Visual Restoration And Circuitry - Retinal Sheet Transplants To Rats With Retinal Degeneration

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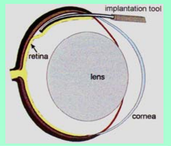
PURPOSE

- **To investigate which cell types are involved in the transplant-induced visual restoration of a degenerating retina.**

Background

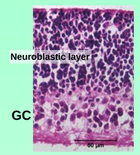
Transplantation of retinal sheets

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

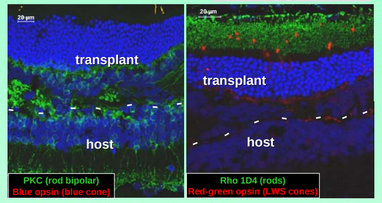


Normal retina

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.

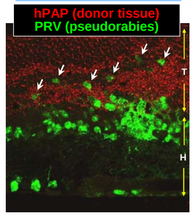


Transplanted S334ter-3 rat with a visual response in the SC. E19 donor, 9 months after surgery. Frozen sections.

Restoration of visual responses by transplants involves synaptic connectivity:

- **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; Yang et al. 2010, *Exp. Eye Res.* 91: 727-738)**
- **Transplant neuronal cells can be labeled from the visually responsive site in the brain by trans-synaptic tracing (using labeled neurotropic pseudorabies virus) (Seiler et al., *Eur. J. Neurosci* 2005, 2008)**
- **Transplant processes invade the host retina and form synapses in the host inner plexiform layer (Seiler et al., *Eur J. Neurosci*. 2010)**

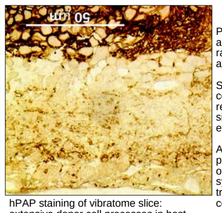
Trans-synaptic label in transplant



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

Extensive invasion of donor cell processes into host

Seiler et al., *Eur. J. Neurosci*., 2010

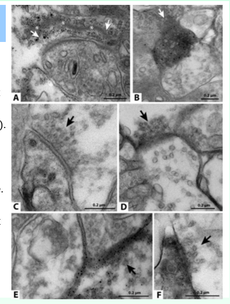


hPAP staining of vibratome slice: extensive donor cell processes in host inner plexiform layer

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.



Background summary: **Which specific cell types are involved in the circuitry between transplant and host retina?**

Methods

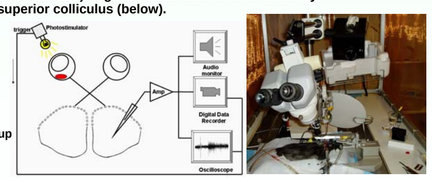
1. Transplantation

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

Rat eyes were imaged by 3D-Ocular Coherence Tomography (OCT) 0.4 - 2.2 months after surgery to determine transplant placement and layering (*Seiler et al. 2010b*). Thirteen transplanted rats were selected and sacrificed at 2.3 – 8.5 months after transplantation (age 3.4 – 9.9 months). Eight of these rats were recorded by electrophysiology for visual responses in the superior colliculus (below).

2. SC recording

Multiunit visual responses were recorded from the exposed superior colliculus (SC) with a 40ms full-field illumination stimulus varying from -5.9 to 1 log cd/m^2 (see *Yang et al. 2010, Exp. Eye Res. 91:727-738*).



Recording and stimulation setup

3. Tissue processing

Rats were perfused through the heart into 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.

4. Immunohistochemistry for Molecular Phenotyping

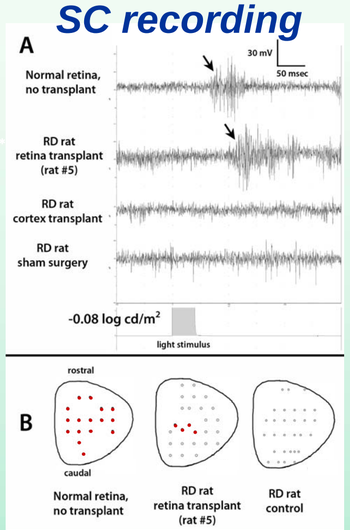
Blocks were thin sectioned at 0.25 µm into serial arrays and probed for aspartate, glutamate, glycine, gluthathione, glutamine, arginine, taurine, GABA, rhodopsin, cone opsin, CRALBP, and DAPI (procedure after Jones et al. 2003 (Comp. Neurol. 464:1-16). http://prometheus.med.utah.edu/~marclab/protocols_CMP.html

RESULTS

1 Overview of experiments

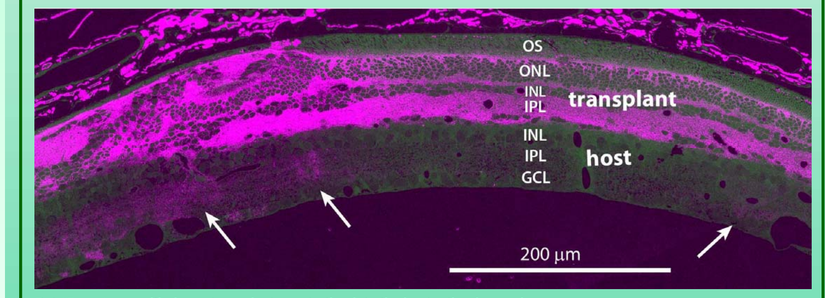
Rat #	Sex	Donor tissue	Age at surgery (mo)	Survival time (mo)	Age at sacrifice (mo)	Organization	Remarks
1	F	E19 retina	1.3	8.5	9.8	Laminated area (7 of 12 slices)	not recorded
2	M	E19 retina + BDNF	1.4	8.3	9.7	Laminated area (6 of 12 slices)	not recorded
3	F	E19 retina + BDNF	1.2	8.4	9.6	Laminated area (3 of 11 slices)	not recorded
4	F	E19 retina + GDNF	0.8	6.7	7.5	Mostly rosettes, laminated area in center of graft (4 of 9 slices)	not recorded
5	F	E19 retina + GDNF	1.1	2.9	4.0	Mostly rosettes, small laminated area (5 of 11 slices)	SC recording, threshold -2.7 log cd/m^2
6	F	E19 retina + BDNF	1.2	8.4	9.6	Laminated area (7 of 11 slices)	not recorded
7	F	E19 retina + GDNF	0.9	4.3	5.2	Laminated area (9 of 13 slices)	SC recording, threshold -2.2 log cd/m^2
8	F	E19 retina + GDNF	1.1	4.3	5.4	Laminated area (5 of 12 slices)	SC recording, threshold -1.5 log cd/m^2
9	F	E19 retina + GDNF	1.1	2.6	3.7	Laminated areas (8 of 11 slices)	SC recording, threshold -2.0 log cd/m^2
10	M	E19 retina	1.1	2.9	4.0	Mostly rosettes, small laminated area (3 of 10 slices)	SC recording, threshold -2.5 log cd/m^2
11	F	E19 retina + BDNF	1.2	3.1	4.3	Mainly rosettes, tiny laminated area (5 of 12 slices)	SC recording, threshold -2.2 log cd/m^2
12	F	E19 retina + GDNF	0.8	4.2	5.0	Rosettes only (7 slices)	SC recording, threshold -0.9 log cd/m^2
13	M	E19 retina + GDNF	1.1	2.3	3.4	Rosettes only (11 slices)	SC recording, threshold -1.5 log cd/m^2

SC recording



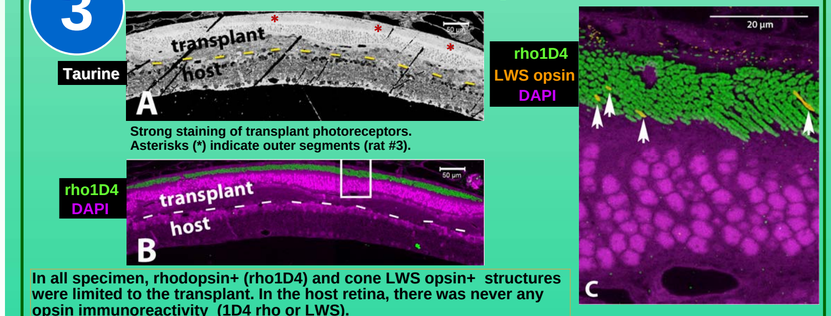
2 Donor cell label (hPAP)

The donor tissue was derived from transgenic rats expressing human placental alkaline phosphatase (hPAP). hPAP was detected histochemically on vibratome slices with BCIP/NBT (Sigma). Slices were embedded in Eponate and sectioned. Magenta = NBT (transplant signal); green = autofluorescence.



Rat #7. Areas with dense transplant penetration into the host retina (arrows).

3 Photoreceptors

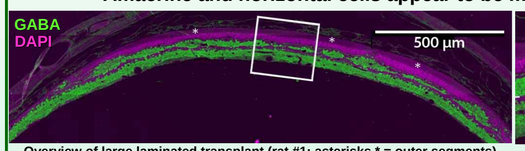


Strong staining of transplant photoreceptors. Asterisks (*) indicate outer segments (rat #3).

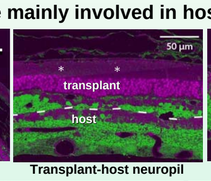
In all specimen, rhodopsin+ (rho1D4) and cone LWS opsin+ structures were limited to the transplant. In the host retina, there was never any opsin immunoreactivity (1D4 rho or LWS).

4 Inner retinal neurons (GABA, glutamate, glutathione)

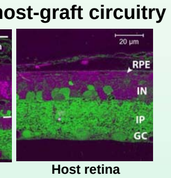
Amacrine and horizontal cells appear to be mainly involved in host-graft circuitry



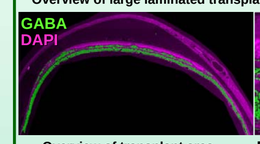
Overview of large laminated transplant (rat #1; asterisks * = outer segments)



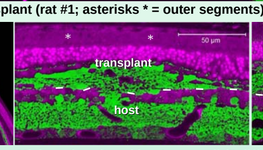
Transplant-host neuropil



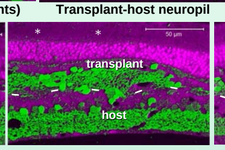
Host retina



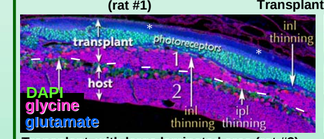
Overview of transplant area (rat #1)



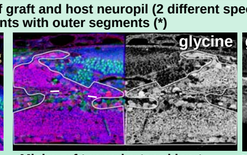
Mixing of graft and host neuropil (2 different specimen; rat #1, rat #2). Transplants with outer segments (*)



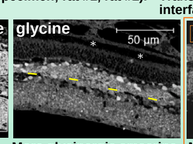
Transplant zone with sparse interfacial processes (rat #2).



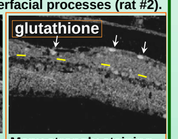
Transplant with large laminated area (rat #3). Transplant inner plexiform layer (ipl) (1) and inner nuclear layer (inl) are thinned in several areas compared to host IPL (2) and INL.



Mixing of transplant and host neuropil at edge of laminated transplant area *(left corner of picture to left)*

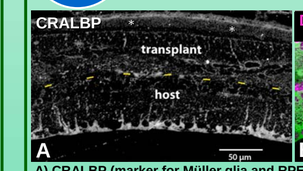


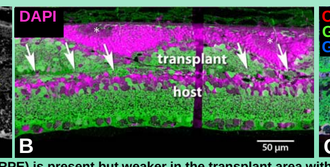
More glycinergic amacrine cells in INL of laminated transplant than in host retina (* = outer segments).

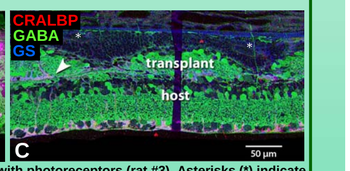


Many strongly staining horizontal cells in transplant (arrows); few cells in host.

5 Glial markers (glutamine synthetase, CRALBP) & neuronal marker (GABA)







A) CRALBP (marker for Müller glia and RPE) is present but weaker in the transplant area with photoreceptors (rat #3). Asterisks (*) indicate presence of outer segments. B,C) Rat #5. Arrows = transplant-host interface. Glial seal on left side (arrow head in C) is interrupted through most of the area, and neural processes crossing the transplant-host interface can be seen.

CONCLUSIONS

- **The host photoreceptors are degenerated. Transplants contain healthy rods & cones with outer segments.**
- **The data indicate that amacrine and horizontal cells of donor and host are mainly involved in the function and communication between transplant and host.**
- **An extensive loss of bipolar cells was observed in the host and to a lesser extent in transplant.**
- **Host and graft neuropil are mixed in many areas, with no glial barriers between transplant and host.**

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)
BCIP – substrate for alkaline phosphatase
LWS – long wavelength (red-green)
CRALBP – cellular retinaldehyde binding protein
GS – glutamine synthetase

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References:

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