

Differential recovery of the electroretinogram, visually evoked cortical potential, and electrically evoked cortical potential following vitrectomy: Implications for acute testing of an implanted retinal prosthesis

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Abstract—To determine the extent to which electrophysiologic tests of the afferent visual pathway are affected by vitrectomy, the procedure was performed in 15 eyes of 11 adult Dutch-belted rabbits. An electroretinogram (ERG), visually evoked cortical potential (VECP), and electrically evoked cortical potential (EECP) were obtained preoperatively and sequentially after surgery. For electrical stimulations, biphasic impulses were delivered to the retina. Post-vitrectomy declines of 49, 25, and 41 percent from the median baseline amplitudes and increases of 13, 18, and 17 percent from the median baseline latency values were found for ERG, VECP, and EECP, respectively. At 90 min, 13 to 30 percent of eyes still had an amplitude more than 10 percent below baseline on at least one of the three tests, whereas 10 to 47 percent of eyes had an abnormal latency more than 10 percent above baseline on at least one of the three tests. Amplitudes were more likely than latencies to return to near baseline, but for eyes that remained subnormal, the decline was greater for amplitudes than latencies. Significant alterations in retinal function, manifested by declines in amplitudes and increases in latencies of the ERG, VECP, and EECP, persist in a large proportion of eyes up to 90 min post-vitrectomy.

Key words: Retinal prosthesis, electroretinogram (ERG), electrically evoked cortical potential (EECP), visually evoked cortical potential (VECP), vitrectomy.

INTRODUCTION

A retinal prosthesis has the potential to restore vision to patients with disease of the outer retina, especially retinitis pigmentosa and age-related macular degeneration [1]. We and others are pursuing this goal using animal [2–5] and human [6,7] retinal stimulation as feasibility steps toward the development of a long-term implantable prosthesis. Both acute [6,8,9] and chronic [7,10,11] retinal stimulation studies related to the development of a retinal prosthesis have been reported.

Surgical introduction of a retinal prosthesis can mechanically damage the implant or the retina, or the saline environment of the eye could damage the microelectronics. Hence it is desirable to test the function of the device and the retina immediately following implantation. Removal of the vitreous humor (i.e., vitrectomy)

Abbreviations: EECP = electrically evoked cortical potential, ERG = electroretinogram, VECP = visually evoked cortical potential.

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would likely be needed to create a surgically well-controlled implantation and to limit postoperative reactions, such as proliferative retinopathy, that could cause vitreous humor traction and retinal detachment [12].

Vitreotomy is known to alter retinal physiology, although this effect is usually temporary and without lasting consequence [13–17]. The effect on retinal function caused by vitrectomy can be tested with the electroretinogram (ERG) or visually evoked cortical potential (VECP) [18,19]. The ERG reflects the electrical activity of the outer (i.e., rods and cones) and middle retina [20,21]. The output cells of the eye, the retinal ganglion cells, do not substantially contribute to the ERG waveform. The VECP reflects electrical activity extending along the entire retino-calcarine pathway (i.e., from the photoreceptors to the primary visual cortex). The amplitude of the VECP derives primarily from activation of the central (i.e., posterior) retina, where there is a relatively high concentration of retinal neurons. Performed together, the ERG and VECP provide a physiological survey of afferent visual function.

An electrically evoked cortical potential (EECP) can be obtained similarly to light-evoked potentials, except that the stimulus is electrical. Hence, the EECP can be used to assess retinal health and device efficacy following implantation of a prosthesis that uses electrical stimulation to activate neurons. Depending on the specific design of a prosthesis, a vitrectomy may be needed to implant the device. Knowing that vitrectomy temporarily alters light-induced retinal responsiveness, we wished to explore the degree to which vitrectomy might also alter electrically induced retinal responsiveness.

MATERIALS AND METHODS

Animals

Thirteen Dutch-Belted rabbits (1.2–2.0 kg) were studied. Two animals that developed a retinal detachment during surgery were excluded; therefore, results from 11 rabbits were included in this study. We obtained data from the second eyes of each animal when possible, but for a variety of technical reasons we only obtained data from 15 eyes in these 11 animals. The protocol for this research was approved by the animal care committee of the Massachusetts Institute of Technology. All animals were treated in accordance with the Association for

Research in Vision and Ophthalmology resolution on the use of animals in research.

Surgery

Animals were initially anesthetized with diazepam (1 mg/kg) and ketamine (35 mg/kg) given intramuscularly and then maintained with 2-percent isoflurane inhalation. Adhesive tape was extended across facial and cranial bones to the base of the surgical table to enhance the stability of positioning of the head with respect to the micromanipulator (see below). A closed vitrectomy to remove the core vitreous humor (which typically required 5–10 min) was performed with a vitrector (Storz Premiere™, model DP2072). The height of the infusion bottle (BSS PLUS, Alcon Co.) generally was 60 cm above the head. Intraocular pressure, measured with a pneumotonometer (Tono-pen®XL Tonometer Mentor®), was maintained between 15 and 20 mm Hg by adjustment of the volume of infusion fluid and height of the infusion bottle. A micromanipulator was used to hold a 125 µm diameter electrode in the mid-vitreous. After the baseline EECP was recorded, the electrode was removed, and the length that had been positioned intraocularly was measured in microns with the micromanipulator as the electrode was withdrawn. After surgery, the intraocular stimulating electrode was reintroduced (using the same angle of insertion) to the original depth. Some variation in the relative positions of the retina and electrode, as occur with systolic/diastolic pulsations of the retina, was unavoidable. ERGs, VECPs, and EECPs were obtained 15, 30, 45, 60, and 90 min post-vitrectomy.

Electrophysiological Recording Methods

Pupils were dilated with 0.8-percent tropicamide, 5.0-percent phenylephrine hydrochloride, and 1-percent atropine sulphate. Each trial consisted of 100 consecutive, computer-averaged stimulations. Two trials (i.e., a total of 200 stimulations) were made for each test and for each time period. The average amplitude of the two trials is the reported value. Noise recordings were obtained by identical methods, except that the light or electrical stimulus did not reach the animal. Ambient illumination was 480 lumen/ft² (i.e., photopic condition).

For the ERG, recordings were made under photopic conditions because we wanted the same recording conditions before and during surgery, and surgery required bright light. The corneal recording electrode was a modified Machemer magnifying vitrectomy lens (Storz, Oph-

thalmic Co.) [22]. The common reference was on an ear. The retina was stimulated with a Grass PS22 photostimulator (Mini Ganzfeld, model PSD22D) positioned 2 cm above the cornea. The photostimulator flash intensity setting was 2 (equal to 2.75 lumen s/ft², for a 10 μ s flash), and flash frequency was 2 Hz. The b-wave amplitude was measured from the negative peak of the a-wave to the positive peak of the b-wave.

For the VECP, the same photic stimulus and technique were used, except that the recording electrode (500 μ m platinum wire) was placed supradural over the occipital cortex. Another electrode, which served as a reference, was 3 mm in front of the bregma and 2 mm to the right of the sagittal suture. The reported amplitude was measured as the largest peak-to-trough excursion within 50 ms after the stimulus.

For the EECP, a micromanipulator was used to hold a 125 μ m diameter, insulated, platinum/iridium electrode (F. Haer Co.) approximately 1 mm from the retina. The return electrode was a 25-gauge insulated wire that was placed in the retrobulbar space. Biphasic impulses (1 mA, 2 ms) were delivered with a current source by an isolated programmable stimulator (Coulbourn Instruments, Bionic Technologies, LLC). The reported amplitude was measured as the largest peak-to-trough excursion within 10 to 30 ms after the stimulus [23].

Data analysis

Amplitude and latency values were normalized for all three testing methods by assignment of a value of 100 to the baseline recording for each test result for each animal. Across all animals, the median of the normalized values for each testing period was plotted. Non-paired Student *t*-tests were used to measure significance by testing the non-normalized values for each time period against the non-normalized baseline values.

RESULTS

Figure 1 shows a typical series of ERG, VECP, and EECP recordings that were obtained from one animal. **Figure 2a** shows the normalized median amplitudes for ERG, VECP, and EECP obtained during and after vitrectomy from all animals. In general, by the end of a vitrectomy there was a statistically significant decrease in the amplitudes of the ERG, VECP, and EECP ($p < 0.001$ for all, using non-normalized values^{*}). The amplitudes then

progressively increased toward baseline over the next 60 to 90 min. In **Figure 1**, the two traces shown for each test condition are each averaged waveforms of 100 stimulations (see MATERIALS AND METHODS) that were obtained consecutively. Noise recordings were obtained by performing 100 consecutive stimulations without allowing the light or electrical stimulus to reach the animal. The scale of the horizontal axis is the same for all graphs, including the “noise” recordings. Electrical shock artifacts are evident in all the lower traces of electrically evoked waveforms (but not the “noise” recording, seen to the far left of **Figure 1**). **Figure 2b** also shows the normalized median latencies for the three testing paradigms from all animals. In **Figure 2a** and **2b** error bars equal 1 standard deviation. Error bars are not provided for baseline values, since the data at the other time periods were normalized to the baseline. In general, by the end of vitrectomy there was a statistically significant increase in the latencies of the ERG and VECP, but not the EECP ($p < 0.006, 0.01, \text{ and } 0.3$, respectively, using non-normalized values).

ERG amplitudes returned to at least 90 percent of the baseline value by 60 min in 11 of 15 eyes. Of the eyes that remained below this standard, the average decline in amplitude was 28 percent by 60 min. At 90 min post-vitrectomy, two more eyes reached 90 percent of the baseline, and two eyes (of the 15) did not. VECP amplitudes returned to at least 90 percent of the baseline value by 60 min in 9 of 15 eyes. Of the eyes that remained below this standard, the average decline in amplitude was 32 percent by 60 min. At 90 min post-vitrectomy, three more eyes reached 90 percent of the baseline, and three eyes (of the 15) did not. EECP amplitudes returned to at least 90 percent of the baseline value by 60 min in 6 of 10 eyes. Of the eyes that remained below this standard, the average decline in amplitude was 18 percent by 60 min. At 90 min post-vitrectomy, one more eye reached 90 percent of the baseline, and three eyes (of the 10) did not.

ERG latencies returned to at least 90 percent of the baseline value by 60 min in 10 of 15 eyes. Of the eyes that remained below this standard, the average increase in

^{*}Non-normalized, median values are used for the statistical analyses for amplitudes and latencies because use of a normalized value of 100 for baseline measurements produces an artificial standard deviation of 0, which inflates the statistical power of the *t*-test when post-baseline values are compared to baseline results.

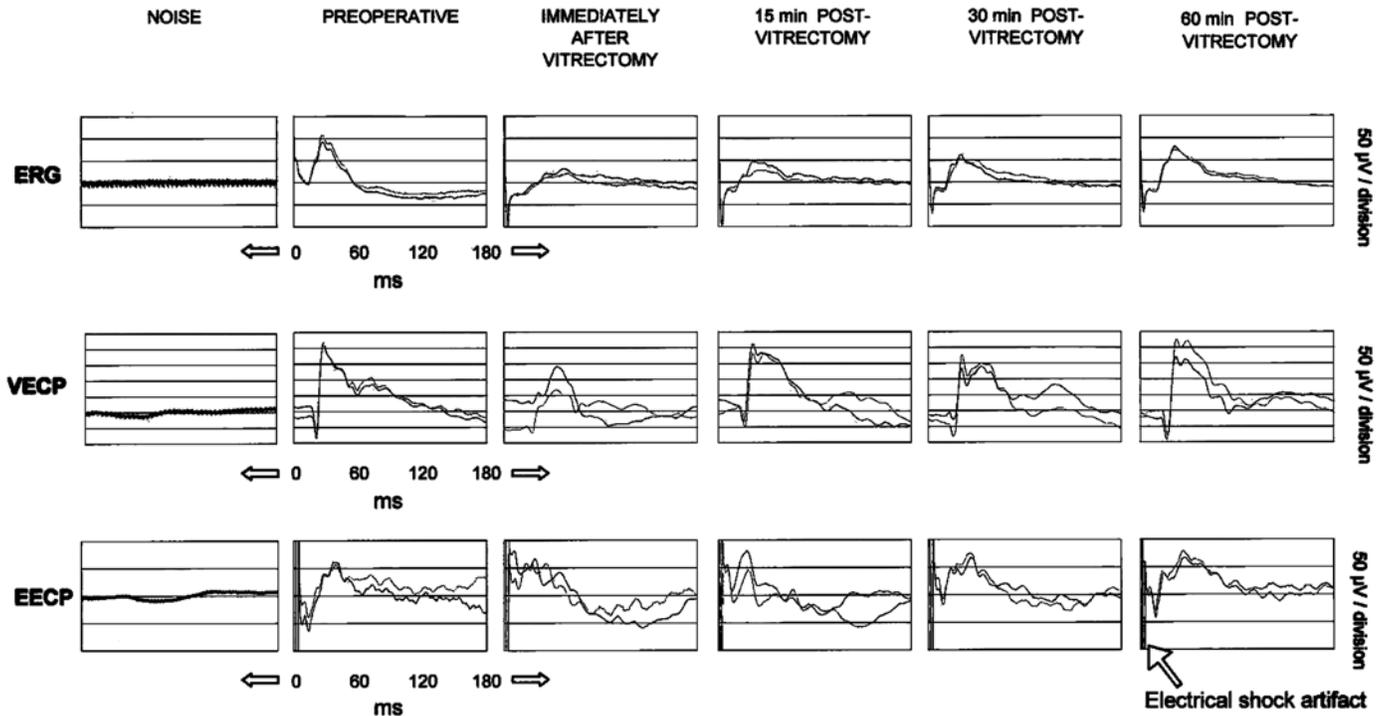


Figure 1.

Preoperative and sequential postoperative ERG, VECP, and EECp waveforms in one animal, showing decrease in amplitudes after surgery and the recovery of signals to roughly baseline by 60 min for each testing method.

latency was 13 percent by 60 min. At 90 min post-vitrectomy, no additional eyes reached 90 percent of the baseline. VECP latencies returned to at least 90 percent of the baseline value by 60 min in 8 of 15 eyes. Of the eyes that remained below this standard, the average increase in latency was 21 percent by 60 min. At 90 min post-vitrectomy, no additional eyes reached 90 percent of the baseline. EECp latencies returned to at least 90 percent of the baseline value by 60 min in 9 of 10 eyes. The tenth eye had a latency 10 percent above its baseline value at 60 min (a recording at 90 min was not obtained for this eye because of an unexplained increase in electrical noise).

DISCUSSION

Vitrectomy was associated with significant alteration in retinal responsiveness. Post-vitrectomy, we found declines of 49, 25, and 41 percent from the median baseline ERG, VECP, and EECp amplitudes, respectively. We also found increases of 13, 18, and 17 percent from the median baseline ERG, VECP, and EECp latencies,

respectively, at the end of vitrectomy. At 90 min post-vitrectomy, 13 to 30 percent of the eyes still had amplitudes that were more than 10 percent below baseline on at least one of the three tests. At the same time point, 10 to 47 percent of the eyes had an abnormal latency that was more than 10 percent above baseline on at least one of the three tests. In general, amplitudes were more likely than latencies to return to near baseline, but for those eyes that remained subnormal, the percentage of decline was greater for amplitudes than for latencies.

The frequency and magnitude of these alterations in retinal function after vitrectomy in rabbits are quite significant. For the purpose of prospective evaluation of device and retinal function post-implantation, "baseline" values for the ERG, VECP, and EECp should not be sought at the end of surgical implantation. The delay that is required to reach a true baseline (i.e., the point at which retinal function could be expected to approach preoperative values) probably cannot be prescribed, since the amount of surgery and the degree of manipulation will vary in accordance with methods used by each group. At the minimum, however, our results suggest that

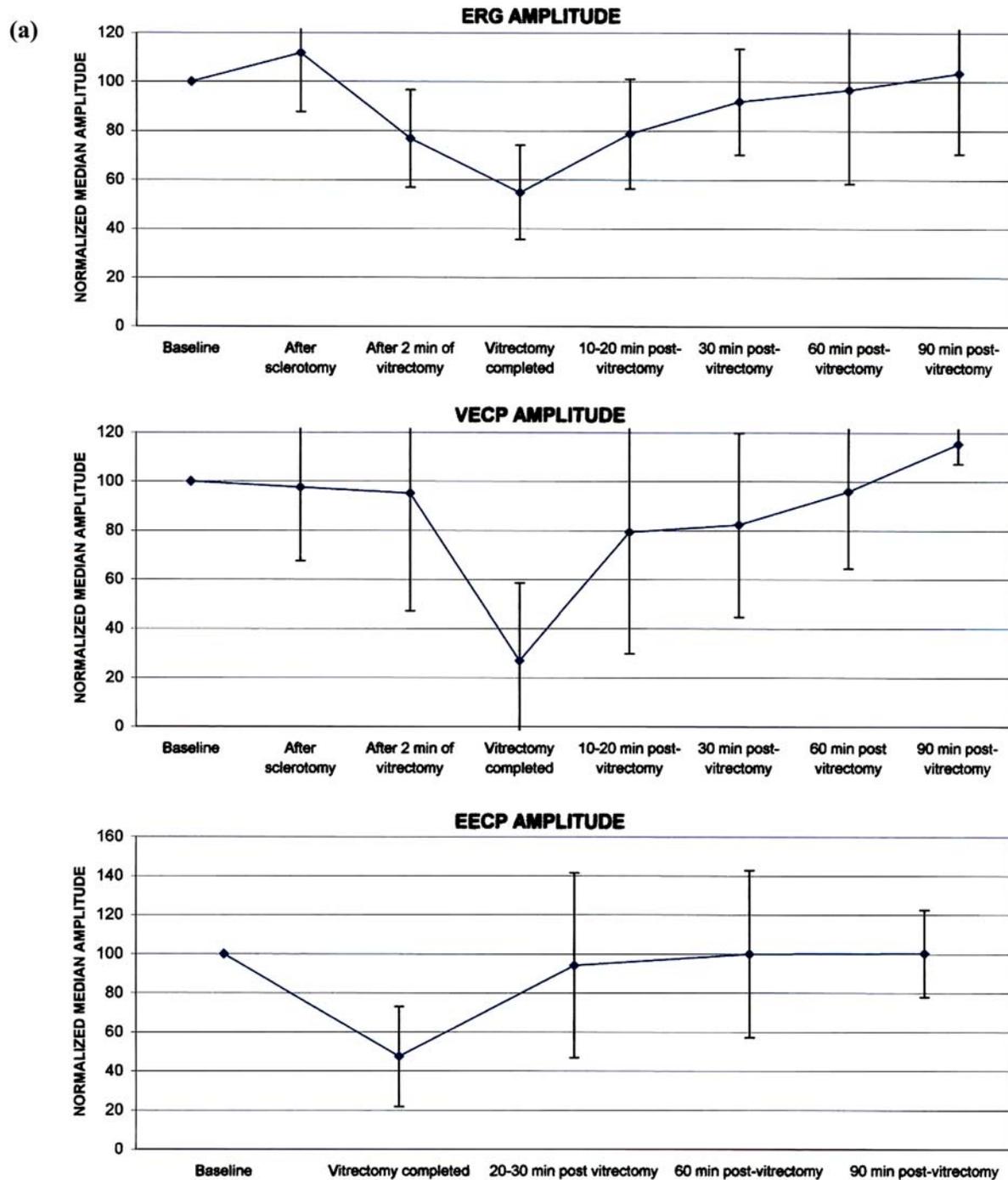


Figure 2a.

Normalized, median amplitudes of ERG, VECP, and EECp before and after vitrectomy for all animals.

a period of at least 90 min must elapse post-vitreotomy to begin to obtain values that could serve the purpose of the prospective assessment of biocompatibility or device

function. Our findings are consistent with Hesse's suspicion that vitrectomy might explain high stimulation thresholds following array implantation in cats [10].

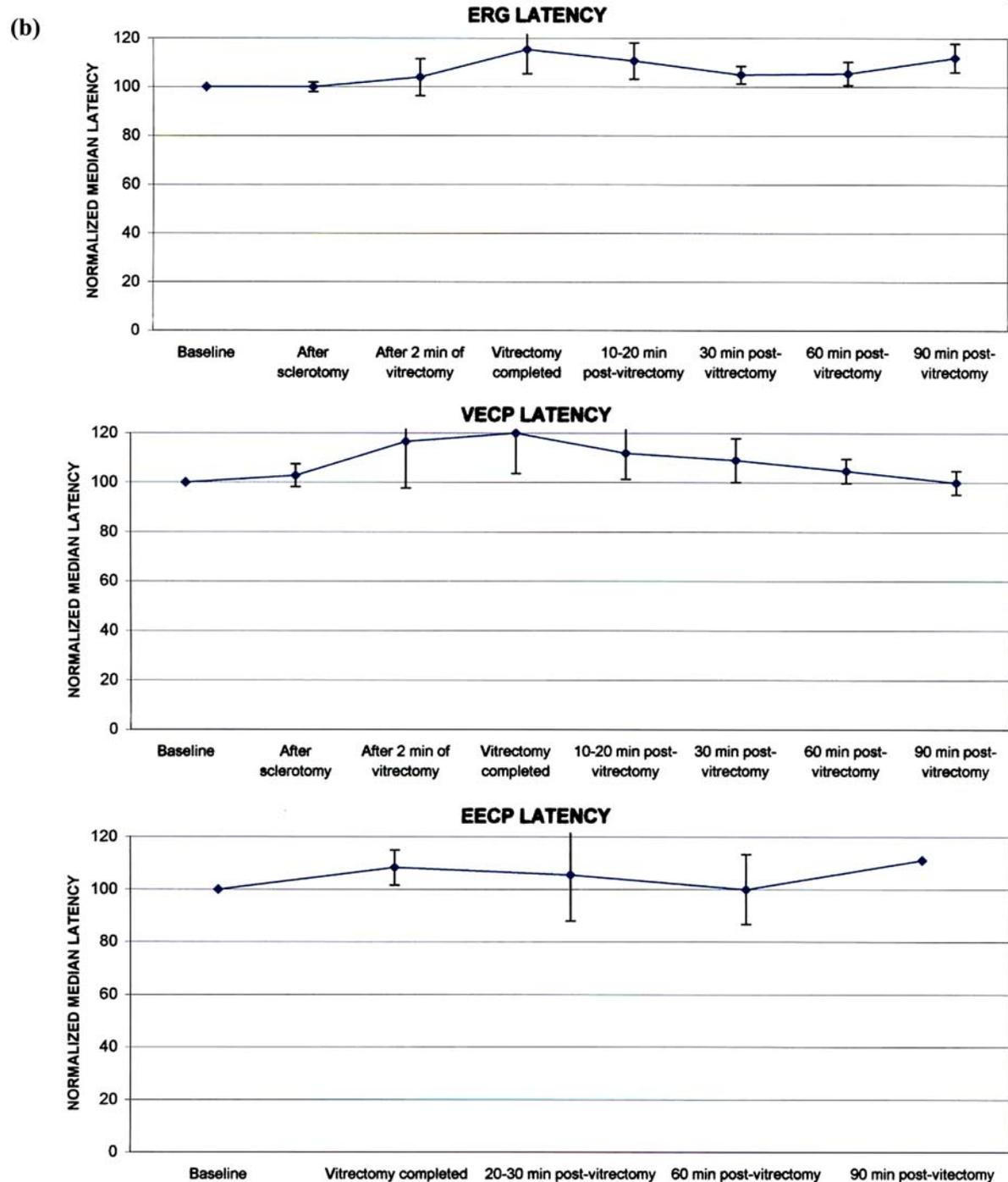


Figure 2b.
Normal median latencies of ERG, VECP, and EECp before and after vitrectomy for all animals.

Several factors could account for the temporary decline in ERG amplitude and increase in ERG latency following vitrectomy. Temperature of infusion fluid

(which is cold relative to the body), composition of the infusion fluid, type and level of general anesthesia, inappropriately high or low intraocular pressure, and surgical

complications such as retinal detachment could individually or collectively contribute to the decrease in amplitude [13,17,24–31,32]. An effect of this type has been observed in humans following vitrectomy, with decreased ERG amplitudes for up to five days [15,16]. The explanation for this prolonged period of subnormal ERG is not known.

The effect of temperature on the infusion fluid has been examined by several investigators. Horiguchi et al. demonstrated in humans that room-temperature infusion fluid can lower ERG amplitudes and that the reduced responsiveness was not caused by light adaptation, phototoxicity, or removal of the vitreous humor [17]. Lachapelle et al. also provided evidence that subnormal temperature of the eye alone can compromise the ERG [25]. There is no evidence that room-temperature infusate causes permanent damage to the retina, which explains the current surgical standard of not pursuing the considerable effort that would be required to maintain intraocular homeothermia during vitrectomy. The practice of using cold infusion fluid is also supported by findings of Tamai et al., who showed that warmer fluids (38 °C) cause more retinal edema post-vitreotomy and post-elevation of intraocular pressure than does room-temperature or cooler fluids (22 and 8 °C) [33,34]. Further, Jabbour et al. found that local ocular hypothermia (down to 7 °C) in experimental open-sky vitrectomy in rabbits reduced intraocular bleeding, fibrin production, and postoperative inflammation [35].

The composition of infusion fluids also influences the potential for damage to the retina, lens, or cornea [36]. In our study we used BSS PLUS™, because its pH and composition (bicarbonate and other ions) causes less alteration of the ERG [13,26,27]. BSS PLUS™ also protects blood-retinal barrier function [37]. Other commercially available solutions have also been found to be similarly beneficial [38,39].

Our study revealed reduced VECP amplitudes and increased latencies during and after vitrectomy. Unlike the ERG, the latency and amplitude of the VECP fluctuate significantly in relation to the depth of general anesthesia [40]. We used isoflurane, which, like halothane and enflurane, can reduce VECP amplitudes at higher concentrations [41]. We used a level of anesthesia just above that needed to perform eye surgery, and we doubt that anesthesia significantly lowered VECP amplitudes. Rather, we suspect that the temperature of the infusate reduced the VECP amplitudes, since cold fluid reduces outer and middle retinal activity (as measured by the ERG), which

would then diminish the activity of the inner retinal neurons that ultimately drive occipital cortical responses measured as the VECP. Similar dynamics probably affected our EECp recordings. No other studies report the effect of vitrectomy on electrically evoked potentials.

CONCLUSION

Electrophysiology of the afferent visual pathway is a useful adjunct for the assessment of retinal biocompatibility and function of a retinal prosthesis after implantation. These physiologic measures must be interpreted with consideration of the alteration in retinal responsiveness that occurs with vitrectomy.

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