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Neuroprotective Dose Response in RCS Rats Implanted with Microphotodiode Arrays

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Abstract

Purpose—Neuropreservation of retinal function and structure in RCS rats following implantation of a microphotodiode array (MPA) has been shown in previous studies(Pardue et al. 2005a; Pardue et al. 2005b). Since microphotodiodes produce electrical currents in proportion to the intensity of incident light, increased light exposure may result in greater neuroprotective effects. Our previous studies suggested that the frequency of light exposure to electroretinogram (ERG) flash stimuli might provide increased neuroprotection. Thus, in this study, we examined the dose response of subretinal electrical stimulation by exposing RCS rats implanted with MPAs to variable durations and combinations of two different lighting regimens: pulsing incandescent bulbs and xenon stimuli from an ERG Ganzfeld. While incandescent light regimens did not produce any significant differences in ERG function, we found significantly greater dark-adapted ERG b-wave amplitudes in RCS rats that received weekly versus biweekly ERGs over the course of 8 weeks of follow-up. These results suggest that subretinal electrical stimulation may be optimized to produce greater neuroprotective effects by dosing with periodic higher current.

1. Introduction

Implantation of a subretinal MPA has been shown to preserve retinal function and structure in RCS rats (Pardue et al. 2005a; Pardue et al. 2005b). In these studies, RCS rats were implanted at 21 days of age and then followed for 8 or 17 weeks. All animals were housed in a normal 12:12 light cycle (~100 lux) and then exposed to ERG flash stimuli such that the 8 week group received weekly testing with a long protocol (17steps) and the 17 week group received biweekly testing with a short protocol (9 steps). The implanted RCS rats with 8 weeks of weekly ERG testing showed significantly greater ERG b-wave amplitudes and greater photoreceptor counts; whereas, the implanted RCS rats from the 17 weeks group did not show any functional or morphological benefits. Since microphotodiodes produce electrical currents in proportion to the intensity and wavelength of incident light, we hypothesize that this difference in neuroprotection is due to the increased current output from the MPA device in the 8 week group undergoing weekly ERG with a long protocol. Furthermore, exposing the eye to greater light levels and therefore increased electrical current may produce greater neuroprotective effects. Here, we examined the dose response of subretinal electrical stimulation by exposing RCS rats implanted with electrically active

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MPAs to variable durations and combinations of two different light regimens: pulsing incandescent bulbs and flashes from xenon bulbs of an ERG Ganzfeld.

2. Materials and Methods

2.1 Implantation of RCS rats with MPAs

Dystrophic RCS rats, originally obtained from Matthew LaVail of the University of California, San Francisco were used in this study. RCS rats (n=28) were implanted monocularly at 21 days of age with an MPA device, as previously described (Ball et al. 2001). Briefly, the rats were anesthetized with ketamine (60 mg/kg)/xylazine (7.5 mg/kg) and the pupils dilated with 1% tropicamide. After preparing a sterile field, the eye was rotated inferiorly and a 1.5 mm incision made through all layers of the orbit, approximately 2 mm from the superior limbus. Saline was placed on the eye and a localized retinal detachment formed over the course of 5 minutes. The implant was then gently inserted into the subretinal space and confirmation of the implant location made by fundus examination.

The MPA device was a 0.5 mm diameter, 25 micron thick silicon disc that contained a series of photodiodes with iridium oxide surface electrodes (9μm × 9μm), as previously described (Chow et al. 2001; Chow et al. 2002). The spectral responsivity of the MPA device ranged from 300 to 1400 nm, peaking at ~840 nm (Chow et al. 2001; DeMarco et al. 2007). For the stimuli used here, the current output varied depending on the light stimuli from environmental to incandescent to xenon flash stimuli (see Table XX.1).

2.2 Light Dosing

Implanted RCS rats (n=24) were divided into three different groups to received incandescent light exposure (350 cd/m2 at 0.25 Hz) on a customized rack. Rats were exposed to chronic (12 hrs/day, 7 days/week; n=5)), daily (1hr/day, 7days/week; n=8), or weekly (1 hr/week; n=11) treatments. The “light rack” consisted of 4 inch elongated incandescent bulbs hung 6 inches apart around the circumference of the normal rodent shoebox caging. The bulbs were placed to optimize exposure to the rats inside the cage. All animals were exposed to the incandescent lighting at the same time of the day using an automatic timer. Rats were provided with food and water and could roam freely in the cages. For animals with hourly light exposure, their behavior was monitored and recorded by an observer every 10-15 minutes.

2.3 Retinal Function Testing

ERGs were recorded from each rat to measure retinal function. All incandescent light exposure groups had biweekly ERGs. However, 6 rats that were exposed to 1 hour/week incandescent lighting had ERG testing weekly. ERG recordings were performed as previously described (Pardue et al. 2005b). Briefly, rats were dark-adapted overnight, anesthetized with ketamine (60 mg/kg)/xylazine (7.5 mg/kg), and pupils dilated (1% tropicamide, 1% cyclopentolate). Dark and light-adapted ERGs were recorded using a 9 step intensity series (biweekly ERGs) or a 17 step intensity series (weekly ERGs). Scotopic stimuli ranged from 3.9×10^{-4}-137 cd s/m^2, while photopic stimuli ranged from 0.15-75 cd s/m^2.

2.4. Morphological Assessment of Photoreceptor Numbers

After 8 weeks of follow-up, all RCS rats were euthanized (anaesthetic overdose) and both eyes were enucleated and fixed in 2% paraformaldehyde/2.5% glutaraldehyde. Following dehydration in a graded alcohol series and embedding in plastic resin (Embed 812/DER 736; Electron Microscopy, Fort Washington, PA), vertical sections were cut at 0.5 microns and stained with toluidine blue. Sections through the center of the implant were photographed

such that 8 locations across the retina were imaged (Pardue et al. 2005b). Photoreceptor nuclei counts were made in each location based on five separate measurements.

3. Results

3.1 Effects of Light Exposure on Retinal Function in Implanted RCS Rats

ERG responses in rats exposed to variable incandescent lighting regimens were not significantly different. Figure X.1A shows representative ERG waveforms from rats at 2 and 8 weeks postop. As seen previously, retinal function in the RCS rats decreased rapidly from 2 to 8 weeks post-implantation and was nearly unrecordable at 8 weeks post-implantation (11 weeks of age) (Pardue et al. 2005b). At 2 weeks after implantation, the chronic exposure group had significantly larger ERGs, however, this difference was diminished by 8 weeks post-op. Figure X.1B shows the maximum b-wave amplitude in response to the brightest flash for each timepoint. No statistical differences were found.

In contrast, frequency of exposure to the xenon flash affected retinal function. Figure X.1C shows that implanted RCS rats undergoing weekly ERG testing had larger ERG waveforms at 2 and 8 weeks post-op. Examination of the max b-wave response (Figure X.1D) shows that the weekly ERG response were greater throughout the testing period (Main effect of two-way repeated ANOVA, $F(1, 43) = 7.33$, $p= 0.024$).

3.2 Effect of Light Exposure on Photoreceptor Survival in Implanted RCS Rats

Exposure of the implanted RCS rats to different light regimens did not affect the number of photoreceptors present at 8 weeks after implantation. The average number of photoreceptors present in rats exposed to chronic incandescent lighting was 91.66 ±74.64 (ave ±stdev) vs daily with 117.33 ±33.67 and weekly with 86.08 ±28.92. Implanted RCS rats that received weekly ERGs also did not have significant more photoreceptors 156.14 ±60.82 compared to biweekly ERGs 86.08 ±28.92; although there was a trend for greater numbers of photoreceptors.

4. Discussion

The current output estimated from the MPA device in response to different lighting conditions increased from sub-microamp with environmental lighting to 1800x larger with incandescent lights to as much as 560,000x greater with xenon flashes. While current output changes greatly over these lighting conditions, our findings show that increasing the incandescent lighting exposure did not result in greater preservation of retinal function. However, increasing exposure to xenon flashes from biweekly to weekly significantly increased retinal preservation in the implanted RCS rats.

While we have calculated the current output from the MPA device based on in vitro recordings in a chamber (see Table X.1), these measurements do not necessarily accurately predict the current output in the cage environment. The light intensity varies within the cage depending on how far the animal is from the light source. Additionally, since there is a gradient current output from the MPA in response to light and animals were free to roam around the cage during the light exposure, it is unknown if the MPA received full light exposure during periods of light dosing. During the light exposure period, particularly with chronic incandescent light exposure, rats slept greater than 50% of the time. The dose of incident light reaching the subretinal implant during the exposure time is likely to be less than the actual output of the light source.

Xenon flashes presented with the ERG testing ranged from 3.9×10^{-4} to 137 cd/m^2 and potentially increased current to the implant from 0.01 to 1680 μA/cm^2. The increased preservation of retinal function with weekly ERGs may be due to the pulses of very high current (3-5 per intensity) or may be due to the increasing current output in a stepwise fashion that was produced from the MPA device as increasing intensities were presented. It should also be noted that the weekly ERG recording provided a higher "dose" of current earlier (1 week post-implantation) in the course of degeneration. It is possible that this exposure to high levels of current at 1 week post-implantation upregulated growth factors, such as fibroblast growth factor 2 (Ciavatta et al. 2009) that could increase the survival of photoreceptor. It does appear that the preservation of retinal function in the weekly ERG group is due to current output from the subretinal device since prior studies have shown that naïve rats or rats with inactive devices receiving weekly ERGs did not have preserved photoreceptor function (Pardue et al. 2005a; Pardue et al. 2005b).

While prior studies have shown that subretinal electrical stimulation preserves photoreceptor at 8 weeks after implantation with an MPA device (Pardue et al. 2005b), our experiments did not show any increased survival of photoreceptors with weekly vs biweekly xenon flash exposure. This may indicate that neurotrophic factors upregulated by the subretinal current may provide support to surviving cells, but not stop apoptotic pathways.

One complication of this study is that white light was used to increase current output from the MPA device. White light can have detrimental effects on photoreceptor survival. While the light levels used here were below those used to induce light damage models of retinal degenerations, it may have confounded our results by increasing photoreceptor death in the degenerating retina (Reme 2005). Future studies will use infrared light to stimulate MPA devices since the MPA is sensitive at these wavelengths and infrared has not been reported to be damaging to the retina.

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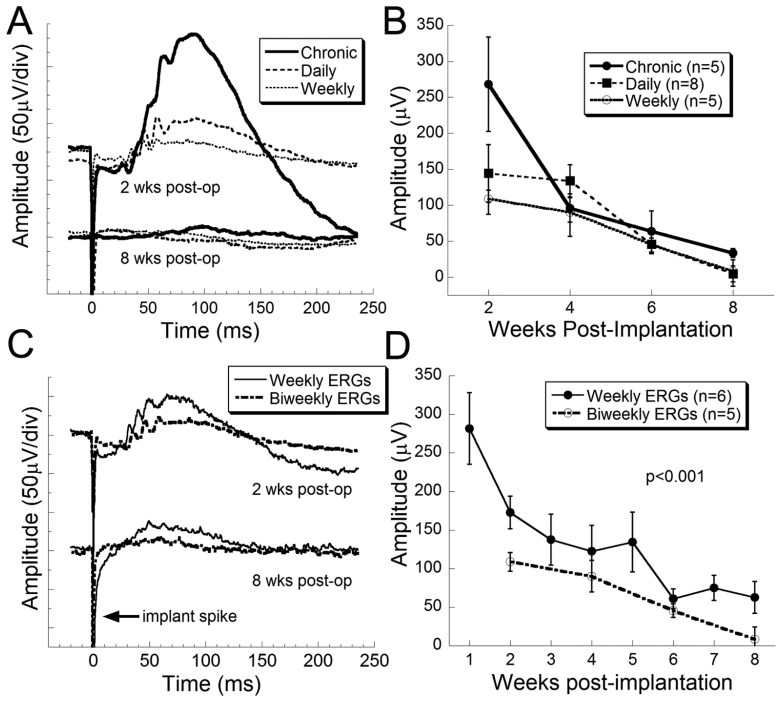

Fig. 1.

ERGs recorded from implanted RCS rats while exposed to different lighting conditions. A) Representative waveforms to bright flashes (137 cd s/m^2) from implanted RCS rats exposed to chronic, daily or weekly incandescent light exposure. B) Average max dark-adapted b-wave to a bright flash from implanted RCS rats exposed to incandescent lights. No significant differences were seen between groups. C) Representative waveforms to bright flashes (137 cd s/m2) from implanted RCS rats that received ERG testing every 2 weeks or weekly. D) Average max dark-adapted b-wave amplitude across weeks of implantation in implanted RCS rats exposed to different frequencies of xenon flashes. RCS rats receiving weekly ERGs had significantly larger b-wave amplitudes across the testing period (p=0.024)

Table 1

Current output from the MPA device in response to different light stimuli.

	Environmental Light	Incandescent Light	Xenon Flash
Wavelength (nm)	500-650	400-1000	400-1400
Light Intensity (cd/m^2)	4.5 to 86	350	3.9×10^{-4} to 137
Stimulus Current (μA/cm^2)	3.0×10^{-3} to 0.3	5.5	0.01 to 1680