Retinal Progenitor Sheet Transplants to Rats with Retinal Degeneration – Circuitry between Transplant and Host

Magdalene J. Seiler[1A,1B], Bryan W. Jones[2], Robert B. Aramant[1A], Robert E. Marc[2], Hans S. Keirstead[1A, 1B]

[A]Anatomy & Neurobiol/Reeve-Irvine Res Ctr, [B]Sue & Bill Gross Stem Cell Research Center, [1]UC Irvine, Irvine, CA;
[2]Ophthalmology, Moran Eye Center, Univ. of Utah, Salt Lake City, UT.

Program # 837.10
Board # L18

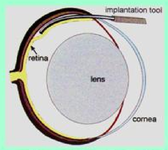
PURPOSE

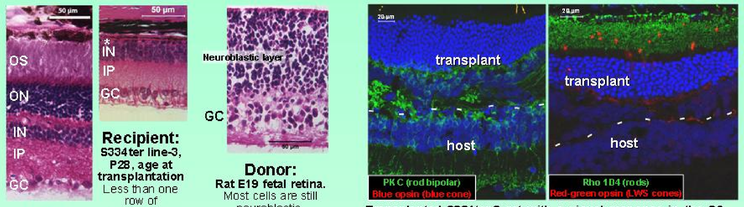
- To investigate the transplant and host circuitry in long-term retinal progenitor sheet transplants that can restore visual function in rats with retinal degeneration.

Background

Transplantation of retinal sheets

Sheets of retinal progenitor cells can be transplanted to the subretinal space of different rodent retinal degeneration models and develop lamination like a normal retina.





Normal retina

Recipient: S334ter line-3, P28, age at transplantation. Less than one row of photoreceptors (cones*).

Donor: Rat E19 fetal retina. Most cells are still neuroblastic, no lamination.

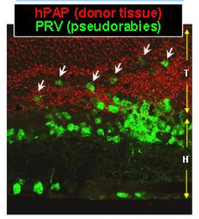
Transplanted S334ter-3 rat with a visual response in the SC. E19 donor, 9 months after surgery. Frozen sections.

Transplants can restore visual responses:

- Transplants preserve optokinetic responses (Thomas et al. 2004, *J Neurosci Meth*, 138:7-13)
- Light stimulation of the eyes can be recorded in the brain according to the retinotopic map (e.g., Seiler et al. 2008, *Eur J. Neurosci*, 28:208-220)
- Transplant neuronal cells can be labeled from the visually responsive site in the brain by trans-synaptic tracing (using labeled neurotropic pseudorabies virus)
- Transplants invade the host retina with processes which form synapses in the host inner plexiform layer (Seiler et al., *Eur J. Neurosci.* in press)

Trans-synaptic label in transplant

hPAP (donor tissue)
PRV (pseudorabies)



PRV (green) - labeled cells in transplant (red), 52 hours after virus injection into the visually responsive site in SC

Extensive donor cell process outgrowth into host

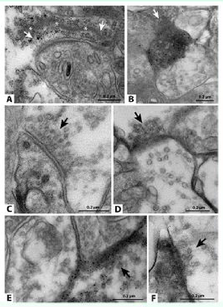
Seiler et al., *Eur. J. Neurosci.*, in press

hPAP staining of vibratome slice: extensive donor cell processes in host inner plexiform layer

Pictures on the right are from 4 different rats (6.1 – 10.5 mo. after transplantation).

Staining for donor cell label hPAP, recognizable as silver grains in the electron microscope.

Arrows indicate a presynaptic element of an apparent synapse between transplant and host cell.



We need to know which specific cell types are involved in the circuitry between transplant and host retina.

Methods

1. Transplantation

S334ter-line-3 rats with fast retinal degeneration received E19 retinal progenitor sheet transplants derived from hPAP (human placental alkaline phosphatase) expressing rats at the age 0.8 - 1.4 months. Some donor retinas were coated with BDNF microspheres or GDNF microspheres before implantation *(Seiler et al. 2008)*.

Rat eyes were imaged by 3D-Ocular Coherence Tomography (OCT) 0.4 - 2.2 months after surgery to determine transplant placement and layering.

Fourteen transplanted rats (2 retina-only, 7 retina + GDNF, 5 retina + BDNF transplants) were selected for this analysis, based on Ocular Coherence Tomography (OCT) results, and sacrificed at 2.3 – 8.5 months after transplantation (age 3.4 – 9.9 months).

2. Tissue processing

Rats were perfused through the ascending aorta with 2.5% glutaraldehyde, 1% paraformaldehyde, 3% sucrose, 1mM $MgSO_4$ in 0.1M phosphate buffer. Eye cups were postfixed overnight after removal of the cornea and embedded in 4% agarose for vibratome sectioning. Selected vibratome slices were flat embedded in Eponate.

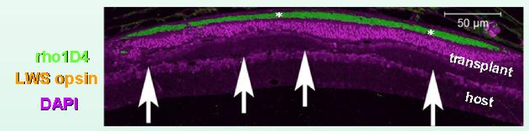
3. Immunohistochemistry for Molecular Phenotyping

Blocks were sectioned into serial ultrathin datasets and probed for aspartate, glutamate, glycine, gluthathione, glutamine, arginine, taurine, GABA, rhodopsin, cone opsin, CRALBP, and DAPI (procedure after Jones et al. 2003 (Comp. Neurol. 464:1-16). http://prometheus.med.utah.edu/~marclab/protocols_CMP.html

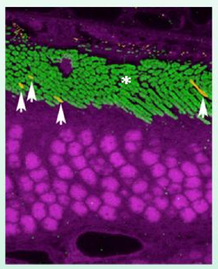
RESULTS

1 Photoreceptors (rods and cones)

In all specimen, rhodopsin+ and cone opsin+ structures were limited to the transplant region. There was never any opsin immunoreactivity (1D4 rho or LWS) in the host retina.

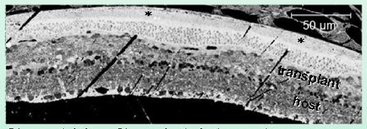


Arrows indicate the distal margin of the host retina. Transplant outer segments indicated by *.



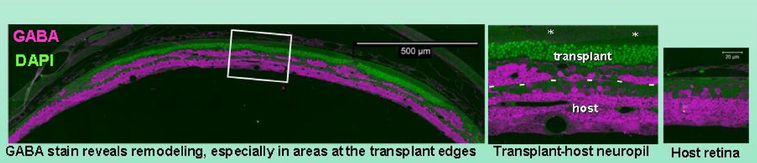
Arrows: cone outer segments (only found in transplant area)

Taurine



Strong staining of transplant photoreceptors. The transplant and host both display normal taurine levels

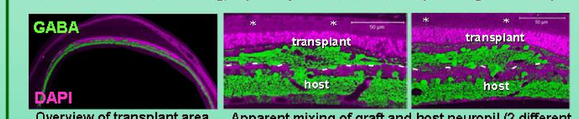
2 Inner retinal neurons (GABA, glutamate, glutathione)



GABA stain reveals remodeling, especially in areas at the transplant edges

Transplant-host neuropil

Host retina



Overview of transplant area

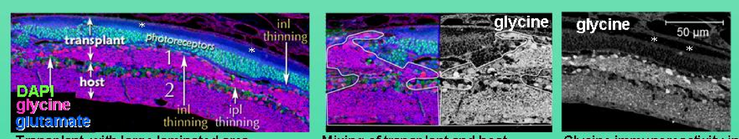
Apparent mixing of graft and host neuropil (2 different specimen). Transplant with outer segments (*)

Transplant zone with sparse interfacial processes.

Normal glutamate and glycine levels in transplant and host, but remodeling. Outer segments (*)

Host retina

Many horizontal cells in transplant (arrows)



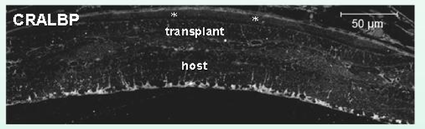
Transplant with large laminated area. Transplant inner plexiform layer (ipl) (1) and inner nuclear layer (inl) are thinned in several areas; compared to host ipl (2) (arrows).

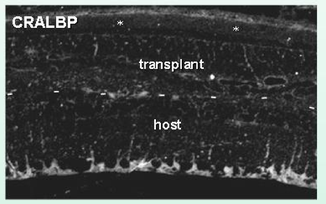
Mixing of transplant and host neuropil at edge of laminated transplant area

Glycine immunoreactivity in laminated area with outer segments (*)

The transplant and host both display normal GABA, glutamate, glycine and glutathione levels. There is extensive remodeling, especially in regions of the transplanted retina at the transplant edges that has lost its photoreceptors.

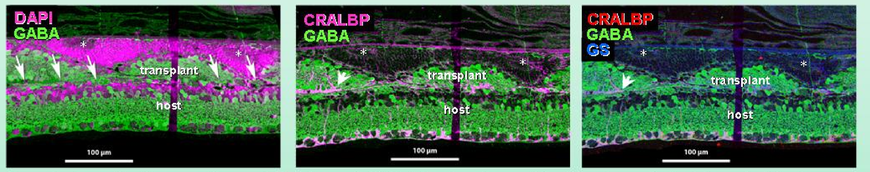
3 Glial markers (glutamine synthetase, CRALBP) and neuronal marker (GABA)





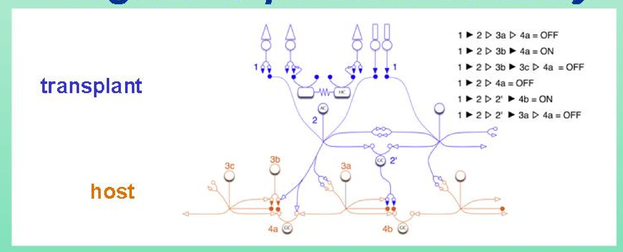
CRALBP (marker for Müller glia and RPE) is present but weaker in the transplant area with photoreceptors. Asterisks (*) indicate presence of outer segments.
CRALBP is much stronger in the remodeled, more disorganized transplant areas (not shown on this picture).

In the region with two inner plexiform layers, there is often a wide glial seal between the host and transplant. However it is clearly interrupted in spots and there are processes passing between host and transplant.



Transplant area showing some disorganization, imaged with 3 different marker combinations. Arrows indicate transplant-host interface. Glial seal on left side (arrow head in middle and right picture) is interrupted through most of the area, and neural processes crossing the transplant-host interface can be seen.

4 Diagram of possible circuitry



CONCLUSIONS

- Transplants contain healthy rods and cones with outer segments whereas the host photoreceptors are degenerated.
- Loss of neurons, including bipolar cells, was observed in the transplant inner nuclear layer.
- In many areas, host and graft neuropil are mixed with no glial barriers between transplant and host.
- Donor and host amacrine cells (glycine, GABA) are likely the main cell types involved in the communication between transplant and host.

ACKNOWLEDGEMENTS:
Supported by Lincy Foundation (MJS, RBA, HSK); NIH Grants EY02576 (RM), EY015128, EB005832 (RM), EY014800 Vision Core (RM), Research to Prevent Blindness Career Development Award (BWJ).
MJS and RBA have a proprietary interest in the implantation instrument and procedure.
We thank Fengrong Yan and Lakshmi Patil for technical assistance.

Abbreviations:
H – Host
T – Transplant
GC – Ganglion cell layer
IP – inner plexiform layer
IN – inner nuclear layer
ON – outer nuclear layer
OS – outer segments
RPE – retinal pigment epithelium
hPAP – Human placental alkaline phosphatase (donor marker)
CRALBP – cellular retinaldehyde binding protein
GS – glutamine synthetase

References:

- Strettoi et al. 2003, *Vision Research* 43:867–877
- Jones et al. 2003, *Comp. Neurol.* 464:1-16
- Jones & Marc 2005, *Exp. Eye Res.* 81:123-137
- Marc et al. 2007, *IOVS* 48:3364–3371
- Seiler et al. 2008, *Exp Eye Res.* 86:92-104
- Seiler et al. 2008, *Eur J. Neurosci*, 28:208-220
- Seiler et al., *Eur J. Neurosci*, in press