Longterm stabilization of regenerating adult retinal ganglion cells

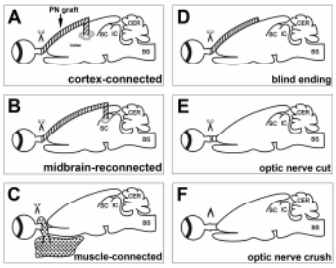
Carolin Chiwitt[1], Tobias Stupp[1], Johannes Seeger[2] and Solon Thanos[1]

[1] Dept. of Experimental Ophthalmology, University Eye Hospital Muenster
[2] Institute of Veterinary Anatomy, University of Leipzig

Introduction

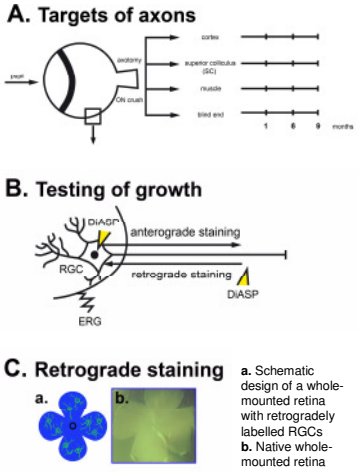
Adult retinal ganglion cells (RGCs) can regenerate their cut axons within peripheral nerve (PN) grafts used to "*bypass*" the distal optic nerve stump. We examined the longterm stabilization of these ganglion cells by guiding their regenerating axons into different termination areas.

Experimental groups



Six different groups were used. **A)** The optic nerve (ON) was connected with "neutral" cortical tissue. **B)** Connection with the major visual target in the midbrain. **C)** Connection with a "foreign" tissue such as muscle. **D)** "Blind ending" peripheral nerve graft. **E)** axotomy group. **F)** Crush group. Twelve rats were used in each group.

Experimental setup



a. Schematic design of a whole-mounted retina with retrogradely labelled RGCs
b. Native whole-mounted retina

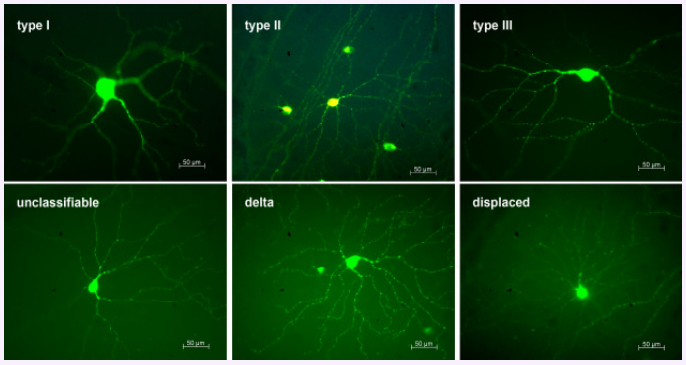
Methods

The optic nerve (ON) of adult rats was completely cut intraorbitally and its ocular stump was connected with different visual target areas (cortex, midbrain) or with non-visual areas (e.g. muscle). Further controls were groups with only cut without graft, ON crush and blind ending grafts. The function of the retina was regularly examined with electroretinogramm (ERG). At 1, 6 and 9 months postsurgery, regenerating or only axotomized or crushed ganglion cells were retrogradely labelled with 4-(4-(didecylamino)styryl)-*N*-methylpyridinium iodide (4-Di-10-ASP) and quantified morphometrically (confocal laser and electron microscopy). Furthermore, the retrogradely labelled RGCs were categorized into three classes (I, II, III) based on morphological criteria. Anterograde staining with intravitreally injected 4-Di-10-ASP was applied as well.

Results

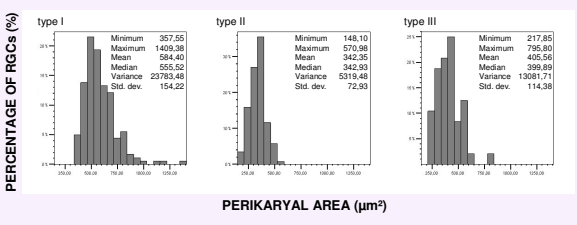
Regenerating RGCs remain stable up to 9 months after grafting at the optic nerve…

1. Ganglion cell typification

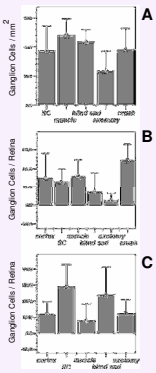


2. Cell size & morphology

type I	584,40 ± 154,22 µm²	large tetra- to pentagonal somata with thick dendrites arising from one of the corners
type II	342,35 ± 72,93 µm²	small cells with 3 or 4 thin primary dendrites
type III	405,56 ± 114,38 µm²	bipolar cells with 2 thick dendrites emerging from the 2 poles of the cell
unclassifiable	313,25 ± 107,29 µm²	RGCs with altered morphologies
delta	517,63 ± 164,50 µm²	a subtype of type I with more tortuous thinner branches
displaced	388,22 ± 186,60 µm²	located in the inner nuclear layer of the retina

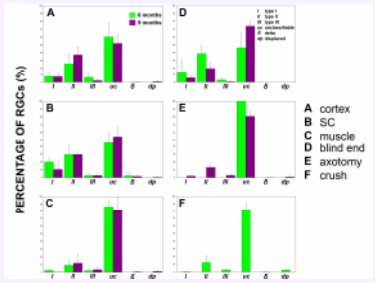


3. Quantification of surviving cells

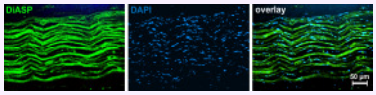


Diagrams show the number of RGCs that were retrogradely labelled with 4-Di-10-ASP 1 month **(A)**, 6 months **(B)** and 9 months **(C)** postsurgery. Error Bars show Mean ± 1,0 SD. Bars show Means.

4. Distribution of cell types

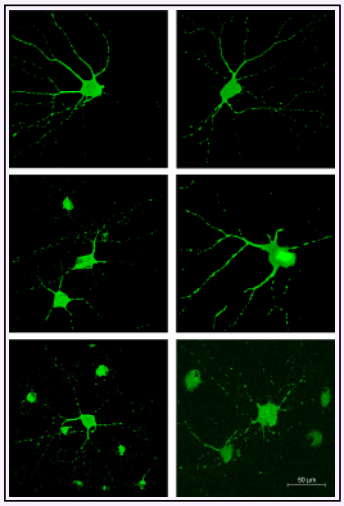


5. Anterograde staining of axons

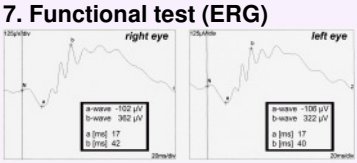


Longitudinal section of a transplanted peripheral nerve graft. Axons grown through are stained with 4-Di-10-ASP. DAPI stains nuclei of glial cells.

6. Confocal laser microscopy

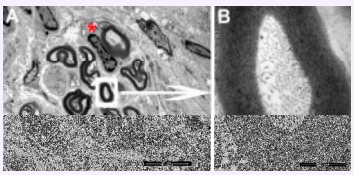


7. Functional test (ERG)



Scotopic Electroretinogramm 4 months postsurgery. LED Flash Intensitiy -20dB, 0,2 Hz. The retina of the left eye is reconnected to the midbrain and its ERG shows a still existing integrity of the retinal function.

8. Electron microscopy



Electron microscopical pictures of a cross section of a transplanted sciatic nerve graft. **A.** A group of myelinated axons. ✱ marks the nucleus of a peripheral myelin bulding Schwann-cell. **B.** In a higher magnification the lamellar structure of the myelin sheath is visible.

Conclusions

Adult ganglion cells of the rat can be reconnected with visual centres using a peripheral nerve "*bypass*". This reconnection stabilizes the cells at morphological and functional levels for a long time.

Contact: chiwitt@uni-muenster.de