CHAPTER 15

Axonal Regeneration from Injured Neurons in the Adult Mammalian Central Nervous System

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I. INTRODUCTION

A failure of axons to grow extensively through damaged central nervous system (CNS) tissues is a common consequence of injury to the brain and spinal cord of adult mammals. By contrast, axons severed in the peripheral nervous system (PNS) can successfully regenerate when they become associated with Schwann cells and other peripheral nerve components. At the turn of this century Cajal expressed his belief that the failure of central fibers to regrow *"derives from external conditions, the presence or absence of auxiliary factors that are indispensable to the regenerative process"* (Ramón y Cajal, 1928, pp. 1–2, italics added) and after observing with Tello (1911) and others (Leoz & Arcuate, 1914) that peripheral nerve grafts inserted into the brain became temporarily innervated he added, *"sprouts, wandering through the scar, can be nourished and oriented by the cells of Schwann that are forming bands of Büngner in the grafted nerves"* (Ramón y Cajal, 1928, p. 589, italics added).

Since these pioneering studies, other investigators have made use of similar transplantation techniques to assess the influence of the nonneuronal environment on axonal regeneration and to test the capacity of CNS neurons to initiate and sustain fiber growth along a substrate normally only present in peripheral nerves (Clark, 1942; Horvat, 1966; Kao, Chang, & Bloodworth, 1977). The recent advent of anatomical techniques for tracing the origin and termination of nerve fibers has helped establish that under the experimental conditions created by the grafting of nerve segments into the adult rat CNS, a heterogeneous

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population of central neurons can indeed regrow lengthy fibers (Aguayo, Benfey, & David, 1983; Aguayo, David, Richardson, & Bray, 1982; Richardson, Issa, & Aguayo, 1982). These findings have been interpreted as a proof of (1) a capacity of certain adult CNS neurons to extend their axons in a manner that resembles their growth during development, and (2) the permissive or stimulating effects on axonal elongation of nonneuronal components of the "denervated" PNS.

In addition, the use of peripheral nerve transplants to investigate CNS regeneration has also shown that

1. Certain nerve cells in the adult brain and spinal cord can regrow axons for distances equivalent to those of the long tracts and association fibers that normally join widely separated regions of the neuraxis (Aguayo et al., 1983; David & Aguayo, 1981).
2. The axons of some CNS neurons may regrow to greater lengths than they attain in the intact animal (Aguayo, Björklund, Stenevi, & Carlstedt, 1984; Benfey & Aguayo, 1982; Benfey, Buenger, Vidal-Sanz, Bray, & Aguayo, in press).
3. Various neuronal populations differ in their responsiveness to injury and grafting (Benfey et al., in press; Bray, Benfey, Buenger, Vidal-Sanz, & Aguayo, 1985; Friedman & Aguayo, in press; So & Aguayo, 1985).
4. Many, if not all, of the central axons that grow into these grafts arise from injured neurons rather than by sprouting from undamaged cells (Friedman & Aguayo, in press; So & Aguayo, 1985. Moreover, central axons that have already grown along transplanted PNS segments may regenerate again if severed in the graft (David & Aguayo, 1985).
5. Graft innervation from certain neurons may be dramatically enhanced by a remote lesion to other processes of the same cells (Richardson & Issa, 1984a).
6. When tested electrophysiologically, some neurons that have regenerated axons discharge spontaneously and can be activated transynaptically by natural or electrical stimulation of their afferents. Deficient responses of other neurons with regenerated axons may be the result of alterations in synaptic afferent connectivity due to axotomy (Munz, Rasminsky, Aguayo, Vidal-Sanz, & Devor, in press; Rasminsky, Aguayo, Munz, & Vidal-Sanz, 1985).

Several of the above-listed observations suggest that many central axons extending along PNS grafts are *"behaving in their growth, ramifications, orientation, energetic progress, etc., exactly like the sprouts of the central stump of a cut nerve"* (Ramón y Cajal, 1928, pp. 740, italics added).

The present chapter describes briefly the experimental methods, results, and interpretation of recent studies where peripheral nerve segments were grafted into the CNS of adult rodents to investigate the potential of central neurons to

elongate axons after injury. This review does not attempt to examine the many important contributions of other research strategies to the study of neuronal regeneration. The reader will find them discussed in recent publications (Björklund & Stenevi, 1984; Cotman, 1978; Kao, Bunge, & Reier, 1983; Nicholls, 1982) and in other chapters of this book.

II. NERVE TRANSPLANTATION AS AN EXPERIMENTAL TOOL FOR THE STUDY OF AXONAL REGENERATION FROM THE CNS

A. Dynamic Changes in Transplanted PNS Segments

Peripheral nerve grafts have been inserted into different regions of the rat CNS to provide central axons with a neuron-free path composed of living Schwann cells and the other cellular and noncellular components of the PNS. For this purpose a segment of autologous sciatic nerve is excised and one or both of its tips inserted into the brain or spinal cord (Fig. 15-1). When removed for transplantation, segments of peripheral nerve undergo a series of changes that are characteristic of Wallerian degeneration (Fig. 15-2):

1. Axons and myelin degenerate rapidly.
2. Schwann cells divide frequently for approximately 2 weeks, the greatest number of mitoses taking place 2–3 days after the interruption of axons (Bradley & Asbury, 1970).
3. Schwann cells remain aligned in longitudinally arranged columns (Büngner bands).
4. Each of these columns is surrounded by the continuous "tube" of basal lamina that enclosed the previous nerve fiber.
5. "Denervated" Schwann cells may secrete a number of different molecules (Bunge & Bunge, 1981; Carey & Bunge, 1981; Skene & Shooter, 1983), some of which are reported to stimulate neurite growth *in vitro* (Richardson & Ebendal, 1982; Varon, Manthorpe, & Williams, 1983–1984).
6. Other nonneuronal components of the original nerve (e.g., fibroblasts, collagen matrix) also undergo poorly understood modifications within excised PNS segments.

It is possible that axonal regrowth along grafts and distal stumps of transected nerves is facilitated by these and other changes that follow axonal degeneration. The basal lamina-bound, chainlike arrangement of the Schwann cells constituting Büngner bands may provide axonal growth cones with a continuous path made of modified cell surfaces and noncellular matrices as well as a unique relay system for the supply of needed factors along the entire course of the nerve or graft.

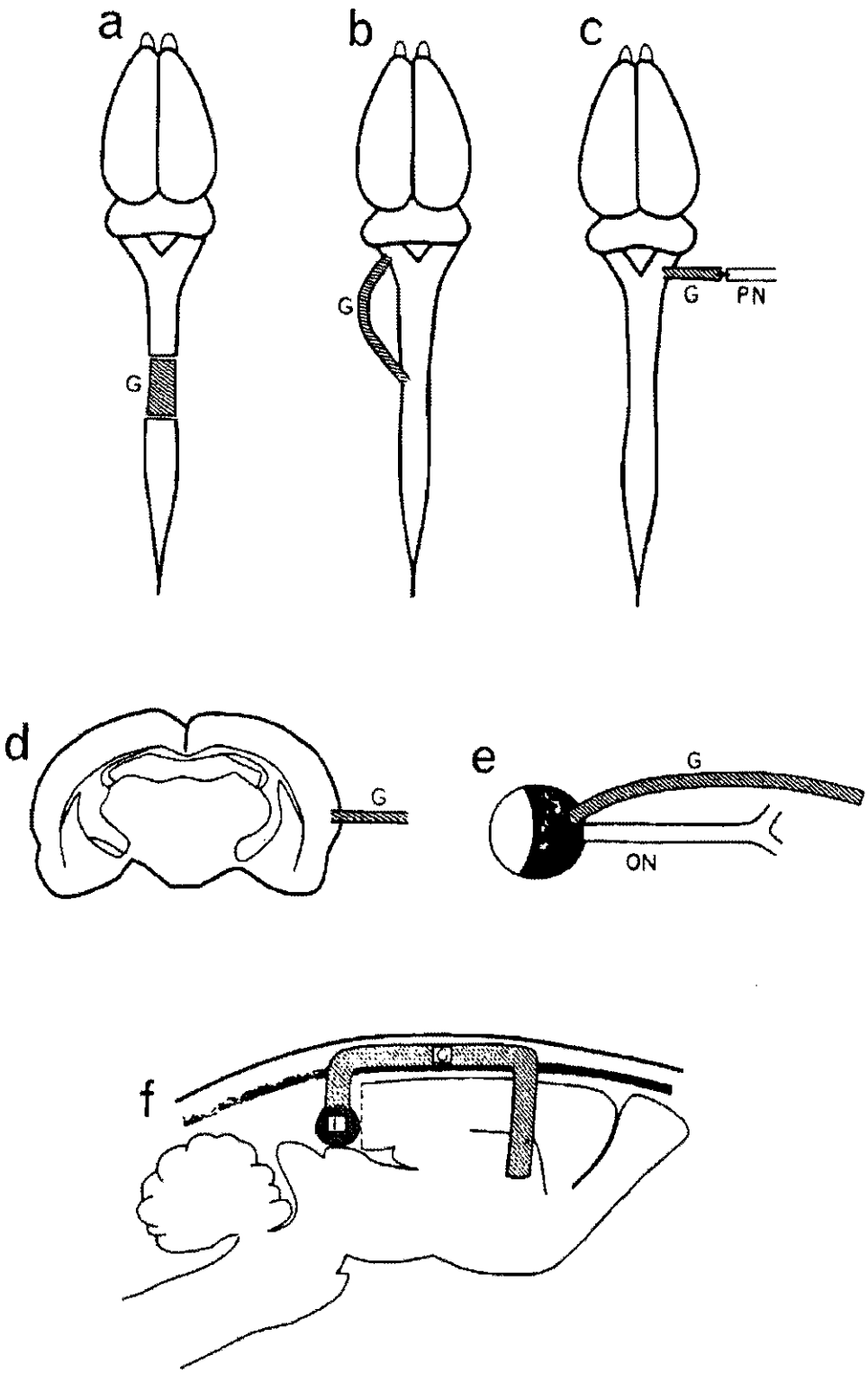

FIG. 15-1. Schematic representation of the different ways in which peripheral nerve segments (G) can be transplanted into the CNS of the adult rat: (a) Joining the cut ends of the transected spinal cord. (b) Bridging two widely separated regions of the neuraxis. (c) Connecting the CNS to other tissues, such as a peripheral nerve (PN). (d) As conduits for the unidirectional growth of fibers arising from the cerebral hemispheres or retina (e). (f) As a reservoir of transplanted fetal CNS neurons whose growth is channeled into the adult brain.

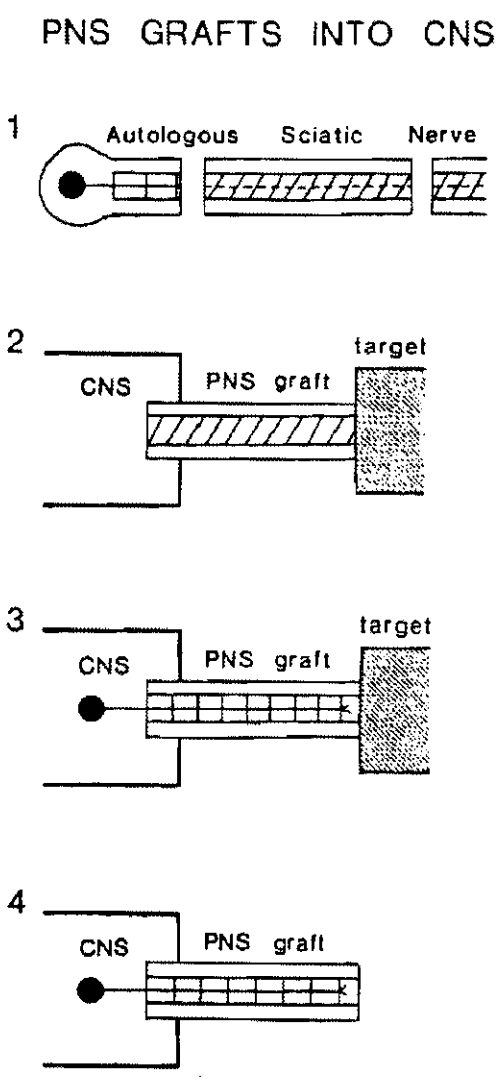

FIG. 15-2. Diagrammatic representation of the changes that follow the grafting of PNS segments into the CNS and other tissues: (1) A segment of peripheral nerve is excised and transplanted. The axons within the PNS segment degenerate, whereas the Schwann cells divide and remain aligned in columns. (2) One end of the graft is inserted into the CNS and the other is linked to a different region of the neuraxis or connected to a selected peripheral target (muscle, nerve, etc.). (3) Injured CNS axons grow through the graft toward the target. (4) Some grafts are left blind-ended. (Modified from Aguayo, Benfey, & David, 1983.)

B. Documentation of the Size, Source, Course, and Termination of Central Axons Regenerating through Grafts

Light- and electron-microscopic examination of PNS grafts at different intervals of up to 1 year after the transplantation of 1–7-cm-long nerve segments into the CNS showed that they had become innervated by myelinated and unmyelinated axons. Fibers within the graft are ensheathed by Schwann cells, basal lamina, and collagen, and their myelin structure is characteristic of the PNS. The diameter of regenerated myelinated fibers varied in the different types of experiments; the thickest fibers, observed in grafts inserted into the region of the dorsal column nuclei of the dorsal medulla oblongata, were 8 μm thick (Munz et al., in press).

Because most central neurons that innervate these grafts are located in an area near the graft tip (Fig. 15-3) it has been possible to take advantage of this predictable distribution of the responding cells to test for axonal regrowth from

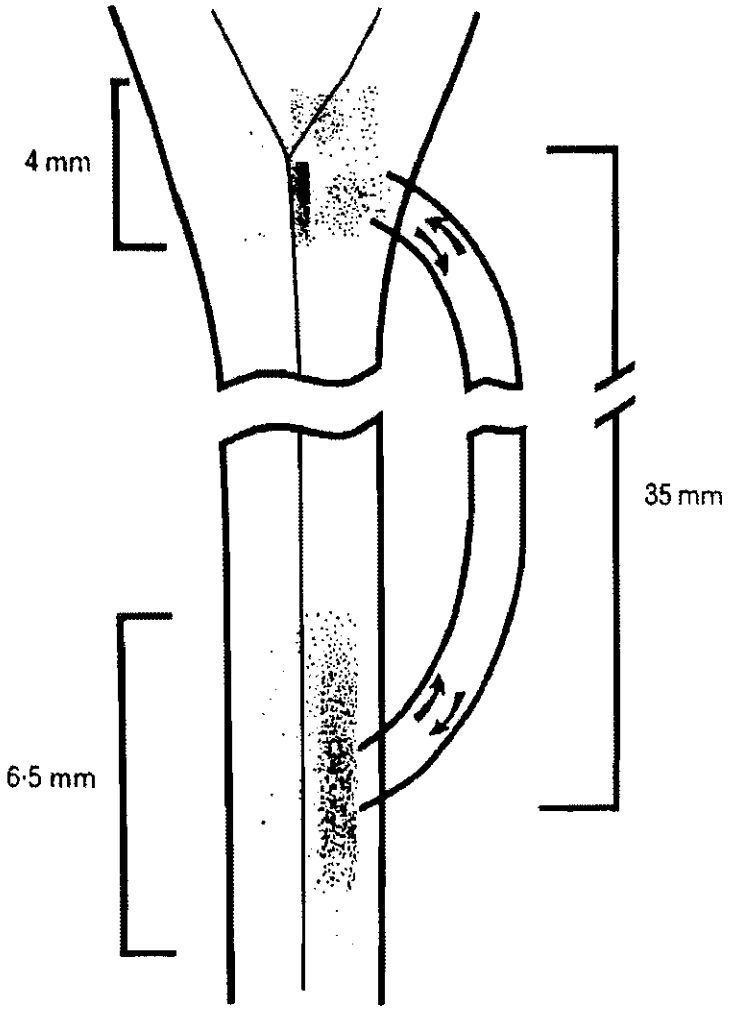

FIG. 15-3. Schematic representation of the distribution of 1472 neurons (dots) labeled retrogradely by HRP applied to the graft in seven rats with PNS "bridges" linking the medulla oblongata and the upper thoracic spinal cord as in Fig. 15-1b. Labeled cells are clustered near the sites of graft insertion. The axons of these neurons regenerated in both directions along these 3.5-cm-long grafts. (Reproduced from David & Aguayo, 1981.)

neurons within many different regions of the rat brain and spinal cord (Aguayo et al., 1983). Anatomical evidence for the central origin of the axons that grew into these grafts was usually obtained with retrograde and anterograde tracing methods. Neuronal somata were retrogradely labeled with horseradish peroxidase (HRP) (Benfey & Aguayo, 1982; David & Aguayo, 1981; Richardson, McGuinness, & Aguayo, 1980), or fluorescent dyes such as True Blue and Nuclear Yellow (David & Aguayo, 1985; Friedman & Aguayo, in press; So & Aguayo, 1985), applied to the cut peripheral end of the graft. Anterograde axonal labeling after the injection into the CNS of tritiated proline and leucine mixtures (Richardson et al., 1984; Richardson, Issa, & Shemie, 1982; Richardson, McGuinness, & Aguayo, 1982) or the application of HRP to cut fibers (David & Aguayo, 1981) furnished information on axon terminals. In addition, fluorescent and immunohistochemical techniques helped visualize the entire

projection of special types of neurons innervating grafts (Aguayo et al., 1984). Single or multiple projections from individual nerve cells were sought by combinations of different fluorescent dyes applied to axons in the graft or brain (Friedman & Aguayo, in press; So and Aguayo, 1985).

Electrophysiological recording of the excitation or inhibition of certain brainstem and cortical neurons projecting axons into the grafts in response to natural or electrical stimulation of their afferents was also considered an indication of regrowth from the CNS (Munz et al., in press; Rasminsky et al., 1985).

C. Technical Advantages

The use of long PNS segments and the insertion of one or both tips of the graft into selected CNS regions facilitates (*a*) the study of regenerative responses in many parts of the brain and spinal cord, (*b*) the determination of the precise location, size, and number of the cells that regrow lengthy axons, (*c*) the anatomical study of synaptic connectivity and transmitter content of these cells by a combination of retrograde labeling from the graft and ultrastructural (B. Friedman and A. J. Aguayo, unpublished) and immunohistochemical (Benfey et al., in press; Bray, Benfey, Buenger, Vidal-Sanz, & Aguayo, 1985) studies of labeled neurons, (*d*) the selection of the course and distance to be covered by growing axons, (*e*) the measurement of the length of the regenerated fibers, (*f*) the separation of sources and targets of axonal growth, an advantage for electrophysiological studies and reliable anatomical tracing, (*g*) the bridging of gaps, (*h*) the investigation of the termination and possible synaptogenesis of regenerated central axons, (*i*) the care and survival of laboratory animals also, because neither spinal transection nor large brain lesions are required for many of these long-term experiments (Fig. 15-1).

D. Present Limitations of These Techniques

In designing and interpreting the results of PNS transplantation experiments it is important to be aware of the following:

1. The CNS neuronal populations recruited into growth are but a small sample of the cells that either neighbor or project across the sites of injury and grafting.
2. Most of the CNS neurons that regenerate axons into PNS grafts inserted into small incisions made in the spinal cord (David & Aguayo, 1981) or brain (Benfey & Aguayo, 1982) are near the graft tip. Grafts implanted into larger lesions that interrupt spinal long tracts can be innervated by more distant neurons situated several centimeters from the graft (Richardson & Issa, 1984a; Richardson et al., 1984)..
3. Not all nerve cells tested have grown axons into grafts (Aguayo et al., 1983; Benfey et al., 1984; Bray et al., 1985; Friedman & Aguayo, in press; So & Aguayo, 1985).

4. Because the retrograde tracers used to identify most of the cells whose axons extend into these grafts are applied at 1 cm or more from the site of grafting into the CNS, cells with shorter axons may not be labeled.
5. As a rule, PNS grafts are also invaded by axons of peripheral nerves in nearby meninges, blood vessels, spinal roots, etc.
6. The target regions to which grafts are connected may also be a source of graft innervation. As a result, grafts often contain a two-directional population of axons (David & Aguayo, 1981).
7. Although central axons may regenerate for several centimeters along PNS grafts, their reentry into the CNS is limited to only a few millimeters; there is no evidence of an enhancement of axonal penetration into the CNS.
8. Thus far there is no proof that regenerating CNS axons make synaptic connections with neurons in the regions of the brain and spinal cord to which they are guided.

III. CELLS OF ORIGIN OF AXONS REGENERATING INTO PNS GRAFTS

New neuroanatomic tracing techniques have made it possible to document the location, size, and number of the neurons whose axons grow along PNS grafts inserted into different regions of the adult rat brain and spinal cord. Some of these neurons are (*a*) in Layers II–VI of the somato-sensory (Benfey & Aguayo, 1982; Vidal-Sanz, Rasminsky, & Aguayo, 1984), motor (Horvat & Aguayo, unpublished), and visual cortices (Buenger & Aguayo, 1983), (*b*) the ganglion cells of the retina (So & Aguayo, 1985), (*c*) the mitral and tufted cells of the olfactory bulb (Friedman & Aguayo, in press), (*d*) in the basal ganglia (Benfey & Aguayo, 1982), (*e*) in the thalamus (mainly the reticular nucleus) (Benfey et al., in press), (*f*) in the hippocampus, dentate nucleus, amygdala, claustrum (Aguayo et al., 1983), and hypothalamus (Benfey et al., in press), (*g*) in the deep cerebellar nuclei, but not in the cerebellar cortex (Dooley & Aguayo, 1982), (*h*) in many brainstem nuclei (Aguayo et al, 1983; Munz et al., in press), (*i*) in all laminae of the spinal cord (David & Aguayo, 1981; Richardson et al., 1984; Richardson, McGuinness, & Aguayo, 1982).

The neurons shown to be capable to renewed growth were not only present in different sites of the CNS but the size of their somata was representative of the wide range observed in normal animals. In the spinal cord (David & Aguayo, 1981; Richardson et al., 1980), cerebral cortex (Benfey & Aguayo, 1982), and retina (So & Aguayo, 1985), where they have been measured, the soma-size histograms of labeled neurons regenerating axons into grafts resembled those of comparable normal cells.

Regenerating cells also varied in their geometric configuration. Axonal regrowth into grafts has been proven from CNS neurons with multibranched axons such as those in the substantia nigra of the mesencephalon (Benfey & Aguayo, 1982) and the reticular nucleus of the thalamus (Benfey et al., in press)

as well as from the retina (So & Aguayo, 1985), where the axons of the ganglion cells do not normally branch (Drager, Edwards, & Barnstable, 1984; Ramón y Cajal, 1955).

A detailed study of the putative transmitter and peptide content of these neurons has not been undertaken, but the combined use of retrograde markers and immunohistochemistry has helped document a vigorous axonal regrowth from GABAergic neurons in the reticular nucleus of the thalamus (Benfey et al., in press). Histofluorescence, characteristic of monoamines, was shown to be present in the soma and processes of mesencephalic neurons several months after their transplantation from fetal brains into PNS "bridges" that linked with the striatum (Aguayo et al.; 1984). These observations suggest that adult and fetal CNS neurons growing long axons through PNS grafts express their transmitter content under these experimental conditions. The location of other neurons suggests further the transmitter heterogeneity of the different populations of nerve cells that are capable of axonal regeneration, for example, norepinephrine in those of the locus ceruleus (Munz et al., in press), serotonin in the cells of the raphe (Richardson et al., 1984), dopamine in the substantia nigra (Benfey & Aguayo, 1982), and vasopressin and oxytocin in the supraoptic and paraventricular nuclei of the hypothalamus (Benfey et al., in press). Similar assumptions are probably justified for several other groups of CNS neurons already shown to regenerate axons into PNS grafts.

There is thus far no indication, therefore, that the cells proven to innervate these grafts belong to an exclusive subgroup of neurons, uniquely capable of regenerative growth. However, conclusions as to the existence of a general neuronal capacity for axonal regeneration in the CNS are not presently justified because the cells identified in these experiments are but a small part of the entire population of the brain and spinal cord of these animals. Indeed, the largest number of intrinsic CNS neurons known to have regrown long axons in a single animal was in a small portion of a PNS-grafted retina, where 2257 ganglion cells were counted in a whole-mount preparation labeled from the graft (So & Aguayo, 1985); these neurons constituted approximately 15% of the ganglion cells that normally populate such a retinal area. In other cell groups, such as those in Clarke's column and the red nucleus, less than 2% of available cells regenerated axons (Richardson et al., 1984).

The overall yield of neurons proven to grow long axons into grafts may be influenced by the grafting methods and tracing techniques used in some of these experiments and also by (*a*) local conditions at the site of grafting, (*b*) the distance between cell somata and the graft, (*c*) injury to one or more axons, and (*d*) special neuronal characteristics. The latter two will be discussed in greater detail in Sections IV and V.

A. Local Conditions at the Site of Grafting

While axonal regrowth of transected and resutured peripheral nerves may appear to be more successful than that from central neurons into PNS grafts,

the difference may not necessarily derive from intrinsic growth deficits of CNS neurons but may be partly determined by unfavorable conditions in the surrounding tissues. The interface created between the CNS and peripheral nerve grafts is unlikely to match the favorable apposition of two similar peripheral nerve stumps. Adverse conditions at the CNS-graft interface likely resemble those caused by damage to normal CNS–PNS junctions. The effect of changes at these natural borders is illustrated by the response of motor axons, known to regenerate in cut peripheral nerves, but many of which are unable to grow when injured intraspinally near the ventral roots (Risling, Cullheim, & Hildebrand, 1983), presumably because most cut fibers retract further into the CNS and become engulfed by rapidly proliferating spinal glia. A few central axons, however, traverse these boundaries and regenerate successfully through the PNS environment of ventral roots or grafts.

B. Distance between Cell Soma and Graft

The number and distribution of regenerating central neurons is also influenced by the location and size of the lesion made for grafting. The largest number of cells regenerating axons was usually situated wtihin a few millimeters of the graft tip but fell rapidly with increasing distance from the site of graft insertion (Fig. 15-3). Because, as discussed in Section IV, damage to central fibers appears to be a prerequisite for graft innervation, some of the differences observed in the experiments under consideration likely reflect the injury or sparing of axonal projections by the various grafting procedures. Indeed, axonal growth from the more distant neurons was not demonstrated in experiments where grafts were inserted with minimal operative damage (David & Aguayo, 1981), but became apparent after complete or partial cord sections that interrupted the long spinal tracts (Richardson et al., 1984; Richardson, McGuinness, & Aguayo, 1982). Graft innervation by damaged nerve cells may also depend on the timing and levels of activation of some of the intrinsic neuronal mechanisms regulating growth. In the injured neurons, the closeness of their somata to the axotomy site and graft may lead to a faster and more effective neuronal response to signals relayed retrogradely (Grafstein, 1975; Lieberman, 1974; Singer, Mehler, & Fernandez, 1982). Such short distances may enable these cells to establish contacts with released molecules (Nieto-Sampedro et al., 1982; Richardson & Ebendal, 1982; Riopelle, Boegman, & Cameron, 1981; Skene & Shooter, 1983; Varon et al., 1983–1984) or surface-bound graft components within critical time periods (Fig. 15-4). Furthermore, putative trophic substances released by grafts or tissue damage may only reach the somata of nearby receptive damaged cells in suitable concentration. Some of these various circumstances may help explain the limited axonal regrowth from distant cell bodies into most of the grafts.

Specific examples of the inverse relationship between graft innervation and the distance between the site of injury and the soma of neurons are:

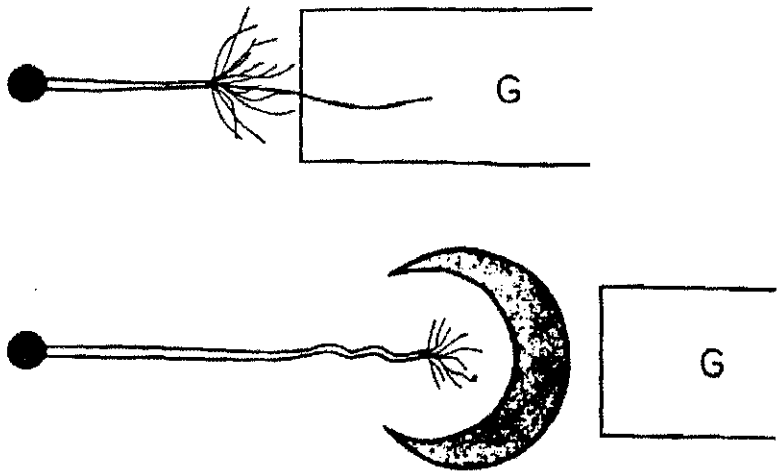

FIG. 15-4. Hypothetical responses of central neurons whose somata are near (top) or more remote from (bottom) PNS grafts (G). Injured axons from nearby cells are more successful in growing into the graft, presumably because their earlier response to axotomy allows them to reach the graft within critical time periods, avoiding the interposition of central neuroglial elements (*) from the injured site.

1. PNS segments grafted intracranially into transected optic nerves were not innervated by a substantial number of regenerating axons (Politis & Spencer, 1982; Richardson, Issa, & Shemie, 1982), whereas fibers from many ganglion cells grew readily into grafts transplanted directly into the retina (So & Aguayo, 1985).
2. The cortico-spinal tract did not grow into grafts in the midthoracic spinal cord (Richardson, Issa, & Shemie, 1982) but processes from pyramidal cells in the fifth layer of the motor cortex regenerated into nearby grafts inserted superficially into the cerebrum (Horvat & Aguayo, unpublished).
3. When PNS grafts were inserted at high-cervical, low-cervical, midthoracic, or lumbar levels of the spinal cord in order to investigate regeneration in long spinal tracts, some fibers of several of the major descending and ascending tracts grew into the cervical grafts. However, long descending fibers rarely regenerated in midthoracic or lumbar grafts and axons ascending from lumbar spinal segments usually failed to enter high cervical grafts (Richardson et al., 1984).

Whatever mechanisms are responsible for the striking differences in the response of central neurons whose axons are injured near or far from the cell body, it is worthwhile noting that such marked positional effects on the number of regenerating axons have not been described in transected and rejoined peripheral nerves. This suggests that the injured CNS environment surrounding the cut nerve fibers may play a determining role in preventing axonal growth cones from entering nearby grafts, the most vulnerable fibers being those that retract the most or respond too slowly or too weakly to the axotomy challenge (Fig. 15-4). However, the recent demonstration of a hundredfold enhancement

in the innervation of PNS grafts inserted in the high cervical spinal cord by the long ascending spinal axons from lumbar primary sensory neurons whose peripheral axons in the sciatic nerve were also cut (Richardson & Issa, 1984a), implies that some growth-limiting aspects of the neuronal and nonneuronal interactions that take place at the sites of CNS injury and PNS grafting can be overcome by an appropriate induction of intrinsic mechanisms within nerve cells. After such double lesion, the enhancement of spinal axonal regrowth resulted in more than 50% of the cells in some L5 ganglia extending into the cervical grafts (Richardson & Issa, 1984a). Although it is not known if the greater innervation of these distant grafts is the result of an earlier and accelerated regrowth of long ascending spinal axons, changes in the speed of the initiation and the rate of the outgrowth have been reported with other forms of enhancement by repeated axotomy (Grafstein & McQuarrie, 1978).

IV. AXONAL REGROWTH FROM INJURED NEURONS

The regrowth of axons from the brain and spinal cord of grafted animals could result either from the extension of nearby nerve fibers of undamaged neurons (Goldberger & Murray, 1978; Liu & Chambers, 1958; Raisman & Field, 1973) or, alternatively, from cells whose axons or collaterals were severed at the time of grafting (Fig. 15-5). As stated above, recent evidence indicates that axotomized neurons may be the main source of the new fibers that grow along these grafts. Detailed studies of the distribution of the cells of origin of regrowing axons in PNS-grafted spinal cords (Richardson et al., 1984), olfactory bulbs (Friedman & Aguayo, in press), and retinas (So & Aguayo, 1985) (Fig. 15-1e), have established that neurons whose axons normally traverse the site of injury and grafting are more likely to innervate these grafts. Proof of damage to these cells has now been obtained with combinations of anatomical tracers. Taking advantage of the different colors and cellular localization of two fluorescent dyes, True Blue and Nuclear Yellow, these retrogradely transported labels were used to determine whether CNS neurons innervated grafts exclusively or also remained connected to their normal targets in the brain, the latter being an indication of graft innervation by collateral growth from nonaxotomized neurons. It was shown that most mitral and tufted cells in the olfactory bulb (Friedman & Aguayo, in press) and all ganglion cells in the retina (So & Aguayo, 1985) (Fig. 15-6) that extended fibers into the graft had lost their axonal projections to the lateral olfactory tract and optic nerve, respectively. Furthermore, in the group of olfactory bulb experiments it was possible, by varying the time of dye application to the graft and lateral olfactory tract, also to establish that the loss of normal olfactory projections through the tract was not due to a gradual axonal retraction related to a lengthy fiber growth into the graft, but was the result of axotomy at the time of grafting into the bulb (Friedman & Aguayo, in press). These findings suggest strongly that sprouting from uninjured nerve cells is not an important source of axonal growth along

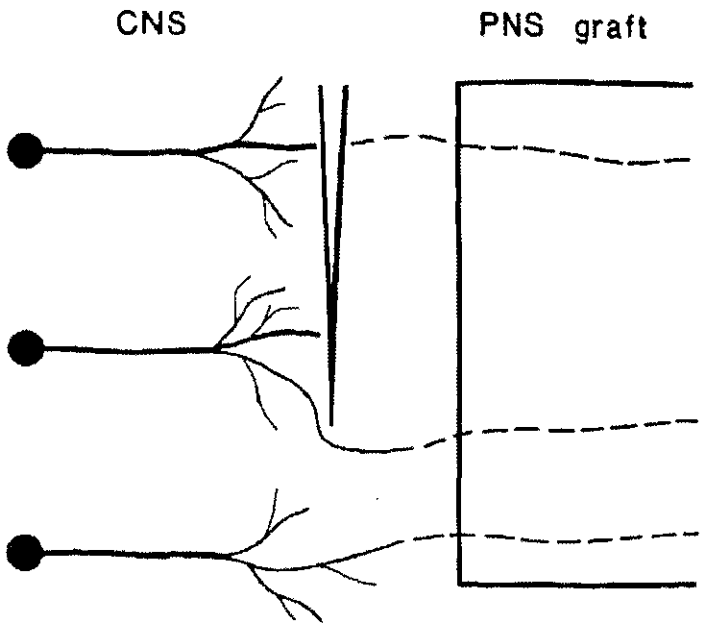

FIG. 15-5. Possible sources of graft innervation from the CNS: injured neurons whose main axon (top) or collaterals (middle) are severed at the time of grafting or nearby undamaged nerve cells (bottom).

these grafts. Moreover, because the axons of intact ganglion cells do not normally branch within the retina (Drager et al., 1984; Ramón y Cajal, 1955), it can be assumed that the regenerated axons that innervate intraocular grafts (So & Aguayo, 1985) are an outgrowth of cut fibers and not an extension from undamaged proximal collaterals of axotomized neurons.

The demonstration that most axons within grafts arise by regeneration from damaged CNS neurons helps explain why many of the cells regrowing axons are located in regions that either project through or are near the sites of injury and grafting.

Retrograde degenerative changes are generally believed to be more severe when axons are cut close to the cell body of neurons with few proximal collaterals (Lieberman, 1974). It is noteworthy, however, that axons of ganglion cells, normally unbranched in the retina (Drager et al., 1984; Ramón y Cajal, 1955), regenerate more vigorously in response to axotomy and grafting near their perikaryon (So & Aguayo, 1985) than when distal lesions are inflicted in the optic nerve (Politis & Spencer, 1982; Richardson et al., 1984). Furthermore, during the time period studied (4–11 weeks) the ganglion-cell-sized-histograms of the cells extending axons into PNS grafts inserted in the retina closely resembled the size distribution of intact retinal cells. Because retrograde degenerative changes are commonly seen after axotomy in the retina, optic nerve, and tract (Goldberg & Frank, 1980; Graftstein & Ingoglia, 1982; Richardson, Issa, & Shemie, 1982), and because the regenerated ganglion cells can be assumed to project exclusively into the graft (So & Aguayo, 1985), it is conceivable that the cells whose axons enter these grafts are protected from the early degenera-

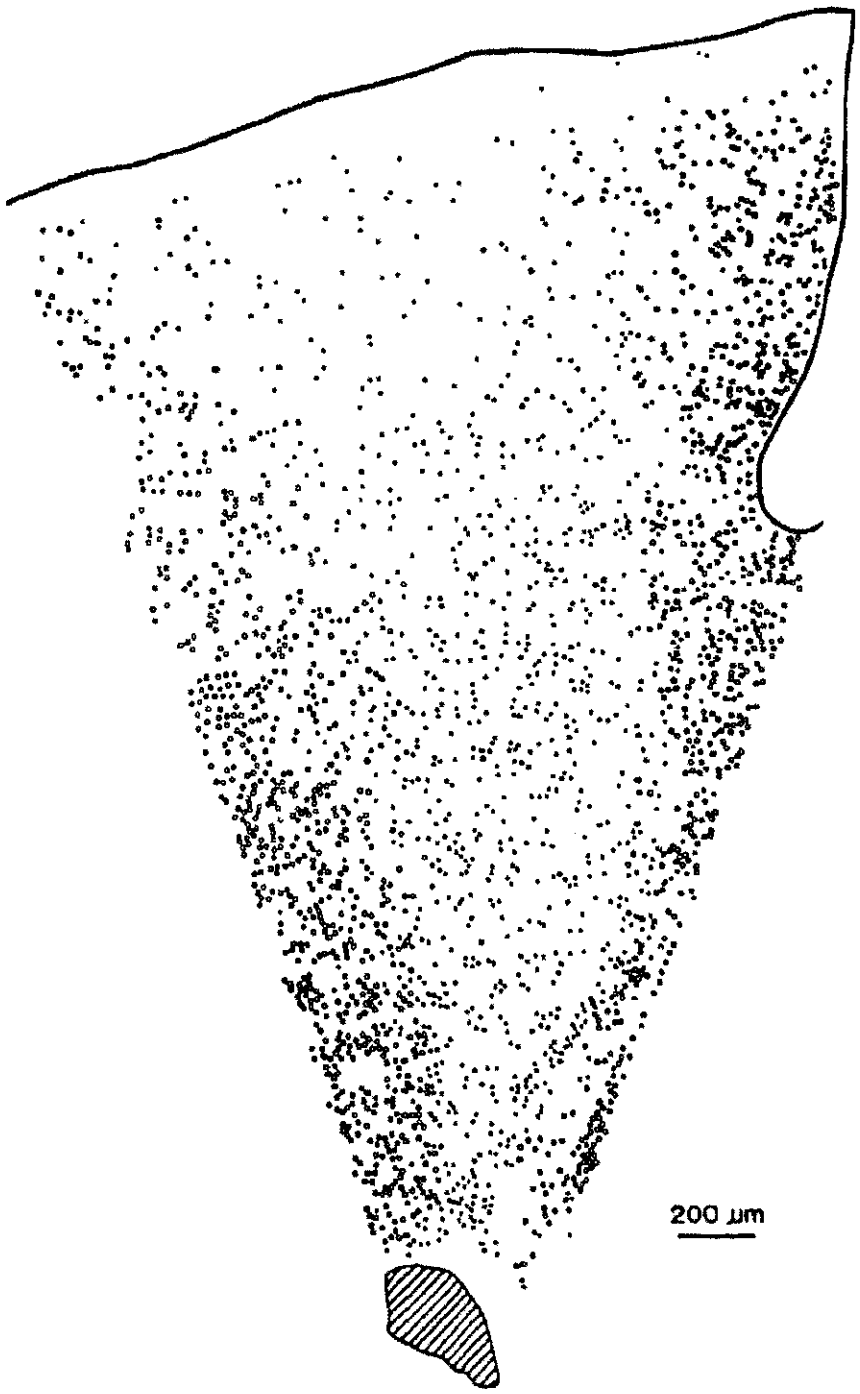

FIG. 15-6. Partial diagram of a whole mount of a PNS-grafted retina where True Blue (TB) was applied to the graft and Nuclear Yellow (NY) to the contralateral optic tract to determine if fibers regenerating into the graft originated from axotomized retinal ganglion cells or from sprouting by cells that retained their projections to the brain. TB-labeled cells (dots) were confined to a triangular zone that fanned out from the site of implantation of the graft (hatching). NY-labeled cells (open circles) were distributed throughout the rest of the retina. No double-labeled cells were found in this and other similar preparations. (Modified from So & Aguayo, 1985.)

tive effects of axotomy. Measurements of perikaryal diameters in PNS-grafted spinal cord (David & Aguayo, 1981; Richardson et al., 1980), brainstem (Richardson et al., 1984), and cerebrum (Benfey & Aguayo, 1982) also gave no indication of a predominance of small-sized neurons among the different cells of origin of the axons that regrew into grafts. A shift of these histograms toward the smaller diameters would have been expected with neuronal atrophy. Conversely, PNS grafts inserted intracranially into transected optic nerves are not innervated appreciably by optic nerve fibers and the mere presence of these distal grafts did not seem to prevent the gradual loss of axons in the ocular stump of the optic nerve (Richardson, Issa, & Shemie, 1982). If axonal regrowth into PNS grafts and neuronal survival are eventually shown to be related it would suggest that conditions created by the reinnervation of these grafts can satisfy the metabolic needs of certain injured CNS neurons. Such graft-dependent beneficial effects may only be temporary because in one group of animals where PNS grafts were accurately placed into the reticular nucleus of the thalamus, a region known to be the source of vigorous regenerative axonal growth into PNS grafts, no cells were retrogradely labeled when HRP was applied to the peripheral end of the graft more than 1 year after grafting (Benfey et al., in press). One of many possible explanations for this finding is that the neurons that initially extended axons along these blind-ended grafts (Fig. 15-2d) eventually degenerated. Such outcome, known for axotomized cells that fail to make synaptic contacts with their target tissues (Kawamura & Dyck, 1981; Lieberman, 1974; Purves & Nja, 1978), is considered an indication of a sensitive interdependence between neurons and their fields of innervation.

The global and dynamic nature of neuronal responses to axotomy is further underlined by the remarkable regrowth of spinal axons arising from dorsal root ganglia whose peripheral axons were also damaged (Richardson & Issa, 1984a). Under these circumstances nerve cells that do not usually innervate grafts inserted several centimeters away from their cell soma were found to grow in large numbers into PNS grafts after a second axonal injury at a peripheral site very distant from its spinal terminals. As indicated by Richardson and Issa (1984a), this enhancement of axonal growth by a simultaneous distant injury suggests axotomy activates mechanisms controlling growth responses of the entire neuron. Interestingly, the enhancement of the spinal response of these bipolar neurons appears to be also under the influence of conditions in their peripheral stump because the regrowth into the graft was less marked when peripheral axons were interrupted by nerve crush rather than by the cutting and removal of a segment from the sciatic nerve (Richardson & Issa, 1984a). The enhancement observed resembles the effects of other injury-related phenomena that lead to changes in neuronal geometry and growth: conditioning (Grafstein & McQuarrie, 1978; McQuarrie & Graftstein, 1973), where the two lesions are separated in time rather than space, and the "pruning effect," where developing neurons extend certain branches to compensate for the permanent loss of others (Schneider, Jhaveri, Edwards, & So, in press). It is not known if intrinsic CNS neurons can also be induced to regenerate in greater numbers by maneuvers

similar to those used by Richardson and Issa (1984a) to stimulate the growth of spinal axons from dorsal root ganglia but, as discussed elsewhere, the vigorous response observed after nerve grafts are inserted near the projections of multibranched nerve cells in the brain could possibly be the result of similar mechanisms (Benfey et al., in press).

Both the demonstration that most if not all axons regenerating into grafts arise from damaged neurons (Friedman & Aguayo, in press; So & Aguayo, 1985) and the enhanced innervation of intraspinal PNS grafts by a lesion of a distant process of a nerve cell (Richardson & Issa, 1984a) indicate that intrinsic neuronal mechanisms activated by injury (Graftstein & McQuarrie, 1978; Singer et al., 1982) are capable of initiating neuronal responses that lead to an extensive outgrowth if fibers encounter appropriate conditions in their environment.

V. DIFFERENCES IN NEURONAL RESPONSES TO INJURY AND GRAFTING

It has become apparent from some of these experiments that certain neuronal characteristics may influence regenerative responses to injury and PNS grafting. The following are examples of a variability that may depend upon the specific properties and requirements of certain neuronal groups.

A. The Reticular Nucleus

When peripheral nerve grafts were inserted into different regions of the thalamus, more than 80% of the nerve cells whose axons extended into the grafts arose from one thalamic subdivision: the reticular nucleus (Benfey et al., in press; Bray et al., 1985; Fig. 15-7). In the rat, the reticular nucleus of the thalamus (RNT) is a narrow sheet of GABAergic neurons that envelops the rostral and lateral surfaces of the dorsal thalamus. These cells have multibranched axons that apparently project exclusively to the various other thalamic nuclei (Houser, Vaughn, Barber, & Roberts, 1980; Jones, 1975; Oertel et al., 1983). There are several possible explanations for the propensity of RNT neurons rather than cells from other thalamic regions to regenerate into the PNS grafts:

1. The Type of Neurotransmitter

The thalamus, where virtually all GABAergic nerve cells are confined to the RNT, provides a unique opportunity to compare the regeneration from GABAergic and non-GABAergic cells. In other studies it had been shown that GABAergic reinnervation occurs in lesioned entorhinal regions (Nadler, Cotman, & Lynch, 1974) as well as in irides inserted into the hippocampus or striatum (Emson, Björklund, & Stenevi, 1977). However, transmitter type is likely not to be the only factor influencing the propensity for regrowth, because

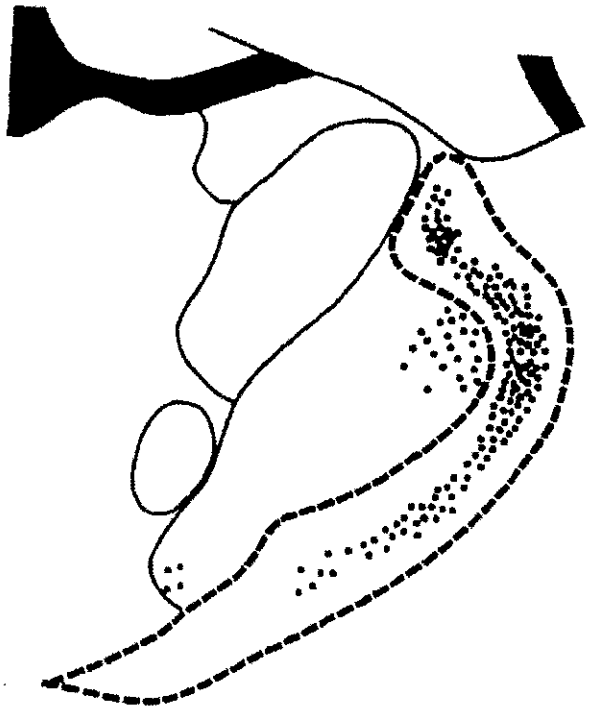

FIG. 15-7. Diagram of a coronal section of the rat thalamus showing the position of 180 neurons retrogradely labeled from a PNS graft. Most labeled cells are within the reticular nucleus (outlined by broken line).

axons of neurons containing transmitters other than GABA also regenerate vigorously in grafts. Furthermore, no regrowth has been observed into similar grafts from cerebellar Purkinje cells, a population of neurons that is also GABAergic (Dooley & Aguayo, 1982).

2. Neuronal Geometry

A marked regrowth has been documented from other populations of neurons with diffusely branched axons that contain transmitters such as dopamine (Aguayo et al., 1984; Björklund, Dunnett, Stenevi, Lewis, & Iversen, 1980), noradrenaline (Björklund, Segal, & Stenevi, 1979; Olson, Seiger, & Stromberg, 1982), or acetylcholine (Gage, Dunnett, Stenevi, & Björklund, 1983). If the mechanisms that initiate regrowth are triggered by injury and transported retrogradely to the perikaryon (Cragg, 1970; Grafstein, 1975; Lieberman, 1974; Singer et al., 1982) the simultaneous injury of several axonal branches may, by augmenting the stimulus, enhance regeneration from these multibranched neurons (Bray et al., 1985; Richardson & Issa, 1984a). The enhanced regeneration of the central axons from dorsal root ganglia by transection of their peripheral fibers (Richardson & Issa, 1984a) argues in favor of this possibility. However, once again this may not be the only explanation for these responses because ganglion cells, whose axons do not ramify in the retina (Drager et al., 1984), regenerate in large numbers when PNS grafts are inserted directly into the eye (So & Aguayo, 1985). Finally, because of the stated inverse relation-

ship that exists between regeneration and the separation of neuronal somata and graft, the short length of the RNT fibers may also facilitate their extension into these grafts.

3. Neuronal Survival

It is possible that other projection neurons of the thalamus are more susceptible to retrograde degeneration than cells of the RNT. Such susceptibility to mechanical injury by projection cells in the different nuclei of the thalamus (Barron, Means, & Larsen, 1973) and the vigorous regrowth from RNT neurons may explain the described differences in the populations of thalamic neurons that innervate grafts but gives us yet no clear indication as to the cellular basis for these neuronal responses.

B. Other Neuronal Populations

Similar comments are relevant to further examples of apparent selectivity in the responses of other neuronal populations. Peripheral nerve grafts inserted into the olfactory bulb (Friedman & Aguayo, in press) or retina (So & Aguayo, 1985) became innervated by the axons of damaged olfactory mitral and tufted cells or retinal ganglion cells, respectively. However other neuronal types in the olfactory bulb or retina do not appear to give rise to a similar growth of nerve fibers. Perhaps the absence of regrowth from these local circuit cells reflects a lack of regenerative capacity, cell loss caused by the grafting procedure, or an inability of these smaller neurons with either short or no axons to grow sufficient distances along these grafts to reach the site of application of the tracers used in these studies.

On the other hand, although the denervated PNS segments used as grafts appear to have become a reservoir of trophic substances that satisfy, both *in vivo* and *in vitro*, certain requirements for the growth of fibers from several populations of peripherally and centrally projecting nerve cells (Aguayo et al., 1983; Richardson & Ebendal, 1982; Varon et al., 1983–1984), it is not known if these substances can meet the needs of all CNS neurons. The specificity of some known trophic effects, such as those mediated by nerve growth factor (Levi-Montalcini, 1983), suggests different growth factors are necessary to influence the wide spectrum of cells that populate the nervous system. It seems possible, therefore, that some of the inequalities in the neuronal responses described above may also depend on the availability or not within the graft of molecules that are optimal for the survival and growth of each of the nerve cell types tested.

The anatomical demonstration of a variability in the regrowth of axons from different neuronal populations provides but a hint of the multiplicity of influences that shape neuronal responses to injury in the adult mammalian brain. Other approaches will be needed to define the relative roles of the different intrinsic and environmental determinants.

VI. COMBINATIONS OF FETAL NEURONAL TRANSPLANTS AND PNS GRAFTS

The transplantation of embryonic CNS tissues into the brain and spinal cord of newborn and adult animals is followed by the survival and differentiation of many of the grafted neurons and the extension of nerve fibers into the host CNS. Moreover, certain functional abnormalities improve after the grafting of fetal nerve cells into the brains of some mutant, aged, or injured laboratory animals (Björklund & Stenevi, 1984). Although axons from the transplanted fetal neurons penetrate and branch extensively in the host CNS, most of these fibers only reach regions within a few millimeters of the neural graft. As a result, it has been necessary to place these neuronal transplants near the tissues whose innervation is intended. To circumvent this limitation, the fetal neuronal transplants have recently been combined with PNS grafts to enhance and guide the growth of their axons toward more distant cell populations in the adult host brain (Aguayo et al., 1984) or peripheral tissues (Bernstein, 1983; Richardson & Issa, 1984b). In one group of experiments (Aguayo et al., 1984), mesencephalic fetal grafts placed extracerebrally over the host colliculus were linked by means of a 2-cm PNS bridge to the striatum of adult rats whose normal nigrostriatal projection had been previously destroyed (Fig. 15-1f). One end of the PNS bridge was attached to the fetal cells, while the main span was placed extracranially beneath the scalp and led rostrally into the denervated striatum through a burr hole in the skull. It was shown that many axons from the monoaminergic cells in the implant grew the entire length of the PNS conduits and that some of these fibers penetrated and branched in the striatum, a normal target of these axons. Fluorescent fibers within the grafts had few branches, whereas those that entered the brain formed a multibranched terminal network. Furthermore, the total length (approximately 2 cm) reached by some of the axons of grafted dopaminergic neurons was considerably greater than that of the normal nigrostriatal projection of mature, intact rats (approximately 6–7 mm). It is not known if the marked lengthening of the unbranched fibers within the graft is mainly accomplished through a reorganization of the overall geometry of these normally diffusely branched neurons or, more likely, also by a substantial increase in cell volume. Similar increases in axonal length, observed in dopaminergic, GABAergic, and other neurons of the mature rat brain (Benfey & Aguayo, 1982; Benfey et al., in press), underline the remarkable plasticity of fetal and adult CNS mammalian neurons.

This new experimental approach provides an opportunity to explore further certain aspects of neuronal growth and connectivity in the mammalian nervous system. It is feasible to attempt, for instance, (1) to transplant other "foreign" neurons to sites located extracerebrally or within the CNS, to provide new sources for the innervation of remote targets across long PNS grafts, (2) to place neuronal grafts as solid pieces (Bernstein, 1983; Richardson & Issa, 1984b) or as selected cells from *in vitro* cultures (Doering & Aguayo, 1984) into the PNS in order to study their capacity and requirements for differentiation, growth, and

connectivity, (3) to permit the further testing of the mechanisms underlining the functional influences of the fetal grafts on host animals (Gage et al., submitted). Such investigations may be facilitated in these preparations by a wider separation of the source and target of axonal growth and the possibility of monitoring, blocking, or stimulating the activity of axons within these long and superficially located PNS bridges.

VII. SOME FUNCTIONAL CHARACTERISTICS OF CNS NEURONS REGENERATING AXONS INTO PNS GRAFTS

To investigate in adult rats the afferent connectivity and discharge patterns of CNS neurons that regenerate axons along peripheral nerve grafts their unitary electrical activity was recorded from small nerve strands teased from PNS grafts inserted into the region of the dorsal column nuclei of the medulla oblongata or the somatosensory cortex (Munz et al., in press; Rasminsky et al., 1985; Keirstead, Vidal-Sanz, Levesque, Rasminsky, & Aguayo, submitted). Electrophysiological studies, carried out 2–6 months after grafting showed spontaneous centrifugal impulse traffic along axons in these strands. Furthermore, the activity of some units was induced, enhanced, or inhibited transsynaptically by natural or electrical stimulation within peripheral receptive fields. However, while many axons could be activated by stimulating electrodes applied directly to the graft, other units were neither spontaneously active nor responsive to stimulation from afferents in the periphery. These experiments suggest that while some of the CNS neurons that project axons into these grafts display a normal or near normal spontaneous and induced activity, the function of other regenerating neurons may be reduced or altered.

The reason why many of the neurons regenerating axons into these grafts do not respond transsynaptically to the stimulation of sensory afferents is unknown. One of several explanations (see Rasminsky et al., 1985) is that only a small number of the cells tested will normally respond to the types and sites of stimulation used in these studies. A more intriguing alternative, however, is that their unresponsiveness derives from deafferentation and axotomy-induced alterations in neuronal connectivity. Deafferentation may result from the severing of afferent fibers by the grafting procedure, while axotomy is known to alter neuronal excitability retrogradely and also to cause the shedding and reorganization of connections to and from axotomized cells (B. Friedman & A. J. Aguayo, unpublished; Mendell, 1984). Because both local CNS damage and axotomy of neurons that regenerate axons have been established in the grafting experiments, it is likely that both circumstances influence the functional responsiveness of the cells tested. Furthermore, in the animals studied, the peripheral tips of the grafts were blind-ended. This may have also contributed to the reduced responsiveness of some of the cells examined physiologically, because the restoration of afferent synapses upon axotomized neurons appears to be contingent

upon the reestablishment of target contacts by such cells (Mendell, 1984; Purves & Lichtman, 1978).

Although it is not yet known if the axons regenerating along PNS grafts are capable of making connections with cells in the tissues to which they are guided, the study of the input and output properties of their cells of origin in the CNS should provide new insights into the various effects of injury on neuronal circuitry.

VIII. ADDITIONAL COMMENTS AND SPECULATIONS

The results of the studies reviewed above suggest that the long-range extension of central axons into the PNS grafts that are inserted into the adult rat CNS depends upon (1) injury to the neuron and (2) the presence in the graft of nonneuronal components of the PNS that have been deprived of their normal contact with axons. Because in the nongrafted but injured adult mammalian CNS there is no substantial elongation of regenerating axons, it can be assumed that axotomy alone can only induce a local outgrowth from the stump or collaterals of the injured cell. The conversion of such early local responses into long-range axonal extension must therefore depend on environmental conditions that permit or facilitate elongation. On the other hand, because the PNS grafts appear to become innervated almost exclusively by injured cells, it is likely that released putative growth-promoting molecules and surface components of these grafts are effective only for those neurons whose regulatory mechanisms have also been signaled appropriately by intrinsic membrane changes and/or retrogradely transported axonal messages consequent to the injury. The demonstration that a heterogeneous population of CNS neurons responds successfully to such intrinsic and extrinsic influences indicates there are nerve cells in the adult mammalian CNS that are capable of responding to injury by replicating normal developmental growth processes that lead to the formation of the short and long fiber projections that link cells within the brain and spinal cord. Furthermore, the marked increase in the number of central fibers that can be induced to regenerate into PNS grafts by a concomitant lesion to another process of these injured neurons (Richardson & Issa, 1984a) suggests the possibility that other cells in the nervous system may also be endowed with metabolic reserves capable of supporting a more vigorous and effective regenerative response than hitherto suspected for these experiments. The reshaping and unusual lengths attained by some of the fibers that grow along PNS grafts underlines remarkable remodeling effects of the environment that surrounds these axons as well as the pliability of certain neurons in the mature mammalian brain.

It is puzzling that the regrowth capabilities of both peripheral and central neurons are not expressed when their axons are injured within the CNS. Molecules with neurotrophic effects have been isolated from wounds in the

nongrafted brain even in the absence of appreciable axonal regeneration (Nieto-Sampedro et al., 1982), and it seems possible that fiber elongation in the mature CNS is, among other reasons, either prevented by local inhibitory effects or requires additional conditions such as the presence of other molecules and an appropriate glial or matrix substrate. In this regard it is interesting that axonal growth along CNS glia is possible on immature cells cultured *in vitro* (Lindsay, 1979; Noble, Fok-Seang, & Cohen, 1984) as well as during development (Silver & Sidman, 1980) or within fetal brain transplants (Björklund & Stenevi, 1984). Axonal elongation is, however, curtailed in denervated (Reier, Stensaas, & Guth, 1983), damaged (Guth, 1974; Windle, 1956), or transplanted (Aguayo et al., 1978; Weinberg & Spencer, 1979) adult neuroglia, suggesting that the nonneuronal environment of the CNS undergoes changes with maturation or injury that affect long-range axonal extension. It is possible that some of the differences in properties of the immature and adult neuroglial environment of the CNS reflect a maturational need to narrow the range of neuronal fluctuations in connectivity in developmentally established neuronal circuitries. In the mature brain, neuroglia and other elements may play a role in the limiting of such fluctuations to circumscribed, short-range changes in the extent of neuronal processes and the formation of synapses; it is conceivable that the determinants of such restricting influences may also curtail regrowth within the injured adult brain.

A greater awareness of the remarkable plasticity of neurons in the adult mammalian CNS and the availability of new research techniques should provide the means to understand these issues better. Currently, the molecular basis of the interactions that determine the success or failure of axonal extension after injury to the PNS and CNS is now being investigated in several laboratories. Molecules within the microenvironment and targets of neurons (Barde, Edgar, & Thoenen, 1983; Levi-Montalcini, 1983; Varon et al., 1983–1984), sheath cell surfaces, and the extracellular matrix (Carbonetto, 1984; Lander, Tomaselli, Calof, & Reichardt, 1983; Letourneau, Ray, & Bernfeld, 1980; Matthew & Patterson, 1983) can be isolated, identified, and tested to define their role in axonal regeneration. Some of these molecules should eventually prove to assist the reconstitution of neuronal connectivity by influencing the expression of genes (Graftstein & McQuarrie, 1978; Skene & Willard, 1981), growth cone activities (Bray & Bunge, 1973; Lasek, McQuarrie, & Wujek, 1981), axonal guidance (Gundersen & Barrett, 1980), and synaptogenesis. The identification of the molecular mechanisms underlining these cellular events remains the main key to the development of strategies for the repair of the damaged nervous system.

"From this it may be inferred that, if experimental neurology is some day to supply artificially the deficiencies in question, it must accomplish these two objects: it must give to the sprouts, by means of adequate alimentation, a vigorous capacity for growth; and, place in front of the disoriented nerve cones and in the thickness of the tracts of the white matter and neuronic foci, specific orienting substances" (Ramón y Cajal, 1928, p. 738, italics added).

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