Neuroprotective Effect of Subretinal Implants in the RCS Rat

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Purpose. Retinal prosthetics have been designed to interface with the neural retina by electrically stimulating the remaining retinal circuits after photoreceptor degeneration. However, the electrical stimulation provided by the subretinal implant may also stimulate neurotrophic factors that provide neuroprotection to the retina. This study was undertaken to determine whether electrical stimulation from a subretinal photodiode-based implant has a neuroprotective effect on photoreceptors in the RCS rat, a model of photoreceptor degeneration.

Methods. Eyes of RCS rats were implanted with an active or inactive device or underwent sham surgery before photoreceptor degeneration. Outer retinal function was assessed with electroretinogram (ERG) recordings weekly until 8 weeks after surgery, at which time retinal tissue was collected and processed for morphologic assessment, including photoreceptor cell counts and retinal layer thickness.

Results. At 4 to 6 weeks after surgery, the ERG responses in the active-implant eyes were 30% to 70% greater in b-wave amplitude than the responses from eyes implanted with inactive devices, those undergoing sham surgery, or the nonsurgical control eyes. At 8 weeks after surgery the ERG responses from active-implant eyes were not significantly different from the control groups. However, the number of photoreceptors in eyes implanted with the active or inactive device was significantly greater in the regions over and around the implant versus sham-surgical and nonsurgical control eyes.

Conclusions. These results suggest that subretinal electrical stimulation provides temporary preservation of retinal function in the RCS rat. In addition, implantation of an active or inactive device into the subretinal space causes morphologic preservation of photoreceptors in the RCS rat until 8 weeks after surgery. Further studies are needed to determine whether the correlation of neuropreservation with subretinal implantation is due to electrical stimulation and/or a mechanical presence of the implant in the subretinal space. (*Invest Ophthalmol Vis Sci.* 2005;46:674–682) DOI:10.1167/iovs.04-0515

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Supported by the Rehabilitation Research and Development Service of the Department of Veterans Affairs and by Optobionics Corporation.

Submitted for publication May 10, 2004; revised October 20, 2004; accepted November 5, 2004.

Disclosure: **M.T. Pardue**, Optobionics Corp. (F); **M.J. Phillips**, Optobionics Corp. (F); **H. Yin**, Optobionics Corp. (F); **B.D. Sippy**, Optobionics Corp. (F); **S. Webb-Wood**, Optobionics Corp. (F); **A.Y. Chow**, Optobionics Corp. (E, I); **S.L. Ball**, Optobionics Corp. (F)

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Retinal diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration, are major causes of blindness due to photoreceptor degeneration. Although current treatment options are few, many treatments for these forms of blindness, including gene therapy,[1–3] neurotrophic factors,[4,5] transplantation of retinal tissue,[6,7] and artificial devices,[8–11] are being investigated. Although each approach is different, many may be complementary. Neurotrophic factors or pharmacologic agents, for example, could be used in conjunction with gene therapy.[12–14]

Retinal prosthetics are designed to stimulate the remaining visual pathways in retinas undergoing photoreceptor degeneration by changing the membrane potential of cells within the electrical field of the device in response to light. Despite the many technological obstacles to producing a mechanical device that interfaces with the neural retina, potential advantages to this approach include (1) the potential applicability to all forms of photoreceptor degeneration; (2) the avoidance of ethical issues related to transplantation of fetal retinal tissue; and (3) the ability to create spatial information with an electrode array. Currently, there are two designs: The epiretinal implant rests on the vitreous side of the retina,[9,15] whereas the subretinal implant is placed in the subretinal space between the retinal pigment epithelium (RPE) and the remaining neural retina.[8,11] Until now, much effort has been focused on the level and/or pattern of stimulation[15,16] needed to produce a recognizable visual signal, mainly concentrating on whether the electrical stimulation produced by the prosthetic can evoke a visual sensation in a patient or activity in the visual cortex.[10,17,18] However, in addition to directly stimulating retinal neurons, we propose that the electrical stimulation produced by the visual prosthetic may upregulate neurotrophic factors, resulting in a protective effect on the remaining retinal circuitry.

Electrical stimulation has been associated with neuroprotection through the enhancement of neurotrophic factors in several different neural tissues. Although the exact mechanism is unclear, there are convincing data that link electrical stimulation with the upregulation of neurotrophins in the central and peripheral nervous system.[19–22] Chronic stimulation is usually applied; however, even acute periods of stimulation may be beneficial, as demonstrated by enhanced survival of axotomized ganglion cells after a single 1-hour episode of transcorneal stimulation (Morimoto T, et al. *IOVS* 2003;44: ARVO E-Abstract 109).

Although electrical stimulation has been shown to promote ganglion cell survival (Morimoto T, et al. *IOVS* 2003;44:ARVO E-Abstract 109),[23] direct evidence linking electrical stimulation of the eye and upregulation of neurotrophins in the retina has not been shown. However, there is a large body of literature describing the presence of growth factors in the eye and the ability of these factors to rescue or protect the retina from injury or disease.[4,5,12–14] Thus, applying electrical stimulation to the retina may upregulate endogenous levels of neurotrophins and thereby induce a protective effect on the diseased retina.

In this study, we examined the neuroprotective effect provided by a subretinal device when implanted in a rodent model of photoreceptor degeneration. With any surgical manipulation, some rescue due to injury-induced neurotrophins is possible.[24–26] To distinguish the neuroprotective effect induced by stimulation from the subretinal implant versus effects from surgical manipulation or a foreign body in the subretinal space, the eyes of the RCS rat model were implanted with an active or an inactive implant or underwent sham surgery. This report describes the retinal function and photoreceptor preservation in each experimental group when the RCS rats received the implants at the onset of photoreceptor degeneration.

METHODS

Animals and Experimental Design

RCS rats, originally obtained from a breeding colony maintained by Matthew LaVail (University of California San Francisco) were maintained as a homozygous breeding colony at the Atlanta VA Medical Center. In the RCS rat, a mutation in the *Mertk* gene[27] impairs phagocytosis of the outer segment by the retinal pigment epithelium (RPE),[28,29] resulting in photoreceptor degeneration. All animals were housed in a 12-hour light–dark cycle. All animal procedures were approved by the local animal care and use committee and conform to the standards of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Fifteen RCS rats were used in the study. In the pigmented RCS rat model used, degeneration starts at approximately 12 days, and the photoreceptors are completely degenerated by approximately 77 days.[30] In this study, all rats received implants at 3 weeks of age, near the beginning of the disease process. Thirteen rats had the active device implanted in the right eye, whereas the left eye was implanted with the inactive device ($n = 4$), underwent a sham procedure ($n = 5$), or had no surgery ($n = 4$). Two RCS rats served as completely naïve animals with no surgery in either eye. To measure outer retinal function, electroretinogram (ERG) recordings were made before implantation and every subsequent week for 8 weeks. Immediately after the eighth week of recording, the rats were killed and the eyes enucleated for histologic processing and photoreceptor cell counts.

Implant Design and Surgery

Active and inactive implants were provided by Optobionics, Inc. (Naperville, IL). The artificial silicon retina (ASR) device was a 25-μm-thick, 1-mm-diameter silicon disc comprising a series of photodiodes with iridium oxide surface electrodes (9×9 μm), as previously described.[31,32] The back side of each disc was uniformly covered with iridium oxide. Inactive devices (Optobionics, Inc.), identical in size, were oxidized on both sides.

Each 1-mm ASR device is covered by 1200 photodiodes. These diodes respond to incident light in the range of 500 to 1100 nm.[32] The rats were housed under fluorescent lighting (25–200 lux) in shoe-box cages positioned in a rack. Most of the energy in the cage environment fell between 500 and 650 nm, with a mean irradiance of 0.1 to 10 μW/cm^2. Within this range of wavelengths, the ASR device responds at ~0.3 ampere/watt during bench-top testing in a phosphate-buffered saline environment. Thus, stimulus currents from the ASR device are estimated to range from several nanoamps per square centimeter to 1 μA/cm^2, modulated at 120 Hz, depending on where the animal is positioned in the cage with respect to the light source. Therefore, the specific illumination level on the retina and the ASR may be quite variable and was not directly measured, but was instead predicted from the known environmental lighting levels and in vitro ASR output measurements. Implant electrical activity was confirmed in each rat by the presence of an implant spike in the full-field ERG recording and/or the recording of an implant spike at the corneal surface in response to a 250-ms infrared LED flash.

As described previously, implants were surgically placed in the subretinal space of RCS rats.[33] Briefly, animals were anesthetized with a ketamine (60 mg/kg)/xylazine (7.5 mg/kg) mixture and placed in a sterile field. The eye was then rotated inferiorly by using a traction suture placed at the limbus. A 1.2-mm incision was made in the globe through the sclera, RPE, and retina and into the vitreous. A drop of saline was placed on the eye and the retina was allowed to detach naturally from the RPE over the next 5 minutes. The implant was then gently inserted into the subretinal space, and confirmation of placement under the retina was made by fundus examination. The localized retinal detachment then resolved in 1 to 2 weeks, as confirmed by the retinal function recovery and fundus examination. Sham surgeries consisted of the same surgical procedure, except no device was placed under the retina.

ERG Testing

After overnight dark adaptation, rats were anesthetized (ketamine 60 mg/kg and xylazine 7.5 mg/kg), the corneas were anesthetized with 0.5% tetracaine and the pupils dilated with 1% cyclopentolate and 1% tropicamide. Responses were recorded from the corneal surface of both eyes simultaneously using silver wire loop electrodes. Platinum needle electrodes placed in the cheek and tail served as the reference and ground, respectively. Under dark-adapted conditions, a 10-step intensity series from 0.001 to 10 cd-s/m^2 was presented to both eyes. With increasing intensity, the interstimulus interval increased from 2 to 70 seconds. Responses of 3 to 10 flashes were averaged to generate a waveform at each flash intensity. To isolate cone-mediated responses, eyes were light adapted for 10 minutes with a 20 cd/m^2 adapting field. Cone ERGs were then obtained by using increasing flash intensities (0.78–20 cd-s/m^2) presented at 2.1 Hz, with 25 flashes averaged for each intensity. All responses were filtered from 0.03 to 1000 Hz with response acquisition coordinated using a signal-averaging system (Diagnosys Espion, Littleton, MA).

Histologic Examination

At 8 weeks after surgery, animals were euthanatized with an intracardial injection of saturated potassium chloride while under deep anesthesia. Twenty eyes were immersion fixed overnight in 2% paraformaldehyde and 2.5% glutaraldehyde (nine active-implant, four inactive-implant, two sham-surgical, and five nonsurgical eyes). The remaining 10 eyes were fixed for immunohistochemical analysis, which will be described elsewhere. After overnight fixation, the cornea and lens were removed, leaving a posterior eye cup with the implant in situ, which was dehydrated through a graded series of alcohols and then embedded in plastic resin (Embed 812/DER 736; Electron Microscopy Sciences, Fort Washington, PA). Vertical sections were cut through the implant and retina with a diamond knife (Diatome Histo Knife; Electron Microscopy Sciences) at 0.5 μm and stained with toluidine blue.

A section through a central area of the implant was identified for each eye, and digital images were taken at 10 locations across the retina (see Fig. 5A). In each eye, five separate measurements were made for each location and then averaged as the representative measurement for that retinal location. From each digital image, measurements of retinal layer thickness and counts of photoreceptor nuclei were made with an image-analysis computer program (UTHSCSA Image Tool, available at http://ddSdx.uthscsa.edu/dig/itdesc.html).

Data Analysis

ERG waveform measurements were made of the b-wave amplitude from the trough of the a-wave to the peak of the b-wave (Fig. 1A). When the high-frequency oscillatory potentials (OPs) were prominent on the leading edge of the b-wave, these wavelets were visually smoothed, and the highest point of the large positive wave was measured as the b-wave. After the b-wave decreased to zero, the scotopic threshold response (STR) was visible and was measured from baseline to the trough of the STR (Fig. 1A). The STR was measured only after all

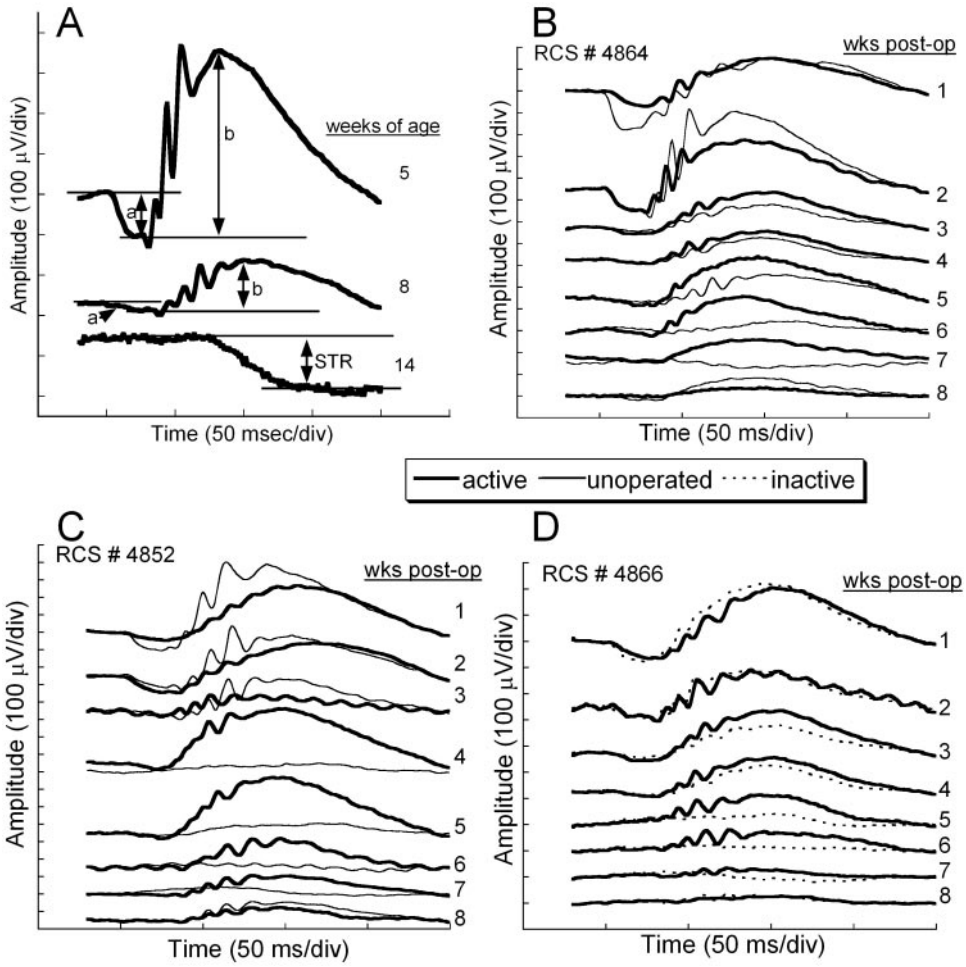

FIGURE 1. (**A**) Representative dark-adapted ERG waveforms from the RCS rats in response to a bright-intensity flash (10 cd-s/m^2) at various ages. The measurement of the a- and b-wave or STR is indicated on each waveform. Note the drastic change in ERG function as photoreceptor degeneration progressed. (**B–D**) The maximum ERG response from three representative RCS rats was recorded weekly for 8 weeks. In each panel, the responses of the eyes of one rat are superimposed. *Thick lines*: active-implant eyes; *thin lines*: opposite eyes, which were either nonsurgical (**B**, **C**) or implanted with the inactive device (**D**). Each waveform is the average of three to five responses to the same flash intensity (10 cd-s/m^2). Immediately after surgery, the responses from implanted eyes were reduced. However, by 4 to 5 weeks after surgery the responses of the active-implant eye were larger than those of the opposite eye in each treatment group. The responses shown in (**C**) are from rat 4852, which demonstrated the largest differences between the nonsurgical and active-implant eyes. In fact, the response from the active eye recovered at 4 weeks after surgery compared with early time points in both amplitude and implicit time. The key applies to graphs **B–D**.

positive waves had disappeared. Thus, the gradual change in ERG waveform is plotted as a continuum from b-wave to STR.

Amplitude measurements were examined over time, using repeated-measures ANOVA across the four treatment groups: nonsurgical, active implant, inactive implant, and sham surgery. Histologic data from each defined location across the retina were compared between treatment groups with a repeated-measures ANOVA. Post hoc comparisons were performed between groups when appropriate.

RESULTS

Retinal Function

Figure 1 shows representative, dark-adapted ERG waveforms at each postoperative week, using the highest flash intensity (10 cd-s/m^2). Each graph presents data from a single RCS rat over the experimental period. For each time point, the responses from the two eyes are superimposed. Each waveform is the average of three to five responses to a 10 cd-s/m^2 flash. Figures 1B and 1C illustrate responses from two RCS rats (4864 and 4852) in which one eye was implanted with the active device (thick line) and the opposite eye was not implanted (thin line). Figure 1D shows responses from RCS rat 4866, in which active (thick line) and inactive (dotted line) devices were implanted.

In RCS rat 4864, shown in Figure 1B, the negative a-wave and positive b-wave were larger in the nonsurgical eye at 1 week after surgery. At 2 weeks after surgery the ERG recordings from the implanted eye were still reduced compared with the nonsurgical eye. A decrease in response amplitude at the first and second week after surgery was seen in all eyes that underwent surgery, regardless of whether it was a sham procedure or implantation with the inactive or active device. Thus, this reduction in amplitude appeared to be due to surgical manipulation. At 3 and 4 weeks after surgery, the b-wave amplitude of the implanted eye was slightly larger than all the other groups. Although overall responses decreased over time due to the photoreceptor degeneration, at weeks 5 to 7 after surgery, the eyes implanted with the active device still had larger b-wave amplitudes. At 8 weeks after surgery, the amplitude in the active eye was actually less than that in the opposite, nonsurgical eye.

Figure 1C shows the responses from RCS rat 4852, which had the active device implanted in the right eye and no surgery in the left eye. The responses from this rat are shown as an example of the largest functional preservation in all the RCS rats studied. Whereas the eye with the active device had reduced responses at postsurgical weeks 1, 2, and 3, by 4 to 5 weeks after surgery, the responses of the active-implant eye were significantly larger than the nearly flat response recorded from the nonsurgical eye. In fact, this response from the active-implant eye was larger, with a faster implicit time than in all the previous weeks, suggesting enhanced retinal function. At 6 to 7 weeks after surgery, the responses of the active-implant eye decreased, and by 8 weeks they were slightly smaller than in the nonsurgical eye.

Figure 1D shows representative waveforms from RCS rat 4866 implanted with the active (thick trace) and inactive (dotted trace) device in the right and left eye, respectively. In this case, both eyes underwent surgery, and thus no differences

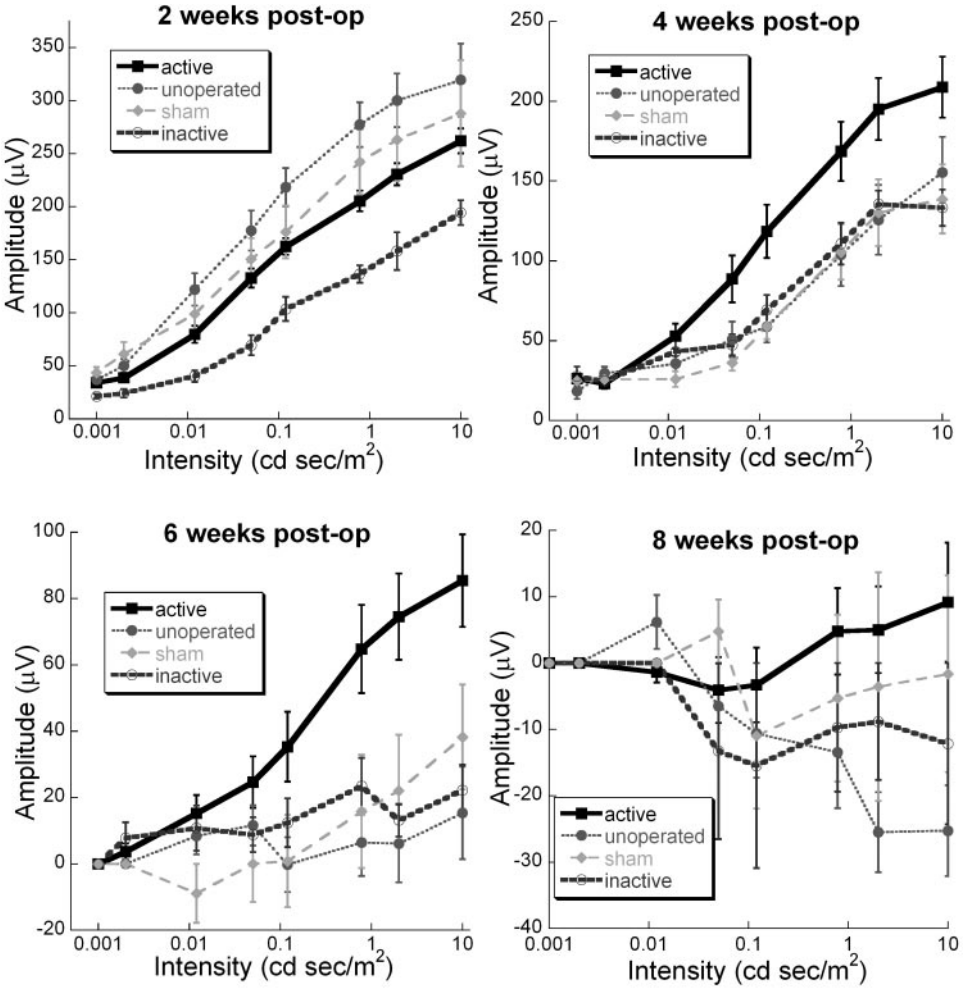

FIGURE 2. Intensity–response functions at 2, 4, 6, and 8 weeks after surgery (±SEM). Each line represents the average amplitude across a dark-adapted intensity series in each treatment group. Although no differences were seen at 2 weeks after surgery, the active-implant eyes had significantly larger responses at 4 and 6 weeks after surgery that then become indistinguishable from those of the other treatment groups at 8 weeks after surgery. Note the change in amplitude scale between panels (n = 13 active-device, 4 inactive-device, 5 sham-surgical, and 4 nonsurgical eyes).

were observed in the first 2 weeks after surgery. Starting at 3 weeks after surgery, the active-implant eye had larger responses than the inactive-implant eye, until 7 weeks after surgery. At 8 weeks after surgery, no differences were detected between the responses of the active- and inactive-implant eyes.

To demonstrate these differences more clearly, Figure 2 presents intensity response functions for all 15 rats used in the study at 2, 4, 6, and 8 weeks after surgery (Fig. 2). An examination of the average b-wave amplitude across the dark-adapted intensity series clearly shows differences between the treatment groups. At 2 weeks after surgery, no significant differences in ERG waveforms were noted between the nonsurgical, active-device, and sham-surgical eyes. In comparison, the ERGs of inactive-implant eyes were significantly smaller ($F_{(3,27)} = 7.07$, $P < 0.001$).

At 4 weeks after surgery, the responses of the active-implant eyes were significantly larger than the responses from all the other treatment groups ($F_{(1,28)} = 9.78$, $P < 0.004$). Between 4 and 6 weeks after surgery, the responses in the sham-surgical, inactive-implant, and nonsurgical eyes decreased by 80%, whereas the responses of the active-implant eyes declined by only 60%. At 6 weeks after surgery, the responses in the active-implant eyes were four times larger than the responses in the other treatment groups ($F_{(1,28)} = 13.01$, $P < 0.001$) indicating preserved retinal function in eyes with subretinal electrical activity. Note that the inactive implant did not appear to provide any functional benefit.

At 8 weeks after surgery, the responses of the active-implant eyes were near zero, whereas only the negative STR was visible in the other treatment groups. At 8 weeks after surgery, the differences between the active-implant eyes and the other control groups were no longer significant ($F_{(3,26)} = 1.52$, $P = 0.23$).

To gain a perspective of retinal function in the treatment groups over time, we plotted the peak amplitude of the dark-adapted ERG response to the brightest flash presented across the 8 weeks for all rats in the study (Fig. 3). As expected, the responses of the nonsurgical eyes declined rapidly over the 8-week testing period. At 1 and 2 weeks after surgery, the responses of eyes undergoing surgery were all reduced. However, from 3 to 5 weeks after surgery, the responses of the active-implant eyes were relatively preserved compared with the other treatment groups. The rate of retinal function loss was slower in the active-implant eyes until 5 weeks after surgery, when the responses were still larger than those of the other treatment groups but were declining at approximately the same rate. By 8 weeks after surgery, no differences were detected between treatment groups. The repeated-measures ANOVA indicates a significant interaction between the treatment groups over time ($F_{(10.59,91.80)} = 2.68$, $P < 0.006$), due to the transient preservation of responses in the active-implant group from postsurgical weeks 3 to 7. However, overall, implantation of the active device did not provide statistically significant preservation across all time points, as determined by testing the main effect of treatment ($F_{(3,26)} = 1.76$, $P = 0.18$). Post hoc simple effects indicate that the active ASR group had significantly higher amplitudes than the nonsurgical eyes from weeks 5 to 8 (Student's t-test, $P < 0.01$). The responses of the

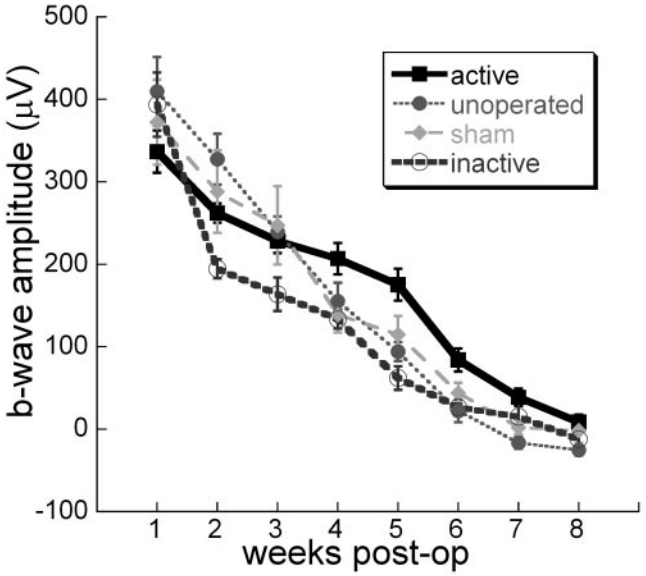

FIGURE 3. Average maximum dark-adapted ERG response (±SEM) plotted over the weeks after surgery. The RCS rat has a progressive photoreceptor degeneration that was clearly identifiable in all treatment groups by the decline in b-wave response. However, the active-implant eyes had significantly greater retinal function than the other treatment groups between 4 and 7 weeks after surgery (n = 13 active-implant, 4 inactive-implant, 5 sham-surgical, and 4 nonsurgical eyes).

active-device group were significantly higher than those of the sham-surgical group at weeks 6 and 7 (Student's t-test, $P < 0.05$) and those of the inactive-ASR group at weeks 2 to 6 (Student's t-test, $P < 0.05$). Thus, implantation with the active ASR provided temporary preservation of retinal function in the RCS rat compared with nonsurgical control eyes, as well as eyes undergoing sham surgery or implanted with the inactive device.

Retinal Morphology

Analyzing retinal morphology across the retina provided a map of photoreceptor preservation in relation to the implant site, which was located in the superior retina. Figure 4 illustrates the retinal morphology at 8 weeks after implantation in specific retinal locations. The top micrographs show retinas from the superior region of an RCS rat with the active device implanted in the right eye (Figs. 4A, 4C) and no surgery in the left eye (Figs. 4B, 4D). In this superior region, only one sparse layer of photoreceptors (arrows) was present in the nonsurgical eye (Fig. 4B), whereas the active-implant eye had four to six rows of photoreceptors remaining (Fig. 4A). Figure 4C shows the retina directly overlaying the implant. Because the implant was sectioned with the tissue to minimize any disruption of morphology that might be caused by removal of the implant, implant debris can be seen as small black particles in the subretinal space. In areas directly over the implant, four to six layers of photoreceptors remained intact, with some photoreceptor inner and outer segments present (Fig. 4C). This is in contrast to the few necrotic photoreceptors present in a similar region in the nonsurgical eyes (Fig. 4D). In rats in which one eye was implanted with the active device and the opposite eye with the inactive device, the differences in the number of photoreceptors was not distinguishable, as shown in Figures 4E and 4F. Thus, implantation of inactive devices also resulted in preservation of photoreceptor cells at 8 weeks after surgery. In the inferior portion of the retina, few photoreceptors remained at 11 weeks of age. Implantation with the active (Fig. 4G) or inactive (Fig. 4H) device did not provide any preservation of photoreceptors in this region of the eye.

Figure 5A is a retinal section of an eye with the active implant at 8 weeks after surgery. For quantitative comparison of the number of photoreceptor nuclei across the retina, we compared 10 adjacent regions of the retina, as illustrated in Figure 5A. Because the ASR devices were always implanted in the superior retina, only three 0.5-mm regions could be quantified above the implant. The remaining seven regions spanned the area over and inferior to the implant. Figure 5B demonstrates a threefold increase in the number of photoreceptors over the implant and in the superior region of the eyes implanted with the active or inactive device after 8 weeks of implantation. This preservation of photoreceptors was significant only in the regions superior to or overlying the implant in the active- and inactive-implant eyes when compared with sham-surgical or nonsurgical control eyes (repeated-measures

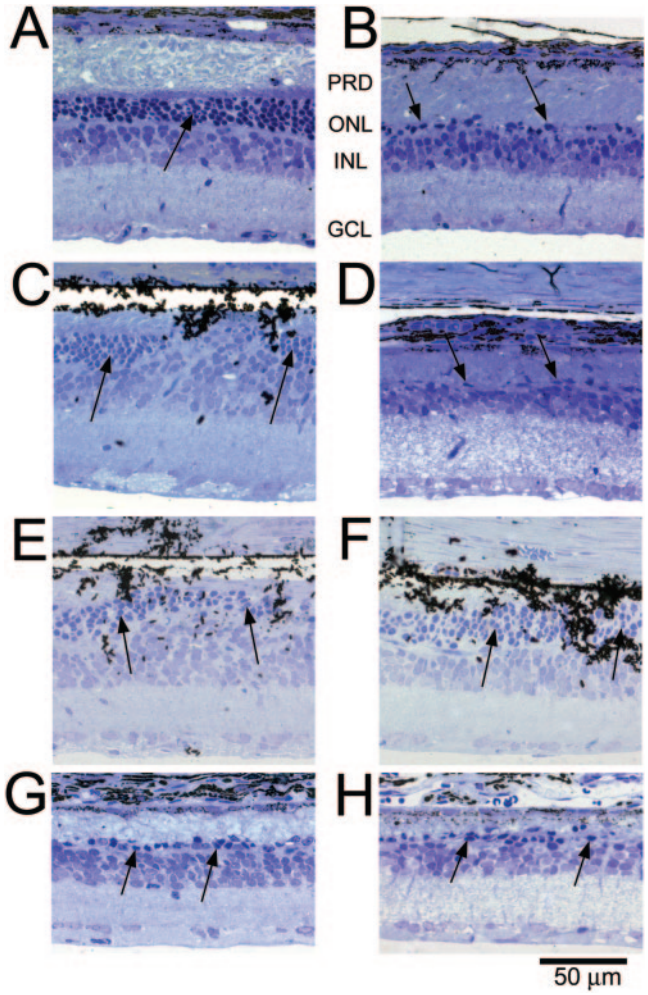

FIGURE 4. Retinal micrographs from RCS rats at different locations across the retina. The superior retina in an active-implant (**A**) and a nonsurgical (**B**) eye; retina directly overlaying the implantation region in an active-implant (**C**) and nonsurgical eye (**D**); retina directly over the implant in an (**E**) active- and an (**F**) inactive-implant eye; inferior retina in an (**G**) active- and an (**H**) inactive-implant eye. *Arrows*: photoreceptor cell nuclei. The *dark* particles are the remnants of the ASR device after sectioning with a diamond knife. PRD, photoreceptor debris.

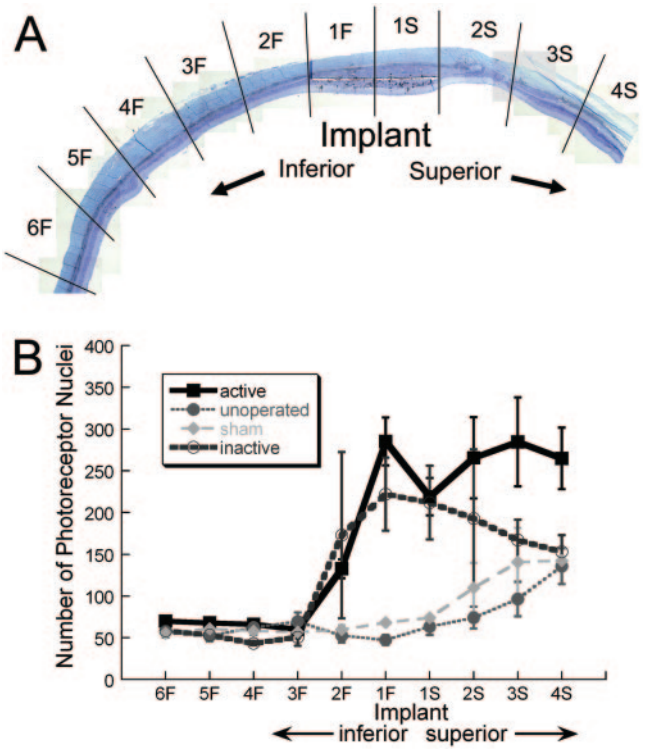

FIGURE 5. (**A**) Micrograph illustrating the 10 retinal locations in which retinal measurements were taken in each eye. The implant is located at 1S to 1F. (**B**) Average number of photoreceptor nuclei (±SEM) versus retinal location for active-ASR, nonsurgical, sham-surgical, or inactive-ASR at 8 weeks after surgery. The numbers of photoreceptors in the active and inactive groups were significantly increased directly overlaying and superior to the implant (n = 9 active-ASR, 4 inactive-ASR, 2 sham-surgical, and 5 nonsurgical eyes).

ANOVA; $F_{(17.80,83.05)} = 2.35$, $P = 0.005$). Although there was a trend toward more photoreceptor preservation in the active- versus inactive-implant eyes in the superior regions, it did not reach statistical significance ($F_{(1,9)} = 0.95$, $P = 0.36$). The protective effect induced by the presence of the active or inactive device did not extend past 1 mm inferior to the implant, with no differences detected in photoreceptor nuclei numbers in areas inferior to the implant among the treatment groups.

To determine the effect of subretinal implantation on the inner retina, thickness measurements were made for each layer across 10 areas of the retina (Fig. 6). The outer nuclear layer (ONL), which contains the photoreceptor nuclei, reflected the same increase in the number of photoreceptor nuclei in the superior retina as shown in Figure 5. As with photoreceptor numbers, ONL thickness was significantly greater in the superior region of the eye ($F_{(6.05,96.75)} = 3.18$, $P < 0.000$), and the eyes with active or inactive devices had the thickest ONL ($F_{(3,16)} = 6.80$, $P < 0.004$). The outer plexiform layer (OPL) was thicker directly over the implant ($F_{(8.31,132.89)} = 3.75$, $P < 0.000$). However, no significant differences were seen between treatment groups. For inner nuclear layer (INL), inner plexiform layer (IPL), and ganglion cell layer (GCL) thicknesses, no significant differences between treatment groups were found. There were, however, differences in thickness across the retina in each of these layers, so that the superior region was thinner (INL: $F_{(5.86,93.78)} = 4.06$, $P < 0.01$; IPL: $F_{(3.93,59.01)} = 7.53$, $P < 0.001$; GCL: $F_{(4.46,22.65)} = 12.58$, $P < 0.001$).

DISCUSSION

Preservation of Retinal Function from Subretinal Implants

These results indicate that when implanted subretinally, before photoreceptors have completely degenerated, an electrically active ASR device provided temporary preservation of retinal function in RCS rat retina. This preservation of function was not found in sham-surgical, inactive-implant, or nonsurgical eyes from 4 to 7 weeks after surgery.

A single-flash full-field ERG response, as recorded herein, results from the combined activity of all the active outer retinal cells. Thus, it is not possible to detect very small changes in a localized area of the retina. However, large areas of retinal disruption result in reduced responses. Immediately after implantation surgery, we expected to have some reduction in ERG responses, due to the creation of a retinal detachment caused by the presence of the 1.0-mm implant in the small rat eye. Although a portion of the retina reattaches, as evidenced by the final histologic appearance of the photoreceptors, the presence of the solid disc implant creates a permanent localized retinal detachment.[34] The reduced responses recorded from the eyes with the inactive implants at 2 weeks after surgery demonstrate such a situation. Based on this hypothesis, the eyes implanted with the active device should also show reductions in ERG amplitudes. However, at this time point, the active-implant responses were similar to those from the sham- and nonsurgical eyes, suggesting that the electrical activity from the implant may be providing some benefit to retinal function.

Histologic Preservation from Subretinal Implants

The histologic analyses indicate preservation of photoreceptors in areas immediately over and surrounding subretinal implants at 8 weeks after surgery. Furthermore, at this postoperative time, corresponding to 12 weeks of age, preservation levels were comparable between RCS retinas implanted with active versus inactive devices. The full-field flash ERGs used in this study to assess retinal function were panretinal recordings yielding a mass response from all the cells of the retina. Thus, a focal ERG from the region directly over and surrounding the implant may be more sensitive in recording functional preservation. Because the histologic data indicated photoreceptor preservation localized to the region around the implant, it is possible that greater functional preservation could be detected with a focal or multifocal[35] ERG recording.

Data from non- and sham-surgical eyes indicate a greater number of photoreceptors in the superior portion of the retina, in agreement with previous reports on the RCS rat that have noted a gradient of degeneration across the retina.[30] LaVail and Battelle[30] reported that degeneration in the RCS rat was slower in the superior portion of the eye, even when pigmented RCS rats were raised in darkness. This suggests that there are inherent differences in the regions of the eye. However, this natural preservation of photoreceptors in the superior retina does not account for the significant number of photoreceptors remaining in the superior retina in eyes implanted with the ASR device, but may suggest that the superior retina is more susceptible to neuroprotective effects.

Effect of Electrical Stimulation

The hypothesis that low-level electrical stimulation produced by a subretinal implant could produce a neuroprotective effect came from a Food and Drug Administration–approved phase I Safety and Feasibility Study.[18] In this study, 10 patients with RP with varied levels of visual acuity underwent implantation with

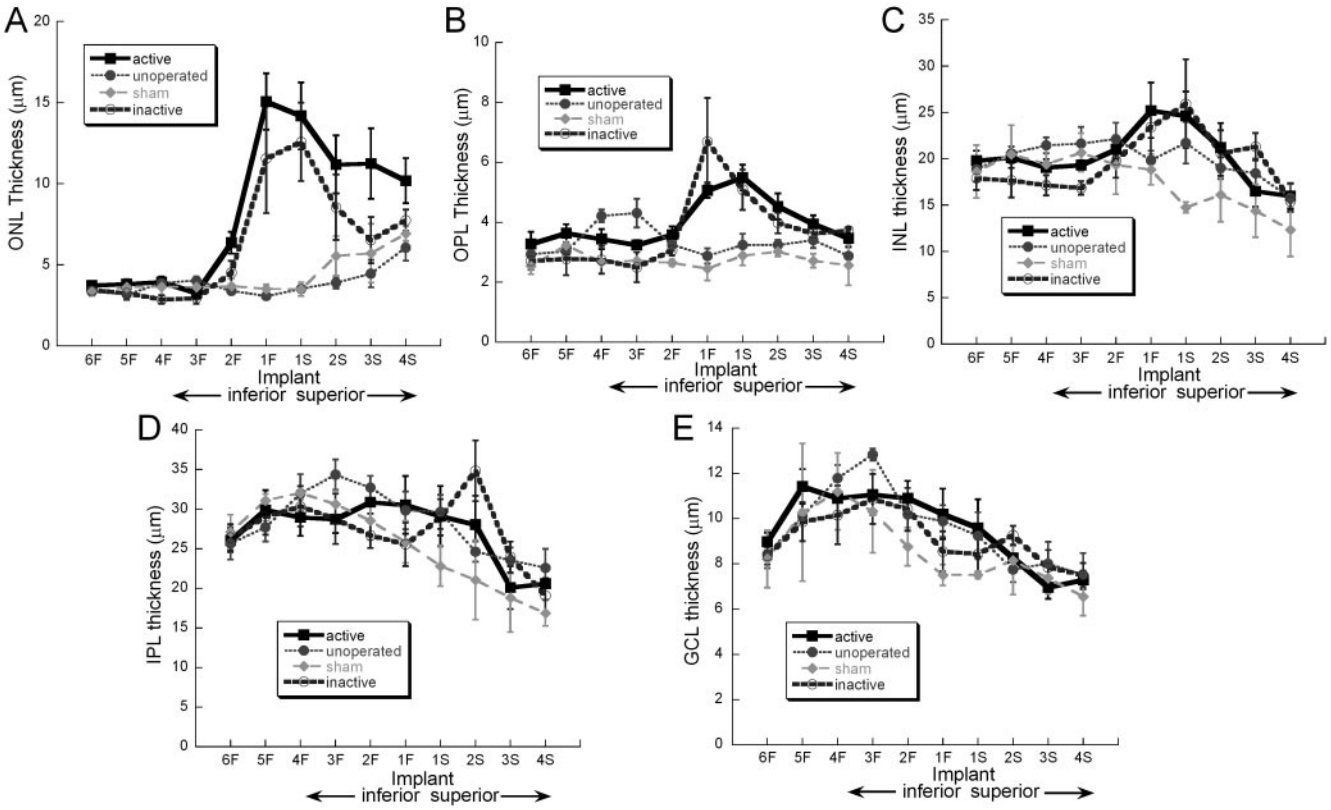

FIGURE 6. Plotting of retinal layer thickness at 8 weeks after surgery across the 10 retinal locations shown in Figure 5A: (**A**) ONL, (**B**) OPL, (**C**) INL, (**D**) IPL, and (**E**) GCL thickness (n = 9 active, 4 inactive, 2 sham-surgical, 5 nonsurgical eyes).

a subretinal ASR device. The ASR device was implanted into the temporal periphery in each patient. In follow-up examinations, the patients reported improvement in visual function in areas that were distinctly different from the implant sites.[18] These improvements first developed from several weeks to months after implantation and have persisted now for up to 3.5 years of follow-up (Chow AY, et al. *IOVS* 2004;45:ARVO E-Abstract 3398). Based on these clinical results, the hypothesis that electrical stimulation applied to the subretinal space causes a neuroprotective effect was investigated by comparing the effect of subretinal implantation of active versus inactive devices.

The exact mechanism of how electrical stimulation is able to provide a neuroprotective effect is unclear. However, studies of motor axons have shown that electrical stimulation enhances regeneration of the nerve[36] and increases the expression of brain-derived neurotrophic factor (BDNF).[37] These results provide a solid link between electrical stimulation and the upregulation of neurotrophins. Furthermore, BDNF has been shown to modulate synaptic plasticity.[38–40] In the eye, electrical stimulation has been used to promote ganglion cell growth and regeneration in vitro.[41] In vivo experiments have demonstrated increased survival of ganglion cells after electrical stimulation is applied to the optic nerve of cats[42] or rats.[23] In fact, even a 1-hour treatment of electrical stimulation applied to the cornea has been shown to be neuroprotective to axotomized ganglion cells in the rat (Morimoto T, et al. *IOVS* 2003;44: ARVO E-Abstract 109). These studies provide evidence that electrical stimulation from a device such as the ASR could provide neuroprotection by increasing local levels of neurotrophic factors that confer neuroprotection.

Growth factors clearly provide a neuroprotective effect in the eye (see Ref. 43 for review). A simple piercing wound through the eyeball causes the upregulation of basic fibroblast growth factor (FGF-2), BDNF, and ciliary neurotrophic factor (CNTF) in a localized region, which temporarily protects the retina from degeneration due to light-induced damage[24,44] or hereditary retinal disease.[4] In addition, neurotrophic factors such as CNTF, BDNF, FGF-2, and interleukin 1β have been injected into the eye directly[4,5] or through viral vector delivery systems[13,45–47] as treatments for degenerative retinal diseases. Such delivery techniques result in a widespread cellular preservation of the retina, not just an area localized to the injection site.[4,13,47] Furthermore, a dry needle inserted into the subretinal space can produce greater rescue effects than one inserted intravitreally,[47] suggesting a possible role of the RPE in the underlying protective mechanism. Similarly, laser lesions to the RCS retina can produce preservation of photoreceptor function and morphology that is correlated with the disruption of RPE.[48] However, the rescue effects produced by these various methods are temporary, lasting no more than 3 months.[4,13,26,48]

The use of electrical stimulation to induce the upregulation of endogenous growth factors in the eye is an obvious step. In the present model, electrical stimulation was applied through the subretinal implant directly to degenerating photoreceptor cells. This may result in the upregulation of neurotrophins to enhance synaptic plasticity between the photoreceptor and second-order neurons. In addition, in the subretinal space the implant contacts a second possible source of growth factors, the RPE.[47,48] This approach has the advantage of providing chronic stimulation for a long-term effect, as well as stimulating endogenous levels of trophic factors that would avoid any toxic effects.

The results of this study indicate that a subretinal implant, like other neurotrophic therapies also does not provide long-term functional preservation as measured with the ERG. However, the presence of photoreceptors at 8 weeks after surgery may be indicative of functional vision, although this has not

been tested. In the patient studies, as mentioned, improvements have persisted for 1.5[18] to 3.5 years (Chow AY, et al. *IOVS* 2004;45:ARVO E-Abstract 3398), suggesting some lasting benefit from the subretinal implant.

Applicability of the Neurotrophic Effect in Models of RP

RCS rats have been used extensively as a model of photoreceptor degeneration in studies investigating growth factors. The RCS rat is a valid model of human disease, since mutations in the homologous gene have been found in human RP patients.[49] However, the RCS mutation may produce a hyperneurotrophic response compared with other retinal mutations. Whereas a sham surgery can produce a neurotrophic effect in the RCS,[50] an S334ter transgenic rat does not show the same neuroprotection at similar postoperative time points.[51] Additionally, laser photocoagulation therapy causes preservation of photoreceptors in RCS rats but not in rds mice.[52]

In our studies using normal animal models,[33,34] implantation of the subretinal implant caused a localized photoreceptor degeneration in the area immediately overlaying the implant. However, in the RCS model, the photoreceptors immediately over the implant were preserved compared with areas more distal to it. At this time, it is not clear whether this susceptibility to preservation is specific to the RCS rat retina or is a general characteristic of retinal degenerations. Thus, to characterize fully a possible neurotrophic effect due to the ASR, further experiments with other models of retinal degeneration are needed.

Electrical Stimulation versus Foreign Body Effect

Separating a neuroprotective effect caused by ASR stimulation from a foreign body response is critical to understanding any visual improvements in animal models or clinical trails. In this study, the morphologic data from eyes implanted at 3 weeks of age with inactive devices are similar to that measured in eyes with active devices. These data were collected at 8 weeks after surgery and suggest that the preservation of photoreceptors is substantially due to the mechanical presence of the ASR device in the subretinal space. It is important to note that sham-surgical eyes showed no preservation of photoreceptors at this time point, whereas those in which a foreign body was chronically present elicited a much larger response.

ERG recordings suggest that electrical stimulation from the implant may produce some temporary improvement in retinal function. The responses recorded from eyes implanted with active ASRs had significantly higher amplitude ERG b-waves at 4 and 6 weeks after surgery than did those from sham-surgical, nonsurgical, or inactive-implant eyes (Fig. 2). Thus, at these time points, the active implant appeared to enhance retinal function.

Whereas differences between treatment groups are no longer significant at 8 weeks after surgery, it is interesting to note that the responses of the active-implant eyes did not have a clear STR. The STR is a small corneal negative wave generated by the inner retina that is recorded in the normal retina in response to very dim flashes.[53] In RCS rats, the STR is recorded near the end of the course of photoreceptor degeneration when a large-amplitude b-wave is no longer present.[54] The presence of the STR in RCS rats between 8 and 12 weeks of age may indicate that the inner retina is still functioning. At 8 weeks after surgery, in the control treatment groups, ERG recordings were dominated by an STR. In comparison, ERG b-wave responses masked the STR in active-implant eyes suggesting preservation of photoreceptor function.

In conclusion, the subretinal implantation of an ASR device delays photoreceptor degeneration in the RCS rat. Similar morphologic results in eyes implanted with the inactive device suggest that this effect may be due to the presence of a foreign body in the subretinal space. ERG measurements, however, suggest that the active device provides better preservation of function than does the inactive device. It may be possible to obtain even greater protective effects from subretinal electrical stimulation by increasing the output from the ASR by (1) altering the ASR design to increase current levels or (2) driving existing ASRs to produce higher levels of stimulation by increasing environmental light levels. However, caution would have to be taken in the former experiments, since many retinal degeneration models are more susceptible to light-induced damage. Future studies will concentrate on increasing the neuroprotective effect from active devices, determining the duration of this protective effect, and investigating possible mechanisms of neuroprotection.

Acknowledgments

The authors thank Neal Peachey for insightful conversations and valuable comments on the manuscript.

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