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Superior colliculus responses to light – preserved by transplantation in a slow degeneration rat model

Biju B. Thomas[a,1], Magdalene J. Seiler[a,b,1,2], SriniVas R. Sadda[a], Robert B. Aramant[a,*,1,2]

[a]*Department of Ophthalmology, Doheny Eye Institute, University of Southern California, 1450 San Pablo St, Los Angeles, CA 90033, USA*
[b]*Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA*

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Abstract

Purpose. To determine whether retinal transplantation can preserve visual responses in the superior colliculus (SC) of the S334*ter*-line-5 rat, a transgenic model for slow photoreceptor degeneration, which is more similar to human retinitis pigmentosa than the fast degeneration line 3 S334*ter* rat.

Methods. Visual responses to a light flash were recorded in the SC. Rats that had received embryonic day (E) 19–20 fetal retinal sheet transplants at the age of 26–30 days were tested at the ages of 200–254 days. Controls were age-matched rats without surgery and with sham surgery. As a baseline, in no-surgery line-5 rats, the temporal pattern of visual sensitivity loss was evaluated electrophysiologically in the SC from 60 days up to one year of age.

Results. In untreated S334*ter*-line-5 rats, decline in visual sensitivity in the SC was parallel to the photoreceptor loss. At 109 day of age, a relative scotoma developed in the area of the SC corresponding to the nasal retinal region. At 200–254 days of age, the majority of the SC was devoid of any light-driven responses. In contrast, at this time point, transplanted rats with 'good' retinal grafts with normal lamination had visual responses in the caudal region of the SC, the area corresponding topographically to the transplant location in the retina. In these rats, the various parameters of SC responses such as the latency of the onset of the visual response, the response peak amplitude and the consistency of the visual response were significantly different from the control groups (no-surgery, sham surgery, 'poor' transplants) and were more comparable to normal albino rats, however, with a slightly longer latency (70–90 vs. 30–50 msec).

Conclusions. Fetal retinal sheet transplantation showed a long-term rescue effect on visual function in this animal model of slow photoreceptor degeneration.

Keywords: retinal transplantation; retinal degeneration; transgenic rats; electrophysiology; superior colliculus

1. Introduction

Retinal degenerations are devastating causes of progressive vision loss and blindness. Common examples of diseases primarily affecting the photoreceptors or the retinal pigment epithelium (RPE) are retinitis pigmentosa (RP) (Santos et al., 1997; Grover et al., 1998; Humayun et al., 1999) and age related macular degeneration (AMD) (Allikmets, 1999; Gorin et al., 1999; Yates and Moore, 2000). Animal models with different rates of retinal degeneration have been used to study these diseases (Aramant and Seiler, 2002). The transgenic S334*ter* rat, which carries a mutant human rhodopsin, is a well-established model of photoreceptor degeneration (Steinberg et al., 1997) and is available in five distinct lines (3, 4, 5, 7 and 9) with different characteristic rates of retinal degeneration. We have used two of these lines, the fast degenerating S334*ter*-line-3 (Sagdullaev et al., 2003) and the slow degenerating line-5 (this report).

In many outer retinal degenerative diseases, the inner retina is initially relatively well preserved (Drager and Hubel, 1978; Eisenfeld et al., 1984; Santos et al., 1997; Humayun et al., 1999), but remodeling of the inner neural retina takes place following the degeneration of

* Corresponding author. Dr Robert B. Aramant, Department of Ophthalmology, Doheny Eye Institute, University of Southern California, 1450 San Pablo St, Los Angeles, CA 90033, USA.
E-mail address: raramant@dohenyeyeinstitute.org (R.B. Aramant).
[1] Previously at Department of Ophthalmology and Visual Sciences, University of Louisville School of Medicine, Louisville, KY 40202, USA.
[2] Previously at Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40202, USA.

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the photoreceptors (Strettoi and Pignatelli, 2000; Jones et al., 2003; Marc et al., 2003; Strettoi et al., 2003). The generally slow, progressive nature of the degenerations, and the relative preservation of the inner retina provide potential opportunities to intervene and preserve or restore vision by means of appropriate treatment strategies. Some of these strategies are aimed at preserving visual responses by preventing further photoreceptor loss. For example, trophic factors can prevent photoreceptor loss for a limited period in certain degeneration models (LaVail et al., 1992, 1998; Lund et al., 2001). Another approach is gene therapy, transfection of retinal cells using vectors carrying appropriate wild type enzyme or growth factor genes (review: Dejneka et al., 2003). Various kinds of donor cells have also been transplanted with the aim of visual rescue (review: Lund et al., 2001; Aramant and Seiler, 2002). However, all rescue strategies must be used before degeneration has progressed to a stage of significant photoreceptor loss.

After this stage, lost photoreceptors need to be replaced by healthy retinal cells (Silverman and Hughes, 1989; Del Cerro et al., 1991; Seiler and Aramant, 1998; Aramant et al., 1999; Kwan et al., 1999; Seiler et al., 1999; Aramant and Seiler, 2002). This laboratory has focused on introducing sheets of fetal retina into the subretinal space using a device that allows gentle placement of the fragile donor tissue as a flat sheet with the proper orientation/polarity. These sheets develop a normal lamination pattern in a variety of rat models of RP (Seiler and Aramant, 1998; Aramant et al., 1999). Recently we have demonstrated that transplantation of fetal retinal sheets restore visual responses in the superior colliculus (SC) of the Royal College of Surgeons (RCS) rat (Woch et al., 2001), and transgenic S334*ter* rats with fast retinal degeneration (Sagdullaev et al., 2003).

The present, more challenging, study used S334*ter*-line-5 rats that are more comparable to human RP because of their slow rate of photoreceptor degeneration. The residual host photoreceptors make it difficult to detect the effect of the transplant. However, this model can give more information about the long-term potential beneficial effect of transplantation on retinal degeneration. To use this model in a practicable time frame, an albino strain was chosen because previous experience indicated that a pigmented strain would degenerate too slowly, with rods still present at 8 months of age (data not shown).

The effects of transplantation on visual function were evaluated by electrophysiological recordings from the SC. The SC receives a direct retinal input that is topographically organized corresponding to the areas of the retina (Siminoff et al., 1966). Physiological changes take place in the visual centers of the brain parallel to the spatial and temporal progression of the photoreceptor degeneration in the retina. In RCS rats, the progression of retinal degeneration is reflected by a gradient decline of sensitivity in the SC from the temporal to the nasal visual field (Sauve et al., 2001) whereas in the rd mouse the sensitivity decline is in a central to peripheral gradient (Drager and Hubel, 1978). The analysis of the visual responses in the S334*ter*-line-5 rat is complicated by the fact that visual responses from host cone photoreceptors remain normal up to 100 days of age, and only slowly deteriorate after that.

The aim of this study was to investigate whether transplantation of fetal retinal sheets could have a beneficial effect by delaying the loss of visual responses in the SC in this rat model of RP.

2. Material and methods

2.1. Animals

In all experimental procedures, the animals were treated according to the NIH guide for the care and use of laboratory animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, under a protocol approved by the University of Louisville. All efforts were made to minimize animal suffering and to use only the minimum number of animals necessary to produce reliable scientific data. Heterozygous S334*ter*-line-5 rats were produced by Chrysalis DNX Transgenic Sciences, Princeton, NJ, USA, and generously provided by Dr M.M. LaVail, UCSF, San Francisco, CA, USA. Heterozygous S334*ter*-line-5 rats were bred with normal Sprague–Dawley albino rats. The offspring was tested by PCR for the presence of the transgene. Electrophysiological recordings were made in the SC of untreated S334*ter*-line-5 rats from 60 to 351 days of age to establish baseline data. Recordings also were made in S334*ter*-line-5 rats with retinal transplants, sham surgery or normal albino rats. In all recordings, the experimenters were blind to the rat's experimental condition.

All of the procedures used in these experiments have been described in detail elsewhere (Woch et al., 2001; Sagdullaev et al., 2003) and will be briefly described.

2.2. Transplantation

Donor retinal tissue was obtained from pigmented Long-Evans rat fetuses at embryonic day 19–20 (E19–E20). The retina was dissected free of surrounding tissues and a piece (average size $0{\cdot}6 \times 1{\cdot}2$ mm^2) was placed into the subretinal space in the superior nasal quadrant of 13 anaesthetized S334*ter*-line-5 rats aged between postnatal day (P) 26 and 30, using a custom-built insertion tool (procedure as described in Seiler and Aramant, 1998; Aramant et al., 1999). The sham surgical procedure was identical to that used in transplantation with the exception that only the vehicle solution was delivered into the subretinal space.

2.3. Electrophysiology

For electrophysiological assessment of visual responses in the SC, rats were dark-adapted overnight and then the eyes were covered with a hood of aluminium foil.

They were anaesthetized by intraperitoneal injection of xylazine/ketamine (37·5 mg kg^{-1} ketamine and 5 mg kg^{-1} xylazine). The gas inhalant anesthetic (1·0–2·0% halothane in 40% O_2/60% N_2O) was administered via an anesthetic mask (Stoelting Company, Wood Dale, IL, USA). Rats were mounted in a stereotactic apparatus, a craniotomy was performed and the SC was exposed. Multi-unit visual responses were recorded extracellularly from the superficial laminae of the SC using nail polish coated tungsten microelectrodes (custom made in our laboratory). Recording sites (200–400-μm apart) covered the full extent of the SC with the exception of its medial area, which was located just under the superior sagittal sinus. At each recording location, 16 presentations of a full-field strobe flash (1300 cd m^{-2}, Grass model PS 33 Photic stimulator, W. Warwick, RI, USA), positioned 30 cm in front of the rat's eye, were delivered to the contralateral eye. An interstimulus interval of 5 sec was used. Up to 32 presentations were performed when the response was inconsistent or when there was no response. All electrical activity was recorded using a digital data acquisition system (Powerlab; ADI Instruments, Mountain View, CA, USA) 100 msec before and 500 msec after the onset of the stimulus and all responses at each site were averaged. Blank trials, in which the illumination of the eye was blocked with an opaque filter, also were recorded at each site. At the end of each recording session, stereotactic coordinates of the electrode penetrations were plotted on a graph paper and superimposed on the diagrammatic sketch of SC to display the area covered by the electrode.

Several properties of the visual responses were analysed including (1) response onset latency-defined as the point at which a clear, prolonged (>20 msec) increase in the light-evoked activity could be measured above background (which was determined using the 100 msec of activity preceding the light flash); (2) consistency of the response-defined as the standard deviation (S.D.) of the response onset latencies from a single recording site and (3) peak response amplitude-defined as the largest excursion peak to peak in the averaged response. The response onset latency for each recording site was then marked on the graph paper.

Since residual responses could be recorded from different areas of the SC even up to 300 days of age, detailed evaluation of visual responsive units was restricted to the caudal region of the SC. The degeneration process begins in this area and corresponds to the area in the retina where the transplant is placed. For statistical comparison of the various properties of the visual responsive units among various experimental groups, values obtained from a single recording site at the caudal region that produced the best response (shortest latency) were used.

2.4. Histology

At the end of the recording session, animals were killed with an overdose of halothane, eyes were either immersed in Bouin's fixative and embedded in paraffin, or fixed in 4% paraformaldehyde in 0·1 M Na-phosphate buffer, infiltrated with sucrose, and frozen in Tissue Tek on dry ice. Transverse sections of the retina were cut, mounted on to slides, and stained with hematoxylin–eosin (H&E). A series of sections through the full extent of each transplant was evaluated at the light microscopic level.

S-antigen immunoreactivity was used to identify the residual photoreceptors in the host retina as well as photoreceptors in the transplant. Briefly, deparaffinized sections were washed with phosphate-buffered saline and incubated for 30 min in 20% horse serum. The sections were incubated with a mouse monoclonal antibody against S-antigen (clone A9C6) (Donoso et al., 1985) at a dilution of 1:20 000 overnight at 4°C and the binding of the primary antibody was detected using the Vector Elite ABC kit for mouse antibodies (Vector Laboratories, Burlingame, CA, USA). The antibody A9C6 is specific for rods and blue cones (Donoso et al., 1985). Alternatively, frozen sections were blocked in 20% goat serum, and incubated with a 1:1000 dilution of A9C6 overnight. After washing, the slides were incubated in a 1:100 dilution of Rhodamine X-anti mouse IgG (Molecular Probes, Eugene, OR, USA), and coverslipped with DAPI-containing Vectashield mounting medium (Vector Labs) for fluorescence. Sections were analysed using a Zeiss confocal microscope.

2.5. Statistics

Statistical comparisons were made using Fisher exact probability test and one way analysis of variance (ANOVA) with subsequent post hoc tests, using a statistics package of GraphPad Software, Inc., San Diego, CA, USA.

3. Results

3.1. Photoreceptor degeneration in S334ter-line-5 rats

The temporal pattern of photoreceptor degeneration was determined qualitatively by examining the morphology of the outer retina (outer nuclear and outer plexiform layer) of untreated line-5 rats between P30 and P351. The normal albino rats provided a baseline for comparing the degenerative state of the retina in the albino line-5 rats. In normal albino rats (Fig. 1), the retina contains an outer nuclear layer (ONL) of photoreceptor cell bodies 10–12 cells thick. Representative photomicrographs of transverse sections from transgenic rat retinas between P44 and P254 (Fig. 1) show a progressive loss of photoreceptors in the ONL starting at the age of P44.

With increasing age, the ONL gradually became indistinct. In the degeneration process, photoreceptor loss occurred more rapidly in the dorsal (superior) retina than the ventral (inferior) region. At P30, the thickness of the ONL in line-5 rats appeared normal (data not shown). By P44, the ONL of transgenic rats became slightly thinner (7–8 cell

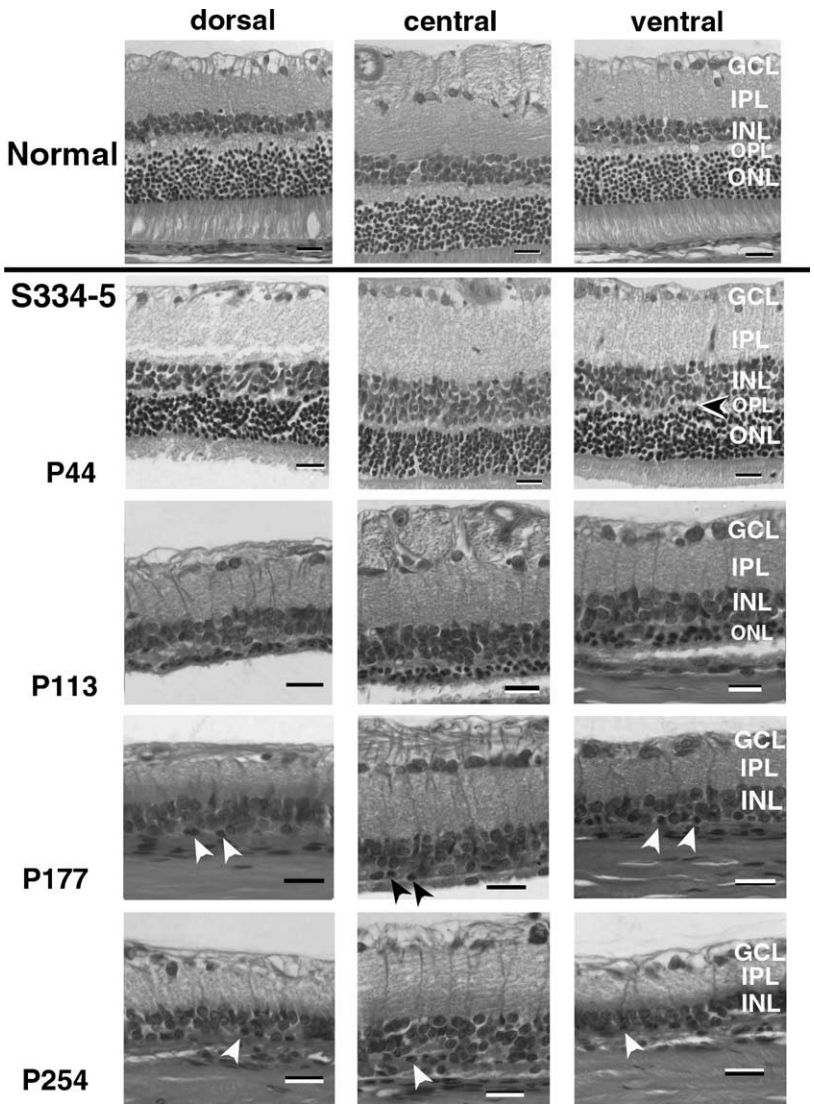

Fig. 1. Histology of the retinal degeneration of albino heterozygous S334*ter*-line-5 rats. In a normal rat, 10–12 rows of photoreceptors are found in the ONL. In the S334*ter*-line-5 rat ($n = 31$), at each age, the retinal degeneration progresses faster in the dorsal retina. At P44, some reduction of the ONL to 7–8 rows is recognizable in the dorsal retina, whereas the ventral retina is still almost normal (9–10 rows). At P113, the ONL consists only of a single row photoreceptor cell bodies in the dorsal retina, and 2–3 rows in the central and ventral retina. At P177, only scattered photoreceptors (arrowheads) are found dorsally, and one row of photoreceptors ventrally. Few cone photoreceptors (arrowheads) are recognizable at P254. 5-μm paraffin section, hematoxylin–eosin staining. GCL, ganglion cell layer; IPL, inner nuclear layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer. Scale bar, 20 μm.

layer in the dorsal retina, 9–10 cell layer ventral) (Fig. 1). Further loss of photoreceptors was evident at P61, with only 1–2 layers of photoreceptor cell bodies remaining in the dorsal retina at P91 (data not shown). At P113, only a single layer was evident in the dorsal retina, whereas 2–3 layers were still found in the ventral retina. By P177, only scattered photoreceptor nuclei remained in the ONL, both dorsal and ventral. In the severely photoreceptor-depleted retinas at P177 and 254, both inner nuclear and the ganglion cell layer appeared to be thinning.

3.2. Visual evoked neural activity of SC

The deterioration of visual responses in the SC of line-5 rats at different stages in the course of retinal degeneration was characterized both spatially and temporally. Representative maps of the SC of a normal albino rat as well as line-5 rats of selected ages are shown in Fig. 2. The sensitivity levels across the visual field are expressed by the mean latencies of the visual responses as a function of their location across the SC. In a normal albino rat, all regions of the SC have responses with short visual latencies. At P109, transgenic rats developed a scotoma (shaded area) in the mid-caudal SC (topographically corresponding to the nasal retina), where no light-evoked activity was elicited. In addition, responses from other SC regions also showed longer mean latencies (<70 msec). As the animals became older and degeneration progressed, the scotoma expanded out towards the rostral SC through the medial and lateral areas. A nasal-temporal positive gradient in the visual

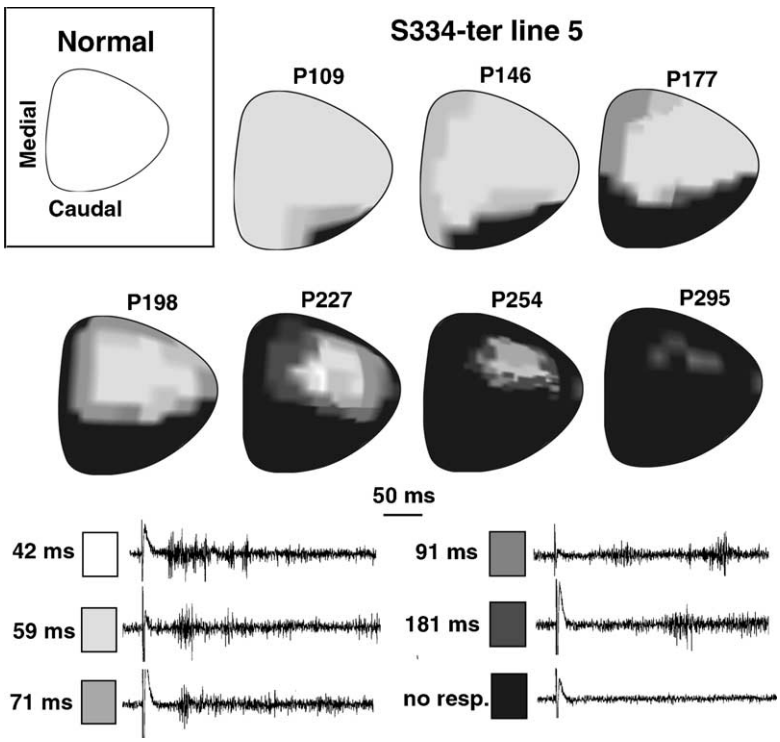

Fig. 2. Progressive loss of visual responses in the superior colliculus (SC). Each figure of the left represents a schematic diagram of average latencies of visual responses as a function of location across the SC of a normal albino rat and S334*ter*-line-5 rats at postnatal ages (P): 109, 146, 177, 198, 227, 254 and 295 days. The shading of the diagrams (white to black) represents increasing mean response latencies. In a normal rat, response latencies in the SC are less than 50 msec. In the S334*ter*-line-5 rat, responses in the SC were normal up to the age of P100 (data not shown). At P109, a small area with no visual response was located in the extreme caudal region of SC and with increasing age, this scotoma increases in size until a steep decline in visual activity could be observed at P254. At the bottom, examples of representative multi-unit visual responses are shown, with the black representing areas where activity could not be distinguished from background.

sensitivity (as manifested by response onset latency) was observed across the SC. Visual sensitivity began to decline around P100, and then rapidly from P200 until P254 (Fig. 2) when the majority of the SC was devoid of any visual activity (except for some spontaneous firing). This decline in visual sensitivity in transgenic rats was concomitant with the photoreceptor degeneration. Weak residual activities could be recorded from some locations in the rostral edges of the SC even up to P295 (Fig. 2).

3.3. Superior colliculus responses following retinal transplantation

Transplanted rats, tested between the ages of 212–254 days, were divided into two groups on the basis of histological evaluation: good transplants (transplants with normal lamination pattern, $n = 8$) and poor transplants (transplants with poor lamination pattern, $n = 5$). Activity in the caudal SC (which normally has no activity after P200 in the absence of a graft), the location corresponding to the transplant site, was evaluated for the quality of the response. Based on data from normal albino rats, responses with <90 msec ('short') onset latencies are assumed to be of better quality.

All rats with 'good' transplants (8/8) had 'short' latency responses, a statistically higher percentage than sham surgery (3/10) animals ($p < 0{\cdot}0007$, Fisher exact probability test) and no-surgery (2/11) rats ($p < 0{\cdot}004$) (Fig. 3).

Fig. 4 shows a scatter plot of the distribution of mean onset latency from the best visually responsive (shortest response onset latency) site as a function of its peak

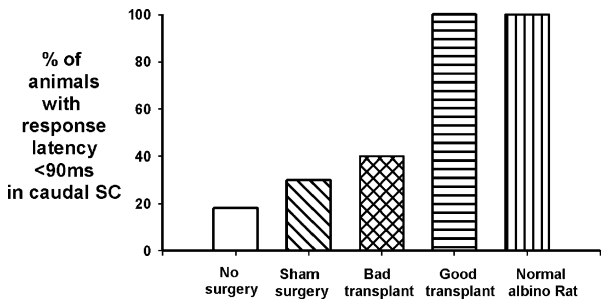

Fig. 3. Percentage of rats demonstrating comparatively short latency responses (<90 msec) in the caudal SC, topographically corresponding to the placement of the transplant in the retina, in the different experimental groups: no-surgery ($n = 11$), sham surgery ($n = 10$), 'good' transplant ($n = 8$), 'poor' transplant ($n = 5$) and normal albino ($n = 5$) rats. Data are represented as mean ± S.E. All rats were tested between the ages of 212 and 254 days. 'Good' transplant vs. no-surgery, $p < 0{\cdot}001$; 'good' transplant vs. sham surgery, $p < 0{\cdot}001$ (Fisher exact probability test).

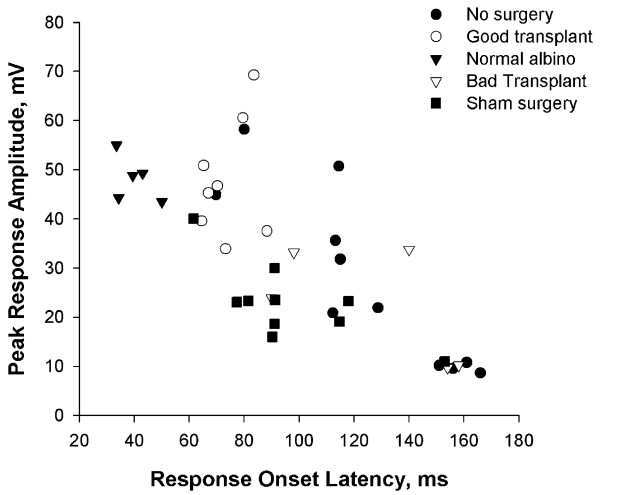

Fig. 4. Distribution of mean onset latency from the best visually responsive site (shortest response onset latency) plotted as a function of its peak amplitude in all rats among the various experimental groups: no-surgery (●; $n = 11$), 'good' transplant (○; $n = 8$), 'poor' transplant (▽; $n = 5$), sham surgery (■; $n = 10$), and normal albino (▼; $n = 5$). All rats were tested between the ages of 212 and 254 days. Statistically significant differences: (a) 'good' transplant latency vs. no-surgery latency, $p < 0{\cdot}01$; (b) 'good' transplant latency vs. 'poor' transplant latency, $p < 0{\cdot}05$; (c) 'good' transplant amplitude vs. no-surgery amplitude, $p < 0{\cdot}05$; (d) 'good' transplant amplitude vs. sham surgery amplitude, $p < 0{\cdot}01$; (e) 'good' transplant amplitude vs. 'poor' transplant amplitude, $p < 0{\cdot}05$. [a,b: Dunn's multiple comparison test; c–e: Bonferroni multiple comparison test].

amplitude in all S334*ter*-line-5 rats belonging to no-surgery, good transplant, poor transplant, sham surgery group, and normal albino rat. Table 1 presents the corresponding latency and amplitude values. Comparing the onset response latencies, a non-parametric ANOVA (Kruskal–Wallis test) showed a significant group effect (kw = 14·8, $p < 0{\cdot}002$), and a post hoc test (Dunn's multiple comparison test) showed significant differences between the good transplant group and the no-surgery and 'poor' transplant groups.

Comparing the peak response amplitudes, a one way ANOVA showed a statistically significant group difference ($p < 0{\cdot}002$) and the subsequent post hoc test (Bonferroni multiple comparison test) revealed a significantly higher response amplitude for the good transplant group when compared with no-surgery ($p < 0{\cdot}05$), 'poor' transplant ($p < 0{\cdot}05$) and sham surgery ($p < 0{\cdot}01$) rats.

The consistency of the onset latency of the visual response from the caudal SC was also assessed. The consistency of the visual response is expressed in terms of S.D. of the response onset latencies. In line-5 rats with good retinal transplants, the consistency of the visual response (S.D. of the response onset latencies) was 2·9, which was close to that observed in normal albino rats (2·3). In contrast to this, the consistency of the visual response in the poor transplant group and sham controls was 18·6 and 19·6, respectively. A non-parametric ANOVA revealed a highly significant group effect (kw = 16·8, $p < 0{\cdot}001$), and a post hoc test (Dunn's multiple comparison test) showed a significant difference between rats with 'good' transplants and all retinal degenerate control rats ($p < 0{\cdot}01$ vs. no-surgery, $p < 0{\cdot}01$ vs. sham surgery, $p < 0{\cdot}05$ vs. poor transplants).

Fig. 5 shows schematic diagrams of the responses in the SC in the no-surgery, sham surgery, and 'good' transplant groups at the age of 225–250 days. Only the 'good' transplant group had good quality responses in the caudal SC.

3.4. Histologic evaluation

Transverse sections of the retina of different groups of rats were stained using hemotoxylin and eosin (data not shown). To evaluate the presence of photoreceptors in the host and control rat retinas and the transplant, sections were stained for S-antigen immunoreactivity (Fig. 6). Retinal transplants showed varying degrees of organization ranging from excellent lamination (Fig. 6(A),(B)) to rosette formation (data not shown). Out of 13 transplanted rats, eight had transplants with normal laminar morphology. They had different cellular and synaptic layers, and photoreceptors with inner and outer segments. This group included transplants with rosettes. These rats were termed as having 'good' transplants. In the 'poor' transplant group, transplants had a poor lamination pattern with rosettes and scattered photoreceptors (not shown). S-antigen reactivity in the host retina was compared among the various experimental groups and various animal ages (Fig. 6).

Table 1
Onset latencies and peak amplitudes of best responsive SC site in different experimental groups (age 200–250 days)

		Latency of best responsive SC site (msec)		Peak amplitude of best responsive SC site (mV)	
Experimental group	*n*	Range	Mean ± S.D.	Range	Mean ± S.D.
Normal albino rat	5	33·5–50·0	40·1 ± 6·8	43·5–55·0	48·2 ± 4·7
'Good' transplant	8	64·5–88·3	73·9 ± 8·9	33·95–69·3	48·0 ± 12·0
'Poor' transplant	5	90·0–160·8	125·7 ± 29·3	10·25–33·8	22·2 ± 11·8
Sham surgery	10	61·5–185·0	96·7 ± 25·0	11·0–40·1	22·8 ± 8·0
No-surgery line-5	11	69·8–210·5	121·2 ± 28·2	10·2–58·3	27·7 ± 17·8

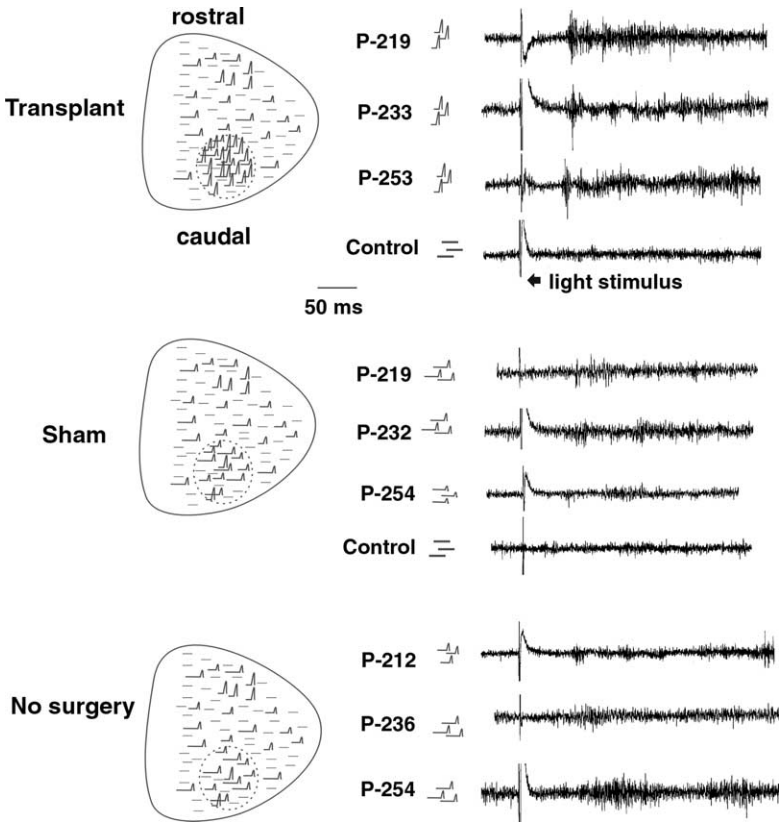

Fig. 5. Schematic diagrams of response onset latency and peak response amplitude of visual responses recorded from the caudal SC (area represented by dotted circle) of all the 'good' transplant ($n = 8$), sham surgery ($n = 10$) and no-surgery ($n = 11$) S334*ter*-line-5 rats at the age of 212–254 days. Different signs in the diagram represent the quality of the visual responses on the basis of response onset latency and peak response amplitude. Representative multi-unit visual responses of three rats belonging to each experimental group are also illustrated. Good quality responses in the caudal SC are only found in the good transplant group.

Regardless of experimental group and age, scattered cells that were reactive to S-antigen (presumably cones) could be found in the host retina. Upon qualitative examination, host cone photoreceptors did not appear to be better preserved in the area of the transplant (Fig. 6). However, a subtle difference cannot be excluded.

4. Discussion

4.1. Spatiotemporal pattern of visual loss in S334ter-line-5 rats

In S334*ter*-line-5 rats, the first sign of photoreceptor degeneration was evident not until after the age of 45 days, unlike some other rodent RP models which show more early and rapid rates of degeneration (Drager and Hubel, 1978; Liu et al., 1999; Sauve et al., 2001; Sagdullaev et al., 2003). The photoreceptor degeneration in line-5 rats advances faster in the dorsal retina whereas in RCS rats it is more advanced in the ventral retina (Dowling and Sidman, 1962; LaVail et al., 1975; Sauve et al., 2001). A different pattern of retinal degeneration in a central to peripheral temporal gradient was reported for some human patients with RP (Grover et al., 1998), rd mice (Carter-Dawson et al., 1978) and cats with taurine deficiency-induced photoreceptor degeneration (Jacobson et al., 1987).

Although only a few layers of cell bodies remain in the ONL at P113 in line-5 rats, the subsequent loss of the remaining cone photoreceptors takes place more slowly. Deterioration of visual function in the SC is manifested by longer response onset latencies and a gradient of sensitivity loss from nasal to temporal visual field. The lengthening of the response onset latency is considered as a diagnostic manifestation of a progressive form of RP (Berson, 1993) and has also been reported for two other rat models (Sauve et al., 2001; Woch et al., 2001; Sagdullaev et al., 2003). A comparable naso-temporal gradient in visual sensitivity across the SC observed in RCS rats is believed to be due to the differential distribution of retinal ganglion cells (Sauve et al., 2001). Our study also indicates that some loss of retinal ganglion cells occurred at late stages of retinal degeneration in the line-5 rat.

The visual sensitivity loss in the SC of line-5 rats takes place slowly even after the majority of the photoreceptors are degenerated. A similar pattern is also observed in RCS

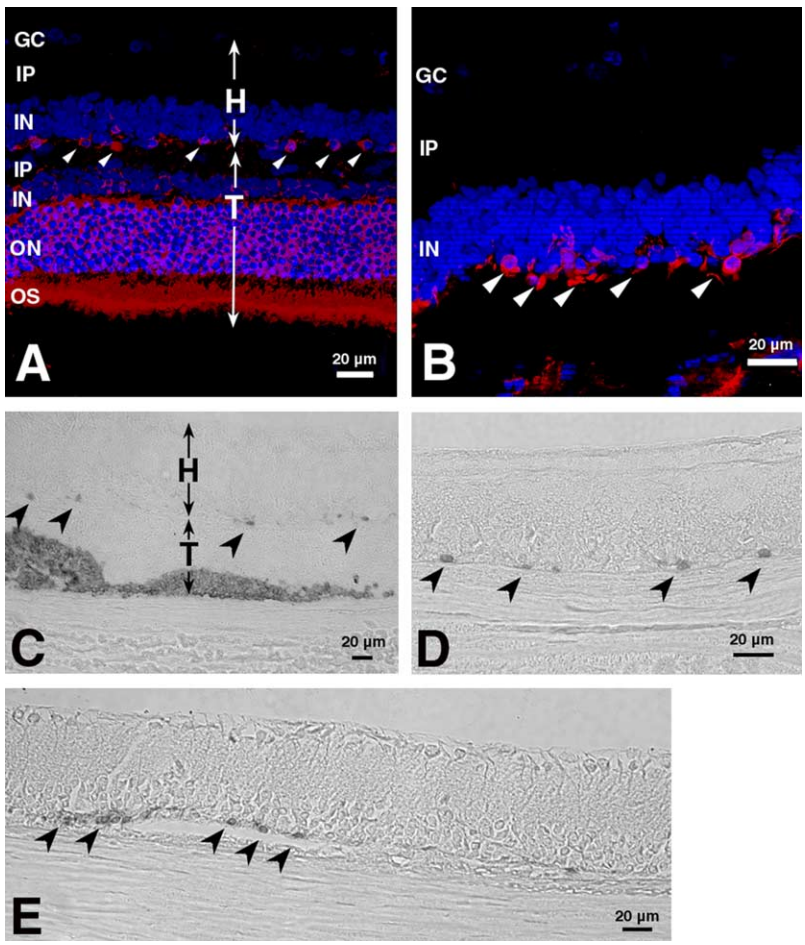

Fig. 6. Morphology of retinas of S334*ter*-line-5 rats with retinal transplants. (A) Confocal image stack (7·4-μm thick) of S-antigen staining (red) and DAPI stain for nuclei (blue) from a 'good' transplant (T) shown along with host retina (H) from an S33*ter* line-5 rat with visual responses, recorded 196 days after transplantation, age 225 days (frozen section). Arrowheads indicate S-antigen-immunoreactive cones in the host retina overlying the transplant. (B) Cones in host retina outside transplant area (arrowheads). Note that this image is slightly more enlarged than (A), which needs to be taken into account for comparison of the density of cones. There is no apparent difference in the cone density of the host retina between (A) and (B). (C–E) S-antigen staining on paraffin sections, using diaminobenzidine as chromogen (black): (C) Transplant surgery rat, that showed a 'good' response in the SC, age 248 days. Few cones in host retina overlying the transplant (arrowheads); (D) sham surgery rat, age 232 days; and (E) no-surgery rat, age 254 days. Arrowheads point to residual cones. Scale bars, 20 μm. GC, ganglion cell layer; IP, inner plexiform layer; IN, inner nuclear layer; OP, outer plexiform layer; ON, outer nuclear layer.

rats (Sauve et al., 2001). However, in rd mice (Drager and Hubel, 1978) and line-3 transgenic rats (Sagdullaev et al., 2003), two other models with faster photoreceptor degeneration, the visual sensitivity loss is more in accordance with the temporal pattern of photoreceptor degeneration. Interestingly, in line-5 rats, the rapid loss of visual sensitivity is observed only after the photoreceptor degeneration is almost complete (P190). At P254, comparatively short latency responses (<90 msec) are observed only from the mid-rostral SC (corresponding to the temporal retina), although weak residual responses can be recorded from other areas. The persistence of some visual responses even after the completion of photoreceptor degeneration has been ascribed to slow degeneration processes that may have enabled compensatory mechanisms to take effect, e.g. the ectopic synapse formation of rod bipolar cells with cones (Cicerone et al., 1979; Peng et al., 2000).

4.2. Restoration of vision by transplantation of fetal retinal sheets

Comparatively short latency responses were observed in the caudal SC of all the line-5 rats with 'good' retinal transplants, exhibiting normal lamination (8/8), whereas a significantly smaller number of sham surgery (3/10) and no-surgery rats (2/11) exhibited such responses. This suggests that short latency ('good') visual responses recorded from the SC of the transplanted rats are confined to an area corresponding to the placement of a well-organized graft in the retina, and these responses are functionally different from the residual responses observed from several areas in the SC of control rats.

In transplanted rats, the peak response amplitude and the consistency of the response are more similar to that of the normal albino rats than to the other control groups,

suggesting that visual responses in transgenic line-5 rats can be maintained at a level approaching that of normal rats. However, the response onset latency is nonetheless longer than normal in transplanted rats. This may be attributed to retinal remodeling and a reduced number of ganglion cells in the host retina consequent to retinal degeneration (Marc et al., 2003). An increase in response onset latency can also be due to an abnormal wiring, an increase in the number of synapses between transplant photoreceptors and the host compared to normal retina. A similar increase in response onset latency was observed after transplantation in RCS rats (Woch et al., 2001) and S334*ter*-line-3 rats (Sagdullaev et al., 2003).

The present study also shows a correlation between the quality of the visual response and the organization of the graft because 'poor', disorganized transplants had no effect on visual responses in the SC.

Another finding of this study is the observation that visual responses can persist for a substantial period of time after neural retinal transplantation. All the rats with good retinal transplants maintained their visual activity in the transplanted area almost at the level of normal rats at least up to the age of 253 days. Preservation of cortically dependent visual activity up to 245 days of age has recently been reported for RCS rats (Coffey et al., 2002). Future studies may be directed at improving the quality of the transplants and the physiological mechanisms underlying the long-term preservation of visual function.

4.3. Visual activities in the SC of control rats

The various groups of age-matched control rats used in this study had developed a large scotoma in the caudal SC at the time of electrophysiological recording. The visual responses recorded from the caudal region of the sham surgery rats and rats with 'poor' transplants were qualitatively similar, but inferior to the responses recorded from the same area of transplanted rats. This supports the contention that transplants with a normal laminar morphology can develop a more efficient functional interaction with the host retina and produce long-term visual preservation. Three sham surgery rats and two untreated rats also exhibited comparatively short latency responses in the caudal SC. However, in all of these rats, visual responses with short onset latency could be recorded from other SC areas too. Subsequent morphological examination revealed residual photoreceptors in the retina. Thus, the comparatively short latency responses found in the caudal SC of certain control rats is considered to be due to individual variations in the progression of visual loss as reported for the RCS rats (Sauve et al., 2001).

4.4. Mechanism underlying the visual restoration

The present study shows that following retinal transplantation, visual responses in the SC can be preserved long-term in a rat model of retinal degeneration. Biochemical evidences exist to show that normal phototransduction processes can take place in the transplanted retinal sheets (Seiler et al., 1999) and electrophysiological studies have shown that in vitro light-dependent electrical activity can originate from the surface of fetal aggregate subretinal grafts (Adolph et al., 1994). Further, there are reports that neural connections from the retina to the SC and visual cortex can be maintained long after the photoreceptors have degenerated (Drager and Hubel, 1978; Eisenfeld et al., 1984; Santos et al., 1997; Humayun et al., 1999). The effect observed in our study may be explained by one or two mechanisms: (1) the formation of functional connections between the transplant and the host, and/or (2) a trophic effect of the transplant on remaining host cones.

Previously it was demonstrated that embryonic aggregate epiretinal transplants can grow neural processes and form synapses within the host retina (Aramant and Seiler, 1995). Furthermore, in intact sheet subretinal transplants, neural processes appear to cross the interface between transplant and host indicating that synaptic connections could potentially form (Seiler et al., 2001; Aramant and Seiler, 2002). Limited synaptic connection between the transplant and host retina has also been suggested by transsynaptic tracing studies from the host brain to the transplant (Aramant et al., 2000). The existence of physical connections (but not necessarily synapses) between a retinal transplant and a normal host retina has also been demonstrated in a rabbit model (Ghosh et al., 1999). Nonetheless, despite this evidence to suggest that connections may form, the mechanism of visual preservation in the present study cannot be determined conclusively. Another potential explanation for the observed result is a rescue effect on remaining host photoreceptors. Mohand-Said et al. (1998) demonstrated that diffusible trophic factors produced by rods can prevent cone degeneration. There are also reports about rescue effects of retinal transplants on rod photoreceptors by RPE transplants (Li and Turner, 1988; Lopez et al., 1989), growth factors (Faktorovich et al., 1990, 1992; LaVail et al., 1992; Unoki et al., 1994; Masuda et al., 1995; Perry et al., 1995; Cayouette and Gravel, 1997; LaVail et al., 1998; Chong et al., 1999), and even sham surgery (Wen et al., 1995; Humphrey et al., 1997; Schraermeyer et al., 1999). However, there are several reasons that argue against the possibility of a rescue effect as the cause of improved visual responses in the line-5 rats in this study. First, the visual responses in the caudal SC were observed only in a very small area of the SC precisely corresponding to the placement of the graft in the retina. If a trophic effect of the transplant on host photoreceptors was the cause, it should have an effect on a larger retinal area, as has been observed in rd mice with rod photoreceptor transplants (Mohand-Said et al., 1997). Second, based on S-antigen immunoreactivity, no difference was observed in the number of remaining cone photoreceptors near the transplant site and other areas of the host

retina. Finally, good quality visual responses were recorded only from rats in which the transplant maintained normal laminar morphology. The morphological integrity of the transplant, however, is less likely to influence the release of any 'rescuing' trophic factors. In other words, if rescue was the principal mechanism, one would also expect to see a rescue effect in the 'poor' transplant group.

In summary, the present investigation demonstrates that near normal visual responses are preserved in the transplanted area of line-5 rats up to 254 days of age. These results give a stronger support than previous studies in other retinal degeneration models that retinal transplantation might be a potential beneficial treatment for patients with retinal degeneration.

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