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Degenerative and Regenerative Responses of Injured Neurons in the Central Nervous System of Adult Mammals

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Degenerative and regenerative responses of injured neurons in the central nervous system of adult mammals

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SUMMARY

In adult mammals, the severing of the optic nerve near the eye is followed by a loss of retinal ganglion cells (RGCs) and a failure of axons to regrow into the brain. Experimental manipulations of the non-neuronal environment of injured RGCs enhance neuronal survival and make possible a lengthy axonal regeneration that restores functional connections with the superior colliculus. These effects suggest that injured nerve cells in the mature central nervous system (CNS) are strongly influenced by interactions with components of their immediate environment as well as their targets. Under these conditions, injured CNS neurons can express capacities for growth and differentiation that resemble those of normally developing neurons. An understanding of this regeneration in the context of the cellular and molecular events that influence the interactions of axonal growth cones with their non-neuronal substrates and neuronal targets should help in the further elucidation of the capacities of neuronal systems to recover from injury.

INTRODUCTION

The re-establishment of interrupted connections between nerve cells in the injured central nervous system (CNS) should entail the reiteration of events responsible for the formation of neural circuits in embryonic and early post-natal life. Indeed, the survival of damaged neurons, the extension of axons towards appropriate targets and the eventual formation of functional synapses are essential steps in both development and regeneration. Certain populations of axotomized neurons in adult non-mammalian vertebrates such as fish and amphibians recapitulate developmentally expressed programmes and regenerate lengthy axonal projections that restore lost functions. Conversely, in adult mammals recovery from CNS injury is hindered by the loss of many damaged nerve cells and the failure of axons to regrow when they are severed.

The presence in the mature CNS of a greater number and variety of neurons and glia than in the embryo, the intricate course of many axonal projections, and the greater separation between neuronal groups that results from the increase in the size of the organism are all potential obstacles to the restoration of neural connectivity in the adult animal. In addition, there is increasing evidence that growth-inhibiting molecules in glial cells (Schwab 1990), changes in the extracellular matrix (Reichardt & Tomaselli 1991), modifications in the function of neuronal receptors (Rutishauser 1986) and even the formation of premature aberrant synapses with cells near the site of damage (Bernstein & Bernstein 1971) may play roles in preventing axonal regrowth in the injured CNS of adult mammals.

Because there is no substantial re-extension of interrupted CNS axons in adult mammals, it has not been possible to determine if developmental cues that might guide axonal projections to their specific targets are retained in the mature CNS or are overridden by growth-inhibiting circumstances. Further, it needs to be determined if the successful elongation of interrupted axons can result in the appropriate reconnection of the number of neurons required for function.

In this article, we review experiments showing that CNS neurons can respond to manipulations of the milieu of their cut axons and support lengthy axonal regrowth that culminates in the formation of new functional synapses in the injured brain. For these studies, cellular and extracellular components of the peripheral nervous system (PNS) have been used to provide a favourable substrate for growth and guidance of regenerating CNS axons to the proximity of selected neuronal targets in distant regions in the brain.

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AXONAL REGENERATION FROM INJURED CNS NEURONS IN ADULT RODENTS

To investigate *in vivo* the role of the non-neuronal environment in the success or failure of axonal regeneration from injured CNS neurons, the glial substrate present in the adult mammalian brain or spinal cord was replaced by non-neuronal components of the PNS (Aguayo 1985). The experimental strategy applied in these studies consisted of by-passing portions of the CNS of laboratory animals with transplanted, axon-free, peripheral nerve (PN) segments whose distal end was either left unconnected or used as a bridge to reach CNS targets (figure 1).

Recent studies of the survival, regrowth, and reconnection of axotomized CNS neurons have focused on the retinal ganglion cells (RGCs) that normally project to the superior colliculus (SC) by way of the optic nerve and tract (for review, see Aguayo *et al.* (1990)). In adult Sprague–Dawley rats or Syrian hamsters, an autologous segment of PN was attached to the ocular stump of the optic nerve (ON) transected near the eye. In some of these animals, the remainder of the PN graft was left blind-ended beneath the scalp. The purpose of these experiments was to study the survival of RGCs (Villegas-Pérez *et al.* 1988*a*) or the growth of axons in the absence of terminal connectivity with a CNS target (Vidal-Sanz *et al.* 1987). In other animals, the distal end of the graft was inserted into the SC (Vidal-Sanz *et al.* 1987; Carter *et al.* 1989*a*), the region of the rodent mesencephalon that normally receives most retinal afferents.

The regeneration of cut RGC axons along the PN graft and their formation of terminals in the SC was documented by anterogradely labelling the axonal projections of RGCs with neuroanatomical tracers (horse-radish peroxidase (HRP), tritiated amino acids or fluorescent dyes). The PN graft and the SC were then surveyed by light and electron microscopy to identify axons or axon terminals containing these markers. In addition, electrophysiological responses to visual stimulation of the retina were sought in both the RGC axons and in the SC to characterize functional correlates of the regeneration of RGC axons and the formation of RGC synapses in the SC (Keirstead *et al.* 1985, 1989).

Certain molecular events that accompany the injury and regrowth of these axons were investigated in similarly prepared animals. These include the expression of selected proteins in RGC neurons and the role of extracellular matrix and cellular components of the PNS and CNS in axonal growth and neuronal survival.

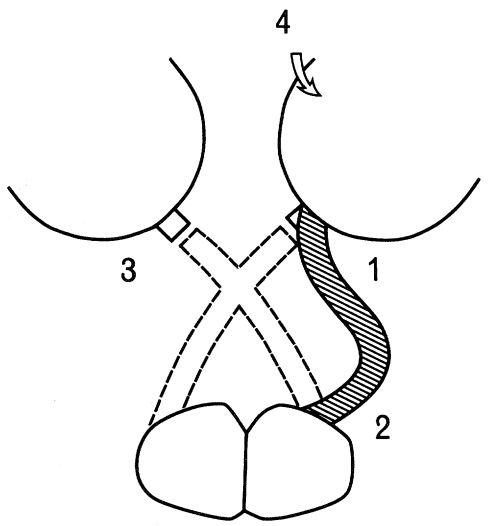

Figure 1. Experimental reconnection of the retina and the superior colliculus (SC) in adult rats or hamsters. 1. One optic nerve was transected near the eye and its orbital stump sutured to a 3–4 cm segment of autologous peroneal nerve (cross-hatched). 2. After 6–8 weeks, the end of the graft was inserted into the SC. 3. The contralateral ON was transected to increase the extent of denervation in the targeted SC. 4. After different survival times, orthogradely transported tracers were injected into eye or the SCs were tested for electrophysiologic responses to light flashed onto the retina. Reproduced with permission from Aguayo *et al.* (1990).

NEURONAL DEATH AFTER AXOTOMY

Many neurons die after axotomy, particularly when axons are severed close to the cell body (for review, see Lieberman (1974)). Paradoxically, axotomy near the cell soma is also a requisite for CNS axons to grow into PN grafts (Aguayo 1985). Although densities of surviving RGCs fell to 18% at 1 month (Villegas-Pérez *et al.* 1988*b*) and 5% at 3 months after an intraorbital axotomy, approximately 20% of the surviving RGCs regenerated lengthy axons along PN grafts apposed to the ON stump (Villegas-Pérez *et al.* 1988*a*). Conversely, in animals in which the ON was cut nearly 10 mm from the eye, 54% of RGCs survived by 3 months but no axons regrew into the PN grafts (Richardson *et al.* 1982). Thus, under these experimental conditions, the neuronal propensity for axonal regeneration appears to be increased by lesions that cause more severe damage to the cell.

Transection of the ON near the eye, but not at distances of 8–10 mm, also leads to an enhanced immunoreactivity for the growth-associated protein, GAP-43 in RGC somata (Lozano 1988) and a marked increase in the transport of this protein along the proximal stump of the ON (Doster *et al.* 1991). The apparent relationship between successful axonal regrowth into PN grafts and the enhanced expression of GAP-43 suggests that this molecule may be involved in cellular mechanisms that modulate the responsiveness of injured neurons to the substrate components that influence axonal regrowth. The reasons why both phenomena are influenced by axonal lesions near the neuronal soma are not understood.

To investigate the role of the target in the survival of axotomized neurons in the mature CNS, a long-term study of RGC populations was carried out in adult rats with ONs transected at 0.5, 3, 8 or 10 mm from the posterior pole of the eye (Villegas-Pérez *et al.* 1988*b*); each of these lesions permanently disconnected the RGCs from all synaptic targets in the brain. When RGC populations were determined between 15 days and 15 months after ON transection, it was found that

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axotomy not only caused an early, abrupt loss of RGCs but also led to a gradual decrease in the number of these neurons that continued throughout the period of study. The severity and duration of the early, more dramatic, post-axotomy loss of RGCs related closely to the proximity of the site of injury to the neuronal soma (Villegas-Pérez *et al.* 1988*b*) while the more protracted loss progressed at a similar slow rate irrespective of the distance between optic nerve transection and the eye.

During development, the fate of many neurons is dependent on interactions with synaptic targets, assumed to be sources of trophic molecules that influence survival and differentiation (Barde 1989). For retinofugal projections, brain-derived neurotrophic factor (BDNF), one of several proteins related to nerve growth factor (NGF) (Leibrock *et al.* 1989), appears to be involved in this dependency (Johnson *et al.* 1986). The separation of RGCs from the SC and other regions of the brain that are presumed to be sources of BDNF and other molecules may be responsible for the massive loss of RGCs that follows axotomy in new born rodents (see for example, Allcutt *et al.* (1984); Muchnick-Miller & Oberdorfer (1981)). The precise mechanisms that lead to the death of cells deprived of trophic support are not well understood but it has been suggested that NGF deprivation results in the transcription of genes that cause the breakdown of cells (Martin *et al.* 1988).

The findings in the retinas of animals where the ON was cut 8 or 10 mm from the eye indicate that the proportion of RGCs that die as a result of the separation of these neurons from their targets is initially small but that the subsequent loss of RGCs gradually and relentlessly reduces the population of retinal neurons. The slow pace of this process may be related to the less strict dependency of mature neurons on trophic factors (Lindsay 1988), which are assumed to be target-derived. The reasons for the more acute loss of RGCs that are axotomized near their somata are also unclear but mechanisms other than those related to their dependency on trophic support from their targets would seem to be involved. It is unlikely that more extensive interactions between axons and components in the longer optic nerve stumps rescue RGCs that are axotomized further from the retina; even when the ON is interrupted near the chiasma (Richardson *et al.* 1982), axons in the proximal stump of the ON undergo a massive retrograde degeneration that exceeds the loss of RGC somata observed in the retinas with lesions at the same level. Moreover, based on studies of NGF mRNA (Lu *et al.* 1991), interrupted ONs in rats do not appear to be an important source of factors that enhance neuronal survival.

It is of interest that the slow rates of cell loss observed after axotomy of the rat optic nerve closely approximate those reported after severing the vagus nerve and mechanically blocking the regrowth of its peripheral axons (Laiwand *et al.* 1987). These observations suggest that certain patterns of neuronal degeneration are common to neurons that are prevented from making connections with their targets in either the PNS or CNS. They also imply that early molecular events involving the expression of trophic molecules in the proximal stump of interrupted peripheral nerves (Heumann *et al.* 1987) may not assure the permanent survival of axotomized cells whose regrowth and connectivity is prevented.

The documentation of a gradual and prolonged phase of neuronal loss after axotomy also raises the possibility that this pattern of cell death is common to other conditions where terminal contacts with fields of innervation may fail to provide trophic support because of deficits in the availability of such molecules or alterations in their access to neurons.

INFLUENCES OF THE PATH AND TARGET OF AXONS ON NEURONAL SURVIVAL

The extent of RGC loss that follows ON transection close to the eye can be reduced in adult rats by the apposition of a segment of peripheral nerve to the ocular stump of the transected optic nerve (Berry *et al.* 1986; Villegas-Pérez 1988*a*). These effects may be explained by the presence in the peripheral nervous system of several factors that can promote the survival of injured neurons. BDNF, which enhances RGC survival *in vitro* (Johnson *et al.* 1986; Thanos *et al.* 1989), is found in PNs (Barker *et al.* 1990). When PNs are transected, as is done to obtain the nerve grafts used in our experiments, the expression of NGF (Heumann *et al.* 1987) and NGF receptors (Taniuchi *et al.* 1986) is markedly enhanced. Furthermore, it has been reported that the introduction of NGF into the eye increases the survival of axotomized RGCs (Carmignoto *et al.* 1989), an effect that might be related to sequence homologies between NGF and BDNF (Leibrock *et al.* 1989) or to the activation of BDNF receptors by high concentrations of NGF (Rodriguez-Tébar *et al.* 1990). Peripheral nerves also contain other trophic molecules that enhance neuronal survival: fibroblast growth factor (Bähr *et al.* 1989; Sievers *et al.* 1987); ciliary neurotrophic factor (Manthorpe *et al.* 1986; Sendtner *et al.* 1990); and laminin (Edgar *et al.* 1984). Because Schwann cells, fibroblasts, and macrophages (Lindholm *et al.* 1987) participate in the synthesis and regulation of several trophic molecules, the PN grafts used in these studies may meet the specific requirements of a wide spectrum of neurons. However, the ability of PN grafts to enhance neuronal survival is only temporary (Villegas-Pérez *et al.* 1988*a*), an outcome that could be due to the decreased synthesis of trophic molecules such as NGF that occurs as regenerating axons grow along the PN graft (Heumann *et al.* 1987).

Additional observations made *in vivo* have provided further evidence that synaptic interactions may, in the long term, be essential for the maintenance of the normal physiologic activity and anatomic integrity of axotomized neurons. In rats and hamsters in which PN grafts bridged the eye and the SC, we have observed no apparent reduction in the extent of SC re-innervation or synapse formation between 2 and 18 months after introducing the regenerated axons into the SC. Moreover, electrophysiological responses to light recorded in the SC of animals with grafts joining the retina and the SC (Keirstead *et al.* 1989) were

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documented for several months after graft insertion (M. Rasminsky, Y. Sauvé, and D. Carter, unpublished observations). Conversely, in blind-ended PN grafts there was a gradual decline in the number of units that responded to retinal illumination (Keirstead *et al.* 1985, 1988), a finding that is consistent with the ongoing anatomic loss of RGCs that follows axotomy and the attachment of blind-ended PN grafts (Villegas-Pérez *et al.* 1988*b*).

CNS AXONS REGENERATE ALONG NERVE GRAFTS

Within PN grafts, the axons of CNS neurons regrow without branching for distances of 3–4 cm (Aguayo 1985; Vidal-Sanz *et al.* 1987). The growth cones of these axons extend in close apposition to Schwann cells and their basal lamina, an anatomical relationship that suggests such contacts mediate neuronal interactions with these substrates. Along the PN grafts, RGC axons extend at rates of 1–2 mm per day (Cho & So 1987) and can elongate for distances that are approximately twice the length of the normal retino-collicular projection of intact adult rats (Vidal-Sanz *et al.* 1987).

The mechanisms whereby regrowing axons interact with some of the cellular and substrate components of the PNS have been investigated in tissue culture. *In vitro*, two major classes of molecules influence cell growth and survival: growth factors and adhesive molecules. Although there are major overlaps in structure and function, the adhesive molecules have been classified functionally into cell adhesion molecules (e.g. N-CAM, N-Cadherin) and extracellular matrix (ECM) proteins (e.g. laminin). Cell adhesion molecules often act through homophillic binding between neurons and cells such as Schwann cells (for review, see Reichardt (1989)) while the ECM proteins act through heterophillic interactions with receptors called integrins whose function is specified by different alpha and beta subunits (Reichardt & Tomaselli 1991). The availability of antibodies and peptides known to disrupt the function of integrins has stimulated studies on the role of these molecules in regeneration and development. For example, laminin, which is expressed transiently in the ECM of the developing vertebrate CNS (Liesi 1985), also facilitates the regeneration of axons in the injured PNS (Sandrock & Matthew 1987). Interestingly, integrins on developing neurons undergo a functional down-regulation (Reichardt & Tomaselli 1991) that in part involves post-translational modification of these receptors influenced by the connection of neurons with their targets (Cohen *et al.* 1989). Furthermore, integrin function becomes apparent following axotomy of peripheral nerves in adult animals (Toyota *et al.* 1990). Thus, integrins and their ECM ligands that are involved in aspects of neural development (Hedgecock *et al.* 1990) may be recapitulated during regeneration.

Interactions between nerve cells and their non-neuronal environment may regulate both qualitatively and quantitatively the intracellular components that participate in axonal growth. The regulation may occur at several levels ranging from gene expression to protein turnover, assembly, and transport. An example of some of these effects is represented by the spectrum of changes in the transport of cytoskeletal proteins of RGCs following abortive regrowth in the ON or axonal regeneration in PN grafts. In the ocular stumps of intracranially transected ONs not exposed to PN grafts, there was a 10-fold decrease in the transport of tubulin and neurofilaments (McKerracher *et al.* 1990*b*). This decrease in rates of slow transport was not accompanied by comparable changes in fast axonal transport. In contrast, when the ON was cut near the eye and replaced by a PN graft, the rates of tubulin and neurofilament transport doubled and the rate of actin transport decreased (McKerracher *et al.* 1990*a*), a pattern that resembles slow transport in the developing ON (Willard & Simon 1983).

FORMATION OF NEW SYNAPTIC CONNECTIONS IN THE INJURED CNS

In adult rats and hamsters, autologous PN grafts attached to the ocular stump of transected ONs (figure 1) were used as bridges to guide RGC axons to the SC (Vidal-Sanz *et al.* 1987; Carter *et al.* 1989*a*) to determine if axonal regeneration could restore synaptic connections in the CNS. After different time intervals, RGCs were labelled intravitreally with tracer substances and the region of the SC near the end of the PN graft was surveyed by light or electron microscopy. In the animals with bridging grafts connecting the retina and the midbrain, the RGC axons grew into the superficial layers of the SC for up to 500 μm at 2 months (Carter *et al.* 1989*a*) and 1000 μm at 8–10 months (Carter *et al.* 1989*b*). Such limited extension into the SC contrasts sharply with the sustained growth of the RGC axons along the PN grafts.

On penetration into the tectum, the regenerating RGC axons extended towards the superficial laminae of the SC that normally receive most retinal projections and formed normal-appearing terminals and synapses (figure 2) that persisted for the entire period studied: up to 10 months in hamsters and 18 months in rats. Moreover, the proportion of contacts made by the RGC terminals on the dendritic shafts and on the spines of SC neurons closely resembled the normal pattern of post-synaptic neuronal domains (Carter *et al.* 1989*a*). Although this striking predilection of the regenerating RGC axons for certain SC laminae and neuronal domains could be dictated by the extensive tectal denervation caused by ON interruption or by molecular affinities between pre- and post-synaptic elements, studies of the synaptic distribution of RGC axons guided to abnormal targets (Zwimpfer *et al.* 1990) suggest that conditions other than denervation may play a role in determining these synaptic preferences. It has not been determined if the regenerated projections are deployed retino-topically.

The regenerated retino-tectal projections have been shown to exhibit two important functional attributes: the RGCs regenerating axons remain responsive to light and the synapses they form transmit impulses to SC neurons. The responsiveness of these neurons was

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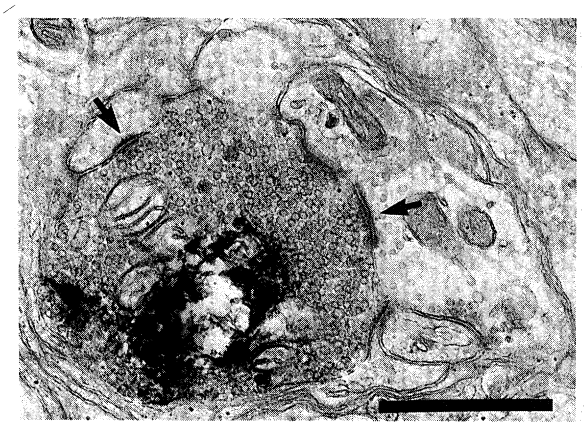

Figure 2. A regenerated retinal ganglion cell (RGC) axon terminal is identified in the superior colliculus by the presence of the reaction product of horse-radish peroxidase orthogradely transported from the eye. Similar in appearance to control RGC terminals, this regenerated terminal contains round vesicles and forms asymmetric synaptic junctions (arrows) with a spine-like process (on the left) and a larger dendritic profile (on the right). Scale bar, 1 μm. Reproduced with permission from Carter *et al.* (1989).

established by demonstrating that, two to three months after the placement of the PN graft, some of the RGCs with regenerated axons fired with 'on', 'off', or 'on–off' bursts of impulses when light was directed onto discrete receptive fields of the retina (Keirstead *et al.* 1985). This finding suggests that following axotomy near their somata and the regrowth of their axons into a PNS environment, the RGCs retained or regained the inputs that mediate these normal responses to light.

Trans-synaptic activity in the vicinity of regenerated terminals was recently demonstrated in the region neighbouring the insertion site of the PN grafts where differentiated RGC terminals and synapses had been observed by electron microscopy. In the superficial 450 μm of the SC, microelectrode recordings revealed both excitatory and inhibitory responses to light flashed into the PN-grafted eye (Keirstead *et al.* 1989). Some of these responses were shown to reflect activity in post-synaptic neurons rather than in regenerated RGC axons that penetrated the SC (Keirstead *et al.* 1989).

A yet unresolved question is the number of contacts that must be made by regenerated RGC terminals to give rise to the activation or inhibition of SC neurons. In contrast to cells such as motor neurons for which each input can contribute only a small fraction of the post-synaptic potential necessary to depolarize the cell to threshold, activation of some nerve cells contacted by RGC axons is thought to be securely mediated by single synapses (Bloomfield & Sherman 1989). In the retinotectal graft preparation, other afferents to the SC are abolished by the transection of the optic nerves and the removal of part of the visual cortex needed to gain access to the SC. It is unclear to what extent the efficacy of the retinotectal trans-synaptic activation observed in these animals is compromised, altered, or perhaps even enhanced by the absence of these other inputs to the SC neurons. However, if single regenerated retinotectal synapses can mediate secure trans-synaptic activation, it is possible that relatively few contacts would be needed in a reinnervated SC to relay visual information.

CONCLUSIONS

The demonstration that functional synapses can be made in the injured adult mammalian CNS as the end-result of a lengthy and circuitous axonal regrowth through these PN-grafts raises the possibility that connections may also be restored spontaneously in the CNS by the short-range regrowth of fibres interrupted near their targets or through the re-establishment of connections between nerve cells, such as interneurons, whose somata and cellular appendages are close to each other. Thus interneurons and the terminals of longer axons may express growth capacities and synaptic predilections within a narrow radius that is defined by their immediate glial or neuronal environment. While these capacities for axonal growth and synaptogenesis may be retained in the adult CNS, it is difficult to envisage how the developmental conditions that guide a multitude of axonal projections to their respective fields of innervation and avoid inappropriate targets would be fully replicated in the injured CNS. However, the precision with which many injured neurons in the brain and spinal cord of adult amphibians and fish re-establish lost connections provides an encouraging argument for pursuing further studies of regeneration in the central nervous system of mammals aimed at a better understanding of the mechanisms that underline these phenomena.

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REFERENCES

Aguayo, A. J. 1985 Axonal regeneration from injured neurons in the adult mammalian central nervous system. In *Synaptic plasticity* (ed. C. W. Cotman), pp. 457–484. New York: Guilford Press.

Aguayo, A. J., Carter, D. A., Zwimpfer, T., Vidal-Sanz, M. & Bray G. M. 1990 Synaptic connections made by axons regenerating in the CNS of adult mammals. *J. exp. Biol.* **153**, 199–224.

Allcutt, D., Berry, M. & Sievers, J. 1984 A quantitative comparison of the reactions of retinal ganglion cells to optic nerve crush in neonatal and adult mice. *Devl Brain Res.* **16**, 219–230.

Bähr, M., Vanselow, J. & Thanos, S. 1989 Ability of adult rat ganglion cells to regrow axons in vitro can be influenced by fibroblast growth factor and gangliosides. *Neurosci. Lett.* **96**, 197–201.

Barde, Y-A. 1989 Trophic factors and neuronal survival. *Neuron* **2**, 1525–1534.

Barker, P., Acheson, A., Pareek, S., Miller, F. D. & Murphy, R. A. 1990 Detection of brain-derived neurotrophic factor (BDNF)-like biological activity and mRNA in sciatic nerve fibroblasts and Schwann cells. *Soc. Neurosci. Abstr.* **16**, 1136.

Bernstein, J. J. & Bernstein, M. E. 1971 Axonal regeneration and formation of synapses proximal to the site of lesion following hemisection of the rat spinal cord. *Expl Neurol.* **30**, 336–351.

Berry, M., Rees, L. & Sievers, J. 1986 Regeneration of

axons in the mammalian visual system. *Expl Brain Res. Suppl.* **13**, 18–33.

Bloomfield, S. A. & Sherman, S. M. 1989 Dendritic current flow in relay cells and interneurons of the cat's lateral geniculate nucleus. *Proc. natn Acad. Sci. U.S.A.* **86**, 3911–3914.

Carmignoto, G., Maffei, L., Candeo, P., Canella, R. & Comelli, C. 1989 Effect of NGF on the survival of rat retinal ganglion cells following optic nerve section. *J. Neurosci.* **9**, 1263–1272.

Carter, D., Bray, G. M. & Aguayo, A. J. 1989*a* Regenerated retinal ganglion cell axons can form well-differentiated synapses in the superior colliculus of adult hamsters. *J. Neurosci.* **9**, 4042–4050.

Carter, D., Bray, G. M. & Aguayo, A. J. 1989*b* Extension and persistence of regenerated retinal ganglion cell axons in the superior colliculus of adult hamsters. *Soc. Neurosci. Abstr.* **15**, 872.

Cho, E. Y. P. & So, K-F. 1987 Rate of regrowth of damaged retinal ganglion cell axons regenerating in a peripheral nerve graft in adult hamsters. *Brain Res.* **419**, 369–374.

Cohen, J., Nurcombe, V., Jeffrey, P. & Edgar, D. 1989 Developmental loss of functional laminin receptors on retinal ganglion cells is regulated by their target tissue, the optic tectum. *Development* **107**, 381–387.

Doster, K. S., Lozano, A. M., Aguayo, A. J. & Willard, M. 1991 Expression of the growth-associated protein GAP-43 in adult rat retinal ganglion cells following axon injury. *Neuron* (In the press.)

Edgar, D., Timpl, R. & Thoenen, H. 1984 The heparin binding domain of laminin is responsible for its effects on neurite outgrowth and neuronal survival. *EMBO J.* **3**, 1463–1468.

Hedgecock, E. M., Culotti, J. G. & Hall, D. H. 1990 The UNC-5, UNC-6 and UNC-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in C. elegans. *Neuron* **4**, 61–85.

Heumann, R., Lindholm, D., Bandtlow, C., Mayer, M., Radeke, M. J., Misko, T. P., Shooter, E. & Thoenen, H. 1987 Differential regulation of nerve growth factor (NGF) and NGF-receptor mRNA in the rat sciatic nerve during development, degeneration, and regeneration; role played by macrophages. *Proc. natn Acad. Sci. U.S.A.* **84**, 8735–8739.

Johnson, J. E., Barde, Y-A., Schwab, M. & Thoenen, H. 1986 Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. *J. Neurosci.* **6**, 3031–3038.

Keirstead, S. A., Vidal-Sanz, M., Rasminsky, M., Aguayo, A. J., Levesque, M. & So, K-F. 1985 Responses to light of retinal neurons regenerating axons into peripheral nerve grafts in the rat. *Brain Res.* **359**, 402–406.

Keirstead, S. A., Rasminsky, M., Vidal-Sanz, M. & Aguayo, A. J. 1988 Functional studies of central nervous system neurons regenerating axons into peripheral nerve grafts. In *The current status of peripheral nerve regeneration* (ed. T. Gordon, R. B. Stein & P. A. Smith), pp. 143–155. New York: Alan R. Liss.

Keirstead, S. A., Rasminsky, M., Fukuda, Y., Carter, D. A., Aguayo, A. J. & Vidal-Sanz, M. 1989 Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons. *Science, Wash.* **246**, 255–258.

Laiwand, R., Werman, R. & Yarom, Y. 1987 Time course and distribution of motoneuronal loss in the dorsal motor vagal nucleus of guinea pig after cervical vagotomy. *J. comp. Neurol.* **256**, 527–537.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Herenger, B., Masiakowski, P., Thoenen, H. & Barde, Y-A. 1989 Molecular cloning and expression of brain-derived neurotrophic factor. *Nature, Lond.* **341**, 149–152.

Lieberman, A. R. 1974 Some factors affecting retrograde neuronal responses to axonal lesions. In *Essays on the Nervous System* (ed. R. Bellairs & E. G. Gray), pp. 71–105. Oxford: Clarendon.

Liesi, P. 1985 Laminin immunoreactive glia distinguish regenerative adult CNS systems from non-regenerative ones. *EMBO J.* **4**, 2505–2511.

Lindholm, D., Heumann, R., Meyer, M. & Thoenen, H. 1987 Interleukin-1 regulates synthesis of nerve growth factor in non neuronal cells of rat sciatic nerve. *Nature, Lond.* **330**, 658–659.

Lindsay, R. M. 1988 Nerve growth factors (NGF, BDNF) enhance axonal regeneration but are not required for survival of adult sensory neurons. *J. Neurosci.* **8**, 2394–2405.

Lozano, A. M. 1988 The expression of the growth-associated protein GAP-43 in the injured and regenerating retinal ganglion cells of the adult rat. Ph.D. Thesis, McGill University.

Lu, B., Yokoyama, M., Dreyfus, C. & Black, I. 1991 NGF gene expression in actively growing brain glia. *J. Neurosci.* (In the press.)

Manthorpe, M., Skaper, S. D., Williams, L. R. & Varon, S. 1986 Purification of adult rat sciatic nerve ciliary neuronotrophic factor. *Brain Res.* **367**, 282–286.

Martin, D. P., Schmidt, R. E., DiStefano, P. S., Lowry, O. H., Carter, J. G. & Johnson, E. M. Jr. 1988 Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *J. Cell Biol.* **106**, 829–844.

McKerracher, L., Vidal-Sanz, M. & Aguayo, A. J. 1990*a* Slow transport rates of cytoskeletal proteins change during regeneration of axotomized retinal neurons in adult rats. *J. Neurosci.* **10**, 641–648.

McKerracher, L., Vidal-Sanz, M., Essagian, C. & Aguayo, A. J. 1990*b* Selective impairment of slow axonal transport after optic nerve injury in adult rats. *J. Neurosci.* **10**, 2834–2841.

Muchnick-Miller, N. & Oberdorfer, M. 1981 Neuronal and neuroglial responses following retinal lesions in the neonatal rats. *J. comp. Neurol.* **202**, 493–504.

Reichardt, L. F., Bixby, J. L., Hall, D. E., Ignatius, M. J., Neugebauer, K. M. & Tomaselli, K. J. 1989 Integrins and cell adhesion molecules: neuronal receptors that regulate axon growth on extracellular matrices and cell surfaces. *Devl Neurosci.* **11**, 332–347.

Reichardt, L. F. & Tomaselli, K. J. 1991 Extracellular matrix molecules and their receptors: Functions in neural development. *Ann. Rev. Neurosci.* (In the press.)

Richardson, P. M., Issa, V. M. K. & Shemie, S. 1982 Regeneration and retrograde degeneration of axons in the rat optic nerve. *J. Neurocytol.* **11**, 949–966.

Rodriguez-Tébar, A., Dechart, G. & Barde, Y-A. 1990 Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* **4**, 487–492.

Rutishauser, U. 1986 Differential cell adhesion through spatial and temporal variations of NCAM. *TINS* **9**, 374–378.

Sandrock, W. W. & Matthew, W. D. 1987 An in vitro neurite promoting antigen functions in axonal regeneration in vivo. *Science, Wash.* **237**, 1605–1608.

Schwab, M. E. 1990 Myelin-associated inhibitors of neurite growth. *Exp. Neurol.* **109**, 2–5.

Sendtner, M., Kreutzberg, G. W. & Thoenen, H. 1990 Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. *Nature, London.* **345**, 440–441.

Sievers, J., Hausmann, B., Unsicker, K. & Berry M. 1987 Fibroblast growth factors promote the survival of adult rat

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

retinal ganglion cells after a transection of the optic nerves. *Neurosci. Lett.* **76**, 157–162.

Taniuchi, M., Clark, H. B. & Johnson E. M. Jr. 1986 Induction of nerve growth factor receptor in Schwann cells after axotomy. *Proc. natn Acad. Sci. U.S.A.* **83**, 4094–4098.

Thanos, S., Bähr, M., Barde, Y-A. & Vanselow, J. 1989 Survival and axonal elongation of adult rat retinal ganglion cells. *Eur. J. Neurosci.* **1**, 19–26.

Toyota, B., Carbonetto, S. & David, S. 1990 A dual laminin-collagen receptor acts in peripheral nerve regeneration. *Proc. natn Acad. Sci. U.S.A.* **87**, 1319–1322.

Vidal-Sanz, M., Bray, G. M., Villegas-Pérez, M. P. & Aguayo, A. J. 1987 Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat. *J. Neurosci.* **7**, 2894–2907.

Villegas-Pérez, M. P., Vidal-Sanz, M., Bray, G. M. & Aguayo, A. J. 1988*a* Influences of peripheral nerve grafts on the survival and regrowth of axotomized retinal ganglion cells in adult rats. *J. Neurosci.* **8**, 265–280.

Villegas-Pérez, M. P., Vidal-Sanz, M., Bray, G. M. & Aguayo, A. J. 1988*b* Retinal ganglion cell death after axotomy is influenced by the distance between the lesion and the neuronal somata. *Soc. Neurosci. Abstr.* **14**, 673.

Willard, M. B. & Simon, C. 1983 Modulations of neurofilament axonal transport during the development of rabbit retinal ganglion cells. *Cell* **35**, 551–559.

Zwimpfer, T., Inoue, H. I., Aguayo, A. J. & Bray, G. M. 1990 Regenerating retinal ganglion cell axons can form synapses with neurons in four different non-retinal targets in the adult hamster. *Soc. Neurosci. Abstr.* **16**, 41.

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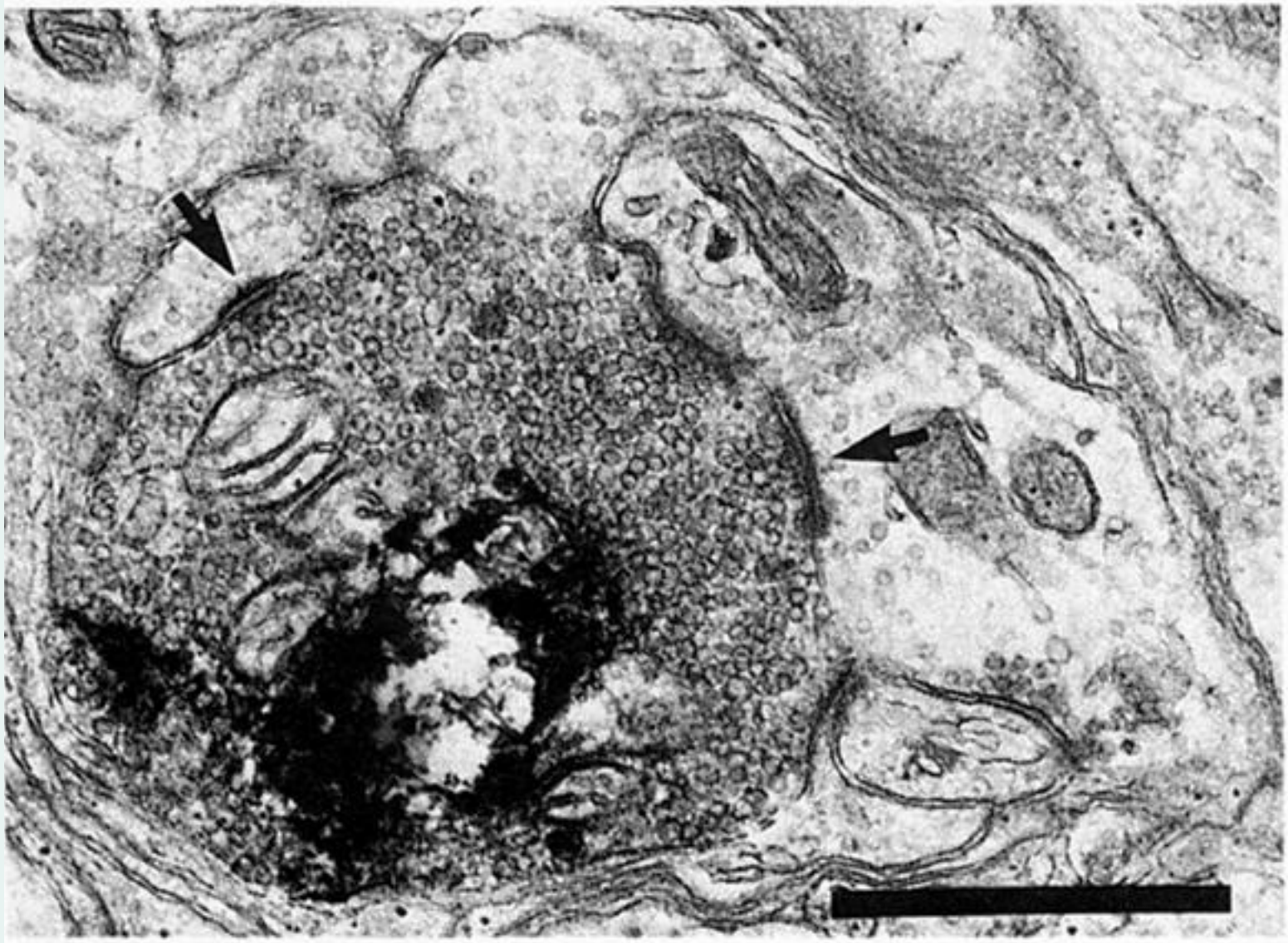

gure 2. A regenerated retinal ganglion cell (RGC) axon
minal is identified in the superior colliculus by the presence
the reaction product of horse-radish peroxidase ortho-
adely transported from the eye. Similar in appearance to
ntrol RGC terminals, this regenerated terminal contains
und vesicles and forms asymmetric synaptic junctions
rrows) with a spine-like process (on the left) and a larger
ndritic profile (on the right). Scale bar, 1 μm. Reproduced
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