Bilateral Alterations of the ERG and Retinal Histology Following Unilateral HSV-1 Inoculation

D. I. Hamasaki,* Richard D. Dix,*† and Sally S. Atherton††

The physiological condition of the retinas of BALB/c mice inoculated unilaterally in the anterior chamber with the KOS strain of herpes simplex virus type 1 (HSV-1) was monitored by ERG recordings. After the ERG recordings, the retinas were examined for histopathological changes. In the inoculated eye, depressed ERGs were recorded on day 2 PI and abolished ERGs on day 4 PI. The changes in the ERGs were complete by day 5–6 PI. Of the 53 inoculated eyes followed for longer than day 6 PI, four (7.5%) remained normal, 30 (56.6%) had reduced ERGs and 19 (35.8%) had abolished ERGs. In the contralateral eyes, the first changes were noted on day 8 PI, and abolished ERGs were recorded on day 9 PI. Of the 55 contralateral eyes followed for longer than 10 days, 15 (27.3%) remained normal, four (7.2%) had reduced ERGs and 36 (65.4%) had abolished ERGs. The percentage of eyes with depressed ERGs was significantly higher in the inoculated than in the uninoculated eyes, and the percentage of eyes with abolished ERGs was significantly higher in the uninoculated eyes than in the inoculated eyes. The histopathological alterations were different for the two eyes. In the inoculated eyes, the changes were mainly in the outer retina, with characteristic folds in the photoreceptor and outer nuclear layer interspersed with normal appearing retina. The pigment epithelium was also abnormal. In the uninoculated eyes, the changes began in the inner retina but rapidly spread to all layers of the retina. This panretinal necrosis accounted for the higher percentage of abolished ERGs in the uninoculated eyes. The differences in the alterations of the ERG and the histopathological changes may be related to the underlying mechanism of action of the HSV-1 during the evolution of the experimental retinopathy.

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there are two waves of virus reaching the eye: the initial wave of virus is found on day 1 PI, but the virus does not replicate and the virus titer remains low; a later wave occurs on day 7 PI, the titer of which increases to a second peak on day 10 PI.

The earlier studies used histopathological methods to assess the damage to the retinas induced by the HSV-1. Histopathological methods have also been used to assess the retinal damage induced by other types of virus, eg, feline leukemia virus,7 blue tongue virus,8 mumps virus,9 and lymphocytic choriomeningitis virus.10 The question arises regarding the physiological condition of the retinas during the viral infection. Tremain and Ikeda reported that the latencies of the neural responses from the superior colliculus were longer in mice infected with the Semliki Forest virus.11 They attributed the longer latencies to the demyelination of the optic nerve fibers which is known to be produced by this virus.

For our study, we used the electroretinogram (ERG) to monitor the physiological state of the retina, and we shall show that inoculation of HSV-1 leads to alterations of the ERG of both eyes. The time course and degree of the alterations, however, were different for the two eyes: in the ipsilateral eyes, over 90% of the eyes had altered ERGs and changes could be noted by day 2 PI; in the contralateral eyes, approximately 70% of the eyes had reduced ERGs and changes began at day 8 PI and were complete by days 9–10 PI. The histopathological changes were different for the two eyes and were present in all eyes which showed changes in the ERG.

Materials and Methods

Animals

All experiments were conducted on adult (9–12 weeks) BALB/c mice obtained from Taconic Farms (Germantown, NY). The mice were held in standard cages and fed Lab Chow (Purina). The animals were kept on a 12:12 light:dark schedule.

The treatment and procedures used in this study conformed to the ARVO Resolution on the Use of Animals in Research.

Inoculation

Under IP chloral hydrate anesthesia (0.36 mg/g body weight), the right eye was proposed and 1.5 × 10⁵ PFU/μl of the KOS strain of HSV-1 was injected into the anterior chamber as described in detail earlier.12 The contralateral eye was not sham-injected. For controls, animals were either sham-injected in one eye with the same amount of carrier solution, or had their crystalline lens deliberately ruptured in one eye. In both groups, the ERG and retinal histology were not altered.

Electroretinography (ERGs)

ERGs were picked up by a wick electrode placed on the cornea of mice anesthetized with IP chloral hydrate. The indifferent electrode was placed subcutaneously on the top of the head, and the animal was grounded by another electrode placed subcutaneously in the neck region.

The ERGs were amplified and displayed on an oscilloscope with a time constant of 0.2 seconds. The high frequency cutoff was set at 1 Kc. The amplified responses were fed from the oscilloscope to a Nicolet (Madison, WI) 1072 Signal Averager, and 16 responses were averaged with a dwell time of 4 msec. The averaged ERGs were plotted on a MFE 715M X-Y plotter. The responses were also sent from the oscilloscope to a Hewlett Packard 3964A analogue tape recorder (Palo Alto, CA) for permanent storage of the individual responses. The b-wave amplitudes were measured from the trough of the a-wave to the peak of the b-wave.

Stimulus

The stimulus light was obtained from a 150 W quartz-halogen light bulb. The light was collected and focused onto a 3 mm diameter fiber optic bundle. The fiber optic bundle was brought into the Faraday cage and placed 1 cm from the eye. Under these conditions, the stimulus can considered to be a ganzfeld stimulus.

The irradiance of the unattenuated stimulus was 2.91 × 10⁵ μW/cm² (United Detector Technology Radiometer; Santa Monica, CA), and neutral density filters were used to reduce the intensity. Stimulus intensities will be referred to by the value of the neutral density filter used to attenuate the full intensity stimulus. A Uniblitz (Rochester, NY) shutter was used to deliver 250 msec pulses at an interstimulus interval of 5 seconds.

Procedure

The anesthetized animal was taped down to a metal plate and one of the eyes taped open. The plate was placed on a heating pad to keep the animal warm. After the electrodes were placed and the fiber optic bundle adjusted for uniform illumination of the eye, a response was recorded with a 2.0 neutral density filter. This initial ERG not only demonstrated that everything was aligned but also gave an indication of the physiological status of the retina.

The animal was then dark-adapted for 30 min, and responses were recorded beginning with a 5.0 log unit
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Fig. 1. Averaged ERGs recorded from the eye of a normal mouse. Sixteen responses were averaged with a dwell time of 4 msec. The numbers on the left represent the value of the neutral density filter used to reduce the full intensity stimulus. At 0, irradiance = 2.91 \times 10^3 \mu W/cm^2. The ERG shown at the bottom was recorded before dark adaptation, and was recorded with a 2.0 neutral density filter. Calibration; 100 \mu V for I = 5.0 to 3.0 and 200 \mu V for I = 2.0 to 0; 100 msec.

filter. Preliminary experiments on normal eyes showed that this intensity was close to the threshold for normal eyes. The stimulus intensity was increased in 0.5 log unit steps for filters 5.0 to 3.0, and then in 1.0 log unit steps to the full intensity stimulus.

Histology

At the conclusion of the ERG recordings, the animals were deeply anesthetized and perfused with glutaraldehyde (5% in 0.1 M phosphate buffer) through the heart. The enucleated eyes were kept overnight in glutaraldehyde in a refrigerator. On the following day, the anterior segment with the lens was cut off and discarded. The posterior segment was sectioned into smaller pieces and embedded in epon. Two micron sections were cut and stained with toluidine blue.

Some of the retinas were embedded in paraffin in order to examine a larger area of the retina. Sections from these blocks were stained with hematoxilin and eosin.

Photomicrographs were taken with a Zeiss microscope.

Results

ERG and Histology of Normal Eyes

The responses recorded from the eye of a normal mouse are shown in Figure 1. The numbers on the left represent the value of the neutral density filter used to reduce the full intensity stimulus (at 0, irradiance = 2.91 \times 10^3 \mu W/cm^2). A small, long latency b-wave was recorded at intensity 5.0. With increasing intensities, there was an increase in the b-wave amplitude and a decrease in the latency. It should be noted that the gain was reduced by a half at I = 2.0. The a-wave was first seen at intensity 2.0. The mouse ERG was characterized by very prominent oscillatory potentials (OPs) which were first seen when the stimulus intensity was 1.0 to 1.5 log units above threshold. Other than the prominent OPs, the ERGs of mice were typical of rod-dominated retinas.

The amplitude of the b-wave was measured from responses such as these, and intensity-response curves plotted. The results from ten normal eyes are shown in Figure 2A. These curves demonstrate the variation in the b-wave amplitude between animals. The means and standard error of the means (SEM) for these data are shown in Figure 2B. The stimulus intensity required to elicit a 25 \mu V b-wave was 4.30 log units, and the maximum amplitude b-wave was 473 \mu V.

Previous studies have shown that approximately 30% of the uninoculated eyes remain histologically normal throughout the experimental period. Intensity-response curves from four uninoculated eyes which had normal amplitude ERGs throughout the experimental period (last tested on days 17 to 20 PI) and had normal histological appearance of the retinas are shown in Figure 3. The numbers in the brackets represent the PI day on which the ERGs were recorded. Thus for the eye shown in A, ERGs were recorded on PI days 6, 8, 10, 11, 12, 14 and 17. These data are presented here to show the variation in the amplitude of the b-waves when they are recorded from the same eye on different days. They also demonstrate that anesthetizing and recording the ERGs
on successive days do not depress or otherwise alter the ERGs.

A photomicrograph of a normal uninfected mouse retina is shown in Figure 4. The histological architecture of the mouse retina is typical of rod-dominated retinas.

**ERGs and Histopathology of the Inoculated Ipsilateral Eyes**

ERGs were recorded from 53 inoculated eyes. An example of one type of change induced in the ERGs of the ipsilateral eyes is shown in Figure 5. When first tested on day 3 PI, there was a general depression of all components of the ERG without any preferential loss of one component. The threshold stimulus intensity was elevated to 3.00 log units, and the maximum amplitude of the b-wave was reduced to 260 μV. When tested again on day 6 PI, only a weak response of abnormal shape was elicited at the higher intensities (1.0 and 0). On day 21 PI, a response could not be elicited from this eye, i.e., the ERG was abolished. These findings demonstrate that HSV-1 can lead to the abolition of all responses from the inoculated eye.

A more common finding is illustrated in the responses recorded from another inoculated eye (Fig. 6). When first tested on day 4 PI, the threshold stimulus intensity was higher at 3.5 to 3.0 log units, and the maximum b-wave amplitude was reduced to 140 μV. All components of the ERG were depressed. The ERGs at day 7 PI were slightly smaller than at 4 days,
Fig. 4. Photomicrograph of a normal mouse retina, original magnification ×200. PE = pigment epithelium; OS and IS = outer and inner segment of photoreceptors; ONL = outer nuclear layer; INL = inner nuclear layer; IPL = inner plexiform layer; GCL = ganglion cell layer.

Fig. 5. Averaged ERGs recorded from an inoculated ipsilateral eye on days 3, 6 and 21 PI. Sixteen responses were averaged with a dwell time of 4 msec. The numbers to the left of each ERG represent the value of the neutral density filter used to attenuate the full intensity stimulus. The recordings labelled "B" are the baseline noise levels. The ERGs shown at the bottom of the days 3 and 21 PI recordings were recorded before dark adaptation with a 2.0 neutral density filter. Calibration: 100 μV; 100 msec.

Fig. 6. Averaged ERGs recorded from an inoculated eye on days 4, 7 and 11 PI. Sixteen responses were averaged with a dwell time of 4 msec. The numbers to the left of each ERG represent the value of the neutral density filter used to attenuate the full intensity stimulus. The recordings labelled "B" are the baseline noise levels. The ERGs shown at the bottom of the days 4 and 11 PI recordings were recorded before dark adaptation and was recorded with a 2.0 neutral density filter. Calibration: 200 μV; 100 msec.

but no further reduction was noted at 11 days. Thus, in this eye, the inoculation of HSV-1 led to a reduction but not abolition of all components of the ERG.

The question then arose as to when the changes in the ERG can be first detected in the inoculated eyes. The ERGs recorded from two ipsilateral eyes at day 2 PI are shown in Figure 7. In both eyes, the threshold stimulus intensity was elevated to 3.0 log units, and the maximum b-wave amplitude was reduced to 190 and 200 μV. As in the previously shown ERGs, all components were depressed. Four other eyes were tested on day 2 PI, and the means and SEM of the b-wave amplitudes for these six eyes are shown in Figure 8 along with the mean intensity-response curve for the normals. As in the individual ERGs, the mean data showed that the threshold was higher (3.10 log units) and the maximum b-wave amplitude was reduced (200 μV). An analysis of variance (with the Newman-Keuls multiple comparison procedure) showed that the responses at day 2 PI were significantly weaker than the normals (P < 0.001).

Similar ERG recordings were obtained from six eyes tested on days 3, 4, and 5 PI. The means and SEM of these results also are shown in Figure 8. There was no significant change between days 2 to 3 PI, and between days 4 and 5 PI (P > 0.05). However, the decrease between days 2 and 4 was significant (P < 0.05).

The maximum b-wave amplitude (V_{max}) was reduced by approximately 60% on days 2–3 PI and by 80% on days 4–5 PI. However, the stimulus intensity (I_{50}) required to elicit a b-wave of one-half the V_{max}...
was reduced only by approximately 0.50 log units for days 2 through 5 PI. Thus, the herpesvirus has altered the \( V_{\text{max}} \) more significantly than the sensitivity of the retina.

The mean and SEM of the b-waves obtained from ten ipsilateral retinas recorded after day 10 PI (days 10–32) are shown in Figure 9 along with the data for the eyes tested on day 5 PI. An analysis of variance showed that the differences were not significant \( (P > 0.05) \). These results indicate that the changes in the ERG of the ipsilateral eyes are essentially complete by day 5 PI.

The alteration of the ERGs of the ipsilateral eyes were classified as normal, reduced or abolished after the changes had run the full course \( (PI > 5–6 \text{ days}) \). Of the 53 ipsilateral eyes followed for longer than 6 days, four \( (7.5\%) \) remained normal, 30 \( (56.6\%) \) had reduced ERGs and 19 \( (35.8\%) \) had abolished ERGs (Table 1). These results demonstrate that following the inoculation of this dosage of the KOS strain of HSV-1 into the anterior chamber, there is a reduction or abolition of the ERG in 92.4% of the inoculated eyes.

Photomicrographs of retinas obtained at various stages of the retinopathy in the inoculated eyes are shown in Figure 10A–D. A characteristic finding in the retinas with reduced ERGs was the presence of...
focal areas of retinal folds (Fig. 10C, D). Between these folds, the layering of the retina was preserved and, except in those eyes with abolished ERGs, the photoreceptors appeared intact. On day 2 PI (Fig. 10A), an early stage of the folds was noted as a bending of the outer nuclear layer (ONL) vitreally, thus increasing the area between the external limiting membrane (ELM) and the pigment epithelium (PE). The outer segments of the photoreceptors were distorted over the folds, and the PE cells were swollen and vacuolated. The ERGs from this eye were reduced (Fig. 7, right column). Under higher magnification, the ELM could be seen to bend and run parallel with the ONL. The inner nuclear layer (INL) was either displaced vitreally and laterally so that the thickness of the INL varied from two to 12 nuclei in the region of the folds. However, the nuclei stained uniformly and appeared normal otherwise. The ganglion cells (GC) also appeared normal.

The folds were deeper on day 4 PI (Fig. 10B) in a retina whose ERG was abolished. The material in the cleft of the folds is probably disintegrating outer segments as it stained similarly to the outer segments of the photoreceptors in normal retinas. The outer segments of the photoreceptors at the edges and in other regions of the retina were short and distorted. Two large vacuoles lie vitread of an intact Bruch’s membrane within the PE cells. The vacuoles appeared empty with the toluidine blue stain. Such vacuoles and other alterations of the PE cells were usually seen overlying the folds during the early stages. The ELM could be followed to the edge of the folds but could not be seen within the folds. The INL at the bottom of the fold is two to three layers thick, but on either side of the folds, the INL is 10 to 12 nuclei thick.

A photomicrograph of a retina with reduced ERGs (see Fig. 6) at day 11 PI is shown in Figure 10C. The fold was deeper and extended through the INL. The ELM was seen to run up to the edge of the fold but could not be seen within the fold. In other, less deep folds from this same section, the ELM could be traced throughout the fold. The PE cells appeared intact and showed no signs of any earlier pathology. Within the cleft, disintegrating outer and inner segments were present, and in the regions adjacent to the folds, the photoreceptors were markedly abnormal. An isolated group of nuclei belonging to the ONL can be seen in the INL. The staining characteristics of the INL appeared normal.

At day 21 PI, there was very little organized material in the clefts of the folds (Fig. 10D). The ERGs from this retina were abolished (see Fig. 5). The PE cells overlying the fold appeared to be normal, and the photoreceptors adjacent to the folds and in other regions of the retina were markedly abnormal. The INL and GC stained normally.

Histopathological changes were found in all eyes with depressed ERGs.

### ERGs and Histopathology of the Uninoculated Contralateral Eyes

ERGs were recorded from 55 uninoculated eyes. An example of the type of changes induced in the ERGs of the contralateral eyes is shown in Figure 11. On day 8 PI, there was a slight elevation of the threshold to 4.00 log units and a decrease in the maximum amplitude of the b-wave to 240 μV. The ERG was abolished 24 hr later (day 9 PI), and was still abolished when tested again on days 12 and 20 (not shown PI).

Because Atherton and Streilein have shown that the viral titers in the contralateral eyes did not increase until day 7 PI, systematic ERG recordings from the contralateral eyes were not begun until day 6 PI. The intensity-response curves for eyes tested on days 6, 7, 8 and 9 PI are shown in Figure 12. The letters in the brackets denote the eventual outcome of these eyes, ie, the ERG response at PI day > 10. The code for the letters is presented in the legend to the figure.

On day 6 PI, ERGs were recorded from five eyes, and all of the intensity-response curves were comparable to those of normal eyes. Two of the eyes had abolished ERGs when tested six days later on day 12 PI. One eye remained normal throughout the testing period (normal ERGs on PI day 17), and two eyes were prepared for histology. Examination of the results on day 9 PI showed that the intensity-response curves were either in the normal range (n = 3) or were completely flat, ie, ERG abolished (n = 4). The three eyes with large amplitude ERGs remained normal throughout the experiment (last tested on days 17, 17 and 20 PI). The ERGs from one of the eyes which had a flat intensity-response curve are shown in Figure 11, day 9 PI.

On day 8 PI, the intensity-response curves fell into two groups. Of the four curves in the normal range, three remained normal throughout the experimental period (tested on days 17, 17 and 20 PI), and one was

### Table 1. Effect of HSV-1 on the ERG

<table>
<thead>
<tr>
<th>ERG</th>
<th>Ipsilateral</th>
<th></th>
<th>Contralateral</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
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<td>7.2</td>
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<tr>
<td>Abolished</td>
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<td>35.8</td>
<td>36</td>
<td>65.4</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>99.9</td>
<td>55</td>
<td>99.9</td>
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</table>
Fig. 10. Alteration of retinal histology of the HSV-1 inoculated eyes at different postinoculation days. (A) = day 2 PI, X240; (B) = day 4 PI, X300; (C) = day 11 PI, X315; (D) = day 21 PI, X378. Abbreviations as in Figure 4.
Fig. 11. Averaged ERGs recorded from an uninoculated, contra-

lateral eye on days 8, 9 and 12 PI. Sixteen responses were averaged

with a dwell time of 4 msec. The numbers to the left of each ERG

represent the value of the neutral density filter used to attenuate

the full intensity stimulus. The recordings labelled "B" are the baseline

noise levels. The ERGs at the bottom of the days 8 and 12 PI

recordings were recorded before dark adaptation with a 2.0 neutral

density filter. Calibration: 200 μV for I = 0 to 2.0 on day 8 PI, and

100 μV for all others; 100 msec.

Fig. 12. Intensity-response curves obtained from the contra-
lateral eyes on days 6, 7, 8 and 9 PI. The letter in the brackets denotes

the eventual outcome of the eye after it had runs its course, ie, PI

> 10 days. The code is: N = normal, F = flat or abolished ERG, H

= eyes prepared for histology after ERG recordings, and D = ani-

mal died before it could be tested again.

preparing for histology. This retina appeared histologically normal. Among the eyes with the weaker in-

tensity-response curves, one had no response on the

next day (see day 9 PI) and the other eye was prepared

for histology (see Fig. 13A).

On day 7 PI, all of the intensity-response curves

(six) were comparable to those of normal eyes. One of

these eyes remained normal throughout the experi-

mental period (last tested on day 15 PI), three eyes

had no response, ie, abolished ERG on PI days 9, 11

and 13, and one eye was prepared for histology. For

the last eye, the animal died before the ERGs could

be repeated. These data show that on day 7 PI, it was

not possible, from an examination of the intensity-

response curves, to differentiate the eyes that would

remain normal from those which would eventually

have abolished ERGs.

Of the 55 contralateral eyes followed for longer

than 10 days, 15 (27.3%) eyes remained normal, four

(7.2%) eyes had reduced responses and 36 (65.5%)

eyes had abolished ERGs (Table 1). Thus in the con-

tralateral eyes, 72.7% of the eyes had reduced ERGs,

which is significantly lower than the percentage

(92.5%) of affected ipsilateral eyes (P < 0.015). An

examination of only the affected eyes showed that the

percentage of eyes with abolished ERG was signifi-

cantly (P < 0.005) higher in the contralateral eyes

(90%, 36/40) than in the ipsilateral eyes (38.8%,

19/49).

Examples of the alterations in the retinal histology

observed in the uninoculated eyes are shown in Fig-

ure 13A–D. The first day on which reduced ERGs

were recorded was on day 8 PI, and photomicro-

graphs of two retinas on day 8 PI are shown in Figure

13A and B. In Figure 13A the retina from an eye with

reduced ERGs (see intensity-response curve in Fig.

12, day 8 PI) is shown. There was preservation of the

layering of the retina and the outer region of the ret-

ina was normal with normally staining photorecep-

tors. The only sign of abnormality at the light micro-

scopic level was seen as an increased granularity of

the chromatin material in some of the nuclei in the

INL and of the GC (arrows).

The other day 8 PI retina had abolished ERGs and

showed greater histopathological changes (Fig. 13B).

The layers of the retinas could still be distinguished

but there was increased granularity of the chromatin

material in the nuclei, and in many there was a dis-

placement of the chromatin material to the edges of

the nuclei in both the INL and in the GC. The photo-

receptors and PE cells (not shown) were also altered

in this retina.

At day 9 PI (Fig. 13C), the ONL abutted against the

PE and the photoreceptors were not present. The

ERGs from this retina were abolished (see intensity-
Fig. 13. Alteration of retinal histology of the uninoculated eyes at different post-inoculation days. (A) = day 8 PI, ×625; (B) = day 8 PI, ×528; (C) = day 9 PI, ×800; and (D) = day 11 PI, ×650. Abbreviations as in Figure 4.
response curve in Fig. 12, day 9 PI). Most of the nuclei in the INL were devoid of chromatin material and appeared as empty circles. The nuclei of the ONL were intermixed with those of the INL which made it difficult to differentiate these two layers. There was also increased granularity of the chromatin material in the nuclei of the PE cells.

By day 11 PI (Fig. 13D), the retinal architecture was lost, with nuclei of the ONL and INL intermixed. The ERG was abolished from this retina. The nuclei of the ONL were abutted against the PE cells with no sign of the photoreceptors. The histopathological appearance of the other eye of this animal is shown in Plate 10C, and the ERGs are shown in Figure 6. By day 14 PI, the retina was completely necrotic with complete loss of all retinal architecture.5,14

All of the uninoculated eyes with depressed ERGs had retinal histopathological changes and those with normal ERGs (n = 15) had normal retinas.

Correlation Between the ERG Changes in the Inoculated and Uninoculated Eyes

An examination was made of 35 pairs of eyes which had ERGs recorded from both eyes after the pathological process had run its full course, ie, after day 10 PI (Table 2). There were three inoculated eyes which had normal ERGs and in all three animals, the opposite eyes also had normal responses. In none of the 35 pairs of eyes was there a pair with normal ERGs in the inoculated eyes and depressed ERG in the other eye. These findings would suggest that in the inoculated eyes with normal ERGs the virus did not get to the retina of the inoculated eye.

A comparison of the changes in the two eyes showed that the uninoculated eyes with abolished ERGs were paired with eyes with either reduced or abolished ERGs. There were eight uninoculated eyes with normal ERGs and three of these were paired with inoculated eyes with normal ERGs. For the other five eyes, the ERGs were abolished in four and reduced in one of the paired inoculated eyes. This suggests that the absence of a pathological change in these eyes was not due to the failure of injecting the virus into the other eye.

### Table 2. Correlation between the ERGs of paired eyes

<table>
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<th>Inoculated</th>
<th>Normal</th>
<th>Reduced</th>
<th>Abolished</th>
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<tbody>
<tr>
<td>Normal</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reduced</td>
<td>1</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Abolished</td>
<td>4</td>
<td>0</td>
<td>8</td>
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</table>

Discussion

The results of this study have shown that the ERG changes induced by HSV-1 inoculation into the anterior chamber of one eye were very different for the inoculated and uninoculated eyes. In the inoculated eyes, reduced amplitude ERGs were recorded in 92.5% of the eyes, which was significantly higher than the 72.7% of the contralateral eyes with reduced responses. The percentage of eyes with abolished ERGs, on the other hand, was significantly higher in the contralateral eyes (90%) than in the ipsilateral eyes (38.8%). Another difference between the eyes was the time course of the changes: in the ipsilateral eyes, the changes were seen as early as day 2 PI; in the contralateral eyes, the first changes were noted at day 8 and abolished responses were recorded on days 8 to 9 PI.

At first hand, it might be suggested that the higher percentage of altered ipsilateral eyes is related to the fact that the inoculation was performed in this eye. However, while the peak viral titers in the ipsilateral and contralateral eyes were comparable, the time of peak titers was different.5 In addition, the percentage of eyes (posterior segments) from which viral DNA could be recovered was the same for the two eyes, although the day of peak recovery was different.5 Thus, the significantly higher percentage of inoculated eyes with altered ERGs cannot be explained as resulting from the inoculation of the herpesvirus into this eye.

The histological study of the retinas showed that the pathological changes in the two eyes were also very different. In the inoculated eyes, the changes were mainly in the outer retina and PE. There were focal areas of retinal folds interspersed with regions of normal-appearing retina. Associated with these folds were pathological changes in the PE. The photoreceptors within and adjacent to the folds were degenerated, and in some retinas, the photoreceptors were abnormal throughout the retina. The inner retina appeared to be normal. In the uninoculated eye, on the other hand, changes were first seen in the inner retina as alterations in the chromatin material of the nuclei in the INL and in the GCL. There was then a rapid progression of the changes to include the photoreceptors and the PE. After running its full course, the retina was completely necrotic with fibrous tissue replacing the retina.

The pathological changes in the ipsilateral retina—the retinal folds—has not been described previously in this model of herpetic retinitis although folds in the retina have been described previously in newborn rabbits following subcutaneous inoculation of...
HSV-2. Retinal folds have also been observed following other viral infection of neonatal retinas in a variety of animals. Although it is difficult to "follow" the evolution of the folds by histological studies, it appears that the depth of the folds can increase over the first several days PI. The mechanism for the generation of these folds remains unclear but the juxtaposition of PE changes and the folds might suggest a causal relationship.

The significant higher percentage of abolished ERGs in the uninoculated eyes is probably a reflection of the more severe histopathological changes in the contralateral retinas. In the contralateral eyes, there is complete retinal necrosis so that even the gross structure of the retina is lost (Fig. 13D). This panretinal necrosis can easily account for the abolished ERGs. In the four eyes with reduced ERGs, the retinas were not completely necrotic. In the ipsilateral eyes, the layering of the retina is preserved (Fig. 10A–D), but the retina is not completely normal as characteristic retinal folds were observed in the ipsilateral retinas with reduced ERGs. Between these folds, normal-appearing retina is present. We suggest that the responses arise from these areas of normal retina, and that the folds account for the reduction of the ERGs. In those inoculated eyes with abolished ERGs, the photoreceptors throughout the sections examined were abnormal. However, because of the limited area of the retina examined in histological sections, it is not possible to state that the photoreceptors throughout the entire retina were abnormal. In like manner, it was not possible to correlate the degree of reduction of the ERG with the degree of retinal pathology because of the limited sampling of the retina.

The pathogenesis of the retinopathy of the inoculated and uninoculated eyes has not been established, and two hypotheses have been put forth on the mechanism. The first hypothesis states that the changes result from the direct cytopathic effect of the herpesvirus, and the second hypothesis states that the changes result from the immunological responses of the animal which attack and destroy the virus-infected cells. The differences in the ERG and histopathological changes in the two eyes may indicate that these two mechanisms may be operating in the two eyes.

It should also be considered that initially the herpesvirus may alter the excitability mechanisms of the retinal neurons without altering the morphology of the retinal cells, as has been reported for dorsal root ganglion cells (DRG) in culture. Fukuda and Kurata and Mayer et al have shown that infection of DRG cells by HSV-1 resulted in the loss of excitability with the preservation of the resting membrane potential and the input resistance of the cells. The gross morphology of the cells was not altered at this stage. The depression of excitability was explained as resulting from a decrease in Na+ channel activity similar to tetrodotoxin poisoning. This loss of excitability was induced by two nonsyncytial strains of the HSV-1. In contrast, one syncytial strain of HSV-1 did not alter the excitability of the DRG cells but induced spontaneous action potentials. The KOS strain of HSV-1 used here is a nonsyncytial strain of HSV-1. Thus, if the KOS strain of the HSV-1 is acting in a similar fashion on the retinal neurons, there might be a reduction in the amplitude of the ERG without significant alterations in the morphology of the retina.

As shown, there was a depression of all components of the ERG in both the inoculated and uninoculated eyes. This finding is somewhat unusual inasmuch as alteration of the extracellular environment, eg, pH, PO2, high K+, or the exposure of the retina to toxins usually leads to a preferential loss of the b-wave leaving the late receptor potential. This loss of the b-wave has been interpreted as resulting from the greater sensitivity of the cells in the inner retina to the altered conditions. With HSV-1 infection, however, it appears that either all of the retinal cells are affected or that the photoreceptors are primarily affected, leading to depression of cells fed by the photoreceptors. Immunoperoxidase staining showed that antigen-bearing cells were found in all layers of the retina, and thus the effect of the virus does not appear to be primarily on the photoreceptors.

The use of the ERG has provided new information on the pathological changes in retinal function induced by HSV-1 infection. The ability to follow the time course of the disease and to assess the extent of the loss of excitability may provide additional information on the mechanism of the HSV-1 induced pathology.

Key words: herpes simplex virus type 1, HSV-1, virus, electroretinogram, mouse, histopathology, retinopathy

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