Title
Retinal Transplantation–Induced Recovery of Retinotectal Visual Function in a Rodent Model of Retinitis Pigmentosa

Journal
Investigative Ophthalmology & Visual Science, 44(4)

ISSN
0146-0404

Authors
Sagdullaev, Botir T
Aramant, Robert B
Seiler, Magdalene J
et al.

Publication Date
2003-04-01

DOI
10.1167/iovs.02-0615

Peer reviewed

Retinal Transplantation–Induced Recovery of Retinotectal Visual Function in a Rodent Model of Retinitis Pigmentosa

Botir T. Sagdullaev,[1] *Robert B. Aramant,*[2,3,4] *Magdalene J. Seiler,*[2,3,4] *Gustaw Woch,*[5] *and Maureen A. McCall*[1,2]

Purpose. To map the spatiotemporal decline in retinally driven activity in the superior colliculus (SC) of transgenic S334ter-line-3 rats that express a mutated rhodopsin, which causes photoreceptor degeneration. To determine whether transplantation of fetal retinal sheets into the subretinal space of these rats can recover visual activity in the SC.

Methods. A visual stimulus was presented to the eye, and responses were recorded across the SC of untreated S334ter-line-3 rats aged 28 to 288 days. These data were used to draw a map of the developing scotoma. Intact retinal sheets from embryonic day 19 rats were transplanted into the subretinal space of S334ter-line-3 rats between 21 and 28 days of age. Responses to retinal stimulation were mapped in the SC of transplanted and sham control rats 78 to 163 days after surgery. The morphology of the retinas in all groups was examined.

Results. Photoreceptor cell loss in untreated rats matched the decline in visual activity in the SC. At 28 days, there was a scotoma in the area of the SC that represents the central retina and, by 63 days, it had enlarged to cover the entire retinal representation. Visual responses were evoked in 64% of rats with retinal transplants. These retinally driven responses were confined to a small, contiguous region of the SC that represents the sector of the retina where the transplant was placed. Visual responses were absent in the SC outside this area in transplant recipients and throughout the SC of untreated and sham control rats.

Conclusions. Transplantation of fetal retinal sheets induced recovery of visual activity in the SC in this model of RP. The mechanisms underlying this functional recovery remain to be resolved, but these results suggest that transplantation should be further explored as a therapy for RP. (*Invest Ophthalmol Vis Sci.* 2003;44:1686–1695) DOI:10.1167/iovs.02-0615

From the Departments of [1]Psychological and Brain Science, [2]Ophthalmology and Visual Science, and [3]Anatomical Science and Neurobiology, University of Louisville, Louisville, Kentucky; and the [5]Department of Biology, Temple University, Philadelphia, Pennsylvania.

[4]Current affiliation: Doheny Eye Institute, Department of Ophthalmology, University of Southern California, Los Angeles, California.

Supported by NIH Grant EY08519; the Foundation Fighting Blindness; the Murray Foundation Inc.; the Vitreoretinal Research Foundation, Louisville, KY; the Kentucky Lions Eye Foundation; a grant from the Research to Prevent Blindness; and funds from an anonymous sponsor.

Submitted for publication June 20, 2002; revised October 21, 2002; accepted October 24, 2002.

Disclosure: **B.T. Sagdullaev**, None; **R.B. Aramant**, (P); **M.J. Seiler**, (P); **G. Woch**, None; **M.A. McCall**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Maureen A. McCall, Department of Psychological and Brain Sciences, University of Louisville, Louisville, KY 40292; mo.mccall@louisville.edu.

Retinitis pigmentosa (RP) is a family of inherited retinal degenerations that leads to loss of vision.[1] Anatomically, RP is a progressive decrease in the number of photoreceptor cell nuclei[2,3] with an accompanying loss of the visual field, which ultimately leads to blindness.[1] Although the phenotype is similar across most forms of RP, the underlying cellular mechanisms are diverse and result from various mutations in many genes.[4–7] Most involve mutations that alter the expression of photoreceptor-cell–specific genes, with mutations in the rhodopsin gene accounting for approximately 10% of these.[8] In other forms of the disease, the regulatory genes of apoptosis are altered (for example, *Bax* and *Pax2*).[9,10]

Rodent models have been developed for many of these forms of RP,[11,12] and a number of treatment strategies are in development that attempt to preserve visual function by delaying or arresting photoreceptor degeneration. One focus is on the development of gene therapies, such as the use of antisense oligonucleotides or ribozymes.[7,13,14] These specific gene therapies, although promising, are limited to a particular gene defect and thus probably will be developed for only the most common forms of RP. Furthermore, they must be used before degeneration has progressed to a stage of significant vision loss, and that may not always be feasible. More general approaches to slow or arrest cell death involve transplantation of RPE or other supporting cells[15,16] or administering or inducing the expression of survival factors[17–21] or apoptotic inhibitors.[22,23] However, several recent studies, using adenoassociated virus (AAV)–mediated delivery of growth factors,[24–26] although producing a delay in the loss of photoreceptor nuclei, fail to correlate the morphologic rescue with a maintenance of visual function, as measured by the ERG.[17,25,26]

Replacement of degenerated retinal cells by transplantation of neural retina into the subretinal space is a third therapeutic strategy, which should be applicable to any type of RP and at later stages of degeneration (for reviews see Lund et al.[15] Mohand-Said et al.,[16] and Aramant and Seiler.[27]). Some transplantation approaches involve either aggregates of or dissociated fetal retinal cells[28–32] or intact sheets of fetal retina.[27,33–38] The transplanted neural cells differentiate in the subretinal space,[28–34,39] and their photoreceptors express visual transduction cascade proteins that are modulated by light.[35,40,41] Both morphologic and functional assessments of the effects of transplantation have been made. Some studies have focused on the preservation of rod and/or cone photoreceptors in the dystrophic retina and the identification of trophic factors that arrest or delay their degeneration.[16,20,42–44] Other studies have attempted to determine whether visual activity can be either preserved or recovered by retinal transplants and have used ERG and multiunit electrophysiological and behavioral assays.[28–30,36,45] We have demonstrated that the presence of sheets of fetal retinal transplants correlates with the presence of visually driven activity in the SC of the Royal College of Surgeons (RCS) rat,[36] a model of a rare form of human RP.[46] Because the decline in visually driven activity in the RCS rat is slow and its progression is not strictly from the

central to the peripheral retina,[47] our experiments could not distinguish between transplant-induced maintenance or recovery of visual activity.[36] The experiments presented in the current study were designed to address this question in a different rodent model of human RP, the S334ter-line-3 transgenic rat that expresses an altered human rhodopsin protein (Steinberg RH, Flannery JG, Naash M, ARVO Abstract 3190, 1996). To this end, we mapped retinally evoked activity in the SC of untreated S334ter-line-3 rats at the age of transplantation and found that visually driven responses were absent in the SC within the area that represents transplant placement. Three to 5 months after transplant surgery, retinally evoked visual responses were recordable in most of the rats, in a discrete region of the SC that corresponds topographically to the location of the transplant in the host retina. In contrast, no visually driven activity was evoked anywhere in the SC of age-matched S334ter-line-3 rats with sham surgery, or outside this region, or anywhere in the opposite SC of rats with transplants. Thus, the presence of the transplant induces recovery of visual activity in the SC, a structure that is essential in integrating sensory motor function.[48] If this visual activity can be shown to mediate visual behavior in rodent RP models, then transplantation of intact retinal sheets may prove useful as a therapeutic approach in patients with a variety of forms of RP.

METHODS

In all experimental procedures, the animals were treated according to the regulations in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in compliance with a protocol approved by the University of Louisville. Most of the experimental procedures have been described in detail elsewhere.[33,35,36]

Experimental Animals

Homozygous founder breeding pairs of S334ter-line-3 rats were produced by Chrysalis DNX Transgenic Sciences (Princeton, NJ). Homozygous S334ter-line-3 rats were bred with normal Copenhagen rats (Harlan, Indianapolis, IN) to produce the heterozygous pigmented offspring that were used in all the experiments.

Transplant Tissue Preparation and Transplantation and Sham Surgical Procedures

Donor retinal tissue was obtained from pigmented Long-Evans rat fetuses at embryonic days 19 and 20. The retina was dissected from the surrounding tissues, including the RPE, cut to approximately 0.5 mm^2 and placed into the nozzle of a custom-made implantation device. A small incision (~1 mm) was cut in the pars plana of the host eye, and the implantation device was used to place the transplant in vehicle solution into the subretinal space, in the superior nasal quadrant in all eyes. The use of this device provides not only consistent transplant placement, but also ensures the apposition of the transplant ganglion cell layer to the host retina. The sham surgical procedure was identical with that used in the transplantation, with the exception that only vehicle solution was delivered from the tool into the subretinal space at a similar location and depth.

Electrophysiology

For electrophysiological assessment of visual responses in the SC, animals were anesthetized by intraperitoneal injection of xylazine and ketamine (37.5 mg/kg and 5 mg/kg, respectively). Gas inhalant anesthetic (1.0%–2.0% halothane in 40% O_2/60% N_2O) was administered through a tracheotomy. Rats were mounted in a stereotaxic apparatus, a craniotomy was performed, and both hemispheres of the SC were exposed. Multiunit responses were recorded extracellularly from the superficial laminae of the SC using parylene-coated tungsten microelectrodes (World Precision Instruments, Sarasota, FL) with tip resistances of 1.0 to 1.5 MΩ. Visual responses were assessed in each rat at recording sites 200 μm apart (approximately 72 sites), forming a regular grid across the full extent of the dorsal surface of the SC. In each rat, 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 or 32 presentations of the visual stimulus, a full-field strobe flash (1300 cd-s/m^2, 100-μs duration; Model PS 22 Photic stimulator; Grass Instruments, West Warwick, RI). We used 32 repetitions of the stimulus in all rats with transplants and in the untreated and sham control rats and 16 repetitions in the normal rats. The stimuli were delivered to the eye with the transplant at a 5-second interstimulus interval, all activity was recorded 100 ms before and 500 ms after the onset of the stimulus, and all responses at each site were averaged. Blank (control) trials consisted of the presentation of the visual stimulus with the eye occluded and were recorded at every visually responsive site. Once the response was characterized, the electrode was raised and moved 200 μm to continue to map activity across the surface of the SC. If no visual response was found at a depth of 100 μm, the electrode was lowered through the SC, and activity assessed until a visual response was encountered or until a depth of 900 μm was reached. In practice, however, if no visual response was recorded at the initial depth, it was rare to obtain one deeper.

TABLE 1. Overview of Experimental Animals

Experimental Group	*n*	Age at Surgery (d)	Age at Recording (d)
Control			
Normal pigmented rats	8		84–168
Untreated S334ter-line-3 rats	5		28–29
	4		32–36
	7		39–50
	4		63–65
	5		99–288
S-antigen immunohistochemistry	2		99–119
Sham surgery (S334ter-line-3 rats)	7	21–26	108–297
S-antigen immunohistochemistry	4	25	108–136
Transplants (S334ter-line-3 rats)			
With visual responses	1	21	104
	4	25	103–140
	1	26	189
	1	28	171
S-antigen immunohistochemistry	4	21–28	103–139
Without visual responses	4	21–26	105–301

Experimental Design

Table 1 outlines the ages at surgery and recording and numbers of rats in each experimental and control group. Electrophysiological recordings were made in untreated S334ter-line-3 rats at five different ages to describe the spatial and temporal characteristics of the decline in visual activity in the SC. Recordings also were made in the SC of S334ter-line-3 rats with retinal transplants or in age-matched sham surgery and normal pigmented Long-Evans rats. All animals with transplants or sham surgery were coded, and the experiments were randomized so that the experimenters were blind to the rat's experimental condition. The codes were broken only after all experiments were completed. Untreated transgenic rats were not included in this randomized design because most (n = 20) were younger (65 days), smaller, and identifiable among the rats with transplants or sham surgery. Normal rats were part of a different randomized design, in which we evaluated transplants in RCS rats[36] and, which overlapped with these experiments. Their data served as the control in both studies.

Data Analysis

At every location, the response to each presentation of the stimulus was examined, and an average response was computed. Several response metrics were determined from these original response traces at each visually responsive location. The onset of the visual response was defined as the point at which a clear, prolonged (>20 ms) increase in light-evoked activity was measurable above background, which was determined from the activity in the 100 ms preceding the light flash. The consistency of the response onset was defined as the standard deviation of the response onset latencies within each trial. Peak response amplitude was defined as the maximum excursion of the response.

For each metric at every visually responsive site, a mean and standard deviation were computed. A mean and standard deviation also were computed over all visually responsive sites in each animal, and group means and standard deviations were computed from the means of individual animals. All statistical comparisons were performed using group means and standard deviations. Because no visual responses were recorded in any age-matched, untreated, or sham-surgery S334ter-line-3 rat, two-tailed Student's *t*-tests with a criterion of $P < 0.01$ were used to compare differences between each response metric in S334ter-line-3 rats with successful transplants and normal rats. Because the variances in the response metrics were different between the two groups (see Figs. 4B, 4C), we performed all the *t*-tests with the assumption of unequal variances. Regression analyses were performed for the mean response latency and the peak amplitude as a function of age, and the slopes of the regression lines from transplanted S334ter-line-3 and normal rats were compared using *t*-tests.

Histology

Tissue Processing. At the end of each experiment, eyes were either immersed in Bouin fixative, or animals were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M Na-phosphate buffer. The eyes were removed and subsequently either embedded in paraffin or frozen in optimal temperature cutting compound (Tissue Tek; Sakura FineTek, Torrance, CA). Transverse sections of the retina were cut, mounted onto slides, and stained with hematoxylin-eosin (H&E). A series of sections through the full extent of the transplant were evaluated at the light microscopic level.

S-antigen Immunohistochemistry. In normal animals, S-antigen recognizes both rod photoreceptors and a subpopulation of blue cone photoreceptors.[49] We used S-antigen immunoreactivity in paraffin sections of retinas from transplant-recipient and untreated S334ter-line-3 rats and those with sham surgery to assess the presence of residual photoreceptors. In rats with transplants, we used the same method to assess the presence of photoreceptors in the transplanted retina. Deparaffinized sections were washed with phosphate-buffered saline and incubated for 30 minutes in 20% horse serum. The sections were incubated with a mouse monoclonal antibody against S-antigen (clone A9C6[50]) at a dilution of 1:20,000 overnight at 4°C and the presence of the primary antibody was detected using an avidin-biotin complex kit for mouse antibodies (Elite ABC; Vector Laboratories, Burlingame, CA).

Results

Photoreceptor Degeneration in S334ter-line-3 Rats

The time course of photoreceptor degeneration was determined qualitatively by examining the morphology of the outer retina (outer nuclear [ONL] and outer plexiform [OPL] layers) of untreated S334ter-line-3 rats between postnatal day (P)11 and P264. Figure 1 shows representative photomicrographs of transverse sections of central and peripheral retina from S334ter-line-3 rats between P11 and P28. Sections from both areas show a progressive loss of photoreceptors in the ONL, with degeneration more advanced in central retina at all ages (P11–P18). At P11, the thickness of the ONL in S334ter-line-3 rats appeared similar to normal; however, pyknotic nuclei were evident in central retina. By P14, the ONL of S334ter-line-3 rats was noticeably thinner in both the central and peripheral retina, and a further loss of photoreceptor cells was evident through P28. At P28, the ONL of S334ter-line-3 retinas consisted of one (central) or two (peripheral) rows of photoreceptor cell bodies that never developed inner or outer segments. With increasing age (through P263), the number of cell bodies in the ONL of S334ter-line-3 rats decreased further, and the layer became indistinct.

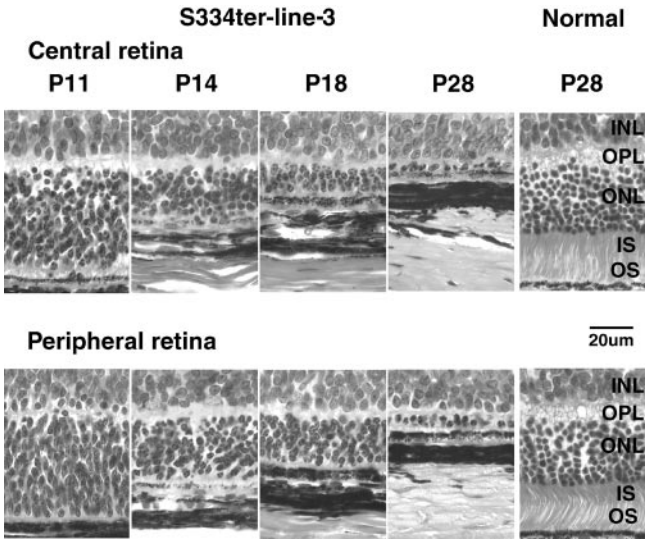

Figure 1. Time course of photoreceptor degeneration in pigmented S334ter-line-3 rats. Images of representative transverse sections of H&E-stained retinas from S334ter-line-3 rats at four postnatal ages and a normal pigmented rat at 28 days. Sections at each age from central and peripheral retina show that photoreceptor degeneration is more advanced centrally. OS, photoreceptor outer segments; IS, photoreceptor inner segments; INL, inner nuclear layer. Scale bar, 20 μm.

Visual Field and Photoreceptor Loss in the SC in S334ter-line-3 Rats

Multiunit electrophysiological recordings in the SC were used to assess the functional state of the retina in control rats (Fig. 2) and in rats with transplants (Fig. 3). The SC was chosen, because it receives a direct retinal input that is topographically organized so that each SC location represents input from a particular part of the retina.[51] At each recording site, responses to every presentation of the stimulus were evaluated and averaged, and a diagram of the visual response latency was constructed over the surface of the SC, using shading to indicate the mean values (Figs. 2, 3, see legends). A summary diagram of the SC in all rats within a group also was constructed from the average onset latency at each site. Sites in which responses had the shortest mean onset latencies (<40 msec) are illustrated in white, whereas locations where activity could not be distinguished from background are shown in black. Control traces (Figs. 2A, 3D) represented the response evoked by presenting the visual stimulus with the eye occluded and indicate that the visually evoked responses were not caused by an unintended auditory or other stimulus cue.

Figure 2 illustrates mean visual latencies across the SC from all control groups: normal rats (Fig. 2A), untreated transgenic rats of various ages (Fig. 2B–D), and transgenic rats after sham surgery (Fig. 2E). In normal rats, responses in all areas of the SC had short visual latencies, although longer latency responses

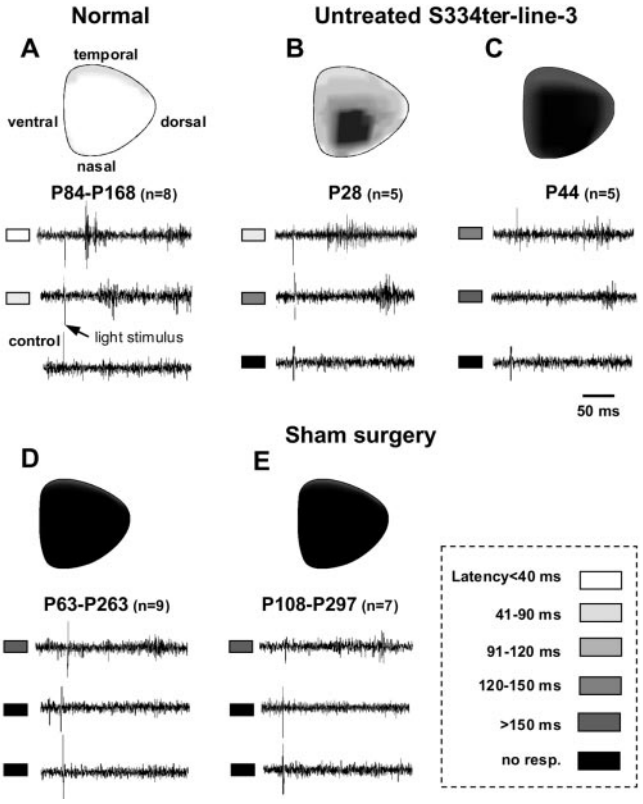

FIGURE 2. Spatiotemporal changes in the mean latency of visual responses in the SC of untreated S334ter-line-3 rats compared with normal and sham-surgery rats. Average maps represent the mean visual latency of all responses in the contralateral SC in control rats: (**A**) normal, (**B–D**) untreated S334ter-line-3 rats at postnatal ages (P28, P44, and P63 or more), and (**E**) sham-surgery rats. Different gray-scale shading on the maps represents increasingly longer latencies and black represents areas with no visual response. The legend indicates the range of latencies represented by shading. no resp., no response. Traces *below* each map illustrate a representative response recorded from one location in one animal in that group. The control trace shows a single sweep that was recorded within a visually responsive area with the light stimulus triggered but the eye occluded.

were found along the rostral edge of the SC. The spatial and temporal decline in visual activity in untreated S334ter-line-3 rats is depicted in maps of animals grouped by age (P28 and P263). At P28 (Fig. 2B), the earliest age recorded, each of the five S334ter-line-3 rats already had a relatively large central scotoma (shaded black) that lacked visual, but not spontaneous, activity. In addition, responses in the SC representing progressively more peripheral retinal locations had increasingly longer mean latencies (70–150 ms). By P44 (Fig. 2C), only visually evoked responses with longer mean latencies (120 to >150 ms) were recordable and were localized along the lateral and rostral borders of the SC, which represent the peripheries of the temporal and dorsal retina. From P63 onward, residual activity was found only on the rostral edge of the SC (Fig. 2D). This decline in visually evoked activity in untreated S334ter-line-3 rats paralleled the degeneration of their photoreceptors spatially, with the central retina more advanced than the periphery. Thus, stimulation of the retina at the time of transplantation (P28), when only cone photoreceptor nuclei are present (Fig. 1) was ineffective in driving visual responses in the SC. The seven age-matched rats with sham surgery showed a pattern of activity that was indistinguishable from that in older untreated transgenic rats (Fig. 2E)—for example, only residual visual activity was recordable at the rostral edge of the SC. Individual maps of the SC in every animal within each control group showed the same pattern as the average map.

Effect of Transplantation on Recovery of Visual Response in the SC of S334ter-line-3 Rats

S334ter-line-3 rats with transplants were divided into two distinct groups, based on assessments of visually driven activity in the SC. In 64% (7/11) of the rats, visually evoked responses could be elicited, and for simplicity we refer to these as rats with successful transplants. In the other 36% (4/11), only residual visually evoked responses were recorded on the rostral edge of the SC (data not shown), which was identical with that seen in untreated rats and rats with sham surgery (Figs. 2D, 2E). In six of the seven rats with successful transplants, recordings were made from alternating symmetrical locations in the two SCs. As is shown in one of the rats (Fig. 3A), all rats had visually evoked responses only in the contralateral SC, which receives input from the eye that received the transplant. In the ipsilateral SC, which receives input from the control eye there were no visually evoked responses, although spontaneous activity was recorded.

In the contralateral SC, all visually evoked responses were located in areas corresponding to the location of the transplant in the superior nasal retinal quadrant. Figures 3B–D show responses in the contralateral SC from three other rats with successful transplants at increasing posttransplantation times. Figure 3E is an enlarged map that represents the average response latencies in all seven rats with successful transplants and Figure 3F shows the location of each of the 20 sites from which visual activity was recorded.

In each of the rats with successful transplants, we found a discrete area in the caudal–lateral SC where retinal stimulation evoked visual responses. In each rat, there was an average of 2.9 visually responsive sites (range: two to four sites), representing approximately 4% of the sites sampled across the SC. These responsive sites always formed a continuous region and were surrounded by sites that were not visually responsive. We compared locations of the individually responsive regions in all the rats with successful transplants with the location of the visual scotoma in untreated transgenic rats at the age of transplantation and found that all were contained within its borders (Fig. 3F, dashed area).

In addition to the onset latency of the visually evoked responses, we measured the consistency of this latency from trial to trial and the peak amplitude of each visual response. Mean results were calculated for each retinal site, each animal, and each experimental group. Figure 4A plots the average response onset latency as a function of average peak amplitude for every responsive site in the SC of rats with successful transplants and at comparable sites in normal rats. The average onset latency was significantly faster in normal rats than in rats with successful transplants (33 ± 3 ms vs. 108 ± 34 ms, respectively; $P < 0.001$) and there was no overlap in these data. The onset latency was also more consistent in normal rats (2.5 ± 1.5 ms vs. 8.8 ± 4.2 ms, respectively; $P < 0.001$). In contrast, there was no significant difference in the mean peak response amplitude between the two groups (125 ± 32 mV vs. 133 ± 18 mV; $P > 0.88$).

To examine changes in transplant-induced visual activity as a function of age of the transplant, we plotted both the mean peak response (Fig. 4B) and mean onset latency (Fig. 4C) of each transplant-recipient and normal rat as a function of age and fitted linear regression lines to these data. Although the slopes of the lines through the data from the two groups are not identical for either metric, *t*-tests showed no statistical differences ($P > 0.12$ and $P > 0.25$, respectively). Although the

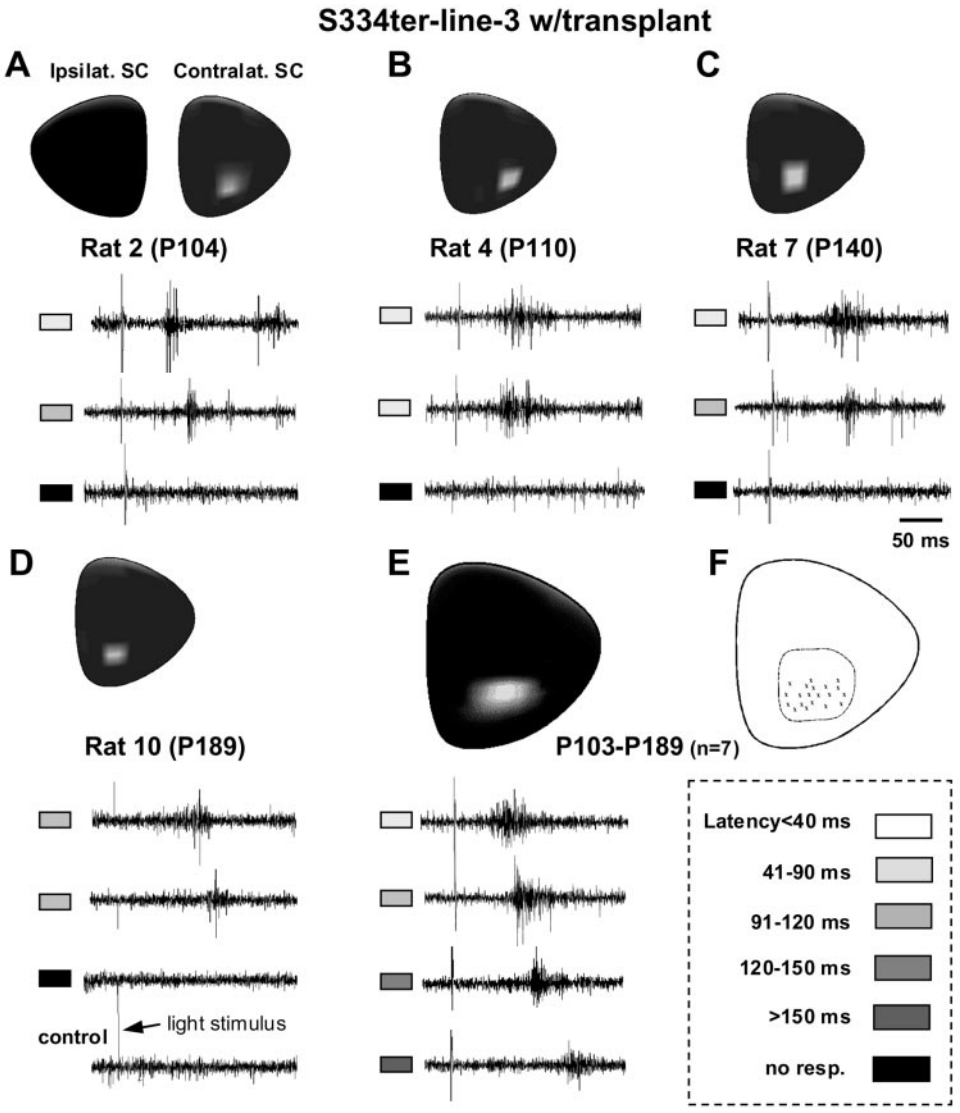

FIGURE 3. Areas of visually evoked activity are found in the SC of S334-ter-line-3 rats with successful transplants. Different degrees of shading depict differences in the mean visual latencies on the maps of the contralateral SC of four individual rats with successful transplants of increasing postsurgical age (**A–D**). Enlarged maps show the average over all seven rats (**E**) and the individual locations of every visually responsive site recorded (**F**). *Dasbed line* in (**F**) outlines the area of the SC from which no visual responses could be evoked in untreated rats at the age of transplantation (P28). In most rats, recordings alternated between the contralateral and ipsilateral SCs, shown in (**A**). The legend and other conventions are the same as in Fig 2.

number of rats at each age was somewhat limited, these data indicate that the response properties of the rats with transplants were not different from those of normal rats over time.

Histologic Evaluations

To evaluate the organization of the transplants, we examined the morphology of the retinas in transverse H&E-stained sections through the transplant and adjacent host tissue (Fig. 5). Retinal transplants exhibited a range of organizations that varied from well laminated (Figs. 5A, 5B; 6A) to transplants containing rosettes (Fig. 5C) and inverted transplants (Fig. 5D). In most S334ter-line-3 rats that exhibited visually evoked responses, the transplants maintained normal laminar morphology, containing all cellular and synaptic layers, and the photoreceptors had inner and outer segments (Figs. 5A, 5B, 6A). Of the four rats with transplants but no visual responses, the transplants had poor lamination patterns and contained only scattered photoreceptor cells (Fig. 5D). Because the restoration of visual responsiveness in the SC could result from photoreceptor rescue,[20,43,44] we assessed the presence of the remaining photoreceptors in the host and control S334ter-line-3 retinas and the photoreceptors in the transplant in retinal sections of S334ter-line-3 rats with successful transplants, for their immunoreactivity to S-antigen (Fig. 6). In the host retinas of transplant-recipient S334ter-line-3 rats (Fig. 6A), in the retinas of untreated S334ter-line-3 rats (Fig. 6B), and in those with sham surgery (Fig 6C), scattered cells that were S-antigen–positive were found. The density of cells reactive to S-antigen was examined qualitatively in these retinas, and there were no obvious differences among the three groups. In addition, we saw no differences in the number of S-antigen–positive cells in areas of the host retina adjoining the transplant and areas remote to it. These data suggest that, with age at transplantation and the survival times used in this study, transplant-induced photoreceptor rescue is not likely to underlie the recovery of visual activity.

DISCUSSION

Temporal and Spatial Loss of Retinal Photoreceptors in Untreated S334ter-line-3 Rats

Photoreceptor degeneration is evident in the central retina of S334ter-line-3 rats at P11, and degeneration progresses through P28 when only a single row of photoreceptors remains. In the peripheral retina, a similar pattern is seen, although the rate of photoreceptor loss is somewhat slower. Still, at P28 only a single row of photoreceptor cells remains. Similar spatial and

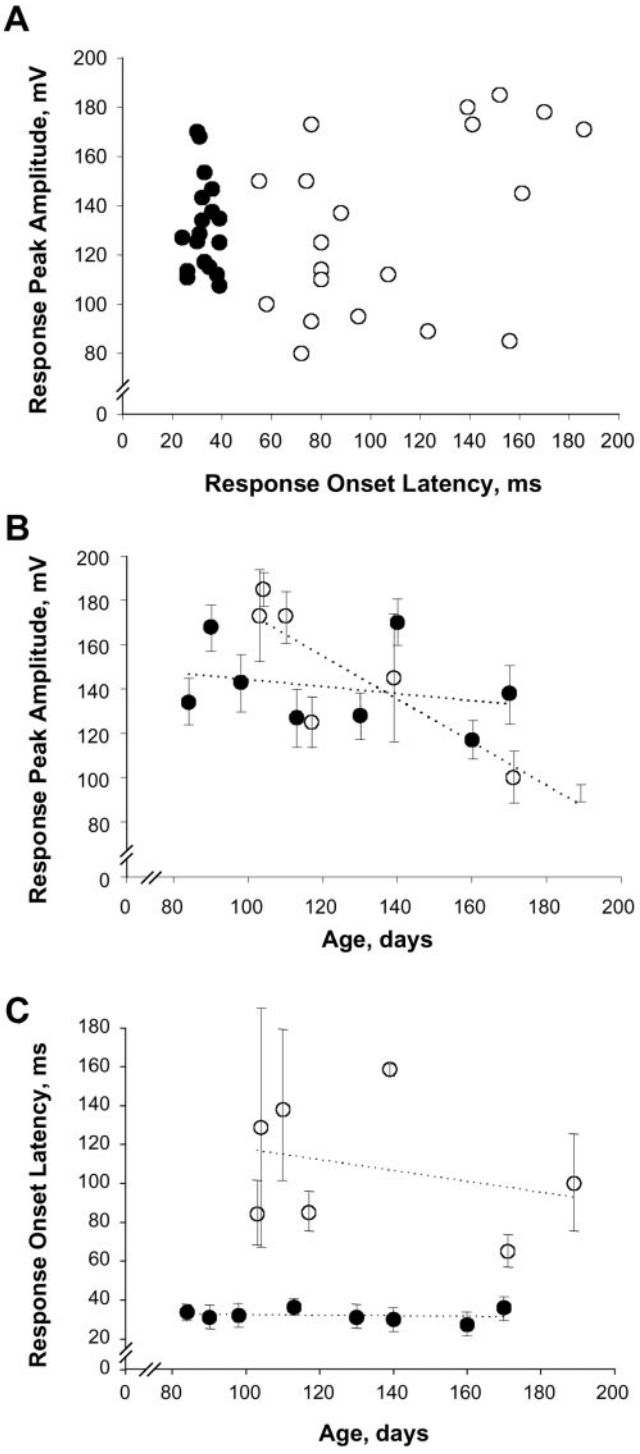

FIGURE 4. (**A**) Mean onset latency from every visually responsive SC location plotted as a function of its peak amplitude for all S334ter-line-3 rats with a successful transplant (○; $n = 20$) and every site within a topographically matched area in the SC in eight normal rats (●; $n = 19$). Mean onset latencies were significantly different between these groups, whereas no significant difference was observed in the amplitude of their peak responses. Maximum peak response (**B**) and mean response onset (**C**) as a function of age at the time of recording from each S334ter-line-3 rat with a successful transplant and each normal rat. T-bar brackets represent the maximum and minimum responses in each animal. Linear regression lines were fit to the data from each group, and *t*-tests showed no significant difference between their slopes.

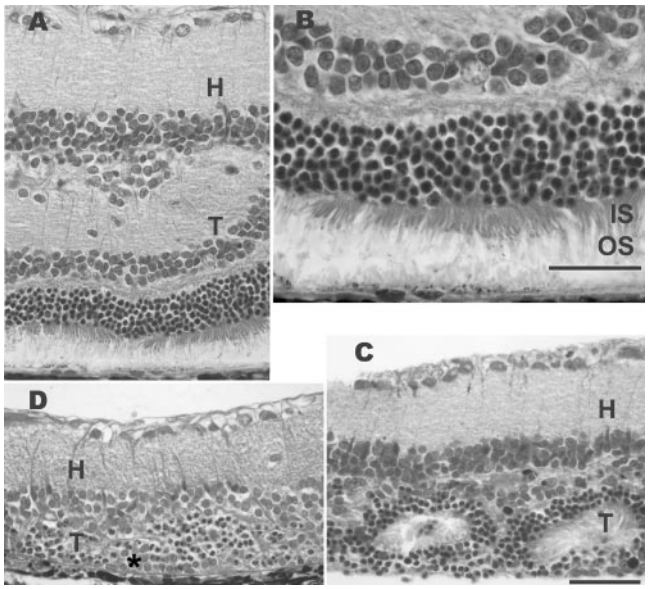

FIGURE 5. Organization of retinal transplants in S334ter-line-3 rats. (**A**) H&E-stained sections from an well-organized transplant (T) and its host retina (H), in a rat with visual responses recorded 85 days after transplantation. (**B**) A higher-power photomicrograph of a portion of this retina illustrates the normal morphology of the inner (IS) and outer (OS) segments of the photoreceptors in the transplant. (**C**) A transplant that formed rosettes within the host retina in a rat with visually evoked responses, recorded 78 days after transplantation. (**D**) An inverted transplant in a rat that had no visual responses. (✶) Displaced inner nuclear layer near the RPE. Scale bars, 30 μm.

temporal patterns of photoreceptor loss have been reported in some patients with RP,[52] as well as in at least two other models of RP: the *rd* mouse[53–55] and cats with taurine-deficiency–induced photoreceptor degeneration.[56] The rate of photoreceptor degeneration that we describe is slower than previously observed in S334ter-line-3 rats, which was essentially complete by P20.[23] The likely explanation is a difference in the pigmen-

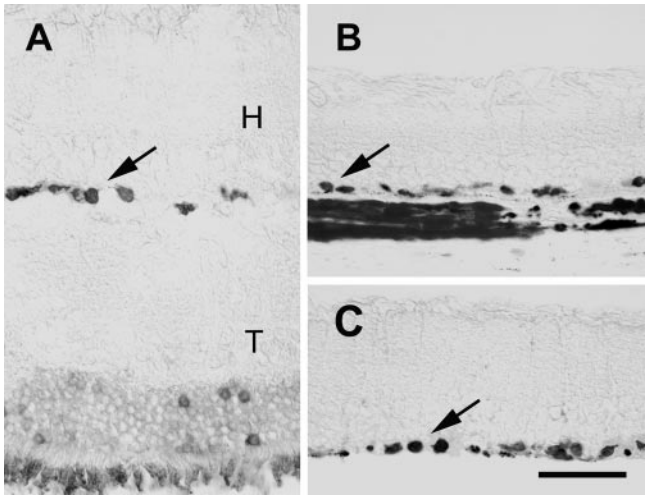

FIGURE 6. S-antigen immunoreactivity in transverse sections through the retina of an S334ter-line-3 rat (**A**) with a transplant (same retina as in Fig. 5A), an age-matched untreated S334ter-line-3 control (**B**), and an S334ter-line-3 sham-surgery control (**C**). There were no obvious differences in the number of S-antigen–positive cells (*arrows*) in the host retina adjacent to the transplant, and in S334ter-line-3 untreated and sham-surgery control rats. Scale bar, 30 μm.

tation of the rats used. The previous study examined the effect of the transgene on unpigmented rats of Sprague-Dawley background, whereas we used a pigmented Copenhagen strain. A similar effect of pigmentation on the rate of photoreceptor degeneration was observed in a transgenic mouse model of RP.[57]

Temporal and Spatial Loss of Visual Activity in the SC of S334ter-line-3 Rats

A loss of visual activity in the SC was evident at P28 and was located in the representation of the central retina. With increasing age, the scotoma expanded outward toward the lateral and caudal portions of the SC, which represent the far periphery of the retina. By P63, only residual visual activity was detectable in the representation of the far peripheral retina, and this activity was maintained through the oldest ages tested. Thus, the loss of visual activity in the SC paralleled photoreceptor loss both spatially and temporally. An identical spatiotemporal pattern of visual field loss was observed in the SC of *rd* mice,[58] which harbor a mutation in a phototransduction cascade protein.[59]

Visual Activity in the SC after Transplantation of Fetal Retinal Sheets

In 64% of the transgenic rats that received transplants, retinally driven visual responses were recordable in the SC contralateral to the eye with the transplant. This visual activity defines a discrete and contiguous region located in rostrotemporal SC that represents the superior nasal quadrant of the retina and location of the transplant. The locations of visually responsive sites were consistent across all the rats in this study and also in sites in RCS rats that received transplants in our previous study.[36] In contrast, areas of visual activity in the ipsilateral SC were absent. This was true even though identical visual stimulation and sampling techniques were used and recordings were alternated across the two hemispheres. Visually responsive areas also were absent in 4 of 11 of the rats that received transplants, age-matched transgenic rats with sham surgery, and untreated rats.

Visual Activity as a Sign of Transplant-Induced Recovery of SC Function

Transplant-induced recovery, rather than maintenance of visual responsiveness, is strongly suggested by these data for a number of reasons. First, untreated S334ter-line-3 rats at the age of retinal transplantation (P28) have only a single row of photoreceptor nuclei in their central retinas. Second, untreated rats at the age of transplantation showed no visual activity in the SC in locations that represent the central retina and the placement of the transplant. Third, each site of visual activity was confined to an area that was within the scotoma at the time of transplantation. Finally, no visual activity was found in any of the age-matched control rats.

Most of the characteristics of the visually evoked responses in the SC of transplant-recipient S334ter-line-3 rats were different from those in the normal rats. In particular, visual response latencies were longer and more variable, although, peak amplitudes were similar. The visual activity that we recorded in S334ter-line-3 rats was similar to the activity that we recorded in RCS rats with transplants.[36] First, the locations of visually responsive activity overlapped between the two groups, as did the average number of visually responsive sites (2.9 vs. 2.7). In addition, the distributions of both visual onset latency and consistency also overlapped between the two transplantation groups (onset latency; 79 ± 11 ms vs. 108 ± 34 ms and response consistency: 4.0 ± 2.0 ms vs. 8.8 ± 4.2 ms).

In the transplantation group, the visually evoked response latency was more than twice the latency in normal rats. A longer latency in transplant recipients may result from increased processing time through both transplanted and host retinas, although a simple increase in retinal thickness should only increase processing time by a factor of less than 2: [transplant + (host − photoreceptors)]. Mean latencies between transplants and normal retinas showed a 2.4-fold increase, which could reflect a reduction, either in the efficacy of the synaptic input or number of inputs driving the visual response. Because Radner et al.[30] report similarly long visual latencies in their retinal ganglion cell recordings, we presume that the effect is retinal and could be analogous to the increase in visual latency that is observed as stimulus intensities are reduced and approach threshold.[60] The same mechanism also could explain the difference in response consistency in transplant recipients, which also is reduced. The similarity in the peak amplitudes across the groups may reflect the all-or-none nature of action potentials. A better understanding of these alterations awaits the challenging experiments to characterize the receptive field properties of single host retinal ganglion cells that provide input to the SC of animals with transplants.

The percentage of transgenic rats with transplants that recovered visual activity is similar to the percentage we reported using the RCS rat (64% and 66%, respectively).[36] Transplantation-induced visual activity also has been reported with the use of retinal aggregates in the *rd* mouse model,[30] although the success rate was somewhat lower (3/10). This difference could be related to either the use of aggregates or the increasing difficulty of transplantation in a smaller eye.

Our qualitative anatomic comparisons between rats with successful and unsuccessful transplants suggests that one important predictor of functional outcome is the morphologic integrity of the transplant. Visual responses were always evoked when transplants were well organized and their photoreceptors were well developed. Disorganized transplants with either poor morphology or an apparent barrier with the host retina did not produce visual responses. That said, there were many transplants with intermediate morphologies and even the presence of rosettes, where there was no correlation with recovery of visual responsiveness. Although the current success rate is good, learning why some transplants show development of normal morphology and others do not is a very important avenue for further refinements in the transplantation technique.

Another important question is the permanence of transplant-induced functional recovery, and our data provide some insight into this question. Forty percent of our transplant-recipient rats (three of seven) retained visual activity for 4 months after surgery. Although a shallow decline in two response measures in rats with successful transplants was observed, it was not significantly different from that in normal controls.

Underlying Mechanisms

These experiments were designed to determine whether transplants can induce the recovery of visual function in the SC. The question of the mechanism(s) that underlie this outcome is complex and, these data speak only to a small component. We assume that in S334ter-line-3 and RCS rats, as in *rd* mice,[58] connections from retinal ganglion cells to the SC and the visual cortex are maintained long after the photoreceptors have degenerated. There are two obvious hypotheses and there is evidence to support each. In the first, light energy is transduced into a neural signal in the photoreceptors of the transplant, and this activity is relayed to the host retina and then to the SC through existing connections. In the second, the trans-

plant provides a trophic factor that recovers the ability of cone photoreceptors in the host to drive visual responses in the SC.

Support for the first hypothesis comes from the observation that in most transplant-recipient S334-ter line-3 and RCS[36] retinas, as well as in retinas with aggregate transplants,[28,30,61] the host and transplant fuse and develop an intermediate plexiform layer. In addition, processes appear to arise from cells in the transplant and cross this layer, where contacts may form (Aramant RB, Seiler MJ, Woch G, ARVO Abstract 528, 2000).[62,63] In support of the trophic factor hypothesis is the observation that transplantation and/or coculture of normal retinas with dystrophic retinas provides widespread preservation of cone photoreceptors, which would ordinarily undergo cell death.[20,43,44] In addition, the fact that both aggregates of photoreceptors and intact retinal sheets induce visual activity in dystrophic host retinas also can be interpreted as evidence in favor of a trophic influence, although different synaptic connections could occur between transplant and host in these two models. Arguing against the trophic hypothesis is that many of our transplants with intact morphology did not induce recovery of visual activity. In addition, our qualitative morphologic observations and those of others[30,36] provide no evidence of transplant-induced preservation of photoreceptor nuclei in the time frame used in the current study, although it is clear that the presence of transplanted normal photoreceptors or application of trophic factors arrest or delay cone photoreceptor degeneration in the short run.[20,42–44] Further, we (in both transgenic and RCS rats with transplants), and Radner et al.[30] observed only a localized recovery and preservation of visual activity induced by the transplant. This observation is more consistent with a mechanism that provides a local influence, rather than a transplant-induced trophic influence, which has been shown to be widespread.[20,43,44] One possibility that our data cannot address is that the transplant provides an initial short-term protective effect that is widespread and that the area shrinks with increasing postsurgical times. If this were the case, the area of localized visual activity would be driven by a small region containing the remaining recovered host cones, which may have been too small to detect with our qualitative observations. To help to discriminate between these hypotheses, both characterizations of visual activity over a wider range of postsurgical ages are necessary required, as are quantitative assessments of photoreceptor nuclei in these models and over the same range of postsurgical times. Finally, it is possible that both mechanisms work in concert to induce the recovery of visual activity. Regardless of the mechanism, our data along with those of Radner et al.[30] and Mohand-Said et al.[20] strongly suggest that retinal transplants are responsible for both recovery and/or preservation of photoreceptor nuclei and visual function.

There are at least two other possible explanations for localized visual activity. First, cells in the transplant could send axons through the host retina and its optic nerve and synapse directly in the SC. However, both we (Seiler MJ, Cuenca N, Aramant RB, Kolb, H, ARVO Abstract 3435, 2002) and others[30] note that few donor ganglion cells survive in the transplant, which means that the "axons" of amacrine and bipolar cells in the transplant would form this projection and make synaptic contacts in the SC. Second, the transplant could cause a release of neurotransmitter that stimulates the cells in the host extrasynaptically. However, it is probable that, if a transmitter were released in a magnitude sufficient to drive cells in the host, it would cause nonspecific excitotoxicity and induce a degeneration in the inner retina, which we did not observe.

Future Considerations

Although the number of cells in the inner retina of dystrophic rodents remains relatively intact,[2,3,64] recent studies show that the dendrites of their postsynaptic cells can undergo morphologic alternations in the absence of photoreceptor input (Gregg RG, Read DS, Peachey NS, Pardue MT, McCall MA, ARVO Abstract 831, 2002).[64–66] Some of these dendritic changes are indicative of a failure to develop normally, whereas others may reflect plasticity and attempts to search out new contacts in the absence of normal synaptic partners. Therefore, it is possible that plastic changes in host bipolar and horizontal cells would induce the establishment of new connections between the host and transplant, if the transplant can provide appropriate guidance signals. In addition, these findings suggest that the timing of transplant placement may be important and that a thorough investigation of the effects of transplants on the postsynaptic dendrites also is needed.

Our data demonstrate that transplants restore visually evoked activity in the SC, and we favor the interpretation that the origin is retinal. However, we still know little about how this comes about. This is a critical question, and characterizing the nature of the visual response at the level of both the host ganglion cells and cells in the SC should help provide a better definition of the types of vision that will be mediated by transplantation. Regardless of the underlying mechanism, these data show that transplantation can be used to induce visual activity, in a wide variety of RP models.

Acknowledgments

The authors thank Matthew M. LaVail, University of California San Francisco, for providing S334ter-line-3 rats; Ron Gregg and Paul DeMarco for critical comments; and Aaron den Dekker, Miranda Messer, Marija Sasek, and Lyndsay Tucker for technical assistance.

References

1. Phelan JK, Bok D. A brief review of retinitis pigmentosa and the identified retinitis pigmentosa genes. *Mol Vis.* 2000;6:116–124.
2. Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retinal Eye Res.* 1998;17:175–205.
3. Humayun MS, Prince M, De Juan E Jr. Morphometric analysis of the extramacular retina from postmortem eyes with retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 1999;40:143–148.
4. Travis GH, Brennan MB, Danielson PE, Kozak CA, Sutcliffe JG. Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (*rds*). *Nature.* 1989;338:70–73.
5. Adler R, Curcio C, Hicks D, Price D, Wong F. Cell death in age-related macular degeneration. *Mol Vis.* 1999;5:31.
6. van Soest S, Westerveld A, de Jong PT, Bleeker-Wagemakers EM, Bergen AA. Retinitis pigmentosa: defined from a molecular point of view. *Surv Ophthalmol.* 1999;43:321–334.
7. Farrar GJ, Kenna PF, Humphries P. On the genetics of RP. *EMBO J.* 2002;5:857–864.
8. Colley NJ, Cassill JA, Baker EK, Zuker CS. Defective intracellular transport is the molecular basis of rhodopsin-dependent dominant retinal degeneration. *Proc Natl Acad Sci USA.* 1995;92:3070–3074.
9. Mosinger OJ, Deckwerth TL, Knudson CM, Korsmeyer SJ. Suppression of developmental retinal cell death but not of photoreceptor degeneration in Bax-deficient mice. *Invest Ophthalmol Vis Sci.* 1998;39:1713–1720.
10. Favor J, Sandulache R, Neuhauser-Klaus A, et al. The mouse Pax2(1Neu) mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye, and kidney. *Proc Natl Acad Sci USA.* 1996;93:13870–13875.
11. LaVail MM. Analysis of neurological mutants with inherited retinal degeneration. Friedenwald lecture. *Invest Ophthalmol Vis Sci.* 1981;21:638–657.
12. Chader GJ. Animal models in research on retinal degenerations: past progress and future hope. *Vision Res.* 2002;42:393–399.

13. Hauswirth WW, LaVail MM, Flannery JG, Lewin AS. Ribozyme gene therapy for autosomal dominant retinal disease. *Clin Chem Lab Med.* 2000;38:147–153.
14. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet.* 2001;28:92–95.
15. Lund RD, Kwan AS, Keegan DJ, Sauve Y, Coffey PJ, Lawrence JM. Cell transplantation as a treatment for retinal disease. *Prog Retinal Eye Res.* 2001;20:415–449.
16. Mohand-Said S, Hicks D, Leveillard T, Picaud S, Porto F, Sahel JA. Rod-cone interactions: developmental and clinical significance. *Prog Retin Eye Res.* 2001;20:451–467.
17. Bok D, Yasumura D, Matthes MT, et al. Effects of adeno-associated virus-vectored ciliary neurotrophic factor on retinal structure and function in mice with a P216L rds/peripherin mutation. *Exp Eye Res.* 2002;74:719–735.
18. LaVail MM, Unoki K, Yasumura D, Matthes MT, Yancopoulos GD, Steinberg RH. Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proc Natl Acad Sci USA.* 1992;89:11249–11253.
19. Steinberg RH. Survival factors in retinal degenerations. *Curr Opin Neurobiol.* 1994;4:515–524.
20. Mohand-Said S, Hicks D, Dreyfus H, Sahel JA. Selective transplantation of rods delays cone loss in a retinitis pigmentosa model. *Arch Ophthalmol.* 2000;118:807–811.
21. Frasson M, Sahel JA, Fabre M, Simonutti M, Dreyfus H, Picaud S. Retinitis pigmentosa: rod photoreceptor rescue by a calcium-channel blocker in the rd mouse *Nat Med,* 1999;5:1183–1187.
22. Davidson FF, Steller H. Blocking apoptosis prevents blindness in Drosophila retinal degeneration mutants. *Nature.* 1998;391:587–591.
23. Liu C, Li Y, Peng M, Laties AM, Wen R. Activation of caspase-3 in the retina of transgenic rats with the rhodopsin mutation s334ter during photoreceptor degeneration. *J Neurosci.* 1999;19:4778–4785.
24. Green ES, Rendahl KG, Zhou S, et al. Two animal models of retinal degeneration are rescued by recombinant adeno-associated virus-mediated production of FGF-5 and FGF-18. *Mol Ther.* 2001;3:507–515.
25. Liang FQ, Aleman TS, Dejneka NS, et al. Long-term protection of retinal structure but not function using RAAV: CNTF in animal models of retinitis pigmentosa. *Mol Ther.* 2001;4:461–472.
26. Lau D, McGee LH, Zhou S, et al. Retinal degeneration is slowed in transgenic rats by AAV-mediated delivery of FGF-2. *Invest Ophthalmol Vis Sci.* 2000;41:3622–3633.
27. Aramant RB, Seiler MJ. Retinal transplantation: advantages of intact fetal sheets. *Prog Retinal Eye Res.* 2002;21:57–73.
28. Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Exp Neurol.* 1999;159:21–33.
29. Coffey PJ, Girman S, Wang SM, et al. Long-term preservation of cortically dependent visual function in RCS rats by transplantation. *Nat Neurosci.* 2002;5:53–56.
30. Radner W, Sadda SR, Humayun MS, et al. Light-driven retinal ganglion cell responses in blind *rd* nice after neural retinal transplantation. *Invest Ophthalmol Vis Sci.* 2001;42:1057–1065.
31. Lund RD, Adamson P, Sauve Y, et al. Subretinal transplantation of genetically modified human cell lines attenuates loss of visual function in dystrophic rats. *Proc Natl Acad Sci USA.* 2001;98:9942–9947.
32. Gouras P, Du J, Kjeldbye H, Yamamoto S, Zack DJ. Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest Ophthalmol Vis Sci.* 1994;35:3145–3153.
33. Seiler MJ, Aramant RB. Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest Ophthalmol Vis Sci.* 1998;39:2121–2131.
34. Aramant RB, Seiler MJ, Ball SL. Successful cotransplantation of intact sheets of fetal retina with retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* 1999;40:1557–1564.
35. Seiler MJ, Aramant RB, Ball SL. Photoreceptor function of retinal transplants implicated by light-dark shift of S-antigen and rod transducin. *Vision Res.* 1999;39:2589–2596.
36. Woch G, Aramant RB, Seiler MJ, Sagdullaev BT, McCall MA. Retinal transplants restore visually evoked responses in rats with photoreceptor degeneration. *Invest Ophthalmol Vis Sci.* 2001;42:1669–1676.
37. Rauer O, Ghosh F. Survival of full-thickness retinal xenotransplants without immunosuppression. *Graefes Arch Clin Exp Ophthalmol.* 2001;239:145–151.
38. Ghosh F, Bruun A, Ehinger B. Immunohistochemical markers in full-thickness embryonic rabbit retinal transplants. *Ophthalmic Res.* 1999;31:5–15.
39. Ghosh F, Juliusson B, Arner K, Ehinger B. Partial and full-thickness neuroretinal transplants. *Exp Eye Res.* 1999;68:67–74.
40. Mirshahi M, Thillaye B, Tarraf M, de Kozak Y, Faure JP. Light-induced changes in S-antigen (arrestin) localization in retinal photoreceptors: differences between rods and cones and defective process in RCS rat retinal dystrophy. *Eur J Cell Biol.* 1994;63:61–67.
41. Brann MR, Cohen LV. Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. *Science.* 1987;235:585–587.
42. Sahel JA, Mohand-Said S, Leveillard T, Hicks D, Picaud S, Dreyfus H. Rod-cone interdependence: implications for therapy of photoreceptor cell diseases. *Prog Brain Res.* 2001;131:649–661.
43. Mohand-Said S, Hicks D, Simonutti M, et al. Photoreceptor transplants increase host cone survival in the retinal degeneration (rd) mouse. *Ophthalmic Res.* 1997;29:290–297.
44. Mohand-Said S, Deudon-Combe A, Hicks D, et al. Normal retina releases a diffusible factor stimulating cone survival in the retinal degeneration mouse. *Proc Natl Acad Sci USA.* 1998;95:8357–8362.
45. del Cerro M, Ison JR, Bowen GP, Lazar E, del Cerro C. Intraretinal grafting restores visual function in light-blinded rats. *Neuroreport.* 1991;2:529–532.
46. Vollrath D, Feng W, Duncan JL, et al. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. *Proc Natl Acad Sci USA.* 2001;98:12584–12589.
47. Sauve Y, Girman SV, Wang S, Lawrence JM, Lund RD. Progressive visual sensitivity loss in the Royal College of Surgeons rat: perimetric study in the superior colliculus. *Neuroscience.* 2001;103:51–63.
48. Glickstein M. How are visual areas of the brain connected to motor areas for the sensory guidance of movement? *Trends Neurosci.* 2000;23:613–617.
49. Nork TM, Mangini NJ, Millecchia LL. Rods and cones contain antigenically distinctive S-antigens. *Invest Ophthalmol Vis Sci.* 1993;34:2918–2925.
50. Donoso LA, Merryman CF, Sery TW, et al. S-antigen: characterization of a pathogenic epitope which mediates experimental autoimmune uveitis and pinealitis in Lewis rats. *Curr Eye Res.* 1987;6:1151–1159.
51. Simon DK, O'Leary DD. Development of topographic order in the mammalian retinocollicular projection *J Neurosci.* 1992;12:1212–1232.
52. Grover S, Fishman GA, Brown J Jr. Patterns of visual field progression in patients with retinitis pigmentosa. *Ophthalmology.* 1998;105:1069–1075.
53. Tansley K. An inherited retinal degeneration in the mouse. *J Hered.* 1954;45:123.
54. Noell WK. Differentiation, metabolic organization, and viability of the visual cell. *Arch Ophthalmol.* 1958;60:702–733.
55. Carter-Dawson LD, LaVail MM, Sidman RL. Differential effect of the rd mutation on rods and cones in the mouse retina. *Invest Ophthalmol Vis Sci.* 1978;17:489–498.
56. Jacobson SG, Kemp CM, Borruat FX, Chaitin MH, Faulkner DJ. Rhodopsin topography and rod-mediated function in cats with the retinal degeneration of taurine deficiency. *Exp Eye Res.* 1987;45:481–490.
57. Naash MI, Ripps H, Li S, Goto Y, Peachey NS. Polygenic disease and retinitis pigmentosa: albinism exacerbates photoreceptor degeneration induced by the expression of a mutant opsin in transgenic mice. *J Neurosci.* 1996;16:7853–7858.

58. Drager UC, Hubel DH. Studies of visual function and its decay in mice with hereditary retinal degeneration. *J Comp Neurol.* 1978; 180:85–114.
59. Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB. Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature.* 1990;347: 677–680.
60. Donner K. Visual latency and brightness: an interpretation based on the responses of rods and ganglion cells in the frog retina. *Vis Neurosci.* 1989;3:39–51.
61. Ivert L, Gouras P, Naeser P, Narfstron K. Photoreceptor allografts in a feline model of retinal degeneration. *Graefes Arch Clin Exp Ophthalmol.* 1998;236:844–842.
62. Aramant RB, Seiler MJ. Fiber and synaptic connections between embryonic retinal transplants and host retina. *Exp Neurol.* 1995; 133:244–255.
63. Ghosh F, Bruun A, Ehinger B. Graft-host connections in long-term full-thickness embryonic rabbit retinal transplants. *Invest Ophthalmol Vis Sci.* 1999;40:126–132.
64. Santos A, Humayun MS, De Juan E. Jr, et al. Preservation of the inner retina in retinitis pigmentosa: a morphometric analysis. *Arch Ophthalmol.* 1997;115:511–515.
65. Strettoi E, Porciatti V, Falsini B, Pignatelli V, Rossi C. Morphological and functional abnormalities in the inner retina of the rd/rd mouse. *J Neurosci.* 2002;22:5492–5504.
66. Peng YW, Hao Y, Petters RM, Wong F. Ectopic synaptogenesis in the mammalian retina caused by rod photoreceptor-specific mutations. *Nat Neurosci.* 2000;3:1121–1127.