J. exp. Biol. 153, 199–224 (1990)
Printed in Great Britain © The Company of Biologists Limited 1990

SYNAPTIC CONNECTIONS MADE BY AXONS REGENERATING IN THE CENTRAL NERVOUS SYSTEM OF ADULT MAMMALS

BY ALBERT J. AGUAYO, GARTH M. BRAY, MICHAEL RASMINSKY, THOMAS ZWIMPFER, DAVID CARTER AND MANUEL VIDAL-SANZ

Center for Research in Neuroscience, The Montreal General Hospital and McGill University, 1650 Cedar Avenue, Montreal, H3G 1A4, Quebec, Canada

Summary

The restoration of connections in the injured central nervous system (CNS) of adult mammals is hindered by the failure of axons to grow back to their natural fields of innervation. Following transection of the optic nerve of adult rodents, the guided regeneration of retinal ganglion cell (RGC) axons along a transplanted segment of peripheral nerve (PN) has shown that these neurones retain their capacities to form well-differentiated synapses in both normal and abnormal targets. The main aim of this review is to describe the anatomical and functional characteristics of some of these connections and to suggest that their terminal distribution and morphology may be the result of a persistence in these targets of molecular determinants that influence normal connectivity in the intact animal.

Introduction

It is generally assumed that the successful regeneration of damaged neural connections requires the recapitulation of several critical steps in development, namely the guided growth of axons towards correct targets and the formation of functionally appropriate synapses. The replication of these events in the injured adult CNS must be carried out, however, under conditions that are markedly different from those present in embryogenesis and soon after the birth of the animal. This review of a selected group of recent experiments in adult mammals attempts to highlight some of the similarities and differences observed between events that can lead to the restoration of synaptic connections in the injured nervous system and those that take place during normal development.

Development of neuronal connections

In the developing organism, the growth and branching of axons and dendrites gives rise to the intricate networks that comprise the adult CNS. The circumstances that lead the extending processes of neurones to their targets are

Key words: synaptogenesis, axotomy, visual system, retina, regeneration, nerve grafts.

unknown, but complex axon–substratum and cell–cell interactions are thought to play key roles in the formation of neuronal assemblies that subserve specific functions (Easter *et al.* 1985; Edelman, 1987; Rutishauser and Jessel, 1988; Lumsden and Keynes, 1989). Groups of differentiating neurones acquire different positions along the developing neuraxis and subserve different roles. While most cells become interneurones that only synapse with their neighbours, many others must extend their axons towards more distant targets. Matrix substrata, cell surface components and diffusible factors acting on receptive growth cones may stimulate or inhibit the elongation of these axons and influence: (a) their overall direction and course; (b) the confinement of terminal arborizations to specific clusters, laminae or columns of cells; and (c) the establishment of synaptic contacts on appropriate dendrites, axons or somata.

Before and after birth, neural networks are modified further by the natural death of nerve cells, the disappearance of redundant fibres, changes in dendritic trees (Purves *et al.* 1986), competitive interactions between different afferents and the shifting and strengthening of terminal connections caused by functional activity (Hubel and Wiesel, 1970; Shatz and Sretavan, 1986; Fraser and Perkel, 1990). Thus, short-range growth and rearrangements in connectivity, generated by changes in arborizations and synapses, may continue to be made long after birth.

In the embryo, some of these events may be facilitated by the short distance that usually separates most neurones from their targets and the time differences in the genesis, migration and neurite outgrowth normally exhibited by the various classes of nerve cells that populate the nervous system. In addition, it is obvious that many CNS neurones persistently support a gradual elongation of their axons in harmony with the growth of the entire organism, an extension that surpasses many times that occurring earlier in development, when most of the neural circuitry is first established. While the capacity of many neurones to extend axons and reorganize dendritic and axonal arborizations appears to be retained into adulthood, there is, nevertheless, no evidence that these capacities lead to major changes in the direction or position of any established long axonal projection in the adult mammalian CNS.

In the mature animal, constraints on fluctuations in connectivity to the narrow range covered by dendrites and axon terminals could reflect an evolutionary need to maintain the main configuration of developmentally established circuits once neurogenesis is completed and no new fibre tracts or long association fibres are generated. Recent studies of two growth-inhibiting proteins expressed postnatally by differentiated oligodendrocytes (Schwab, 1990) suggest one possible mechanism that may normally curtail fibre tract changes in the white matter while permitting the local reorganization of connections in the grey matter. Plasticity in the grey matter, rich in terminal arborizations, neuronal somata and dendrites, could be influenced locally by repulsive and affinity-determined molecular interactions (Bonhoeffer and Gierer, 1984; Müller *et al.* 1990) that eventually lead to the formation of stable synapses upon appropriate classes of cells. Because terminal synaptogenesis may limit fibre extension (Bernstein and Bernstein,

1971), neurone–neurone interactions could also help confine the connections made by axonal arborizations to groups of cells near the sites reached by projecting fibres.

In the hypothetical scheme described, an early stage of directed axonogenesis, largely dependent on interactions with non-neuronal substrata, makes it possible for the axons of certain neurones to reach the more distant regions of the neuraxis that harbour their specific targets. In mammals, the completion of this stage of development appears to be associated with changes in the glial environment that halt new fibre elongation into the white matter but allow established projections to lengthen during the gradual postnatal growth of the organism. In the neuropile of cortical and non-cortical regions of the grey matter, the selection of postsynaptic neurones and their cellular domains (somata or dendrites) by projecting axons and by interneurones would be regulated locally by surface molecules that induce and secure synapses but continue to allow narrow fluctuations in local connectivity. The expression of these molecules would be modulated by activity and by competitive interactions (Easter *et al.* 1985; Shatz and Sretavan, 1986; Edelman, 1987; Debski *et al.* 1990). Thus, during postnatal maturation the neuronal capacities to sustain axonal growth and to form connections would be moderated by radial interactions with the non-neuronal environment and by influences from the target, mediated by terminals and synapses.

Disruption of neuronal circuitries by injury

Serious injuries to the brain or spinal cord are commonly followed by persistent histological alterations and deficits of function. In mammals and other amniotes, the anatomical and functional abnormalities generated by such injuries change with time, but normal function is seldom restored. Some of the effects of CNS injuries entail: (a) the immediate death of neuronal and non-neuronal cells at the site of damage; (b) the interruption of axons and dendrites; (c) the rapid or protracted loss of axotomized neurones whose somata are some distance from the lesion (Villegas-Pérez *et al.* 1988, 1989; Sofroniew *et al.* 1990; and (d) changes in molecular components of damaged or denervated non-neuronal tissues where demyelination and reactive gliosis are well-recognized histological changes (Gasser *et al.* 1986; Finklestein *et al.* 1988; Perry and Gordon, 1988; Reier *et al.* 1989; Stoll *et al.* 1989; Kreutzberg *et al.* 1990).

Because lost neurones other than those in the olfactory sensory epithelium are not spontaneously replaced in mammals, recovery from injury in the peripheral or central nervous system is largely dependent on: (a) the survival of a critical number of damaged cells; (b) the successful regrowth of interrupted axons; (c) the guidance of these axons to their normal fields of innervation; (d) the branching and distribution of axon terminals within appropriate sectors; and (e) the establishment of functional contacts capable of influencing postsynaptic cells in a predictable and beneficial fashion.

In the mammalian CNS, the failure of cut axons to regrow is a major obstacle to

the restoration of neural connectivity. This lack of axonal re-extension in the brain and spinal cord contrasts with the successful regeneration of cut axons into peripheral nerve stumps, rich in Schwann cells, extracellular matrix components and other non-neuronal elements that express trophic factors (Richardson and Ebendal, 1982; Heumann *et al.* 1987*a*,*b*), and critical surface molecules (Carbonetto *et al.* 1987; Reichardt *et al.* 1989). The impressive regrowth that follows peripheral nerve transection, however, often leads to no significant recovery of useful function (Mackel *et al.* 1983), presumably because many regrowing fibres reach inappropriate targets after extending along Bungner bands previously occupied by other axons (Horch and Burgess, 1980). Thus, while axonal extension is an unavoidable step in the repair process, it does not ensure the restoration of connections with functionally and topographically appropriate targets. As in development, pathway guidance to the discrete fields of innervation that contain the normal postsynaptic targets of projection neurones may be critical to the more subtle local synaptic discriminations made subsequently by axons within such territories.

From *in vivo* studies in adult rodents, it has been proposed that axotomized CNS neurones retain their capacity to initiate and sustain axonal regrowth but that conditions in the mature CNS inhibit or fail to promote fibre elongation after injury (David and Aguayo, 1981). The presence of various inhibitory glial components (Pesheva *et al.* 1989; Schwab, 1990; Snow *et al.* 1990), a deficit of growth-promoting molecules (Carbonetto *et al.* 1987; Sandrock and Matthew, 1987) and even the formation of premature synapses with neurones near the site of damage (Bernstein and Bernstein, 1971) may all contribute to limit axonal regrowth in the injured CNS.

Certain experimental modifications (Schnell and Schwab, 1990; Schwab, 1990) and substitutions (see below) of the growth-hindering glial environment of the CNS provide a novel opportunity to determine if neuronal connections can be restored by the regrowth of severed axonal projections to selected regions of the brain or spinal cord of adult mammals.

An experimental *in vivo* strategy to determine if synaptogenesis can follow axonal regeneration in the central nervous system

The search for restored CNS connectivity as a result of axonal regeneration has been made possible by the demonstration that many different classes of axotomized CNS neurones are capable of regrowing lengthy axons when the glial substratum present in the adult CNS is replaced by a transplant of non-neuronal components of PN (see Aguayo, 1985). The experimental strategy used in these *in vivo* studies involves by-passing portions of the CNS *via* a grafted, axonless PN segment capable of facilitating and guiding the regrowth of the cut central axons to remote CNS targets. Using these techniques, regenerating axons can be led to the vicinity of their normal fields of innervation or to regions that are not normally subserved by these projections.

The presence of long regenerated central axons within accessible PN 'bridges' that extend extracranially (Vidal-Sanz *et al.* 1987; Carter *et al.* 1989*a*) or extraspinally (David and Aguayo, 1981) for several centimetres before re-entering the CNS facilitates the study of axonal growth and connectivity by anatomical and electrophysiological techniques, minimizing undue spread of tracers or ambiguities concerning effective sites of electrical stimulation. For such technical reasons and because axotomy near the neuronal somata is a pre-requisite for regeneration into the PN grafts (see below), these experiments have concentrated on the responses of long fibre projections axotomized far away from their fields of innervation. It is hoped, however, that evidence obtained from these studies may also stimulate ideas on the possible capacities for growth and reconnection that may reside in the larger population of interneurones that makes up the CNS.

This review focuses on studies of regeneration from one axonal projection, the retinofugal system of adult rodents, where the regrowth of severed retinal ganglion cell (RGC) axons along a PN graft linking the eye and the superior colliculus (SC) has resulted in the formation of well-differentiated RGC synapses in the SC (Vidal Sanz *et al.* 1987; Carter *et al.* 1989*a*). In parallel investigations, the synapses of these regenerated RGC axons were also shown to be capable of mediating trans-synaptic electrophysiological responses from neurones in the SC after the retina was stimulated by light (Keirstead *et al.* 1989). Finally, PN grafts were used to investigate terminal differentiation and synaptogenesis in experiments where regenerating central axons were guided to abnormal targets in the CNS (Zwimpfer *et al.* 1989, 1990). Descriptions and comments pertinent to these studies are given in the paragraphs that follow.

Regenerating retinal ganglion cell axons form synapses in the superior colliculus

In adult hamsters and rats, an autologous PN graft measuring 2–4 cm in length was attached to the ocular stump of optic nerves transected within the orbit (Vidal-Sanz *et al.* 1987; Bray *et al.* 1988; Carter *et al.* 1989*a*). Approximately 6 weeks later, the distal end of the graft was inserted into the ipsilateral SC (Fig. 1) and the contralateral optic nerve (ON) was cut intraorbitally to eliminate all normal retinotectal connections.

The PN grafts were attached to optic nerves cut intraorbitally because axotomy near the neuronal perikaryon appears to be a pre-requisite for the growth of RGCs and other CNS axons into peripheral nerve grafts (see Aguayo, 1985). This puzzling association between axonal regeneration into these grafts and the distance that separates the axotomy site from the cell somata may also influence other cellular responses to axotomy, including the enhanced expression of the growth-associated protein GAP-43 (Lozano *et al.* 1987; Lozano, 1988). Indeed, following ON transection near, but not far from, the eye in adult rats, there is an augmented GAP-43 immunoreactivity in RGC somata and axons and an increase in the axonal transport of the GAP-43 protein (Lozano, 1988). The reason why axonal regrowth into PN grafts and enhanced GAP-43 expression both occur

under these circumstances remains unclear; a possible explanation is that GAP-43 is somehow involved in a chain of injury-induced cellular events that modulates the receptiveness of growth cones to molecules in the non-neuronal environment. The enhanced expression of these and other molecules in nerve cells injured near the cell body may play a role in the establishment of the neurone–substratum interactions required to sustain axonal elongation when conditions in the axonal environment are propitious. Possible repressor molecules within the long stump that remains when the ON is severed far from the retina could also inhibit GAP-43 expression in distally axotomized RGCs.

The proximal location of an axonal injury, however, also causes a massive retrograde degeneration of retinal ganglion cells. In rats, approximately 85% of the RGCs are lost 15 days after cutting the ON immediately behind the eye (Villegas-Pérez *et al.* 1988). From these decimated retinas, nearly 20% of the surviving RGCs regenerate their axons along the entire 3–4 cm length of the PN grafts (Villegas-Pérez *et al.* 1988). Conversely, after intracranial lesions of the ON that are 10 mm from the eye, approximately two-thirds of the RGCs survive for 15 days after axotomy (Villegas-Pérez *et al.* 1989). This larger population of retinal

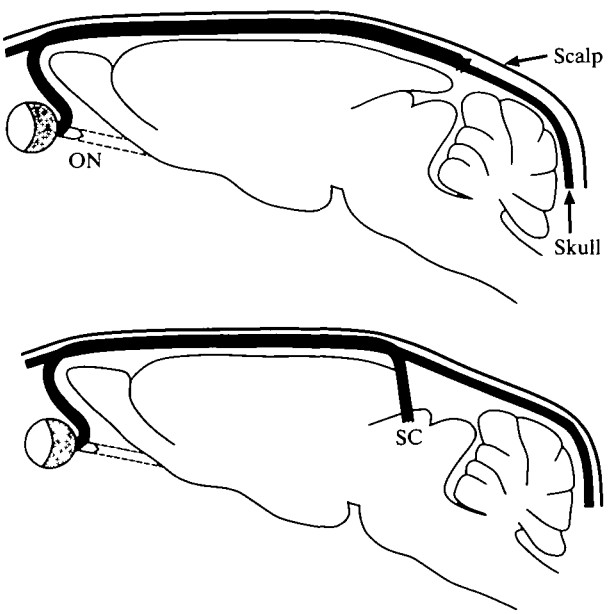

Fig. 1. Method used to implant peripheral nerve (PN) segments (cross-hatched) as bridges between the retina and the superior colliculus (SC) in adult rats or hamsters. (A) One end of an autologous PN segment was attached to the orbital stump of the optic nerve (ON) transected immediately behind the eye. The other end of the PN graft was left under the scalp over the skull. (B) 8–10 weeks later, the unconnected tip of the PN graft was implanted into the SC. After survival times varying from 2 to 18 months, orthogradely transported tracers were injected into the vitreous chamber of the eye to identify regenerated retinal ganglion cell axons and their terminals. (Reproduced with permission from Vidal-Sanz *et al.* 1987.)

neurones, however, gives rise to no significant regrowth of axons into PN grafts placed intracranially, away from the RGC somata (Richardson *et al.* 1982). Thus, under these experimental conditions, the neuronal propensity to regenerate cut axons bears a curious relationship to lesions that cause more serious damage to these cells. It is assumed that the retrograde degeneration of these neurones substantially diminishes the pool of viable cells that can give rise to axonal regeneration.

In the animals with PN grafts joining the eye and the dorsal mesencephalon, fluorescent tracers, horseradish peroxidase (HRP) or tritiated amino acids were injected intravitreally to label the regrowing axons, as well as their arborizations and terminals. Retinal axons, isolated from each other by their Schwann cell and basal lamina ensheathment, were shown to have extended without branching for distances that were nearly twice as long as those of the normal retinotectal projections found in the intact adult animal (Vidal-Sanz *et al.* 1987; Carter *et al.* 1989*a*).

In the hamsters, light and electron microscope studies of 758 labelled terminals from RGC axons that extended into the SC between 6 and 8 weeks after insertion of the PN graft into the SC were compared with 698 retinotectal terminals from intact hamsters. It was documented that: (i) the regenerating RGC axons penetrated the SC for distances of up to 500 μm and arborized in the superficial layers of the SC that normally receive most retinal projections; (ii) the ultrastructural features of the newly formed RGC terminals, including their content of round vesicles and pale mitochondria, resembled normal presynaptic structures; and (iii) the characteristics of their postsynaptic contacts, virtually all of which were axo-dendritic and asymmetrical (Gray type I), were also strikingly similar to those of normal retinocollicular connections (Carter *et al.* 1989*a*).

These findings in hamsters, together with similar investigations in the rat (Fig. 2) (Vidal-Sanz *et al.* 1987; Bray *et al.* 1988), demonstrate that regenerating axons do not necessarily stop growing at the interface of the PN grafts and the CNS, but can penetrate the damaged CNS tissue and form arborizations and synapses within the nearby grey matter. This extension of the regenerating RGC axons into the SC was slight in comparison to the lengths they had covered along the PN grafts. However, the penetration of the RGC axons into the SC for up to 500 μm from the tip of the graft approximated the lengths of the arborizations made by the longest retinal afferents within the normal SC of these rodents (Sachs and Schneider, 1984). Because the SC is a small, dome-shaped structure whose retino-recipient neurones are normally near the pial surface, this short extension into the grey matter should have permitted the RGC axons to reach cells in most regions of the SC that normally receive retinal inputs. Thus, the small numbers of RGC axons emerging from the graft, rather than the limited extension of the incoming fibres, may account for the poor re-innervation observed in the SC of these animals.

It is of interest that the RGC axons that regenerated into the SC arborized preferentially within the superficial layers of the superior colliculus. Since the PN

grafts ended in or near the stratum opticum (SO), incoming axons could have grown dorsally into the stratum griseum superficiale (SGS) or ventrally into the stratum griseum intermediale. However, the vast majority of the identified axon terminals observed were in the SO and SGS, the main retino-recipient layers of the normal SC. In normal rodents, most retinal inputs to the SC come from the contralateral eye and terminate mainly in the SGS and the SO, while the smaller ipsilateral projection ends principally in the SO (Chalupa and Rhoades, 1979; Frost *et al.* 1979; Jen *et al.* 1984). When, in these experiments, the regrowing RGC axons were all directed *via* a PN graft to the ipsilateral SC, the synapses made by these axons were not concentrated in the SO but extended into the SGS, which is normally a target of the contralateral retinal inputs. Such a broadened deployment of ipsilateral RGC axons can also be induced experimentally in immature hamsters (Schneider, 1973; Mooney *et al.* 1985). Similarly, following ON section, the goldfish retinotectal system, which normally projects contralaterally, can regrow and innervate the main retino-recipient layers of the ipsilateral tectum if this tectum is deprived of its normal retinal input (Meyer, 1978).

In the SC of the animals with retinocollicular PN grafts, and in those of the controls, virtually all RGC terminals that showed synapses contacted dendritic spines or shafts rather than cell bodies. While this apparent preference may only

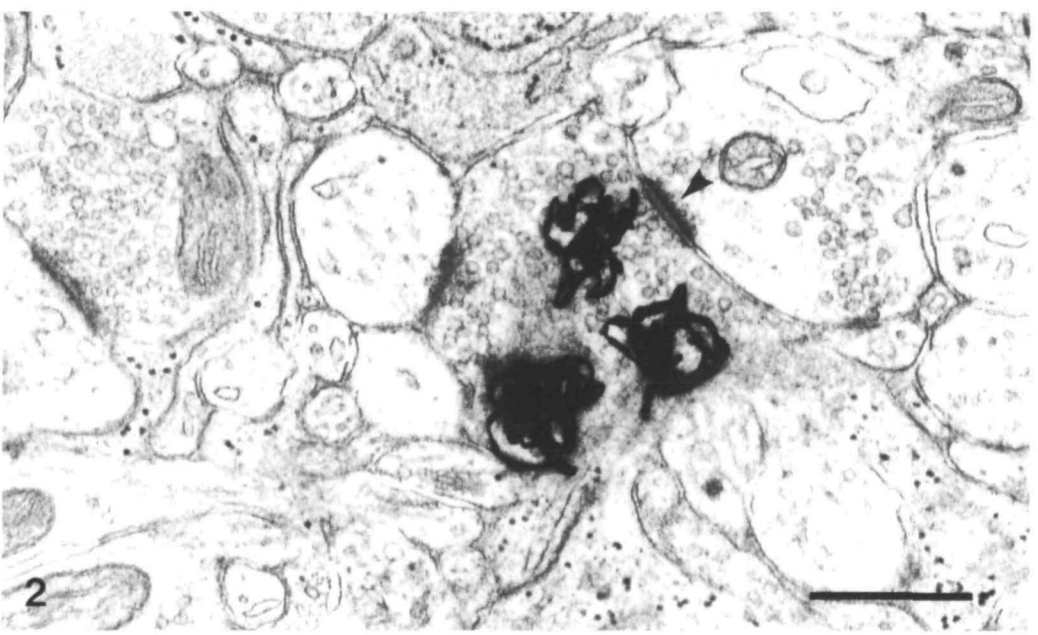

Fig. 2. Electron micrograph of a regenerated retinal ganglion cell terminal in the stratum griseum superficiale of the superior colliculus (SC) of a rat 16 months after the retina and the SC were connected by a peripheral nerve graft. In this autoradiograph, a terminal, which contains spheroidal vesicles and forms an asymmetrical contact (arrowhead) with a dendritic shaft, is labelled with silver grains after the injection of [^{3}H]leucine/proline into the eye. Scale bar, 0.5 μm.

reflect the high density of dendrites in the grey matter of the SC, the observation that approximately 60% of the synapses made by normal and regenerating RGC–SC terminals are on spines rather than on the shafts of dendrites (Carter *et al.* 1989*a*) argues against a purely random selection of synaptic targets (Fig. 3). A predilection for precise positions in the dendritic tree is also observed after neural transplantation (Sotelo and Alvarado-Mallart, 1987; Sotelo, 1989) and when sprouting is induced by a partial deafferentation of inputs (Lewis and Cotman, 1982). This affinity of the axonal terminals for certain neuronal domains suggests a discrete, patch-like expression of molecules in postsynaptic neurones that selectively stabilizes presynaptic contacts. Such determinants of the regional distribution of synaptic contacts may not only be expressed during development and regeneration but may also play a role in the maintenance of connectivity during normal synaptic turnover and the rearrangement of synaptic connections that follows sprouting (Raisman and Field, 1973; Cotman and Nieto-Sampedro, 1984). As occurs during development and after sprouting, the availability of such sites on the surface of the targeted neurone may be a determinant of the arrangement of the contacts made by regenerating axons and target cells. It may be useful to consider the possibility that mechanisms akin to those involved in specifying the location of synapses at the neuromuscular junction also play a role in determining the selective distribution of synapses between neurones (Poo and Young, 1990; Magill-Solc and McMahan, 1990). Because regenerated RGC synaptic contacts have been found in the SC for periods of up to 18 months in rats and 9 months in hamsters, it seems likely that these retinotectal connections are permanently restored (Bray *et al.* 1988; Carter *et al.* 1989*b*).

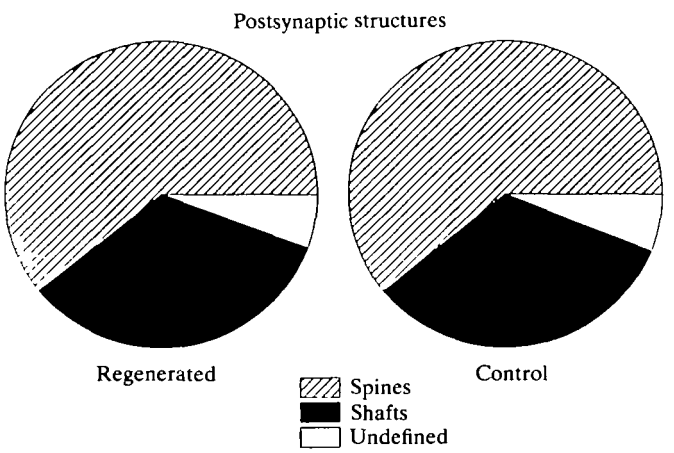

Fig. 3. Postsynaptic domains of neurones in the superior colliculus contacted by 901 regenerated and 471 control retinal ganglion cell terminals. In addition to the illustrated distributions on dendritic shafts and spines, which were similar for the regenerated and control axon terminals, one of the regenerated terminals formed a synapse on the cell body of a neurone.

Anatomical characteristics of the synapses made by regenerated retinal ganglion cell axons that regrow into the superior colliculus

Morphometric ultrastructural studies of the synaptic specializations generated in the SC by the RGC terminals and estimates of the ratio of synaptic contacts to the perimeter of each terminal indicated that both were similar to those of normal retinotectal connections. The preservation of the ratio between the number of RGC synaptic contacts made within the neuropile of the SC and the perimeter of their terminals may be an indication that the overall size of such terminals is influenced by the number of contacts. It is less likely, however, that such ratios might denote that the amount of membrane formed by a terminal dictates the number of synaptic contacts that is generated.

Some of the regenerated terminals were larger and had longer synaptic junctions than controls. Although the size of axon terminals appears to be smaller during development than in the mature CNS (Kalil *et al.* 1986), large terminals have been reported in other adult vertebrates where synaptogenesis is known to occur as a result of regeneration or sprouting. In the goldfish optic tectum, for example, regenerating retinotectal terminals are initially large and form longer synaptic contacts (Radel and Yoon, 1985). Furthermore, while the regeneration of axon terminals and synapses has not been studied previously in the adult mammalian CNS, larger terminals and longer, more frequent, synaptic contacts per terminal have been documented in studies of the collateral sprouting of intact neurones in partially denervated areas of the CNS (Raisman, 1969; Matthews *et al.* 1976; Chen and Hillman, 1987; Murray *et al.* 1987; Steward *et al.* 1988) and in experiments where afferent fibre populations are decreased (Sotelo, 1975; Hillman and Chen, 1985).

In the goldfish, in which a full complement of RGC axons may eventually re-innervate the optic tectum following ON crush (Murray *et al.* 1982), the size of the regenerated retinotectal terminals is reported to return to normal approximately 1 year after denervation (Murray and Edwards, 1982; Radel and Yoon, 1985; Hayes and Meyer, 1988). An overall tendency towards smaller terminals has also been observed in the SC approximately 9 months after the insertion of the caudal end of the PN grafts into the SC of hamsters (D. Carter, G. M. Bray and A. J. Aguayo, unpublished observations). Morphometric studies of RGC terminals in rats, 16–18 months after PN grafting, have shown no difference between the size of the regenerated terminals and age-matched controls (M. Vidal-Sanz, A. J. Aguayo and G. M. Bray, unpublished observations). These overall findings suggest that, although the developmental maturation of neuronal connections involves an enlargement of the terminals, regenerated terminals may follow a different pattern typified by a gradual reduction in their size.

While there are obvious differences between the normal and the regenerated retinotectal projections we have studied, particularly with regard to the number of RGC axons that innervate the SC, the evidence described here suggests that the patterns of terminal arborization as well as the morphology and preferential distribution of synapses to superficial layers of the SC made by individual

regenerated RGC axons resemble those of retinofugal axons in the SC of rodents during: (a) the development of the visual system (Sachs *et al.* 1986); (b) the re-innervation that follows optic tract lesions in the immediate postnatal period (So *et al.* 1981); and (c) the transplantation of foetal retinas to the midbrain of immature hosts (Hankin and Lund, 1987).

Competitive interactions between different RGC axons, believed to play an important role in the formation of neural connections (Easter *et al.* 1985), are unlikely to have influenced the distribution of arborizations and synapses in these animals because so few RGC axons reached the SC. It is not known, however, if the preferences of RGC axons for certain SC laminae and neuronal domains are dictated by the extensive tectal denervation caused by the severing of the optic nerves and the unavoidable damage to the occipital lobes and the SC that is inflicted by the insertion of the PN grafts into the dorsal midbrain of these animals. Studies of the synaptic distribution of RGC axons guided to an abnormal target, the cerebellum, suggest the possibility that conditions other than those that are a direct result of denervation may play a role in determining synaptic distribution (see below).

A gradual loss of RGCs follows axotomy in the optic nerve and also the regrowth of RGC axons into blind-ended PN grafts (Villegas-Pérez *et al.* 1988, 1989). This protracted cell loss may result from a persistent disconnection of these neurones with targets that are their main source of trophic support. Because, in rats and hamsters in which the PN grafts were used to bridge the eye and the tectum, no apparent fall in the number of RGC arborizations in the SC has been observed during studies that span most of the life of these animals (D. Carter, G. M. Bray and A. J. Aguayo, unpublished observations), it is likely that the restored RGC connections in the SC may ensure RGC survival.

The studies of axonal regeneration into the SC of adult rodents reviewed here have not encompassed several other important aspects of RGC regeneration. These include determining more precisely the number and size of the arborizations formed, the classes of SC neurones that are re-innervated, and whether there is a retinotopic order to the deployment of the RGC axons that reach the SC. While other vertebrates such as amphibians and fish appear to be capable of restoring lost retinotectal connections with a great deal of accuracy, it remains to be shown that axonal regeneration in adult mammals can replicate the pathfinding patterns and the terminal connectivity that they achieve during normal development. In our experiments, the massive loss of RGCs that follows proximal axotomy in the optic nerve has imposed serious limitations on the search for answers to some of these questions.

Electrophysiological studies of afferent and efferent connectivity of axotomized retinal ganglion cells whose axons regenerate along peripheral nerve grafts

Axonal injury not only disrupts connections with target cells innervated by the damaged axons but may also lead to loss or modification of function of the

axotomized neurones themselves. In addition to the dramatic retrograde cell loss that can follow axotomy, there may be changes in afferent connectivity, patterns of dendritic arborization or membrane properties of damaged neurones (Purves and Lichtman, 1985; Purves, 1975; Mendell *et al.* 1976; Thanos and Aguayo, 1988). For this reason, as a prelude to physiological studies of synaptic connectivity in the retinocollicular grafts, the physiological properties of RGCs were investigated after regeneration of their axons into PN grafts that were not connected to a CNS target (Keirstead *et al.* 1985). This was accomplished by teasing axons from PN grafts inserted into the retina and recording the responses in these axons to visual stimulation of the retina.

The responses to visual stimulation of some of the RGCs with regenerated axons were indistinguishable from those characteristic of RGCs in the intact animal (Fig. 4). These cells responded to light with on, off or on–off responses, and had receptive fields of sizes similar to those of normal RGCs, in some cases accompanied by a suppressive surround organization like that seen in some RGCs in intact animals. This responsiveness of RGCs with regenerated axons indicates

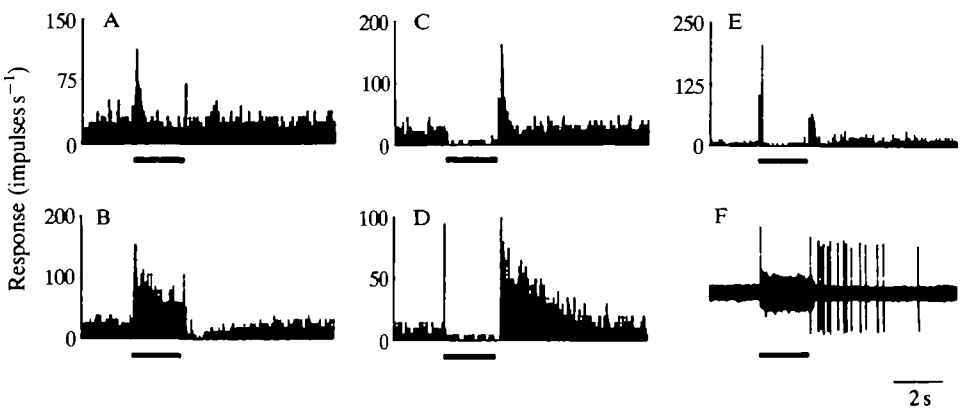

Fig. 4. Responses to light recorded from the regenerated axons of retinal ganglion cells. (A–E) Peristimulus time histograms of unitary responses to visual stimuli presented during the 2 s period indicated by the bars. In some histograms artefacts related to shutter opening and closing are seen in bins at the onset and offset of illumination. (A,B) On responses with transient (A) and sustained (B) responses to illumination. (C,D) Off responses with depression of firing during illumination and transient (C) and more protracted (D) increases in firing at the cessation of illumination. (E) An on–off response with brief increases in firing at both the onset and offset of illumination. (F) Simultaneous recordings from two regenerated retinal ganglion cell axons teased from a single fascicle. During the time indicated by the black bar, a circle of light 2° in diameter was presented on a tangent screen within the rat's visual field. The low-amplitude unit discharged in response to the onset of light and its discharge was sustained throughout the 2 s of illumination (on response); the large-amplitude unit discharged in response to cessation of illumination (off response). The centres of the receptive fields for these two units were identical. (Reproduced with permission from Keirstead *et al.* 1985.)

that at least some of the RGCs that regenerate axons into PN grafts are able to retain, or regain, enough of their normal inputs to ensure function.

The number of visually responsive units appeared to decline with time, fewer units being found in grafts examined 25–28 weeks after insertion in the retina than in those examined 9–11 weeks after graft insertion. Almost no visually responsive units could be found in animals with grafts implanted for 44–48 weeks, despite the fact that the grafts still contained viable axons responsive to electrical stimulation (Keirstead *et al.* 1988). It is unclear whether this reflects simply the continuing attrition of the RGC population following axotomy (Villegas-Pérez *et al.* 1988) or whether some surviving RGCs with regenerated axons lose their ability to respond to light, perhaps because of changes in afferent connections or membrane properties. A similar loss with time of functioning cells with regenerated axons was found in a study of the function of brainstem neurones that had regenerated axons into PN grafts (Gauthier and Rasminsky, 1988). For both the RGCs and the brainstem neurones, the regenerating axons were blind-ended and it is possible that long-term viability and/or function of the regenerating cells was compromised because of the absence of axonal contact with an appropriate target. As noted above, retinocollicular synapses, once established following regeneration of RGC axons through PN grafts, appear to be stable for many months, implying the survival of afferent RGCs. Nonetheless, it will be important to establish explicitly if RGC survival and function are enhanced by axonal contact with appropriate or inappropriate targets.

To assess the function of synapses formed by regenerating RGC axons, hamsters with PN grafts directed from the eye to the ipsilateral SC were studied electrophysiologically 15–18 weeks after graft insertion into the SC (Keirstead *et al.* 1989) (Fig. 5). Within the superficial 450 μm of the SC, in the vicinity of graft insertion, units were found that responded with either excitation or inhibition to flashes of light directed towards the retina. The proof that at least some of these units were postsynaptic to the afferent regenerated RGC axons established that the regenerated synapses observed in the morphological studies were indeed functional and capable of mediating trans-synaptic responses to light. Light-responsive units tended to be found in clusters within a few hundred micrometres of one another, consistent with the limited morphological observations so far made of the patterns of arborization of regenerating RGC axons.

The responses to light of most of the units definitively identified as postsynaptic were much less vigorous than that characteristic of retinal ganglion cells with regenerated axons or of visually responsive neurones in the superficial layers of the SC in the intact animal. However, these responses may not have been representative of all reinnervated SC neurones. The electrophysiological criterion used to identify visually responsive units as postsynaptic would have failed to distinguish between responses in securely innervated SC neurones and responses in the terminals of regenerated RGC axons. This criterion was thus strongly biased towards identification of SC neurones that were insecurely rather than securely contacted by regenerated RGC axons and there may, consequently, have been a

large class of briskly responsive SC neurones that could not be identified as such. It is thus not possible to make generalizations concerning the response properties of reinnervated SC neurones.

Little information is available concerning the extent or pattern of innervation of either intact or reinnervated SC neurones necessary to ensure either normal or attenuated responsiveness to light. Individual synaptic contacts of regenerated RGC axons on SC neurones appear remarkably normal at an ultrastructural level, but comparisons cannot be made between reinnervated and intact neurones regarding the extent of reinnervation of individual SC neurones by their afferents. Although it is known that neurones in the retino-recipient laminae of the rodent SC each receive several thousand synaptic contacts (Albers *et al.* 1990), it is not known how many of these derive from direct retinal input, how many contacts per cell reflect input from individual RGC afferents and how many retinocollicular synapses need to be activated against the background of many other visual and non-visual inputs in order to displace the membrane potential of the cell to

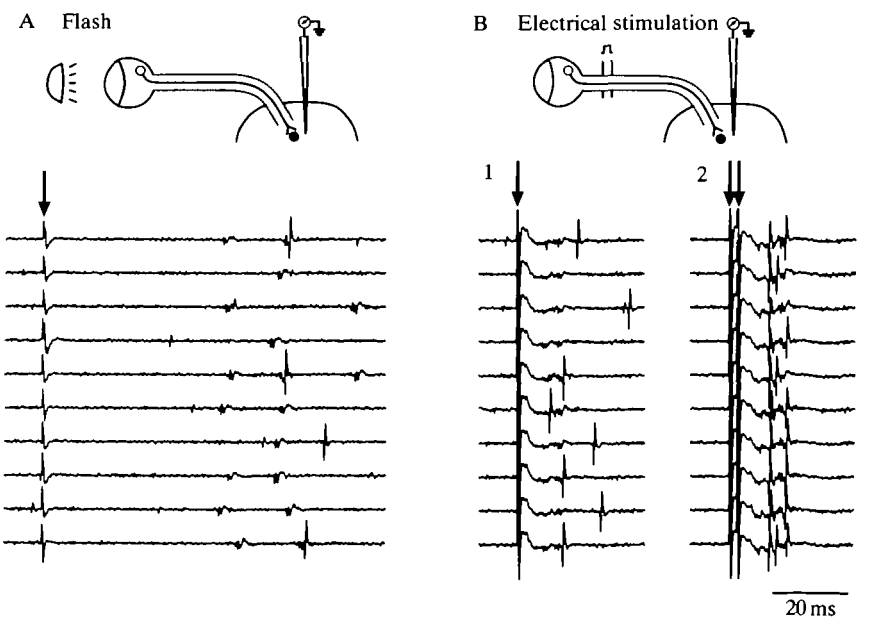

Fig. 5. Recordings from the superior colliculus (SC) of a hamster with a peripheral nerve (PN) graft directed from the eye to the SC. (A) A single unit 250 μm below the surface of the SC responds to light flashes to the eye (arrow) with a single spike on four of ten successive trials. (B) The same unit responds erratically with inconstant latency to (traces 1) single electrical stimuli (arrow) delivered to the PN graft but responds with a more constant latency, often with multiple spikes, to (traces 2) paired electrical stimuli (arrows) of the same intensity. This pattern of response, reflecting postsynaptic summation of subthreshold EPSPs, is inconsistent with that anticipated from an RGC axon and thus identifies this unit as an SC neurone. This criterion of exclusion would fail to distinguish responses in securely excited SC neurones from responses in RGC axon terminals. (Reproduced with permission from Keirstead *et al.* 1989.)

threshold. Other areas giving rise to major inputs to the superficial layer of the SC, such as the visual cortex, the lateral geniculate nucleus and the pretectal nuclei, are themselves deprived of their visual inputs as a result of transection of the optic nerves and are no longer able to exert their influence on SC neurones when a PN graft replaces the optic nerve. Formation and function of synaptic connections between regenerated RGC axons and SC neurones thus occurs in a context substantially altered from that in the intact animal. It will not be surprising if the response properties of the reinnervated neurones prove to differ substantially from those of neurones in the intact animal.

It remains an open question to what extent synaptic activation of SC neurones by regenerated retinocollicular synapses can be translated into behavioural function. Experiments in which foetal retinas are grafted onto neonatal midbrain indicate that the synaptic connections formed between grafted RGCs and midbrain neurones can mediate pupillary responses (Klassen and Lund, 1989) and Pavlovian conditioned responses (Coffey *et al.* 1989) in response to visual stimulation in the absence of any obvious pattern of spatial organization of tectal reinnervation by the transplanted retina (Galli *et al.* 1989). However, such responses are unlikely to demand the precise retinotopy that characterizes the normal pattern of innervation of the tectum by the retina. It remains to be determined if any retinotopic order can be re-established in the SC of adult rodents following regeneration of RGC axons through PN grafts joining the eye and the tectum.

Regenerating retinal ganglion cell axons also make synapses when guided to the cerebellum

The restoration of useful function by the regrowth of severed axons may depend not only on the re-establishment of a critical number of appropriate connections but also on the avoidance or elimination of synapses with abnormal targets (Sperry, 1943; Schneider *et al.* 1985). The guiding of regenerating central axons to anatomically inappropriate regions of the CNS *via* PN grafts provides an opportunity to investigate in adult mammals the freedoms and constraints that govern the establishment of synaptic interactions. For this purpose, the regrowth of retinal axons was directed to the cerebellar vermis (Zwimpfer *et al.* 1989), a CNS target that does not normally receive any direct inputs from the eye either during development (Frost, 1984) or in the mature animal. In these experiments, two important components of the regenerative response, axonal elongation and pathway finding, were deliberately rearranged to force axons into a region of the CNS that is both foreign to and distant from the natural RGC targets. The initial objective was to determine if RGC axons would grow and arborize in the cerebellum. It was anticipated that such uncharacteristic growth would permit new investigations on the conditions that influence presynaptic differentiation and postsynaptic preferences within the territory reached by an anomolous growth of axons.

In these experiments (Zwimpfer *et al.* 1989), one end of an autologous PN segment was grafted to the ocular stump of the ON as described above. The other end of the graft was extended caudally beyond the tectum and inserted superficially into lobules VI and VII on the right side of the cerebellar vermis. To assess the effects of denervation of the cerebellar cortex on the formation of RGC synapses, mossy and climbing fibres were interrupted by transecting the right middle and inferior cerebellar peduncles (group A hamsters); this procedure was omitted in other similarly grafted hamsters (group B). 1–9 months after the insertion of the PN graft into the cerebellum, RGC terminals were labelled by an intravitreal injection of HRP similar to that used in the studies of the retinocollicular connections described above.

Labelled RGC axons regrew along these grafts and penetrated the cerebellum for distances of up to 650 μm. The penetration and overall branching characteristics of the RGC–cerebellar axons were not noticeably different from those observed in the SC when RGC axons reach the tectum *via* a PN graft (Carter *et al.* 1989*a*). A striking feature of the RGC axons that arborized within the cerebellum was that they avoided the white matter and extended preferentially into the granule cell layer (GCL). Although RGC axons occasionally grew to the level of Purkinje cell somata, they generally avoided the molecular layer (ML).

By electron microscopy, it was possible to determine that the RGC arborizations made synapses with cerebellar neurones (Fig. 6). In hamsters from both groups A and B, 172 labelled retinocerebellar terminals were found in the GCL, while only 12 were observed in the ML. Most RGC terminals made synapses on granule cell dendrites; only one synapsed on a granule cell soma. The few synapses in the ML were axodendritic, probably contacting Purkinje cells. The retinocerebellar connections had the ultrastructural characteristics of well-differentiated, asymmetrical (Gray type I) axodendritic synapses. These retinocerebellar connections appeared to be persistent as they were present up to 9 months after insertion of the PN graft. We do not know if the RGC–cerebellar synapses made in the adult animal can mediate function.

A remarkable feature of many of the RGC terminals that synapsed in the cerebellum was their large size. Their average area was at least three times greater than that of either normal or regenerated RGC terminals in the SC and it approximated that of normal mossy fibre terminals (Palay and Chan-Palay, 1974). The mean size of the retinal terminals in the cerebellum did not change significantly during the period of study (1–9 months after the caudal end of the graft was inserted into the cerebellum). While most normal mossy fibres contained dark mitochondria, those within retinocerebellar terminals were pale, resembling the mitochondria of normal and regenerated retinotectal projections (Carter *et al.* 1989*a*).

Fewer retinocerebellar arborizations were found in group B animals with intact cerebellar pedunculi, but the extensive denervation of the cerebellar cortex caused by the severing of climbing and mossy fibre inputs (group A hamsters) did not appear to influence either the predominant distribution of RGC axons and

synapses to the GCL or the mossy-fibre-like appearance and size of the terminals made by the RGC axons in the cerebellum. The preference for the GCL observed in animals in both groups suggests that afferent denervation may not be the main determinant of the terminal distribution of these axons.

Although the formation of these abnormal connections in the cerebellum raises the worrying possibility that axons regenerating in the adult CNS will indiscriminately synapse on any nerve cells they encounter, the apparent predilection of the retinocerebellar terminals for dendrites in the granular layer of animals with and without an extensive denervation of the cerebellar cortex also suggests that the RGC axons may exhibit preferences for certain layers and neuronal domains, a feature that is reminiscent of the regrowth observed previously in the SC (Carter *et al.* 1989*a*).

The existence of certain affinities between RGC axons and cerebellar granule cells is supported by several additional investigations and theoretical arguments. Indeed, studies of the position of the tip of the PN graft in the cerebellum did not favour the possibility that the preferential growth of the RGC axons to the GCL is merely the result of graft location. In addition, while RGC axons avoided the cerebellar white matter, where putative inhibitors of fibre adhesion and growth

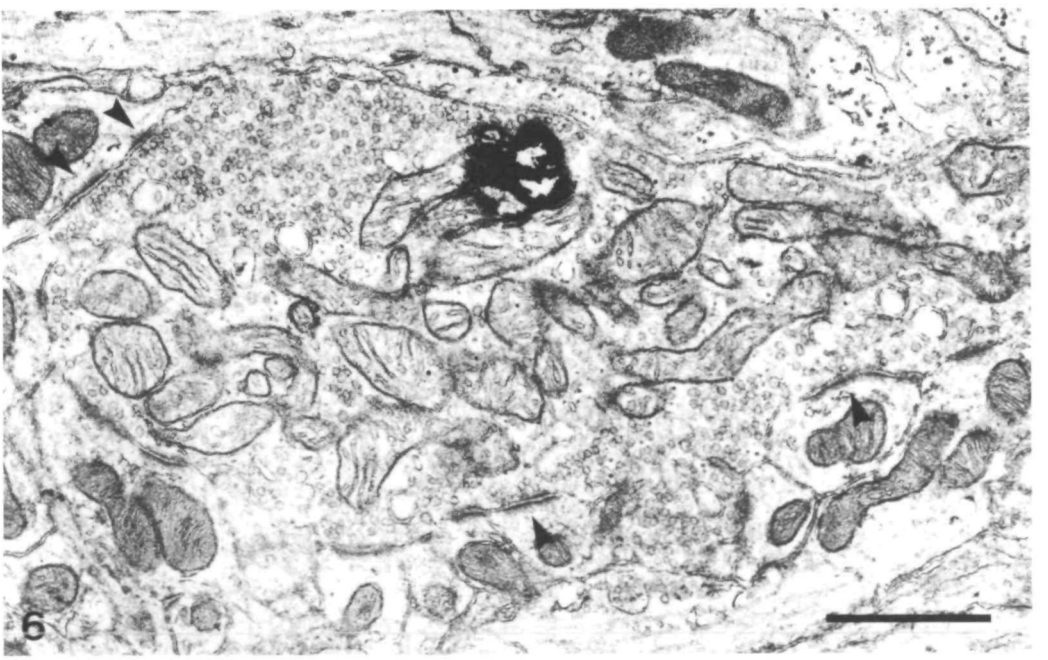

Fig. 6. A horseradish-peroxidase-labelled terminal within the granule cell layer (GCL) formed by a retinal ganglion cell axon that had grown along a peripheral nerve graft connecting the retina and the cerebellum. This terminal contains numerous pale mitochondria and spherical vesicles, and forms asymmetrical synapses (arrowheads) with dendrites in the GCL. Scale bar, 1 μm. (Reproduced with permission from Aguayo *et al.* 1990.)

(Caroni and Schwab, 1988; Schwab, 1990) may be expressed, they did not favour the molecular layer, which is totally lacking in myelin. Sprouting of climbing fibres, known to occur in the ML of the mammalian cerebellum after a partial lesion of the inferior olive (Rossi *et al.* 1989), could, if sprouting were to occur exclusively in this layer, favourably compete for the synaptic sites of Purkinje cell dendrites in the molecular layer and thus divert the incoming RGC fibres. This possibility also seems an unlikely explanation for the preference of RGC terminals for the GCL. The demonstration that climbing fibres can be induced to sprout within the ML of the adult rat (Rossi *et al.* 1989) suggests that the preference of RGC axons for the GCL is not a result of the ML acting as a mechanical barrier to fibre ingrowth.

A more attractive explanation for these synaptic preferences is that the retino-recipient layers of the superior colliculus and the granule cell layer of the cerebellum express common molecules that play a role in their recognition by RGC growth cones. While no evidence is yet available to explain this predilection, candidates for this role could include neurotransmitters, receptors and trophic factors. The postulated role of transmitters in recognition and synaptogenesis in the adult mammalian CNS is not well understood (Lipton and Kater, 1989) but certain excitatory amino acids or related peptides may be transmitters in both cerebellar mossy fibres (Somogyi *et al.* 1986; Olson *et al.* 1987) and in RGCs (Anderson *et al.* 1987). Certain receptors, such as the *N*-methyl-D-aspartate (NMDA) receptor, may also be involved in neuronal recognition, as suggested by studies of the amphibian retinotectal system and other neuronal projections in different animal species (Dębski *et al.* 1990). While NMDA receptors are plentiful in the granule cell layer, where they are expressed primarily on dendrites of granule cells (Olson *et al.* 1987), they are less numerous within the ML (Monaghan and Cotman, 1985) and do not appear to be present on mature Purkinje cells (Olson *et al.* 1987). In contrast, NMDA receptors are abundant in the SC and many other areas of the adult mammalian CNS (Greenamyre *et al.* 1985). Pertinent examples of the role of trophic factors in establishing and sustaining anomalous connections are the effects of nerve growth factor (NGF) application, or its denervation-related local release, on the anomalous invasion of the CNS by peripheral sympathetic fibres (Levi-Montalcini, 1976; see Crutcher, 1987). While the role of NGF in the retina of mammals is unclear, brain-derived neurotrophic factor (BDNF) has been shown both to support the survival and growth of RGCs and to be present in the SC (Johnson *et al.* 1986). Although the presence of BDNF itself has not been reported in the cerebellum of rodents, progress in the sequencing of the BDNF gene (Leibrock *et al.* 1989) has led to the demonstration that, in the cerebellar cortex, mRNAs for BDNF are preferentially distributed in the GCL (Hofer *et al.* 1990).

The peculiar morphology of the RGC–cerebellar terminals is an additional issue of interest. Because they are large and synapse predominantly with dendrites in the granular layer, many of these RGC terminals resemble normal mossy fibre endings in the cerebellar cortex. One possible explanation of their size is that

inappropriate contacts in any abnormal target may generate unusually large terminals, well beyond the increases in size observed among the regenerated RGC fibres in the SC. This possibility is being explored by determining the characteristics of abnormal synaptic contacts made by RGCs in other inappropriate CNS regions of the brain to which axons are guided *via* PN grafts (Zwimpfer *et al.* 1990). An alternative explanation is that pre- and postsynaptic interactions reshape the regenerated terminals to fit the standard morphology of normal regional inputs. The latter hypothesis is consistent with the conclusions drawn by other investigators who have studied the ultrastructural characteristics of the abnormal connections established by axons after the ablation of their normal targets at critical periods of neural development (Kalil and Schneider, 1975; Kalil and Behan, 1987; Campbell and Frost, 1988). In such experimental circumstances, the size and shape of the terminals formed resembled those of the normal terminals found within these targets.

The postulated influence of interactions between pre- and postsynaptic constituents on the geometry of axon terminals is further highlighted by the known disparities that exist between terminals arising from the same neurone but reaching different cells or separate domains of the same neurone. Such a diversification of synaptic morphology was noted by Ramón y Cajal (1909) in the cochlear nucleus and subsequently described for connections in this (Morest, 1968) and various other neuronal systems. Other examples of such normal diversity include the synapses made by normal retinal efferents sharing connections in different brain targets (Lund, 1969; Campbell *et al.* 1984)

General comments

Developmental associated changes in the cellular and molecular components of the CNS are thought to curtail the regrowth of axons in the injured CNS of mammals. In the retina of adult rodents, the replacement of the optic nerve by a peripheral nerve graft enhances, at least temporarily (Villegas-Pérez *et al.* 1988), the survival of axotomized retinal ganglion cells, facilitates and guides the elongation of these central axons to distant targets (Vidal-Sanz *et al.* 1987) and permits the formation of new synapses in the regions of the CNS to which they are guided (Vidal-Sanz *et al.* 1987; Bray *et al.* 1988; Carter *et al.* 1989*a*; Zwimpfer *et al.* 1989).

Experiments described in this review indicate that, under conditions that permit axonal regrowth, injured CNS neurones display a remarkable capacity to initiate and sustain the renewed extension of their cut fibres. The guiding of these regenerated axons to regions of the CNS that are either the natural (the superior colliculus) or unnatural (the cerebellar cortex) targets for retinal projections results in the formation of terminal arborizations and synapses.

In the SC, the distribution of the regenerated RGC arborizations and synapses does not appear to be totally haphazard. Indeed, their terminations are restricted to the normal retino-recipient layers of the SC and synapses are made on the

neuronal domains selected by normal retinotectal projections. Moreover, with time, regenerated synapses attain normal morphological characteristics and can mediate the trans-synaptic activation of neurones in the SC when the retina is stimulated by light.

As described previously for peripheral axons and for immature central projections that are forced to terminate in unusual targets (see Purves and Lichtman, 1985), the RGCs of these adult animals also appear competent to make synapses upon anatomically inappropriate neurones. In the cerebellum, peculiarities in the distribution and differentiation of the RGC terminals suggest that there may also be preferences in the synaptic selections made by the regenerating RGC fibres that penetrate the cortex.

Our overall findings, as well as those of other investigators (see other chapters in this volume), suggest that connectivity in the CNS may be established and maintained by graded affinities, functional activities and molecular interdependencies that are possibly shared by discrete populations of neurones in different regions of the neuraxis. During development, spatially restricted and time-dependent events that involve the expression of specific substratum components, the fasciculation of related axons and the presence of gradients of trophic molecules (Rutishauser and Jessel, 1988) may guide axons to their normal fields of innervation and thus narrow the final selection of their postsynaptic partners to the cells encompassed by terminal arborizations.

The resistance of the mature CNS to axonal extension has forced us to create an experimental artifice to bypass these tissues in order to recreate interactions between the sources and targets of certain central axonal projections. It does now appear that, upon their arrival at such targets, the regenerated axons are subjected to conditions that determine the formation of terminal arborizations and the differentiation of synapses, an indication of the likely persistence in the neuropile of the injured CNS of adult mammals of the molecular determinants of these phenomena.

The demonstration that functional synapses can be made in the injured adult mammalian CNS as the end result of an extensive and circuitous axonal regrowth *via* the PN grafts raises the possibility that connections may also be restored spontaneously in the CNS by the short-range regrowth of axons interrupted near their targets or through the re-establishment of connections between nerve cells, such as interneurones, whose somata and cellular appendages are close to each other. Thus, interneurones, and the terminals of longer axons, may express growth capacities and synaptic predilections within the narrow radius defined by their immediate glial and neuronal environment. While these capacities for axonal growth and synaptogenesis may be retained in the adult CNS, it is difficult to conceive how the time- and substratum-dependent developmental conditions that guide a multitude of axonal projections to their normal fields of innervation, while avoiding inappropriate targets, can be fully replicated in the injured CNS to permit the restoration of immensely complex circuits. The remarkable precision and order with which many injured neurones in the brain and spinal cord of amphibian

and fish re-establish damaged axonal projections provides an encouraging argument for the continuation of studies of regeneration in the central nervous system of mammals. It will be important to determine if suppression of the effects of putative molecules that inhibit axonal growth in the CNS of adult mammals (Schwab, 1990) leads not only to an extensive re-elongation of cut axons but also to a re-expression of the substratum conditions responsible for their guidance.

The technical assistance of M. David, J. Laganière, S. Shinn, J. Trecarten and W. Wilcox is gratefully acknowledged. DC, TZ and MV-S were supported by Medical Research Council fellowships. The Medical Research Council of Canada, the Multiple Sclerosis Society of Canada, the Spinal Cord Research Foundation, the Daniel Heumann Fund for Spinal Cord Research and the M. Wiesberg Vision Regeneration Fund provided financial support. The laboratories in which many of the studies reviewed here were carried out are part of the Canadian Network for the Study of Neural Regeneration and Functional Recovery.

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. AND BRAY, G. M. (1990). Axonal regeneration and synapse formation in the injured CNS of adult mammals. In *Brain Repair* (ed. A. Björklund, A. J. Aguayo and D. Ottoson), pp. 251–272. London: Macmillan Press.

ALBERS, F. J., MEEK, J. AND HOFMANS, T. G. M. (1990). Synaptic morphometry and synapse-to-neuron ratios in the superior colliculus of albino rats. *J. comp. Neurol.* **291**, 220–230.

ANDERSON, K. J., BORJA, M. A., COTMAN, C. W., MOFFETT, J. R., NAMBOODIRI, M. A. A. AND NEALE, J. H. (1987). *N*-Acetylaspartylglutamate identified in the rat retinal ganglion cells and their projections in the brain. *Brain Res.* **411**, 172–177.

BERNSTEIN, J. J. AND 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.

BONHOEFFER, F. AND GIERER, A. (1984). How do retinal axons find their targets on the tectum? *Trends Neurosci.* **7**, 378–381.

BRAY, G. M., VIDAL-SANZ, M. AND AGUAYO, A. J. (1988). Regenerated retino-collicular synapses eighteen months after substitution of the optic nerve by a peripheral nerve graft in adult rats. *Soc. Neurosci. Abstr.* **14**, 654.

CAMPBELL, G. AND FROST, D. O. (1988). Synaptic organization of anomalous retinal projections to the somatosensory and auditory thalamus: Target-controlled morphogenesis of axon terminals and synaptic glomeruli. *J. comp. Neurol.* **272**, 383–408.

CAMPBELL, G., SO, K.-F. AND LIEBERMAN, A. R. (1984). Normal postnatal development of retinogeniculate axons and terminals and identification of inappropriately-located transient synapses: Electron microscope studies of horseradish peroxidase-labelled retinal axons in the hamster. *Neuroscience* **13**, 743–759.

CARBONETTO, S., EVANS, D. AND COCHARD, P. (1987). Nerve fiber growth in culture on tissue substrata from central and peripheral nervous systems. *J. Neurosci.* **7**, 610–620.

CARONI, P. AND SCHWAB, M. E. (1988). Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. *J. Cell Biol.* **106**, 1281–1288.

CARTER, D., BRAY, G. M. AND 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. AND 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.

CHALUPA, L. M. AND RHOADES, R. W. (1979). An autoradiographic study of the retinotectal projection in the golden hamster. *J. comp. Neurol.* **186**, 561–570.

CHEN, S. AND HILLMAN, D. E. (1987). Enlargement of synapses in the striatum following partial deafferentation. *Soc. Neurosci. Abstr.* **13**, 1663.

COFFEY, P. J., LUND, R. D. AND RAWLINS, J. N. P. (1989). Retinal transplant-mediated learning in a conditioned suppression task in rats. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7248–7249.

COTMAN, C. W. AND NIETO-SAMPEDRO, M. (1984). Cell biology of synaptic plasticity. *Science* **225**, 1287–1294.

CRUTCHER, K. A. (1987). Sympathetic sprouting in the central nervous system: a model for studies of axonal growth in the mature mammalian brain. *Brain Res. Rev.* **12**, 203–233.

DAVID, S. AND AGUAYO, A. J. (1981). Axonal elongation into PNS 'bridges' after CNS injury in adult rats. *Science* **214**, 931–933.

DEBSKI, E. A., CLINE, H. T. AND CONSTANTINE-PATON, M. (1990). Activity-dependent tuning and the NMDA receptor. *J. Neurobiol.* **21**, 18–32.

EASTER, S. S., PURVES, D., RAKIC, P. AND SPITZER, N. C. (1985). The changing view of neural specificity. *Science* **230**, 507–511.

EDELMAN, G. M. (1987). *Neural Darwinism. The Theory of Neuronal Group Selection*. New York: Basic Books Inc.

FINKLESTEIN, S. P., APOSTOLIDES, P. J., CADAY, C. G., PROSSER, J., PHILIPS, M. F. AND KLAGSBRUN, M. (1988). Increased basic fibroblast growth factor (bFGF) immunoreactivity at the site of local brain wounds. *Brain Res.* **460**, 253–259.

FRASER, S. E. AND PERKEL, D. H. (1990). Competitive and positional cues in the patterning of nerve connections. *J. Neurobiol.* **21**, 51–72.

FROST, D. O. (1984). Axonal growth and target selection during development: retinal projections to the ventrobasal complex in neonatal Syrian hamsters. *J. comp. Neurol.* **230**, 576–592.

FROST, D. O., SO, K. F. AND SCHNEIDER, G. E. (1979). Postnatal development of retinal projections in Syrian hamsters: A study using autoradiographic and anterograde degeneration techniques. *Neuroscience* **4**, 1649–1677.

GALLI, L., RAO, K. AND LUND, R. D. (1989). Transplanted rat retinae do not project in a topographic fashion on the host tectum. *Expl Brain Res.* **74**, 427–430.

GASSER, U. E., WESKAMP, G., OTTEN, U. AND DRAVID, A. R. (1986). Time course of the elevation of nerve growth factor (NGF) content in the hippocampus and septum following lesions of the septohippocampal pathway in rats. *Brain Res.* **376**, 351–356.

GAUTHIER, P. AND RASMINSKY, M. (1988). Activity of medullary respiratory neurons regenerating axons into peripheral nerve grafts in the adult rat. *Brain Res.* **438**, 225–236.

GREENAMYRE, J. T., OLSON, J. M. M., PENNEY, J. B. AND YOUNG, A. B. (1985). Autoradiographic characterization of *N*-methyl-D-aspartate-, quisqualate- and kainate-sensitive glutamate binding sites. *J. Pharmac. exp. Ther.* **233**, 254–263.

HANKIN, M. H. AND LUND, R. D. (1987). Role of the target in directing the outgrowth of retinal axons: Transplants reveal surface-related and surface-independent cues. *J. comp. Neurol.* **263**, 455–466.

HAYES, W. P. AND MEYER, R. L. (1988). Normal and regenerating optic fibers in goldfish tectum: HRP–EM evidence for rapid synaptogenesis and optic fiber–fiber affinity. *J. comp. Neurol.* **274**, 516–538.

HEUMANN, R., KORSCHING, S., BANDTLOW, C. AND THOENEN, H. (1987*a*). Changes of nerve growth factor synthesis in non-neuronal cells in response to sciatic nerve transection. *J. Cell Biol.* **104**, 1623–1631.

HEUMANN, R., LINDHOLM, D., BANDTLOW, C., MEYER, M., RADEKE, M. J., MISKO, T. P., SHOOTER, E. AND THOENEN, H. (1987*b*). 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.

HILLMAN, D. E. AND CHEN, S. (1985). Plasticity in the size of presynaptic and postsynaptic

membrane specializations. In *Synaptic Plasticity* (ed. C. W. Cotman), pp. 39–76. New York: Guilford Press.

HOFER, M., PAGLIUSI, S. R., HOHN, A., LEIBROCK, J. AND BARDE, Y.-A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO J.* **9**, 2459–2464.

HORCH, K. W. AND BURGESS, P. R. (1980). Functional specificity and somatotopic organization during peripheral nerve regeneration. In *Nerve Repair and Regeneration* (ed. D. L. Jewett and H. R. McCarroll), pp. 105–109. St Louis: C. V. Mosby Co.

HUBEL, D. H. AND WIESEL, T. N. (1970). The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol., Lond.* **206**, 419–436.

JEN, L. S., SO, K.-F. AND WOO, H. (1984). An anterograde HRP study of the retinocollicular pathways in normal hamsters and hamsters with one eye enucleated at birth. *Brain Res.* **294**, 169–173.

JOHNSON, J. E., BARDE, Y.-A., SCHWAB, M. AND THOENEN, H. (1986). Brain-derived neurotrophic factor supports the survival of cultured retinal ganglion cells. *J. Neurosci.* **6**, 3031–3038.

KALIL, R. E. AND BEHAN, M. (1987). Synaptic reorganization in the dorsal geniculate nucleus following damage to visual cortex in newborn or adult hamster. *J. comp. Neurol.* **257**, 216–236.

KALIL, R. E., DUBIN, M. W., SCOTT, G. AND STARK, L. A. (1986). Elimination of action potentials blocks the structural development of retinogeniculate synapses. *Nature* **323**, 156–158.

KALIL, R. E. AND SCHNEIDER, G. E. (1975). Abnormal synaptic connections of the optic tract in the thalamus after midbrain lesions in newborn hamsters. *Brain Res.* **100**, 690–698.

KEIRSTEAD, S. A., RASMINSKY, M., FUKUDA, Y., CARTER, D. A., AGUAYO, A. J. AND VIDAL-SANZ, M. (1989). Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons. *Science* **246**, 255–258.

KEIRSTEAD, S. A., RASMINSKY, M., VIDAL-SANZ, M. AND 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), pp. 143–155. New York: Alan Liss.

KEIRSTEAD, S. A., VIDAL-SANZ, M., RASMINSKY, M., AGUAYO, A. J., LEVESQUE, M. AND SO, K.-F. (1985). Responses to light of retinal neurons regenerating axons into peripheral nerve grafts in the rat. *Brain Res.* **359**, 402–406.

KLASSEN, H. AND LUND, R. D. (1989). Retinal graft-mediated pupillary responses in rats; restoration of a reflex function in the mature mammalian brain. *J. Neurosci.* **10**, 578–587.

KREUTZBERG, G. W., GRAEBER, M. B., RAIVICH, G. AND STREIT, W. (1990). Neuron–glial relationship during regeneration of motoneurons. In *Regulation of Gene Expression in the Nervous System*, vol. 59, *Neurology and Neurobiology* (ed. A. M. Stella, J. de Vellis and J. R. Perez-Polo), pp. 333–341. New York: Wiley–Liss Inc.

LEIBROCK, J., LOTTSPEICH, F., HOHN, A., HOFER, M., HENGERER, B., MASIAKOWSKI, P., THOENEN, H. AND BARDE, Y.-A: (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* **341**, 149–152.

LEVI-MONTALCINI, R. (1976). The nerve growth factor: Its role in growth, differentiation and function of the sympathetic adrenergic neuron. *Prog. Brain Res.* **45**, 235–258.

LEWIS, E. R. AND COTMAN, C. W. (1982). Mechanisms of septal lamination in the developing hippocampus revealed by outgrowth of fibers from septal implants. III. Competitive interactions. *Brain Res.* **233**, 29–44.

LIPTON, S. A. AND KATER, S. B. (1989). Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. *Trends Neurosci.* **12**, 265–270.

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. PhD thesis, McGill University, Montreal, Canada.

LOZANO, A. M., DOSTER, S. K., AGUAYO, A. J. AND WILLARD, M. B. (1987). Immunoreactivity to GAP-43 in axotomized and regenerating retinal ganglion cells of adult rats. *Soc. Neurosci. Abstr.* **13**, 1389.

LUMSDEN, A. AND KEYNES, R. (1989). Segmental patterns of neuronal development in the chick hindbrain. *Nature* **337**, 424–428.

LUND, R. D. (1969). Synaptic patterns of the superficial layers of the superior colliculus of the rat. *J. comp. Neurol.* **135**, 197–208.

MACKEL, R., KUNESCH, E., WALDHÖR, F. AND STRUPPLER, P. (1983). Reinnervation of mechanoreceptors in the human glabrous skin following peripheral nerve repair. *Brain Res.* **268**, 49–65.

MAGILL-SOLC, C. AND MCMAHAN, U. J. (1990). Synthesis and trasnsport of agrin-like molecules in motor neurons. *J. exp. Biol.* **153**, 1–10.

MATTHEWS, D. A., COTMAN, C. AND LYNCH, G. (1976). An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat. II. Reappearance of morphologically normal synaptic contacts. *Brain Res.* **115**, 23–41.

MENDELL, L. M., MUNSON, J. B. AND SCOTT, J. G. (1976). Alterations of synapses on axotomized motoneurones. *J. Physiol., Lond.* **255**, 67–79.

MEYER, R. L. (1978). Deflection of selected optic fibers into a denervated tectum in goldfish. *Brain Res.* **155**, 213–227.

MONAGHAN, D. T. AND COTMAN, C. W. (1985). Distribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. *J. Neurosci.* **5**, 2909–2919.

MOONEY, R. D., KLEIN, B. G., SZCZEPANIK, A. M. AND RHOADES, R. W. (1985). Extensive recrossing of retinotectal axons after neonatal unilateral superior collicular lesions in hamster. *Devl Brain Res.* **19**, 297–313.

MOREST, D. K. (1968). The collateral system of the medial nucleus of the trapezoid body of the cat, its neuronal architecture and relation to the olivo-cochlear bundle. *Brain Res.* **9**, 288–311.

MÜLLER, B., STAHL, B. AND BONHOEFFER, F. (1990). *In vitro* experiments on axonal guidance and growth-cone collapse. *J. exp. Biol.* **153**, 29–46.

MURRAY, M. AND EDWARDS, M. A. (1982). A quantitative study of the reinnervation of the goldfish optic tectum following optic nerve crush. *J. comp. Neurol.* **209**, 363–373.

MURRAY, M., SHARMA, S. AND EDWARDS, M. A. (1982). Target regulation of synaptic number in the compressed retinotectal projection of goldfish. *J. comp. Neurol.* **209**, 374–385.

MURRAY, M., WU, L. F. AND GOLDBERGER, M. E. (1987). Spared root deafferentation of cat spinal cord: Anatomical recovery. In *Effects of Injury on Trigeminal and Spinal Somatosensory Systems* (ed. L. M. Pubols and B. J. Sessle), pp. 261–271. New York: Alan Liss.

OLSON, J. M. M., GREENAMYRE, J. T., PENNEY, J. B. AND YOUNG, A. B. (1987). Autoradiographic localization of cerebellar excitatory amino acid binding sites in the mouse. *Neuroscience* **22**, 913–923.

PALAY, S. L. AND CHAN-PALAY, V. (1974). *Cerebellar Cortex*. New York: Springer-Verlag.

PERRY, V. H. AND GORDON, S. (1988). Macrophages and microglia in the nervous system. *Trends Neurosci.* **11**, 173–277.

PESHEVA, P., SPIESS, E. AND SCHACHNER, M. (1989). J1–160 amd J1–180 are oligodendrocyte-secreted nonpermissive substrates for cell adhesion. *J. Cell Biol.* **109**, 1765–1778.

POO, M. AND YOUNG, S. H. (1990). Diffusional and electrokinetic redistribution at the synapse: a physicochemical basis of synaptic competition. *J. Neurobiol.* **21**, 157–168.

PURVES, D. (1975). Functional and structural changes of mammalian sympathetic neurones following interruption of their axons. *J. Physiol., Lond.* **252**, 429–463.

PURVES, D., HADLEY, R. D. AND VOYVODIC, J. T. (1986). Dynamic changes in the dendritic geometry of individual neurons visualized over periods of up to three months in the superior cervical ganglion of living mice. *J. Neurosci.* **6**, 1051–1060.

PURVES, D. AND LICHTMAN, J. W. (1985). *Principles of Neural Development*. New York: Sinauer Associates Inc.

RADEL, J. D. AND YOON, M. G. (1985). Time-course of ultrastructural changes in regenerated optic fiber terminals of goldfish. *Brain Res.* **342**, 168–171.

RAISMAN, G. (1969). Neuronal plasticity in the septal nuclei of the adult rat. *Brain Res.* **14**, 25–48.

RAISMAN, G. AND FIELD, P. M. (1973). A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei. *Brain Res.* **50**, 241–264.

RAMÓN Y CAJAL, S. (1909). *Histology du Systeme Nerveux de l'Homme et des Vertebres*. Vol. I, pp. 754–838.

REICHARDT, L. S., BIXBY, J. L., HALL, D. E., IGNATIUS, M. J., NEUGEBAUER, K. M. AND TOMASELLI, K. J. (1989). Integrins and cell adhesion molecules: Neuronal receptors that regulate axon growth on extracellular matrix and cell surfaces. *Devl Neurosci.* **11**, 332–347.

REIER, P. J., ENG, L. F. AND JAKEMAN, L. (1989). Reactive astrocyte and axonal outgrowth in the injured CNS. In *Neural Regeneration and Transplantation, Frontiers of Clinical Neuroscience,* vol. 6 (ed. F. J. Seil), pp. 183–209. New York: Alan Liss.

RICHARDSON, P. M. AND EBENDAL, T. (1982). Nerve growth activities in the rat peripheral nerve. *Brain Res.* **246**, 57–64.

RICHARDSON, P. M., ISSA, V. M. K. AND SCHEMIE, S. (1982). Regeneration and retrograde degeneration of axons in the rat optic nerve. *J. Neurocytol.* **11**, 949–966.

ROSSI, F., WIKLUND, L., VAN DER WANT, J. J. L. AND STRATA, P. (1989). Climbing fibre plasticity in the cerebellum of the adult rat. *Eur. J. Neurosci.* **1**, 534–547.

RUTISHAUSER, U. AND JESSELL, T. M. (1988). Cell adhesion molecules in vertebrate neural development. *Physiol. Rev.* **68**, 819–857.

SACHS, G. M., JACOBSON, M. AND CAVINESS, V. S., JR. (1986). Postnatal changes in arborization patterns of murine retinocollicular axons. *J. comp. Neurol.* **246**, 395–408.

SACHS, G. M. AND SCHNEIDER, G. E. (1984). The morphology of optic tract axons arborizing in the superior colliculus of the hamster. *J. comp. Neurol.* **230**, 155–167.

SANDROCK, A. W. AND MATTHEW, W. D. (1987). Identification of a peripheral nerve neurite growth-promoting activity by development and use of an *in vitro* bioassay. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6934–6938.

SCHNEIDER, G. E. (1973). Early lesions of superior colliculus: Factors affecting the formation of abnormal retinal projections. *Brain Behav. Evol.* **8**, 73–109.

SCHNEIDER, G. E., JHAVERI, S., EDWARDS, M. A. AND SO, K-F. (1985). Regeneration, re-routing, and redistribution of axons after early lesions: Changes with age, and functional impact. In *Recent Advances in Restorative Neurology*, vol. 1, *Upper Motor Neuron Functions and Dysfunction* (ed. J. Eccles and M. Dimitrijevic), pp. 291–310. New York: Karger.

SCHNELL, L. AND SCHWAB, M. E. (1990). Axonal regeneration in the rat spinal cord produced by antibody against myelin-associated neurite growth inhibitors. *Nature* **343**, 269–272.

SCHWAB, M. E. (1990). Myelin-associated inhibitors of neurite growth. *Expl Neurol.* **109**, 2–5.

SHATZ, C. J. AND SRETAVAN, D. W. (1986). Interactions between retinal ganglion cells during the development of the mammalian visual system. *A. Rev. Neurosci.* **9**, 171–207.

SNOW, D. M., LEMMON, V., CARRINO, D. A., CAPLAN, A. I. AND SILVER, J. (1990). Sulfated proteoglycans and astroglial barriers inhibit neurite outgrowth *in vitro*. *Expl Neurol.* **109**, 111–130.

SO, K. F., SCHNEIDER, G. E. AND AYRES, S. (1981). Lesions of the brachium of the superior colliculus in neonate hamsters: Correlation of anatomy with behaviour. *Expl Neurol.* **72**, 379–400.

SOFRONIEW, M. V., GALLETLY, N. P., ISACSON, O. AND SVENDSEN, C. N. (1990). Survival of adult basal forebrain cholinergic neurons after loss of target neurons. *Science* **247**, 338–342.

SOMOGYI, P., HALASY, K., SOMOGYI, J., STORM-MATHISEN, J. AND OTTERSEN, O. P. (1986). Quantification of immunogold labelling reveals enrichment of glutamate in mossy and parallel fibre terminals in cat cerebellum. *Neuroscience* **19**, 1045–1050.

SOTELO, C. (1975). Synaptic remodeling in mutants and experimental animals. In *Aspects of Neural Plasticity* (ed. F. Vital-Durand and M. Jeannerod), pp. 167–190. Paris: INSERM.

SOTELO, C. (1989). Purkinje cell replacement by intracerebellar neuronal implants in an animal model of heredodegenerative ataxia: An overview. In *Neuronal Grafting and Alzheimer's Disease* (ed. F. Gage, A. Privat and Y. Christen), pp. 34–42. New York: Springer Verlag.

SOTELO, C. AND ALVARADO-MALLART, R. M. (1987). Reconstruction of the defective cerebellar circuitry in adult Purkinje cell degeneration mutant mice by Purkinje cell replacement through transplantation of solid embryonic implants. *Neuroscience* **20**, 1–22.

SPERRY, R. W. (1943). Effect of 180 degrees rotation of the retinal field on visuomotor coordination. *J. exp. Zool.* **92**, 263–279.

STEWARD, O., VINSANT, S. L. AND DAVIS, L. (1988). The process of reinnervation in the dentate

gyrus of adult rats: An ultrastructural study of changes in presynaptic terminals as a result of sprouting. *J. comp. Neurol.* **267**, 203–210.

STOLL, G., TRAPP, B. D. AND GRIFFIN, J. W. (1989). Macrophage function during Wallerian degeneration of rat optic nerve: Clearance of degenerating myelin and Ia expression. *J. Neurosci.* **9**, 2327–2335.

THANOS, S. AND AGUAYO, A. J. (1988). Changes in dendrites of adult rat ganglion cells regenerating axons into peripheral grafts. In *Post-lesion Neural Plasticity* (ed. H. Flohr), pp. 129–138. Heidelberg: Springer-Verlag.

VIDAL-SANZ, M., BRAY, G. M., VILLEGAS-PÉREZ, M. P., THANOS, S. AND 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. AND AGUAYO, A. J. (1988). 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. AND AGUAYO, A. J. (1989). The distance of axotomy from the neuronal cell body influences the rate of retrograde degeneration but not long-term survival of retinal ganglion cells (RGCs). *Soc. Neurosci. Abstr.* **15**, 457.

ZWIMPFER, T., AGUAYO, A. J., BRAY, G. M. AND LAWRENCE, D. G. (1989). Synapse formation by regenerating retinal ganglion cell axons directed into an inappropriate target (the cerebellar cortex) in adult hamsters. *Soc. Neurosci. Abstr.* **15**, 458.

ZWIMPFER, T. J., INOUE, H. I., AGUAYO, A. J. AND 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** (in press).