Unilateral Injury to the Adult Rat Optic Nerve Causes Multiple Cellular Responses in the Contralateral Site

Nicole Bodeutsch,[1] Heike Siebert,[1] Catherine Dermon,[2] Solon Thanos[1]

[1] Department of Experimental Ophthalmology, School of Medicine, University of Münster, Domagkstraße 15, 48149 Münster, Germany

[2] Department of Biology, Laboratory of Physiology and Neurobiology, University of Crete, Vasilika Vouton 2208, 714 09 Heraklion, Crete, Greece

Received 22 April 1998; accepted 3 August 1998

ABSTRACT: This study was undertaken to examine whether unilateral injury to one optic nerve (ON) elicits a response in the microglia, neuroglia and ganglion cells of the retina and ON of the contralateral site as well. Bilateral activation of the transcription factor c-*jun* could be immunohistochemically detected in the ganglion cell layer 2 days after crush and later. Microglial cells were detected with the activation-specific antibodies MUC 102 and OX-42. They showed an immediate and clear pattern of activation within the contralateral ON and retina, although this response was less pronounced than in the directly lesioned site. Astrocytes and Müller cells showed a typical up-regulation of glial fibrillary acidic protein in the lesioned retina and only focal but virtually no generalized up-regulation in the contralateral eye. Ganglion cells whose axons had been crushed responded with vigorous axonal growth after 2 days in culture, in addition to exhibiting *in situ* reactions. However, ganglion cells of the contralateral retina responded with a moderate regeneration, too. Growth was less pronounced than in the crushed retina but significantly better than in retinas on untreated animals. The results suggest that unilateral lesion of the optic nerve elicits a defined response in the major cell types of the contralateral retinofugal system. The findings suggest that it is advisable to maintain caution in the use of the contralateral optic nerve and retina as a control in experiments dealing with cellular processes of de- and regeneration. © 1999 John Wiley & Sons, Inc. J Neurobiol 38: 116–128, 1999

Keywords: **retina; optic nerve injury; degeneration; axonal regeneration; sympathetic ophthalmia**

Ganglion cells in the retina (RGCs) of adult rodents are vulnerable to injury of the optic nerve and usually respond with retrograde and anterograde degeneration (Richardson et al., 1982; see Liebermann, 1971, for review of the early literature). However, under certain experimental conditions such as the replacement of the optic nerve with a peripheral nerve graft (Politis and Spencer, 1982; Vidal-Sanz et al., 1987), or explantation of the lesioned retina and culturing *in vitro* (Ford-Holevinski et al., 1986; Bähr et al., 1988; Thanos et al., 1989) adult RGCs can regenerate axons over long distances. This pivotal response of the same cell to different environmental conditions is coinfluenced by interactions with neuroglial cells such as astrocytes (Bovolenta et al., 1997), regeneration-in-

Correspondence to: S. Thanos

Contract grant sponsor: Deutsche Forschungsgemeinschaft; contract grant numbers: Th 386/6-1, Th 386/7-1

Contract grant sponsor: Bundesministerium Für Bildung und Forschung General Secretatiat for Research and Technology (GSRT)

Contract grant sponsor: International Institute of Paraplegia

Contract grant sponsor: International Spinal Research Trust

Contract grant sponsor: BARNEF

© 1999 John Wiley & Sons, Inc. CCC 0022-3034/99/010116-13

hibitory oligodendrocytes (Schwab and Caroni, 1988; Vanselow et al., 1990), and microglial cells (Thanos et al., 1991). In addition, soluble factors such as neurotrophins influence the survival of cells and their regenerative ability *in vivo* (Mey and Thanos, 1993; Mansour-Robey et al., 1994; Wen et al., 1995) and *in vitro* (Thanos et al., 1989, 1993; Fournier et al., 1997; Fournier and McKerracher 1997). Although the number of regenerating ganglion cells can be enhanced to levels of 15–20% of the total axotomized population, most of the cells die, indicating that until now, no factor can ensure their complete rescue and axonal regrowth. The efficacy of regeneration varies among the aforementioned conditions but never approaches complete quantitative regeneration of the optic nerve.

The intracellular mechanisms of the nerve cell response to injury remain unknown. Morphologically, the degenerative response is marked at an early stage with activation of immediate genes such as the transcription factors c-*jun,* KREB, and others (Herdegen et al., 1993). In the downstream cascade, the neurons translocate their nuclei into eccentric positions, enlarge in size, show chromatolytic cytoplasmic changes, and finally disappear at later stages (Liebermann, 1971), with some of the nuclei showing a positive TUNEL reaction (García-Valenzuela et al., 1993). Degenerating cells are cleared away by microglial cells which seem to respond to the lesion and participate in the process of degeneration by mechanisms which are still to be elucidated (Thanos et al., 1991). Astrocytes and retinal Müller cells respond with up-regulation of intermediate filaments such as glial fibrillary acidic protein (GFAP) a sign of activation and proliferate to form a gliotic scar (Fulcrand and Privat 1997; Bignami and Dahl, 1979). The morphological correlates of the regenerative response are the transient expression of transcription factors (Herdegen et al., 1993; de Léon et al., 1995) but pronounced increase of somal size (Mey and Thanos, 1993) and absence of chromatolysis and of nucleus dislocation. These perikaryal features are accompanied by formation of growth cones at the junction to the peripheral nerve graft *in vivo* (Vidal-Sanz et al., 1987) and on the polylysine-laminin substrate in culture (Bähr et al., 1988).

The prominent usage of the RGC as a model in de- and regeneration studies with biochemical and increasingly with molecular biology approaches (McKerracher et al., 1996 for review) requires well-characterized experimental and control conditions. As quoted in the literature, the retina and optic nerve (ON) contralateral to an injured eye are often used as a control both *in vivo* and *in vitro.* However, there are several reasons to assume one or multiple responses in the contralateral site as well. The first is that axons from both optic nerves are in intimate contact while crossing at the optic chiasma. The second is the appearance of a small retino-retinal projection in rodents (Müller and Holländer, 1988) with the corresponding ganglion cells becoming axotomized as well. The third is the lesioning of the optic nerve microvasculature, which may induce a generalised vascular response. In addition, a human disease called sympathetic ophthalmia (Kupper et al., 1993) and an experimental mouse uveitis (Yew et al., 1990; Lam et al., 1996) are aggrevated by the contribution of the contralateral eye to diseases such as intraocular infections and massive traumas, which initially affect only one eye. The present work was undertaken to analyze (a) the response of ganglion cells of the retina contralateral to optic nerve crush. This was done by first monitoring the expression of c-jun *in vivo* in both retinas after unilateral crush and second by determining the number of regrowing axons after explantation and culturing *in vitro;* (b) the response of microglial and perivascular cells by using immunohistochemical markers of their activation; and (c) The astroglial and Müller cell reaction by using antibodies to GFAP.

MATERIALS AND METHODS

Surgery *In Vivo*

A total of 63 young adult Sprague–Dawley rats of both sexes (13 animals) and of the Lister Hooded strain (50 animals) were anesthetized with an intraperitoneal injection of 0.2–0.4 mL ketamine-hydrochloride, (Ketanest; Parke Davis) and 0.1 mL Rombun (Bayer, Leverkusen). To crush the optic nerve, a small incision was made in the temporal conjunctiva of the left eye. A pair of open curved forceps were then inserted behind the eye and squeezed together for about 10–12 s to crush the ON. The animals were returned to their cages. Rats used later for immunohistochemistry or *in vitro* studies for retinal explants were surgically treated in an identical fashion. All surgery was done under microscopic control. The use of animals was in accordance with the national guidelines for animal research with permission from the local authorities.

Immunohistochemistry

ON. At 24 h and 6 days after crush, the ONs were surgically excised, frozen, and cut with the cryostat. MUC 102 antibody, which recognizes two proteins on the surface of activated microglial cells (62 and 70 kD) (Gehrmann and Kreutzberg, 1991), was used for staining. The secondary antibody was visualized with Neufucsin. The Neufucsin color reaction protocol was modified as following:

1× washing with 0.01 *M* phosphate-buffered saline (PBS), 2× with Tris-buffered saline (TBS), pH 7.6, biotinylated immunoglobulin G (IgG) of rabbit anti-mouse for 30 min at room temperature, 2× washing with TBS, avidin–biotin complex for 30 min at room temperature, staining with Neufucsin for 12–15 min at room temperature, counterstaining 2 s with hematoxylin III (Gill) to stain the cell nuclei, 4× washing with H_2O, and 2× with TBS, and coverslipped using Aquatex.

Retina. Five days after crush, the eyes were enucleated and frozen in liquid N_2 for cryostat sections (10 μm thick).

Anti-c-*jun *and Anti C3 receptor (OX-42). The sections were fixed in cold methanol (−20°C) for 5 min, washed three times for 5 min in 10 m*M* PBS, and then blocked for 30 min in 10% fetal calf serum (FCS). The sections were then incubated overnight at 4°C with an antibody against c-*jun* [c-*jun* AP-1 (D); Santa Cruz Biotechnology] at 1:50 dilution [0.1% bovine serum albumin BSA in 10 m*M* PBS] or to OX-42 (complement-3-receptor antibody; Camon Immunological Services, Germany). After washing three times, the second fluorescent antibody to rabbit IgG (Sigma) at a dilution of 1:300 was added for 1 h at room temperature and after washing three times, coverslipped with Mowiol (Hoechst) and photographically documented using a fluorescence microscope equipped with the corresponding filters (Axiophot, Zeiss).

Anti-GFAP. The dilution of anti-GFAP antibody from mouse–mouse hybridoma cells (Boehringer Mannheim) was 1:4. The secondary antibody was labeled with the fluorescence tetramethyl-rhodamine-isothiocyanate (TRITC) anti-mouse IgG (Dianova), at a dilution of 1:100. Double immunofluorescence was performed with combinations of the antibodies.

Explanatation Procedures

After 5 days, the rats were deeply anesthetized with 5 mL 7% chloralhydrate, and both eyes were enucleated. The retinas were removed from the eyes under sterile conditions and flat-mounted onto a nitrocellulose filter (Sartorius), and each was dissected into eight optic disc–oriented, wedgelike pieces with a tissue chopper, and explanted (with the ganglion cell layer facing downwards) in a petriperm dish (Heraeus) coated with poly-D-lysine (MW 502,400–506,200 Da; Sigma; 200 μg/mL overnight at 37°C) and laminin (Boehringer Mannheim; 20 μg/mL, 1 h, 37°C). The retina remained attached to the bottom of the dish using 10-μL drops of Matrigel (Serva) and were cultured (37°C, 5% CO_2, 100% humidity) under serum-free conditions in S4 medium (Bähr et al., 1988). Then, the regenerated axons which grew out of the explant were counted under an inverted phase-contrast photomicroscope after 2, 5, 14, and 28 days in culture. In cultures of ≥5 days, half of the medium was replaced with fresh medium at every third day (Axiovert 35; Zeiss). Statistical comparisons were performed with the two-tailed Student *t* test.

RESULTS

Retinal Cell Responses *In Vivo*

Injury to the optic nerve results in dramatic cellular responses within the ganglion cell layer of the corresponding retina (Richardson et al., 1982; Thanos, 1991). These changes affect all cells of the retina, are devastating for the axotomized neurons, and are well characterized, and thus are not the objective of the present study. However, the ganglion cell layer of the contralateral retina responds with cellular changes, too, although the vast majority of axons are not directly affected by the surgery. Both the axotomized [Fig. 1(a)] and the contralateral retina [Fig. 1(b)] showed clear staining with antibodies to the transcription factor c-*jun,* which is known to be activated after axotomy (Herdegen et al., 1993). As also shown in Figure 1(a), deeper layers of the retina were also immunopositive for c-*jun,* whereas this response was topologically restricted to the ganglion cell layer in the contralateral retina, with fewer cells being immunopositive within the inner nuclear layer (INL) [Fig. 1(b)]. The nontreated control retina remained nonreactive to c-*jun* immunohistochemistry [Fig. 1(c)]. Involvement of the deeper layers of the axotomized retina indicated a transneuronal response of the retinal interneurons and confirmed the high sensitivity of this factor in the detection of posttraumatic activation. The moderate response in the contralateral retina is a new observation and occurred as early as the response within the injured retina, documenting that direct lesioning is not essential to activate immediate-early genes.

The second major population of nonneuronal cells within the retina was composed of astrocytes and cells of Müller, both of which are usually immunoreactive to GFAP. As Figure 2(a) shows, in the normal retina of the rat the staining was strong among the optic fiber layer (OFL) astrocytes and faintly detectable in Müller cells. The retina of the crushed ON showed a clear up-regulation of GFAP in both the astrocytes and Müller cells [Fig. 2(b)]. The retina contralateral to the injury showed a focal but very moderate up-regulation in the population of few astrocytes and virtually no enhanced immunoreactivity of Müller cells [Fig. 2(c)]. This effect was not uniformly distributed across the retina, with some areas being reminiscent of a normal retina as in Figure 2(a) and some regions

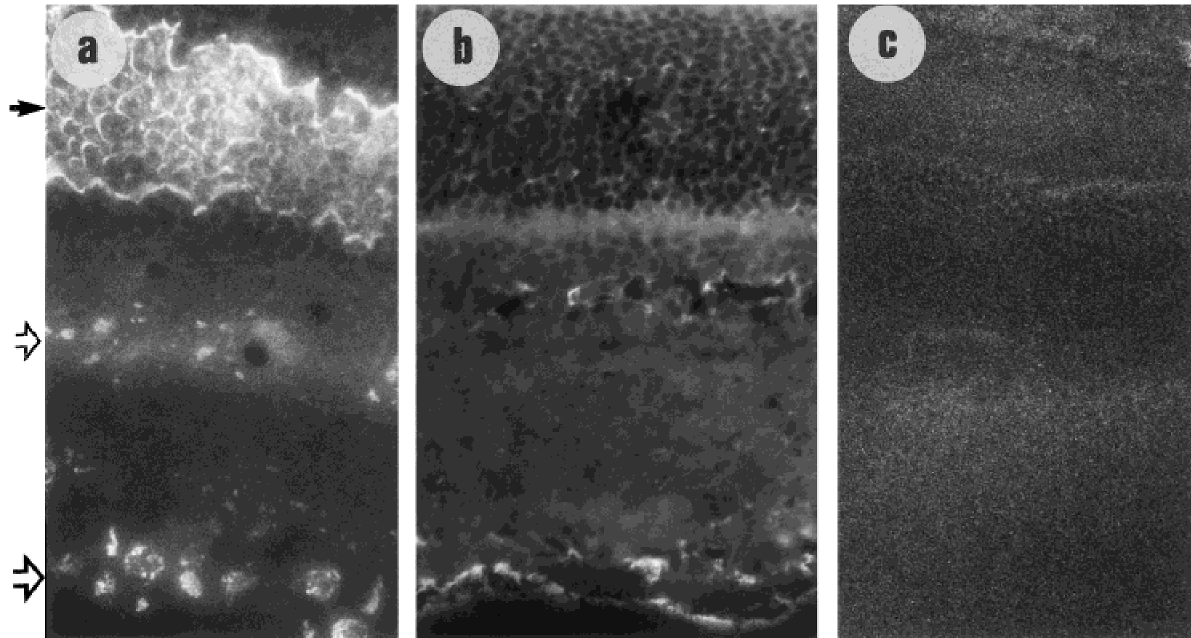


Figure 1 Expression of the transcription factor c-*jun* within the retina. Fluorescence photomicrographs showing the c-*jun* immunoreactivity within the crushed (a) contralateral (b) retina 5 days after optic nerve crush and nontreated control retina (c). As anticipated, immunoreactivity is stronger within the lesioned retina and extends from the ganglion cell layer (large arrowhead) through the whole retinal depth to the photoreceptors (black arrow). The retina contralateral to the crushed nerve shows a clear immunoreactivity within the ganglion cell layer and only moderate reactivity in the inner nuclear layer (small arrowhead). Scale bar = 10 μm.

having labeling similar to that of the crushed retina of Figure 2(b). This may correlate with the fact that the retino-retinally projecting neurons (Müller and Holländer, 1988) were scattered across the retinal surface, inducing only local responses after injury.

The retinal and ON microglial cells are responsible for posttraumatic phagocytosis and removal of cellular debris produced during degeneration (Stoll et al., 1989; Thanos et al., 1991). As investigated to date, they are activated posttraumatically and express a number of major histocompatibility (MHC-I) and II antigens. Figure 3 shows that ON microglial cells were positively stained with the MUC 102 antibody as early as 24 h after unilateral optic crush. However, the labeling was not restricted to the nerve with induced degeneration, but was visible in the contralateral nerve as well [Fig. 3(a,b)]. In addition to activation-specific stain with the antibody, microglial cells displayed ameboid shapes [Fig. 3(e)] which indicated their activation within the optic nerves (Ling, 1981; Murabe and Sano, 1982). By 6 days after unilateral ON injury, both ON, displayed a stronger pattern of microglial activation [Fig. 3(c,d)], although the immunoreactivity was less pronounced in the nonlesioned optic nerve [Fig. 3(c)]. The nontreated control ON did not show MUC 102 labeling [Fig. 3(f)]. A quantification of activated microglial cells at 24 h and 6 and 12 days after axotomy showed that in both nerves, MUC-positive cells increased in number, with the density always higher within the injured nerve (Fig. 4). The number of microglial cells increased over time in both the crushed ON and the ON situated contralateral to this lesion. In the crushed ON, the number of microglial cells were already enhanced 24 h after crush (from ca. 370 cells/mm^2 (control) to ca. 660 cells/mm^2), whereas in the contralateral ON, the number of microglia corresponded to that of the untreated animal (control). Six days after crush, the number of microglial cells in the contralateral ON was increased as well (ca. 480 cells/mm^2; crushed ON ca. 1000 cells/mm^2). Twelve days after the lesion, the number of microglial cells were further increased in both the crushed (ca. 1300 cells/mm^2) and contralateral ON (ca. 840 cells/mm^2, t test; $p < .001$).

In addition to the use of MUC 102 antibodies (Gehrmann and Kreutzberg, 1991), activated micro-

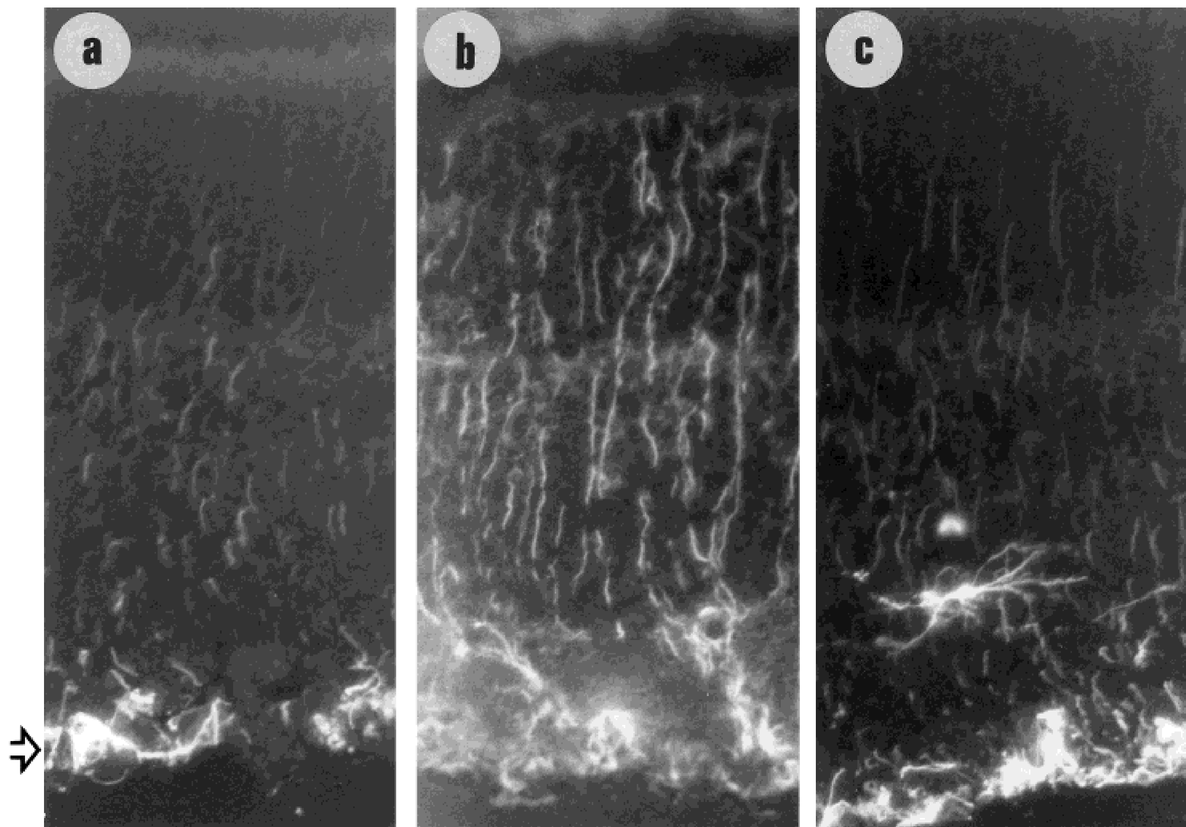


Figure 2 Immunohistochemistry with antibodies to GFAP. (a) Control, untreated retina with positive astrocytes within the OFL (large arrowhead) and punctuate staining along the Müller cells. (b) Labeling of the injured retina 5 days after crush of the ON shows intense labeling both of astrocytes and of Müller cells. (c) Staining of the retina contralateral to the crushed optic nerve with enhanced immunoreactivity for Müller cells compared to the untreated control in (a). Note also the unusual staining of an astrocyte within the IPL. Scale bar = 10 μm.

glial cells can be stained with antibodies to the complement 3 receptor (OX-42) (Hickey and Kimuna, 1988). As anticipated, the crushed optic nerve [Fig. 5(a)] showed a massive response at the retroorbital site of crush. Many microglial cells and perivascular cells [Fig. 5(a,b)] displayed an intense staining close to the optic nerve head [Fig. 5(a)] and in more eccentric retinal positions [Fig. 5(c)]. The retina contralateral to the crushed optic nerve showed a moderate but clear immunoreactivity to OX-42 antibodies, with perivascular cells and microglial cells being localized within the OFL, ganglion cell layer (GCL), and inner plexiform layer (IPL) [Fig. 5(d)]. Double labeling with the antibodies OX-42 [Fig. 5(e)] and c-*jun* [Fig. 5(f)] revealed that the c-*jun*–positive cells were not microglial cells. In addition, double labeling with c-*jun* and GFAP (data not shown) indicated that astrocytes also did not up-regulate c-*jun*.

Axonal Regeneration in Culture

In the serum-free S4 medium, virtually no axons grew out from control retina explanted from completely untreated rats and observed after 2 days in culture ($n = 9$) [Fig. 6(a)]. However, more fibers grew out when the retinal explants were obtained from the retina contralateral to the site of injury ($n = 14$) [Fig. 6(b)]. In contrast to both of these conditions, vigorous axonal growth was observed when the explants were derived from precrushed retina ($n = 14$) and explanted over 2 days in culture [Fig. 6(c)]. Typically, several fasciculated or single axons grew in their original orientation and appeared around the tip of the retinal piece that corresponded to the optic disc *in vivo* [Fig. 6(c)]. As the quantification in Figure 6(d) shows, the number of axons increased from less than two axons per explant [1.89 $\pm$ 0.82 standard deviation (S.D.)] in control tissue to significantly more axons in

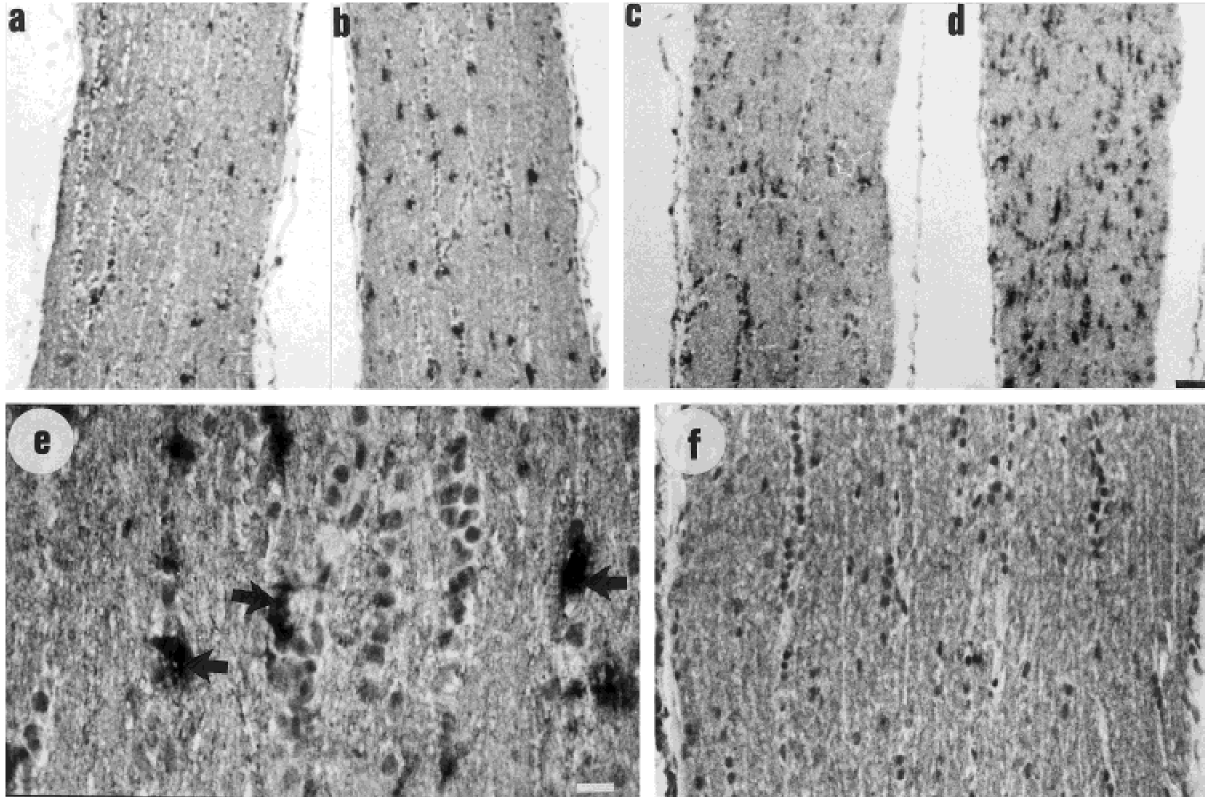


Figure 3 Microglial staining within the optic nerve. Longitudinal section of both optic nerves of a rat with unilateral crush 24 h (a,b) and 6 days (c,d) before. The optic nerve contralateral to the crush (a,c) shows a clear immunoreactivity to the antibody MUC 102 which labels activated microglial cells. At both stages of observation this reactivity is lower than in the crushed optic nerve (b,d). Scale bar = 50 μm. (e) Large magnification through the ON at 6 days after crush shows the nonramified shapes of microglial cells (arrows). Scale bar = 10 μm. (f) Longitudinal section though the optic nerve of a nontreated control rat shows no microglial reactivity with the MUC 102 antibody. Scale bar = 50 μm.

retinal tissue from the contralateral retina (13.71 $\pm$ 5.11 axons; $p > .05$). The axon population in precrushed retinal pieces was much higher (404.93 $\pm$ 98.12) than in the control group and in the contralateral retinal tissue.

For the comparison of different periods of observation, the numbers from eight explants were summed to calculate the total number of axons corresponding to the entire retina. After 5, 14, and 28 days in culture, no difference was observed between the control retina and the retina contralateral to the crushed one. Figure 7 compares the quantification of axons at 5, 14, and 28 days in culture. In control retinas, fewer than 50 axons (n = 25 retinas) were found at 5 days (44.78 $\pm$ 15.1) to increase to 868.48 $\pm$ 161.28 S.D. at 14 days (n = 12 retinas), and to decline to 739.2 $\pm$ 144.50 S.D. at 28 days in culture (Fig. 7). In contrast, 464.77 $\pm$ 123.05 S.D. axons were observed in the crushed retina at 5 days to reach a maximum of 2320.98 $\pm$ 363.305 S.D. at 14 days and decline to 1438.28 $\pm$ 248.29 S.D. at 28 days (Fig. 7). At all stages of assessment, the differences between the control and experimental tissue were significant (Student t test; $p > .005$).

DISCUSSION

The present study confirmed that unilateral injury to the optic nerve induces the well-studied cellular changes in the retina that is directly involved. However, our new finding is that the three major types of cells respond in the unlesioned contralateral retina and ON as well. Ganglion cells respond with activation of immediate-early genes such as c-*jun* and a moderate reactive anabolism, best detectable in explanted cul-

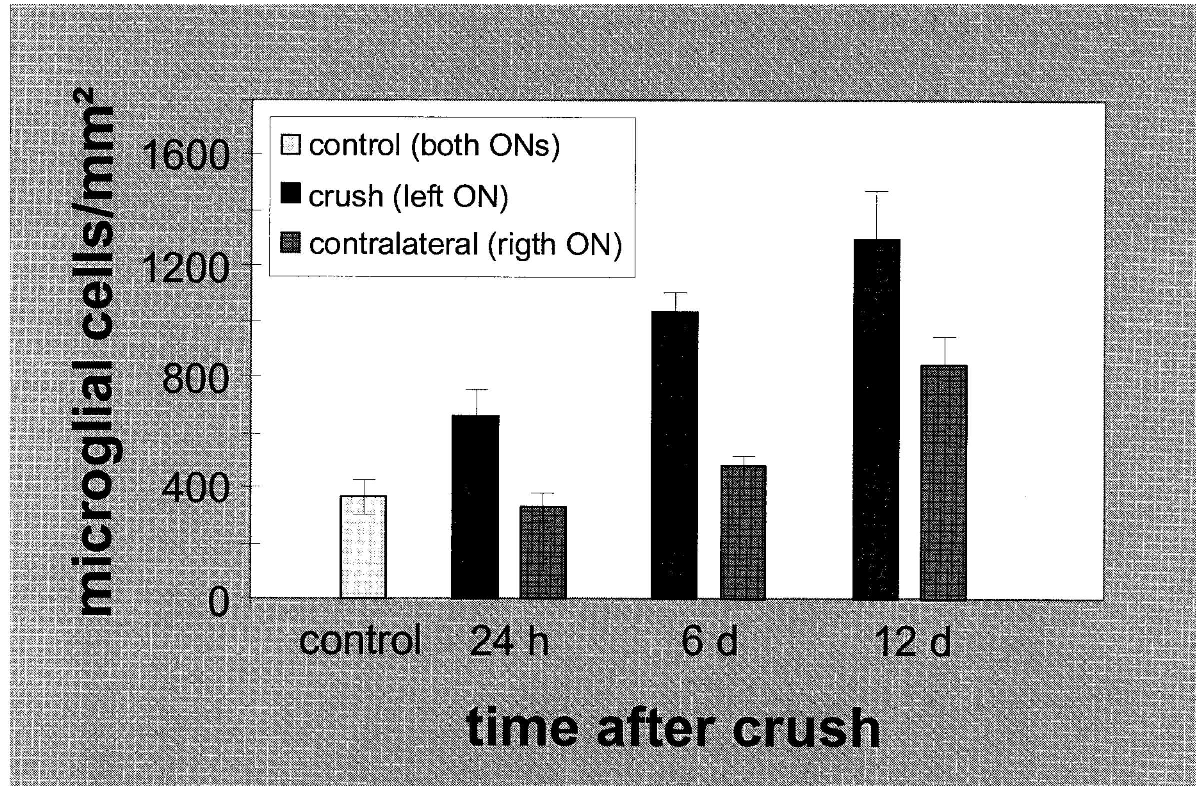
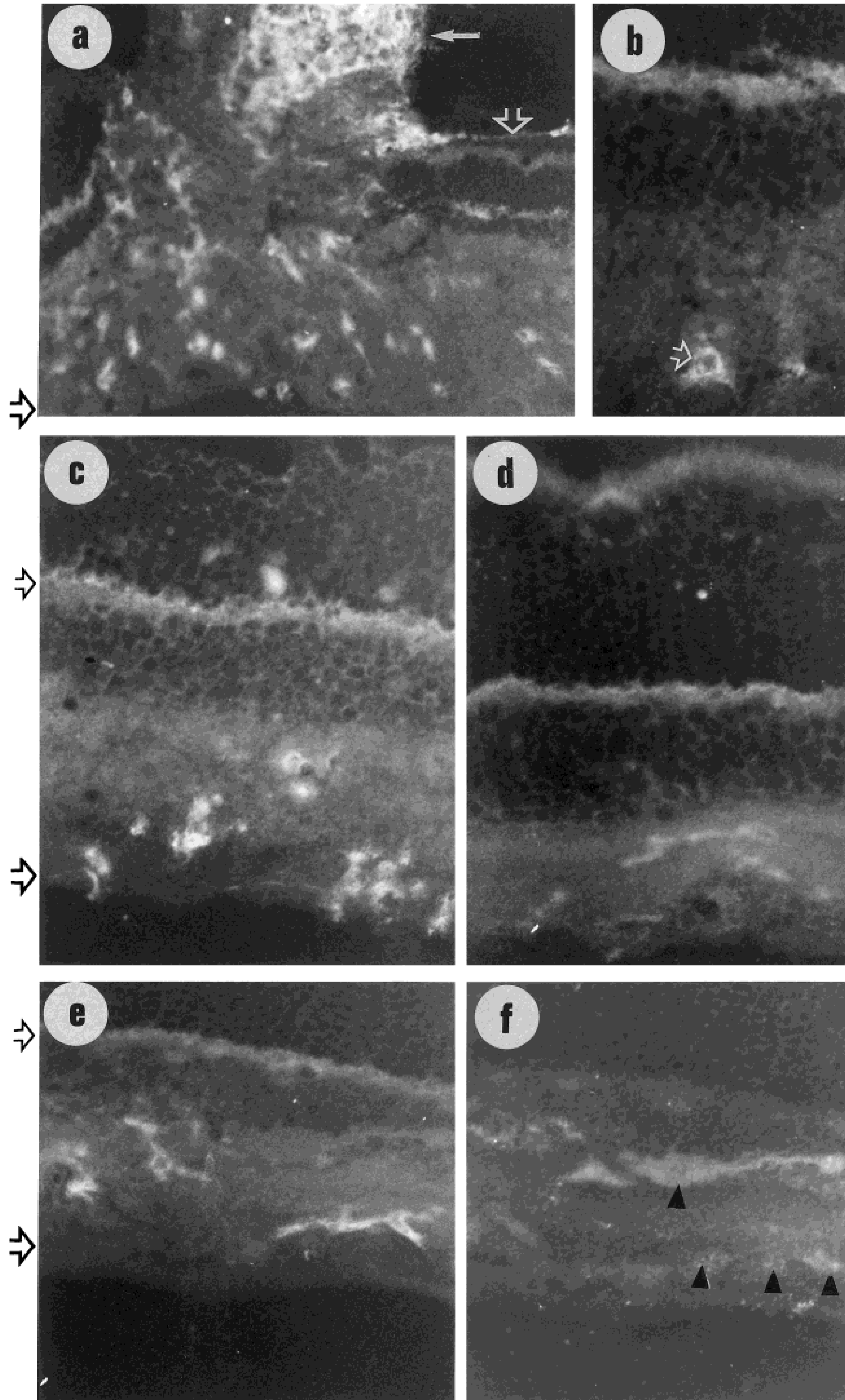


Figure 4 Quantification of microglial cells within the ON. Number of activated microglial cells per square millimeter 24 h and 6 and 12 days after the lesion in longitudinal sections of the ON. The identification of the microglial cells occurred with MUC 102 and AB complex with counterstaining with neufucsin.

tures which showed a significantly increased propensity for regeneration of axons. Microglial and perivascular cells become activated both within the unlesioned ON and the retina. Astrocytes, and in particular Müller cells, responded with only moderate up-regulation of their GFAP content. All these orchestrated changes indicate that the contralateral eye should be used with caution as a control tissue in experimental designs involving models of lesion and degeneration.

The morphological and functional responses of adult ganglion cells to a direct intraorbital axotomy have been well documented (see Liebermann, 1971, for review of early literature). The primary response depends on the method of ON lesion, with the ON crush producing the most subtle changes, probably because the meningeal sheath remained intact (Moore and Thanos, 1996). This method of moderate lesioning has also been applied as preconditioning of retinal tissue which is used 5–6 days later to obtain explants for *in vitro* regeneration experiments (Bähr et al., 1988; Thanos et al., 1989). As confirmed in the

Figure 5 Microglial labeling. (a) Section through the crushed optic nerve (ON) at the optic nerve head with strong immunoreactivity to OX-42 within the nerve itself (arrow) and the retina (arrowhead) 5 days after crush. (b) Enlarged retina microcapillary with perivascular microglial cells in the GCL (arrowhead). (c,d) Comparison of the OX-42 immunoreactivity within the crushed (c) and the contralateral retina (d). (e,f) Double staining with OX-42 (e) and c-*jun* (f) of the same section reveals that c-*jun* is not expressed by the microglial cells, but by ganglion cells (arrowheads) in the GCL, and perhaps in displaced ganglion cells in the INL. For all photographs, large arrowheads mark the OFL; small arrows indicate the level of the OPL. Scale bars = 50 μm for (a), 10 μm for (b–f).


a
b
c
d
e
f

present work, vigorous regrowth of ganglion cell axons can be obtained in this paradigm, indicating that *in vivo* crush induces primarily anabolic changes in the cell bodies. This anabolism is best documented after explantation, resulting in appreciable number of axons which approach almost 1% of the total population even under serum-free conditions provided in the paradigm. In addition, a moderate but significant regeneration of axons was observed from explants taken from the contralateral unlesioned retina. One explanation for onset of metabolism in the unlesioned eye is a retrograde signaling from the optic chiasm, the region of interdigitation between the two nerves while crossing to their contralateral projection areas (Perry, 1979, 1980; Linden, 1987). This signaling of yet unknown nature may be mediated by the anterogradely degenerating axons of the lesioned eye or by humoral factors such as cytokines. A second explanation is the hematogeneous transfer of lesion-induced signals to the contralateral nerve and retina. A first line of evidence speaking for the hematogeneous transfer is the fast staining 2 days after crush of perivascular microglial cells with the activation-specific antibody OX-42 in the contralateral retina.

A further explanation could be provided by the small percentage of retinal axons forming the retino-retinal projection (Müller and Holländer, 1988). Although the number of retinal ganglion cells terminating within the contralateral retina is very low, these are essentially crushed, thus explaining their regrowth after explantation. By assuming that the efficiency of regrowth is about 1% as in the directly crushed retina, these numbers fit well to explain that the retino-retinally projecting cells contribute to the regeneration. The finding is in conformity with the irregular, focal distribution of GFAP up-regulation in astrocytes, but not in cells. This fact does not explain, however, the rather uniform distribution of c-*jun*–positive cells within the ganglion cell layer of the unlesioned eye. It documents on the other hand, that axotomy of these few cells (ca. 0.01% of all RGCs) is sufficient to induce clearly detectable responses seen with the different methods. The expression of c-*jun* has been described to occur only in lesioned motoneurons and retinal ganglion cells (Herdegen et al., 1993; de Léon et al., 1995), and it is surprising to detect this transcription factor in the retina contralateral to the lesioned ON.

The response of microglial cells is in conformity with the role of these cells in neural degeneration. The cells seem to be activated irrespective of the cause of neural disease and express MHC antigens as indicators of their involvement in the antigen-presentation cascade (Giulian et al., 1993). One example with microglial activation in absence of degeneration is the facial nucleus in rats, which is characterized by a transient microglial response (Kreutzberg, 1966). The present demonstration of microglial response in the contralateral nerve and retina indicates the sensitivity of the cell in sensing alterations in the brain, and that activation of microglial cells is not necessarily associated to the direct degeneration of neurons. One of the reasons of the limited activation may be their function as phagocytotic cells to remove the myelin debris and the axons of the few retino-retinally projecting cells (Müller and Holländer, 1988). The second reason may be their function as a surveillance system, which is generally activated throughout the brain as a result of a localized injury. Similarly, the up-regulation of GFAP in neuroglial cells of the retina may be attributed to the interactions between the ganglion cells, the microglial cells, and astroglia. Compared with other central nervous systems, contralateral effects of lesions have been described in cortex ablation experiments in rats (Pearson et al., 1984), in the lateral geniculate body after striate cortex lesions in monkeys (Hendrickson and Dineen, 1982), and in the hypoglossal nerve injury model which results in bilateral cell body changes in the medulla oblongata (Watson, 1968). The mechanisms of cell body responses may be very similar between the different areas of the brain, but remain to be elucidated.

The findings of this study may shed light onto the mechanisms of development of sympathetic ophthalmia, a very rare but one of the most intractable human eye diseases (Kuppner et al., 1993). The clinical manifestation of this disease may be acute or chronic, and involves infiltration of lymphocytes, plasma cells, and epithelioid cells in the choroid, thus resulting in uveitis, particularly in the choroid. In addition, a number of cell-adhesion molecules are expressed indicating lymphocyte activation and immigration (Kuppner et al., 1993). A model of traumatically induced sympathetic ophthalmia has been developed in mice. The model consists of perforation of the eye and lodging of an iron wire in the vitreous, induction of massive intraocular inflammation, and analysis of the contralateral eye with protein chemistry and electrophysiology (Lam et al., 1996). As expected from manifestation of contralateral uveitis, low-molecular proteins appeared in the gels, and cyclosporine treatment suppressed the responses. The present model of isolated injury to the optic nerve is different and primarily affects the retinal ganglion cell axons, leaving the choroid and intraocular structures intact. Its sensitiv-

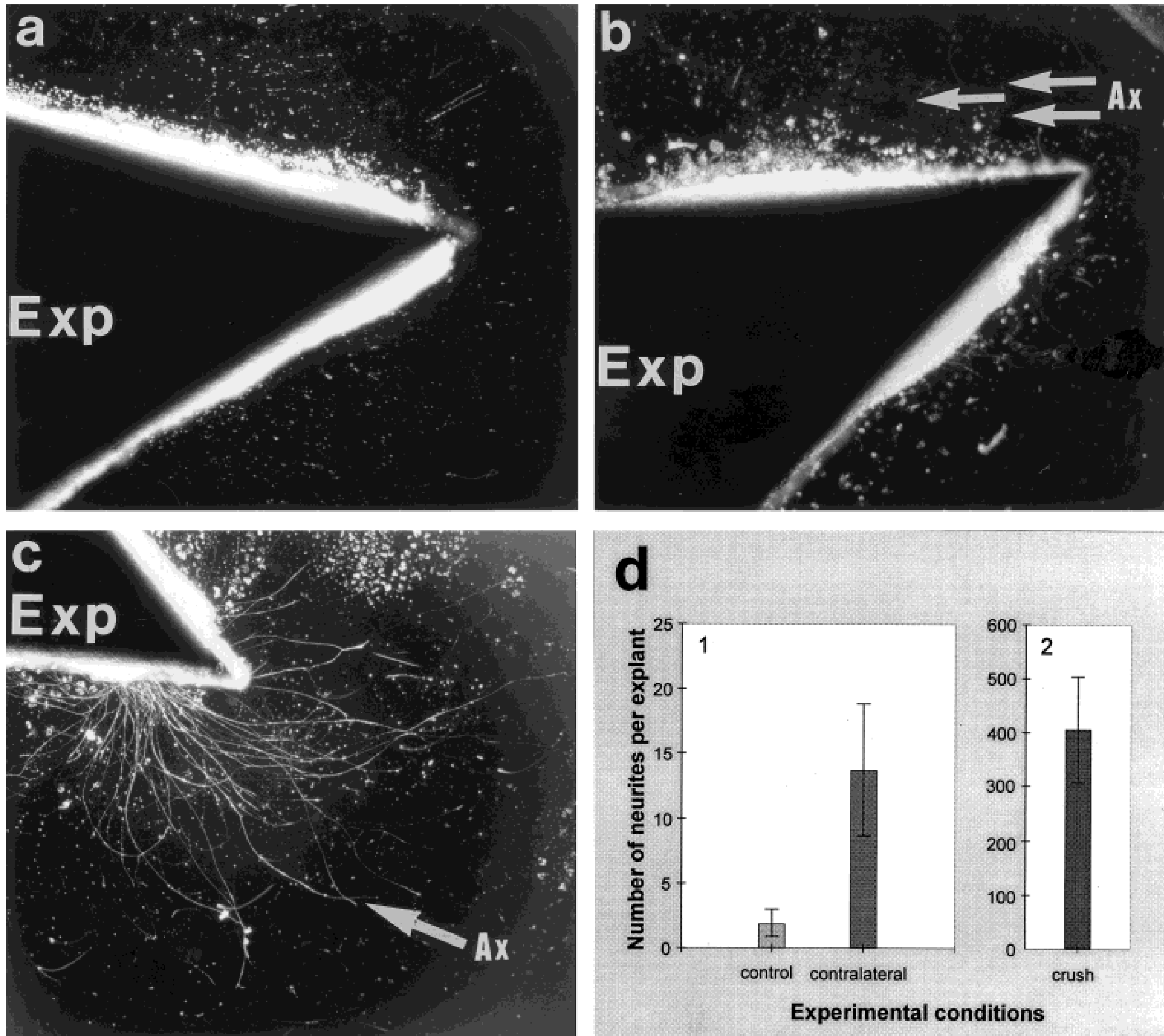


Figure 6 Retinal explants with regenerating axons from (a) control, (b) contralateral to crush, and (c) crushed eyes after 2 days in culture. The black triangles are the nitrocellulose filters carrying the retinal explants (Exp) whose edges are visible as white stripes along the filter edges. Scale bar = 50 μm. (d) Histograms showing the regeneration of ganglion cell axons under different experimental conditions in culture: (1) The different reaction of retinal ganglion cells (RGCs) between retinas situated contralateral to an optic nerve crush and retinas taken from animals without any lesion (control group). In the contralateral group, 14 retinas were used, and 9 in the control group. The criterion of the examination was the number of regenerating axons in the explants. There is a significant difference (*t* test; $p > .05$) between the control retinas and the retinas situated contralateral to an optic nerve crush after 2 days in culture. The axonal outgrowth in the contralateral retinas was sevenfold higher than in the control group. (2) Comparison with the retina situated ipsilateral to the crush. The axonal outgrowth in the ipsilateral retinas were about 30-fold higher than in the contralateral retinas.

ity is documented by the orchestrated response of the various types of cells. Although the role of humoral factors and secondary contribution of the uvea cannot be excluded, the results show that the primary response is initiated within the retina and ON.

In conclusion, the data suggest that the use of the contralateral retina as control tissue in studies of neural degeneration should be considered with caution. This is due to the sensitivity of the system and to cellular responses which are similar to the traumati-

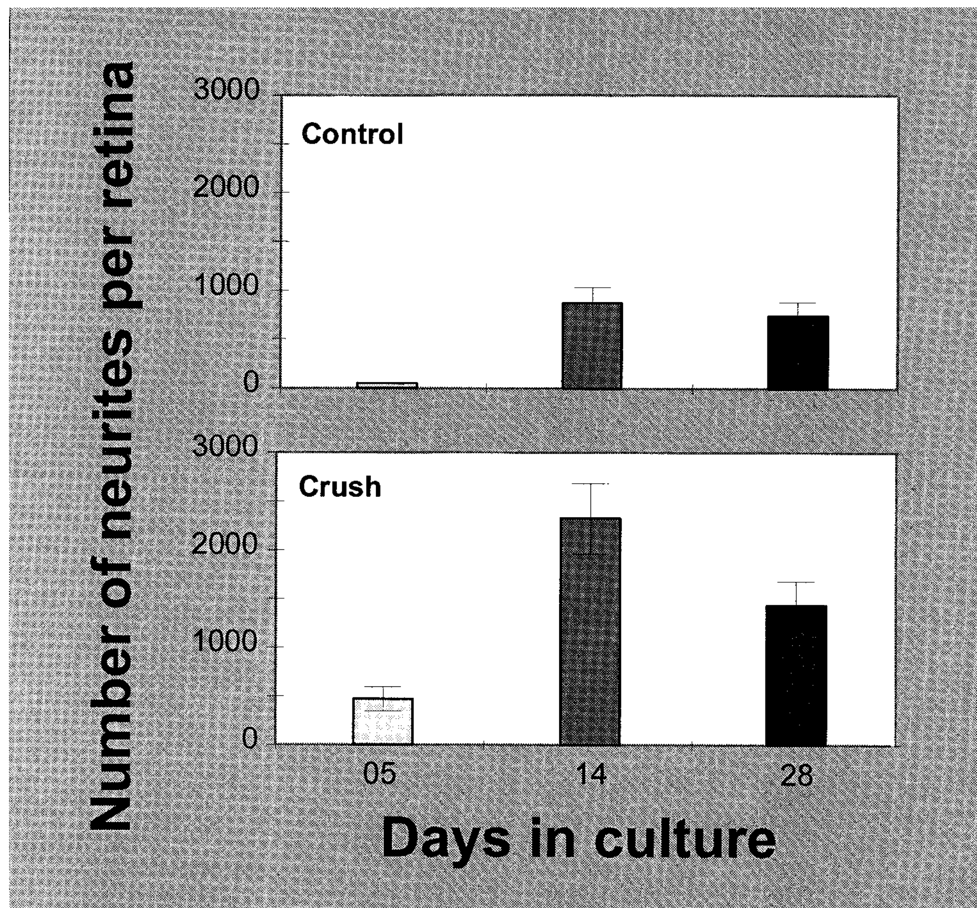


Figure 7 Quantitative comparison between the number of regenerated axons in the retina with a blind crush of the ON 5 days before explantation [Fig. 1(b)] and that of control retinas [Fig. 1(a)] after 5, 14, and 28 days, in culture. In the crush group, 37 retinas were used at 5 day (23 retinas at 14 days, 20 retinas at 28 days, the loss of retinas is explained by contamination). The criterion of the examination was the number of regenerating axons in the explants. The prelesion of the axons 5 days before the explantation of the retina significantly enhanced the number of outgrowing axons *in vitro.* The enhancement of regenerating axons in the noncrushed retina from 5 to 14 days in culture was 20 to 30 times, and of the crushed retina only five times. However, the explants without a crush were unable to reach the number of axons of the explants with a crush. In both groups, the number of axons was diminished 1.1-fold up to 1.7-fold at 14–28 days in culture.

cally induced responses, although less moderate than these.

First indications that the contralateral retina responds to an injury were obtained during a collaboration work with A. Stamatakis from the University of Crete. The authors thank him for helpful discussion at this initial stage of work. The technical assistance of M. Wild, C. Riedinger, and B. Kracht is greatly acknowledged. The project was supported by the Deutsche Forschungsgemeinschaft (Grants Th 386/6-1 and 7-1), the Bundesministerium für Bildung und Forschung (BMBF), and the International Institute of Paraplegia (IRIP), and the International Spinal Research Trust (ISRT), and in the frame of BARNEF (EU-project in BIOMED I).

REFERENCES

Barres BA, Silverstein BE, Corey DP, Chun LL. 1988. Immunological, morphological, and electro-physiological variation among retinal ganglion cells purified by panning. Neuron 1:791–803.

Bähr M, Vanselow J, Thanos S. 1988. *In vitro* regeneration of adult rat ganglion cell axons from retinal explants. Exp Brain Res 73:393–401.

Berkelaar M, Clarke DB, Wang Y-C, Bray GM, Aguayo AJ. 1994. Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. J Neurosci 14:4368–4374.

Bignami A, Dahl D. 1979. The radial glia of Müller and

their response to injury: an immunofluorescence study with antibodies to the glial fibrillary acidic (GFA) protein. Exp Eye Res 28:63–69.

Bovolenta P, Fernaud-Espinosa I, Mendez-Otero R, Nieto-Sampedro M. 1997. Neurite outgrowth inhibitor of gliotic brain tissue: mode of action and cellular localization, studied with specific monoclonal antibodies. Eur J Neurosci 9:977–989.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

de Léon M, Nahin RL, Molina CA, de Léon DD, Ruda MA. 1995. Comparison of c-jun, junB, and junD mRNA expression and protein in the rat dorsal root ganglia following sciatic nerve transection. J Neurosci Res 42:391–401.

Ford-Holevinski TS, Hopkins JM, McCoy JP, Agranoff BW. 1986. Laminin supports neurite outgrowth from explants of axotomized adult rat retinal neurons. Dev Brain Res 28:121–126.

Fournier AE, McKerracher L. 1997. Expression of specific tubulin isotypes increases during regeneration of injured CNS neurons, but not after application of brain-derived neurotrophic factor (BDNF). J Neurosci 17:4623–4632.

Fournier AE, Beer J, Arregni CO, Essagian C, Aguayo AJ, McKerracher L. 1997. Brain-derived neurotrophic factor modulates GAP 43 but not T alpha1 expression in injured retinal ganglion cells in adult rats. J Neurosci Res 47: 561–572.

Fulcrand J, Privat A. 1997. Neuroglial reactions secondary to Wallerian degeneration in the optic nerve of the postnatal rat: ultrastructural and quantitative study. J Comp Neurol 176:189–224.

García-Valenzuela E, Fahmey A, Sharma S. 1993. Axotomy induces apoptotic cell death in adult retinal ganglion cells. Eur J Neurosci 6(Suppl):129.

Gehrmann J, Kreutzberg GW. 1991. Characterisation of two monoclonal antibodies directed against rat microglia. J Comp Neurol 313:409–430.

Giulian D, Vaca K, Corpuz M. 1993. Brain glia release factors with opposing actions upon neuronal survival. J Neurosci 13:29–37.

Hendrickson A, Dineen JT. 1982. Hypertrophy of neurons in dorsal lateral geniculate nucleus following striate cortex lesions in infant monkeys. Neurosci Lett 30:217–222.

Herdegen T, Bastmeyer M, Bähr M, Stürmer C, Bravo R, Zimmermann M. 1993. Expression of JUN, KROX and CREB transcription factors in goldfish and rat retinal ganglion cells following optic nerve lesion is related to axonal sprouting. J Neurobiol 24:528–543.

Hickey WF, Kimura H. 1988. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen *in vivo.* Science 239:290–292.

Kreutzberg GW. 1966. Autoradiographische Untersuchung über die Beteiligung von Gliazellen an der axonalen Reaktion im Facialiskern der Ratte. Acta Neuropathol 7:149–161.

Kuppner MC, Liversidge J, McKillop-Smith S, Lumsden L, Forrester JV. 1993. Adhesion molecule expression in acute and fibrotic sympathetic ophthalmia. Curr Eye Res 12:923–934.

Lam WL, Chow PH, Chik KP, Yew DT. 1996. Bilateral biochemical and physiological retinal changes after unilateral ocular trauma in the mouse. Neurosci Lett 218: 1–4.

Liebermann AR. 1971. The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol 14:49–124.

Linden R. 1987. Displaced ganglion cells in the retina of the rat. J Comp Neurol 258:138–143.

Ling E-A. 1981. The origin and nature of microglia. In: Federof S, Hertz L, editors. Advances in cellular neurobiology, Vol 2. New York: Academic Press. p 33–82.

Mansour-Robaey S, Clarke DB, Wang Y-C, Bray GM, Aguayo AJ. 1994. Effects of ocular injury and the administration of brain-derived neurotrophic factor (BDNF) on the survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci USA 91:1632–1636.

McKerracher L, Chamouc M, Arregui CO. 1996. Role of laminin and integrin interactions in growth cone guidance. Mol Neurobiol 12:95–116.

McKerracher L, Hirscheimer A. 1992. Slow transport of the cytoskeleton after axonal injury. J Neurobiol 23:568–578.

Mey J, Thanos S. 1993. Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats *in vivo.* Brain Res 602:304–317.

Moore S, Thanos S. 1996. Differential increases in rat retinal ganglion cell size with various methods of optic nerve lesion. Neurosci Lett 207:117–120.

Müller M, Holländer H. 1988. A small population of retinal ganglion cells projecting to the retina of the other eye: an experimental study in the rat and the rabbit. Exp Brain Res 71:611–617.

Murabe Y, Sano Y. 1982. Morphological studies on neuroglia: VI. Postnatal development of microglial cells. Cell Tissue Res 225:469–485.

Neukirchen RO, Schlosshauer B, Baars S, Jäckle H, Schwarz U. 1982. Two-dimensional protein analysis at high resolution on a microscale. J Biol Chem 257:15229–15234.

Pearson RC, Sofroniew MV, Powell TP. 1984. Hypertrophy of immunohistochemically identified cholinergic neurons of the basal nucleus of Meynert following ablation of the contralateral cortex in the rat. Brain Res 311:194–198.

Perry VH. 1979. The ganglion cell layer of the retina of the rat: a Golgi study. Proc R Soc Lond B 204:363–375.

Richardson PM, Issa VMK, Shemie S. 1982. Regeneration and retrograde degeneration of axons in the rat optic nerve. J Neurocytol 11:949–966.

Schwab ME, Caroni P. 1988. Oligodendrocytes and CNS myelin are nonpermissive substrates for neurite growth

and fibroblast spreading *in vitro*. J Neurosci 8:2381–2393.

Stoll G, Trapp BD, Griffin JW. 1989. Macrophage function during Wallerian degeneration of rat optic nerve: clearance of degenerating myelin and Ia expression. J Neurosci 9:2327–2335.

Takahashi N, Cummins D, Caprioli J. 1991. Rat retinal ganglion cells in culture. Exp Eye Res 53:565–572.

Thanos S. 1991. The relationship of microglial cells to dying neurons during natural neuronal cell death and axotomy-induced degeneration of the rat retina. Eur J Neurosci 3:1189–1207.

Thanos S, Mey J, Wild M. 1993. Treatment of the adult retina with microglia-suppressing factors retards axotomy-induced neuronal degradation and enhances axonal regeneration *in vivo* and *in vitro*. J Neurosci 13:455–466.

Thanos S, Bähr M, Barde Y-A, Vanselow J. 1989. Survival and axonal elongation of adult rat retinal ganglion cells: *in vitro* effects of lesioned sciatic nerve and brain derived neurotrophic factor (BDNF). Eur J Neurosci 1:19–26.

Vanselow J, Schwab ME, Thanos S. 1990. Responses of regenerating rat retinal ganglion cell axons to contacts with central nervous myelin *in vitro*. Eur J Neurosci 2:121–125.

Vidal-Sanz M, Bray GM, Villegas-Perez MP, Thanos S, Aguayo AJ. 1987. Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in adult rat. J Neurosci 7:2894–2909.

Villegaz-Pérez MP, Vidal-Sanz M, Rasminsky M, Bray GM, Aguayo AJ. 1993. Rapid and protracted phases of retinal ganglion cell loss follow axotomy in the optic nerve of adult rats. J Neurobiol 24:23–36.

Watson WE. 1968. Observations on the nucleolar and total cell body nuclei acid of injured nerve cells. J Physiol Lond 196:655–676.

Wen R, Song Y, Cheng T, Matthes MT, Yasumura D, LaVail MM, Steinberg RH. 1995. Injury-induced upregulation of bFGF and CNTF mRNAs in the rat retina. J Neurosci 15:7377–7385.

Yew DT, Yang QD, Mak NK, Aú CY, Liu WK. 1990. Bilateral retinal response after traumatic injury of one optic nerve in mice. Ophthalm Res 22:67–72.