Experimental Eye Research 128 (2014) 34–42

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Inner retinal preservation in rat models of retinal degeneration implanted with subretinal photovoltaic arrays

Jacob G. Light [a, b], James W. Fransen [c], Adewumi N. Adekunle [a], Alice Adkins [b], Gobinda Pangeni [d], James Loudin [e], Keith Mathieson [e, g], Daniel V. Palanker [e, f], Maureen A. McCall [c, d], Machelle T. Pardue [a, b, *]

[a] Ophthalmology, Emory University, USA
[b] Rehab R&D Center of Excellence, Atlanta VA Medical Center, USA
[c] Anatomical Sciences & Neurobiology, University of Louisville, USA
[d] Ophthalmology & Visual Sciences, University of Louisville, USA
[e] Hansen Experimental Physics Laboratory, Stanford University, USA
[f] Ophthalmology, Stanford University, USA
[g] Institute of Photonics, University of Strathclyde, UK

ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form
9 September 2014
Accepted in revised form 11 September 2014
Available online 16 September 2014

Keywords:
Retina
Prosthetic
Bipolar cells
Amacrine cells
Müller glial cells

ABSTRACT

Photovoltaic arrays (PVA) implanted into the subretinal space of patients with retinitis pigmentosa (RP) are designed to electrically stimulate the remaining inner retinal circuitry in response to incident light, thereby recreating a visual signal when photoreceptor function declines or is lost. Preservation of inner retinal circuitry is critical to the fidelity of this transmitted signal to ganglion cells and beyond to higher visual targets. Post-implantation loss of retinal interneurons or excessive glial scarring could diminish and/or eliminate PVA-evoked signal transmission. As such, assessing the morphology of the inner retina in RP animal models with subretinal PVAs is an important step in defining biocompatibility and predicting success of signal transmission. In this study, we used immunohistochemical methods to qualitatively and quantitatively compare inner retinal morphology after the implantation of a PVA in two RP models: the Royal College of Surgeons (RCS) or transgenic S334ter-line 3 (S334ter-3) rhodopsin mutant rat. Two PVA designs were compared. In the RCS rat, we implanted devices in the subretinal space at 4 weeks of age and histologically examined them at 8 weeks of age and found inner retinal morphology preservation with both PVA devices. In the S334ter-3 rat, we implanted devices at 6–12 weeks of age and again, inner retinal morphology was generally preserved with either PVA design 16–26 weeks post-implantation. Specifically, the length of rod bipolar cells and numbers of cholinergic amacrine cells were maintained along with their characteristic inner plexiform lamination patterns. Throughout the implanted retinas we found nonspecific glial reaction, but none showed additional glial scarring at the implant site. Our results indicate that subretinally implanted PVAs are well-tolerated in rodent RP models and that the inner retinal circuitry is preserved, consistent with our published results showing implant-evoked signal transmission.

Published by Elsevier Ltd.

1. Introduction

Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are leading causes of irreversible blindness worldwide (Hartong et al., 2006). In these diseases, vision loss, regardless of underlying etiology, results from degeneration of retinal photoreceptors. Remodeling of the inner retina occurs in late stages of disease (Jones and Marc, 2005; Marc and Jones, 2003; Marc et al., 2007; Strettoi et al., 2002), but photoreceptor degeneration leaves the neurons and circuitry of the inner retina relatively intact for extended periods of time (Humayun et al., 1999; Jones et al., 2003; Marc and Jones, 2003; Marc et al., 2007; Strettoi et al., 2002, 2003).

One promising approach that targets the remaining retinal circuitry to restore lost vision uses prosthetic devices to

* Corresponding author. Research Service (151Oph), Atlanta VA Medical Center, 1670 Clairmont Rd, Decatur, GA 30033, USA.
E-mail addresses: mpardue@emory.edu, Machelle.Pardue@va.gov (M.T. Pardue).

http://dx.doi.org/10.1016/j.exer.2014.09.004
0014-4835/Published by Elsevier Ltd.

functionally replace photoreceptors. Several different designs and placement strategies are currently being evaluated. Epiretinal placement and stimulation of the retinal ganglion cells (RGC) should require algorithms to selectively achieve information transmission (Jensen et al., 2005; Humayun et al., 2012). Suprachoroidal implants (Cicione et al., 2012; Kanda et al., 2004; Morimoto et al., 2011; Wong et al., 2009; Yamauchi et al., 2005) and subretinal microphotodiode arrays (Chow et al., 2001; Mathieson et al., 2012; Rizzo, 2011; Zrenner et al., 1999) are designed to directly stimulate bipolar cells and theoretically utilize network-mediated retinal stimulation, preserving the integrative properties of second order neurons in the inner plexiform layer (IPL) (Asher et al., 2007; Wang et al., 2012). Other strategies utilize optogenetics to confer light sensitivity to bipolar or RGCs (Bi et al., 2006; Busskamp et al., 2012; Garg and Federman, 2013; Isago et al., 2012; Lin et al., 2008; Tomita et al., 2007) to directly stimulate retinal tissues.

Subretinally placed photovoltaic arrays (PVAs) provide targeted stimulation to the inner nuclear layer (INL) (Fransen et al., 2014) due to their current density distribution and size (Mathieson et al., 2012). Because bipolar cells are interneurons that connect photoreceptors to RGCs they are involved in signal transmission with PVAs. Retention of these cells and formation of a functional retinal–prosthetic interface would aid in visual restoration. For this to occur there must be a high level of biocompatibility between the retina and prosthesis. As such, measures of the integrity of the bipolar cells and other retinal constituents are critical components in evaluating the success of any subretinal prosthetic.

Previous studies have attempted to characterize the condition of implanted and/or electrically stimulated retinal tissue histologically and immunohistochemically (Alamusi et al., 2013; Chow et al., 2001; Pardue et al., 2001; Ray et al., 2009, 2011; Tamaki et al., 2008). However, many of these studies have only examined the effects of certain aspects of the treatment paradigm, such as acute electrical stimulation or biocompatibility of a prosthetic device in wild-type animals that do not exhibit degenerative pathology. In this study, we examined retinal morphology after implantation of two generations of subretinal silicon devices in two RP rat models. We compared a monopolar PVA (mPVA) with no perforations (Chow et al., 2001) to a bipolar PVA (bPVA), which includes bipolar pixels separated by 5 μm gaps (Mathieson et al., 2012). Photovoltaic pixels in monopolar devices have individual active electrodes, but share a common large return electrode on the back side of the implant. Bipolar pixels are composed of 3 photodiodes in series, connected between the active electrode in the center of the pixel and a return electrode surrounding each pixel (Mathieson et al., 2012). All devices in the present study were photoactive. The bPVA gaps enhance proximity of the electrodes to inner retinal neurons and allow diffusion of extracellular milieu through the implant (Adkins et al., 2013; Mathieson et al., 2012). Since the subretinal PVA stimulates retinal neurons that are within close proximity to the electrode (Fransen et al., 2014), we focused our analysis on inner retinal cells that are likely activated by the PVA device. Rod bipolar cells and cholinergic amacrine cells represent well defined populations of cells with robust cellular markers to assess overall inner retinal health. We also assessed glial reaction in tissues within and distal to the implant site from 16 to 26 weeks post-implantation in the S334ter-3 and 4 weeks post-implantation in the RCS rat. Our results suggest that both the mPVA and bPVA designs are well tolerated and preserve the necessary inner retinal circuitry that underlie the transmission of signals to the RGCs and beyond (Fransen et al., 2014).

2. Methods

2.1. Animals and experimental groups

All animal procedures were approved by the Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research. Two models of RP were used: the Royal College of Surgeons (RCS) and S334ter-3 rats from an in-house breeding colony originated from breeders donated by Dr. Matthew LaVail (University of California, San Francisco) (LaVail et al., 1975; Mullen and LaVail, 1976).

The RCS rats ($n = 4$) were implanted binocularly at 4 weeks of age and terminated 4 weeks post-implantation. RCS rats exhibit a moderate rate of photoreceptor degeneration; approximately 50% of the initial ONL thickness was present at the age of implantation (LaVail and Battelle, 1975). Four eyes were implanted with an mPVA device and 4 with a bPVA device. The eyes were divided such that all bPVA-implanted eyes were processed as frozen sections for retinal cross-sections and half the mPVA eyes processed similarly with the remaining prepared as retinal flat mounts.

S334ter-3 rats were implanted monocularly (right eye) with either an mPVA ($n = 4$) or a bPVA ($n = 7$) from 6 to 12 weeks of age and were terminated at 22–32 weeks of age (16–26 weeks of implantation). Monocular implantation accommodated superior colliculus recordings that are reported elsewhere (Fransen et al., 2014). The S334ter-3 is a rapid degeneration model and most photoreceptors had degenerated at the time of implantation (McGill et al., 2012). All S334ter-3 eyes were processed as frozen sections. Additional cross sections were analyzed from three age-matched unimplanted control eyes from each RP strain, as well as 3 eyes from 8-week-old Long Evans wild-type rats acquired from Charles River.

2.2. Overview of devices

Two types of PVA were explored: mPVA and bPVA (Mathieson et al., 2012; Pardue et al., 2005b). mPVA devices, provided by Optobionics, Inc (Glen Ellyn, IL), were fabricated using previously described thin-film fabrication methods (Chow et al., 2001). The mPVA is a 1 mm diameter silicon disk, 25 μm thick, containing 1200 microphotodiodes with active electrodes on one face and a common return electrode on the back, both coated with iridium oxide (Chow et al., 2001). The bPVA device's photovoltaic arrays were composed of triple-diode pixels fabricated on a silicon wafer. Each pixel contains an active electrode in its center and a return electrode at the circumference. Upon illumination with a pulse of light, each pixel generates a bi-phasic pulse of electric current flowing through the tissue between electrodes, primarily stimulating the inner nuclear layer (INL) cells (Fransen et al., 2014). Electrodes were coated in iridium oxide and the details of manufacturing methods of the bPVA were published previously (Wang et al., 2012). Five-μm wide gaps were etched between adjacent pixels for electrical isolation and to improve nutrients flow through the implant (Mathieson et al., 2012). The bPVA device measured 0.8 × 1.2 mm and was 30 μm thick. bPVA devices were left in retinal tissue for histological analysis due to tissue destruction caused by removal.

2.3. Surgical procedure

The surgical methods employed for implantation of the PVAs into the subretinal space have been described previously (Pardue et al., 2005b). Briefly, rats were anesthetized [ketamine (60 mg/kg) and xylazine (7.5 mg/kg)] and placed into a sterile field. A traction suture was made at the superior limbus and the eye was rotated inferiorly. A ~1.0 mm incision was made in the superior