

Function and Structure in Retinal Transplants

Alan R. Adolph¹, Charles L. Zucker¹, Berndt Ehinger² and Anders Bergström²

¹*The Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, USA*

²*Department of Ophthalmology, University of Lund, Lund, Sweden*

SUMMARY

Embryonic mammalian donor retina transplanted into the subretinal space of a mature host develops into a graft with well-organized, but atypical retinal structure. We tested the effect of this organization on rabbit-to-rabbit graft functional properties, isolating the graft to avoid contamination of graft responses by host retinal activity. Transient ON or ON-OFF spike-like responses and local electroretinograms (L-ERGs) were recorded simultaneously via a single electrode on the graft surface. These response components depended on stimulus diameter, sometimes in a way indicating antagonistic center-surround receptive field organization and spatial tuning (43%). Other times, the responses were an increasing function of stimulus diameter which saturated for large spots (57%). Response amplitudes were a monotonically-increasing function of light intensity over the narrow range tested. The L-ERGs were reminiscent of the proximal negative response or M-wave seen in normal retinas, which reflect light-induced amacrine cell activity. Thus, for the first time, we have shown that these subretinal grafts possess light-transduction and complex functional properties like those in normal retinas. They also possess the cellular complement and synaptic microcircuitry needed to form these physiological properties. Therefore, these results demonstrate a functional ability and capacity in transplants that is required if nerve cell

transplantation surgery is to be done with therapeutic aims.

KEY WORDS

retina, transplants, electrophysiology, function, anatomy, ultrastructure

INTRODUCTION

Retinal transplantation as a strategy for restoration or rescue of function in retinas rendered afunctional by disease or trauma is an intriguing idea. There has been rapid growth in research focused on this strategy, partially stimulated by recent transplantation efforts in brain related to neurodegenerative diseases such as Parkinson's, Huntington's, and Alzheimer's /1-3/. Such studies have been hindered by the fact that the specific properties of the inputs, intrinsic processing, and outputs of the systems affected, are not well-defined. This is especially significant when attempting to study specific correlated functional and structural properties of the implanted tissue. In contrast to these studies, the present study utilizes the retina, for which there is good understanding about the inputs (complex light patterns), intra-retinal processing, anatomical microcircuitry, and the output (patterns of ganglion cell activity) /31,32/. Here we demonstrate for the first time, organized, complex physiological function in combination with its underlying neuronal microcircuitry in mammalian sub-retinal transplants.

The use of embryonic or fetal donor tissue permits *in situ* development, differentiation, and persistence in the host retina of most types of replacement retinal elements and the complex circuitry required to process visual stimuli /4,5/. Anatomically, these grafts synaptically integrate

Reprint address:

Alan R. Adolph
Neuroscience Unit
The Schepens Eye Research Institute
20 Staniford Street
Boston, MA 02114, USA

intrinsically and may do so with the (mature) host retina /6/. Second, because of the relative immunological privilege of the retina/CNS and the reduced immunogenicity of fetal donor tissue, rejection has not been problematic in allografts. Standard immunosuppression therapies have even permitted widely disparate xenografting like human donor to rat host /2,5,6/.

In initial attempts at retina to retina transplantation, Turner and Blair /7/ injected fragments of embryonic donor retina into a vitreal locus apposed to a retinal lesion site. The grafts, which fused with the host retina, formed layered, folded sheets with rosettes consisting of circular arrays of photoreceptors (outer segments oriented inward) and outer neural layers /8/. del Cerro *et al.* /9/ injected suspensions of fluorescent-tracer labeled neonate retinal cells into the subretinal space of normal and light-damaged host rats. They observed possible anatomical integration of the differentiated donor cells with host retina, and ultrastructural evidence suggestive of photoreceptor to second-order neuron synaptic contact. A later study of retinal transplantation in rodents by this group demonstrated, using psychophysical methods, possible restoration of retina-dependent function /33/. Sheets of intact photoreceptor layer were also grafted subretinally into light-damaged adult rat retinas by Silverman and Hughes /10,11/. In these experiments already differentiated photoreceptors were grafted to rd mouse and RCS rat retina mutants, and mature human photoreceptors to immunosuppressed, light-damaged adult rats. The grafts survived and showed potential for photo-transduction (opsin immunostaining), development of photoreceptor synapses, and possible involvement in pupillary reflex action /12/.

Several criteria must be satisfied in order for transplanted embryonal retinal tissue to develop into a functional retina within the host. Firstly, the fundamental retinal cell types must develop and mature such that their functionally important components (e.g. photoreceptor outer segment discs, synaptic components, etc.) are present. Secondly, these cells should be organized in a fashion which allows for appropriate cellular connectivity (e.g. photoreceptor synaptic terminals located near bipolar and horizontal cell processes,

and bipolar cell terminals near amacrine and ganglion cell processes). Finally, these neurons must make both functional and appropriate synaptic contacts. We have been examining these issues using specific physiological and anatomical approaches in rabbit donor to rabbit host retinal transplants.

METHODS

Retinas from Dutch-belted rabbits served as donor and host. Mature eyes were relatively large, approximating human and primate eyes in size (ca. 20 mm diam.) and, unlike rodent eyes, in their relative volumes of lens capsule and vitreous cavity, making them easily adaptable for manipulation with ophthalmic microsurgical instruments. Aggregates of embryonic day 15 or 16 (E15 or E16) donor retinas (birthdate, E32), were transplanted to a subretinal locus in the posterior pole of adult male hosts. After development of mature grafts (45 to 60 days post transplant), an isolated graft preparation was set up *in vitro* as a superfused, everted eyecup preparation. The resulting isolated grafts were large (>1mm) and visible under the dissecting microscope.

The transplant surgical techniques are described in detail elsewhere /13/. Briefly, fragments of embryonic donor neural retinas are inserted into a subretinal locus using a flexible Teflon micropipette. The pipette enters the intraocular space via a pars plana approach through a metal guard tube. The guard tube and extendable micropipette are incorporated into a stainless steel surgical handpiece. Donor tissue is ejected from the pipette by hydraulic pressure from a microsyringe. Micropipette manipulation and tissue ejection are viewed through a surgical contact lens.

In the isolation procedure, the host retina is carefully stripped from its underlying RPE/choroid/sclera base, using microforceps and a microsclerotomy knife. The graft remains attached to the base, usually separating cleanly from the retracting host retina. The host optic nerve is transected where it passes through the choroid/RPE surface. This procedure permits complete detachment and removal of the host retina, and leaves a large, highly visible graft anchored to an

underlying host RPE/choroid/sclera base (refer to Fig. 2B).

For electrophysiology, electrodes were epoxy-insulated tungsten with platinum blacked tips, 4-10 megohms resistance (F. Haer & Co.). Amplifier was an Axoprobe-1A with output split into low-pass (LERGs) and high-pass (spikes) frequency bands by a variable frequency, electronic filter (AP Electronics). The electrodes were positioned with a manual micromanipulator (Brinkmann) and stepped using a hydraulic (Kopf) microdrive. Light stimuli were spots formed by aperture stops in the collimated beam of an optical system with tungsten-halogen light source, neutral-density attenuation filters and narrow-band interference filters. Position was steered by orthogonal, galvanometer-driven mirrors. Extracellular spike activity was analyzed as peristimulus spike histograms time-locked to the computer controlled stimulus trigger signal. Fourier analysis of the spike histograms by the computer generated mean amplitude, phase, and variance measures up to N (specifiable) harmonics.

In the *in vitro* retinal preparation, the eye was enucleated under urethane general anesthesia supplemented by local lidocaine anesthesia around the anterior segment of the globe. Animal care and surgical procedure was in accordance with institutional guidelines and approved by the Schepens Eye Research Institute's Animal Care and Use Committee and adhered to the ARVO Resolution on the Use of Animals in Research. The lens and anterior segment were excised, vitreous removed, the eyecup flattened using peripheral radial cuts and everted and positioned over a dome support in the perfusion chamber, and held in position by an O-ring clamp. Ames medium supplemented with Nu-Serum (Becton-Dickinson) was superfused over the retina at ca. 2-5 ml/min. A circulating heater (Lauda) and organ-bath components (Radnotti) were used to maintain bath temperature at 35°C. pH was monitored on-line by a microsensor (Microelectrodes Inc.) and maintained at 7.4 by adjusting the O₂/CO₂ ratio of the gas mixture bubbling through the Ames medium. The physiological condition of the retina was monitored using the recorded ERG responses. Host or graft retinas had stable responses under these conditions for 4 to 6 hours or longer.

For Lucifer Yellow filling, the posterior eyecups with the transplants were lightly fixed in 1% buffered formaldehyde for 30-40 min and washed. The transplants, adherent to the pigment epithelium and choroid, were mounted on a black Millipore filter (type AA, 0.8 micron pore size). The tissue was stained with ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium, 1 µg/ml = 2.5 µM) for 2 mins and then transferred to a specially designed stage chamber in a Zeiss UEM microscope equipped with epifluorescence illumination. The transplants were identified by their red ethidium bromide fluorescence, and cells within them impaled with a Lucifer Yellow-filled (2-4% in distilled water) microelectrode (50-100 megohms). Cells were injected with dye by iontophoretic current (several nanoamperes, pulsed negative for several minutes) while the extent of filling was monitored with the fluorescence microscope. In favorable cases, more than 100 cells could be injected in a single transplant. After the dye injections and photographs in whole mount, the tissue was fixed as described below, serially sectioned, and checked for completeness of host retina removal.

For both light and electron microscopy, retinas were fixed with 1.6% glutaraldehyde plus 1% freshly prepared formaldehyde in 0.1 M sodium phosphate buffer containing 0.15 mM CaCl₂, pH 7.35 for one hour at room temperature, followed by overnight at 4°C. Tissue was then washed, post-fixed for one hour with 2% OsO₄, dehydrated through a graded series of ethanol and embedded in Epon. One micron sections were cut for light microscopy and stained with a mixture of methylene and toluidine blue. For EM, 80 nm silver sections were cut, counterstained with uranyl acetate and lead citrate, and examined with a Philips 410 EM at 80 kV.

RESULTS

The subretinal transplant technique /13/ produces grafts that are well organized morphologically and synaptically, but not identical to normal retinas. There are regions containing "rosettes" of photoreceptors surrounded by appropriately layered retinal elements (Fig. 1). The goal of the research presented in this paper is to

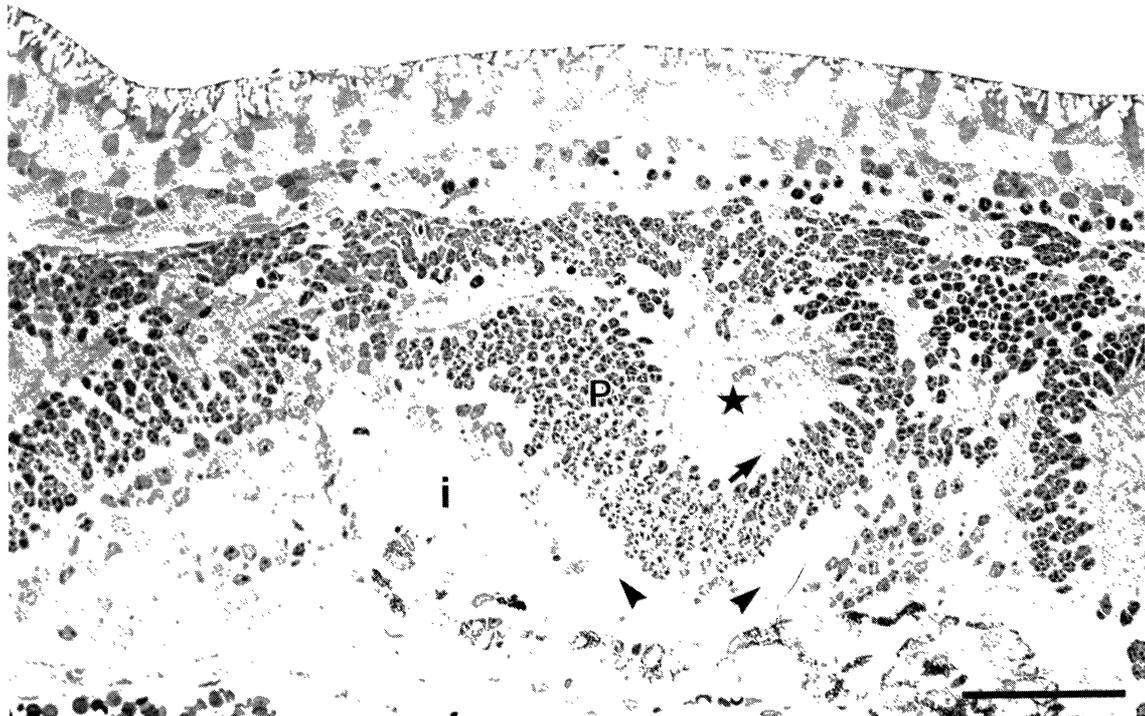


Fig. 1: Seen in the light microscope, transplants contain regions that resemble the normal layers of the adult retina. The grafts are composed of cell layers organized into rosettes with photoreceptor cells oriented toward the rosette lumen. A rosette is composed of a lumen (star) surrounded by an innermost lamina of photoreceptor cells and, to varying degrees, by other retinal layers. An outer limiting membrane (i.e. a zone of tight junctions between the cells) at the level of the photoreceptor cells is also usually seen (arrow). Photoreceptors form the most central cell layer of the rosettes (P). Often, a concentric neuropil similar to an outer plexiform layer (arrow heads) is seen around the rosette center. The space between rosettes is usually filled with cells similar to those of the inner nuclear layer with neuropil regions resembling the inner plexiform layer (i). In contrast to the normal appearance of the host retina, remote from the graft, host photoreceptors largely degenerate where the graft separates these cells from the pigment epithelium. No cellular infiltration nor other signs of inflammation are seen in either host retinas or grafted retinas. (Calibration bar = 50 μm .)

establish that there are light responses and some degree of visual processing in the rabbit subretinal transplants. Evaluation of activity inherent in the graft is complicated in the graft-host complex which results from our transplant procedure, due to the “contaminating” electrical activity derived from the juxtaposed host neural retina. Our initial tests have, therefore, been done under conditions where host activity is eliminated. The transplant is physically and electrically isolated by stripping away the overlying host retina, leaving an island of graft retina sitting on a base of host pigment epithelium (Figs. 2A, B). This preparation is maintained *in vitro* in an organ bath apparatus. In 8 out of 9 experiments, light-driven retinal responses were recorded by micro-electrodes. (In the unsuccessful

experiment, the host lens and vitreous sustained surgical trauma during the transplant procedure. This resulted in cataract formation, massive vitreal reaction and retinal detachment, severely damaging the graft.) In addition, the completeness of graft isolation from the host was confirmed by examining the grafts in serial histological sections in the light microscope. No traces of host retina were identified in the sections.

Focal field responses (L-ERGs) and spike-like activity were recorded simultaneously at various regions on the graft using a platinized, tungsten extracellular microelectrode (Fig. 3). Mapping the response with a light spot showed that the receptive field was restricted to the area on the graft around the electrode tip; there was no measurable response

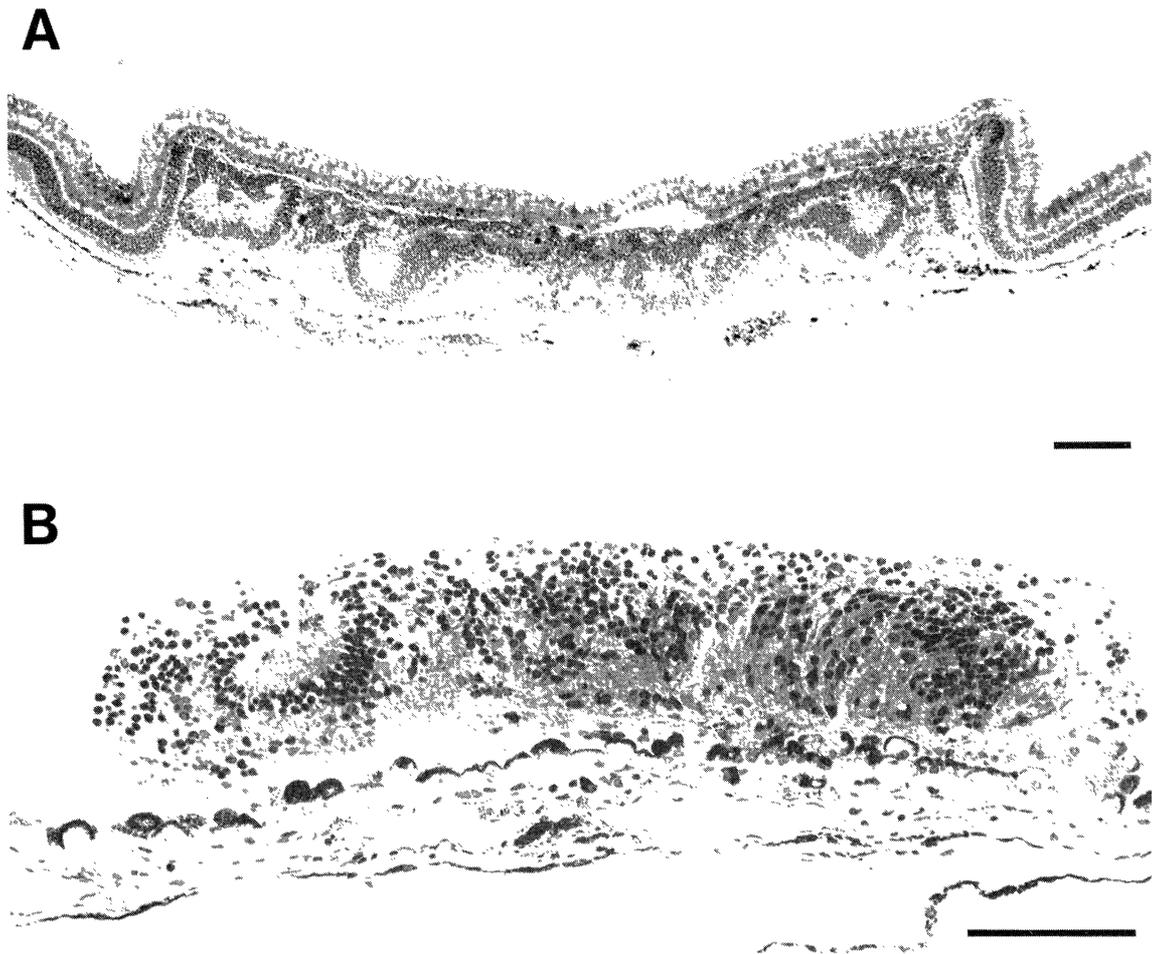
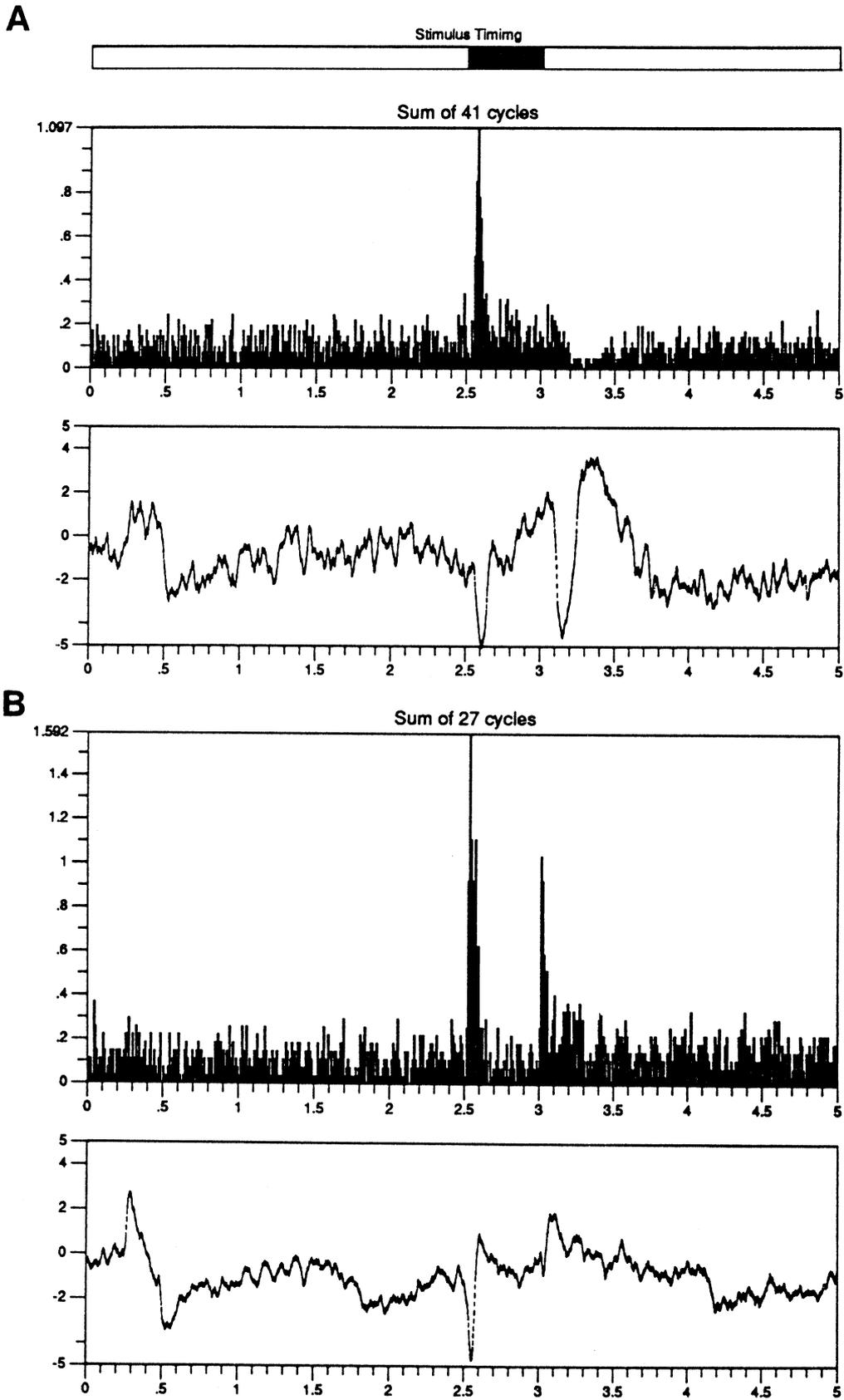


Fig. 2: Low magnification light micrographs of sub-retinal grafts from two different preparations with the overlying ghost retina in place (A), and after physical isolation of the graft (B). It is from such isolated grafts (Fig. 2B) that electrical activity was recorded. Through serial sectioning, we have been able to confirm the completeness of isolation from residual host retina. (Calibration bars = 100 μm .)

to a 250 μm light spot, focused onto regions off the graft or even on it, but distant (>250 μm) from the electrode location (Fig. 4). The time-course of spike-like responses was transient ON or ON-OFF. This may reflect a sampling bias in the experiments or a dearth of direct, transretinal, OFF-only pathways activated under the photopic stimulus conditions. Spike response measures were sometimes maximal at an intermediate stimulus spot diameter (N=4). This may indicate some form of center-surround, receptive-field organization and spatial tuning (e.g. Fig. 5A). However, in other instances (N=3), neither peak frequency of spike-like responses nor local ERG amplitude had this

sharply-peaked dependence on spot size (e.g. Fig. 5B). Both spike-like activity and local ERG amplitude appeared to depend on stimulus intensity (Fig. 5C). For a given stimulus spot size, they increased with light intensity. (One graft in the series of eight from which activity was recorded was only qualitatively evaluated.) Graft local ERGs consisted of varying proportions of transient hyperpolarizations at light-ON and -OFF, such as a proximal negative response or M-wave. In normal retinas, such responses are attributed to large IPL potassium currents which reflect light-induced amacrine cell activity [16,17]. The differing spatial dependence of extracellular spike response pattern



and concurrent local ERG activity (e.g. Figs. 3, 5A) suggests distinct regions of origin within the graft, e.g., spiking amacrine processes, or, possibly, ganglion cells of superficial locus, and amacrine/Müller cell activity in INL/IPL-like regions.

Ultrastructurally, photoreceptor inner and outer segments with connecting cilia and basal bodies appeared well developed /30/. Clusters of photoreceptor terminals frequently exhibited one or several synaptic ribbons with postsynaptic processes arranged in a dyadic or triadic configuration (Fig. 6). Postsynaptic elements at these photoreceptor synapses resembled those belonging to horizontal and bipolar cells. Regions corresponding to the inner plexiform layer exhibited a relatively high synaptic density, with most synaptic terminals being well filled with conventional synaptic vesicles. Occasionally, small and large dense-cored vesicles were seen indicating the presence of biogenic amines and neuropeptides, respectively. Bipolar ribbon synapses were also common, often with postsynaptic processes in a typical dyadic arrangement (Fig. 7A). Amacrine-to-amacrine (Fig. 7B) and amacrine-to-bipolar synapses were common, sometimes in both serial and reciprocal configurations. Gap junctions involving amacrine and bipolar cell processes were also readily identified.

Using intracellular dye filling with Lucifer yellow, we identified retinal neurons with cell body and dendritic morphologies resembling those of all

normal retinal cell types with the possible exception of ganglion cells. Two examples of Lucifer yellow-filled, intragraft, amacrine cells are shown in Fig. 8. The dendritic morphology of the cell in Fig. 8A resembles that described for narrow-field AII-amacrine cells, which in normal rabbit retinas act as interneurons between rod bipolar cells and cone ganglion cells /21/. An example of a wide-field amacrine cell, also found in normal rabbit retinas /22/, is shown in Fig. 8B.

DISCUSSION

The results indicate a high level of function and organization of underlying structure within the transplants. We have shown by light and electron microscopy /25,26/ plus intracellular dye marking /27/ that rabbit retinal grafts develop in a manner that provides distinct retinal regions where specific retinal processing can take place. The transplants contain in abundance the specific cellular and synaptic components which are necessary for phototransduction and for the processing of visual information in a fashion similar to that carried out by normal retinas. The isolated grafts exhibit spike-like activity organized into spatial receptive fields with excitatory centers, and either inhibitory or no surrounds. They also have light-driven local ERGs reflecting amacrine cell (M-wave) and bipolar cell (b-wave) activity. Thus, rabbit retinal grafts contain the neural components and local synaptic circuitry

Fig. 3: Electrical activity in isolated grafts in response to light. The activity recorded by a single extracellular tungsten microelectrode in response to a 0.5 mm diameter light spot focused on the graft surface. The light source was a spectrally-unfiltered tungsten-halogen lamp producing an irradiance of ca. 100 $\mu\text{W}/\text{cm}^2$. Shutter open is indicated by the dark bar in the upper trace in A, and occurs between 2.5 and 3.0 sec of the 5.0 sec peristimulus time frame. Upper data panels in A and B are peristimulus spike histograms to 41 repetitions (A) and 27 repetitions (B) of the stimulus cycle. Spike responses from the band-pass filter are threshold-detected and converted to standard pulses which are processed by the histogram program. The abscissa in all traces is time in seconds. The histogram ordinates are in hundreds of spikes/sec, i.e. peak responses in A is 109.7/sec, and in B is 159.2/sec. Lower data panels in A and B are averaged LERG responses. A 20 μV calibration pulse occurs between 0.25 and 0.5 sec of each response, which is averaged along with the LERG signal. The distortion of the square calibration pulses by the band-pass filtering indicates the degree of distortion of the LERG signal. In A, spike response is ON transient with slight sustained elevation of rate over background spontaneous activity. There is an inhibition of firing at light OFF. The LERG exhibits transient hyperpolarizations at light ON (ca. 20 μV) and OFF (ca. 60 μV). In B, the spike response is transient ON-OFF with no poststimulus inhibition. In contrast to the LERG shown in A, that in B has a distinct hyperpolarizing transient at light ON (ca. 40 μV) and an attenuated one at OFF (less than 10 μV).

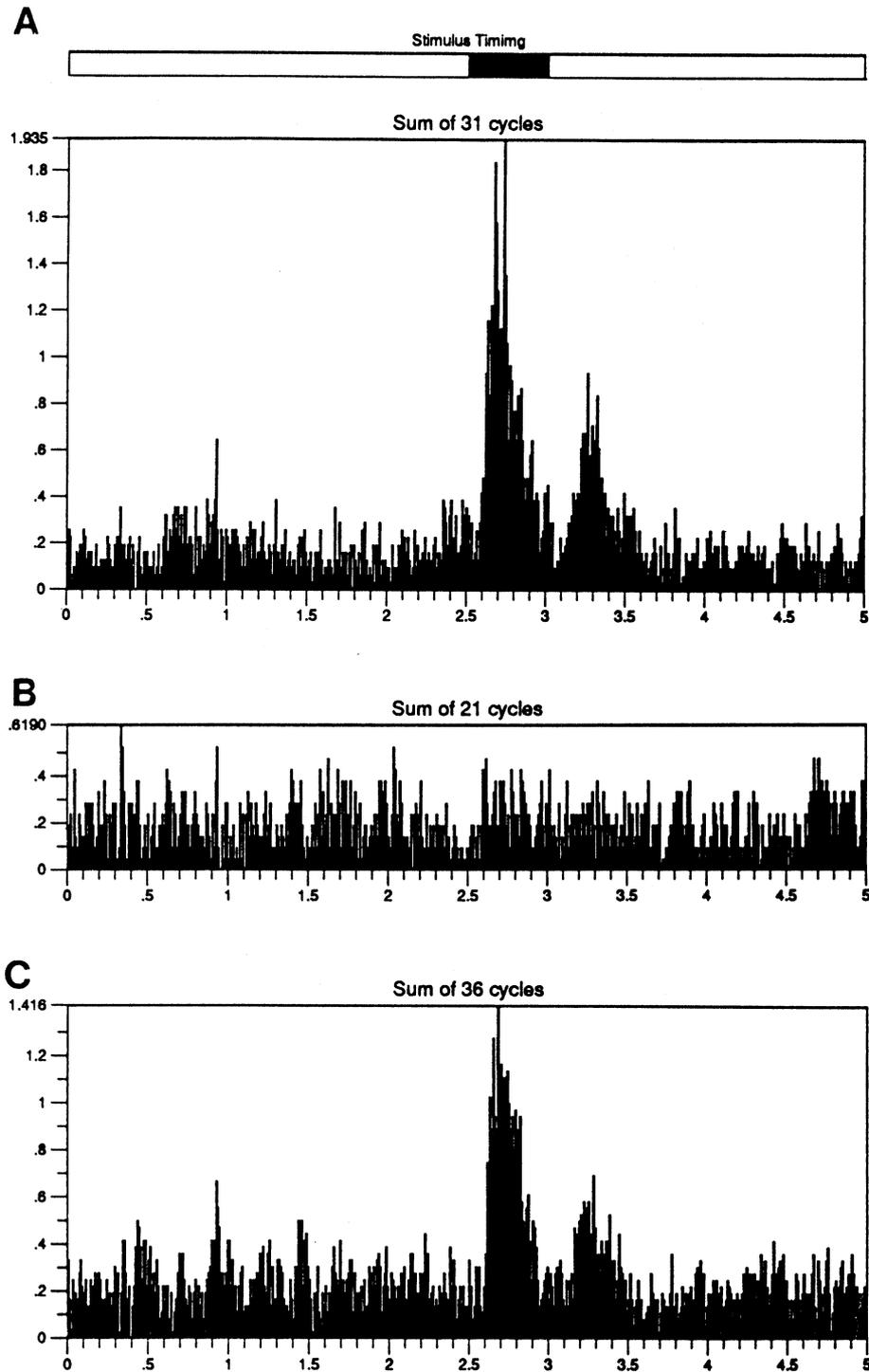


Fig. 4: Spatial localization of spike response by isolated graft to focal light stimulus. A 0.25 mm diameter light spot was used to map spike response receptive field localization. Spike histograms obtained and displayed like the data presented in Fig. 2. (A) ON-OFF response seen when the stimulus spot was centered over the receptive field (RF). The RF center was coincident with electrode tip location. (B) Activity seen when the spot was moved ca. 0.25 mm temporal, but still on the graft. (C) Spot repositioned to RF center resulting in an ON-OFF response like that in (A). A similar no response result was seen when the spot was positioned in each of 3 other locations orthogonal to the RF center and displaced by 0.25 mm. The displaced spot positions included 2 on the fusiform graft (0.25 mm temporal and nasal) and 2 off the graft but on the host RPE (0.25 mm dorsal and ventral).

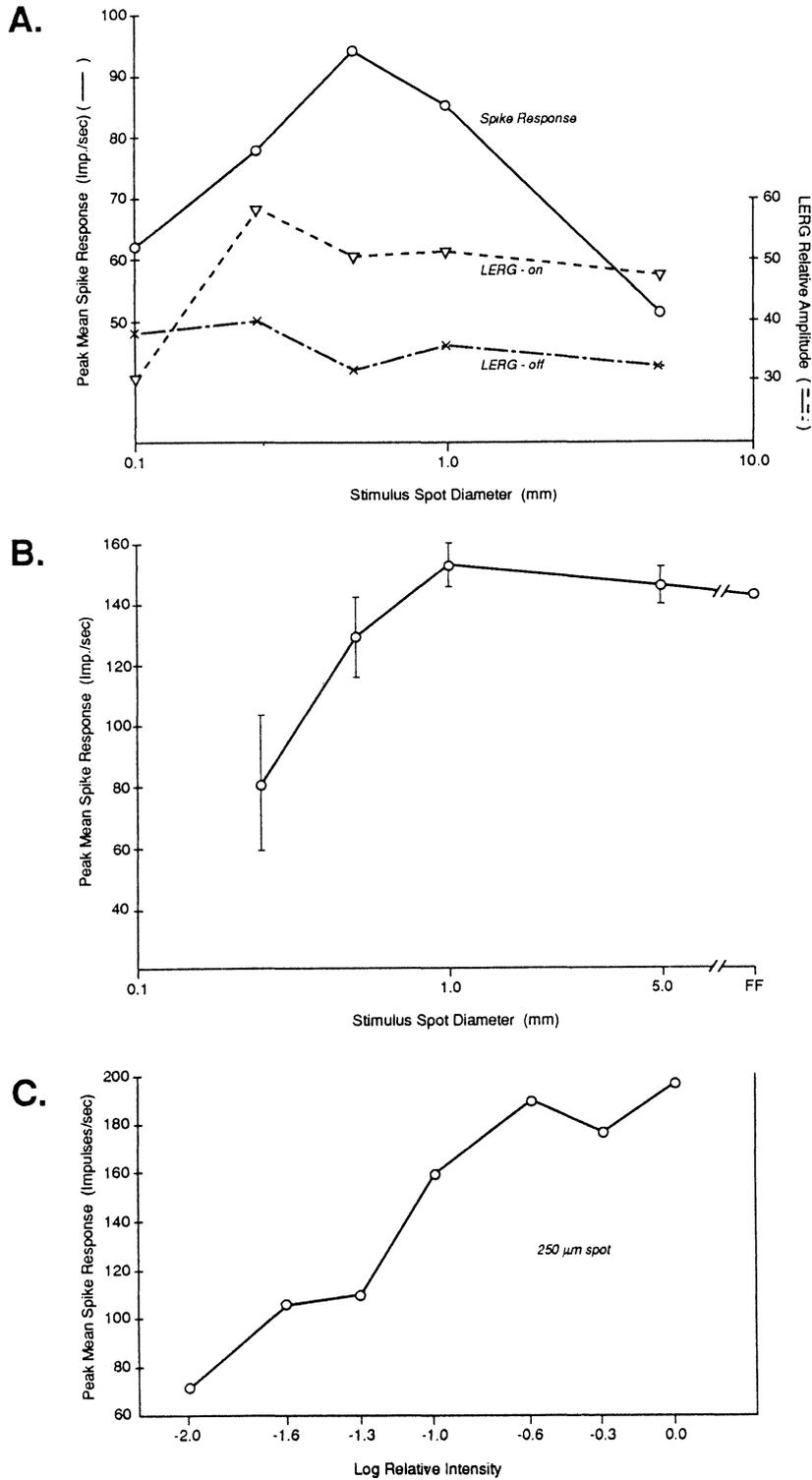


Fig. 5: (A) Response as a function of stimulus diameter. Peak frequency of averaged spike response to repeated light stimuli of indicated diameter with constant intensity. Relative amplitudes of LERG ON- and OFF-transients recorded simultaneously with spike responses. (B) Example of peak frequency of spike response which does not decrease markedly for large diameter stimuli. This may be a receptive field with little, or no, surround inhibitory influences. (C) Spike response as a function of stimulus intensity. Averaged peak spike response at different light intensities with fixed spot diameter (0.25 mm). Attenuation using neutral density filters; maximum irradiance at 0.0 log attenuation ca. 100 $\mu\text{W}/\text{cm}^2$.

necessary for the electrophysiological responses seen in these grafts and may serve for more complex physiological processing of visual information.

A question remains concerning the origin of the organized spike activity within these grafts. It is unlikely that the source of the spike-like activity recorded in the isolated grafts derives from host ganglion cells. Histological examination of the isolated graft preparations indicates the absence of residual host retina, including host ganglion cells (Fig. 2B). Although it is possible that we overlooked host ganglion cells despite examining serial sections through the full extent of the isolated graft preparations, it is highly improbable that this has happened in all the isolated grafts tested (N=9) which gave similar electrophysiological responses (N=8). Also, since we are dealing with subretinal grafts, host ganglion cells would generally be separated from the graft by the rest of the host

retinal layers. Thus any remaining host ganglion cells would likely be embedded in sufficient host retina to make them readily observable upon serial sectioning.

Perhaps ganglion cells developed in the graft are the source of the recorded spike activity. Although this is a more attractive hypothesis than their origin being host ganglion cells, there are some inherent problems with this interpretation. In normal prenatal rabbit retina, ganglion cells outside the visual streak do not begin to differentiate until ca. E20-E24 (those within the streak, even later), significantly later than the age (E15/E16) of the donor retinas /23/. By E30 in normal retina, elongation of ganglion cell axons towards relatively proximate tectal targets has begun within the permissive environment of the optic fiber layer /28/.

Thus, although the E15/E16 donor ganglion cell precursors are not subject to the degenerative influences of axotomization, they do face an

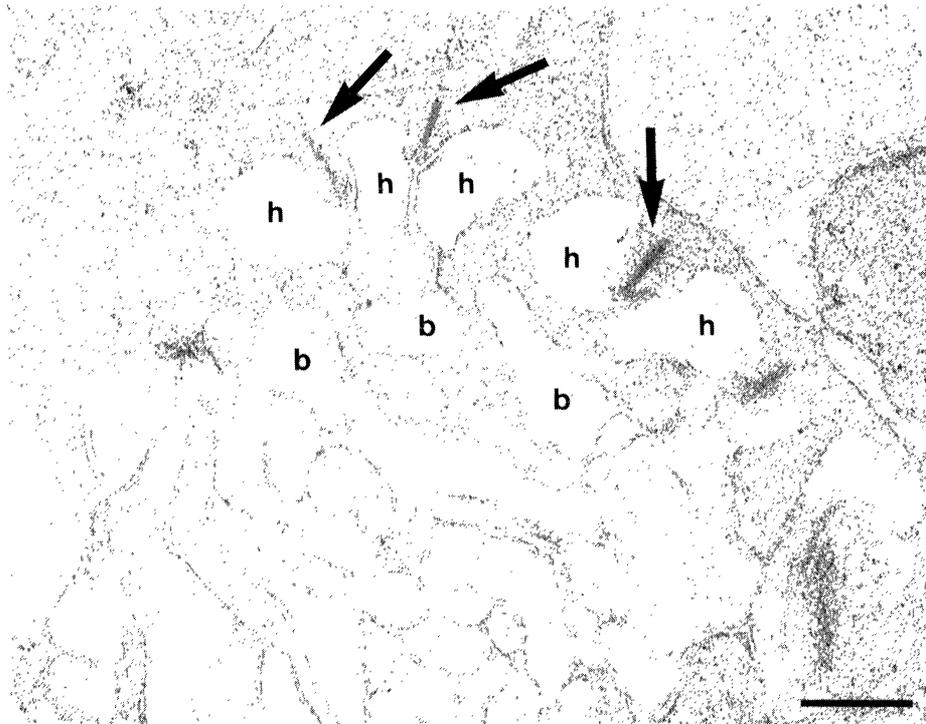


Fig. 6: Electron micrograph of synaptic features common to the outer plexiform layer within rabbit retinal transplants. A photoreceptor synaptic terminal (cone pedicle) is shown with several characteristic synaptic ribbons (arrow), each with postsynaptic horizontal (h) and bipolar (b) cell processes arranged in a typical triadic configuration. Such outer plexiform layer type circuitry underlies the formation of center-surround receptive-field organization and spatial tuning characteristics seen in normal retinas. (Calibration bar=0.5 μm .)

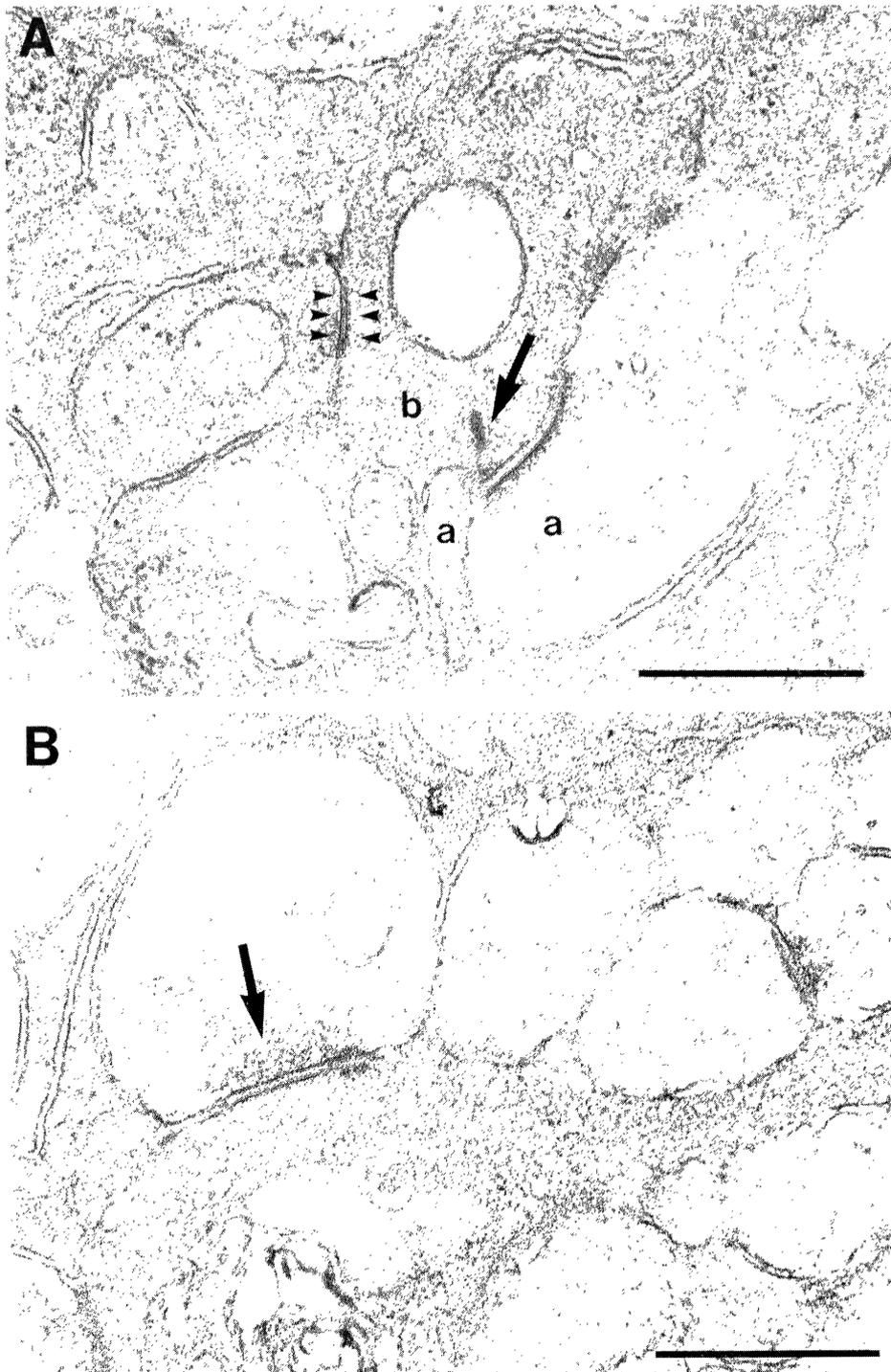


Fig. 7: Electron micrographs of synaptic features common to the inner plexiform layer within rabbit retinal transplants. (A) Shows a bipolar cell synaptic terminal (b), in an inner plexiform region of a graft, exhibiting a synaptic ribbon (arrow) and postsynaptic amacrine cell processes in a typical dyadic arrangement. (B) Shows an example of a conventional amacrine-to-amacrine cell synapse (arrow) frequently found in inner plexiform regions of retinal transplants. Amacrine/inner plexiform layer circuitry underlies transient ON-OFF type responses seen in normal retinas. (Calibration bars=0.5 μ m.)

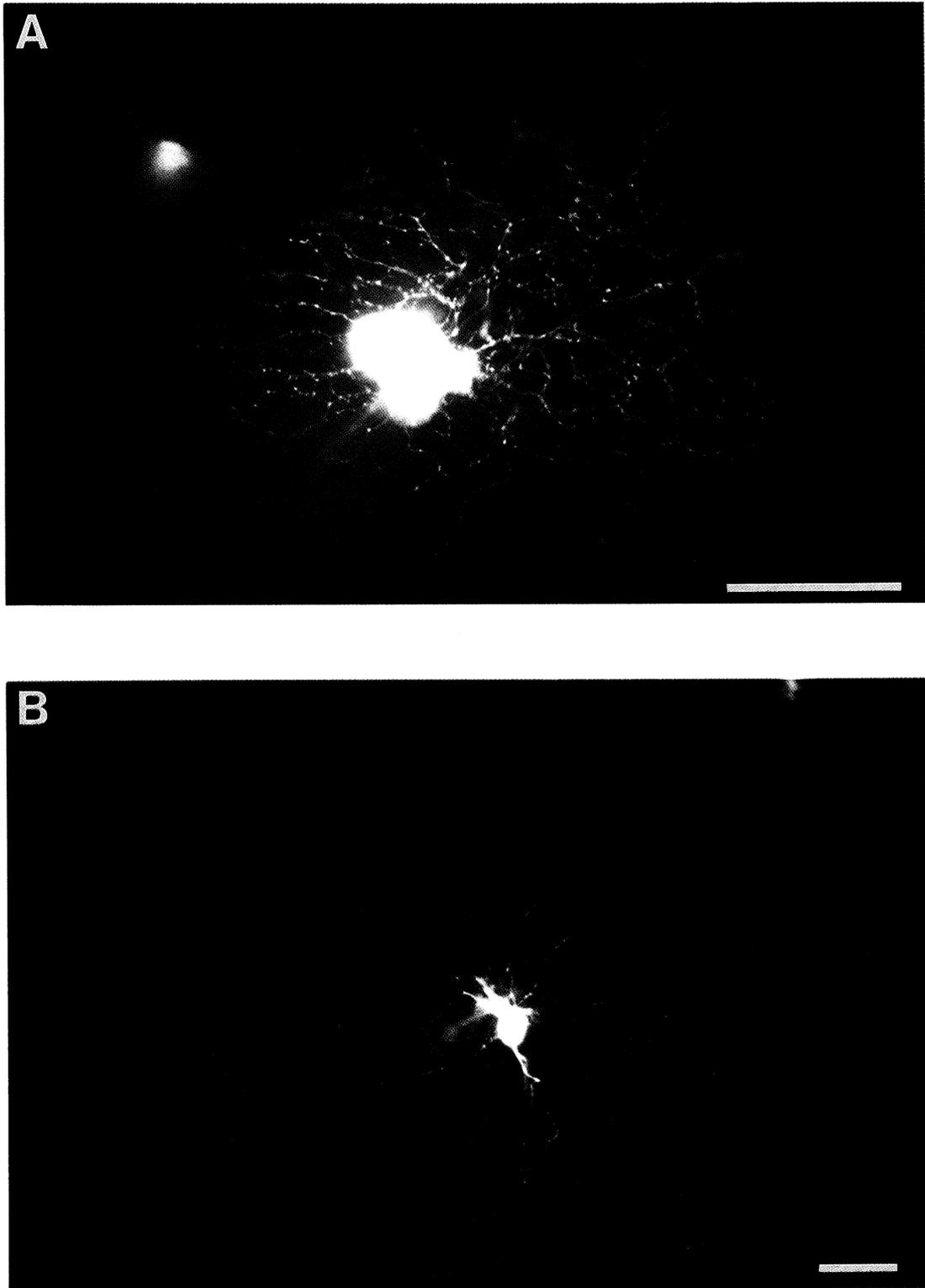


Fig. 8: Intraocular amacrine cells filled with Lucifer yellow. (A) A narrow-field amacrine cell with a dendritic morphology resembling that described for AII-type amacrine cells. (B) Shows an amacrine cell with a wide dendritic field spread. Note that the calibration bars represent 20 μm for both cells.

environment inhibiting axonal regeneration through the subretinal space of the mature host and isolation from a CNS target. In addition, the tectal target which acts trophically and tropically during normal development of ganglion cell axons, is more distant and mature in the host and may not influence donor ganglion cell development in the subretinal graft. In any event, at postnatal day P30-P45 or older, at a time when ganglion cell axons in normal intact retinas have established functional contact with the CNS /24/, the subretinal grafts appear largely devoid of mature ganglion cells identified by both their morphology and a variety of qualitative immunohistochemical markers (e.g. Thy-1, NSE, AB-5) /29/.

Alternatively, amacrine spike activity may be significant in the isolated grafts and therefore responsible for the organized, light-driven, spike responses within the grafts. Our usual findings of M-wave type LERG activity in the isolated grafts and significant populations of LY-labelled amacrine cells in the morphological analyses, suggest that such neurons may be the source of spike activity. In normal, control retinas, a common form of intracellularly recorded amacrine cell response consists of robust spiking superimposed on relatively large, but slower, transient membrane potential depolarizations at light-ON and -OFF /18/. Such spiking activity in wide-field amacrine cells may subserve long-range interactions within the IPL, including surround actions /14,15/, and exhibit a high degree of receptive field organization /18/. Definitive conclusions concerning the origin(s) of the spatially and temporally organized spike activity await the results of intracellular recording and dye-marking experiments.

Although the structure of these graft retinas is atypical of the flat laminar organization in normal retinas and their photoreceptor outer segments do not adjoin host pigment epithelium, the transplants do exhibit light responsiveness and well-organized functional properties. Such functionality may be expected to persist based on the demonstrated long-term persistence of light-driven responsiveness in ectopic retinal transplants to the optic tectum in rodents /19,20/. Those ectopic grafts also develop rosetiform organization of their photoreceptors without pigment epithelial contact.

In summary, there are several points that must be made. Foremost, this paper presents studies of the graft retina that develops from the subretinal transplantation of undifferentiated embryonic retina. The objective was to demonstrate that the retinal graft which develops exhibits the intrinsic anatomy and function spatially and temporally appropriate for use in a therapeutic strategy. For this purpose it was essential that the inherent responses of any host retina be eliminated to avoid any "contamination" of the graft's pristine responses. Graft-driven, spatially and temporally appropriate host optic nerve responses are essential for any possible therapeutic usefulness of retinal cell transplants. That is the subject of another study, currently underway, requiring hosts with defined functional deficits, and techniques to distinguish graft from host cells, processes, and synapses. Results of recent anatomical and physiological experiments in other laboratories, notably those of Silverman *et al.* /12// and Yamamoto *et al.* /34/, using subretinal photoreceptor or RPE allotransplants to appropriate host rodents (e.g. RCS rats and rd/rds mice), report a degree of restoration or maintenance of synaptic integration between graft and host retina, and possible graft-driven host responses. These results lead us to expect some integration at both the outer and inner plexiform levels in our rabbit transplant model analogous to the aforementioned OPL integration in the rodent models.

In conclusion, the research reported in this paper may be valuable in several ways. First, to determine whether a transplantation model, using whole neural retinal donors functionally coupled to their host retinas via longitudinal and lateral preganglion cell synaptic and junctional contacts, is a viable therapeutic strategy. Such a strategy would be applicable in retinas afunctional because of retinopathies in which the ganglion cells and optic nerve link between retina and brain are spared. Second, as a model for the prenatal ontogeny of correlated function and structure in the retina. And third, to examine, based on the apparent disparities between well-organized function in the retinal grafts and their relative anatomical disorder, the role of structural redundancy and necessity for highly ordered organization in the normal retina.

ACKNOWLEDGEMENTS

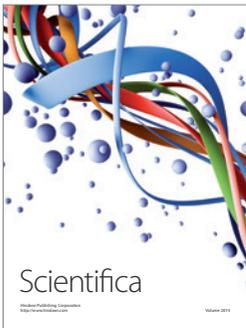
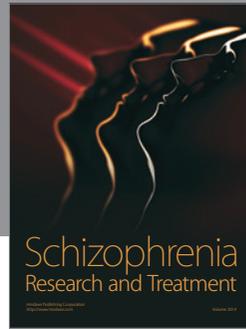
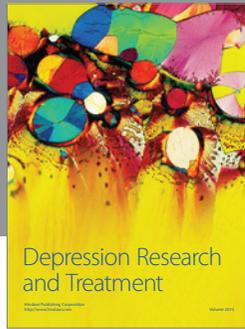
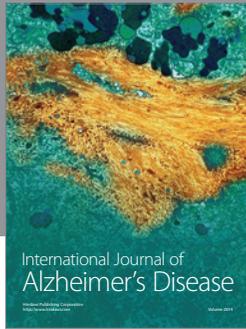
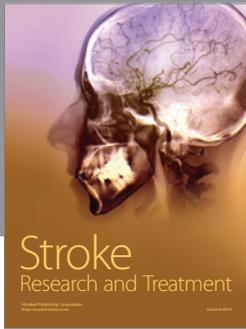
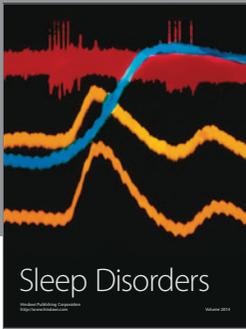
Supported by the Retinal Transplant Program of The Schepens Eye Research Institute, Charles de Gunzburg, Fred L. Emerson Foundation, Dr. S. Williams, Retina Research Foundation, T. and E. Segerfalks stiftelse, Crafoordska stiftelsen, Kronprinsessan Margaretas Arbetsnämnd, T. and R. Söderbergs stiftelser, RP Foundation, Thelma Zoégas fond, The Royal Physiographic Society at Lund, Karin Sandqvists stiftelse, Maggie Stephens stiftelse, Margit Thyselius Foundation, and Swedish Medical Research Council (projects 14X-2321 and 09724).

Thanks to C. LaBlanc and N. Beckmann for technical assistance.

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