Title
Restoration of visual responses following transplantation of intact retinal sheets in rd mice

Journal
Experimental Eye Research, 79(3)

ISSN
0014-4835

Authors
Arai, S
Thomas, BB
Seiler, MJ
et al.

Publication Date
2004-09-01

DOI
10.1016/j.exer.2004.05.013

Peer reviewed

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Eye Research 79 (2004) 331–341
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Restoration of visual responses following transplantation of intact retinal sheets in *rd* mice

S. Arai[a,b,1], B.B. Thomas[a,1], M.J. Seiler[a,c,1], R.B. Aramant[d], G. Qiu[a], C. Mui[a], E. de Juan[a], S.R. Sadda[a,*]

[a]*Doheny Retina Institute, Doheny Eye Institute, Keck School of Medicine, University of Southern California, DEI 3610, 1450 San Pablo Street, Los Angeles, CA 90033-3699, USA*
[b]*Division of Ophthalmology and Vision Science, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan*
[c]*Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*
[d]*Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY, USA*

Received 15 December 2003; accepted in revised form 13 May 2004

Available online 2 July 2004

Abstract

Purpose. To correlate the functional outcomes with histologic findings following transplantation of fetal retinal *sheets* in *rd* mice, and to investigate the mechanisms of visual function restoration.

Methods. Twenty-one postnatal day 31–38 *rd/rd* (C3H/HeJ) mice were transplanted in one eye with retinal sheets (1·0 × 0·4 mm) obtained from embryonic day (E) 17 enhanced-green-fluorescent protein (eGFP) mice. Five mice underwent sham surgery without insertion of tissue. Four to five weeks after transplantation, visual responses to a light flash were recorded across the superior colliculus (SC) in seven eyes of seven transplanted mice that had clear corneas and lenses, and in all five sham surgery mice. Following the SC recording, the eyes were enucleated and processed for immunohistochemistry and examined using confocal microscopy.

Results. In three out of the seven eyes (43%), positive responses were recorded in the SC in an area topographically corresponding to the placement of the transplant in the host retina. No responses were recorded in the untreated eyes of 5-week-old and 9-week-old *rd/rd* mice, and in the 9-week-old sham surgery mice. In contrast, visual responses were recorded over the entire SC in normal eyes. The response onset latencies of the 3 transplanted mice with responses were similar to those of normal control mice. The organization of the graft did not appear to correlate as expected with the electrophysiology results, as eyes with well-organized, laminated grafts showed no response whereas the three light-responsive eyes had rosetted or disorganized grafts. All three light-responsive eyes demonstrated much higher levels of recoverin immunoreactivity in the *host* retina overlying the graft compared with untreated age-matched rd/rd mice.

Conclusion. Restoration of the SC visual response does not appear to depend on a well-organized transplant in the *rd* mouse. Increased recoverin-staining in the host retina in light-responsive animals suggested that host cone rescue was the likely mechanism of vision restoration in this transplant model.

Keywords: retinal transplantation; retinal degeneration; electrophysiology; rescue

1. Introduction

Retinitis pigmentosa (RP) has a prevalence rate of about 1 in 4000 with an estimated 1·5 million affected individuals worldwide (Berson, 1993; Humayun et al., 1999b; Jones et al., 2003; Pacione et al., 2003). Although no proven visually beneficial treatments for patients are currently available, a variety of approaches to preserve or restore vision are under investigation, including neural retinal transplantation (Kaplan et al., 1997; Algvere et al., 1999; Das et al., 1999; Radtke et al., 1999; Humayun et al., 2000; Binder et al., 2002; Radtke et al., 2002; Berger et al., 2003), gene therapy (Bennett et al., 1996, 1998; Ali et al., 2000; Bennett, 2000; Vollrath et al., 2001), pharmacotherapies (Faktorovich et al., 1990; Li et al., 1998; Chong et al., 1999; Caffe et al., 2001) and visual prosthetic devices (Weiland et al., 1999; Humayun et al., 1999a; Margalit et al., 2002; Lakhanpal et al., 2003).

* Corresponding author. Dr S.R. Sadda, Doheny Retina Institute, Doheny Eye Institute, Keck School of Medicine, University of Southern California, DEI 3610, 1450 San Pablo Street, Los Angeles, CA 90033-3699, USA.
E-mail address: ssadda@doheny.org (S.R. Sadda).
[1] These authors contributed equally to the paper.

0014-4835/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
DOI:10.1016/j.exer.2004.05.013

Retinal transplantation was introduced in the 1980s with high expectations (del Cerro et al., 1997; Aramant and Seiler, 2002). Since then, many investigators have reported encouraging results in this difficult field in animal models (Silverman and Hughes, 1989; del Cerro et al., 1991; Silverman et al., 1992; Gouras et al., 1994; Ghosh et al., 1998; Seiler and Aramant, 1998; Aramant et al., 1999; Kwan et al., 1999; Gouras and Tanabe, 2003) and in human patients (Das et al., 1999; Radtke et al., 1999; del Cerro et al., 2000; Humayun et al., 2000; Radtke et al., 2002; Radtke et al., 2004). Several studies have suggested some improvement in visual function as judged from light reflexes, behavioral testing, and electrophysiological responses in blind animal models (del Cerro et al., 1991; Silverman et al., 1992; del Cerro et al., 1995; Kwan et al., 1999; Radner et al., 2001; Woch et al., 2001; Sagdullaev et al., 2003; Thomas et al., 2004). Del Cerro (del Cerro et al., 1991, 1995) studied the suppressive effect of a warning flash on the startle reflex response to acoustic stimuli in light-damaged rats and reported partial restoration of this visually mediated behavior after retinal transplantation. Silverman (Silverman et al., 1992) reported that transplants of photoreceptor sheets restored the pupillary reflex and visually evoked potentials (VEPs) after retinal transplantation in light-damaged rats. Kwan (Kwan et al., 1999) demonstrated that the preference for the dark compartment was restored after retinal cell transplantation to rd/rd mice. Radner (Radner et al., 2001) showed light driven ganglion cell responses after neural retinal transplantation in 3 out of 10 two-week-old rd/rd mice. Lastly, Woch (Woch et al., 2001), Sagdullaev (Sagdullaev et al., 2003), and Thomas et al. (Thomas et al., 2004) demonstrated that light driven superior colliculus (SC) responses could be restored by fetal retinal sheet transplantation in RCS rats, S334ter-3 rats with fast retinal degeneration, and S334ter-5 rats with slow retinal degeneration. However, the physiologic mechanism for the restoration is unclear – whether these responses were due to a rescue effect or by direct replacement of damaged retina with reestablishment of neural circuitry.

Superior colliculus (SC) recording is a well-established method of objectively measuring retinal function (Siminoff et al., 1966). With this method, the visually responsive area can be localized on the topographic map of SC and compared with the position of the transplant in the retina (Woch et al., 2001; Sagdullaev et al., 2003; Thomas et al., 2004). The aim of the present study is to evaluate the correlation between the functional success of retinal transplantation and the histologic findings. In this study, we report the presence of SC responses after retinal trans-plantation of fetal retinal sheets into 5-week (PD 31–38) old rd/rd mice and evaluate the histology using immunohistochemistry.

2. Methods

2.1. Animals

All animals were treated according to the regulations provided by the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and The National Institute of Health Guide for the Care and Use of Laboratory Animals. 21 rd/rd (C3H/HeJ) mice were used as transplant recipients at the age of postnatal day (PD) 31–38. E17 timed-pregnant enhanced-green-fluorescent (eGFP) mice (C57BL/6-TgN(ACTbEGFP)1Osb) were used as the source of donor tissue. Controls included: (1) four 5-week-old (PD 34–35) untreated rd/rd mice (2) three 9-week-old (PD 61–65) untreated rd/rd mice (C3H/HeJ), (3) five 9 wk old (PD 63–65) rd/rd mice with sham surgery on PD 36, and (3) three 9-week-old (PD 63–67) wild-type mice (C57BL/6J).

2.2. Transplantation procedure

The transplantation surgical technique used in this study is an adaptation of our previously described method for rat transplantation (Seiler and Aramant, 1998; Aramant et al., 2002), with modification of the implantation tool to accommodate the smaller mouse eye. To harvest the donor tissue, mice embryos (E17) were removed by caesarean section and fetal retinas were carefully dissected free from surrounding tissues, and then flattened in hibernation medium. The tissue was kept cold until transplantation. The flattened tissue was cut into rectangular pieces (around 1·0 × 0·4 mm) to fit the lumen of the custom-designed implantation tool (US patent#6 159 218).

For transplantation surgery, mice were anesthetized by intraperitoneal injection of a mixture of ketamine (60 mg kg^{-1}) and xylazine (8 mg kg^{-1}). A small incision (0·5–1·0 mm) was created behind the pars plana and a local retinal detachment was produced. The implantation tool was carefully inserted into the subretinal space nasal to the optic nerve and the graft tissue was gently released. Previous histologic studies have demonstrated that this technique allows precise and reliable delivery of tissue to the target host retinal location. This highly reproducible delivery with respect to transplant location has been of importance in subsequent electrophysiologic assessment of the transplant site. Following implantation, the scleral incision was closed with 10–0 sutures. The procedure was the same for sham surgery, except that no tissue was implanted.

2.3. Electrophysiology

Mice eyes frequently develop varying degrees of corneal opacity after transplantation surgery, perhaps due to transient intraocular pressure elevation or other trauma from the transplant procedure. Since any corneal opacity

may attenuate the light stimulus for electrophysiologic testing, only 7 of the transplanted mice with clear corneas and lenses were selected for testing. The electrophysiologist was masked as to the identity of the mice (transplanted or control mice). Superior colliculus (SC) recording of visual responses to a light flash was performed 25–35 days after retinal transplantation at the age of PD 59–76, or 26–28 days after sham surgery on PD 63–65. Animals were dark-adapted overnight and then anesthetized by intraperitoneal injection of xylazine and ketamine (37·5 and 5 mg kg^{-1}, respectively). Gas inhalant anesthesia (1·0–2·0% halothane in 40% O_2/60% N_2O) was administered via an anaesthetic mask (Stoelting Company, Wood Dale, IL, USA). Mice were mounted in a stereotactic apparatus and a craniotomy was performed to expose both hemispheres of the SC. Multi-unit responses were recorded extracellularly from the superficial laminae of the SC using custom-made nail polish-coated tungsten microelectrodes. Recording sites (200–400 μm apart) covered the full extent of the SC with exception of its medial area, which was located just under the superior sagittal sinus. In each mouse, the mapping was performed systematically starting at the medial-caudal corner of the SC and progressing until the rostral edge of the SC was reached. The map was then continued in rows parallel and lateral to the preceding row and in alternating directions, rostrally and caudally, until the lateral-rostral corner of the SC was reached. At each position, the electrode was lowered 100 μm beyond its point of contact with the surface of the SC and responses were recorded to 16 presentations of the visual stimulus, a full-field strobe flash (20 cd m^{-2}, 10-μs duration; Model PS 22 Photic stimulator; Grass Instruments, West Warwick, RI, USA).

2.4. Histology

Immediately following the SC recording, the eyes were enucleated and processed for immunohistochemistry. Most eyes were immersion fixed for 30 min in 4% paraformaldehyde in 0·1 M phosphate buffer (pH 7·4). The anterior segments were removed and the posterior eyecups were fixed in the same solution for an additional 30 min. After fixation, the eyecups were washed in phosphate buffer, dissected out, and postfixed in 30% sucrose/0·1 M phosphate buffer overnight. The specimens were embedded in molds containing Tissue-Tek OTC 4583 Compound (Sakura Finetek, USA, Inc., Torrance, CA, USA) and were frozen in isopentane cooled with dry ice. Ten-micrometre sections were cut on a JUNG CM3000 series microtome cryostat (Leica Microsystems, Bannockburn, IL, USA).

2.5. Immunohistochemistry

Sections were thawed and washed in phosphate buffered saline (PBS), blocked with 20% goat serum in 0·1% triton X-100/1% bovine serum albumin (BSA) in PBS, and incubated with the primary antibodies overnight at 4°C (see Table 1). The sections were rinsed several times with PBS and incubated with secondary antibodies for 30 min. After washing, the slides were cover-slipped with DAPI containing Vectashield™ mounting media (Vector Laboratories, Burlingame, CA, USA) and viewed on a LSM 510 Laser Scanning Confocal Microscope (Carl Zeiss, Oberkochen, Germany). An overview of the antibodies used in this study is shown in Table 1. For quantification of recoverin-staining, the number of recoverin-positive photoreceptor cells (presumed cones) were counted by two independent

Table 1
Reagents for immunohistochemistry

Primary antibodies	Species	Specific for	Dilution	Supplier
Protein kinase C	Rabbit	Rod bipolar cells	1:200	Oxford Biomed., Oxford, MI, USA
Rhodopsin	Rabbit	Photoreceptors (Rods)	1:1000	Plantner (Plantner et al., 1982)
Recoverin	Rabbit	Photoreceptors (cones and rods); cone bipolar cells	1:500	Dizhoor (Dizhoor et al., 1991) McGinnis (McGinnis et al., 1997)
Secondary antibodies				
Rhodamine X anti-rabbit	Goat	Rabbit IgG	1:200	Molecular Probes, Eugene, OR, USA
Miscellaneous				
Biotinylated peanut agglutinin	n.a.	Cone interphotoreceptor matrix; inner and outer plexiform layer	1:1000	Vector Labs, Burlingame, CA, USA
Labelled streptavidin				
Rhodamine X—conj.	n.a.	Biotin	1:200	Molecular probes, Eugene, OR, USA

Table 2
Overview of experiments

Mouse no.	Host age	Donor age	SC responses	Post surgery	Age at SC recording	Graft histology	Comment
5	PD 31	E17	+	28 days	PD 59	Disorganized	
8	PD 35	E17	+	25 days	PD 60	Rosettes	Cell migration, rod bipolar cells crossing transplant–host interface
10	PD 35	E17	–	28 days	PD 63	Laminated	
15	PD 35	E17	+	29 days	PD 64	Rosettes	Cell migration, rod bipolar cells crossing transplant–host interface
19	PD 38	E17	–	38 days	PD 76	Rosettes	Plastic sections; no immunohistochemistry
20	PD 38	E17	–	35 days	PD 73	Rosettes	Clear extension and migration, rod bipolar cells crossing transplant–host interface
25	PD 38	E17	–	33 days	PD 71	Laminated	Rod bipolar cells crossing transplant–host interface
101–105	PD 36	NA	–	27–29 days	PD 63–65	NA	Sham surgery

observers and averaged from at least three fields through the graft per experiment, two fields of the host retina outside the graft and from 3 to 4 fields in analogous retinal regions in the controls. For counting, recoverin-positive photoreceptor cells were distinguished from recoverin-positive cone bipolar cells based on cell morphology and positioning in the host retina. Counts of different experimental groups were statistically compared using a one-way ANOVA, followed by post hoc analysis with a Student–Newman–Keuls test (see Table 3).

3. Results

3.1. Electrophysiology

In three out of the seven recorded eyes (43%), visual responses were detected in the SC (Table 2, Figs. 1 and 2), but only in the area of the SC which corresponded to the retinal area where the grafts were placed. No responses were recorded in 9-week-old sham surgery eyes, and the untreated eyes of 5-week-old (the age of the mice at the time of transplant surgery) and 9-week-old *rd/rd* mice. In contrast, visual responses were recorded over the entire SC in normal eyes. The response onset latencies of the light-responsive transplanted mice were 30–35 msec (32·6 ± 1·39), similar to those of the normal control mice (32·8 ± 1·25 msec) ($P < 0{\cdot}81$).

3.2. Histology

3.2.1. Transplant organization

In all of the seven eyes that had undergone SC recording, transplants were identified as retinal sheets in the subretinal space. To describe the quality and organization of the transplant, a three-category classification system (Seiler and Aramant, 1998) was used in this study, as previously described. 'Laminated' was used to describe grafts with the 'best' (most resembling normal retina) organization with

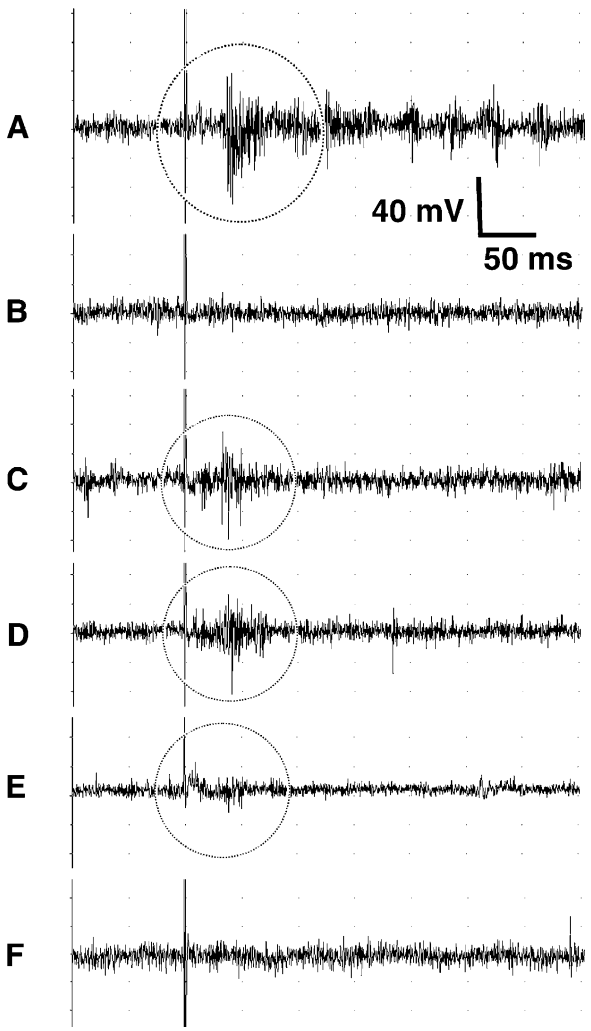
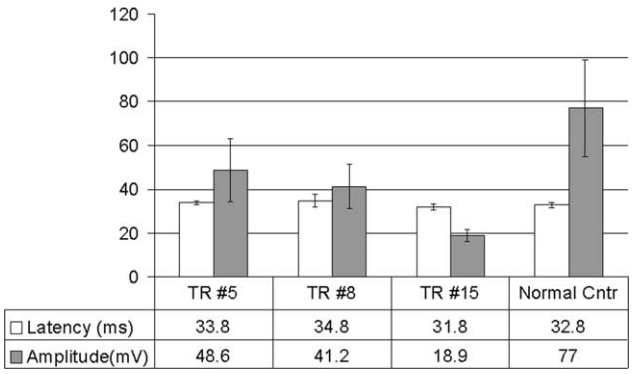


Fig. 1. Examples of superior colliculus recordings from (A) normal mouse; (B) untreated rd/rd control mouse (5 weeks old); transplanted mice (C) #5, (D) #8, (E) #15 with visual responses after transplantation; and (F) sham surgery mouse (similar traces were recorded in all five sham surgery mice). Visual responses were only found in the area of the SC topographically corresponding to the placement of the transplant in the retina. In mouse #15(E), the amplitude was low, but the response was very consistent with the same latency in repeated recordings.



	TR #5	TR #8	TR #15	Normal Cntr
Latency (ms)	33.8	34.8	31.8	32.8
Amplitude(mV)	48.6	41.2	18.9	77

Fig. 2. Latency versus amplitude in the three transplanted mice with visual responses versus the normal control mice: visual responses of transplanted mice have normal latencies, but lower amplitudes compared to control mice.

parallel layers and fully developed photoreceptor outer segments in contact with the host RPE (Fig. 3(A)). 'Rosette' was used to designate the presence of spherical structures with photoreceptors and their outer segments directed to the central lumen of the structure, with inner retinal cells comprising the outer layers of the structure (Fig. 3(B)). The 'worst' category 'Disorganized' was used to indicate that a transplant was comprised of cells that were not arranged in any recognizable layers (Fig. 3(C)).

Electrophysiologic findings did not correlate as expected with the histology. Indeed, among the three tested animals with good visual responses by electrophysiology, the graft was disorganized in one animal (#5), and contained rosettes in the other two, albeit with rhodopsin-positive structures (presumably rudimentary outer segments) at the center of the rosettes (#8, #15). No significant areas of lamination were observed in these grafts. In some areas, however, extension of the GFP-positive (donor) cell processes into the host layers were observed (#8; Fig. 4(A)). Also, some areas showed the crossing of rod bipolar cells between the host and the transplant (#8, #15; Fig. 4(B)). In contrast, the remaining four eyes, which did not exhibit a positive SC response, displayed better graft morphologies with excellent lamination (#10, #25, Fig. 3) in two of the four. There was also some evidence of extension of donor processes and donor cell migration into the host layers in these eyes (#20, Fig. 4(C)) as well.

3.2.2. Host retina

Staining with anti-recoverin, anti-rhodopsin and peanut agglutinin (PNA) was performed to evaluate the status of the remaining photoreceptors in the host retina. Recoverin would be expected to label residual cones and some classes of cone bipolar cells (McGinnis et al., 1997, 1999). Cone bipolar cells and cones can generally be distinguished however, based on cell morphology and positioning within the host retina. In this study, recoverin-positive photoreceptor cells were observed in the untreated 5 and 9-week-old rd/rd control eyes (Fig. 5(A) and (B)), in the host retina in all transplanted eyes (Fig. 5(C) and (G)), and the sham surgery eyes (Fig. 5(H) and (I)). Increased numbers of

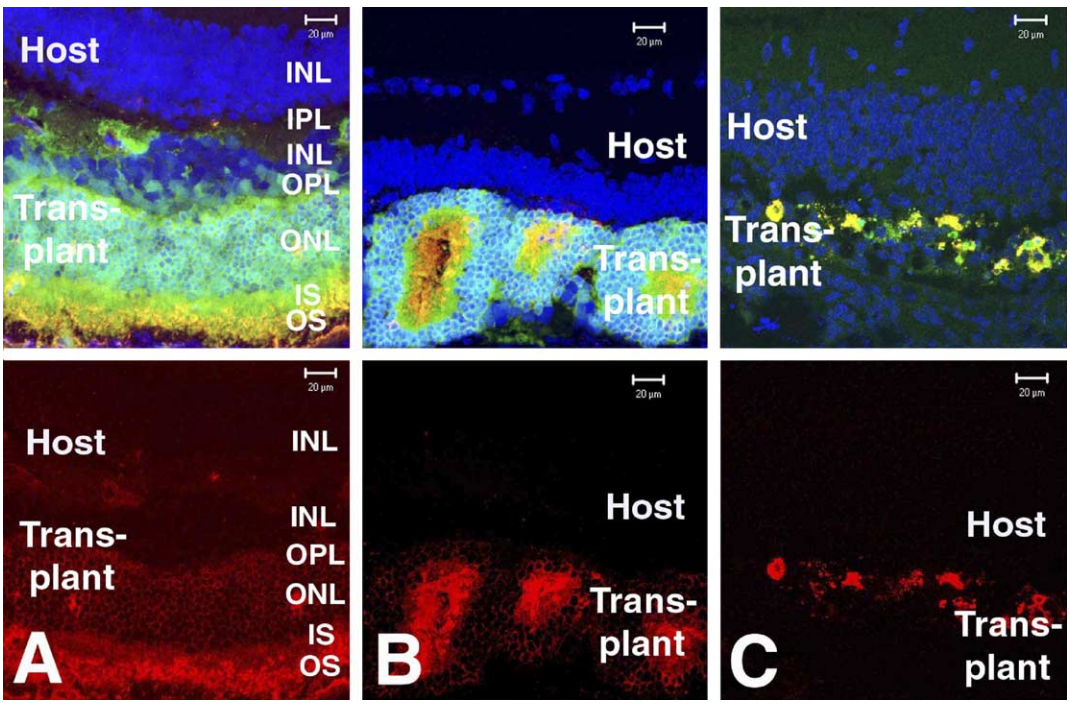


Fig. 3. Rhodopsin staining of transplants. Red, rhodopsin staining for photoreceptors. Green, green fluorescent protein (GFP) for donor tissue; blue, DAPI staining for nucleus. (A) 'Lamination' in mouse #25 (no SC response), (B) 'Rosette' in mouse #15 (positive SC response), (C) 'Disorganized' in mouse #5 (positive SC response). No staining in the host retina.

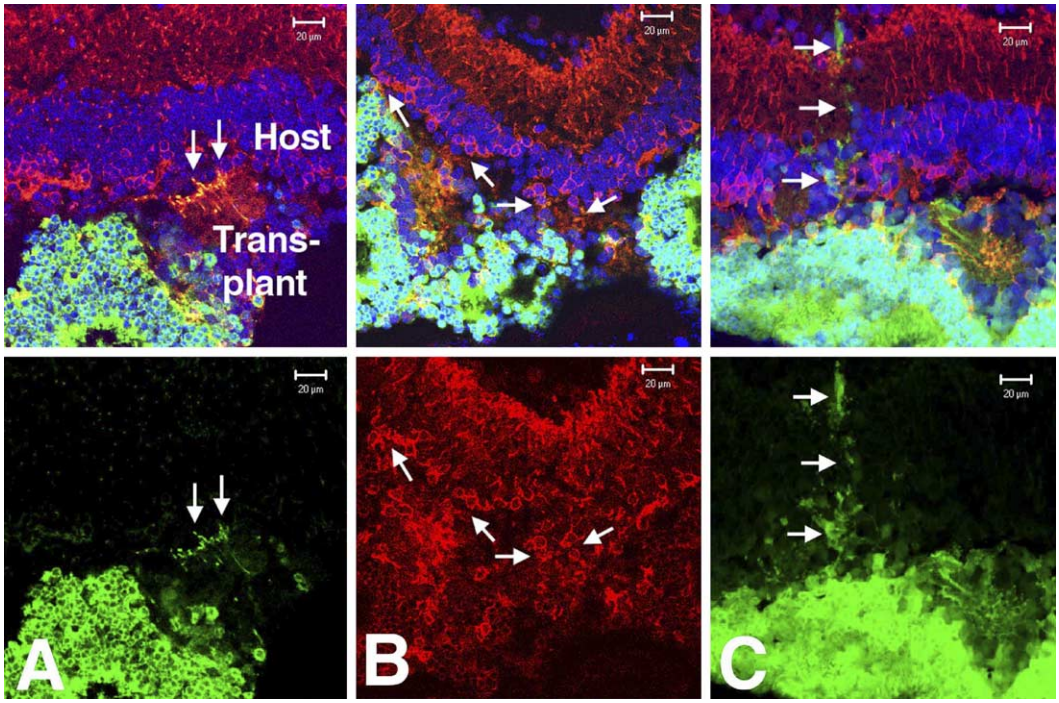


Fig. 4. Graft/host integration. Red, protein kinase C for rod bipolar cells; green, green fluorescent protein (GFP) for donor tissue; blue, nuclear DAPI staining. (A) Extension of GFP-positive (donor) cell processes to the host layers were observed (mouse #8). (B) Bipolar cells from host and transplant made close contact with each other (mouse #15). (C) Clear extension of donor fibers into host retina (mouse #20).

recoverin-positive photoreceptor cells were found in the peripheral ventral retina compared with the central retina. However, since all of the grafts were transplanted in a highly localized region in the central retina (nasal to the disk), comparisons between groups were made only for immunoreactivity in the central retina, comparing only analogous areas among the various groups.

The comparison of recoverin-staining between 5-week and 9-week-old rd mice demonstrated an apparent reduction in positive cells in the 9-week-old animals consistent with a slowly progressive loss of cones (Fig. 5(A) and (B)). In contrast, the host retina overlying the graft in the transplanted animals with positive light-responses clearly demonstrated significantly higher numbers of recoverin-positive photoreceptor cells, compared with the corresponding retinal location of 5-week-old no surgery control rd mice, 9-week-old no surgery control rd mice, 9-week-old sham surgery mice, and 9-week-old control transplanted rd mice without light responses (no statistically significant difference was observed between the latter three groups) (see Table 3; Fig. 5(C) and (E)). Furthermore, surprisingly, one of the light-responsive transplanted eyes (mouse #8) (Fig. 5(C)) showed much higher numbers of recoverin-positive cells compared with untreated 5-week-old *rd/rd* mice (which was the age of mouse at the time of transplantation) (Fig. 5(A)).

The remaining two eyes with positive responses exhibited similar immunoreactivity as the untreated 5-week-old *rd/rd* mice. Outside of the graft, the number of immunoreactive cone photoreceptor cells significantly decreased (Fig. 5(D) and (F)). The transplanted eyes with negative responses showed similar levels of recoverin-positive photoreceptor cells as untreated age-matched (9 weeks old) *rd/rd* eyes (Fig. 5(G)). Sham surgery eyes showed some increase in recoverin-staining (Fig. 5(H) and (I)) compared to no surgery controls, but significantly less than the transplanted eyes with light responses (see Table 3).

PNA-staining was only observed in the outer plexiform layer (OPL) with no evidence of residual host outer segments in transplanted or control eyes (data not shown). Rhodopsin staining failed to reveal any remaining rod cells in the host retina of transplanted eyes or in control eyes, but did stain rod and cone photoreceptors in the transplants (Fig. 3).

4. Discussion

In this study, we evaluated the SC responses in rd mice following sheet retinal transplantation and correlated these observations with the microscopic findings. Previously, Kwan and co-workers reported restoration of visual responses (as demonstrated by a light-dark preference test) following transplantation of a retinal cell suspension in 6–8-week-old rd mice (Kwan et al., 1999). In prior studies,

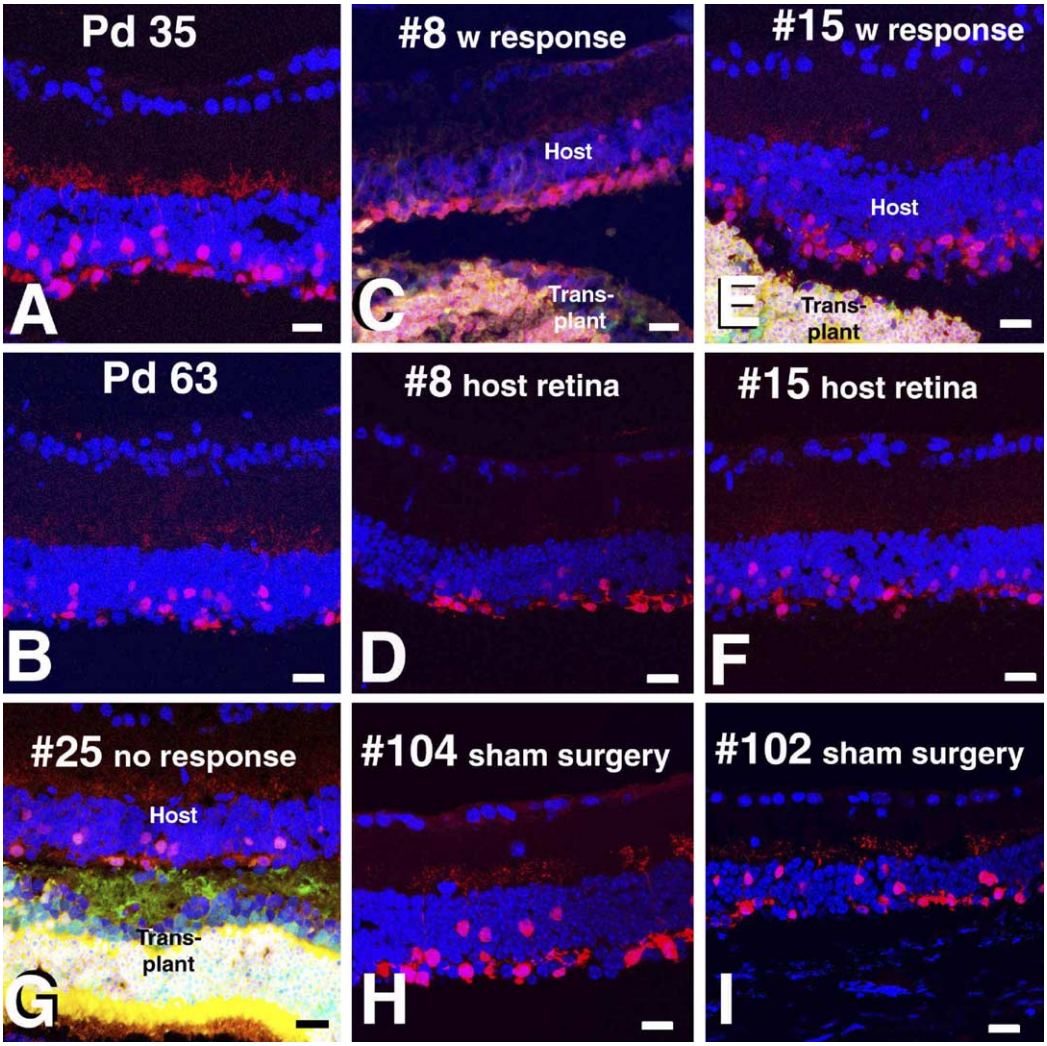


Fig. 5. Recoverin-staining. Red, recoverin; green, green fluorescent protein (GFP) for donor tissue; blue, nuclear DAPI staining. (A) Non-surgery control, *rd/rd*, PD 35: recoverin-staining of midperipheral dorsal retina shows monolayer of surviving cones and some cone bipolar cells. (B) Non-surgery control, *rd/rd*, PD 63: number of surviving cones is much decreased and only a few cones and cone bipolar cells were observed. (C,D) Mouse #8, *rd/rd*, PD 60, 25 days after transplantation. (C) Inside the graft area of the host retina: increased number of cones and a few cone bipolar cells. The space between transplant and host is a processing artifact. (D) Outside the graft area; number of surviving cones is decreased compared with the graft area. (E,F) Mouse #15, *rd/rd*, PD 64, 29 days after transplantation. (E) Inside the graft area of the host retina: increased number of cones and cone bipolar cells. (F) Outside the graft area: number of surviving cones is decreased compared with graft area. (G) Mouse #25, *rd/rd*, PD 71, 33 days after transplantation, no visual responses in SC: inside the graft area of the host retina: only few surviving cones and cone bipolar cells are observed. (H) Mouse #104, *rd/rd*, PD 65, 28 days after sham surgery, no visual response in SC: some increase in cones in dorsal retina. (I) Mouse #102, rd/rd, PD 65, 28 days after sham surgery, no visual response in SC: few cones in dorsal central retina.

we also observed some functional recovery in a small percentage (3/10) of animals (as assessed by retinal surface ganglion cell recordings) following retinal *fragment* transplants in *rd* mice (Radner et al., 2001). We had performed the present study to evaluate the hypothesis that transplantation of intact retinal sheets would improve graft morphology and enhance the functional outcome of the procedure. Because of the small mouse eye, a low percentage of laminated transplants was expected. However, the two (out of seven) laminated transplants still gave enough data for this study. Interestingly, better electrophysiologic responses were observed in mice with apparently poor transplant morphologies although no responses were observed in age-matched sham surgeries. To interpret the significance of these observations, it is important to consider the potential mechanisms by which transplantation could affect the course of visual changes in retinal degeneration models. There are two readily apparent mechanisms. The first mechanism is direct replacement of the damaged retina and reestablishment of functional neural connections with the remaining host retina. Although there is evidence that transplants themselves are functional (Adolph et al., 1994; Seiler et al., 1999) and that some degree of graft-host integration can occur after retinal

Table 3
Recoverin-staining of cones in host retina

Experimental group	Rd no surgery PD 35	Rd no surgery PD 63–67	Sham surgery PD 63–65	Transplant with SC response PD 59–64		Transplant without SC response PD 63–76	
No. of eyes	2	2	5	3 (#5, #8, #15)		3 (#10, #20, #25)	
				Transplant area	Outside transplant	Transplant area	Outside transplant
Microscopic fields counted	3	4	9	16	6	11	3
No. of cones/100 μm of host retina ± S.D.	5·14 ± 1·94	1·77 ± 1·46	4·04 ± 1·82	9·35 ± 4·6*	4·63 ± 1·12	2·64 ± 1·18	5·14 ± 0·75

Rd, rd mouse; PD, postnatal day; SC, superior colliculus. *Significantly higher numbers of host cones in the transplant area of eyes with SC response: versus no surgery (PD 35), $P < 0{\cdot}01$; versus no surgery (PD 63–67), $P < 0{\cdot}001$; versus sham surgery, $P < 0{\cdot}001$; versus outside transplant, $P < 0{\cdot}001$; versus transplant area without response, $P < 0{\cdot}001$.

transplantation (Ehinger et al., 1991; Silverman et al., 1992; Gouras et al., 1994; Aramant and Seiler, 1995; Seiler and Aramant, 1998; Ghosh et al., 1999; Kwan et al., 1999; Gouras and Tanabe, 2003; Zhang et al., 2003), the presence of definite functional synaptic connections has not yet been reported. As a second potential mechanism of action, the transplanted tissue could provide an indirect effect via the release of humoral factors that could prevent or retard the degeneration of the remaining host photoreceptors (Mohand-Said et al., 1997, 1998, 2000; Fintz et al., 2003).

To determine the mechanism for the visual responses observed in our experiments, several points should be considered. First, if synaptic connectivity was the mechanism of restoration, one might expect that the morphology of the graft and the extent of processes extending between the graft and the host would have an effect on the functional outcome. It would seem reasonable that cells organized in regular layers with a normal polarity would have a better opportunity to elaborate processes and make functional contacts with host cells. On the other hand, if a rescue effect on remaining host cones due to the release of diffusible/humoral factors was the primary mechanism, then organization of the cells would likely be less important; only the presence or absence of the tissue in close proximity to the host cones would be relevant. However, the functional effect of laminated versus non-laminated grafts was not equivalent in this study. Rather, laminated grafts appeared to have no effect. One explanation, consistent with a rescue mechanism, is that there may have been a greater distance between the transplant cells producing the 'rescuing factor' (presumably the transplant photoreceptors – specifically rods) and the host cones, as a result of an intervening transplant inner nuclear layer. This well-developed inner nuclear layer, which did not form in the non-laminated grafts, may have presented a relative barrier to the putative diffusible 'rescuing factors'. With respect to the morphologic evidence of connections in light-responsive animals, two of the three (#8, #15) demonstrated evidence of processes extending between the transplant and the host, including apparent areas of contact between transplant and host bipolar cells. However, non-responding animals, including animals with laminated grafts (#20) also showed similar findings. Furthermore, the observation of a few processes between the graft and host does not imply that functional connections have formed. Given these issues, it is not possible to use the morphologic data to support the hypothesis that graft–host connectivity was responsible for the visual responses.

A second line of evidence is also relevant to the discussion of connectivity versus rescue effects. The latency of the visual response observed in this study was similar to that of normal animals. This is in contrast to previous observations in retinal degenerate rat models in which light-responsive transplanted animals demonstrated a marked prolongation in latency (Woch et al., 2001; Sagdullaev et al., 2003). The prolonged latency was postulated to be due to the formation of aberrant circuits between the graft and the host such as transplant bipolar to host bipolar connections. Furthermore, also in contrast to the present study, in the previous studies, most of the light-responsive animals demonstrated a laminated transplant morphology. The normal response latency of the light response observed in our study would suggest that the normal phototransduction and transmission pathways were employed (i.e. photoreceptor → bipolar → ganglion cell) to generate the response. While it is possible that the transplanted photoreceptors formed connections with host bipolar cells (or transplant bipolar cells formed connections with host ganglion cells) and reconstituted a normal pathway, this would seem unlikely given previous experience.

In summary, taken as a whole, the available data suggests that the mechanism of restoration of visual responses in this retinal degeneration–transplantation model is a rescue of remaining host cones. The *rd/rd* mouse is a well-characterized animal model of retinal degeneration and at the age of PD 35, more than 99·7% of rods have

degenerated, leaving a monolayer of cones, without outer segments (Mohand-Said et al., 2000). The number of remaining cone nuclei slowly declines over the ensuing months (Sanyal and Bal, 1973), followed by a slow remodeling of the inner retina (Strettoi and Pignatelli, 2000; Marc et al., 2003; Strettoi et al., 2003). In this study, recoverin immunochemistry was examined in the host retina of the transplanted and control eyes. Since immunoreactivity for rhodopsin and PNA-staining was negative in the host retina, the recoverin-positive cells were expected to be surviving cone cell bodies and cone bipolar cells (McGinnis et al., 1997, 1999). When untreated eyes were compared at the age of 5 and 9 weeks, recoverin-staining was much decreased at the age of 9 weeks, consistent with progression of the retinal degeneration in the absence of a 'rescue' intervention. If cones were simply being rescued by transplants in this animal model, why were no visual responses observed in 5-week-old control, non-transplanted *rd/rd* mice (i.e. mice corresponding to the age at which transplants were performed)? One explanation is that even the residual cones in 5-week-old mice were not functioning properly. Certainly, these residual cone photoreceptors do not possess outer segments. Perhaps, the transplants prevented these cones from dying and restored their phototransduction activity. Interestingly, even these presumed 'rescued' cones do not elaborate outer segments. The ability of cells without outer segments to mediate phototransduction and visual responses, however, has been well documented (An et al., 2002).

Consistent with the presumed rescue mechanism, all of the transplanted eyes with positive visual responses demonstrated significantly higher numbers of recoverin-positive cells overlying the graft, compared with regions of the same eye outside the graft, transplanted eyes with no light response, sham surgery eyes, and untreated 5 and 9-week-old *rd/rd* eyes.

Another possible, but less likely explanation for this observation is that the graft or the transplant surgery stimulated a 'regeneration' of host cone cells. How could this be possible? The existence of retinal progenitor cells within the adult neural retina has been well established (Tropepe et al., 2000). In chickens, retinal progenitor cells in the adult retina, either derived from the ciliary margin (Fischer and Reh, 2000) or from Muller glia (Fischer and Reh, 2001) can be stimulated by trauma or other factors to proliferate. Although this possibility has not yet been shown in mammalian retina, it may be possible that in the animals in our study, the surgical trauma of the transplant surgery or the fetal graft itself led to activation of host retinal progenitor cells. With our existing data, it is not possible to draw any conclusions. Future studies, including BrdU-labelling of the host retina, may help elucidate an explanation to this observation.

Another factor in obtaining visual responses from transplanted mice may have been the age at recording. Responses were obtained from 3 of 4 transplanted mice recorded between postnatal day (PD) 59–64, with surgery between PD 31–35 whereas no responses were seen with the three mice recorded PD 71–76, with surgery at PD 38. Thus, the transplant effect may be transient, or alternatively, if the tissue is transplanted at a more advanced stage of degeneration, it may have no effect.

A limitation of the study is the relatively small number of treated animals, which could undergo electrophysiologic testing for structural versus functional correlation. Although over 20 animals underwent transplantation, only seven maintained clear ocular media (cornea and lens) after the sheet transplant procedure. In most cases, the loss of clarity was due to corneal opacification. Interestingly, such a phenomenon is not observed in rats. The etiology for this opacification is unknown, but may be due to some inadvertent surgical trauma or lengthy operative time. Indeed, retinal *sheet* transplant surgery in the smaller (compared with rats) mouse eye is a technically challenging task, which can require a lot of effort for a single surgery.

In summary, restoration of light driven SC responses (in an area corresponding to the graft location in the retina) were observed in three of the seven eyes of *rd/rd* mice that received subretinal fetal sheet transplants at PD 31–38. The morphology of the graft did not correlate well (or correlated inversely) with the electrophysiology, as animals with well-laminated grafts showed no response, whereas some poorly organized grafts exhibited positive responses. The latency of the SC responses was normal in transplanted light-responsive animals suggesting that the response was due to host cells with a relatively 'normal' retinal circuit. Increased numbers of recoverin-positive cells (including cone cell bodies) were observed in the host retina overlying the graft, compared with age-matched untreated controls. The mechanism of visual restoration is likely due to a rescue effect on host cones, rather than direct replacement of the damaged retina and graft-host connectivity.

Acknowledgements

The authors thank Xiaoji Xu, and Zhenhai Chen for their technical assistance. This work was supported by: The Foundation Fighting Blindness, Foundation for Retinal Research, Michael Panitch Fund for Retinal Research, NIH EY03040 and an anonymous sponsor.

Magdalene J. Seiler and Robert B. Aramant have a proprietary interest in the implantation instrument and procedure.

References

Adolph, A.R., Zucker, C.L., Ehinger, B., Bergstrom, A., 1994. Function and structure in retinal transplants. J. Neural Transplant. Plast. 5, 147–161.

Algvere, P.V., Gouras, P., Dafgard Kopp, E., 1999. Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. Eur. J. Ophthalmol. 9, 217–230.

Ali, R.R., Sarra, G.M., Stephens, C., Alwis, M.D., Bainbridge, J.W., Munro, P.M., Fauser, S., Reichel, M.B., Kinnon, C., Hunt, D.M., Bhattacharya, S.S., Thrasher, A.J., 2000. Restoration of photoreceptor ultrastructure and function in retinal degeneration slow mice by gene therapy. Nat. Genet. 25, 306–310.

An, G.J., Asayama, N., Humayun, M.S., Weiland, J., Cao, J., Kim, S.Y., Grebe, R., de Juan Jr, E., Sadda, S., 2002. Ganglion cell responses to retinal light stimulation in the absence of photoreceptor outer segments from retinal degenerate rodents. Curr. Eye Res. 24, 26–32.

Aramant, R.B., Seiler, M.J., 1995. Fiber and synaptic connections between embryonic retinal transplants and host retina. Exp. Neurol. 133, 244–255.

Aramant, R.B., Seiler, M.J., 2002. Retinal transplantation—advantages of intact fetal sheets. Prog. Retin. Eye Res. 21, 57–73.

Aramant, R.B., Seiler, M.J., Ball, S.L., 1999. Successful cotransplantation of intact sheets of fetal retina with retinal pigment epithelium. Invest. Ophthalmol. Vis. Sci. 40, 1557–1564.

Bennett, J., 2000. Gene therapy for retinitis pigmentosa. Curr. Opin. Mol. Ther. 2, 420–425.

Bennett, J., Tanabe, T., Sun, D., Zeng, Y., Kjeldbye, H., Gouras, P., Maguire, A.M., 1996. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. Nat. Med. 2, 649–654.

Bennett, J., Zeng, Y., Bajwa, R., Klatt, L., Li, Y., Maguire, A.M., 1998. Adenovirus-mediated delivery of rhodopsin-promoted bcl-2 results in a delay in photoreceptor cell death in the rd/rd mouse. Gene Ther. 5, 1156–1164.

Berger, A.S., Tezel, T.H., Del Priore, L.V., Kaplan, H.J., 2003. Photoreceptor transplantation in retinitis pigmentosa: short-term follow-up. Ophthalmology 110, 383–391.

Berson, E.L., 1993. Retinitis pigmentosa. The Friedenwald Lecture. Invest. Ophthalmol. Vis. Sci. 34, 1659–1676.

Binder, S., Stolba, U., Krebs, I., Kellner, L., Jahn, C., Feichtinger, H., Povelka, M., Frohner, U., Kruger, A., Hilgers, R.D., Krugluger, W., 2002. Transplantation of autologous retinal pigment epithelium in eyes with foveal neovascularization resulting from age-related macular degeneration: a pilot study. Am. J. Ophthalmol. 133, 215–225.

Caffe, A.R., Soderpalm, A.K., Holmqvist, I., van Veen, T., 2001. A combination of CNTF and BDNF rescues rd photoreceptors but changes rod differentiation in the presence of RPE in retinal explants. Invest. Ophthalmol. Vis. Sci. 42, 275–282.

Chong, N.H., Alexander, R.A., Waters, L., Barnett, K.C., Bird, A.C., Luthert, P.J., 1999. Repeated injections of a ciliary neurotrophic factor analogue leading to long-term photoreceptor survival in hereditary retinal degeneration. Invest. Ophthalmol. Vis. Sci. 40, 1298–1305.

Das, T., del Cerro, M., Jalali, S., Rao, V.S., Gullapalli, V.K., Little, C., Loreto, D.A., Sharma, S., Sreedharan, A., del Cerro, C., Rao, G.N., 1999. The transplantation of human fetal neuroretinal cells in advanced retinitis pigmentosa patients: results of a long-term safety study. Exp. Neurol. 157, 58–68.

del Cerro, M., DiLoreto Jr, D., Cox, C., Lazar, E.S., Grover, D.A., del Cerro, C., 1995. Neither intraocular grafts of retinal cell homogenates nor live non-retinal neurons produce behavioral recovery in rats with light-damaged retinas. Cell Transplant. 4, 133–139.

del Cerro, M., Humayun, M.S., Sadda, S.R., Cao, J., Hayashi, N., Green, W.R., del Cerro, C., de Juan Jr, E., 2000. Histologic correlation of human neural retinal transplantation. Invest. Ophthalmol. Vis. Sci. 41, 3142–3148.

del Cerro, M., Ison, J.R., Bowen, G.P., Lazar, E., del Cerro, C., 1991. Intraretinal grafting restores visual function in light-blinded rats. Neuroreport 2, 529–532.

del Cerro, M., Lazar, E.S., Diloreto Jr, D., 1997. The first decade of continuous progress in retinal transplantation. Microsc. Res. Tech. 36, 130–141.

Dizhoor, A.M., Ray, S., Kumar, S., Niemi, G., Spencer, M., Brolley, D., Walsh, K.A., Philipov, P.P., Hurley, J.B., Stryer, L., 1991. Recoverin: a calcium sensitive activator of retinal rod guanylate cyclase. Science 251, 915–918.

Ehinger, B., Bergstrom, A., Seiler, M., Aramant, R.B., Zucker, C.L., Gustavii, B., Adolph, A.R., 1991. Ultrastructure of human retinal cell transplants with long survival times in rats. Exp. Eye Res. 53, 447–460.

Faktorovich, E.G., Steinberg, R.H., Yasumura, D., Matthes, M.T., LaVail, M.M., 1990. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 347, 83–86.

Fintz, A.C., Audo, I., Hicks, D., Mohand-Said, S., Leveillard, T., Sahel, J., 2003. Partial characterization of retina-derived cone neuroprotection in two culture models of photoreceptor degeneration. Invest. Ophthalmol. Vis. Sci. 44, 818–825.

Fischer, A.J., Reh, T.A., 2000. Identification of a proliferating marginal zone of retinal progenitors in postnatal chickens. Dev. Biol. 220, 197–210.

Fischer, A.J., Reh, T.A., 2001. Muller glia are a potential source of neural regeneration in the postnatal chicken retina. Nat. Neurosci. 4, 247–252.

Ghosh, F., Arner, K., Ehinger, B., 1998. Transplant of full-thickness embryonic rabbit retina using pars plana vitrectomy. Retina 18, 136–142.

Ghosh, F., Bruun, A., Ehinger, B., 1999. Graft-host connections in long-term full-thickness embryonic rabbit retinal transplants. Invest. Ophthalmol. Vis. Sci. 40, 126–132.

Gouras, P., Du, J., Kjeldbye, H., Yamamoto, S., Zack, D.J., 1994. Long-term photoreceptor transplants in dystrophic and normal mouse retina. Invest. Ophthalmol. Vis. Sci. 35, 3145–3153.

Gouras, P., Tanabe, T., 2003. Survival and integration of neural retinal transplants in rd mice. Graefes Arch. Clin. Exp. Ophthalmol. 241, 403–409.

Humayun, M.S., de Juan Jr, E., del Cerro, M., Dagnelie, G., Radner, W., Sadda, S.R., Constancia Del, C., 2000. Human neural retinal transplantation. Invest. Ophthalmol. Vis. Sci. 41, 3100–3106.

Humayun, M.S., de Juan Jr, E., Weiland, J.D., Dagnelie, G., Katona, S., Greenberg, R., Suzuki, S., 1999a. Pattern electrical stimulation of the human retina. Vision Res. 39, 2569–2576.

Humayun, M.S., Prince, M., de Juan Jr, E., Barron, Y., Moskowitz, M., Klock, I.B., Milam, A.H., 1999b. Morphometric analysis of the extramacular retina from postmortem eyes with retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci. 40, 143–148.

Jones, B.W., Watt, C.B., Frederick, J.M., Baehr, W., Chen, C.K., Levine, E.M., Milam, A.H., Lavail, M.M., Marc, R.E., 2003. Retinal remodeling triggered by photoreceptor degenerations. J. Comp. Neurol. 464, 1–16.

Kaplan, H.J., Tezel, T.H., Berger, A.S., Wolf, M.L., Del Priore, L.V., 1997. Human photoreceptor transplantation in retinitis pigmentosa. A safety study. Arch. Ophthalmol. 115, 1168–1172.

Kwan, A.S., Wang, S., Lund, R.D., 1999. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. Exp. Neurol. 159, 21–33.

Lakhanpal, R.R., Yanai, D., Weiland, J.D., Fujii, G.Y., Caffey, S., Greenberg, R.J., de Juan Jr, E., Humayun, M.S., 2003. Advances in the development of visual prostheses. Curr. Opin. Ophthalmol. 14, 122–127.

Li, T., Sandberg, M.A., Pawlyk, B.S., Rosner, B., Hayes, K.C., Dryja, T.P., Berson, E.L., 1998. Effect of vitamin A supplementation on rhodopsin mutants threonine-17 → methionine and proline-347 → serine in transgenic mice and in cell cultures. Proc. Nat. Acad. Sci. USA 95, 11933–11938.

Marc, R.E., Jones, B.W., Watt, C.B., Strettoi, E., 2003. Neural remodeling in retinal degeneration. Prog. Retin. Eye Res. 22, 607–655.

Margalit, E., Maia, M., Weiland, J.D., Greenberg, R.J., Fujii, G.Y., Torres, G., Piyathaisere, D.V., O'Hearn, T.M., Liu, W., Lazzi, G., Dagnelie, G., Scribner, D.A., de Juan Jr, E., Humayun, M.S., 2002. Retinal prosthesis for the blind. Surv. Ophthalmol. 47, 335–356.

McGinnis, J.F., Stepanik, P.L., Chen, W., Elias, R., Cao, W., Lerious, V., 1999. Unique retina cell phenotypes revealed by immunological

analysis of recoverin expression in rat retina cells. J. Neurosci. Res. 55, 252–260.

McGinnis, J.F., Stepanik, P.L., Jariangprasert, S., Lerious, V., 1997. Functional significance of recoverin localization in multiple retina cell types. J. Neurosci. Res. 50, 487–495.

Mohand-Said, S., Deudon-Combe, A., Hicks, D., Simonutti, M., Forster, V., Fintz, A.C., Leveillard, T., Dreyfus, H., Sahel, J.A., 1998. Normal retina releases a diffusible factor stimulating cone survival in the retinal degeneration mouse. Proc. Nat. Acad. Sci. USA 95, 8357–8362.

Mohand-Said, S., Hicks, D., Dreyfus, H., Sahel, J.A., 2000. Selective transplantation of rods delays cone loss in a retinitis pigmentosa model. Arch. Ophthalmol. 118, 807–811.

Mohand-Said, S., Hicks, D., Simonutti, M., Tran-Minh, D., Deudon-Combe, A., Dreyfus, H., Silverman, M.S., Ogilvie, J.M., Tenkova, T., Sahel, J., 1997. Photoreceptor transplants increase host cone survival in the retinal degeneration (rd) mouse. Ophthalmic. Res. 29, 290–297.

Pacione, L.R., Szego, M.J., Ikeda, S., Nishina, P.M., McInnes, R.R., 2003. Progress toward understanding the genetic and biochemical mechanisms of inherited photoreceptor degenerations. Ann. Rev. Neurosci. 26, 657–700.

Plantner, J.J., Hara, S., Kean, E.L., 1982. Improved, rapid immunoassay for rhodopsin. Exp. Eye Res. 35, 543–545.

Radner, W., Sadda, S.R., Humayun, M.S., Suzuki, S., Melia, M., Weiland, J., de Juan Jr, E., 2001. Light-driven retinal ganglion cell responses in blind rd mice after neural retinal transplantation. Invest. Ophthalmol. Vis. Sci. 42, 1057–1065.

Radtke, N.D., Aramant, R.B., Seiler, M., Petry, H.M., 1999. Preliminary report: indications of improved visual function after retinal sheet transplantation in retinitis pigmentosa patients. Am. J. Ophthalmol. 128, 384–387.

Radtke, N.D., Aramant, R.B., Seiler, M.J., Petry, H.M., Pidwell, D.J., 2004. Vision change after sheet transplant of fetal retina with RPE to a Retinitis Pigmentosa patient. Arch. Ophthalmol. 122, in press.

Radtke, N.D., Seiler, M.J., Aramant, R.B., Petry, H.M., Pidwell, D.J., 2002. Transplantation of intact sheets of fetal neural retina with its retinal pigment epithelium in retinitis pigmentosa patients. Am. J. Ophthalmol. 133, 544–550.

Sagdullaev, B.T., Aramant, R.B., Seiler, M.J., Woch, G., McCall, M.A., 2003. Retinal transplantation-induced recovery of retinotectal visual function in a rodent model of retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci. 44, 1686–1695.

Sanyal, S., Bal, A.K., 1973. Comparative light and electron microscopic study of retinal histogenesis in normal and rd mutant mice. Z. Anat. Entwicklungsgesch. 142, 219–238.

Seiler, M.J., Aramant, R.B., 1998. Intact sheets of fetal retina transplanted to restore damaged rat retinas. Invest. Ophthalmol. Vis. Sci. 39, 2121–2131.

Seiler, M.J., Aramant, R.B., Ball, S.L., 1999. Photoreceptor function of retinal transplants implicated by light-dark shift of S-antigen and rod transducin. Vision Res. 39, 2589–2596.

Silverman, M.S., Hughes, S.E., 1989. Photoreceptor transplantation in inherited and environmentally induced retinal degeneration: anatomy, immunohistochemistry and function. Prog. Clin. Biol. Res. 314, 687–704.

Silverman, M.S., Hughes, S.E., Valentino, T.L., Liu, Y., 1992. Photoreceptor transplantation: anatomic, electrophysiologic, and behavioral evidence for the functional reconstruction of retinas lacking photoreceptors. Exp. Neurol. 115, 87–94.

Siminoff, R., Schwassmann, H.O., Kruger, L., 1966. An electrophysiological study of the visual projection to the superior colliculus of the rat. J. Comp. Neurol. 127, 435–444.

Strettoi, E., Pignatelli, V., 2000. Modifications of retinal neurons in a mouse model of retinitis pigmentosa. Proc. Nat. Acad. Sci. USA 97, 11020–11025.

Strettoi, E., Pignatelli, V., Rossi, C., Porciatti, V., Falsini, B., 2003. Remodeling of second-order neurons in the retina of rd/rd mutant mice. Vision Res. 43, 867–877.

Thomas, B.B., Seiler, M.J., Sadda, S.R., Aramant, R.B., 2004. Superior colliculus responses to light-preserved by transplantation in a slow degeneration rat model. Exp. Eye Res. 79, 29–39.

Tropepe, V., Coles, B.L., Chiasson, B.J., Horsford, D.J., Elia, A.J., McInnes, R.R., van der Kooy, D., 2000. Retinal stem cells in the adult mammalian eye. Science 287, 2032–2036.

Vollrath, D., Feng, W., Duncan, J.L., Yasumura, D., D'Cruz, P.M., Chappelow, A., Matthes, M.T., Kay, M.A., LaVail, M.M., 2001. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. Proc. Nat. Acad. Sci. USA 98, 12584–12589.

Weiland, J.D., Humayun, M.S., Dagnelie, G., de Juan Jr, E., Greenberg, R.J., Iliff, N.T., 1999. Understanding the origin of visual percepts elicited by electrical stimulation of the human retina. Graefes Arch. Clin. Exp. Ophthalmol. 237, 1007–1013.

Woch, G., Aramant, R.B., Seiler, M.J., Sagdullaev, B.T., McCall, M.A., 2001. Retinal transplants restore visually evoked responses in rats with photoreceptor degeneration. Invest. Ophthalmol. Vis. Sci. 42, 1669–1676.

Zhang, Y., Arner, K., Ehinger, B., Perez, M.T., 2003. Limitation of anatomical integration between subretinal transplants and the host retina. Invest. Ophthalmol. Vis. Sci. 44, 324–331.