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Activation of retinal ganglion cells in wild-type and *rd1* mice through electrical stimulation of the retinal neural network

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ABSTRACT

We compared the thresholds and response properties of extracellularly recorded retinal ganglion cells (RGCs) in wild-type and *rd1* mouse retinas to electrical stimulation of the retinal neural network. Retinas were stimulated in vitro with biphasic current pulses (1 ms/phase) applied with a 400-μm diameter, subretinal electrode. Three types of responses were observed in both wild-type and *rd1* RGCs. Type I cells elicited a single burst of spikes within 20 ms following application of the electrical stimulus, type II cells elicited a single burst of spikes with a latency greater than 37 ms, and type III cells elicited two and occasionally three bursts of spikes. For all ages examined, ranging from postnatal day (P) 25 to P186, the thresholds of RGCs were overall consistently higher in *rd1* mice. Median threshold values were 14 and 50 μA in wild-type and *rd1* mice, respectively. We propose that photoreceptors lower the thresholds for activation of RGCs whereas postreceptoral neurons determine the response properties of RGCs to electrical stimuli.

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1. Introduction

Retinal degenerative diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD) cause the loss of light-sensing photoreceptors. Currently no treatment is available to reverse the degenerative process or restore vision in these patients. The preservation of the remaining neural network in patients with RP and AMD provides the opportunity to restore vision by means of an electronic retinal prosthesis.

A discouraging finding from human studies is that the currents required for evoking visual percepts in RP patients is much higher than those needed in healthy individuals (Delbeke et al., 2001; Gekeler, Messias, Ottinger, Bartz-Schmidt, & Zrenner, 2006; Rizzo, Wyatt, Loewenstein, Kelly, & Shire, 2003). There are a number of possibilities that could account for the higher currents in RP patients. First, several studies have reported a significant decrease in the number of RGCs in moderate and severe human RP retinas (Humayun et al., 1999; Santos et al., 1997; Stone, Barow, Humayun, de Juan, & Milam, 1992). If a visual percept requires activation of a minimum number of RGCs, then with fewer surviving RGCs a greater amount of current might be needed to recruit a sufficient number of RGCs. Second, a higher current may be required because of reduced excitability of the remaining individual RGCs. Third, the increased thresholds in RP patients may be due to alterations in the neural retina, either the loss of photoreceptors or remodeling of retina that follows photoreceptor loss (Jones & Marc, 2005). Finally, non-retinal explanations, such as reorganization of visual cortex following long-term visual deprivation (Burton, 2003), are also a possibility.

The primary objective of this study was to test the hypothesis that thresholds of RGCs in degenerate retina are higher than those in healthy normal retina when RGCs are activated through electrical stimulation of the retinal neural network. For this study, we used the *rd1* mouse, which is a well-studied animal model of retinitis pigmentosa (Farber, Flannery, & Bowes-Rickman, 1994).

2. Materials and methods

2.1. Animals and tissue preparation

Seventeen (17) wild-type (C57BL/6 strain) and 18 *rd1* (C3H/HeJ strain) mice were used in this study. The mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were breed and housed in the Animal Research Facility at the Boston VA Healthcare System. Mice were reared on a 12 h light/dark cycle using standard fluorescent lighting. All animal care procedures and experimental methods adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the VA Boston Institutional Animal Care and Use Committee.

On the day of an experiment, a mouse was deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Under normal room lighting, an eye was enucleated, hemisected along the ora serrata, and the retina separated from the choroid. The retina was transferred to a dish containing Ames medium and any remaining vitreous was removed mechanically with fine forceps. The whole-mounted retina was then placed photoreceptor side down in a recording chamber and held in place with a nylon mesh (Fig. 1). Mounted on a fixed-stage upright microscope (Nikon Eclipse E600FN), the retina was perfused (~2 ml/min) with bicarbonate-buffered (pH 7.4)

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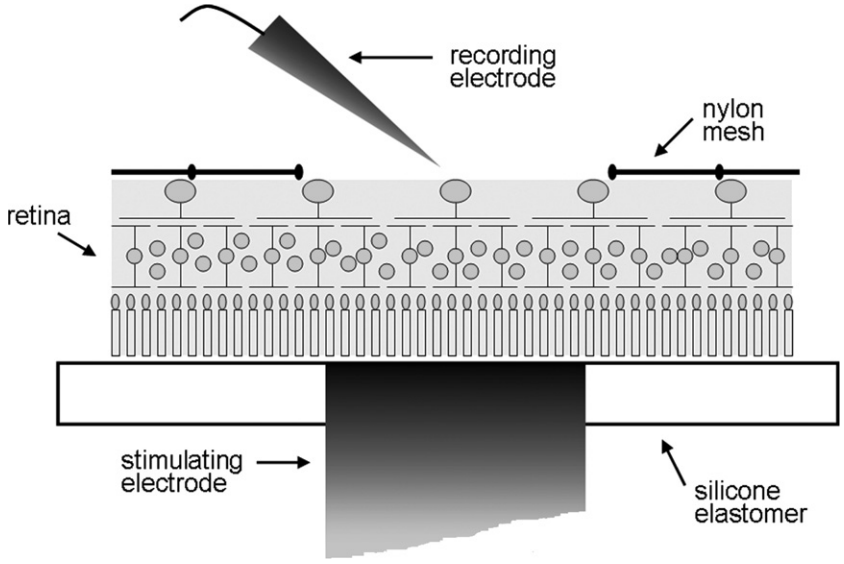

Fig. 1. Schematic representation of the retina-electrode preparation crosssection. A piece of retina (~4 × 4 mm) was centered over the stimulating electrode with photoreceptor side down. The retina was held in place by a nylon mesh, which had a 1-mm diameter hole removed for access of RGCs by the recording electrode. Physiological solution flowed over the top of the retina, through the nylon mesh.

Ames medium (Sigma–Aldrich, St. Louis, MO) supplemented with 2 mg/ml D-(+) glucose and equilibrated with 5% CO_2/95% O_2. An in-line heating device (Warner Instruments, Hamden, CT) was used to maintain recording temperature at 35–36 °C.

2.2. Electrical stimulation and recording

The stimulating electrode was a platinum wire of 400-μm diameter that was embedded in silicone elastomer (Sylgard 184; Dow Corning, Midland, MI), which formed the floor of the recording chamber. The return electrode was located distantly (~2 cm from the stimulating electrode). Electrical stimuli consisted of charge-balanced biphasic current pulses of 1 ms per phase, with the anodal phase preceding the cathodal phase. The interpulse separation was 0.5 ms. All current pulses were delivered at a frequency of 1.0–1.5 Hz through constant-current stimulus isolation units (PSIU-6, Grass-Telefactor, West Warwick, RI) attached to a Grass-Telefactor S88 stimulator. Higher stimulation frequencies were not used in order to avoid depression of RGC responses (Jensen & Rizzo, 2007).

Neuronal activity was recorded with quartz-platinum/tungsten microelectrodes with impedances between 0.7 and 1.3 MΩ (Thomas Recording GmbH, Germany). Recordings were amplified with a differential amplifier (Model XCell-3; FHC, Bowdoin, ME) and digitized on-line with a PC running Spike 2 acquisition and analysis software (version 5; Cambridge Electronic Design Ltd., Cambridge, UK).

During an experiment, the room was illuminated with dim red light to avoid desensitizing mouse cone photoreceptors. However, no attempt was made to maintain the retina in a dark-adapted state. With the aid of red light (>630 nm; tungsten-halogen light source) that was delivered from below (through the microscope condenser), the tip of the recording electrode was visually advanced to the retinal surface with a motor-driven micromanipulator. Recordings were made from RGCs located either directly over the stimulating electrode or within 200 μm of the stimulating electrode. No significant correlation was found between the measured threshold or response of a RGC and the location of a RGC. All data were therefore pooled. In some experiments, the AMPA/kainate receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and the NMDA receptor blocker 2-amino-7-phosphonoheptanoic acid (AP-7) were added to the extracellular bath solution to block excitatory, glutamatergic synaptic transmission. Drugs were purchased from Sigma–Aldrich.

2.3. Light stimulation

Light from a mercury arc lamp illuminated an aperture that was focused on the retina from above through the 4× objective of the microscope. The image produced on the retina was a 250-μm diameter spot, which was centered on the recorded RGC. Interference filters (peak transmission at either 368 or 545 nm) and neutral density filters were inserted in the light path to control the wavelength and intensity of light stimulation. A shutter (Uniblitz, Rochester, NY) was used to control the stimulus duration, which was set to 700 ms. The intensity of the unattenuated light stimulus on the retina was measured with a spectroradiometer (RPS900, International Light) to be 3.5×10^{16} photons/cm^2/s for 368 nm light and 2.5×10^{17} photons/cm^2/s for 545 nm light.

2.4. Data analysis

Thresholds were determined by increasing a subthreshold current until action potentials were elicited more than 50% of the time for five or more consecutive stimulations. Response latencies of individual RGCs were measured using a current set at 2× threshold and were calculated as the mean to the first spike in response to 5–10 presentations of an electrical stimulus. High levels of spontaneous activity that could potentially interfere with correctly determining the latency of a first spike did not occur.

Statistical comparisons between *rd1* mice and wild-type mice were performed with SigmaStat software (version 3.5, Systat Software Inc., Point Richmond, CA), for a statistical significance level of $P < 0.05$. When groups of data were judged to consist of normally distributed data ($P < 0.05$, Kolmogorov–Smirnov test), the data groups were compared by the *t*-test and are presented as means ± SD. Otherwise, data groups were compared by applying non-parametric statistics and are presented as medians.

3. Results

Data were obtained from 43 wild-type RGCs and 50 *rd1* RGCs in mice of ages ranging from postnatal day (P) 25 to P186. We shall first give a description of the electrically evoked responses of RGCs in wild-type and *rd1* mouse retinas. We will then compare the thresholds for activation of RGCs in wild-type mice with those obtained for RGCs in *rd1* mice.

3.1. Characterization of the electrically evoked responses of RGCs

We examined the extracellularly recorded responses of both wild-type and *rd1* RGCs to symmetric biphasic current pulses (1 ms/phase) using currents set at 2× threshold. We found that RGCs could be assigned into three classes based on their response to subretinal stimulation. Type I cells elicited a single burst of spikes within 20 ms following application of the electrical stimulus, type II cells elicited a single burst of spikes with a latency greater than 37 ms following the electrical stimulus, and type III cells elicited two and occasionally three bursts of spikes (Fig. 2). The relative frequencies of the three types were similar in both mouse strains. In wild-type mice, 56% (24 of 43) were type I cells, 28% (12 of 43) were type II cells, and 16% (7 of 43) were type III cells. In *rd1* mice, 54% (27 of 50) were type I cells, 34% (17 of 50) were type II cells, and 12% (6 of 50) were type III cells.