

The OP results are shown in the next series of figures. The peak-to-trough amplitudes of the individual OP wavelets were measured individually so the relative involvement of each component could be assessed. The OP amplitudes measured under scotopic conditions to a blue flash are shown in Figure 7A, and the corresponding implicit times are shown in Figure 7B. For all OPs, the amplitudes for the controls (diagonal lines) are approximately 50% larger than for the diabetics (hatched). There was a significant difference between the amplitudes for the two groups, and a significant interaction (Table 2). The OP implicit times for the diabetics, however, were not significantly delayed, relative to the controls.

The results for the OPs measured to the bright white flash under scotopic conditions are shown in Figure 8. There were significant differences in amplitude (Figure 8A) between the two groups, but no significant interaction (Table 2). The OP implicit times for this condition (Figure 8B) were not statistically different for the two groups.

**Fig. 6.** Averaged photopic b-wave responses to the 30-Hz flicker. The left side of the figure shows the averaged b-wave amplitudes and the right side shows the averaged implicit times. Each pair of histograms shows the results for the diabetics (hatched) and the controls (diagonal stripes). Error bars are  $\pm 1$  standard error.



The results for the OPs measured under photopic conditions are shown in Figure 9. Under these conditions, only three OPs were recordable. Although the OP amplitudes for the diabetics were smaller than the amplitudes for the controls (Figure 9A), this difference was not statistically significant. Consistent with the other OP conditions, there were no significant differences in implicit times between the groups (Figure 9B; Table 2).

To examine the degree of impairment in the individual OPs (ie, OP1-OP4), we calculated the percent loss in amplitude (relative to the amplitude of the control group) for each OP. The percent loss values are shown in Table 3. Larger values indicate greater losses in amplitude. There were no significant differences among the percent amplitude loss for the different OPs for any measurement condition.

### Discussion

#### Selective Impairment of the OPs

This study demonstrates that OP amplitudes are significantly reduced in patients with early diabetic retinopathy. The loss of OP amplitude agrees with previous studies.<sup>1-3,6</sup> In comparison, the amplitudes of the scotopically measured b-waves were normal for our patients, also consistent with previous work.<sup>1-2,7</sup> However, several parameters of the ERG were found

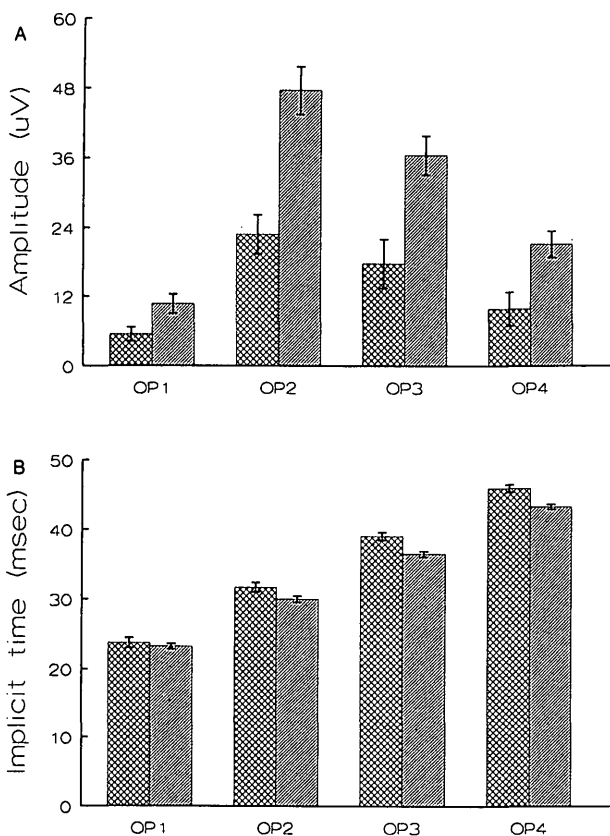


Fig. 7. Averaged OP amplitudes to the scotopic blue flashes. (A) The averaged OP amplitudes; (B) the averaged implicit times. Each pair of histograms shows the results for the diabetics (hatched) and the controls (diagonal stripes). Error bars are  $\pm 1$  standard error.

to be abnormal in our diabetic patients. These parameters include both b-wave amplitudes and implicit times and ERGs measured under scotopic and photopic conditions. Although scotopic b-wave amplitude is not a sensitive indicator of early diabetic retinopathy, other parameters of the ERG, including scotopic b-wave implicit times, indicate the presence of early retinal changes. These ERG parameters were not examined in previous studies of the selective involvement of the OPs.<sup>1-3</sup> Our results indicate that OP amplitudes are not selectively impaired in early diabetic retinopathy, relative to other ERG parameters.

#### Selective Impairment of Photopic Versus Scotopic ERG Parameters

The ERG abnormalities found in our patients were present for both rod dominated (S1 blue) and cone dominated (photopic flicker) conditions, indicating that both systems show losses in early diabetic retinopathy.

The changes in the scotopic ERG in the diabetic patients is corroborated by elevations in psychophysio-

cally measured dark adapted thresholds in the same patients<sup>14-15</sup> and by other studies of dark adaptation in patients with diabetic retinopathy.<sup>18-19</sup>

#### Selective Impairment of Individual OP Wavelets

A significant body of evidence suggests that the individual OPs have different neural generators. For example, individual OPs have different retinal depth profiles, with the earlier OPs arising more proximally within the retina than the later ones.<sup>20</sup> The earlier OPs also have been shown to be sensitive to the disruption of gamma-aminobutyric acid blockers, whereas the later OPs are more vulnerable to glycine antagonists.<sup>21-23</sup> Also, the OPs are differentially affected by disease<sup>24-25</sup> and show different time courses of light adaptation.<sup>26-27</sup> As a result, in the present study we examined the amplitudes of the individual OP wavelets rather than the sum of the OP amplitudes<sup>2-5</sup> or the root mean square Fourier amplitude.<sup>28</sup> The results from this study did not show selective changes in OP amplitude, but did show a uniform reduction for all OPs.

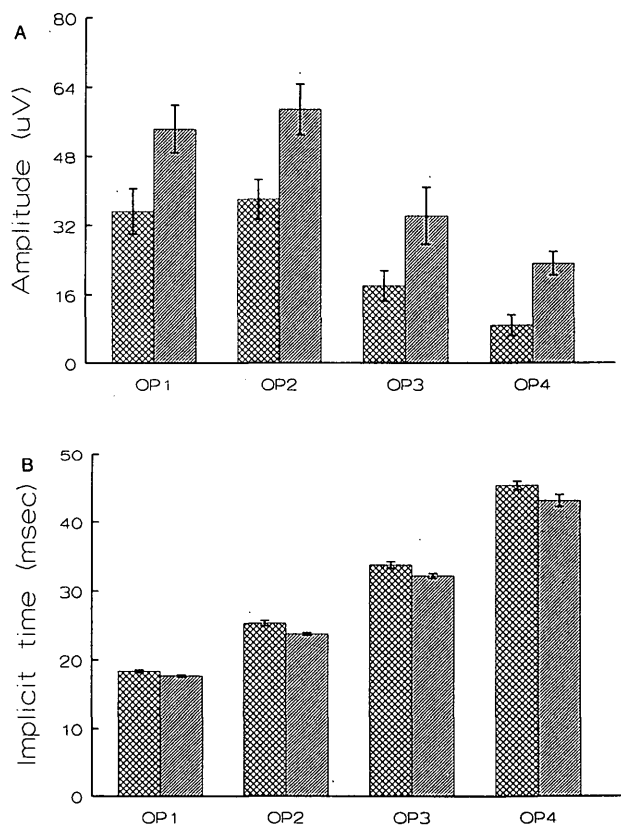


Fig. 8. Averaged OP amplitudes to the scotopic white flashes. (A) The averaged OP amplitudes; (B) the averaged implicit times. Each pair of histograms shows the results for the diabetics (hatched) and the controls (diagonal stripes). Error bars are  $\pm 1$  standard error.

### Site of Retinal Losses in Early Diabetic Retinopathy

Do the results from this study provide any information about the retinal locus of diabetic retinopathy? Previous studies have established that the OPs are impaired in patients with diabetic retinopathy. The OPs have been shown to derive from the inner plexiform layers involving the axon terminals of the bipolar cells, the processes of the amacrine cells, and the dendrites of the ganglion cells.<sup>29</sup>

The results of the present study indicate that additional retinal sites are affected in early diabetic retinopathy. The changes in b-wave amplitudes and implicit times are consistent with defects in the mid-retinal layer.<sup>30</sup> Also, recent evidence suggests that changes in the rod dominated b-wave implicit time may reflect sensitivity losses at the level of the receptors.<sup>16-17</sup> Additional support for photoreceptor changes derives from studies of retinal hypoxia.<sup>31-32</sup> In the cat, the inner retina is relatively unaffected by systemic hypoxia, whereas the outer retina is sensitive to small variations in arterial oxygen tension. This vulnerability of the outer retina to hypoxia is partly a

**Table 3.** Percent loss in oscillatory potential (OP) amplitude (from control)

	OP no.	Percent loss
I. Scotopic blue OP	1	48.9
	2	52.1
	3	51.4
	4	53.3
II. Scotopic white OP	1	35.2
	2	35.4
	3	47.3
	4	61.5
III. Photopic white OP	1	33.6
	2	18.4
	3	20.1

result of the high oxygen consumption of the photoreceptors.<sup>31-32</sup> We are examining patients with advanced diabetic retinopathy to evaluate rod photoreceptor status.

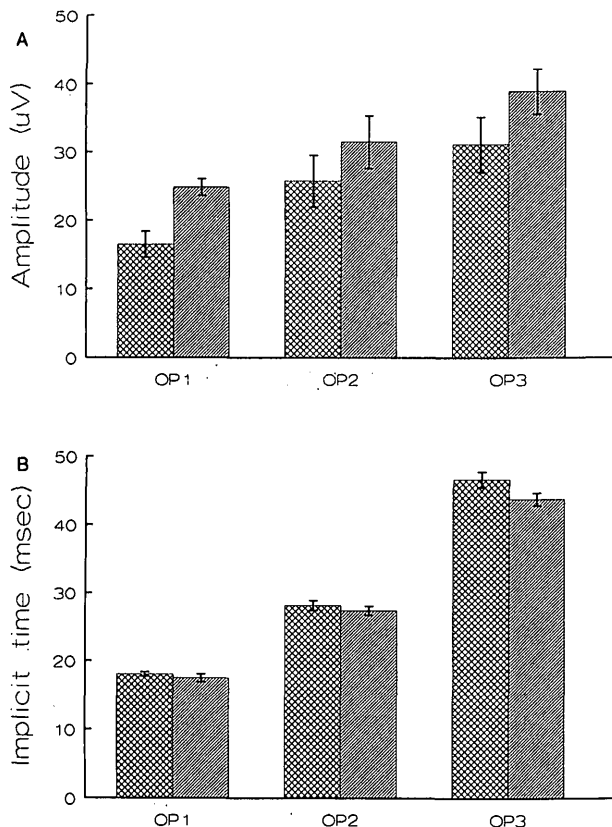
**Key words:** diabetes mellitus, electroretinogram, oscillatory potentials

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**Fig. 9.** Averaged OP amplitudes to the photopic white flashes. (A) The averaged OP amplitudes; (B) the averaged implicit times. Each pair of histograms shows the results for the diabetics (hatched) and the controls (diagonal stripes). Error bars are  $\pm 1$  standard error.

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