Evidence that a-Wave Latency of the Electroretinogram Is Determined Solely by Photoreceptors

Hui Qiu*, Eriko Fujiwara†, Mu Liu‡, Byron L. Lam‡ and D. I. Hamasaki‡

*Department of Ophthalmology, Hamamatsu University Medical School, Hamamatsu, Japan;
†Department of Ophthalmology, Fukuoka University School of Medicine, Fukuoka, Japan;
‡Bascom Palmer Eye Institute, University of Miami, School of Medicine, Miami, FL, USA

Purpose: To identify the retinal cells that determine the a-wave latency of rats.

Methods: Electroretinograms (ERGs) were recorded from the rod-dominated (0.85% cones) retinas of Long-Evans rats following an intravitreal injection of 1 μL of 40 mM 2-amino-4-phosphonobutyric acid to block the activity of the ON pathway of the second order retinal neurons. ERGs were also recorded following an intraperitoneal injection of sodium iodate to destroy the retinal pigment epithelial (RPE) cells. Damage to a large area of the retina was produced by constant light exposure, and focal damage to the retina was induced by argon laser photocoagulation. The effects of age and anesthesia level on the a-wave latency were also determined.

Results: Blocking the activity of the ON pathway of the second order retinal neurons did not alter the a-wave latency, and destroying the RPE cells also did not alter the a-wave latency. Damage to a large area of the retina resulted in prolonging the latency but focal retinal damage did not alter the a-wave latency. The a-wave latency was longer in young rat pups but was adult-like by 18 days. The level of anesthesia had no effect on the latency except at very deep stages.

Conclusions: The a-wave latency is determined solely by the activity of the photoreceptors. A prolonged latency would indicate that the photoreceptors are damaged over a large area of the retina. **Jpn J Ophthalmol 2002;46:426–432** © 2002 Japanese Ophthalmological Society

Key Words: a-Wave latency, 2-amino-4-phosphonobutyric acid, electroretinograms, sodium iodate, rat.

Introduction

In a previous paper, we demonstrated that reliable measurements of the a-wave latency of the electroretinograms (ERGs) can be made, and that the potential level (μV) of the latencies selected was lower than the mean pre-stimulus baseline potential level by 1 and 2 standard deviations (SD).[1] We recommended that the a-wave latency rather than the implicit time (IT) be used to assess the temporal course of the a-wave.

It has been clearly established that the a-wave arises from photoreceptor activity, and it is also known that the later phase of the a-wave is altered by the activity of second-order neurons, eg, bipolar cells. Thus, the IT is determined by the activity of both the photoreceptors and bipolar cells, and the physiological significance of the IT is not known. To be especially meaningful in laboratory and clinical studies, it would be more important to obtain a measure of the time course of the a-wave whose cellular origin is known.

We hypothesized that the latency of the a-wave of the ERG is determined solely by the activity of the photoreceptors. To test this hypothesis, we compared the a-wave latencies of normal rats with rod-dominated (0.85% cones) retinas to that of rats treated with 2-amino-4-phosphonobutyric acid (APB) and to rats treated with sodium iodate. We also determined how other factors such as age, level of anesthesia, and focal or extensive photoreceptor damage affected the a-wave latencies.

Materials and Methods

Experimental Procedures

The experimental procedures were similar to those described in detail in the previous paper.[1] Briefly, experiments were conducted on adult Long-Evans rats weighing approximately 200–300 g. Immunohistochemical methods have shown that the cones make up only 0.85% of the photoreceptors in the rat retina.[2] The rats were housed in standard rat cages with lighting on a 12-hour:12-hour dark:light cycle. All animals used were treated in accordance with the ARVO Resolution on the Use of Animals in Research, and the experimental protocol was approved by the University of Miami Animal Care and Use Committee.

Recording the ERGs

The ERGs were recorded from anesthetized rats with a wick-Ag:AgCl electrode placed on the cornea, and the reference electrode placed subcutaneously on the head. The animals were grounded by a needle placed subcutaneously in the neck region. The signals were fed to a Tektronix A39 preamplifier with the half-amplitude bandpass set at 0.1 Hz to 10 kHz. The output from the preamplifier was displayed on an oscilloscope and also fed to a Biopac MP100 for signal averaging. The sampling rate was 1000 samples/s.

Stimulus

The light for the stimulus was obtained from a 15-V 10A quartz-iodide lamp bulb. The maximum luminance of the stimulus was 2.53 log cd/m^2 and neutral density (ND) filters were used to reduce the full-intensity stimulus. The stimulus intensities were increased in 1.0 log unit steps and are designated by the ND filter used to attenuate the full-intensity stimulus. A pulse generator (S44; Grass Instruments, Quincy, MA, USA) drove the Uniblitz shutter and controlled the stimulus duration at 1 second. Two responses were averaged at the lower stimulus intensities (ND = 6.0 to 4.0) and one response at the higher stimulus intensities (ND = 3.0 to 0). With each increase in stimulus intensity, the recovery period was increased stepwise from 1.5 minutes at the lowest stimulus intensity to 8 minutes at the highest stimulus intensities.

Treatment Protocol

To block the activity of the ON pathway of the second order neurons, 1 μL of 40 mM APB was injected into the vitreous of the left eye.[3] The animal was then set up for recording as usual with 30 minutes of dark-adaptation. The time between the injection of APB and the beginning of the recordings was about 45 minutes, and the recordings for the entire intensity series required approximately 35 minutes. After these recordings, the same amount of lactated Ringer's solution (1 μL) was injected into the right eye, and ERGs were recorded as with the APB-injected eye.

In 1954, Noell reported that an intravenous injection of sodium iodate destroyed the RPE cells in rabbits and monkeys.[4] The physiological consequence of the destruction of the RPE cells was the loss of the c-wave of the ERG. To determine whether the activity of the RPE cells affected the a-wave latency, we injected 10 mg (in 0.2 mL) of sodium iodate intraperitoneally in 2 rats and 15 mg in 1 rat. ERGs were recorded from both eyes of each rat approximately 24 hours later.

To determine whether focal damage to the photoreceptors will alter the a-wave latency or whether damage to a large area of the retina was needed to prolong the a-wave latency, we compared the a-wave latency in rats that were exposed to constant light[5] for 24 and 48 hours (n = 3) to the latency of rats with focal retinal damage induced by laser photocoagulation (n = 4). To produce the focal damage of the photoreceptors, we created approximately 100 focal burns at the posterior pole of the eye with an argon laser. On the following day, the animals were anesthetized and ERGs were recorded as usual.

Procedure

After the animal was set up and the eye was dark-adapted for 30 minutes, ERGs were recorded, beginning with a stimulus intensity that was determined from earlier experiments to be close to the b-wave threshold (ND = 6.0). The stimulus intensity was in-
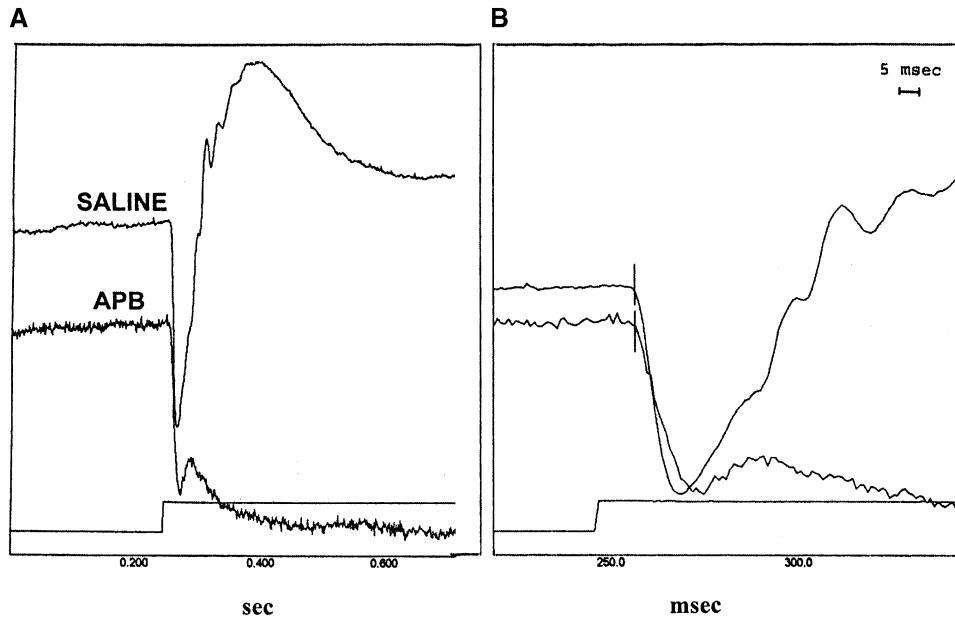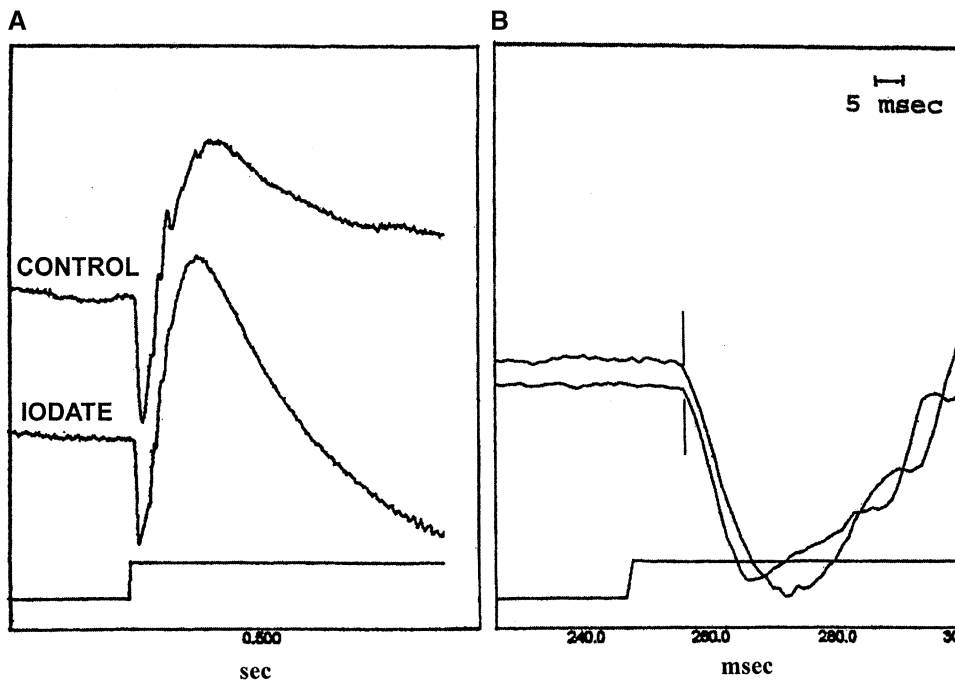
Figure 1. (**A**) Electroretinograms (ERGs) recorded from a rat eye injected intravitreally with 1 mL of 40 mM 2-amino-4-phosphonobutyric acid(APB) and from a control rat eye injected with 1 mL of lactated Ringer solution. Both ERGs were elicited by the full-intensity stimulus. (**B**) The time base has been expanded for the ERGs.

creased in 1.0 log unit steps to the full-intensity stimulus intensity.

The amplitude of the a-wave was measured from the baseline to the trough of the a-wave, and the amplitude of the b-wave was measured from the baseline or trough of the a-wave to the peak of the b-wave. The a-wave latencies and implicit times were measured as described in the previous paper.[1]

Figure 2. (**A**) Electroretinograms (ERGs) recorded from a rat eye 24 hours after an intraperitoneal injection of 10 mg sodium iodate, and from a control eye. Both ERGs were elicited by the full-intensity stimulus. (**B**) The time base has been expanded for the ERGs.
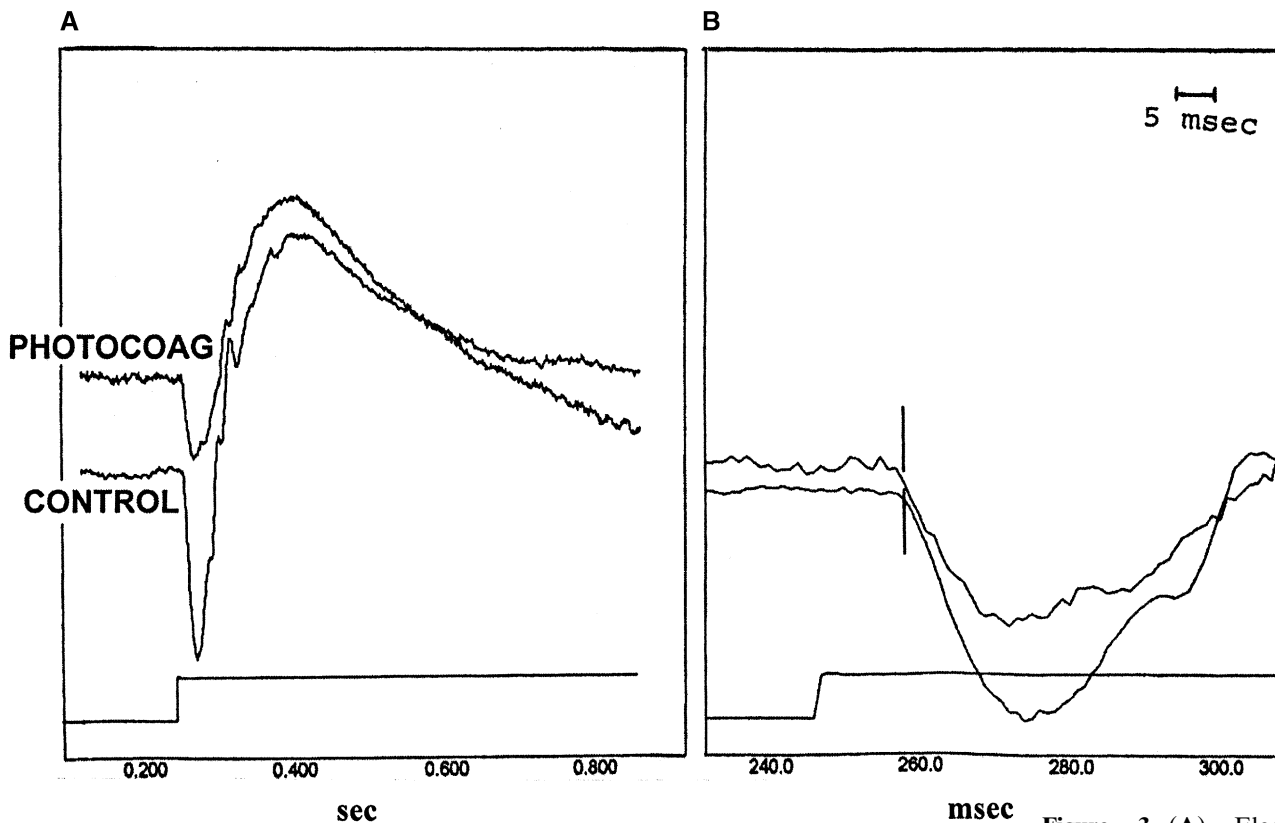
Figure 3. (A) Electroretinograms (ERGs) recorded from a rat eye given approximately 100 focal argon laser burns in the posterior pole 24 hours earlier. The ERG recorded from a control rat eye is shown for comparison. Both ERGs were elicited by the full-in-

Statistical Analysis

The Student unpaired *t*-test was used to examine the statistical significance of any differences. The *P* value < .05 was considered significant.

Results

Effect of APB

The ERGs recorded from an APB-injected eye showed the typical changes, viz, a loss of the b-wave except for a small b-wave at the highest stimulus intensity, leaving only the PIII component. The ERGs recorded from the saline-injected eyes had the same threshold, amplitude, and shape as those recorded from an untreated eye (data not shown).

The ERGs elicited by the full-intensity stimulus (luminance = 2.53 log cds/m^2) from a Ringer's-injected eye and from an APB-injected eye are shown in Figure 1A. The stimulus onset has been aligned to show the time course of the ERGs. The loss of the b-wave can be seen in the APB-injected eye. To demonstrate the a-wave latencies more clearly, the time

base was expanded (Figure 1B), and it can be seen that the a-wave latency of the APB-injected eye was the same as that for the Ringer's-injected eye.

Similar experiments were conducted on 6 pairs of eyes, and the mean a-wave latency was 11.8 ± 1.2 milliseconds for the APB-injected eyes, and 10.8 ± 0.5 milliseconds for the saline-injected eyes. This difference was not significant (*P* > .05).

Effect of Sodium Iodate

The ERG recorded from one of the rats injected with 10 mg iodate and the ERG from a control rat are shown in Figure 2A. Both ERGs were elicited by the full-intensity stimulus. The strong negative-going wave following the b-wave in the iodate-injected eye showed that the positive c-wave, originating from the RPE cells, had been greatly reduced.

To examine the a-wave latencies more clearly, the time base was expanded (Figure 2B). The a-wave latencies of these ERGs are the same and demonstrate that the a-wave latency was not altered by destroying the RPE and abolishing the c-wave.
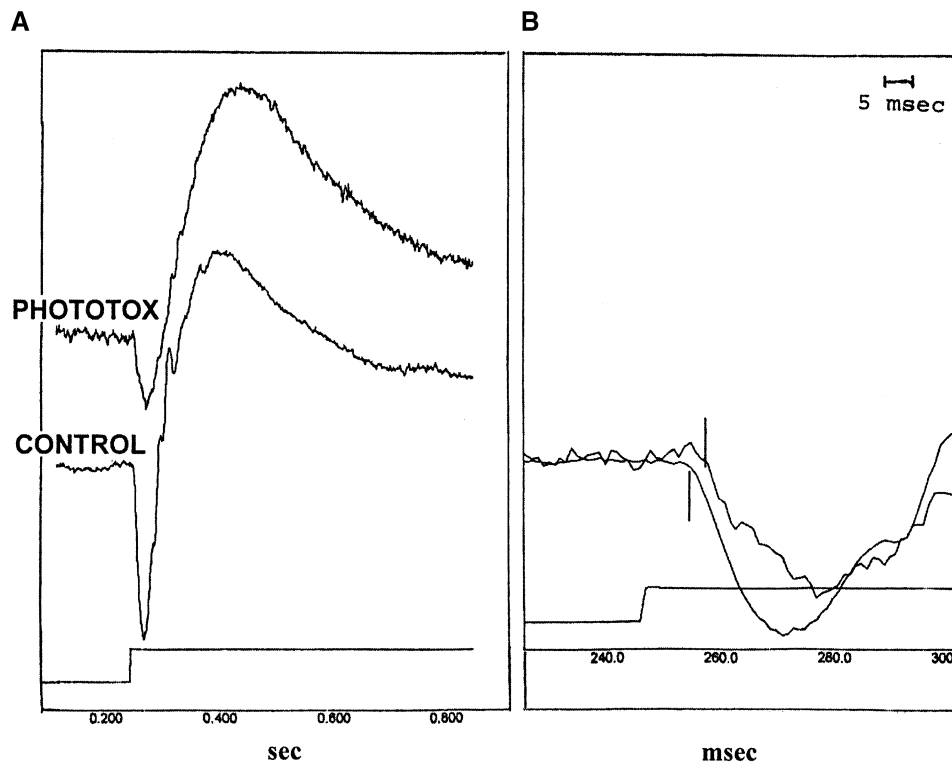
Figure 4. (**A**) Electroretinograms (ERGs) recorded from an eye of a rat that had been exposed to continuous light for 24 hours. The ERG from a control eye is shown for comparison. Both ERGs were elicited by the full-intensity stimulus. Phototox: phototoxicity). (**B**) The time base has been expanded for the ERGs.

Similar results were obtained from the other two animals (1 with 10 mg and the other with 15 mg iodate), although the b-wave was also strongly depressed with the 15 mg dosage.

These two sets of experiments demonstrated clearly that the a-wave latency was not affected by the ON pathway of the bipolar cells and by the activity of RPE cells. Because the cones make up only 0.85% of the photoreceptors in the rat retina, the effect of blocking the OFF pathway with cis-2,3-piperidine-dicarboxylic acid (PDA) was not performed.

Effect of Photoreceptor Damage

In the previous paper, we showed that stimulus intensity and background illumination affected the a-wave latency.[1] The question then arose as to what other factors alter the a-wave latency. Because the a-wave latency is determined by photoreceptor activity, we compared the effects of focal damage to the photoreceptors with the effects of damage to a large area of the retina induced by light toxicity.[5]

The ERGs recorded from an eye with focal retinal lesions is compared to the ERG from a control animal in Figure 3A. Both ERGs were elicited by the full-intensity stimulus. The focal coagulations reduced a- and b-waves to approximately 60% of the control ERG. To examine the a-wave latencies more clearly, the time base was expanded (Figure 3B), and it can be seen that the a-wave latency in the eye with the focal retinal damage was the same as in the control eye. For the four eyes, the mean latency in the eyes with photocoagulation was 10.5 milliseconds, while in the control eyes, the mean latency was 11.0 milliseconds. This difference was not significant (t-test; $P > .05$).

To destroy a large area of the retina, rats were exposed to constant light for 24 (n = 2) or 48 (n = 1) hours. To do this, the pupils of the eyes of the rats were first dilated with 1% atropine ointment, and the rats were placed individually in a clear, shoe-box type of cage and exposed to continuous illumination. The cage was set on a photographic light box. Fluorescent lights were on continuously. The luminance in the box was determined with the photometer detector turned in different directions: in the upward
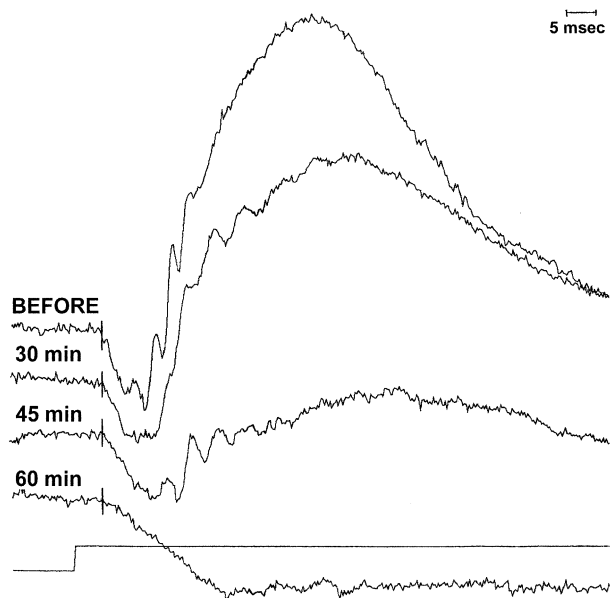
and downward direction, the luminance was approximately 2.55 log cd/m^2, and in the lateral direction, the luminance was 1.6 log cd/m^2. After the continuous exposure, the animals were anesthetized and ERGs were recorded as described.

The ERG recorded from an animal that was exposed for 24 hours is compared to the ERG from a normal rat in Figure 4A. Both ERGs were elicited by the full-intensity stimulus. As expected, the amplitudes of both the a- and b-waves were significantly reduced, and the a-wave latency was prolonged to 12 milliseconds in these exposed eyes. For the eyes exposed to 48 hours of constant light, the ERGs were further reduced and the a-wave latency was increased to 15 milliseconds.

Effect of Level of Anesthesia

Earlier studies have shown that the level of anesthesia can alter the amplitude and IT of the a- and b-waves.[6] This was important because all animal experiments are performed on anesthetized animals, and sedation is used to record ERGs from young children.

After recording the dark-adapted ERGs, a lethal dose of anesthesia was given, and the ERG elicited by the full-intensity stimulus was recorded every 15 minutes until the animal died (Figure 5). During this period, there was a gradual decrease in the amplitude of the b-wave and an increase in the ITs of the a- and b-waves, as has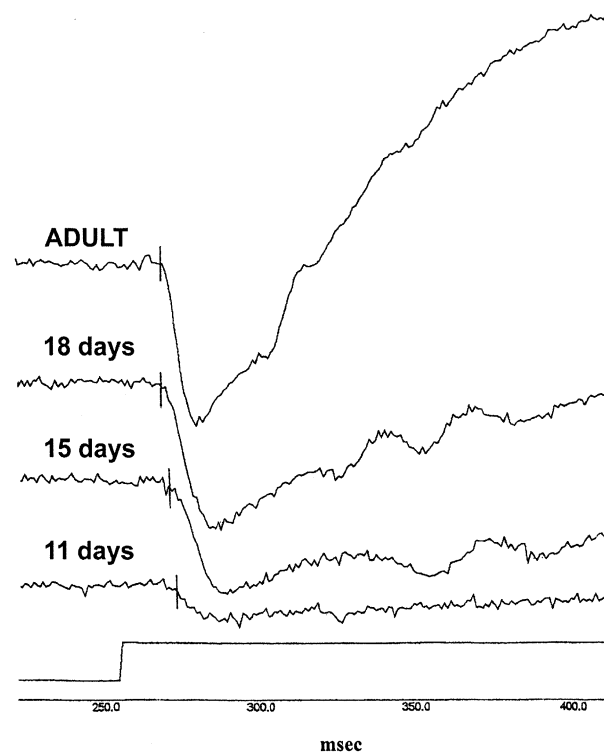 been reported. The latency of the a-wave, on the other hand, did not change even after the b-wave amplitude was approximately equal to the a-wave amplitude.

Effect of Age on the Latency

ERGs were recorded from 4 pups of 11 to 21 days of age. The ERGs elicited by the full-intensity stimulus from an adult is compared with the ERGs recorded from pups of 11, 15, and 18 days of age in Figure 6. At 11 days, the mean latency was 19 milliseconds, and the implicit time of the a-wave could not be determined because the b-wave had not yet developed. At 15 days, the a-wave latency was 16 milliseconds, and at 18 days the latency was 11 milliseconds, which was comparable to the adult. While the a-wave latency was adult-like, the b-wave amplitude was still not adult-like. Similar observations were made for the other 3 animals.

Discussion

These results have shown that blocking the activity of ON-center bipolar cells and abolishing the c-wave did not affect the a-wave latency. Thus, the

Figure 5. Effect of level of anesthesia on a-wave latency. The electroretinograms recorded before and 30, 45, and 60 minutes after a lethal injection of anesthesia.

Figure 6. Effect of age on the a-wave latency. Electroretinograms elicited by the full-intensity stimulus from an 11-, 15-, and 18-day-old rat and an adult rat are shown.

activity in the ON pathway and RPE cells does not affect the a-wave latency. What about the cone-driven, OFF-center hyperpolarizing bipolar cells? As Bush and Sieving showed, blocking these cells by PDA resulted in a change in the a-waves of primates.[7] However, an examination of their Figures 2 and 3 shows that the changes were mainly in the amplitude of the later phases of the a-wave. The a-wave latency, as might be expected, did not appear to be altered. It was only with stroboscopic (microsecond) flashes, that the entire a-wave was affected by PDA. In any case, this question was not relevant as cones make up only about 0.85% of the photoreceptors in the rat retina,[2] and their contribution to the a-waves directly or through the OFF pathway would be minor. Thus, we can conclude that the a-wave latency is determined solely by the activity of the photoreceptors, and any alterations of the a-wave latency must arise from alterations of the photoreceptor transduction process.

Our results showed that focal lesions, which covered approximately one fourth to one third of the retina and reduced the a- and b-waves by up to 50%, did not alter the a-wave latency. However, destruction of a large area of the retina by phototoxicity that also reduced the a- and b-wave amplitude significantly led to an increase in the a-wave latency. These two sets of experiments indicate that changes in the a-wave latency are due to alteration of the photoreceptors over a large area of the retina. For example, we shall show that both the scotopic and photopic a-wave latencies are significantly longer in patients with retinitis pigmentosa (RP).[8] This would indicate that the rods and cones have been affected over a large area of the retina in these RP patients.

We also found that the a-wave latency was not altered significantly by the level of anesthesia. Even after there was a greater than 50% reduction in the amplitude of the b-wave, and the b/a ratio was equal to about 1.0, the a-wave latencies were not altered.

Thus, alterations of the a-wave latencies in animal experiments and also in sedated children cannot be attributed to the anesthesia.

The a-wave latency was also prolonged in very young rat pups. However, at 18 days when the b-waves were still not adult-like, the a-wave latency had attained adult values. These findings indicate that a-wave latencies attain adult values at a very young age.

In summary, we have shown that the a-wave latency is determined solely by the activity of the photoreceptors, and the activity of second-order neurons and RPE cells do not affect the latency. To affect the a-wave latency, a large area of the photoreceptors must be damaged. These findings will help us interpret any changes in the a-wave latencies in laboratory experiments and in patients.

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