Schwann Cell Grafting into the Retina of the Dystrophic RCS Rat Limits Functional Deterioration

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Purpose. To examine whether congenic Schwann cells grafted into the subretinal space of dystrophic Royal College of Surgeons (RCS) rats can prevent photoreceptor loss and maintain visual function.

Methods. Purified neonatal Schwann cells derived from congenic rats were grafted into the subretinal space of 3- to 4-week-old dystrophic RCS rats. Graft placement was confirmed using Schwann cells labeled in vitro with the fluorescent dye Hoechst 33342 or in grafted eyes processed for electron microscopy (48-hour to 1-month survival). At longer intervals, up to 9 months after surgery, animals were examined for photoreceptor survival; preservation of a visual reflex, head-tracking to moving stripes; and preservation of visual receptive fields associated with the region of graft placement.

Results. One week after the graft was performed, Schwann cells had integrated into the subretinal space with little evidence of a reactive response. When screened for head-tracking to moving stripes, Schwann cell–grafted animals performed better than sham-treated or control dystrophic animals. Threshold sensitivity measurements and visual field assessment made by recording from the superior colliculus also showed a significant level of preserved function compared with control animals. Functional rescue was correlated with photoreceptor survival and could be observed for at least 9 months after grafting.

Conclusions. Schwann cells injected into the subretinal space limit functional deterioration and prolong photoreceptor survival. It is suggested that they act by local release of growth factors that either support photoreceptors directly and/or stimulate phagocytosis in RPE cells. (*Invest Ophthalmol Vis Sci.* 2000;41:518–528)

The dystrophic Royal College of Surgeons (RCS) rat shows a progressive loss of photoreceptors that is most marked during the first 3 months of life[1] and is due primarily to a failure of retinal pigment epithelial (RPE) cells to phagocytose shed outer segments efficiently.[2,3] Rod photoreceptor death is initially rapid,[1,4] but cones are also lost, although at a slower rate.[5] With time, secondary degenerative events occur including alterations to the vascular supply to the retina (e.g.,[6–11]), leading to retinal ganglion cell loss and disruption of the inner retina.[10,11] Apart from these anatomic changes, there is a diminution of the pupillary light reflex (PLR),[12] development of a relative scotoma recorded in the superior colliculus,[13,14] and other changes in visual responsiveness.[15] Transplantation of normal RPE cells into the dystrophic RCS retina at an early age has been shown to result in local rescue of photoreceptors[16,17] and to facilitate some degree of functional recovery.[14,18,19] Although the use of nondystrophic RPE cells to replace equivalent defective cells would be the most direct for repair strategies, there are a number of difficulties including providing a plentiful source of cells at the appropriate time, screening for infections, and preventing immune rejection. Nonretinal cell sources that have already been investigated include iris pigment epithelial cells in retinal transplantation studies,[20] and in the central nervous system, grafts of fibroblasts transfected with growth factors have been shown to prevent neuronal degeneration (e.g., Reference 21).

An alternative to cell transplantation has been the injection of growth/survival factors directly into the retinas of dystrophic RCS rats,[22,23] light-damaged rats,[24,25] and various mutant mouse strains with inherited retinal degeneration.[26] These studies reported rescue of photoreceptors in RCS and light-damaged rats, but more variable results in mice, with some mouse strains responding better than others. The mechanism by which the survival of these photoreceptors is regulated by growth factors has yet to be explored. The problem with injection of factors is that physiological doses may not be achieved,[26] and a single injection may be inadequate to give prolonged results. Furthermore, excessive doses of growth factors—for example, basic fibroblast growth factor—can in-

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duce neovascularization and cataract formation.[23] Multiple injections may cause significant damage in their own right. Another approach has been to inject the adenoviral vector Ad-ciliary neurotrophic factor intravitreally into the *rds* mouse eye.[27] This technique has also been shown to prevent photoreceptor death and to increase the responsiveness of rod photoreceptors.

In the present study, we used another approach that combines cell grafting and growth factor delivery by transplanting syngeneic Schwann cells subretinally. Schwann cells produce several factors necessary for photoreceptor survival, including basic fibroblast growth factor,[28] ciliary neurotrophic factor,[29] and brain-derived neurotrophic factor.[30] As a result, they may serve to deliver physiological levels of multiple growth factors in the immediate vicinity of the subretinal space. A ready supply of Schwann cells can be generated from the recipient's own tissues, overcoming the logistic, ethical, and immune complications associated with obtaining RPE cells from donor eyes. We did not use autografts in this experiment, because highly purified populations of Schwann cells (virtually free of fibroblasts[31]) can be more readily generated from early postnatal donors.

METHODS

Donor Cells

Purified neonatal Schwann cells were cultured from the sciatic nerves of congenic neonatal (postnatal day [P]1–P2) RCS rats using a method described in Brook et al.[31] that was modified from that of Brockes et al.[32] The cells were grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Paisley, UK) plus 10% fetal calf serum (Life Technologies). Some cultures were labeled in vitro with filter-sterilized Hoechst 33342 (Sigma, Poole, UK) in DMEM (7.5 μg/ml) for 4 minutes at 37°C and then thoroughly washed in DMEM plus fetal calf serum.

Fibroblasts (208F, a 3t3-like RAT-1 cell line originating from Fischer rats and obtained from the European Collection of Animal Cell Cultures, Porton Down, UK) were grown in the same medium and used as a comparative cell implant for anatomic analysis.

Both cell types were resuspended in DMEM, without serum but with DNase (Type IV; Sigma), before grafting. DNase reduced cell aggregation in suspension by digesting the sticky DNA released from dead cells. The DMEM–DNase medium, without cells, was also used for sham injections.

Transplantation Procedure

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, Home Office (UK) regulations for the care and use of laboratory animals, and the U. K. Animals (Scientific Procedures) Act (1986). Dystrophic pigmented RCS rats (N = 86; 3–4 weeks old) were used as hosts in this study. They were anesthetized with tribromoethanol (230 mg/kg, intraperitoneally), and a further topical anesthetic (Ophthaine; 0.5% proxymetacaine hydrochloride; E. R. Squibb, Hounslow, UK) was applied to the eye. The pupil was dilated using tropicamide (1% Mydriacyl; Alcon, Hemel Hempstead, UK) and the eye proptosed slightly using a broad rubber band. A suture was used to stabilize the eye, and cells or the carrier medium was injected into the subretinal space (dorsotemporal retina) by means of either a 30-gauge steel needle or a fine glass capillary attached by tubing to a 10-μl Hamilton syringe (Wilmad, Reno, NV).

The study was divided into two parts. The first was designed to establish graft placement in, and integration with, the host retina in the early posttransplantation period. Fifteen animals received 2×10^4 Schwann cells in 2 μl carrier medium to the right eye alone (with or without Hoechst label) or sham injections (identical volume of carrier medium including DNase) to the right eye only. For animals to be processed for electron microscopy (i.e., those without Hoechst label) the site of the graft was marked by a suture in the sclera placed just anterior to the site of injection.

The second study was conducted in two parts and designed to assess changes in visual function over time, correlating the results with anatomic preservation. In the first (pilot) part of this study, 11 animals received 2×10^4 Schwann cells in 2 μl carrier medium (high-dose) injections of Schwann cells and 5 had 2×10^3 Schwann cells in 2 μl carrier medium (low-dose) injections into one or both eyes. Fibroblasts (2×10^4) were injected subretinally into one or both eyes of 10 animals, and 8 had sham injections of carrier medium (including DNase) to both eyes. Unoperated eyes served as control eyes for the degree of dystrophy. Animals were screened using PLR, a measure of minimal visual function (described in Reference 12), before electrophysiological assessment for area of visual field rescue and changes in threshold sensitivity. Eyes were processed for anatomic examination at the end of the experiment. In the second part of the study, head-tracking to moving stripes was used as another noninvasive test to screen for transplantation effects. For this experiment, 25 animals received Schwann cell injections to the right eye (high dose, n = 16; low dose, n = 9). Twelve animals had carrier medium plus DNase injected into the right eye. This is an ongoing study, but threshold sensitivity maps have been constructed for some of the animals and the eyes processed for histology.

Experimental Procedures

Anatomy. Animals used for short-term localization of grafts after transplantation received an overdose of sodium pentabarbitone (Euthatal, Rhône Mérieux, Harlow, UK; 200 mg/ml), perfused intracardially with phosphate-buffered saline (PBS) and the eyes removed and frozen in optimal cutting temperature compound (OCT; Tissue-Tek, Miles, Raymond Lamb, London, UK) in liquid nitrogen vapor before sectioning. Survivals were for 48 hours, 1 week, 2 weeks, and 1 month. To confirm that the Hoechst-labeled cells were Schwann cells, the sections were postfixed briefly in 95% alcohol, reacted with monoclonal low-affinity NGFr antibody (1:5; Boehringer–Mannheim, Lewes, UK) overnight at 4°C, and visualized with anti-mouse IgG TRITC-F(ab′)$_2$ conjugate (1:64; Sigma).

For electron microscopy, animals were anesthetized as described, and the eyes were injected directly with 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer plus 0.1% picric acid using a 30-gauge needle. A puncture in the front of the eye provided a drainage hole and relieved any resultant increase in pressure. Immediately after this, the animal was perfused transcardially with PBS followed by the aldehyde mixture. The eyes were excised and left in the same fix overnight. After it was washed in 0.1 M phosphate buffer, the retina was postfixed in 1% osmium tetroxide in

buffer, dehydrated through graded alcohols and propylene oxide to Agar resin (Agar Scientific, Stansted, UK).

After functional testing, rats were given an overdose of sodium pentabarbitone, and perfused intracardially with PBS followed by periodate-lysine-paraformaldehyde.[33] The eyes were excised, embedded in polyester wax, and processed for immunohistochemistry and routine histology.[11]

After blocking with 5% defatted milk in PBS, sections were reacted with monoclonal Rat-401 (for Schwann cells,[34] 1:5; developed by Susan Hockfield and supplied by the Developmental Studies Hybridoma Bank maintained by the University of Iowa, Department of Biological Sciences, Iowa City, under contract NO1-HD-7-3263); monoclonal anti-rhodopsin, N-terminal, 4D2, (for photoreceptors, 1:3000; the generous gift of Robert Molday, University of British Columbia, Vancouver, Canada); monoclonal anti-phosphotyrosine (for microglia, 1:400; Sigma); and polyclonal anti-fibronectin (to indicate the position of fibroblasts, 1:400; Sigma). Antibodies were visualized with appropriate kits (ABC Elite; Vector Laboratories, Peterborough, UK), 1% nickel chloride and 3-3′ diaminobenzidine (Sigma, UK). Sections were counterstained with 0.05% aqueous thionine before they were dehydrated through a graded series of alcohols and mounted (DePeX; Merck–BDH, Lutterworth, UK). Negative control sections were incubated in defatted milk overnight before proceeding with the secondary antibodies as described.

In Vivo Screening of Visual Function. As mentioned above, the PLR was used in the pilot study to screen for visual function. Although pupillometry showed transplant-associated rescue, the high level of sensitivity of this test yielded more variable results, and transplant versus sham effects could not be distinguished until 6 months after transplantation. In the subsequent series head-tracking was used because sham effects were lost by 4 months of age. Accordingly, only the data from head-tracking are presented here.

The head-tracking method is based on an optokinetic test devised by Cowey and Franzini.[35] Animals were placed individually into an enclosed clear plastic container, surrounded by a motorized drum that could revolve clockwise or counterclockwise. Vertical black-and-white lines of three varying widths, subtending 0.125, 0.25, and 0.5 cyc/deg were presented (in a randomized order) to the animal and rotated alternately clockwise and counterclockwise, each for 60 seconds. This stimulated a subcortical reflex, so that a seeing animal involuntarily turned its head, tracking the moving lines. All animals were videotaped so that movements could be scored later. Animals were scored only when the speed of the head turn corresponded with the speed of rotation of the stripes. Habitual and other seemingly randomized movement was excluded from the timing. Light levels were kept constant, and the plastic container was cleaned between animals. Testing was performed over 3 consecutive days by a single observer, and each animal was tested at two different time points.

Results were analyzed using a two-way analysis of variance with one repeated measure.

Electrophysiology

Based on performance during initial screening tests, a number of animals were also studied electrophysiologically (recording from the superior colliculus to define the extent of preserved visual field). Details of the methods used are given elsewhere.[14] Briefly, under urethane anesthesia (1.25g/kg) the test eye was immobilized using 6-0 sutures attached to the frame, and the pupil was dilated with topical tropicamide as before. Corneal clouding was prevented using a noncorrective contact lens. Animals were dark adapted for 1 hour at 0.34 candelas (cd)/m^2 (mesopic range) before recording from the superficial layers of the superior colliculus contralateral to the experimental eye. Single and multiunit recordings were made covering the full extent of the superior colliculus along a rectilinear grid of 200 μm periodicity. In the pilot study, a receptive field (RF) was first defined (180 cd/m^2, spot illumination), and then a light spot (1-second duration) was presented to its center. Presence or absence of responsiveness to standard illumination (1.5 log elevation from background; i.e., 5.8 cd/m^2) was recorded by assembling poststimulus time histograms (5-msec bins) over 30 consecutive stimuli, using interstimulus intervals varying from 3 to 20 seconds to minimize adaptation. Using this defined set of luminance conditions, the scale of the visual field deficit was mapped and showed the development of a partial scotoma.

To assess quantitatively the level of functional rescue across the visual field, a more sensitive test was performed on animals from the head-tracking series. In these, the threshold sensitivity was recorded at each point. This allowed measurement of exact threshold similar to perimetry testing in humans. To obtain field positions at the beginning of the experiment, a light spot 4.5 log units above a baseline luminance of 0.02 cd/m^2 was used. Thereafter, to minimize adaptation, light sources of just above threshold were used to test RF location. From this point the luminance was reduced until threshold was reached. Compilation of visual thresholds at each point produced a map encompassing the whole superior colliculus, presented as the corresponding retinal representation.

All eyes were processed for immunohistochemistry after functional testing.

Results

Anatomic Observations

The location of donor cells and their relation to the cells of the host retina after transplantation was established using the Hoechst-labeled material and the retinas prepared for electron microscopy. Figure 1a shows clusters of Hoechst-labeled cells in the subretinal space 48 hours after surgery. Because it was clear that there was a spread of label into the adjacent photoreceptors and probably the RPE (even though the cells were thoroughly washed in fresh medium after incubation in the dye), a second label was used to confirm donor cell identity. For this purpose low-affinity NGFr antibody was used to identify Schwann cells (Fig. 1b). Using both labels, the clustered cells lying between the photoreceptors and the RPE were identified as aggregated Schwann cells. By 1 week (Figs. 2a, semithin; 2b, electron micrograph) the grafted cells were distributed in a more linear array between the RPE and the photoreceptors, with little indication of any immune or reactive response, and they appeared to be well integrated. There was some deposition of matrix around the cells, and collagen was also present. The latter was presumably produced by intrusive fibroblasts, introduced at the time of grafting either as contaminants in the Schwann cell culture or carried from the sclera by the micropipette. Outer segments were missing from

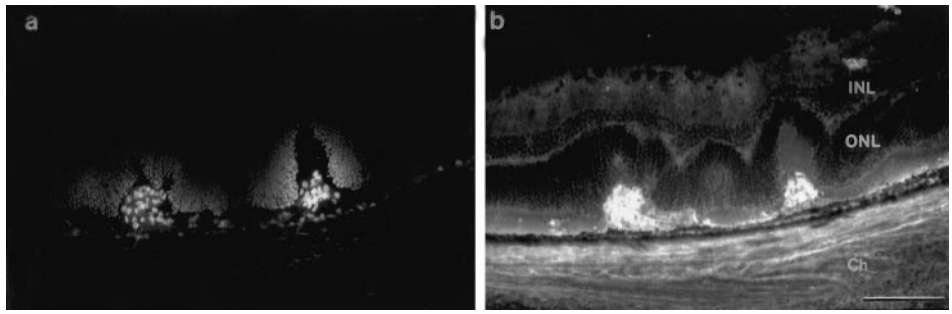

FIGURE 1. Transverse section of dystrophic RCS rat retina 48 hours after transplantation of Schwann cells into the subretinal space. (**a**) Two clusters of Hoechst-labeled Schwann cells. Note spread of dye into photoreceptors and RPE; (**b**) same section reacted with low-affinity NGFr and TRITC. Scale bar, 100 μm. INL, inner nuclear layer; ONL, outer nuclear layer; Ch, choroid.

photoreceptors immediately deep to the graft, but they were present on those photoreceptors adjacent to the graft.

Photoreceptor survival was prolonged by Schwann cells, fibroblasts, and the effects of sham surgery (Figs. 3a, 3b, 3c), compared with results in non–surgically treated dystrophic animals in all the specimens studied at 1.5 to 3 months. The area of rescue was extensive in Schwann cell–grafted retinas (Fig. 3a), and the outer nuclear layer was three to six cells deep. The orderly arrangement of photoreceptors was sometimes disrupted, especially close to the site of injection. In general, Schwann cell–grafted animals showed better preservation of the photoreceptor layers. Inner segments could be identified readily (Fig. 3a), and the presence of outer segments was suggested in rhodopsin-stained sections. This was confirmed by electron microscopy. The survival effect was much more localized in both fibroblast- and sham-injected animals (Figs. 3b, 3c). A comparable area of the dorsal retina in an untreated dystrophic animal of equivalent age is shown in Figure 3d. Only isolated photoreceptors remained, and a zone of debris filled the region between the inner nuclear layer and the RPE.

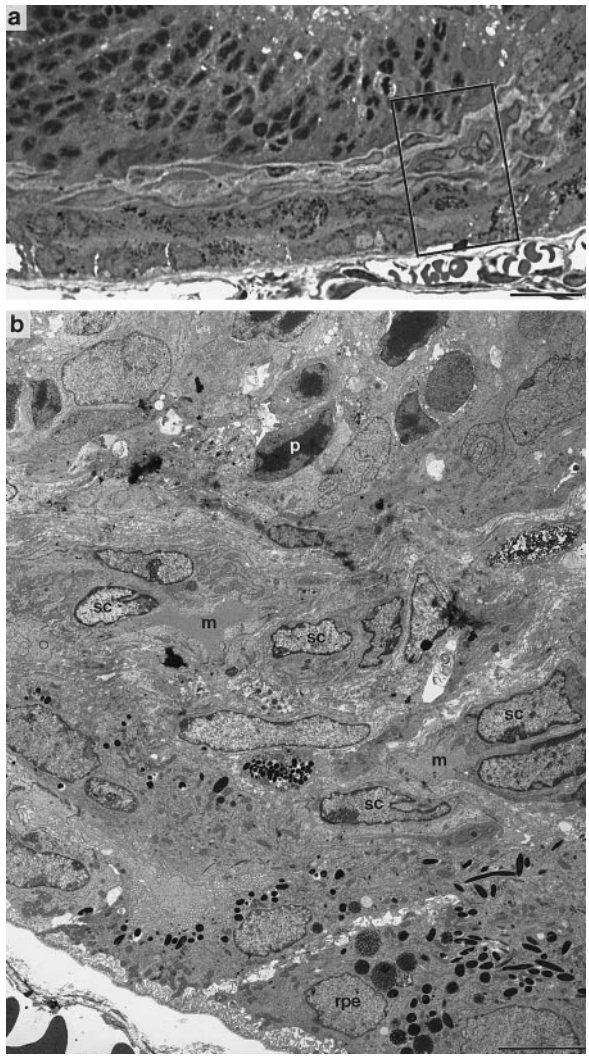

FIGURE 2. Schwann cells in the subretinal space of dystrophic RCS rat retina, 1 week after transplantation. (**a**) Light micrograph of semithin section showing Schwann cells lying between the RPE and the photoreceptor layer. Area outlined approximates to region illustrated in (**b**). (**b**) Electron micrograph showing grafted Schwann cells lying between the RPE (rpe) and the photoreceptor layer. Toluidine blue. Scale bar, (**a**) 20 μm; (**b**) 5 μm. p, photoreceptor; sc, Schwann cell; m, matrix.

Even at 9 months after transplantation, three of five eyes with Schwann cell grafts showed some degree of photoreceptor preservation, particularly in the peripheral retina, and one of these had a substantial area of outer nuclear layer cells that survived (Fig. 4a). In this last animal, the photoreceptor layer was five to six cells deep at best; there were well-organized inner segments and a suggestion of outer segments. The RPE cells associated with the surviving photoreceptors often appeared larger and had a higher pigment density than those in other parts of the retina. The site of graft insertion was marked by a disciform scar (Fig. 4a). Again, surviving photoreceptors in the area around the transplant site had inner and outer segments, but those immediately deep to the graft site or scar did not. Identification of Schwann cells subretinally at longer survivals was more difficult than anticipated. Rat-401 originally had been chosen as a label for Schwann cells in the anatomic study because it had been a good marker for them in the rat brain (see[43]). It was less useful in the retina because reactive Müller cell processes were also Rat-401–positive for some time after surgery. However, in Schwann cell–grafted animals unusual accumulations of small anti-Rat 401–positive cells could be identified adjacent to inner retinal vessels (Fig. 4b) or possibly along retinal ganglion cell axon bundles (Fig. 4c). It was not clear whether their presence in this location was the result of initial placement or secondary migration.

In all the other categories of surgically treated animals at 9 months only isolated photoreceptors remained, and there was no specific survival associated with the disciform scars in either fibroblast- or sham-treated animals. This is in agreement with previous findings from this laboratory.[18]

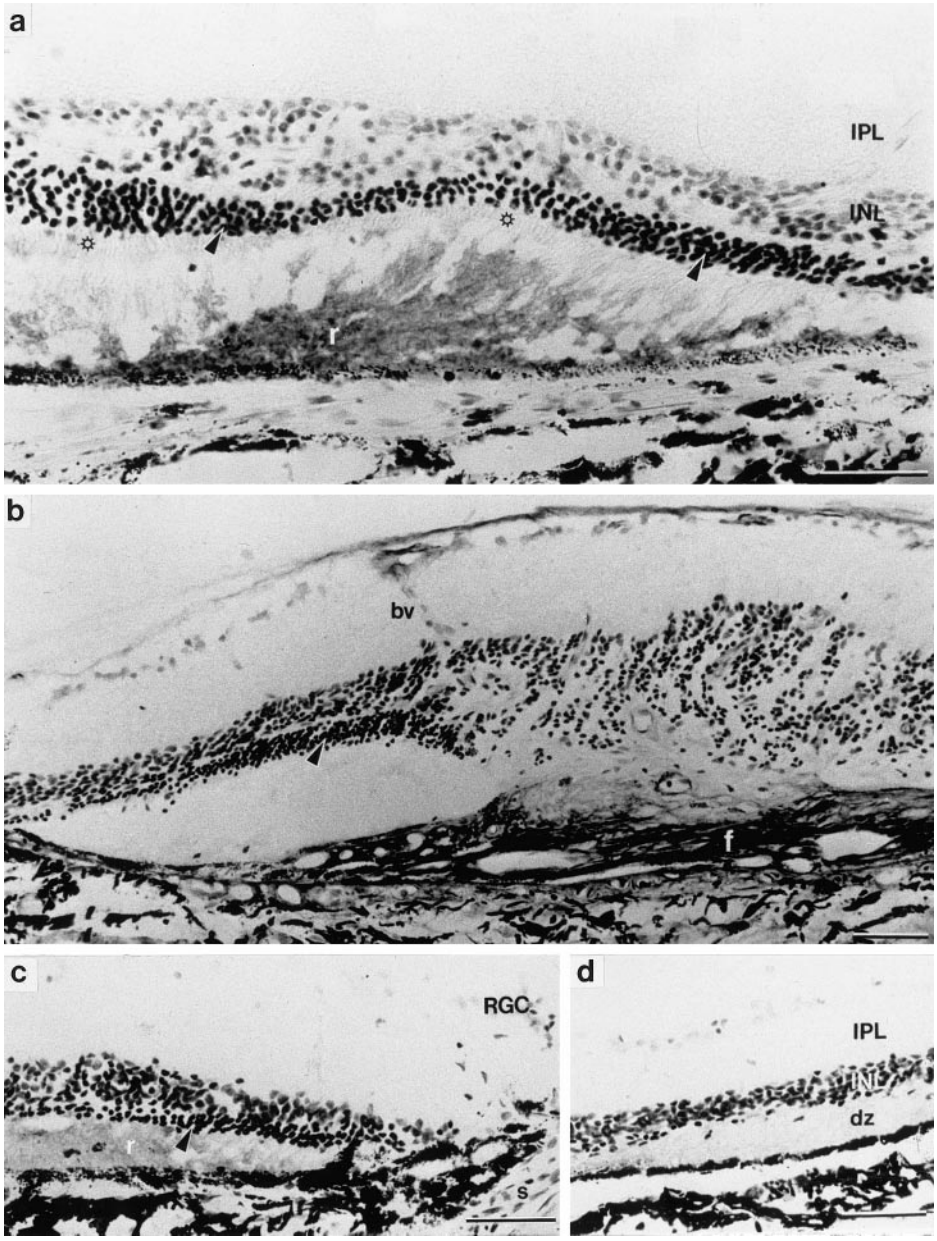

FIGURE 3. Transverse sections of dystrophic RCS rat retina at 3 months after surgery. (**a**) Schwann cell graft in subretinal space. Extensive photoreceptor survival in the vicinity of the graft. *Asterisks*: inner segment zone. (**b**) Fibroblast graft in subretinal space. (**c**) After sham surgery. (**d**) Dystrophic retina in dorsotemporal region, similar to the grafted region seen in (**a**) and (**b**). (**a**, **c**) Anti-rhodopsin plus thionine counterstain; (**b**) anti-fibronectin plus thionine counterstain; (**d**) thionine counterstain. (**a**, **b,** and **c**) *Arrowheads*: photoreceptor layer. s, site of insertion. Scale bars, 50 μm. IPL, inner plexiform layer; INL inner nuclear layer; r, rhodopsin-positive material; bv, blood vessel; f, fibroblasts reacted with anti-fibronectin; RGC, retinal ganglion cell; dz, debris zone;.

Head-Tracking

In preliminary studies, it has been found that dystrophic RCS rats lose head-tracking capability by 8 weeks of age, even though the retina still has a photoreceptor layer several cells thick. However, sham injections prolonged tracking capability for several weeks. In the present study, transplant-receiving and sham-injected rats, were tested at 12 (Fig. 5a) and 16 weeks (Fig. 5b) of age—that is, 8 and 12 weeks after transplantation. Analysis of variance showed significant differences between the groups at the 0.05 level and Fisher's exact probability test showed that high- ($P < 0.01$) and low-dose ($P < 0.01$) Schwann cell–grafted and sham-treated animals ($P < 0.05$) performed significantly better than dystrophic animals and high- ($P < 0.05$) and low–dose ($P = 0.063$) Schwann cell–grafted animals performed better than sham-treated animals. There was no significant difference between the two Schwann cell groups ($P = 0.67$). At 0.5 cyc/deg only Schwann cell–grafted animals showed any following response.

Between 12 and 16 weeks of age, the sham effect was considerably reduced. There was some decline in performance in the transplant group, presumably in part because the sham effect had been lost. In general, animals with high doses of Schwann cells performed better. At 16 weeks high-dose Schwann cell–grafted animals performed significantly better than sham ($P < 0.05$) or dystrophic ($P < 0.01$) animals. However, there was no significant difference between the high- and low-dose Schwann cell groups ($P = 0.27$) or between sham and dystrophic animals ($P = 0.76$).

To summarize, this method provided an effective screen for graft-related effects, at least at shorter survivals, although persistence of a sham effect precluded effective screening until after 12 weeks of age.

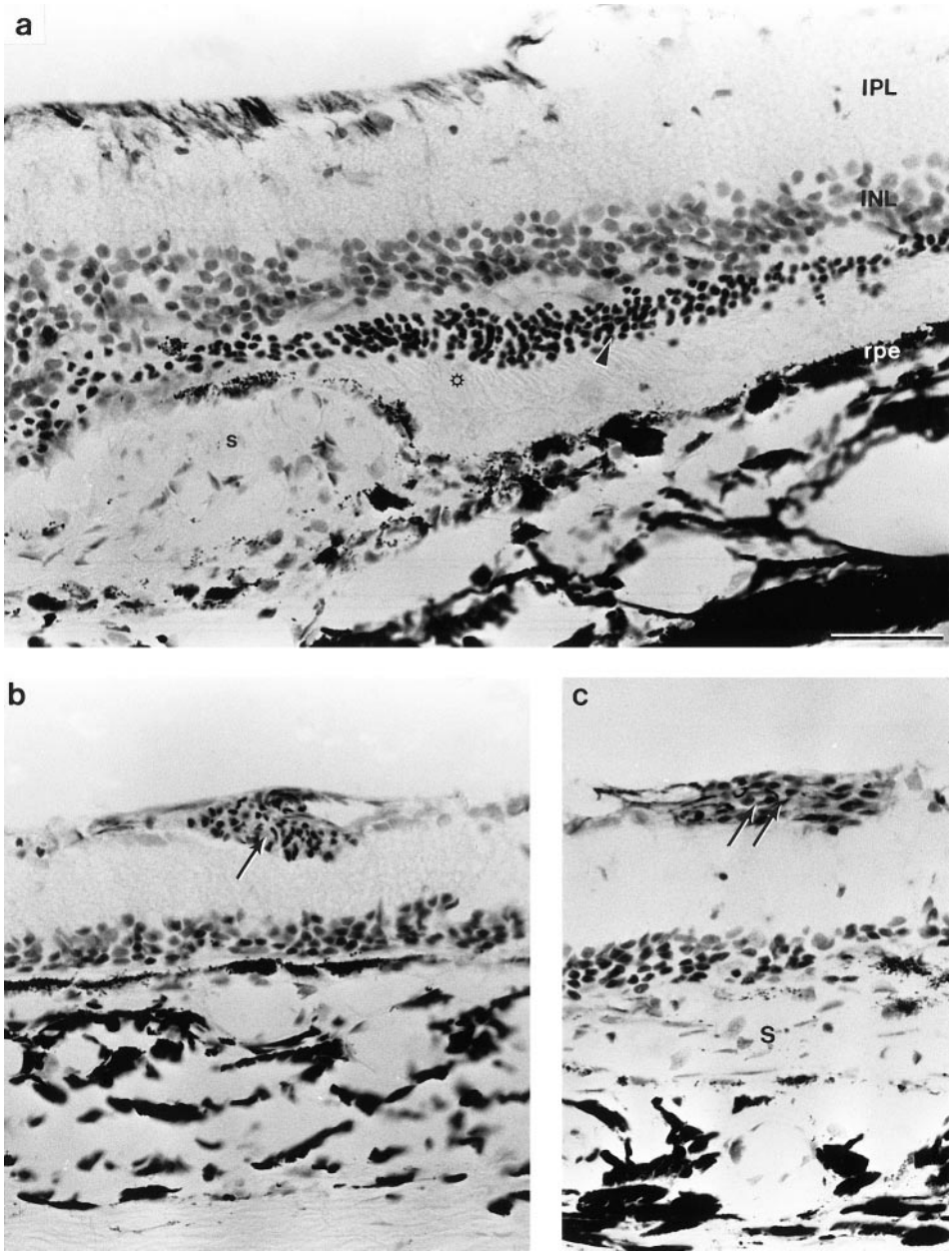

FIGURE 4. Transverse sections of dystrophic rat retina, 9 months after surgery. (**a**) Region of dystrophic dorsal retina where photoreceptors were spared. Photoreceptor segments clearly visible. *Asterisk*: inner segment zone. *Arrowhead*: photoreceptor layer. (**b**, **c**) Region of dystrophic dorsal retina a short distance from the site of graft insertion. No photoreceptors remained, and debris zone resolved. Schwann cells (reacted with anti-Rat 401) associated with inner retinal vessel or retinal ganglion cell axon bundle. Schwann cells presumed to have migrated from site of insertion (s) along basal lamina of blood vessels to present site. Thionine counterstain. Scale bar, 50 μm. (**b**) *Arrow:* inner retinal vessel + Schwann cells; (**c**) *double arrow:* retinal ganglion cell axon bundles (?) plus Schwann cells IPL, inner plexiform layer; INL, inner nuclear layer; rpe, RPE.

Electrophysiology

The retinal input to the superior colliculus represents a virtually unfiltered map of the visual field, changes in which sensitively reflect visual field deterioration in the dystrophic RCS rat.[14] In previous work in which stereotyped recording conditions were used, a partial scotoma was found to develop from the center to the periphery, involving the whole visual field by 6 months of age. With use of the same testing conditions in this experiment, unoperated dystrophic animals showed scotoma development as before, but animals with Schwann cell grafts showed areas of rescue even at 9 months after transplantation. Care was taken at each time point to compare the best performing Schwann cell–grafted animals with the best performing sham-treated animals (based on performance in initial screening by either PLR or head-tracking). In the best sham-injected animal, only two points (open circles) were responsive to focal stimulation at 9 months' survival (Fig. 6d), and approximately 60% of the colliculus was nonresponsive even to whole-field stimulation (dashes). This was very similar to the pattern seen in age-matched unoperated dystrophic rats. A Schwann cell–grafted rat (Fig. 6c) by contrast showed focal responses from 30 points, and surrounding this area of responsiveness, there was a larger area that responded to whole-field stimulation (crosses). Post-stimulus histograms recorded from focal stimulation showed brisk responses after Schwann cell transplantation (Fig. 7c) with amplitudes and latencies to light flashes close to normal levels (Fig. 7a). By comparison, responses from sham-injected animals of similar age were extremely weak (Fig. 7b) with latencies approximately four times greater than those of nondystrophic animals and approximately twice as long as those of Schwann cell–grafted animals. At 3 months after transplantation, a similar field analysis produced a less clear discrimination between sham- and Schwann cell–injected animals (Figs. 6a, 6b). For this reason a quantita-

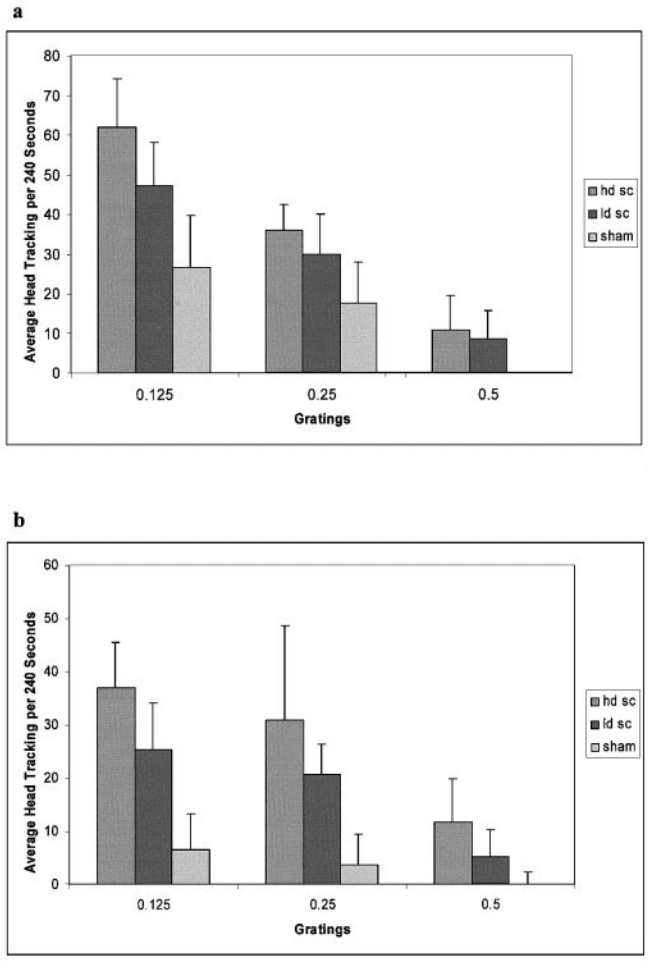

FIGURE 5. Head-tracking responses of high- and low-dose Schwann cell–grafted animals compared with sham-injected animals at (**a**) 12 weeks' survival and (**b**) 16 weeks' survival. Data from dystrophic animals not plotted because the mean was less than 1 second at 12 weeks and zero at 16 weeks.

tive approach was adopted, measuring visual thresholds. This permitted a more sensitive assessment of rescue of function across the visual field. In normal nondystrophic rats (Fig. 8a), thresholds were generally approximately 0.5 log units above a baseline illumination of 0.02 cd/m^2. In dystrophic RCS rats at 3.5 months, the figures were closer to 3.5 log increments over background (Fig. 8b). After sham injections (Fig. 8c), an area was seen close to the site of injection, where thresholds were somewhat lower than those in untreated dystrophic rats and usually extended further from the edge of the colliculus. Schwann cell–grafted animals, by comparison, showed a larger area of lower threshold responses, which extended far beyond the area adjacent to the site of injection (Fig. 8d). The size of the area of lower thresholds correlated in general with head-tracking behavior and with anatomy. The better the field rescue, the larger the area of surviving photoreceptors and the more robust the head-tracking response.

These results show that Schwann cell grafts can preserve good visual responsivity over a significant area of visual field and that this effect is sustained for prolonged periods.

DISCUSSION

This study shows that Schwann cells grafted into the subretinal space rapidly integrate into the tissue environment and can sustain visual function and promote photoreceptor survival for a considerable period. Previous studies have shown that grafts of peripheral nerve segments support neuron survival and axon regrowth in a number of central nervous system locations, including the spinal cord (e.g., Reference 36), retina (e.g., Reference 37), and thalamus.[38] This property is dependent on the presence of viable Schwann cells in the graft,[39,40] and purified populations of Schwann cells transplanted on artificial substrates[41] or grafted as cell suspensions (e.g., References 31,42–44) have also been shown to support cell survival and axon regrowth. Schwann cells injected into the vitreous of the eye promote retinal ganglion cell survival after optic nerve section,[45] and when grafted into the visual cortex of dark-reared rats, they contributed to the normal development of visual response properties.[46] However, although evidence has been presented that Schwann cells survive in the subretinal space of normal rats and do not have deleterious effects in the short term,[47] this is the first report of Schwann cells limiting deterioration of visual function and photoreceptor death in dystrophic RCS retinas over prolonged survival times.

The short-term survival study shows that, after transplantation, Schwann cells rapidly settled in the subretinal space and formed a sheet between the RPE and photoreceptors. We found that the Hoechst label on its own was not a definitive marker for transplanted cells, because RPE cells and photoreceptors took up the dye (presumably derived from Schwann cells that failed to survive transplantation; see also Reference

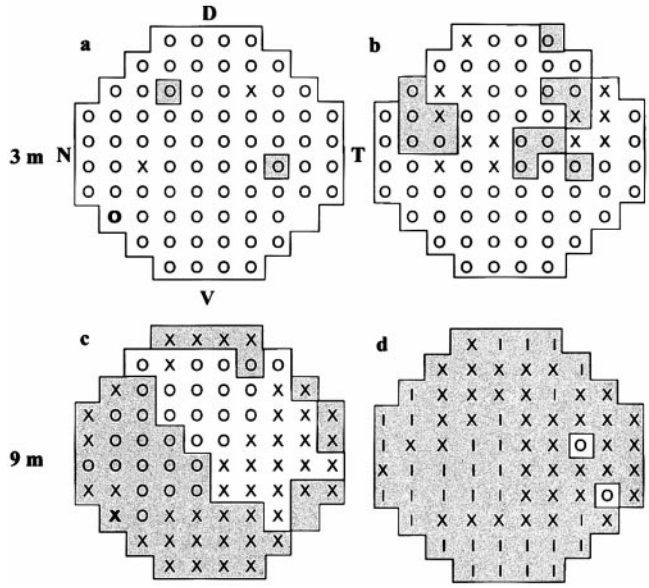

FIGURE 6. Retinal representation of receptive fields recorded in superior colliculus after Schwann cell-grafting (**a, c**) or sham surgery (**b, d**). (**a, b**) Animals 3 months after surgery. (**c, d**) Animals 9 months after surgery. In each instance, animals with the best PLR were selected for electrophysiology. ○: RFs to low light illumination; X surrounded by *gray* background: response recorded but only to whole-field illumination (i.e., relative scotoma); *dashes* with *gray* background: no response. D, dorsal; T, temporal, V, ventral; N, nasal.

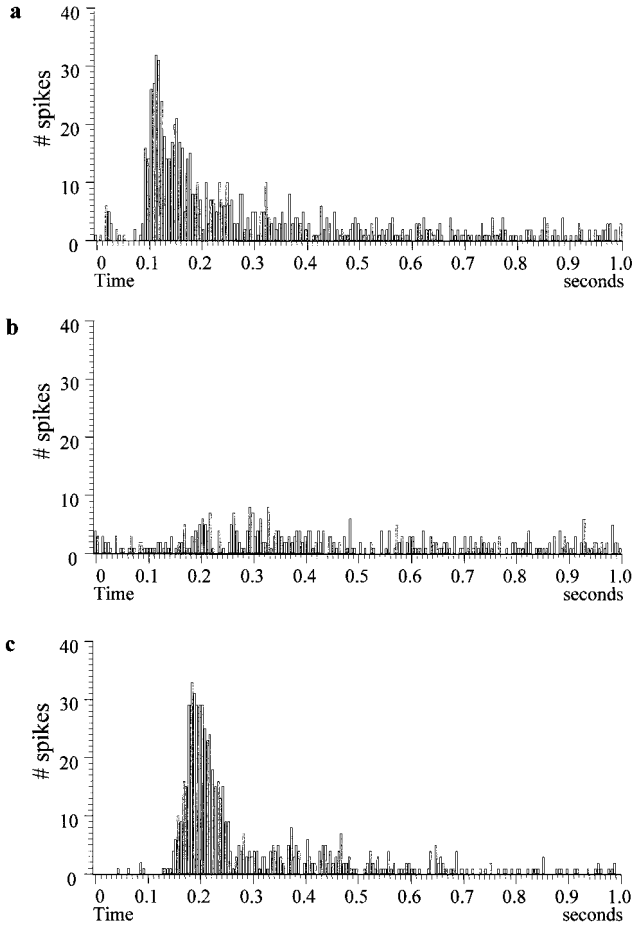

FIGURE 7. Poststimulus responses recorded from neurons in the superior colliculus of: (**a**) nondystrophic RCS rat; (**b**) dystrophic RCS rat with sham surgery; (**c**) dystrophic rat with Schwann cell graft at 9 months' survival. Each recording is the sum of 30 sweeps.

48). However, for short survivals double-labeling with anti-NGFr provides effective confirmation of their presence. The value of Rat-401 as a label for Schwann cells in the reactive retina is limited because it also stains reactive Müller cell processes. Presently, evidence for Schwann cell survival at longer time intervals depends on three observations. First, photoreceptors survive only for prolonged periods in Schwann cell–injected rats; second, where grafts have penetrated the retina, there is evidence of Rat-401–positive cell aggregations on the vitreal surface of the retina; and third, immune rejection of the grafts is unlikely because syngeneic donor cells were used. Ongoing ultrastructural studies will determine the long-term relationship of Schwann cells (with their characteristic cytological features) with surviving photoreceptors.

Throughout the study, there was variability in the level of rescue between animals. This may partly relate to variations in the number of cells introduced since cell reflux at the time of surgery, variations in graft placement ,and migration of donor cells away from the site of grafting. The migratory capacity of Schwann cells, particularly along basal lamina (e.g., associated with blood vessels) has been described before.[31,41,49,50] Post-transplantation apoptosis may also be a factor.

The variability creates difficulties for the analysis of behavioral results. However, the reliability of statistical testing can be greatly enhanced by correlating the degree of photoreceptor survival (assessed anatomically) with behavioral responses. It should be noted that fibroblasts give some degree of functional (using PLR) and anatomic rescue in the short term when compared with the age-matched dystrophic animals, but with time, their effect diminishes. This correlates with other results from this laboratory.[18] Anatomically, layers of surviving photoreceptors or photoreceptor segments were never observed after 9 months' survival in fibroblast-grafted retinas. Similarly for a limited period, and as others have observed,[18,22,24,25] sham surgery results in some protection possibly because of the local release of factors at the site of injury.[24,51,52] The sham effect on visual behavior at 12 weeks of age (8 weeks after transplantation) is considerable, and it may be that the presence of DNase in the carrier medium enhances it further, perhaps by delaying the onset of the microglial response.

The head-tracking test has proven to be an effective measure of visual behavior, at least up to 16 weeks of age, and permits relatively early identification of effective grafts. So far, there is good correlation between head-tracking and electrophysiological results, although the latter technique provides the most sensitive measure of graft efficacy and the area of retina protected. By measuring visual threshold for defined RFs the degree of protection afforded by each treatment can be assessed point to point across the visual field, and data are provided that can be compared with typical human visual field charts.

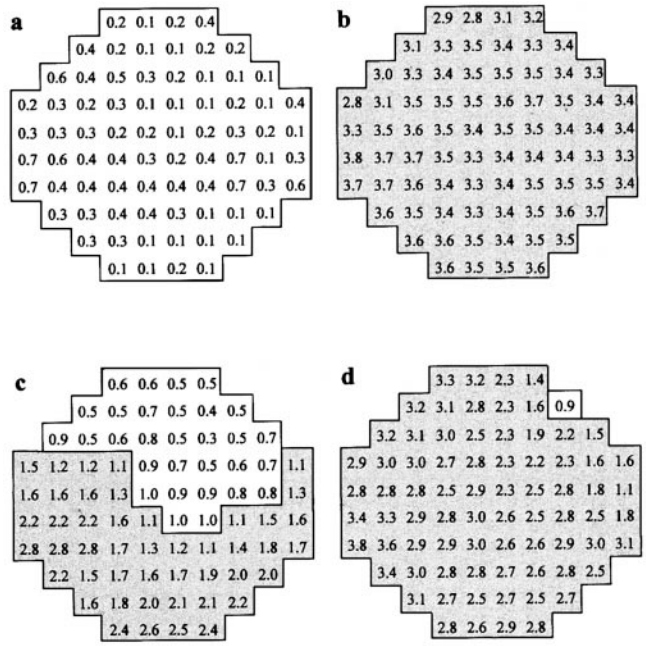

FIGURE 8. Threshold sensitivities recorded across the superior colliculus measured in log candelas per square millimeter. Numbers are relative to a background illumination of 0.02 cd/m^2. All numbers higher than 1.0 cd/m^2 are shaded. Animals aged 16 weeks. (**a**) Normal nondystrophic animal, threshold sensitivities less than 0.5 cd/m^2; (**b**) threshold sensitivities of a dystrophic rat, mostly more than 3.0 cd/m^2; (**c**) Schwann cell–grafted animal with a large area of preservation less than 1.0 cd/m^2 with the remaining area is less than 3.0 cd/m^2; (**d**) animal with sham surgery shows a small area of protection and remaining levels slightly better than those of a dystrophic animal. Dorsal retinal representation is at *top* and temporal is to the *right*, as shown in Figure 6.

The mode of action of the Schwann cells remains to be elucidated. The most likely explanation is that Schwann cells directly support photoreceptors because they produce growth factors known to promote photoreceptor survival,[28,29,54] and single injections of such factors into the vitreous have been shown to preserve the outer nuclear layer, although in theses studies the survival times have tended to be short.[22,25,26] Additionally, such factors are effective in maintaining segments in vitro.[55] Although direct support of photoreceptors by added growth factors is the most likely mechanism of action in animal models in which RPE function appears to be normal, photoreceptors in the RCS rat are lost because the RPE cannot phagocytose outer segments.[3] Because the electrophysiological data indicate that rescued photoreceptors function, it may be expected that outer segment phagocytosis continues after Schwann cell transplantation. It is possible, therefore, that one or more of the trophic factors produced by Schwann cells stimulate the existing dystrophic RPE to phagocytose outer segments more efficiently. It is unlikely that Schwann cells themselves are involved in phagocytosis, because although they are capable of phagocytosing debris,[56] their recognition mechanism is likely to be very specific. Indeed, outer segments are absent in photoreceptors immediately deep to the graft but are present in photoreceptors adjacent to grafts where the photoreceptors maintain their normal relationship with the RPE. The possible effects on Müller cells may also be important because these cells are intimately linked metabolically with photoreceptors. Other possibilities include recruitment of macrophages, which may take over phagocytosis, although our studies so far do not show excessive macrophage–microglial invasion in the region of the grafts, other than those normally observed in the debris zone of the adjacent degenerating retina (see Reference 57). Thus, unless the factors released by Schwann cells alter the rate of microglial activity, it seems unlikely that microglia alone take over the phagocytosis of outer segments, particularly because they do not normally promote significant photoreceptor survival in the dystrophic retina. Microglial end feet have been detected in the photoreceptor layer of the RCS rat at P14, long before there are overt signs of photoreceptor degeneration.[58] Furthermore, a previous study has indicated that transplants of macrophages have no protective effect in RCS rats.[59]

The RCS rat has been used as a model, albeit an indirect one, for age-related macular degeneration. A number of studies have attempted RPE transplantation in patients with age-related macular degeneration but the results so far have not produced firm evidence of improved vision.[60,61]. However, greater success has been achieved with retinal translocation surgery.[62–64]

The Schwann cell approach presents some advantages over other cell transplantation studies by avoiding three important problems: 1) the logistic difficulties of obtaining a large population of suitable donor eyes to provide RPE cells; 2) the risk of disease transfer; 3) immune responses that must be considered when grafting into the central nervous system or other sites such as the subretinal space (e.g.,. References 65–68). Although the present study has involved grafting between animals, grafting within an animal is possible. Autografts would be highly desirable when contemplating the transition of this technique to the clinical setting. Schwann cells could be derived from the patient (e.g., from the sural nerve), and human Schwann cells[69] have been shown to promote axon regeneration in the transected rat spinal cord.[70] Schwann cells also have some advantages over growth factor injections by their continuous delivery at physiological levels and by being targeted in the subretinal space (instead of the vitreous), reducing the risk of complications such as cataract formation. Finally, although the efficacy of Schwann cell grafts may compare with expectations from gene therapy approaches, that technology is still in a developmental stage and is likely to entail delivery of only one growth factor at a time. These observations suggest that the transplantation of Schwann cells might usefully be considered among the range of therapies currently under study to treat retinal degenerative diseases.

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