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THE USE OF PERIPHERAL NERVE GRAFTS TO ENHANCE NEURONAL SURVIVAL, PROMOTE GROWTH AND PERMIT TERMINAL RECONNECTIONS IN THE CENTRAL NERVOUS SYSTEM OF ADULT RATS

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SUMMARY

During both development and regeneration, the survival of neurones and the growth of axons are controlled by inherent neuronal properties, conditions in the axonal environment, and the establishment of appropriately timed and specific functional contacts. To study the effects of extrinsic influences on the survival, growth and connectivity of axotomized neurones in the mature mammalian CNS, we replaced the optic nerve in adult rats with segments of autologous peripheral nerve (PN) and used morphometric techniques, neuroanatomical tracer substances and immunological cell markers to examine retinal ganglion cells (RGCs), their axons in the PN grafts and their terminals in the superior colliculi (SC) of these animals. We observed that: (1) the survival of axotomized RGCs was enhanced by the PN grafts; (2) in the PN-grafted eyes, approximately 20% of the surviving RGCs regrew their axons into the grafts and (3) some of the RGC axons that regenerated along the PN grafts bridging the eye and the tectum re-entered the SC, arborized and made synaptic contacts with tectal neurones. It is not known if the terminal connections established between RGCs and cells in the SC are appropriate, functional or capable of influencing the long-term survival of their cells of origin.

INTRODUCTION

The interruption of fibre pathways by mechanical injury to the nervous system cannot be considered simply as a form of neural disconnection. Such injuries initiate a chain of widespread, complex and time-related responses that not only affect cells that are directly damaged but also alter the structure and function of entire groups of related neurones, glia and the extracellular matrix. In the investigation of strategies to modify these effects of neural injury and to promote recovery, certain insights may be obtained by viewing the responses to injury as a breakdown of processes that regulate neural development and maintenance. In this report, we summarize some of the developmental interactions that lead to the establishment of neural circuitry, review certain effects of axotomy in the mature nervous system, and describe

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experiments aimed at investigating the role of epigenetic influences on neuronal responses to injury in the adult mammalian central nervous system.

THE ESTABLISHMENT OF NEURAL CIRCUITRIES DURING DEVELOPMENT

The formation of the nervous system depends on a series of precisely timed developmental events (for a review, see Purves & Lichtman, 1985): (a) an early phase of neurogenesis that includes cell division, neuronal migration to specified locations within the embryo, and differentiation into specialized cell types; (b) survival – the modulation and curtailment of neuronal death; (c) the guided growth and differentiation of axons and dendrites; (d) recognition of and contact with appropriate targets; (e) synaptic differentiation, including the acquisition of suitable transmitters and the induction of receptors; (f) maturation of the neurone–target relationships to achieve functional connectivity; and (g) the long-term maintenance of neurones and their processes so that they are both permanent and modifiable.

The progression of these orderly processes depends largely on complex and incompletely understood interactions between intrinsic, genetically regulated neuronal properties and extrinsic, epigenetic influences arising from the neural environment. These extrinsic influences may be mediated by secreted molecules and surface interactions with other cells (neuronal and non-neuronal) or the extracellular matrix located along the neurones' pathways or in their targets (for a review, see Edelman, Gall & Cowan, 1985).

NEURAL INJURY AND THE MULTIPLE EFFECTS OF AXOTOMY

In this section, we consider the diverse effects of axotomy as perturbations of the orderly developmental interactions that are the basis of the mature nervous system and review the extent to which neuronal responses to injury are able to recapitulate developmental events.

Retrograde effects on neurones

Interruption of axons leads to a spectrum of retrograde changes that affect proximal axons and the neuronal perikarya. The perikaryal changes range from chromatolysis to death of the axotomized neurones, particularly if the lesion is near the cell body (for a review, see Lieberman, 1974). In mammals, neuronal degeneration due to axotomy can be prominent in the CNS (Lieberman, 1974; Barron, 1983) but also follows injury to peripheral nerves (Aldskogius, Barron & Regal, 1980; Aldskogius & Risling, 1982; Tessler *et al.* 1985). In a recent study in which retrogradely transported tracers were used to identify retinal ganglion cells (RGCs) in adult rats, there was a loss of at least 90% of these neurones by 1 month after transection of the optic nerve near the eye (Villegas-Pérez, Vidal-Sanz & Aguayo, 1986; M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation). In contrast, when RGCs were transected intracranially, the retrograde loss of retinal neurones was delayed (Misantone, Gershenbaum & Murray, 1984).

Other examples of CNS neurones that undergo extensive retrograde death following axotomy are the thalamic projection neurones (Lieberman, 1974; Barron, Means & Larsen, 1973) and the cholinergic cells of the nucleus basalis (L. R. Williams *et al.* 1986).

Axotomy can also lead to retrograde and orthograde transneuronal changes. For example, when peripheral nerves are cut in adult animals, there are changes in the synaptic contacts on motor neurones (for a review, see Mendell, 1984) and rearrangements in the representations of sensory receptive fields in the spinal cord (Devor & Wall, 1978, 1981*a*,*b*) and the cerebral cortex (Kaas, Merzenich & Killackey, 1983).

Orthograde effects on neurones and other targets

In addition to degeneration of the distal segments of transected axons and their synaptic terminals, axonal interruption causes trans-synaptic changes in the target tissues, whether they are other neurones, sensory receptors or effector organs. For example, denervated skeletal muscle progressively atrophies and shows major changes in the pattern of distribution of its acetylcholine receptors (Fambrough, 1981). Within the CNS, the interruption of neural circuitry can be followed by replacement of the lost presynaptic terminals by new terminals from local neurones (Murray, Battisti & Goldberger, 1986) leading to enhancements of certain transmitter types, such as those that are GABAergic (Houser, Lee & Vaughn, 1983).

Changes in the non-neuronal environment

In the peripheral nervous system, there are striking cellular (for a review, see Bray, Rasminsky & Aguayo, 1981) and molecular changes in the distal segments of interrupted nerves. These include the proliferation (Bradley & Asbury, 1970) and dedifferentiation of Schwann cells, the appearance of macrophages, the secretion of nerve growth factor (NGF) and other trophic factors (Riopelle, Boegman & Cameron, 1981; Richardson & Ebendal, 1982; Varon, Manthorpe & Williams, 1983/1984; Abrahamson, Wilson & Rush, 1986), the expression of NGF receptors (Taniuchi, Clark & Johnson, 1986) and the appearance of apolipoprotein-E (Skene & Shooter, 1983; Muller, Ignatius, Hangen & Shooter, 1986).

In the CNS, most of the dividing cells distal to a transecting lesion are astrocytes and microglia rather than oligodendrocytes (Skoff, 1975). Furthermore, the proliferating astrocytes appear to belong to the specific subclass that expresses the type I antigenic phenotype; astrocytes that express the type II antigenic phenotype were not detected after optic nerve transection, suggesting that these cells depend on axons for their long-term survival (Miller *et al.* 1986). Although it has been postulated that the proliferation of astrocytes after CNS injury is an impediment to the regeneration of interrupted axons (Windle, 1956; Reier, Stensaas & Guth, 1983), Manthorpe, Rudge & Varon (1986) suggest that these cells may be the source of the neurotrophic activity that can be extracted from injured CNS tissue after delays of several days (Nieto-Sampedro *et al.* 1982, 1983; Manthorpe *et al.* 1983).

EXPERIMENTAL MODIFICATION OF THE EFFECTS OF AXOTOMY

Recovery from neural injury in the CNS of adult mammals is typically limited because, under ordinary circumstances, few neurones replicate the events of normal development. Except in the olfactory sensory epithelium (Graziadei & Monti Graziadei, 1979), cell division does not replace lost neurones, the failure of axotomized neurones to survive can be substantial, and the regenerative responses of most axotomized central neurones are restricted to short-range changes in neuronal connectivity and synapse organization (Raisman, 1985). Thus, strategies have been devised to overcome these limitations to regeneration by manipulations that introduce extrinsic modifiers of neuronal responses.

Axonal regeneration in the central and peripheral nervous systems

Effective regeneration after axotomy depends on: survival of the axotomized neurones; axonal growth by the sprouting of axons from the proximal nerve stumps and their subsequent elongation and guidance to proper targets; axonal ensheathment and differentiation; the re-establishment of connectivity through synaptic contacts with target cells and, finally, the loss of inappropriate connections or redundant axon branches.

In adult mammals, extensive axonal regrowth and terminal reconnectivity are limited to certain types of injury in the peripheral nervous system (PNS). Following crush injury in the peripheral nervous system, the sequence of events listed above can lead to a nearly complete restoration of nerve fibre structure (Cragg & Thomas, 1964; Devor & Govrin-Lippmann, 1979; Diamond & Jackson, 1980) and function (Burgess & Horch, 1973; Burgess, English, Horch & Stensaas, 1974; Dykes & Terzis, 1979), presumably because, with this type of injury in which the Schwann cell basal lamina is not disrupted, many columns of dedifferentiated Schwann cells remain aligned to direct the regenerating axons to their appropriate targets. However, after complete transection of peripheral nerves, and in spite of surgical apposition of the proximal and distal nerve stumps, functional recovery is incomplete (Horch & Burgess, 1980), probably because axons miss their specific targets as a result of the misalignment of the Schwann cell columns disrupted at the site of injury.

In certain anamniotes, CNS neurones are able to elongate their injured axons and accomplish a successful restoration of connectivity and function. If the optic nerves are transected in the adult goldfish, for example, retinal ganglion cells survive, regenerate their axons and reform synapses in the optic tectum with a restoration of visual function (for a review, see Grafstein, 1986). In mammals, however, interruption of the optic nerve or other CNS pathways leads to abortive axonal sprouting without axonal elongation so that target neurones remain permanently disconnected. Furthermore, the retrograde loss of axotomized neurones, which can be substantial with lesions near the neuronal perikarya (Villegas-Pérez *et al.* 1986; M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation),

imposes important limitations on the potential for structural regeneration and functional recovery in both the CNS and the PNS.

A potential for the regeneration of CNS axons

In spite of the devastating effects of axotomy on CNS neurones, there is experimental evidence that their responses to injury can be modified by external influences to replicate some of the processes that characterize the formation of neuronal circuitries during development as well as the reconstitution of certain injured systems in other vertebrates.

Neurone survival

Following axotomy, neuronal survival can be enhanced *in vivo* in the CNS by: (a) the administration of specific molecules such as NGF (L. R. Williams *et al.* 1986; Hefti, 1986; Kromer, 1987) to rescue the magnocellular neurones of the nucleus basalis after lesions of the fimbria-fornix; (b) the grafting of foetal neurones to replace the natural target of the lateral geniculate nucleus (Haun & Cunningham, 1984) or the red nucleus (Bregman & Reier, 1986) in neonatal rats; (c) the apposition of peripheral nerve (PN) grafts to the transected optic nerves of adult rats (Berry, Rees & Sievers, 1986; Villegas-Pérez *et al.* 1986; M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation).

Axonal sprouting

Examples have been reported in which local axonal growth for distances of several millimetres has occurred after small injuries near cell bodies in the retina (Leoz Ortin & Arcaute, 1914; Goldberg & Frank, 1980; McConnell & Berry, 1982; So, Xiao & Diao, 1986) or spinal cord (Risling, Cullheim & Hildebrand, 1983; Havton & Kellerth, 1987). Furthermore, the introduction into other regions of the CNS of iridis or foetal neural tissues (Björklund & Stenevi, 1984) has provided evidence of the responsiveness of mature central neurones to conditions in their immediate environment. However, neither the sprouts of nerve cells indigenous to the adult host CNS nor those arising from transplanted foetal neurones have elongated successfully for the distances required to restore major projections within the brain and spinal cord, an indication that substrate conditions within the mature CNS of mammals either fail to promote substantial elongation or exert inhibitory effects on neurite extension.

Axonal elongation from axotomized CNS neurones

Further studies in which PN grafts were used as 'bridges' between different regions of the CNS (David & Aguayo, 1981; Vidal-Sanz *et al.* 1987) established that cells in the mature mammalian CNS were capable of sustaining lengthy neurite growth when their interrupted axons were provided with a propitious environment (for a review, see Aguayo, 1985). In these experiments, the PN grafts probably

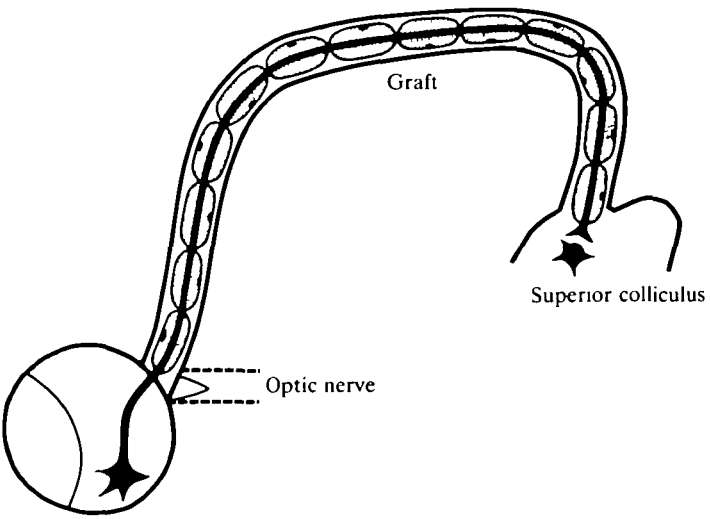

Fig. 1. Diagram of a peripheral nerve graft joining the superior colliculus and the optic nerve, transected near the eye in an adult rat. Axons of retinal ganglion cells grow into the grafted nerve segment, become ensheathed by Schwann cells and elongate through the graft for distances of 3–4 cm, but when they re-enter the superior colliculus their extension is limited to less than 1 mm.

provided a unique chain-like arrangement of sheath cells within basal lamina tubes that offer axonal growth cones the plasma membranes and matrix surfaces as well as the secreted molecules needed for a continuous extension towards distant targets (Fig. 1). Under such experimental conditions some of the axotomized CNS neurones grew for distances even greater than those they accomplish during normal development in the intact animal (Benfey & Aguayo, 1982; So & Aguayo, 1985; Vidal-Sanz, Villegas-Pérez, Cochard & Aguayo, 1985; Vidal-Sanz *et al.* 1987).

Terminal connectivity

In neural transplantation experiments, it has been documented that adult neurones remain receptive to the new inputs that originate from nearby grafted immature nerve cells (Sotelo & Alvarado-Mallart, 1986; Clarke, Gage, Nilsson & Björklund, 1986). Moreover, when PN grafts containing axons regenerating from the transected optic nerves of adult rats were inserted into the denervated superior colliculi, synaptic connections were reformed (Vidal-Sanz *et al.* 1987). Further studies are necessary to determine if these reformed synapses are functional, appropriate or sustained.

Thus, the axotomized CNS neurones in adult mammals are able to respond to a variety of external influences that affect their survival, the elongation of their axons, and the redevelopment of their terminal connections. To this extent, therefore, th effective reconstitution of injured neural tissues has involved a recapitulation of some of the original developmental processes.

EFFECTS OF PN GRAFTS ON CELL SURVIVAL, AXON GROWTH AND TERMINAL CONNECTIVITY OF RETINAL GANGLION CELLS AXOTOMIZED IN ADULT MAMMALS

The retina contains a well-characterized population of neurones whose arrangement is particularly suited for anatomical, functional and molecular studies of the regenerative capacities of the CNS. We have investigated the effects of PN grafts on the survival, axonal regrowth and terminal connectivity of RGCs axotomized in adult Sprague-Dawley rats by transecting the optic nerve (ON) and replacing it with an autologous segment of peroneal nerve (Fig. 1; Vidal-Sanz *et al.* 1987). Three groups of grafted rats and appropriate controls were examined: (1) to determine the overall responses of retinal neurones to axotomy and PN grafting by applying retrogradely transported tracer substances to the distal ends of the PN grafts; (2) to study the survival of RGCs in PN-grafted and non-grafted retinas by using morphometric techniques, double-labelling with retrogradely transported fluorescent markers, and immunological markers and (3) to identify the growth and possible connectivity of RGC axons that had grown along the grafts by examining the PN grafts and brainstems after injection of orthogradely transported tracers into the PN-grafted eyes.

Regrowth of axotomized retinal ganglion cells

In rats in which a PN graft was attached to the orbital stump of an optic nerve transected near the eye, horseradish peroxidase (HRP) was applied to the blind-ended distal end of the graft after 8–10 weeks and the retinas were examined 2 days later for retrogradely labelled RGCs (Fig. 2), between 1 and 11% (mean 3·3%, $N = 20$) of the original normal population of RGCs (estimated to be 110000; Perry, 1981) grew along the graft for 3–4 cm (Vidal-Sanz *et al.* 1985, 1987). However, in experiments in which two different fluorescent tracers were used to estimate the relative extents of survival and regrowth, it was found that as many as 20% of the surviving RGCs had grown their axons to the end of the PN grafts (Villegas-Pérez *et al.* 1986; M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation). Because not all regrowing axons may have reached the site of application of these tracers (the end of the grafts) and some may not have incorporated the tracers used for these studies, the incidence of axonal regeneration among surviving RGCs may be even higher.

Using the orthograde transport of HRP, rhodamine isothiocyanate (RITC) or tritiated amino acids to label axons regenerating from the PN-grafted eyes, labelled axon profiles were identified along the entire lengths of the PN grafts and in the superior colliculus (SC). Although axon growth rates showed considerable variation, the fastest growing retinal fibres extended at rates of 1·3 mm day^{-1} for distances that were nearly double those of the normal retino-collicular projections in adult rats (Trecarten *et al.* 1986). Within the PN grafts, the labelled tips of the regenerating RGCs, which were similar to the growth cones seen on the optic nerves of immature birds (Thanos & Bonhoeffer, 1983) and mammals (R. W. Williams, Bastiani, Lia &

Chalupa, 1986), were apposed to the outer surfaces of Schwann cells and often contacted the Schwann cell basal lamina in a manner that resembled the extension of peripheral nerve fibres. Interestingly, the rates of regrowth of the adult rat retinal axons in these grafts were lower than those observed in regenerating peripheral nerves (Forman & Berenberg, 1978) but similar to those of regenerating retinal axons in anamniotes (Grafstein, 1986) and in the developing optic nerves of immature rodents (Lund & Bunt, 1976). It can be concluded from these observations that while the PN environment permits or enhances the expression of axonal growth potentials, there are intrinsic mechanisms peculiar to different classes of neurones that regulate the rates at which such axons extend.

Effects of PN grafts on retinal ganglion cell survival

Approximately half of the neurones in the ganglion cell layer of the retinas of adult rodents die after axotomy (Grafstein & Ingoglia, 1982; Misantone *et al.* 1984; Allcutt, Berry & Sievers, 1984; Berry *et al.* 1986; Villegas-Pérez *et al.* 1986;

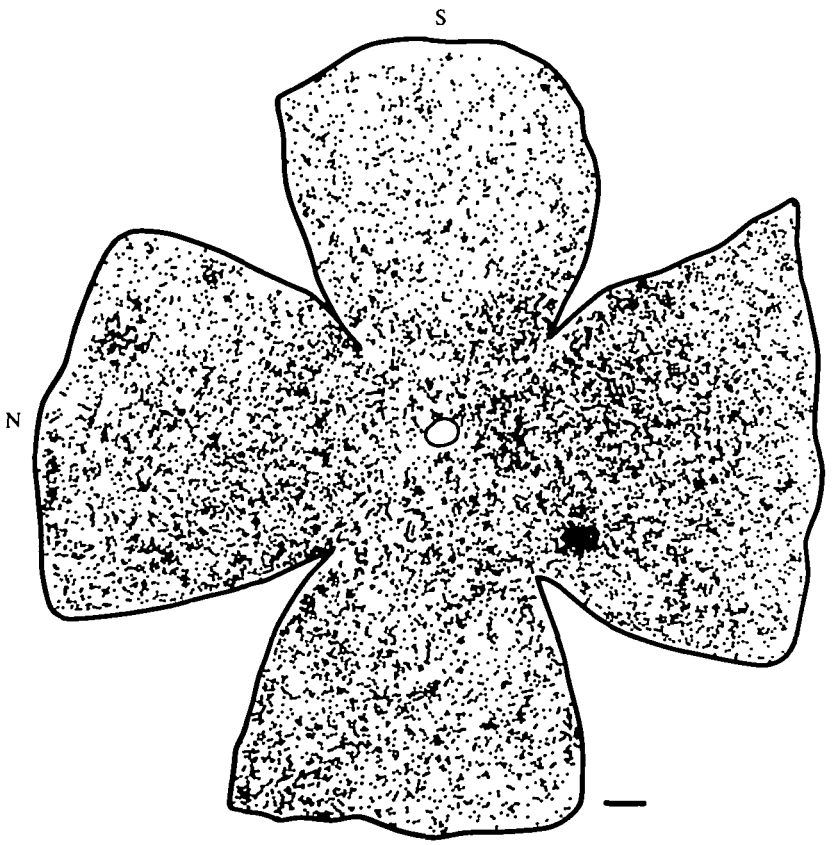

Fig. 2. Drawing of a flattened retinal whole-mount showing the location of ganglion cells (dots) retrogradely labelled with horseradish peroxidase applied to the distal end of an autologous peripheral nerve segment grafted to the optic nerve. S, superior; N, nasal. Scale bar, 500 μm.

M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation). However, assessments of cell death based only on counts of the total numbers of surviving neurones in the ganglion cell layer do not distinguish between the ganglion cells and the many axonless amacrine cells present in this layer (Perry, 1981; Linden & Perry, 1983). Moreover, estimates based on size criteria alone are inaccurate because of the overlap between large amacrine cells and small RGCs (Perry, 1981). Thus, we used 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (diI), a retrogradely transported tracer that remains in neurones for long periods (Honig & Hume, 1986), to estimate the densities of surviving RGCs in axotomized and PN-grafted retinas (Villegas-Pérez *et al.* 1986; M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation). Examined from 15 days to 3 months after axotomy, the densities of surviving RGCs in the retinas of these animals were two- to four-fold greater in the retinas with PN grafts. In retinas examined after longer intervals (6 and 9 months), the effects of PN grafts were assessed by identifying intraretinal RGC axons by their immunoreactivity to RT97, a monoclonal antibody to 200 kDa neurofilaments (Anderton *et al.* 1982). In such preparations, there was a striking enhancement of RGC axon preservation in the PN-graftedretinas (M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation).

These results imply that early interactions between RGCs and the PN grafts mitigate the retrograde effects of axotomy on some of these retinal neurones by inducing the mature RGCs to mount the metabolic responses to axotomy that permit them to survive injury and to regrow lengthy axons. Although the mechanisms responsible for these effects are unknown it is possible that neuronal survival may not necessarily require retinal axonal extension into the grafts but may be mediated by molecules released by graft components acting on RGCs soon after injury (M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation).

Terminals of RGC axons that enter the superior colliculus

The terminals of RGC axons that had grown along the PN grafts and entered the SC were visualized by the orthograde transport of RITC or HRP (Vidal-Sanz, Bray & Aguayo, 1986; Vidal-Sanz *et al.* 1987). Some regenerating axons penetrated the PNS–CNS interface at the tips of these grafts and extended into the SC for distances up to 500 μm. The marked differences in the extent of axonal elongation in the PNS milieu of the grafts and that of the target CNS undoubtedly reflect the decisive influence of local substrates on axonal elongation.

Within the SC, the RITC- or HRP-labelled axon terminals consisted of single fibres with little branching or multiple-branched arborizations up to 300 μm in length. Using electron microscopy, small HRP-labelled axon profiles were observed within the territories of the arborizations seen using light microscopy. Some of these profiles contained synaptic vesicles and contacted unlabelled neural structures, usually dendrites; a few of these contacts showed pre- and postsynaptic specializations (Vidal-Sanz *et al.* 1986, 1987). Since the tracer-injected eyes and the targeted regions of the CNS were only linked by axons that had extended along the grafted PN bridges, the labelled profiles found in the SC are presumed to be RGC axon

terminals. Although it has been demonstrated that axotomized RGCs whose axons have grown into PN grafts retain or regain their electrophysiological responses to light (Keirstead *et al.* 1985), it remains to be determined if such activity can be relayed trans-synaptically to neurones in the SC.

SOME COMMENTS AND CAVEATS

Although some of our results suggest that grafted segments of peripheral nerve can enhance the survival of the axotomized neurones, promote and guide the elongation of their axons and permit the formation of new synapses, many questions need to be explored before it can be concluded that axonal regeneration in adult mammals can lead to the restoration of neuronal circuitry. In particular, two questions need to be pursued further.

(1) *Will the survival of axotomized neurones, initially enhanced by the PN grafts, persist?* In this regard, there is already experimental evidence to suggest that the viability of regenerating neurones that are prevented from forming terminal connections may not be sustained indefinitely by the contact of their axons with the non-neuronal environment of peripheral nerve. Even in the PN-grafted retinas examined 9 and 12 months after axotomy, the course of many of the surviving RGC axons had become distorted and irregular (M. Vidal-Sanz, M. P. Villegas-Pérez & A. J. Aguayo, unpublished observations) and there was a progressive loss of their cells of origin (M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo, in preparation). Furthermore, axons that had regenerated into PN grafts from the retina (Keirstead *et al.* 1985) or the brainstem of adult rats (P. Gauthier & M. Rasminsky, in preparation) have shown decreasing responsiveness to physiological stimuli after periods of several months. Finally, when neurones from the foetal neopallium are transplanted into peripheral nerves of adult rats and isolated from their connections with the rest of the CNS, they undergo protracted cytoskeletal changes that resemble those observed in the brains of ageing animals (Doering & Aguayo, 1987). Thus, it remains to be determined if such late morphological and functional effects on axotomized/PN-grafted neurones can be prevented by the establishment of synaptic contacts with the target tissues to which they are guided.

(2) *Will regrowing axons replicate the synaptic arrangements that are essential to sensory and other systems that depend for their functioning on orderly placed synapses?* Although our working hypothesis has been that the elongation and guidance of axons to their targets, accomplished by the use of the PN grafts, could permit recognition phenomena that determine selective synaptogenesis, there is yet no evidence that such essential processes can be replicated in adult mammals. Because retinal projections are retinotopically arranged, it may now be possible to explore this question further in animals with PN bridges joining the eye and the superior colliculus.

It has also become increasingly apparent that under usual circumstances the CNS environment that surrounds injured axons offers little support for the survival of axotomized neurones. In the experiments of Villegas-Pérez *et al.* (1986) and

M. P. Villegas-Pérez, M. Vidal-Sanz, G. M. Bray & A. J. Aguayo (in preparation), nearly 90 % of all RGCs died soon after cutting of the optic nerve; a similar loss has been reported in adult rats among the cholinergic neurones of the nucleus basalis following the sectioning of the fimbria-fornix (Hefti, 1986; L. R. Williams *et al.* 1986; Kromer, 1987). However, although conditions in the injured CNS did not substantially protect either the RGCs or the nucleus basalis neurones from undergoing retrograde degeneration, both these populations of cells have proved capable of overcoming some of the effects of injury if they are provided with critical trophic molecules or tissues that presumably contain such molecules. One of the circumstances that have limited further studies of the connectivity and function of regenerated central axons is the fact that only a few neurones extend along the PN bridges and re-enter the CNS (David & Aguayo, 1981; Vidal-Sanz *et al.* 1987). The present studies provide a clear indication for the need to develop strategies aimed specifically at increasing the viability of injured neurones as a potential source of regenerated axons that may reinnervate their targets.

The experimental use of transplanted non-neuronal tissues as a source of epigenetic influences that can modify neuronal responses to injury in the adult mammalian nervous system has helped to document a remarkable responsiveness of mature nerve cells to changes in their environment. Because the establishment of appropriate neural circuits during development is contingent upon sequential neuronal events that are precisely timed and occur in concert with complex changes in the various cells and matrices of the non-neuronal milieu, the harnessing of these neuronal regenerative potentials for purposes of neural repair will depend on our ability to understand the mechanisms whereby genomic expression can be influenced by these variable epigenetic conditions. In the final analysis, it may turn out that the appropriate and orderly manipulation of extrinsic conditions in the environment of these remarkably plastic nerve cells will represent the most difficult challenge.

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