Light-Driven Retinal Ganglion Cell Responses in Blind *rd* Mice after Neural Retinal Transplantation

Wolfgang Radner,[1] *Srinivas R. Sadda,*[1] *Mark S. Humayun,*[1] *Satoshi Suzuki,*[1] *Michelle Melia,*[1,2] *James Weiland,*[1] *and Eugene de Juan, Jr*[1]

Purpose. Light-elicited retinal ganglion cell (RGC) responses after fetal neural retinal transplantation have not been demonstrated in animal or human subjects blind from outer retinal degeneration, despite apparent morphologic success. This study was designed to test the hypothesis that the functional success of retinal transplantation may be enhanced by using a young host retina (13 days old).

Methods. At postnatal day (P)13 C3H/HeJ (*rd/rd*) retinal degenerate mice received a subretinal transplant, in one eye only, of neural retinal tissue isolated from newborn normal C57/BL6J mice. Between 33 and 35 days after transplantation, local electroretinograms (ERGs) and ganglion cell responses were recorded directly from the retinal surface using a differential bipolar surface electrode. Measurements were performed both with and without light stimulation. Similar recordings were also performed in age-matched eyes subjected to sham transplantation, in control eyes that were not subjected to surgery, and in animals eyes that underwent transplantation at 8 weeks of age. After the recordings, the eyes were processed for light and transmission electron microscopy.

Results. Three of 10 mice showed bursts of ganglion cell action potentials (ON response only) as well as recordable intraocular ERGs over the transplant in response to 1-second and 200-msec light stimuli. Light-driven ganglion cell responses could not be recorded in areas outside the transplant in all transplant-recipient eyes, age-matched control eyes, and sham-transplantation eyes. Light responses also could not be recorded in animal eyes that received transplants at an older age (8 weeks). Electron microscopic examination confirmed the presence of photoreceptor outer segments in the areas affected by transplantation.

Conclusions. This study demonstrates the presence of light-driven ganglion cell responses after subretinal transplantation in a retinal degenerate model. This finding may reflect functional integration of the transplant with the host, but a rescue effect on remaining host photoreceptors cannot be ruled out. The findings suggest, however, that modification of host parameters, such as host age, may be important approaches for improving the functional success of retinal transplantation. (*Invest Ophthalmol Vis Sci.* 2001;42:1057–1065)

From the [1]Wilmer Ophthalmological Institute and [2]Wilmer Biostatistics Center, The Johns Hopkins Medical Institutions, Baltimore, Maryland.

Supported by the Foundation Fighting Blindness, the Grousbeck Family Foundation, and the Foundation for Retinal Research.

Submitted for publication April 19, 2000; revised July 31 and November 21, 2000; accepted December 15, 2000.

Commercial relationships policy: N.

Corresponding author: Eugene de Juan, Jr, Department of Ophthalmology, Wilmer Ophthalmological Institute, Johns Hopkins Hospital, 721 Maumenee, 600 North Wolfe Street, Baltimore, MD 21287. edejuan@jhmi.edu

The success of organ transplantation procedures (such as heart, kidney, and liver transplantation) has sparked interest in the transplantation of neural tissue as a potential treatment for certain neurologic disorders (such as Parkinson's disease and spinal cord injuries).[1,2] Although Royo and Quay[3] reported the first successful intraocular neural retinal transplantation procedure in 1959, significant interest in retinal transplantation as a means to restore visual function did not develop until the mid-1980s.[4,5] Since that time, a number of investigators have demonstrated that the morphologic structures necessary for phototransduction can develop in transplanted retinal tissue and that long-term survival of these transplants can be achieved in the subretinal space without immunosuppression.[6–12]

A key requirement for successful retinal transplantation is that the transplanted retinal tissue integrate functionally with the remaining host retina. For this reason, the functional capabilities and functional integration of neural retinal transplants have been the focus of study for a number of research groups. Adolph et al.[13] detected electrophysiological function in retinal transplants that were isolated from the host retina and noted that retinal transplants could respond to light, even when they were organized as rosettes. In addition, in well-developed retinal transplants, Seiler et al.[10] observed light-dependent distributional changes of proteins involved in phototransduction. Moreover, a number of histologic studies have suggested the presence of possible synaptic connections between the host retina and transplanted retinal tissue.[6–17] Despite this morphologic evidence, the presence of functional integration has been difficult to establish. Del Cerro et al.[18,19] used the suppressive effect of a warning light flash on the startle reflex response to acoustic stimuli in light-blinded 344 Fisher rats to investigate functional integration after retinal transplantation. They observed partial restoration of this visually mediated behavior after retinal transplantation,[18] but no recovery after sham injection or injection of retinal cell homogenates or cerebellar cells.[19] In addition, Kwan et al.[20] observed a restoration of dark preference behavior at low luminance levels after transplantation in the *rd* mouse. Interpretation of behavioral changes in animals, however, can be very difficult and imprecise. In the light-blinded Fisher rat, Silverman et al.[21] found some recovery of the pupillary reflex and visually evoked potentials (VEPs) after neural retinal transplantation. The pupillary reflex, however, is not a good predictor for retinal recovery, because there is no correlation between the size of the remaining photoreceptor population and the extent of pupillary constriction.[9] Although a VEP is a more objective measure, detection of a full-field flash VEP does not establish that the observed response is coming from the precise area of the transplantation.

To enhance the possibility of synaptic integration between the host retina and the transplant, attempts have been made to modify the transplant by using fetal neural retinal tissue with the presumed advantages of greater synaptic plasticity and less immunogenicity.[22,23] In addition, some investigators have attempted to remove the inner retinal layers of the transplant with a vibratome,[24] in hope of reducing a potential anatomic barrier to integration.

Despite the considerable attention directed to modification of the transplant, host characteristics have not been well studied. Gouras et al.[6] were the first to suggest that a glial barrier derived from remaining host Müller cells may hinder the formation of connections between the graft and host retinal cells. Another explanation for the absence of functional integration, however, may be a reduced ability of mature host retinal neurons to form new synapses. The goal of our work was to determine whether the functional success of retinal transplantation can be enhanced by the use of a less mature or younger host retina. In this study, we report the presence of light-driven retinal ganglion cell (RGC) responses after transplantation of normal newborn mouse retina into young (13-day-old) retinal degenerate (*rd*) mice.

Materials and Methods

Colonies of C3H/HeJ (*rd/rd*; Jackson Laboratories, Bar Harbor, ME) and C57/Bl6 (wild-type, Jackson Laboratories) were maintained at the Wilmer/Woods animal facility at the Johns Hopkins Hospital. Thirteen-day-old C3H/HeJ mice underwent transplantation in one eye only of neural retinal tissue isolated from the eyes of newborn (postnatal day [P]0) C57/Bl6 mice. Animals were anesthetized by intraperitoneal injection of a mixture of ketamine (60 mg/kg; Ketalar; Parke Davis, Morris Plains, NJ) and xylazine (8 mg/kg; Phoenix Pharmaceutical, Inc., St. Joseph, MO). Additional topical anesthesia was provided with 1% proparacaine hydrochloride drops (Alcon, Fort Worth, TX). Eyes were dilated with 1% tropicamide (Alcon). Transplantations of small retinal aggregates were performed in one eye only, at the equator (at the 12 o'clock position), according to the technique developed by Lazar and del Cerro.[25] A 27-gauge, partially sheathed butterfly needle connected to a microsyringe (containing the retinal microaggregate suspension) served as the delivery system. Under direct visualization through the pupil with a stereomicroscope, the needle was inserted through the sclera with the bevel facing the surgeon. The needle was rotated without changing its angle until the tip was in the subretinal space. The tip was advanced slightly to elevate the retina, and 2 to 4 μl of the retinal microaggregate suspension was injected into the subretinal space. Successful transplantation was confirmed by direct visualization using a coverslip on the cornea and a surgical microscope. After successful transplantation, the whitish retinal transplants were readily identified posterior to the injection site, under the detached host retina. Before the RGC recordings, fundoscopy was again performed (in transplant-recipient eyes only) to confirm the location of the transplants and to exclude any iatrogenic trauma to the retina caused by the procedure. For control eyes, we used 10 age-matched untreated animals and 10 age-matched animals subjected to sham-transplantation, in which the identical transplantation procedure was performed but with subretinal injection of Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) only (no retinal tissue). Ten animals also received transplants at 8 weeks of age. All animals were managed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the experimental protocol was approved by the Johns Hopkins University School of Medicine Institutional Review Board.

Recording of In Vivo RGC Responses and ERGs

RGC and electroretinogram (ERG) recordings[13] were performed 33 to 35 days after retinal transplantation. At this age (P46–P48), in a non–transplant-recipient *rd* mouse, the rod photoreceptor degeneration is essentially complete. Animals were dark adapted for at least 4 hours and then anesthetized with the same medications and dosages used for transplantation. A 30-gauge needle was used to make a small incision into the cornea just anterior to the limbus. Sodium hyaluronate (Healon GV; Pharmacia & Upjohn, Columbus, OH) was injected into the anterior chamber through the incision site to maintain intraocular pressure. The cornea was excised, and an intracapsular lensectomy was performed. The retinal surface was exposed for electrophysiological testing by dissecting the vitreous with sodium hyaluronate. All these preparative procedures were performed under dim red light illumination. Body temperature was maintained at 35°C with a feedback-controlled heating pad.

For differential RGC recordings, the tips (separated by 200 μm) of two 125-μm diameter paralyene-coated tungsten electrodes (A-M systems, Everett, WA) were positioned on the retinal surface using a microscope and a micromanipulator (MX-100; Newport; Irvine, CA). The ground electrode was placed in the animal's mouth. A halogen light source was used as the light stimulus, and the eyes were directly illuminated (full-field illumination; intensity: 5×10^5 cd/m^2) using a fiber optic (6-mm diameter) positioned at a distance of 1.0 to 1.5 cm from the retina. The onset of the light stimulus was at 0.1 second of the recording. One-second and 200-msec light stimuli were used for the recordings. Ten animals each were used for RGC recordings in the four experimental groups: the transplant host retinal areas (three recording locations) of transplant-recipient eyes posterior from the visible injection site; the areas uninvolved in the transplantation (three recording locations) of these eyes on the side opposite the transplant (at 6 o'clock); the corresponding areas (12 [two locations] and 6 o'clock [one location]) of control and sham-transplantation eyes. In each group, a total of three separate locations within a given experimental host retinal area were sampled.

Ten cycles (1 cycle = 1.6 seconds) of RGC responses were recorded at each location with the light turned either on or off (shutter sound only). Light-driven RGC responses were recorded from a normal B57/Bl6 mouse before each experimental session to confirm that all recording equipment was functioning properly. The examiners and the electrophysiologist were not masked to the transplant location or the treatment group assignment. Transplants were always performed at the same location, and it was usually easy to identify the transplant location during microscopic inspection while positioning the electrodes for the recordings.

The output of the recording electrodes was fed into a differential AC preamplifier (band-pass filter, 300-2000 Hz; gain, 10-fold; EX - 1; Dagan, Minneapolis, MN) that was connected to another AC amplifier (gain, 2000-fold; AM502; Tektronix, Beaverton, OR). Total gain was 20,000-fold. For ERG recordings, an identical setup was used, but with the band-pass filter of the amplifiers set at 0.3 to 300 Hz. A speaker and an oscilloscope were connected to the system to allow both audio and video monitoring of the recording conditions and the RGC responses. The output of the amplifier was fed into a computer (466 MHz; Pentium II microprocessor; Intel, Mountain View, CA). Data were acquired synchronously to the shutter action with a custom-written software program (LabView National Instruments, Austin, TX) and analyzed using another program (MatLab; (MathWorks, Natick, MA). Thresholds were then set manually for each file. For the construction of peristimulus time histograms (PSTHs), spikes were counted only if the amplitude was at least twice the amplitude of baseline. When a light response could be recognized with the speaker and recorded on the computer, it was verified by at least 10 consecutive recordings, either with the shutter action only (light turned off) or light stimulation.

For statistical analysis, the spike counts from 10 consecutive recordings were summed, transformed into logs, and then summed across the three locations for each experimental area (transplant-recipient eyes had six recording locations, three for the transplant area and three for the nontransplant area). Thus, after log transformation and summing, for the purpose of statistical comparisons, 10 animal eyes were used for each experimental group and area. All comparisons of spike counts between groups were made using the two-tailed unpaired *t*-test, with the exception of the comparison between nontransplant and transplant areas (spontaneous activity) of the same eye, for which the two-tailed paired *t*-test was used. Using a Bonferroni adjustment for multiple comparisons, $P < 0.0083$ was considered statistically significant (testing the hypothesis that there was a differ-

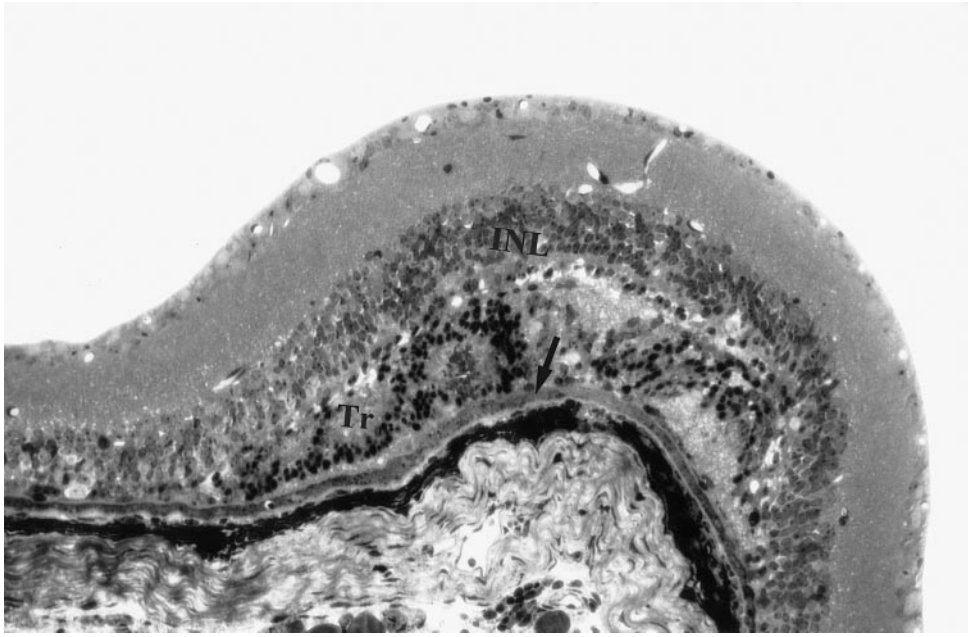

FIGURE 1. Transplant in the eye of a 46-day-old *rd* mouse (mouse 7). Transplanted cells (Tr) are present between the host inner nuclear layer (INL) and RPE (*arrow*). Light microscopy, ×100.

ence in number of spikes recorded during the stimulus interval among the different experimental groups).

For histologic investigations, animals were killed with an intraperitoneal injection of pentobarbital (200 mg/kg). The eyes were then enucleated and immersion fixed with 3% glutaraldehyde in 0.1% 1 M phosphate buffer for 24 hours. For light and electron microscopy, specimens were postfixed in 1% veronal acetate-buffered osmium tetroxide, dehydrated in a series of graded ethanols, and embedded in plastic. For transmission electron microscopy, ultrathin sections were stained with uranyl acetate and lead citrate.

RESULTS

Histology

Light and electron microscopic examination revealed the presence of transplanted retinal tissue in the subretinal space in the eyes of two of the animals that exhibited light-driven RGC responses (Figs. 1, 2). In the third positive animal, the specimen was lost during histologic processing. The transplants were organized into rosettes (Fig. 2A) or small sheets of retinal cells that contained photoreceptors with well-developed outer segments (Fig. 2C). In some areas, the photoreceptor outer segments were in contact with the host retinal pigment epithelium (RPE; data not shown). At the inner aspects of the photoreceptors, near the transplant–host interface, ribbon-type and conventional synapses with synaptic vesicles were observed (Fig. 2D). It was not possible to determine whether the synapses were between transplant cells or between transplant and host neurons. Nontransplant areas in the transplant-recipient eyes uniformly showed only a few remaining cones, but no evidence of outer segments (Fig. 2B). The control nonsurgical eyes (including the fellow eyes of the light-responding transplant recipients) and the control sham-transplantation eyes similarly showed no outer segments and only rare cone nuclei. In both transplant-recipient and control eyes, the inner nuclear and ganglion cell layers were well preserved in all animals.

Electrophysiology

At ages P46 to P48 (33–35 days after transplantation), light-driven RGC responses could be recorded in 3 of the 10 transplant-recipient *rd* mice. The bursts of light responses were clearly identified with the speaker, and the RGC action potentials (which were typically much greater than twice the noise–baseline amplitude) were confirmed on both the oscilloscope and the computer. Light-driven RGC responses were robust and reproducible with both 1- and 0.2-second light stimuli (Figs. 4, 5). To investigate RGC activity in adjacent locations surrounding the light-responsive areas (over the transplant), recordings were performed from at least five different positions at a distance of approximately 500 μm from the light-responsive locations. None of these adjacent regions showed any light response.

In contrast to the ON and OFF RGC responses to a 1-second light stimulus in a normal C57/Bl6 mouse (Fig. 3), the transplant-recipient eyes of these *rd* mice showed ON, but no clearly detectable OFF, responses (Figs. 4A, 4D). No light-driven RGC responses of any kind were recorded in the nontransplant areas of the surgically treated eyes, the untreated control eyes, and the sham-treated eyes. Fisher's exact test was performed to compare the frequency of positive (light-responsive) eyes between the transplant area group and the sum of the other control groups, and the difference was found to be significant (two-tailed unpaired *t*-test; $P = 0.012$). Spontaneous RGC activity (i.e., random RGC firing unassociated with light stimulation), however, was recorded in all groups (Fig. 6). The level of spontaneous RGC activity in any eye or location (including the transplant areas), however, was much lower than the level of light-driven RGC activity (compare Figs. 4D and 4E). RGC activity over the transplant area in nonresponsive transplant recipients with the light on was virtually identical with the spontaneous activity in these animals (i.e., there was no increase in baseline activity when the light was on).

The PSTH for the 1-second light in light-responsive retinal locations showed a maximum RGC spike count at 0.2 seconds after the onset of the light stimulus and a subsequent progressive return of spike activity to baseline (Figs. 4A, 4D). A 200-msec light stimulus also caused a maximum RGC spike count after 0.2 seconds but with a reduction to baseline level 0.2 seconds later (Figs. 5A, 4D). No change in the RGC spike count was observed in the shutter-only mode when the light was turned off (Figs. 4E; 5E).

PSTHs representing the average spike count per location (Figs. 6A through 6D) in the four control groups were calculated as an average of 300 recordings. For each experimental group, recordings were obtained from 10 eyes, with a total of 30 recordings obtained from three retinal locations (10 recordings per location). The same eyes were used for the transplant area and the nontransplant area of a transplant-recipient group. In the transplant locations (Fig. 6A), the spontaneous activity (recorded in the shutter-only mode with the light source turned off) led to an average RGC spike count of 107 ± 118.9 (log units: 5.17 ± 1.57) spikes per location (spk/loc) ± SD This spontaneous activity was significantly higher ($P < 0.004$) than the activity observed in the nonsurgical control eyes (Fig. 6C; 13.9 ± 8 spk/loc [log units: 3.22 ± 0.62]), but was not significantly higher ($P = 0.15$), although there was a trend, than in the nontransplant locations of the same transplant-recipient eyes (42.9 ± 38.9 spk/loc [log units: 4.35 ± 1.14]; Fig. 6B). In sham-transplantation eyes (Fig. 6D), the RGC activity (111.5 ± 119.3 [log units: 5.36 ± 1.32]) did not significantly differ from the spontaneous activity of the transplant areas of the transplant-recipient eyes ($P = 0.78$). Animal eyes subjected to transplantation at 8 weeks of age showed a similar level of spontaneous activity in the transplant areas as sham-transplantation eyes, but no light-driven responses.[26]

Local light-driven ERGs were also recorded from the host retinal surface over the light-responsive locations determined by the RGC recordings. A 1-second light stimulus was used. The appearance of a retinal surface ERG is very different from a corneal ERG, with a negative deflection appearing as the predominant component.[13] The negative deflection amplitude of the ERG waveform from light-responsive locations was 90 μV (Fig. 7A), 69% of the amplitude (130 μV) observed in a normal C57/Bl6 mouse (Fig. 7B) recorded at the beginning of the session the same day. Local retinal surface ERGs were not recordable (i.e., were flat) from the transplant-recipient eyes that did not show light-driven RGC activity. Local ERGs also were not recordable from nontransplant areas of all transplant-recipient eyes, nor from any of the other control groups.

Discussion

The purpose of this study was to explore whether host factors, such as host age, contribute to the functional success of neural retinal transplantation. To assess for functional success, we used RGC recordings as our measurement technique. Although previous investigators have measured local ERGs over the graft,[13] such activity is not sufficient to establish that functional connections have developed. The a-wave of the ERG is derived

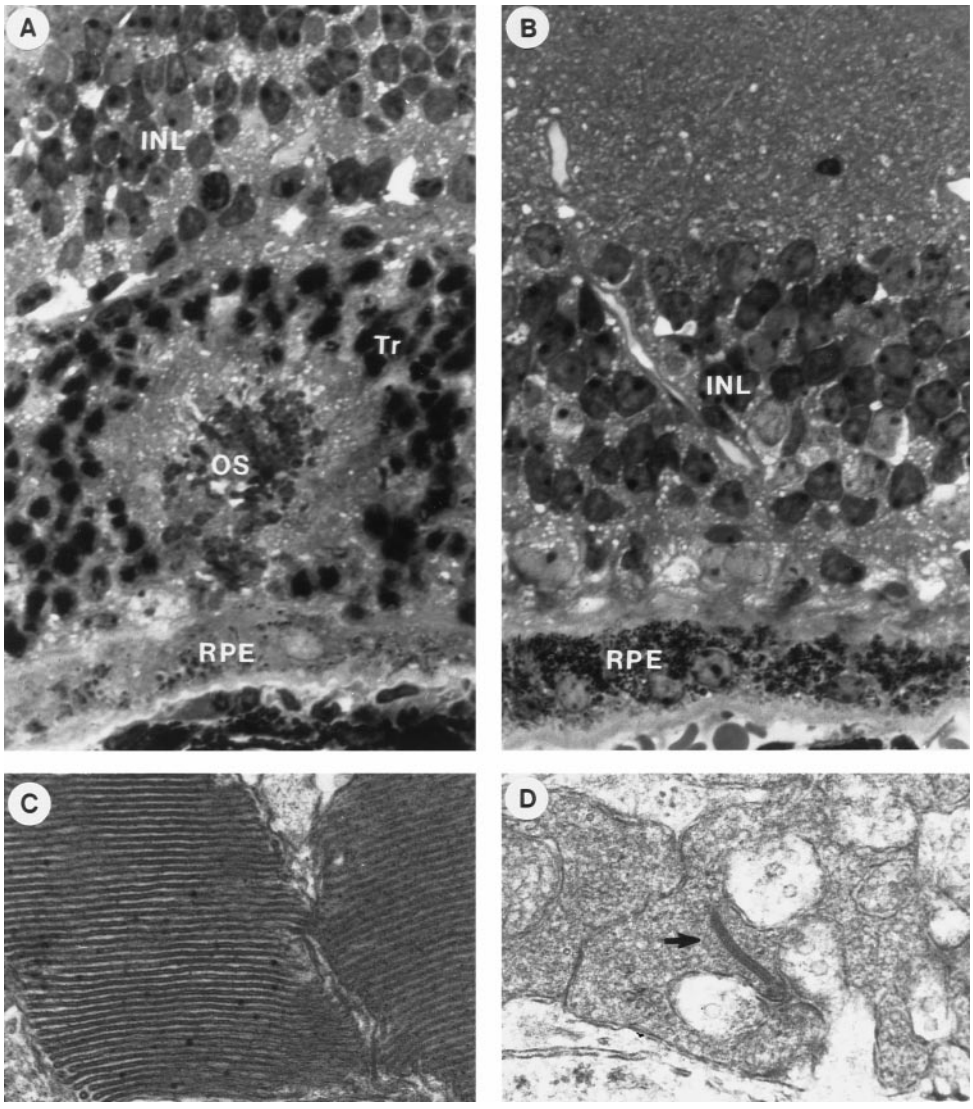

Figure 2. Transplant in the same 46-day-old *rd* mouse eye (mouse 7) at higher magnification. (**A**) The transplant (Tr) cells were present between the host inner nuclear layer (INL) and the host RPE. Rosettelike structures containing photoreceptor outer segments (OS) were present. (**B**) Nontransplant area of the same eye showing extensive degeneration and loss of photoreceptors. The well-preserved INL is shown directly adjacent to the RPE. (**C**) Transplant photoreceptor OS demonstrated the characteristic stacked-disc arrangement. (**D**) Ribbon-type synapse (*arrow*) was present within the transplant. Light microscopy: (**A**, **B**) ×400; transmission electron microscopy: (**C**) ×40,000; (**D**) ×10,000.

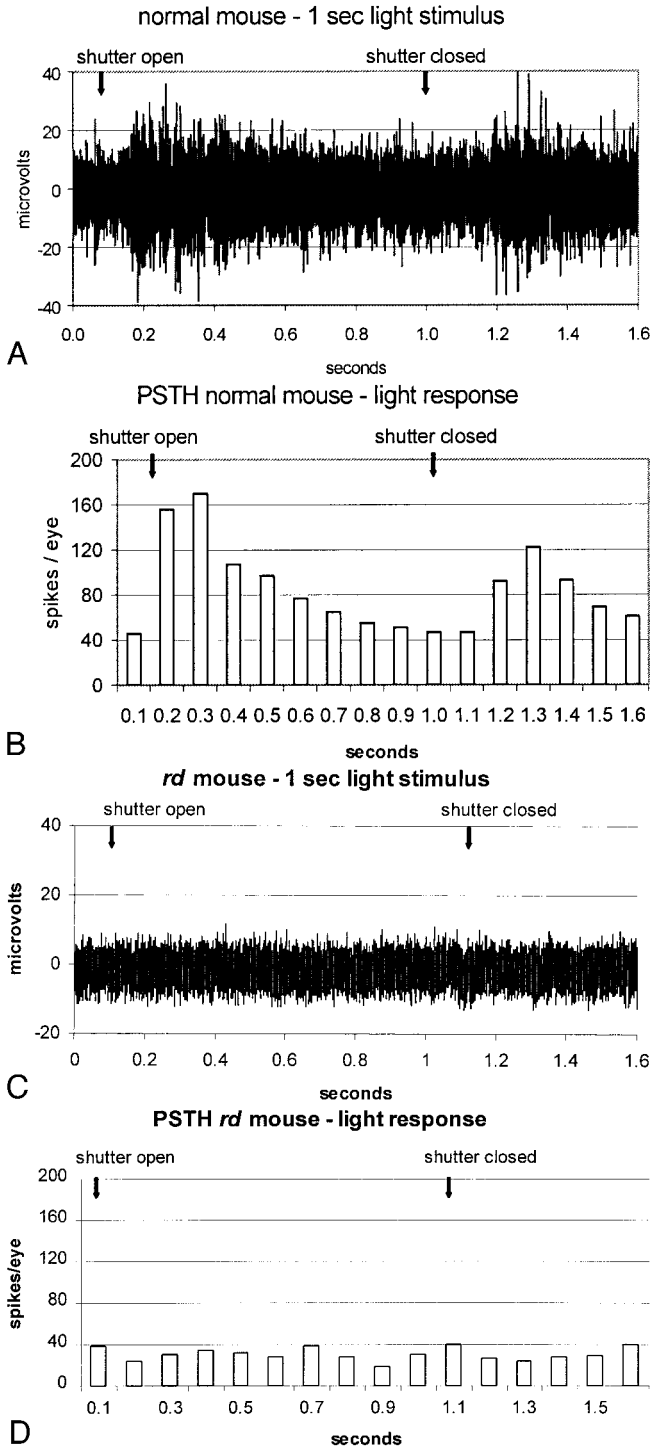

FIGURE 3. RGC responses to a 1-second light stimulus in a normal 7-week-old C57/Bl6 mouse (**A**, **B**) and in a control 7-week-old *rd* mouse without surgery (**C**, **D**). (**A**) Raw data from the normal mouse show a burst of increased RGC activity after the light was turned on (shutter open). (**B**) PSTH (grouping spikes into 0.1-second intervals) of 10 cycles recorded with the light turned on in the normal mouse eye shows evidence of increased RGC activity, both after the light was turned on (ON response) and after it was turned off (OFF response). In the *rd* mouse, there was no increase in RGC activity after light stimulation (**C**, **D**).

from the photoreceptors, and the "b-wave" is derived from bipolar cell activity modified by Müller cells. Because the transplanted cells may differentiate into bipolar cells as well as photoreceptors, the transplant alone may produce both components of the ERG. Host and transplant bipolar cells cannot be distinguished by the ERG. If responses were measurable from the host RGCs, however, this would suggest that true connections have formed between the transplant and the host retina. Connectivity can also be studied by measuring responses from higher order centers, such as the superior colliculus[27] or the visual cortex[28] itself, as described by Craner et al. Recordings from the visual cortex (such asVEPs) have the advantage of providing information that is more representative of actual visual perception. Full-field flash VEPs, however, do not have the precise spatial selectivity of RGC responses, which allow direct assessment of function over the transplant itself.

Most neuronal cells in the retina, such as the photoreceptors, bipolar cells, and horizontal cells, show graded responses. Only RGCs and some amacrine cells are known to show spike responses (action potentials).[29] With our recording technique, the differential bipolar retinal surface electrodes are carefully advanced with a micromanipulator, until the electrodes just contact the retinal surface. Both visual and audio cues are used to monitor the electrode approach. With this method, unintentional penetration of the retinal surface is nearly impossible. Without retinal penetration, we have not been able to record responses from deeper retinal layers. Thus, it is unlikely that the spike responses are from the deeper amacrine cells and are more likely a manifestation of RGC activity.

In this study, we demonstrated the presence of light-driven RGC responses (ON response only) over the graft 5 weeks after transplantation of normal newborn retinal tissue into the subretinal space of 3 of 10 *rd* mice aged 13 days. A light response could not be elicited in any control eye or nontransplant area. In addition, we could not elicit light responses from *rd* mice that received transplants at 8 weeks of age or older.[26] A reason for this apparent age-dependent effect may be the higher synaptic plasticity of the developing retinal tissue in younger mice. After transplantation of fetal rat retinal tissue over newborn rat superior colliculi, McLoon et al.[30] demonstrated survival of the retinal transplants and found histochemical evidence for synaptic connections between graft and host tissue. Furthermore, Lund and Coffey[31] performed functional experiments on newborn rats that received, from day 12 to 15 embryos, neural retinal transplants that were placed over their midbrains. Although the retinal transplants were organized as rosettes, the transplanted tissue made preferential axonal innervations into visual centers (i.e., the superior colliculus), and photic stimulation of the transplanted tissue caused reproducible pupillary constriction responses. In addition, learned behaviors were modified by photic inputs received through the transplanted tissue. Thus, by using fetal retinal tissue and a young host, Lund and Coffey demonstrated functional connections between graft and host neural tissue.

Fetal retinal tissue has been suggested as a suitable source of donor tissue because of its presumed greater plasticity and lower antigenicity compared with adult retinal tissue.[22,23] Despite these apparent advantages, functional integration of fetal retinal tissue transplanted into the subretinal space has not been demonstrated. In Lund and Coffey[31] and in our work, a young host animal was also used. In both studies, the findings suggest that functional connections may have developed between the host and the graft. This observation is consistent with our hypothesis that a younger host retina containing less well-differentiated cells may be more receptive to synaptic contacts, thereby enhancing functional integration with the transplant.

There are other possible explanations, however, for the apparent preservation of RGC responses in some of the transplant-recipient *rd* mice in our study. Mohand-Said et al.[32] demonstrated an apparent remote protective effect of the

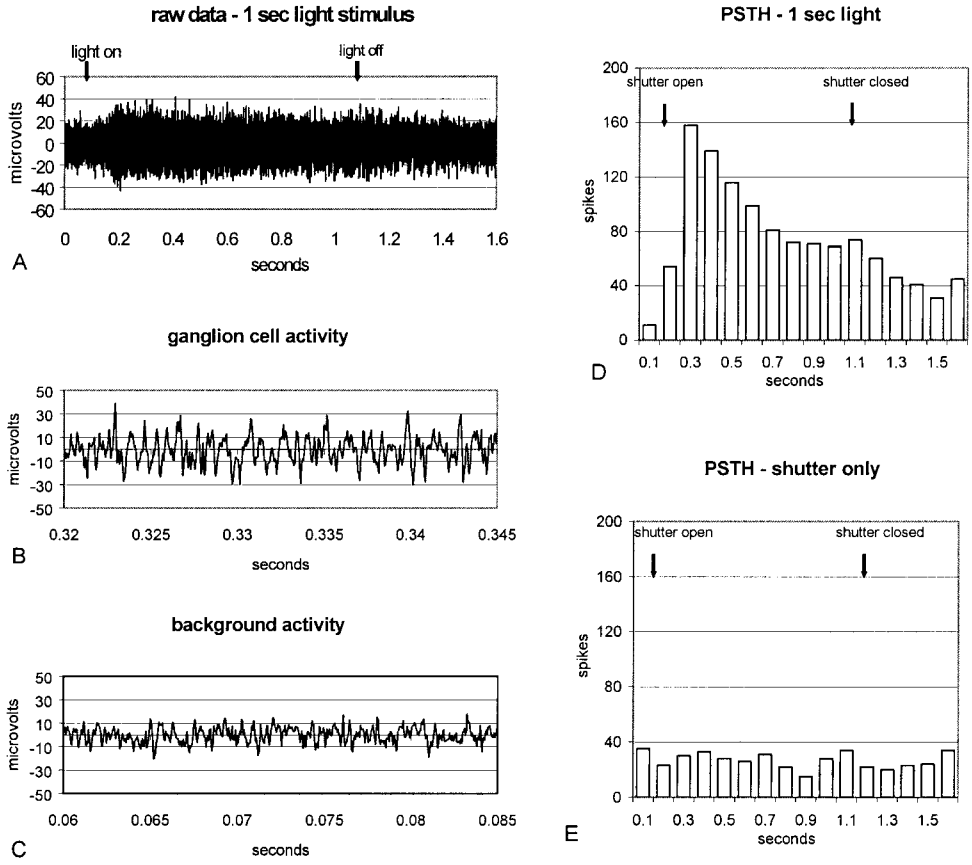

FIGURE 4. RGC response to a 1-second light stimulus in mouse 7 (a light-responsive transplant-recipient mouse). (**A**) Raw data show a burst of RGC activity. RGC activity began to increase at approximately 0.1 second after the onset of the light stimulus. (**B**) Typical ganglion cell spikes were seen within the burst, with spike amplitude well above background (**C**). PSTHs of 10 cycles recorded either with the light turned on (**D**) or the light turned off (**E**). The shutter opened at 0.1 second for 1 second. RGC spike counts reached a peak at 0.2 seconds after the onset of the light stimulus and then decreased continuously, returning to background level during the 1 second of light. (**E**) No light-driven changes of the RGC spike number were found when the light was turned off in the shutter-only mode.

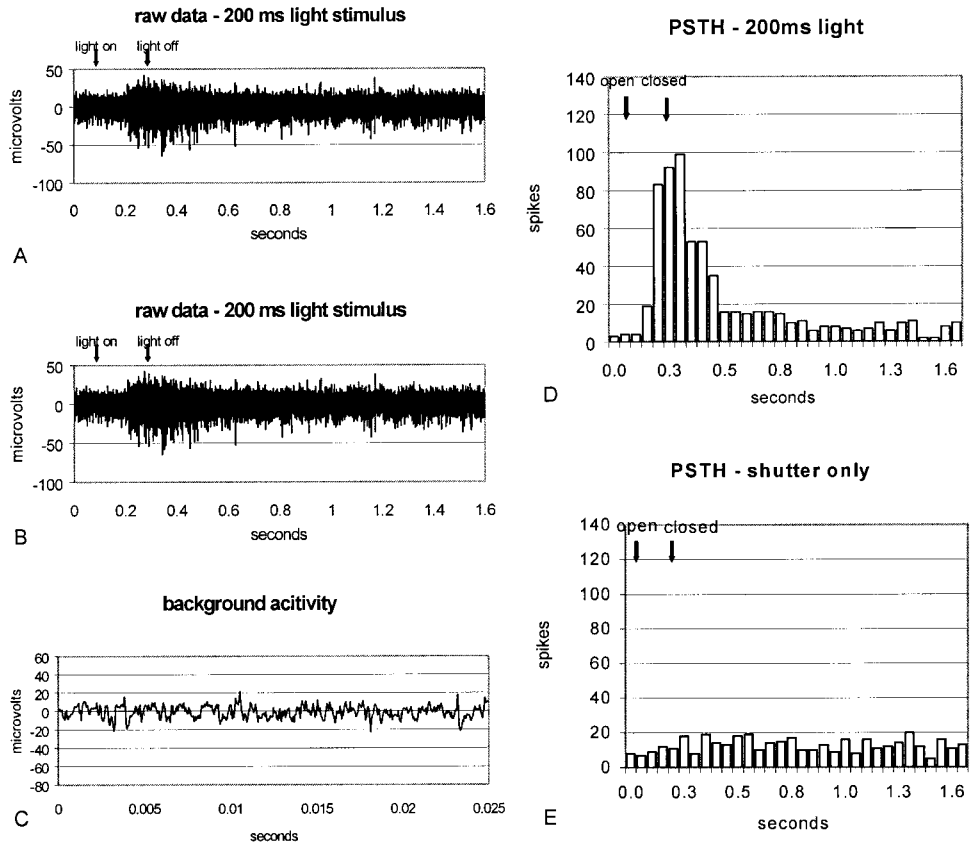

FIGURE 5. RGC response to a 200-msec light stimulus of another light-responsive transplant-recipient *rd* mouse (mouse 8). (**A**) Raw data show a burst of RGC activity. The RGC activity increased at approximately 0.1 second after the onset of the light stimulus (at 0.1 second). (**B**) Typical ganglion cell spikes can be found within the burst, with spike amplitudes well above background (**C**). PSTHs of 10 cycles recorded either with the light turned on (**D**) or the light turned off (**E**). The shutter opened at 0.1 second. The RGC spike count reached a peak at 0.1 second after the onset of the light stimulus and returned to background level 0.2 seconds later. (**E**) No change in RGC spike activity was found in the shutter-only mode when the light was turned off.

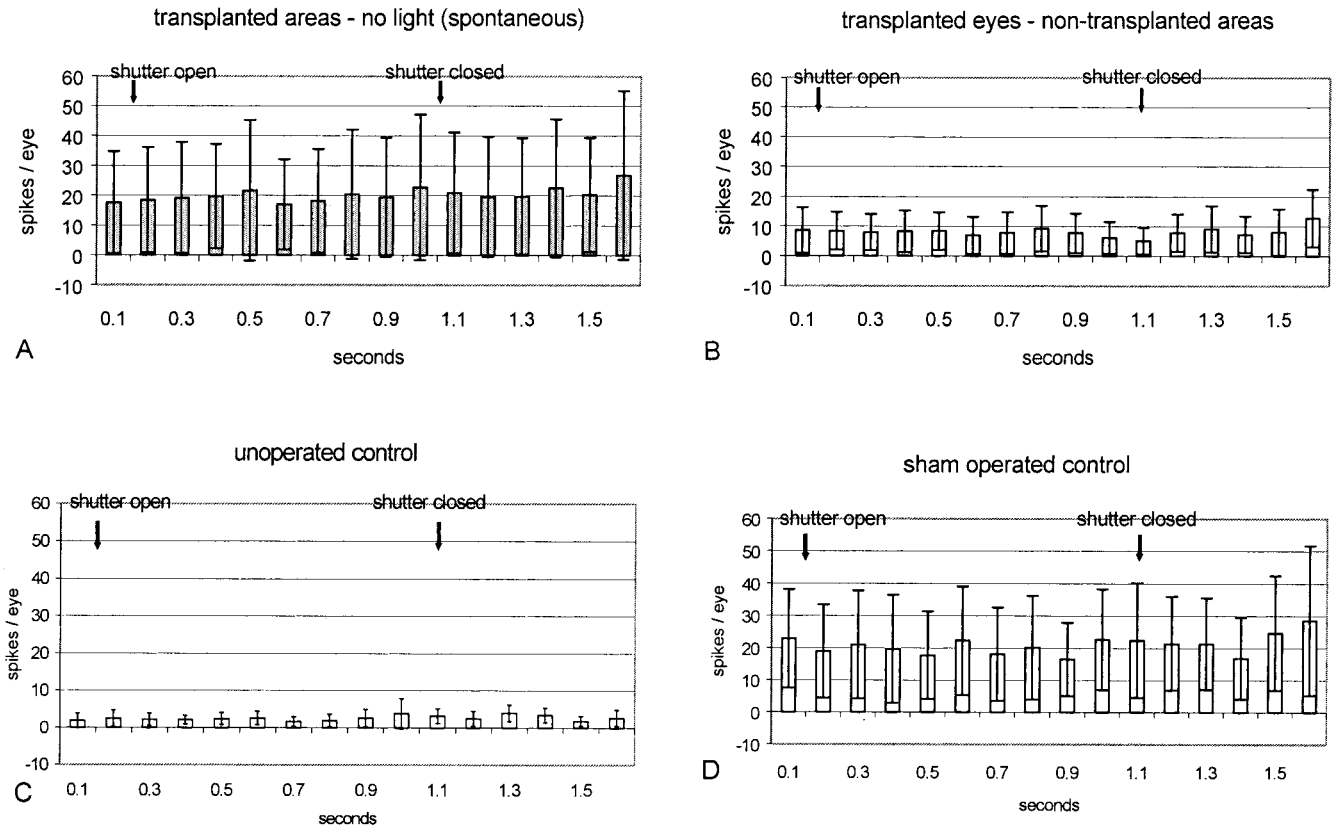

FIGURE 6. Comparison of spontaneous RGC activity among control groups. (**B**, **C**, and **D**) Average PSTHs are shown with the 1-second light stimulus turned on, because there was no significant difference in RGC activity with the light turned on or off ($P = 0.2$). (**A**) Averaged PSTH showing the spontaneous activity in the transplant areas (shutter-only mode with the light turned off). The spontaneous activity is significantly higher than that obtained from the nontransplant areas of the transplant-recipient eye (**C**; $P < 0.001$) and the nonsurgical control eyes (**B**; $P < 0.001$). In sham-transplantation eyes (**D**) the RGC activity did not differ from the spontaneous activity of the transplant areas (**A**; $P = 0.68$). Error bars, SD.

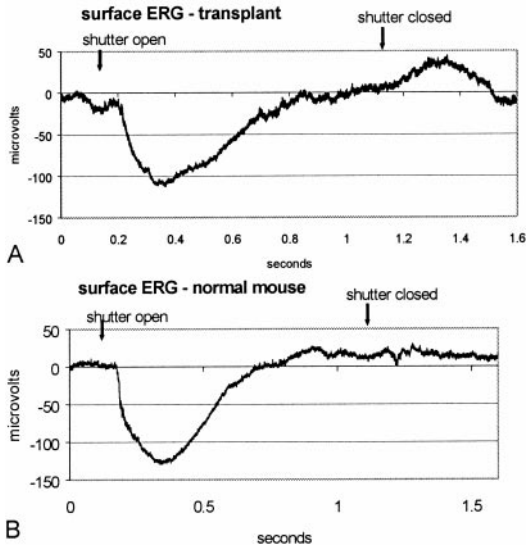

FIGURE 7. Surface ERGs (averaged from five cycles) from (**A**) the host retinal surface of the light-responsive transplant area of *rd* mouse 7 and from (**B**) the retinal surface of a normal C57/Bl6 mouse. A 1-second light stimulus was used. The negative deflection amplitude (90 μV) of the transplant area (**A**) was 69% of that obtained from the normal mouse (130 μV).

transplant on the host retina that leads to survival of remaining photoreceptor cells. They found increased survival of cones in the central quadrants after transplantation in the mid- and far periphery. In our experiments there were still a few cone nuclei remaining in both age-matched control animals and in the 7-week-old transplant recipients. There was no difference, however, in the number of cone nuclei between control eyes and the nontransplant areas of the transplant-recipient eyes. Because the donor tissue was not labeled, it was not possible to reliably distinguish between host and transplant cones. Consequently, it was not possible to compare the number of host cones within and outside the transplant area. Nonetheless, no photoreceptor outer segments could be identified in the control eyes or the nontransplant areas of the transplant-recipient eyes. Indeed, photoreceptor outer segments have never been reported in *rd* mice of this age.[33,34] In the light-responsive transplant areas, however, we found photoreceptor outer segments that showed a well-developed stacked-disc configuration (Fig. 2C). No outer segments were found outside the transplant area, and no light response was elicited from any nontransplant region. Thus, if photoreceptor rescue was responsible for preservation of light responsiveness in these transplant-recipient eyes, the effect was highly localized and regional, in contrast to the remote effect reported by Mohand-Said et al.[32]

It is also possible that residual host cone cells without outer segments contributed to the light response that was observed. The *rd* mouse harbors a mutation in the gene (*rd/rd*) that encodes the rod cGMP phosphodiesterase.[35] The retinal degeneration starts at P10, with near total loss of

the rods by P21. For unknown reasons, the cones are also severely affected as well and degenerate subsequently.[36] Corneal ERGs cannot be recorded after 4 weeks of age.[35] Although the remaining cones do not have outer segments after P21, Dräger[37] was able to record a light-driven decrease in superior colliculus activity for several weeks after birth. These responses were only detectable after stimulation of a peripheral ring of retina, and a dense scotoma was noted centrally. The physiologic mechanism or origin of these responses, however, is unclear. One hypothesis is that because the outer retinal degeneration progresses centrifugally (i.e., from the center to the periphery), some rods or cones may remain near the ora serrata. We have never found, however, any histologic evidence for photoreceptor outer segments, even in the retinal periphery. In addition, the strong bursts of light-driven RGC activity could only be recorded over the transplant areas, which were located posterior to the equator.

Another explanation for the preservation of ganglion cell responses may be an effect of the surgical procedure itself. An increase in spontaneous RGC activity was observed in both transplant-recipient and sham-transplantation animals. The cause for the increased spontaneous activity is uncertain, but it may have been due to some increased release of neurochemically active mediators as a result of the surgical manipulation. Only transplant-recipient eyes, however, exhibited light-dependent activity, and the magnitude (spike count) of the light response was markedly higher than the spontaneous activity. This finding suggests that the effect was due to the transplanted tissue itself.

Another possible explanation for the light-driven RGC responses is that they are derived from ganglion cells originating in the transplant. Although we have never histologically observed a ganglion cell layer in our transplants, it is possible that some ganglion cells develop. Our recording technique, however, appears to sample only highly localized responses. We are not able to record the graded responses of bipolar cells unless we penetrate deep into the retina. Thus, because we are recording from the retinal surface, we would not expect to record from the transplant ganglion cells unless they have migrated into the host ganglion cell layer. Future investigations using labeled transplanted tissue may help explore this possibility.

In summary, we report the preservation of light-driven RGC responses in 3 of 10 eyes of *rd* mice that received subretinal newborn retinal transplants at only 13 days of age. This effect was not observed in control animals or animals that received transplants at an older age. Although the effect may be due to factors other than functional integration, the finding suggests that modification of host parameters (such as host age in this case) may be important in improving the functional success of retinal transplantation. Further study of host parameters, including efforts designed to improve the efficacy of transplantation into older animals, appears warranted.

References

1. Borlongan C, Sanberg P, Freeman T. Neural transplantation for neurodegenerative disorders. *Lancet.* 1999;353:29–30.
2. Young W. Spinal cord regeneration. *Science.* 1996;273:451–458.
3. Royo P, Quay W. Retinal transplantation from fetal to maternal mammalian eye. *Growth.* 1959;23:313–336.
4. del Cerro M, Gash DM, Rao GN, et al. Intraocular retinal transplants. *Invest Ophthalmol Vis Sci.* 1985;26:1182–1185.
5. Gouras P, Flood M, Kjeldbye H, Krebs W. Transplantation of cultured human retinal cells to monkey retina. *Ann Acad Brasil Cienc*. 1984;58:431–443.
6. Gouras P, Du J, Kjeldbye H, et al. Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest Ophthalmol Vis Sci.* 1994;35:3145–3153.
7. 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.
8. Ghosh F, Johansson K, Ehinger B. Long-term full-thickness embryonic rabbit retinal transplants. *Invest Ophthalmol Vis Sci*. 1999; 40:133–142.
9. Del Cerro M, Lazar E, DiLoreto D. The first decade of continuous progress in retinal transplantation. *Micros Res Tech*. 1997;15:130–141.
10. 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.
11. del Cerro M, Humayun MS, Sadda SR, et al. Histologic correlation of human neural retinal transplantation. *Invest Ophthalmol Vis Sci.* 2000;41:3142–3148.
12. Ehinger B, Bergström A, Seiler M, et al. Ultrastructure of human retinal cell transplants with long survival times in rats. *Exp Eye Res.* 1991;53:447–460.
13. Adolph A, Zucker C, Ehinger B, Bergström A. Function and structure in retinal transplants. *J Neural Transplant Plast*. 1994;5:147–161.
14. Turner J, Seiler M, Aramant R, Blair J. Embryonic retinal grafts transplanted into the lesioned adult retina. *Prog Brain Res*. 1988; 78:131–139.
15. Turner J, Blair J. Newborn rat retinal cells transplanted into a retinal lesion site in adult host eyes. *Brain Res*. 1986;391:91–104.
16. Aramant R, Seiler M. Fiber and synaptic connections between embryonic retinal transplants and host retina. *Exp Neurol*. 1995; 133:244–255.
17. Zucker C, Ehinger B, Seiler M, et al. Ultrastructural circuitry in retinal cell transplants to rat retina. *J Neural Transplant Plast.* 1994;5:17–29.
18. del Cerro M, Ison JR, Bowen GP, et al. Intraretinal grafting restores visual function in light-blinded rats. *Neuroreport.* 1991;2:529–532.
19. del Cerro M, DiLoreto D, Cox C, et al. Neither intraocular grafts of retinal cell homogenates nor live non-retinal neurons produce behavioral recovery in rats with light-damaged retinas. *Cell Transplant.* 1995;4:133–139.
20. Kwan AS, Wang S, Lund RD. Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Exp Neurol*. 1999;159: 21–33.
21. Silverman M, Hughes S, Valentino T, Liu Y. Photoreceptor transplantation: anatomic, electrophysiologic, and behavioral evidence for the functional reconstruction of retinas lacking photoreceptors. *Exp Neurol.* 1992;115:87–94.
22. Widner H, Brundin P. Immunological aspects of grafting in the mammalian central nervous system: a review and speculative synthesis. *Brain Res.* 1988;472:287–324.
23. Aramant R, Seiler M, Turner J. Donor age influences on the success of retinal transplants to adult rat retina. *Invest Ophthalmol Vis Sci*. 1988;29:498–503.
24. Kaplan H, Tezel T, Berger A, et al. Human photoreceptor transplantation in retinitis pigmentosa: a safety study. *Arch Ophthalmol.* 1997;115:1168–1172.
25. Lazar E, del Cerro M. A new procedure for multiple intraretinal transplantation into mammalian eyes. *J Neurosci Methods*. 1992; 43:157–169.
26. Radner W, Suzuki S, Humayun M, et al. Increased spontaneous ganglion cell activity after neural retinal transplantation [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1999;40:**S**160. Abstract nr 2426.
27. Lawrence JM, Sauve Y, Keegan D, et al. Schwann cell grafting into the retina of the dystrophic RCS rat limits functional deterioration. *Invest Ophthalmol Vis Sci.* 2000;41:518–528.
28. Craner S, Radel J, Jen L, et al. Light evoked cortical activity produced by illumination of intracranial retinal transplants: experimental studies in rats. *Exp Neurol.* 1989;104:93–100.

29. Eliasof S, Barnes S, Werblin F. The interaction of ionic currents mediating single spike activity in retinal amacrine cells of the tiger salamander. J Neurosci. 1987;7:3512–3524.
30. McLoon L, Lund R, McLoon S. Transplantation of reaggregates of embryonic neural retinae to neonatal rat brain: differentiation and formation of connections. *J Comp Neurol.* 1982;205: 179–189.
31. Lund R, Coffey P. Visual information processing by intracerebral retinal transplants in rats. *Eye.* 1994;8:263–268.
32. Mohand–Said S, Hicks D, Simonutti M, et al. Photoreceptor transplants increase host cone survival in the retinal degeneration (rd) mouse. *Ophthalmic Res*. 1997;29:290–297.
33. Blanks J, Adinolfi A, Lolley R. Photoreceptor degeneration and synaptogenesis in retinal degenerative (rd) mice. *J Comp Neurol*. 1974;156:95–106.
34. Faber D, Flannery J, Bowes–Rickman C. The rd mouse story: seventy years of research on an animal model of inherited retinal degeneration. *Prog Retinal Eye Res.* 1994;13:31–64.
35. Bowes C, Danciger M, Baxter L, et al. Retinal degeneration in the rd mouse is caused by a defect in the β subunit of rod cGMP-phosphodiesterase. *Nature.* 1990;347:677–680.
36. LaVail M, Matthes M, Yasumura D, Steinberg R. Variability in rate of cone degeneration in the retinal degeneration (rd/rd) mouse. *Exp Eye Res.* 1997;65:45–50.
37. Dräger U, Hubel D. Studies of visual function and its decay in mice with hereditary retinal degeneration. *J Comp Neurol*. 1978;180: 85–114.