A different retinal glia response to optic nerve injury/ lipopolysaccharide administration in hooded and albino rats

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Abstract

Despite a massive degeneration of retinal ganglion cells (RGC) after optic nerve crush (ONC) in hooded rats only a minor increase in retinal glial fibrillary acidic protein (GFAP)-immunoreactivity was found in the inner retina. Interestingly, a combination of ONC with the administration of the proinflammatory agent lipopolysaccharide (LPS) but not LPS alone induces increased GFAP-immunoreactivity. In contrast albino rats showed elevated GFAP-immunoreactivity in response to both, LPS-administration and ONC with no further increase after a combination of both. These data demonstrate significant differences in retinal glia responsiveness between hooded and albino rats after optic nerve lesions. © 2001 Elsevier Science B.V. All rights reserved.

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A common feature of reactive astrogliosis in the injured brain is the increased expression of GFAP [17]. After axonal injury of periphal nerves it has been shown that microglial and astroglial cells are rapidly activated close to the axotomized neurons [15]. In line, following motor axon injury astrocytes rapidly upregulate the gap junction protein connexin-43 and GFAP [1]. Concomitantly, microglial cells proliferate and migrate towards the axotomized neuron perikarya. After injury of central axons, however, the glial cell responses around the affected neuron perikarya appears to be minimal or absent, unless neuron degeneration occurs [1]. In contrast microglia proliferates and astrocytes upregulate GFAP along central axons undergoing anterograde, Wallerian, degeneration.

After graded ONC cellular degeneration of RGC occurs mainly between day 5 and 14 post injury [3], which is comparable to the degeneration process after optic nerve transection at equal distance to the optic nerve head [21], although the number of degenerating RGC after ONC is slightly lower than after a complete crush [3,18,22]. It is widely accepted that the functional properties of activated glia cells have important impact on neuronal survival, axon regeneration, and plasticity. To further characterize the process of RGC degeneration we examined the regulation of retinal glial GFAP-expression in response to graded ONC in hooded and albino rats.

Male hooded rats from the PVG and BDE/Han strain (age 12 weeks/purchased from Moellegard; Berlin; F.R.G.; Harlan Winkelmann, Borchen, F.R.G.) or albino rats from the Sprague Dawley (local breeding facilities of the Leibniz-Institute for Neurobiology) and Wistar strain (Harlan Winkelmann, Borchen, F.R.G) were utilized in all experiments. The rats were anesthetized with a mixture of Ketamin and Rompun (50 mg/kg i.p. and 10 mg/kg i.p.) and then the conjunctiva was incised laterally. ONC was performed as described previously [3,18]. After crush, a local antibiotic was applied into the cut at the conjunctiva, to prevent infections. For the LPS experiments rats were slightly anesthetized with Halothan (chamber inhalation, approx. 3% v/v), and then a single dose of bacteriotoxin...
(100 µg LPS of Salmonella typhimurium, (Sigma, Deisen- 
hofen, F.R.G.)) was injected into one paw. The following 
systemic fever reaction lasted for approx. 2–3 days. In 
experiments with combined crush/LPS administration 
injections were made 3 days post-crush. Retinas were 
processed for GFAP-stainings 1 week after the crush 
lesion. For the Xenon irradiation experiments rats were 
positioned under a modified incident light microscope 
with dual Xenon and Halogen illumination (Zeiss Axiotech 
100). After using the incident Halogen illumination for 
focussing the optics on the retina, the microscope was 
switched to a spot (XBO 75, 80/20 beamsplitter) for 20 s, 
cause local retinal lesions with 200–300 µm in diameter 
by Xenon-induced coagulation. A total of 3–6 animals was 
used for each experimental condition (Control: 3–4; 1 
week post-crush: 6; LPS: 4–5; LPS/crush: 4–6).

GFAP and OX-42 immunostainings were performed 
after 4% paraformaldehyde immersion fixation of the 
retinae, both with cryostat sections and retinal 
wholemounts. Following previously published immuno-
fluorescence protocols [12,14], monoclonal mouse anti-
GFAP antibody (Sigma/clone GA-5) was used at a 
dilution of 1:400 and monoclonal mouse anti RAT-CD11b 
(Serotec-Camon/clone OX-42) at a dilution of 1:250. As 
secondary antibody, CY3 conjugated anti-mouse IgG was 
used for direct fluorescence detection (Sigma, Deisen-
hofen, F.R.G.). Control experiments were performed by 
omission of the primary antibody. For western blot analy-
sis retina protein homogenization was performed as 
described previously [14,18]. Three cyrostat sections (40 
µm) were cut from the inner part of retinal flat mounts and 
processed for homogenization.

As reported previously GFAP-immunostainings in 
normal control retinas reveals label on astrocytes in the 
ganglion cell layer (GCL) and a faint staining in the inner 
nuclear layer (INL) which is most likely associated with 
Müller glia [5] (Fig. 1). No apparent difference in staining 
intensity was found between different rat strains (data not 
shown). In hooded rats ONC lead to no obvious change in 
the staining pattern as compared to control retinas (Fig. 1). 
One week post-injury a slightly enhanced fluorescence 
signal in the GCL was found (Fig. 1b). A similar result 
was obtained after GFAP-stainings in retinal flat mounts of 
crushed retinas (Fig. 2). Most of the GFAP-immunosignal 
was detected in close proximity to endothelial cells but no 
change in the staining intensity was found 1 week after 
crush in comparison to controls (Fig. 2b). Xenon irradia-
tion of the retina, however, leads to a pronounced induc-
tion of GFAP-positive reactive macroglia in close proximi-
ty to the lesion site (Fig. 2c), with a dense network of

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**Fig. 1.** GFAP-immunopositive glial cells in normal and injured retinae after ONC and LPS treatment in PVG (A–D) and Sprague Dawley rats (G–H). (A) In cryostat sections of control tissue from hooded rats GFAP-label was mainly associated with the GCL. (B) Optic nerve crush leads to no detectable 
increase of GFAP-immunoreactivity 1 week post-injury. (C) Similarly no apparent alterations were found in LPS treated hooded rats. (D) A combined 
treatment of LPS and ONC induces dense macroglial processes immunopositive for GFAP in the IPL, INL and ONL (see arrows) and a more intense label 
in the GCL. (E) No difference in GFAP-label was found in control albino rat retina as compared to hooded rats. (F) Optic nerve crush in albino rats lead to 
a clear increase in GFAP-staining intensity in the GCL and to the induction GFAP-positive processes in the outer retina. (G) LPS administration had a 
similar effect on GFAP-label in the outer retina of the albino rat as ONC. (H) No further enhancement was apparent after a combined LPS/ONC treatment 
in albino rats. OX-42-immunoreactivity in the GCL of PVG (I+K) and Wistar rats (J+L). In control animals (I+J) only faint label is found in the inner 
retina, whereas 1 week after ONC (K+L) in both rat strains an increase of OX-42 immunoreactivity is seen in the GCL. GCL: Ganglion cell layer; INL: 
Inner nuclear layer; ONL: Outer nuclear layer. Scale bar is 50 µm.
processes in the penumbra of the lesion. Thus, mechanical injury in the hooded rat retina induces reactive gliosis and the employed staining method was suitable to detect GFAP-upregulation. No staining was found in control experiments after omission of the primary antibody (data not shown).

The GFAP-immunostaining results were confirmed by western blot analysis of tissue homogenates from the inner retina of hooded rats (Fig. 3) where no increased GFAP-immunoreactivity was found within 2 weeks post-injury. Since the peak of RGC degeneration is around 1 week post-injury [3] no clearcut correlation could therefore be established between cell death in the GCL of hooded rats and macroglial GFAP expression.

In contrast, ONC in albino rats resulted in clearly elevated GFAP-immunoreactivity in the inner retina 1 week post-crush (Fig. 1). Intense label of glial processes in the inner plexiform layer (IPL) were clearly visible and the staining intensity of putative Müller endfeet and astrocyte somata in the GCL was elevated homogenously throughout the retina (Fig. 1), a finding further confirmed by western blot analysis of protein homogenates from the inner retina (Fig. 3).

Most interestingly, we found that a subsequent LPS treatment after ONC in rats of the PVG strain lead to intense label of Müller glia processes in the IPL, INL, GCL and in part also in the outer nuclear layer (ONL) (Fig. 1c). This staining pattern was not found in retinas of sham crushed hooded rats treated with LPS (Fig. 1), indicating that only the combination of axonal injury and the LPS induced inflammatory response results in glial upregulation of GFAP (Fig. 1). Again a clear strain difference between hooded and albino rats was found after LPS treatments. Albino rats exhibited increased GFAP-staining of retinal glia after LPS administration which was of similar intensity as compared to ONC (Fig. 1). Hence, a combined LPS/ONC treatment had no further impact on GFAP-positive macroglia in these retinas. Interestingly, the strain differences observed in the macroglia response to ONC were not found after staining with OX-42 1 week post-crush (Fig. 1i–1), indicating that activated microglia is present in the inner retina in hooded and albino rats after the lesion.

During a series of studies on the retinal glia response to graded ONC we observed strain differences between hooded versus albino rats in retinal GFAP-expression. A similar difference was observed after i.p. administration of LPS. No difference between both strains, however, was seen in the intensity of OX-42 staining, a marker of

Fig. 2. GFAP-immunostainings in retinal flat mounts of hooded rats. (A) In control tissue most of the GFAP-immunosignal is localized in proximity to putative blood vessels. (B) ONC does not lead to an increase of GFAP-immunoreactivity or an altered localization of the immunosignal. (C) A local Xenon-irradiation induces a meshwork of intense GFAP-fluorescence signals close to the glia scar at the lesion site (see arrows). BV: Blood vessel. Scale bar is 50 μm.
activated microglia, in response to ONC. As outlined above, it is assumed that the severity of neuronal degeneration after central nerve axotomy determines the extent of reactive astrogliosis [1]. Therefore, the minor response of rats from the PVG and BDE/HAN strain despite an elimination of more than one third of RGC within 5–7 days following nerve crush [3,22], which approximates ≈40,000 cells in the GCL, was unexpected. Thus, although RGC degeneration occurs in hooded and albino rats to a similar extent, the macroglia response is to a surprising degree strain-dependent. Taken together these data suggest that a different threshold for reactive gliosis exists in albino and hooded strains.

One possible reason for this strain difference is a lower susceptibility to or a lower production of inflammatory agents in the PVG and BDE/HAN strain as compared to Sprague Dawley and Wistar rats. The bacterial endotoxin LPS is a proinflammatory agent that causes experimental uveoretinitis in the susceptible Lewis rat strain but not in the poorly susceptible Brown–Norway and Long Evans rats, from which both PVG and BDE/HAN derive [6]. Although no ocular inflammation was observed in albino animals, it seems that the potential inflammatory/pyrogenic effect of the endotoxin is sufficient to induce increased GFAP-immunoreactivity in the albino but not the hooded rat retina. Most interestingly, we found that combined ONC and LPS administration leads to a massive GFAP-upregulation in all retinal layers of pigmented rats. This points to a possible cooperative mechanism and part of this mechanism could be a potentiation of a minor inflammatory response in hooded rats after ONC by the bacterial endotoxin. It is, therefore, conceivable that phagocytosis of degenerating RGC in hooded as compared to albino rats is accompanied by an attenuated inflammatory response and that inflammatory agents might be important stimuli for Müller glia to become reactive after ONC.

A possible explanation for the relatively minor astrogliosis response in both, hooded and albino strains, could be that astrogliosis is not involved in the clearance of cellular debris in the GCL. Phagocytosis of degenerating RGC during retinal development is accomplished by Müller glia and microglia [8]. Moreover, in contrast to many other species astrogliosis in murine and rat retina is mainly attached to blood vessels and to a much lesser extent to axon bundles [7,9]. Accordingly, Müller glia, which fulfills important functions in neurotransmitter uptake at glutamatergic synapses of RGC [16] and which is supposed to be in cell–cell contact to RGC [9,21] reacts more prominently with an increased GFAP expression after ONC and LPS in albino and combined ONC/LPS in hooded rats. This is also in accordance to previous reports who found a similar increase in retinal Müller glia GFAP-immunoreactivity of albino rats and rabbits after optic nerve transection [4,10,19].

Thus, activation of retinal glia from different rat strains seems to exhibit differential susceptibility to inflammatory stimuli and to optic nerve lesions. Moreover, degeneration of RGC after axon injury alone is not sufficient to induce reactive macroglia but other, probably inflammatory stimuli are necessary, to induce increased GFAP immunoreactivity in the retina. Therefore it seems that a differential production of or susceptibility to inflammatory agents and not solely RGC degeneration could determine the macroglia response to ONC. In consequence, care should be taken in the interpretation of data from different rat strains concerning retinal macroglia activation after axon injury.

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