Retinal Transplants Restore Visually Evoked Responses in Rats with Photoreceptor Degeneration

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

Purpose. To assess whether transplantation of intact sheets of fetal retina with retinal pigment epithelium (RPE) into a retina with photoreceptor degeneration restores visually evoked responses.

Methods. Sheets of fetal retina with RPE were transplanted into the subretinal space of Royal College of Surgeons (RCS) rats at 37 to 69 days of age. Sixty-three days to 10 months after transplantation, multiunit visual responses were recorded in the superior colliculus (SC) of transplanted rats, age-matched untransplanted rats, and rats with sham surgery.

Results. In 19 of 29 RCS rats with transplants, visually evoked responses were recorded from and restricted to a small area of the SC that corresponds topographically to the portion of the retina in which the transplant was placed. Outside of this area, no visual responses were evoked. Visually evoked responses were never recorded in age-matched, nontransplanted RCS rats. Visually evoked responses were recorded in 6 of 13 RCS rats with sham surgery, but these responses were significantly different from responses in rats with transplants.

Conclusions. These results demonstrate that this transplantation technique restores visually evoked responses in the brain. Although the underlying mechanism is unknown, we propose that the central visual response results from increased synaptic efficacy within the host retina. If it can be established that functional connections between the transplant and the host retina produce the effect, then it would indicate that the technique could be explored as a therapeutic strategy in some diseases of retinal degeneration. (*Invest Ophthalmol Vis Sci.* 2001;42:1669–1676)

Many progressive, blinding eye diseases involve a selective degeneration of the photoreceptor cells, which transduce light energy to a neural signal.[1,2] Morphologic examinations of these retinas suggest that in the absence of most photoreceptors, the circuitry of the inner retina remains relatively intact,[3] although changes have been noted in bipolar cells and their inputs.[4–8] Because these blinding eye diseases result from mutations in different genes,[9] treatments will require the development of either generally applicable strategies or specific individual therapies. For example, a general strategy that has been devised overexpresses trophic factors in the retina and arrests or delays photoreceptor degeneration.[10,11]

The Royal College of Surgeons (RCS) rat is one model of retinal degeneration and has been used extensively to assess treatments for photoreceptor degeneration. Injection of bFGF[12] and transplants of various tissues, such as dissociated retinal pigment epithelium (RPE) cells,[13,14] iris pigment epithelial cells,[15] and Schwann cells,[16] delay degeneration of photoreceptors if transplantation is performed before postnatal day 28 (P28). A similar delay results from sham surgery.[17–19] However, sham surgery and these generic transplantation strategies, which rely on the release of trophic factors, have no therapeutic effect if the surgery is performed after P38.[20] Thus, when photoreceptor degeneration is advanced, therapies that rely on trophic support appear to be of limited value because they cannot replace the degenerated cells.

Whether retinal transplantation can be used as a therapeutic approach in photoreceptor degeneration depends on the ability of the transplant to make functional connections that are capable of evoking visual responses in the host. Several studies provide data that suggest that functional connections may form after transplantation. Embryonic retinas transplanted to the superior colliculus (SC) of newborn hosts make functional connections with the SC and drive a pupillary reflex.[21–24] Retinal aggregate transplants, dissected from the host eye in which they matured, drive a local light-evoked electroretinogram in vitro.[25] Injections of dissociated fetal retinal cells into the subretinal space of light-damaged rats appear to mediate a visually evoked behavior.[26] Postnatal photoreceptor sheets transplanted into the eye of light-damaged rats may result in visually evoked potentials (VEPs) in the cortex, although interpretation of these data is not straightforward because the age at which transplantations were performed was not reported.[27] In a mouse model of retinal degeneration, *rd*, implantation of retinal microaggregates affects a light–dark behavior preference.[28] Taken together, these results suggest a beneficial effect of transplantation on restoration of vision. However, none of these studies directly demonstrates that either a physiologically significant connection arises between the transplant and the existing circuitry of the host retina or that visual responses are retinotopically localized in a brain structure that receives direct input from retinal ganglion cells.

Aramant and Seiler[29–31] have developed a retinal transplantation technique in which an intact sheet of fetal retina is transplanted with or without its RPE into a degenerated host retina. The transplanted fetal retina develops a normal lamination pattern and, several components of the normal visual transduction cascade are modulated by light in its photoreceptors.[31] Processes arise from cells in the transplant and appear to cross into the host retina,[32] and cells in the transplant can be transsynaptically labeled by retrogradely transported virus injected into the SC.[33]

To determine whether retina/RPE transplantation results in visually evoked responses in a central visual structure, we placed transplants in RCS rats 1.2 to 2.1 months of age, by which time their photoreceptors are irreversibly damaged[20]

From the Departments of [1]Ophthalmology and Visual Sciences, [2]Anatomic Sciences and Neurobiology, and [3]Psychological and Brain Sciences, University of Louisville, Kentucky.

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

Submitted for publication March 30, 2000; revised July 26, 2000 and January 30, 2001; accepted February 13, 2001.

Commercial relationships policy: P (RBA, MJS); N (all others).

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

TABLE 1. Overview of Animals Used in the Experiments

Experimental group	*N*	Age at Recording (months)	Age at Surgery (months)	Time after Surgery (months)
Controls				
Normal pigmented rats	8	2.8–5.6	n.a.	n.a.
Normal albino rats	4	2.1–7.1	n.a.	n.a.
RCS rats	6	2.0–4.4	n.a.	n.a.
RCS rats: sham surgery	13	3.6–12.7	1.2–1.5	2.1–11.5
S-antigen immunohistochemistry	8	3.6–8.0	1.2–1.5	2.1–5.4
RCS rats: cortex transplant	3	5.8–7.1	1.2–1.7	4.3–5.4
Transplants				
RCS rats with transplants and visual responses	19	3.3–8.1	1.2–2.1	1.8–6.8
S-antigen immunohistochemistry	10	3.4–8.1	1.4–1.9	1.9–6.8
RCS rats with transplants and without visual responses	10	3.1–10.7	1.5–2.3	1.4–8.4
S-antigen immunohistochemistry	2	3.2–7.6	1.5	1.7–6.1

n.a., not applicable.

and recorded multiunit visually evoked responses in the SC. With this approach, the site of each visually responsive area could be localized on the topographic map of the SC[34] and compared with the position of the transplant in the retina. The data presented here demonstrate that cotransplantation of fetal retina/RPE restores visually evoked postsynaptic responses in the SC of the RCS rat. These responses are elicited only in regions of the SC that topographically match the retinal area in which the transplant is placed. Visual responses also could be evoked in the SC of rats with sham surgery, although these responses are both quantitatively and qualitatively different from those driven by the transplant. Thus, our results show that the presence of the transplants produces an effect on visual activity. If this activity is directly related to visual perception, then transplantation of sheets of retina/RPE could be useful as a therapeutic approach to maintain or restore light perception in patients with retinal degenerative diseases.

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 the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Transplant Tissue Preparation

The procedure has been described in detail elsewhere.[29,30] Donor retinal tissue was obtained from embryonic day (E)19 to E20, pigmented Long-Evans rat fetuses. Donor eyes were incubated in dispase (Collaborative Biomedical Products, Bedford, MA), and the retina with its attached RPE was dissected free of surrounding tissues and embedded in 0.4% MVG alginate (Pronova, Oslo, Norway). Some control rats received transplants of fetal cortex. For this condition, donor cortex was removed from the same aged embryos and cut into thin sections by hand.

Transplantation Procedure

In anesthetized rats, a small incision (~1 mm) was cut behind the pars plana of the host eye, and the transplant (either retina or cortex) was placed into the subretinal space, in the superior nasal quadrant of the host, using a custom-made implantation tool.

Sham Surgery

The surgical procedures for the sham surgeries were identical with those used in transplantation with the exception that the tool did not contain any tissue when it was placed into the subretinal space.

Experimental Animals

Electrophysiological recordings were made in the SC of 29 albino RCS rats with retina/RPE transplants. Recordings also were made in four groups of age-matched controls: 12 normal rats (8 pigmented Long-Evans rats and 4 albino Sprague–Dawley); 6 nontransplanted albino RCS rats; 13 RCS rats with sham surgery and 3 RCS rats with fetal occipital cortex transplants (Table 1). Transplanted rats were 3.6 to 10.7 months of age at the time of recording, which was 1.4 to 8.4 months after transplantation. Control rats were 2 to 12.7 months of age. A subset of these rats (6 with sham surgery and 2 with retinal transplants) was recorded with the experimenters blind to their experimental group.

Verification of Transplant Placement

Placement of the transplants was evaluated after each surgery. The pupil was dilated with a corneal application of atropine, and a fundus examination was performed. In all animals, the pigmented transplant was localized to the nasal/dorsal quadrant of the albino host retina close to the optic disc. After electrophysiological recording, placement of the transplant also was verified histologically.

Electrophysiology

Surgical Preparation. Anesthesia was induced with 4% halothane mixed with room air, followed by an intraperitoneal injection of a mixture of xylazine/ketamine (37.5 mg/kg ketamine and 5 mg/kg xylazine) in sterile saline. A tracheostomy was performed to enable artificial ventilation. The femoral vein was cannulated for drug and saline infusions. Blood pressure was monitored via a cannula in the femoral artery, which was attached to a pressure transducer (model BP-1; World Precision Instruments [WPI], Sarasota, FL). During the recordings, the rats were paralyzed by a combination of pancuronium bromide (0.1 mg/kg/h) and curare (0.01 mg/kg/h) in saline (0.8 ml/h) and artificially ventilated with 1.0% to 2.0% halothane in 40% oxygen/60% nitrous oxide. The level of halothane was adjusted to maintain blood pressure between 60 and 80 mm Hg. The end-tidal CO_2 level was maintained at 2.8% to 3.1%. The pupils were dilated by topical application of 1% atropine sulfate, and the corneas were protected with artificial tear ointment. Each rat was mounted in a stereotaxic apparatus, a parietal craniotomy was performed, and the SC was visualized by removing the overlying cortex by suction.

Electrophysiological Recording. Multiunit visual responses were recorded extracellularly from the superficial laminae of the SC using commercially available metal electrodes (WPI) whose resistances were between 1.0 and 1.5 MΩ. The electrode was positioned at the surface of the SC using stereotaxic coordinates with references to

lambda and the edges of the exposed SC. In each animal, multiple electrode penetrations were performed, and the electrode was advanced through the SC using a hydraulic microdrive (Kopf Instruments, Tujunga, CA). The first penetration was positioned at the caudal end of the SC in a location that matched the topographic area of the retina containing the transplant. Subsequent penetrations moved rostrally by 200-μm steps until the anterior pole of the SC was reached. A second, parallel row of penetrations was positioned 200 μm laterally and moved from rostral to caudal. At each position, the electrode was lowered 100 μm beyond its point of contact with the surface of the SC, 16 to 32 presentations of a full-field visual stimulus were performed, and the responses were recorded using a digital data acquisition system (Powerlab; AD Instruments, Mountain View, CA). The electrode was then either lowered through the SC in 50-μm steps, and visual responses were noted or it was moved to continue to map the extent of the visually responsive area. Multiunit signals were recorded, amplified, and filtered from 200 and 16,000 Hz (Fintronics Bioamplifier, Orange, CT), displayed on a Tektronics model 5103 storage oscilloscope (Beaverton, OR), and monitored via an audio monitor. Blank trials, in which the photostimulator was blocked by a light-tight cover also were recorded to establish the baseline activity level at each site. On penetrations where no visual response could be elicited, activity was sampled up to a depth of 900 μm.

Visual Stimulation. A full-field strobe flash (1300 cd/m^2) was delivered to the eye using a photostimulator (model PS 22 Photic stimulator; Grass, West Warwick, RI), positioned 30 cm in front of the rat's eye. An interstimulus interval of 5 seconds was used.

Response Analysis. Input from two channels was simultaneously acquired: one channel represented the external trigger from the photostimulator and the second the extracellular visual signal elicited by the stimulus. A pretrigger, 100 msec before the onset of the light flash, initiated each 500-msec sweep. The onset of the visual response was defined as the point at which a clear, prolonged (>20 msec) increase in activity could be measured above the background activity. The latency of the response for each sweep was measured by positioning two cursors, one at the onset of the stimulus artifact and the second at the onset of the visual response. Mean latencies and SDs were computed both for each recording site, for all sites within each animal, and for all animals within each group. Peak response amplitudes were measured from the averaged sweeps at each visually responsive position in each animal. The peak response was defined as the largest excursion peak to peak in this response.

Histology

Tissue Processing. At the end of each recording experiment animals were perfused transcardially with saline followed by fixative. The eyes were removed, and the eyecups were postfixed and subsequently either embedded in epon or paraffin or frozen in Tissue-Tek. Transverse sections of the retina were cut, mounted onto slides, and stained with either hematoxylin–eosin or toluidine blue. A series of sections through the full extent of the transplant were evaluated by light microscopy.

S-antigen Immunohistochemistry. The presence of S-antigen immunoreactivity was analyzed in retinal paraffin sections from 12 rats that received retinal transplants (n = 10 rats with visual responses and n = 2 rats without visual responses), 8 rats that received sham surgery and 3 RCS rats without transplants (Table 1). Deparaffinized sections were washed with phosphate-buffered saline (PBS) and incubated for 30 minutes in 20% horse serum. The sections then were incubated with a monoclonal antibody against S-antigen (clone A9C6; gift of Larry A. Donoso[35]) at a dilution of 1:20,000 overnight at 4°C. After several washes with PBS, the binding of the primary antibody was detected using the Vector Elite ABC kit for mouse antibodies (Vector Laboratories, Burlingame, CA).

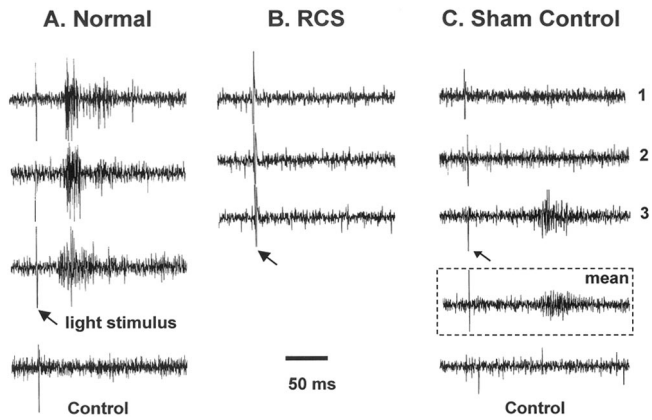

Figure 1. Representative multiunit electrophysiological recordings from the SC of controls. *Traces* are single sweeps (250-msec duration) from individual recording sites in the SC of three different rats in each control condition. *Arrows,* the stimulus artifact. (**A**) In the normal Long-Evans rats, short latency, visually evoked responses were elicited from sites over the entire SC. (**B**) In untransplanted RCS rats, all which were recorded at ≥4 months of age; no visual responses were recorded from any location in the SC. In addition, no visual responses were recorded in the SC of any of the RCS rats with fetal cortex transplants (data not shown). (**C**) In the majority of the rats with sham surgery, no visual responses were recorded from any site in the SC. *Traces 1* and *2*: individual sweeps from visually unresponsive locations in two rats. In 6 of 13 sham surgery controls, long-latency, weak visual responses were recorded; *trace 3*: individual sweep of a response and (mean) the average visual response from that location. Control: for each recording site, responses were recorded with the light source covered. No visual responses were elicited in this condition, which indicates that all other responses originate in the retina.

Results

Recordings were made from cells in the SC of RCS rats with retina/RPE transplants and also in age-matched controls, which included the following groups: normal rats (Long Evans or Sprague–Dawley), RCS rats without transplants, RCS rats with sham surgery performed, and RCS rats with fetal cortex transplants (Table 1; see Methods).

Normal Controls

Normal pigmented rats represent the control for the transplanted retina. In these rats, both single and multiunit visual responses were recorded in response to a full-field flash (Fig. 1A) and receptive fields were mapped onto a tangent screen, using a hand held lamp (Keeler, Broomall, PA). These data (not shown) provided our stereotaxic map and were essentially identical with published maps of the rat SC. Unpigmented rats represent the control for the RCS retina. There was no difference in the responses in the SC of the pigmented and unpigmented controls (see Fig. 4).

Rats with Fetal RPE/Retina Transplants

In 66% of the rats with transplants (19/29), a multiunit visual response could be evoked in a small, localized area of the SC (Figs. 2A, 2B and 3A, 3B) that corresponded to the topographic area of the retina containing the transplant (Figs. 2A and 3A). In 5 of these 19 rats, both SC were exposed and recordings were alternated in symmetrical positions in both hemispheres (Figs. 3A, 3B). No visually evoked responses were found anywhere in the ipsilateral SC, regardless of whether the transplanted or the nontransplanted eye was stimulated (Figs. 3A, 3B).

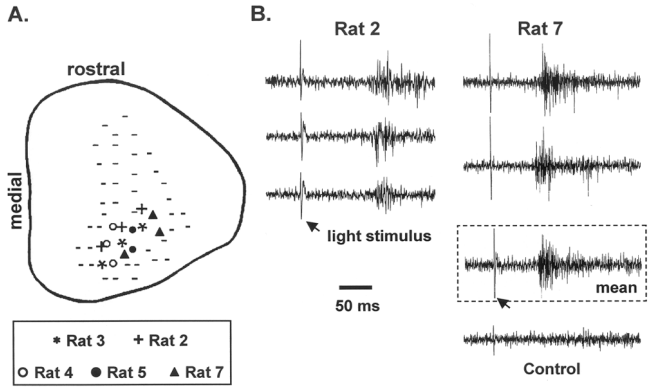

FIGURE 2. Representative multiunit electrophysiological recordings in visually responsive areas of the SC, contralateral to the eye that received a retina/RPE transplant. (**A**) Locations of all visually evoked responses elicited in five rats with retina/RPE transplants (distinguished by different symbols) and some of the surrounding recording sites from which no visual response could be elicited (−). The visually responsive areas in the SC in the caudal/lateral quadrants correspond to the retinotopic representation of the transplant in the nasal/dorsal retina. (**B**) *Traces* are single sweeps from several visually responsive recording sites in the SC in rat 2 and rat 7, recorded 3.5 to 4.8 months after transplantation, respectively. The onset of their multiunit visual response has a latency of 125 and 69 msec, respectively. An average of 16 sweeps at one location also is shown (mean). Conventions and control recordings are described in Figure 1.

Untransplanted and Sham Controls

In all the RCS rats without transplants (6/6; Fig. 1B), in all those with fetal cortex transplants (3/3), and in the majority of rats with sham surgery (7/13), no visual responses could be evoked in any portion of the SC. In all these rats, spontaneously active units were encountered in most penetrations. In 6 of 13 of the RCS rats with sham surgery, visually evoked responses could be detected in the SC (Fig. 1C, traces 1 and 2). These responses had small peak amplitudes and long latencies (Fig. 4), and the response latencies were less consistent. In 4 of these 6 rats, these visual responses were elicited over the entire dorsal surface of the SC. In the other 2 rats, visual responses were elicited within the visual projection of the surgical site.

Response Onset Latency

The latency of the onset of each visual response was determined for all groups of rats. Figure 4 plots the onset latency for individual recording sites as a function of its peak amplitude for normal pigmented and unpigmented controls (filled and open diamonds, respectively); transplanted rats (filled circles), and sham controls (open triangles). The latencies from normal controls were taken from sites in an area of the SC comparable to that in transplanted rats. In normal controls, onset latency ranged from 24 to 52 ms with a mean ± SD of 35 ± 8 ms, which is within the range of previously published data.[36,37] In contrast, onset latency in transplanted rats ranged from 67 to 103 ms, with a mean of 79 ± 11 ms and showed no overlap with normal controls. Onset latency in sham controls ranged from 66 to 166 ms with a mean of 118 ± 37 ms. Mean latencies also were computed for each animal, and an overall mean was computed. These values are similar to those computed over all cells. An ANOVA was performed and showed a significant group effect ($F = 79.3$; $P < 0.001$), and a post hoc test (Bonferroni) showed a significant difference between the rats with transplants and the sham controls ($P < 0.001$).

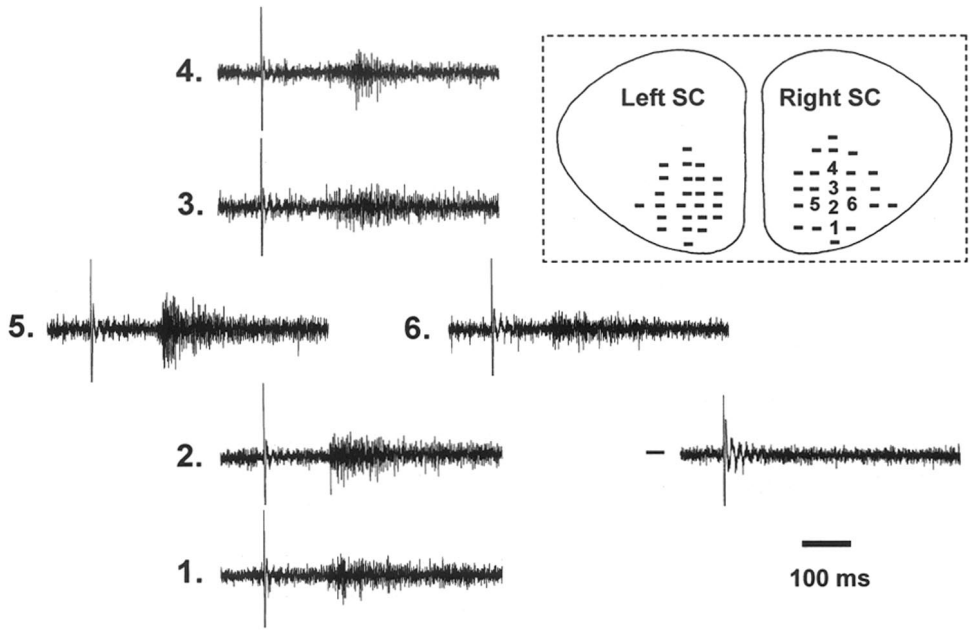

FIGURE 3. Representative multiunit electrophysiological recordings from the SC of one of the six RCS rats with a retina/RPE transplant in which both hemispheres were sampled. *Inset*: numbered locations (1 through 6) indicate visually responsive locations in the SC in an RCS rat, which was recorded 6.8 months after transplantation. The visually unresponsive sites surrounding these sites in the same hemisphere and the unresponsive sites from symmetrical locations in the opposite hemisphere also are indicated (−). Visual responses are confined to a small area of the SC, contralateral to the eye that received the transplant, which correspond to the retinotopic location of the transplant in the retina. In all other locations in this hemisphere and in all locations in the ipsilateral SC, no visual response could be evoked. (1 through 6) *Traces* are single sweeps from each of the visually responsive recording sites in the SC (number corresponds to location). One sweep (−) shows the typical activity recorded in a visually unresponsive location.

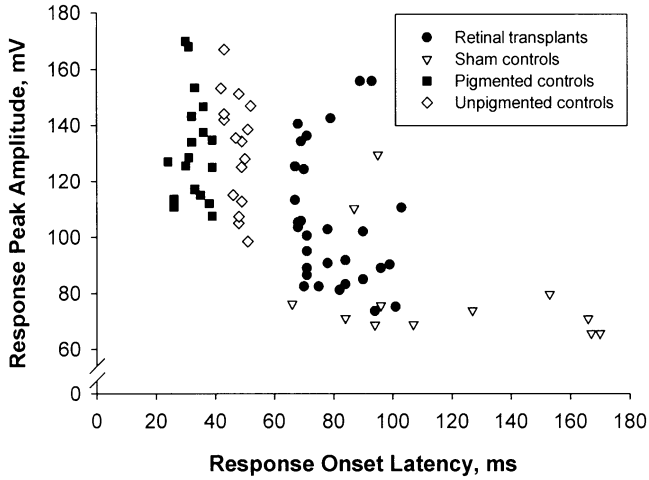

FIGURE 4. Scatter plot of the distribution of onset latency of the visual response plotted as a function of its peak amplitude. Peak amplitudes represent the amplified signal (800×) that was fed to the data acquisition/analysis programs. Data from responses at individual sites in 16 of 19 rats with retina/RPE transplants and visual responses (●; $n = 37$ sites), all 6 sham controls with visual responses (▽; $n = 12$ sites). Data from topographically matched areas in 8 normal, pigmented (■; $n = 18$) and 4 normal, unpigmented (◇; $n = 16$) controls. In three transplanted rats quantitative data were not available for analysis. Analyses of variance with subsequent post hoc comparisons showed that both onset latency and peak amplitude are significantly different in the RCS rats with sham surgery compared to rats with transplants ($P < 0.001$).

Peak Response

For each normal, transplant, and sham control rat, the peak amplitude of each response was determined and is plotted in Figure 4. In normal controls, peak responses ranged from 98.5 to 170 mV with a mean of 130 ± 19 mV. Peak responses in transplanted rats ranged from 75 to 156 mV with a mean of 108 ± 22 mV and in sham controls ranged from 66 to 130 mV with a mean of 80 ± 20 mV. An ANOVA showed a significant group effect ($F = 18.2$; $P < 0.001$), and a post hoc test (Bonferroni) showed that there was a significant difference between the rats with transplants and the sham controls ($P = 0.002$).

Consistency of Response Onset Latency

We assessed the consistency of the onset latency of the visual response by measuring the difference between its shortest and longest response latency within a given sequence of 16 stimulus presentations. In normal controls, the mean difference was 9 ± 3 ms. In rats with transplants, this difference was 14 ± 5 ms and in the sham controls the difference was 24 ± 4 ms. An ANOVA was performed, a significant group effect found ($F = 30.1$ $P < 0.001$), and a post hoc test (Bonferroni) showed a significant difference between the rats with transplants and the sham controls ($P < 0.001$).

Histologic Evaluation

Qualitative histologic evaluations of transverse retina sections were performed for all RCS rats with transplants, for 10 sham controls and for 2 cortical transplant controls. Transplants were examined for laminar and overall morphologic organization and whether their photoreceptors had developed outer segments. In addition, both the host retina of eyes with transplants and RCS control retinas were examined for the presence and number of S-antigen–positive cells, indicating the continued presence of host photoreceptor cells.

The retinal transplants exhibited a range of organizations that varied from well laminated to transplants containing rosettes (Figs. 5A through 5C). In general, the majority of animals with transplants that elicited a visual response maintained a normal laminar morphology that contained all cellular and synaptic layers. Cell bodies in the outer nuclear layer (ONL) of the transplants had characteristic photoreceptor morphology, although, their outer segments tended to be short or absent (Figs. 5B, 5C). Further, the inner retina of the host adjoining the transplant did not differ morphologically from the inner retina outside the transplant area and, in general, appeared intact (Fig. 5D). No defined photoreceptor layer could be found in the retinas of any of the controls (i.e., untransplanted, sham surgery, and cortical transplanted retinas), although scattered cells with morphologic characteristics of cones could be found. The density of S-antigen–reactive cells was examined in transverse sections of seven retinas with transplants and revealed no obvious differences in the density of cells (presumably residual cones) in the host retina inside versus outside the transplant area (Figs. 6A, 6B). In addition, no differences in the density of S-antigen–immunoreactive cells were observed in any of the RCS controls. In contrast, the transplants had large areas of immunoreactive photoreceptors in their outer nuclear layers.

DISCUSSION

In 66% of RCS rats transplanted with intact sheets of fetal retina/RPE into the subretinal space visually evoked activity was restored in the SC. In these rats, the visual responses were restricted to a small area of the SC that corresponds to the retinotopic location of the transplant. In the ipsilateral (control) SC, no visually evoked activity remained. In addition, visual responses could not be recorded in age-matched RCS rats without transplants, nor could visual activity be evoked if fetal cortex is transplanted instead of retina. Thus, the presence of a retina/RPE transplant was required to restore visually evoked activity in the SC. In 46% of our RCS rats with sham surgery alone, visual responses also were found. When compared with the responses recorded in rats with retina/RPE transplants, the responses in the sham surgery controls had significantly lower peak amplitudes and longer onset latencies, and these response latencies were relatively inconsistent. In addition, in most of these controls the responses also were not topographically organized.

In contrast to the rats with retinal/RPE transplants that showed visually evoked activity, in the other 34% we found no visual activity. The reason for this difference within our rats with transplants is unknown. As in previous studies,[29,30] the rats studied here, with and without visual activity, exhibited a range of morphologic organization, which varied from well laminated to those containing rosettes. In the majority of the retina/RPE transplanted rats, the transplants consisted of a normal lamination pattern and included an ONL with photoreceptors. In some, the photoreceptors also had outer segments. In the host retina there was no defined ONL, although lamination in the inner retina appeared intact.[38] The morphology of the inner retina of all the RCS rats, with and without transplants, with sham surgery and with fetal cortex transplants also was similar. A qualitative examination using S-antigen immunohistochemistry showed no differences in the numbers of positive cells in the host retina adjoining and outside the transplant area. Similarly, no obvious differences in the number of positive cells were seen across the three groups of RCS controls.

The presence of visual activity in some of our sham controls raises issues regarding the mechanisms underlying the visually

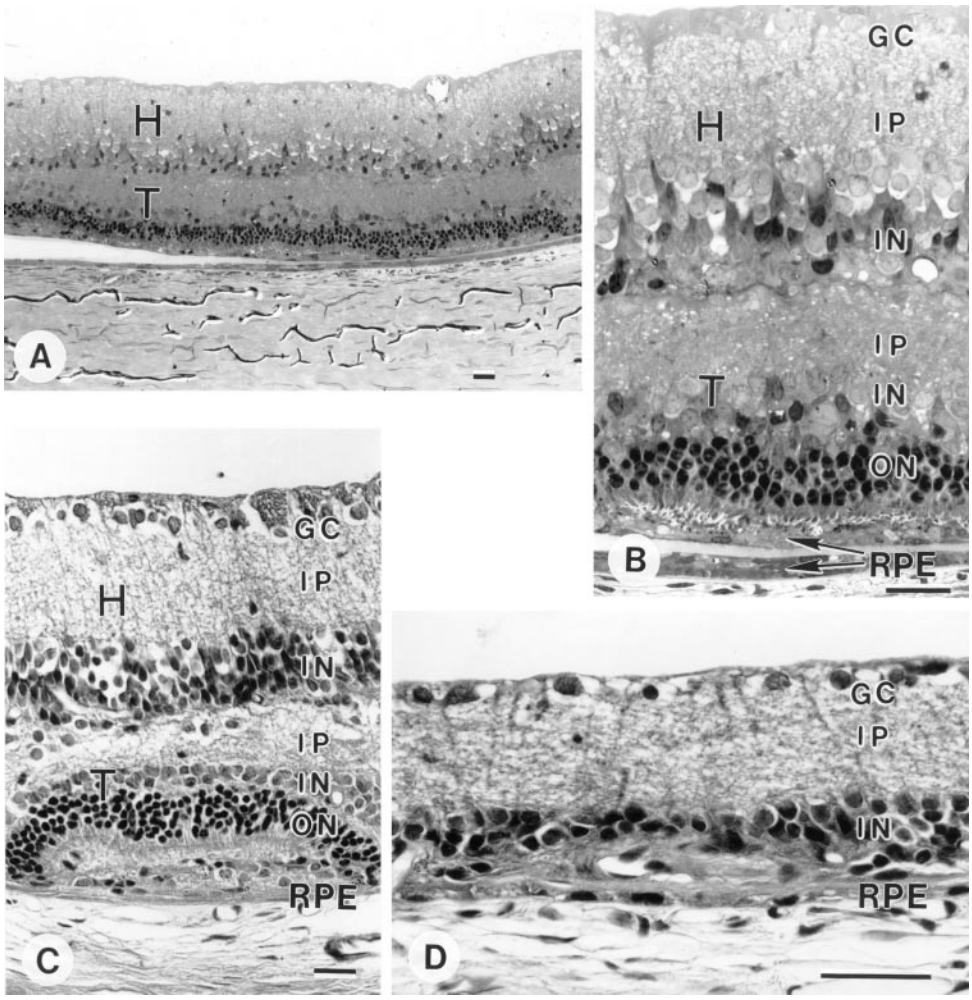

FIGURE 5. Morphology of retinas transplanted with an E19 rat retina/RPE transplant in two RCS rats with visual responses recorded in the SC. (**A**) Low-power photomicrograph of a 1-μm-thick epon section, stained with toluidine blue for rat 2, 3.5 months after transplantation (see Fig. 2B for recordings). A large area of a transplant (T) that exhibits good lamination is shown along with its (H) host retina. (**B**) Enlargement of a portion of the photomicrograph shown in (**A**). Note the integration between transplant and host retinas and that transplant photoreceptors have short outer segments. *Arrows,* the transplant and host RPE, which are separated by a narrow gel-filled space. (**C**) Photomicrograph of an 8-μm-thick paraffin section, stained with hematoxylin-eosin in rat 6, 4.7 months after transplantation. This transplant, although relatively small, also was well laminated, and its photoreceptors had short outer segments. (**D**) High-power photomicrograph of the retina shown in (**C**) outside the area of the transplant. An outer nuclear layer in the host retina is not apparent, although other retinal lamina are evident. GC, ganglion cell layer; IP, inner plexiform layer; IN, inner nuclear layer; ON, outer nuclear layer; RPE, retinal pigment epithelium. Scale bars, 20 μm.

evoked responses in the SC of transplanted rats. A key question is whether the presence of the transplant maintains already existing connections in the host by means of a trophic factor or whether the transplant restores function by formation of new synapses with the host. The presence of visually evoked responses in the sham controls suggests that surgical intervention, via trophic factor release, can maintain visual responses in the SC. Previous transplantation studies also have shown that surgery itself effects the rate of photoreceptor degeneration or loss of visual function.[17,18] This effect of surgical intervention is probably related to the induction of trophic factor expression.[39] In addition, both in vitro[40] and in vivo[14,16,28,41,42] studies indicate that RPE, Schwann cell, and dissociated rod photoreceptor transplants provide trophic factors that delay or arrest the degeneration of host photoreceptors or of visual function. The differences that we observe in both the quantitative and qualitative aspects of the visual responses between the sham controls and rats with retinal transplants suggest that the presence of functional connections between the transplant and the host retina or a factor released from the transplant is responsible for the more robust visual responses. Li and Turner[20] showed that the timing of RPE transplantation into

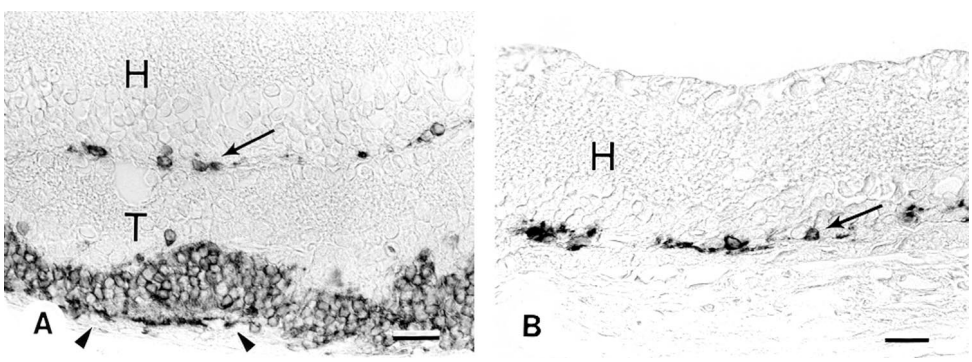

FIGURE 6. Representative example of S-antigen immunoreactivity in sections through the retina of rat 6 (see also Fig. 5C). (**A**) In this retina, numerous S-antigen–positive cells are found in the outer nuclear layer of the transplant (T), whereas a small number of S-antigen–positive cells, presumably cones (*arrow*) are found in the host (H) along its border with the transplant. In the transplant, the S-antigen–positive cells had short outer segments (area between *arrowheads*). (**B**) In the same retina, outside the area with the transplant, scattered S-antigen–positive cells (*arrow*) can be seen along its border with the RPE. No obvious differences in the density of S-antigen–positive cells were seen in the host retina within and outside the area of the transplant. Thus, there is no evidence of host photoreceptor rescue by the transplant. Scale bars, 20 μm.

RCS rats is a critical factor in delaying the normal course of photoreceptor degeneration. After 38 days of age, RPE transplantation had no effect. Our RCS rats received fetal retina/RPE transplants at 37 to 69 days of age. Therefore, it is unlikely that the response in the SC of the transplanted rats is a simple delay of degeneration in the host related to the presence of the transplant. In addition, in our transplantation model this effect is not reflected in host photoreceptor salvage, again suggesting restoration via an increase in synaptic efficacy of the host circuitry. Thus, the visual responses in the SC are most likely to result from functional interactions between the transplant and existing host circuitry. This hypothesis is supported further by the observation that cells in the transplant are labeled after injections of a retrogradely and transynaptically transported pseudorabies virus[43] into the visually responsive area of the SC of transplanted rats.[33] The existence of physical connections between a retinal transplant and a normal host retina also has been demonstrated in a rabbit model.[44] An alternative explanation for this result is that the transplants send axons through the host optic nerve directly to the SC. The former explanation, however, is more likely for several reasons. First, the visual response in the SC in our transplanted rats is topographically organized. In contrast, projections to the SC from either fetal retina transplanted to the brain[45] or from retinal ganglion cell axons regenerating through a peripheral nerve graft to the SC are not topographically organized.[46–48] Second, the response properties that we record in the SC of transplanted rats, while significantly different from normal rats are considerably more robust than the responses in the sham controls. Taken together these data provide stronger support for the hypothesis that visual activity in the SC is restored as a result of the presence of the retinal transplant and its connections to the host retina, rather than a simple trophic effect. Another explanation for the restored response is a release of excitatory neurotransmitter from the transplant that stimulates cells in the host extrasynaptically. We believe this scenario is unlikely because the neurotransmitter reuptake system in the transplant should remove the transmitter before it can diffuse to the host retina. If the reuptake system was defective, then transmitter release would produce a prolonged excitatory response in both the transplant and the host retina, which should produce excitotoxicity and retinal degeneration. We have not observed either lengthened visual responses in transplanted rats or any nonspecific degeneration.

The presence of the retinal transplant in the eye causes the restoration of the visual responses in the SC either by direct synaptic connections with the host retina or via specific retinal trophic factors that enhance remodeling of the host circuitry or a combination of the two. This could occur by a mechanism similar to that shown in two other models of retinal degeneration.[7,8] At this point, our data support the hypothesis that the visual responsiveness results from an increase in synaptic efficacy due to connections from the transplant to the host. However, these data cannot definitively prove that only one mechanism is responsible.

To overcome the complication of the effect of surgical intervention in the RCS rats, we are currently investigating the use of another model of retinal degeneration, the S334-ter line 3 transgenic rat for use in this transplantation paradigm. In this model, the degeneration results from the photoreceptor-specific expression of a truncated form of human rhodopsin,[49,50] rather than the RPE defect in the RCS rat. Preliminary data indicate that in S334-ter line 3 transgenic rats with transplants visual responses similar to those reported here are seen, but sham surgery does not result in any visually evoked responses in the SC in these rats.[51]

Although we have demonstrated that RCS rats with retina/RPE transplants show significantly better responses in the SC than in rats with sham surgery, we cannot predict the level of visual function that will be associated with these responses. It is likely that the increased responsiveness found in the animals with transplants will be reflected in their heightened ability to discriminate light and dark over that found in RCS rats either without transplants or with sham surgery. Should this prove to be the case, retinal transplantation could increase light perception in individuals with advanced photoreceptor degeneration, which could significantly improve their quality of life.

Acknowledgments

The authors thank Nigel Cooper, Ronald G. Gregg, Henry Kaplan, and Neal Peachey for critically reviewing the manuscript; Lyndsay Tucker, Kelly Volk, Marija Sasek, and Miranda Messer for their technical assistance; and Norman D. Radtke (Audubon Hospital, Louisville) for his interest and support.

References

1. Stone J, Maslim J, Valter-Kocsi K, et al. Mechanisms of photoreceptor death and survival in mammalian retina. *Prog Retin Eye Res.* 1999;18:689–735.
2. Voaden MJ. Retinitis pigmentosa and its models (Abstract). *Prog Retin Eye Res.* 1990;10:293.
3. Papermaster DS, Windle J. Death at an early age. Apoptosis in inherited retinal degenerations. *Invest Ophthalmol Vis Sci.* 1995; 36:977–983.
4. Fletcher EL, Kalloniatis M. Neurochemical architecture of the normal and degenerating rat retina. *J Comp Neurol.* 1996;376:343–360.
5. Hanitzsch R, Zeumer C, Lichtenberger T, Wurziger K. Impaired function of bipolar cells in the Royal College of Surgeons rat. *Acta Anat.* 1998;162:119–126.
6. McCall MA, Gregg RG, Merriman K, Goto Y, Peachey NS, Stanford LR. Morphological and physiological consequences of the selective elimination of rod photoreceptors in transgenic mice. *Exp Eye Res.* 1996;63:35–50.
7. Peng Y-W, Hao Y, Petters RM, Wong F. Ectopic synaptogenesis in the mammalian retina caused by rod photoreceptor-specific mutations. *Nat Neurosci.* 2000;3:1121–1127.
8. Strettoi E, Pignatelli V. Modifications of retinal neurons in a mouse model of retinitis pigmentosa (In Process Citation). *Proc Natl Acad Sci USA.* 2000;97:11020–11025.
9. Inglehearn CF. Molecular genetics of human retinal dystrophies. *Eye.* 1998;12:571–579.
10. Cayouette M, Gravel C. Adenovirus-mediated gene transfer of ciliary neurotrophic factor can prevent photoreceptor degeneration in the retinal degeneration (*rd*) mouse. *Hum Gene Ther.* 1997;8: 423–430.
11. Chong NH, Bird AC. Management of inherited outer retinal dystrophies: present and future. *Br J Ophthalmol.* 1999;83:120–122.
12. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. *Nature.* 1990;347:83–86.
13. Li LX, Turner JE. Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. *Exp Eye Res.* 1988;47:911–917.
14. Lopez R, Gouras P, Kjeldbye H, et al. Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. *Invest Ophthalmol Vis Sci.* 1989;30:586–588.
15. Rezai KA, Kohen L, Wiedemann P, Heimann K. Iris pigment epithelium transplantation. *Graefes Arch Clin Exp Ophthalmol.* 1997; 235:558–562.
16. Lawrence JM, Sauve Y, Keegan DJ, et al. Schwann cell grafting into the retina of the dystrophic RCS rat limits functional deterioration. Royal College of Surgeons. *Invest Ophthalmol Vis Sci.* 2000;41: 518–528.

17. Humphrey MF, Chu Y, Mann K, Rakoczy P. Retinal GFAP and bFGF expression after multiple argon laser photocoagulation injuries assessed by both immunoreactivity and mRNA levels. *Exp Eye Res.* 1997;64:361–369.
18. Wen R, Song Y, Cheng T, et al. H. Injury-induced upregulation of bFGF and CNTF mRNAs in the rat retina. *J Neurosci.* 1995;15: 7377–7385.
19. Schraermeyer U, Kociok N, Heimann K. Rescue effects of IPE transplants in RCS rats: short-term results. *Invest Ophthalmol Vis Sci.* 1999;40:1545–1556.
20. Li L, Turner JE. Optimal conditions for long-term photoreceptor cell rescue in RCS rats: the necessity for healthy RPE transplants. *Exp Eye Res.* 1991;52:669–679.
21. Klassen H, Lund RD. Retinal graft-mediated pupillary responses in rats: restoration of a reflex function in the mature mammalian brain. *J Neurosci.* 1990;10:578–587.
22. Lund RD, Coffey PJ. Visual information processing by intracerebral retinal transplants in rats. *Eye.* 1994;8:263–268.
23. Radel JD, Kustra DJ, Das S, Elton S, Lund RD. The pupillary light response: assessment of function mediated by intracranial retinal transplants. *Neuroscience.* 1995;68:909–924.
24. Radel JD, Kustra DJ, Lund RD. The pupillary light response: functional and anatomical interaction among inputs to the pretectum from transplanted retinae and host eyes. *Neuroscience.* 1995;68: 893–907.
25. Adolph AR, Zucker CL, Ehinger B, Bergstrom A. Function and structure in retinal transplants. *J Neural Transplant Plast.* 1994; 5:147–161.
26. 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.
27. Silverman MS, Hughes SE, Valentino TL, Liu Y. Photoreceptor transplantation: anatomic, electrophysiologic, and behavioral evidence for the functional reconstruction of retinas lacking photoreceptors. *Exp Neurol.* 1992;115:87–94.
28. Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Exp Neurol.* 1999;159:21–33.
29. Seiler MJ, Aramant RB. Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest Ophthalmol Vis Sci.* 1998;39: 2121–2131.
30. 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.
31. 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.
32. Seiler MJ, Aramant RB, Ball SL. Bipolar and ganglion cells in intact-sheet retinal transplants–potential transplant/host interactions [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1999;40(4):**S**730. Abstract nr 3858.
33. Aramant RB, Seiler MJ, Woch G. Retinal transplants form synaptic connections with host retinas with photoreceptor degeneration–demonstrated by transsynaptic tracing from host brain [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2000;41(4):**S**101. Abstract nr 528.
34. Siminoff R, Schwassmann HO, Kruger L. An electrophysiological study of the visual projection to the superior colliculus of the rat. *J Comp Neurol.* 1966;127:435–444.
35. 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.
36. Dyer RS, Annau Z. Flash evoked potentials from rat superior colliculus. *Pharmacol Biochem Behav.* 1977;6:453–459.
37. Hetzler BE, Melk AM. Effects of ketamine, naloxone, and physostigmine on flash evoked potentials in rat superior colliculus. *Pharmacol Biochem Behav.* 1989;32:511–518.
38. Mullen RJ, LaVail MM. Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. *Science.* 1976;192:799–801.
39. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Basic fibroblast growth factor and local injury protect photoreceptors from light damage in the rat. *J Neurosci.* 1992;12:3554–3567.
40. 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.
41. Li L, Sheedlo HJ, Turner JE. Retinal pigment epithelial cell transplants in retinal degeneration slow mice do not rescue photoreceptor cells. *Invest Ophthalmol Vis Sci.* 1993;34:2141–2145.
42. Sauve Y, Klassen H, Whiteley SJ, Lund RD. Visual field loss in RCS rats and the effect of RPE cell transplantation. *Exp Neurol.* 1998; 152:243–250.
43. Aston-Jones G, Card JP. Use of pseudorabies virus to delineate multisynaptic circuits in brain: opportunities and limitations [In Process Citation]. *J Neurosci Methods.* 2000;103:51–61.
44. 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.
45. Lund RD, Hankin MH, Sefton AJ, Perry VH. Conditions for optic axon outgrowth. *Brain Behav Evol.* 1988;31:218–226.
46. Vidal-Sanz M, Bray GM, Villegas-Pérez MP, Thanos S, Aguayo AJ. Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. *J Neurosci.* 1987; 7:2894–2909.
47. Vidal-Sanz M, Bray GM, Aguayo AJ. Regenerated synapses persist in the superior colliculus after the regrowth of retinal ganglion cell axons [published erratum appears in *J Neurocytol.* 1992;21:234]. *J Neurocytol.* 1991;20:940–952.
48. Sauve Y, Sawai H, Rasminsky M. Functional synaptic connections made by regenerated retinal ganglion cell axons in the superior colliculus of adult hamsters. *J Neurosci.* 1995;15:665–675.
49. Steinberg RH, Flannery JG, Naash M, et al. Transgenic rat models of inherited retinal degeneration caused by mutant opsin genes [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1996;37(3):**S**698. Abstract nr 3190.
50. Steinberg RH, Matthes M, Yasamura D, et al. Slowing by survival factors of inherited retinal degenerations in transgenic rats with mutant opsin genes [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):**S**226. Abstract nr 1069.
51. Sagdullaev BT, Aramant RB, Seiler MJ, Woch G, , et al. . Retinal transplantation restores visual responses in a transgenic rat model of retinal degeneration. *Soc Neurosci Abstr.* 2000;26:567.