though LGd neurons received morphologically normal synaptic input from the single remaining eye. This study further emphasizes that the binocular competition critical for the initial formation of the normal visual system does not require visual experience since it exercises its influence before birth. Finally, the dependence of normal development on prenatal binocular competition is selective; segregation of the LGd into magno- and parvocellular moieties and the laminar distribution of their terminals in the cerebral cortex developed normally in all experimental animals. This example of how an error in development of a single structure can alter distant but related structures in the complex primate brain may offer insight into various abnormalities of lamination or connections that occur in congenital malformations in humans.

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12. The smaller size of the LGd in the monkeys enucleated at later gestational ages may be due to neuronal atrophy, loss of neurons, or both. This effect may be related to the higher dependence of already committed, more mature LGd neurons on the proper retinal input.
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## Axonal Elongation into Peripheral Nervous System "Bridges" After Central Nervous System Injury in Adult Rats

Abstract. *The origin, termination, and length of axonal growth after focal central nervous system injury was examined in adult rats by means of a new experimental model. When peripheral nerve segments were used as "bridges" between the medulla and spinal cord, axons from neurons at both these levels grew approximately 30 millimeters. The regenerative potential of these central neurons seems to be expressed when the central nervous system glial environment is changed to that of the peripheral nervous system.*

The inability of axons to elongate for more than a few millimeters within the damaged central nervous system (CNS) is believed to be a fundamental feature of the failure of regeneration in the brain and spinal cord of adult mammals. On the other hand, axons from transected peripheral nerves successfully regrow over long distances when they become associated with Schwann cells in the distal nerve stump or in a nerve graft. Although many mechanisms are probably involved (*1*), there is increasing evidence that differences in the capacity of certain damaged axons to elongate in the CNS and peripheral nervous system (PNS) are more dependent on the environment in which these axons are located than upon intrinsic properties of neurons. This hypothesis, proposed by Cajal (*2*), has received additional support from recent studies. (i) Experiments in adult mammals have demonstrated that, although PNS axons, with a known capacity to regenerate, fail to lengthen in a milieu of CNS glia (*3*), the axons from intrinsic CNS neurons grow into peripheral nerve segments transplanted into the transected spinal cord (*4*). (ii) Tissue culture investigations show that Schwann cells, other nonneuronal cells, and factors in the culture media exert various trophic influences on neurons (*5*). Using a new experimental model, we provide evidence that axons from nerve cells in the injured spinal cord and brainstem can elongate for unprecedented distances when the CNS glial environment is replaced by that of peripheral nerves.

In adult Sprague-Dawley rats weighing between 250 and 350 g, segments of autologous sciatic nerve 35 mm long were used as "bridges" between the medulla oblongata and the lower cervical or upper thoracic spinal cord (Fig. 1A). These "bridges" were placed extraspinally in the subcutaneous tissues along the back of the animal. One end of the graft was inserted through a laminectomy into the dorsolateral spinal cord, and the other end was introduced into the lower medulla through a small opening made across the craniocervical junction. We ensured the penetration of the graft endings into the CNS by using a glass rod with a 150-μm tip. Retaining 10-0 sutures were placed at both ends of the graft. The grafting procedure resulted in local damage at the site of insertion of the nerve into the dorsal brainstem and spinal cord, but the rest of the neuraxis was left intact. Because axons from spi-

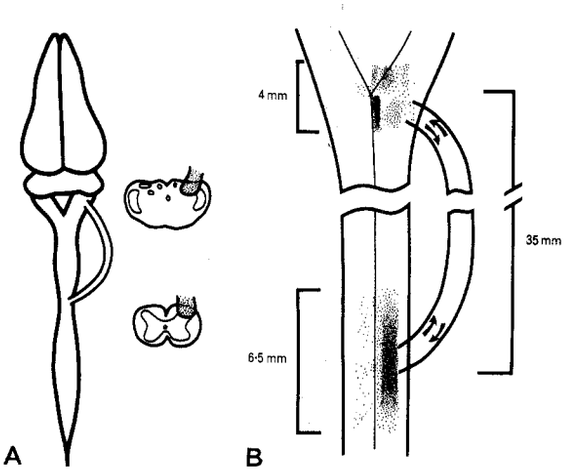


Fig. 1. (A) Diagram of the dorsal surface of the rat CNS, showing a peripheral nerve "bridge" linking the medulla and the thoracic spinal cord. Cross sections depict the region where the ends of the nerve graft were inserted. The origin of axons innervating the graft was determined by retrograde labeling with HRP. Axonal elongation was measured between the site of HRP application and that of the labeled cells in the CNS. For this purpose, when neurons were sought in the medulla, the tracer was applied after sectioning the nerve at a level situated approximately 30 mm from the brainstem and 5 mm from its caudal insertion into the cord (group A, Table 1). Conversely, when the growth of axons from the spinal neurons was assessed, the graft was cut close to the brainstem (group B, Table 1). The short stumps of these nerve grafts were also used for anterograde labeling. (B) Approximate rostrocaudal position of 1472 labeled CNS neurons (dots) demonstrated in seven grafted rats. In the brainstem the territory occupied by 450 of these cells extended along 4 mm, whereas 1022 labeled neurons were scattered along a 6.5-mm segment of the spinal cord.


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nal roots may also innervate these grafts (*4*), the two ipsilateral dorsal root ganglia neighboring the site of the graft insertion were avulsed. Animals survived without apparent neurologic deficit and were killed by systemic perfusion of fixative between 22 and 30 weeks after grafting (*4*). At autopsy, the ends of the grafts were found to be in gross continuity with the brainstem and spinal cord. The light and electron microscope cross-sectional appearance of the midportion of each graft was similar to that of a regenerated peripheral nerve and contained numerous myelinated and unmyelinated fibers ensheathed by Schwann cells.

In seven rats, the cells of origin and the termination fields of axons traveling in the regenerated graft were determined by means of retrograde and anterograde transport of horseradish peroxidase (HRP) (*6*) applied to the tips of the transected graft (Table 1, groups A and B). Two additional animals were used only for the study of the terminal course of fibers. In all rats the HRP was applied to the graft extraspinally, thereby minimizing the possibility of a spurious labeling of neurons due to interstitial spread of the tracer into the CNS. In addition, we investigated extracellular diffusion of the label in three control rats (Table 1, group C) by crushing the graft with jeweler's forceps 5 mm from the rostral and caudal attachments of the "bridge" and applying HRP to the nerve in its midportion. For both retrograde and anterograde studies, HRP (20 percent, Sigma VI) was applied to the tips of the cut nerve grafts in small Gelfoam pads for 1 to 2 hours. The exposed portion of the nerve was laid over a Parafilm sheet and covered with Vaseline to avoid direct contamination of the tissues by the tracer. Rats were killed 24 to 48 hours after HRP application. Serial cryostat sections, 20 to 40 μm thick, were obtained from the brainstem and spinal cord and reacted with tetramethylbenzidine and $H_2O_2$ (*4*, *6*).

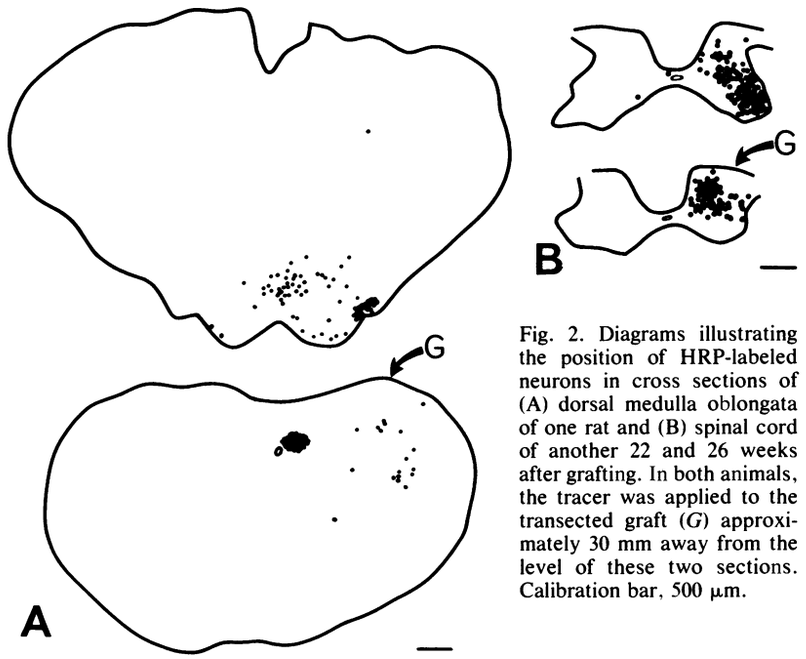


Fig. 2. Diagrams illustrating the position of HRP-labeled neurons in cross sections of (A) dorsal medulla oblongata of one rat and (B) spinal cord of another 22 and 26 weeks after grafting. In both animals, the tracer was applied to the transected graft (*G*) approximately 30 mm away from the level of these two sections. Calibration bar, 500 μm.

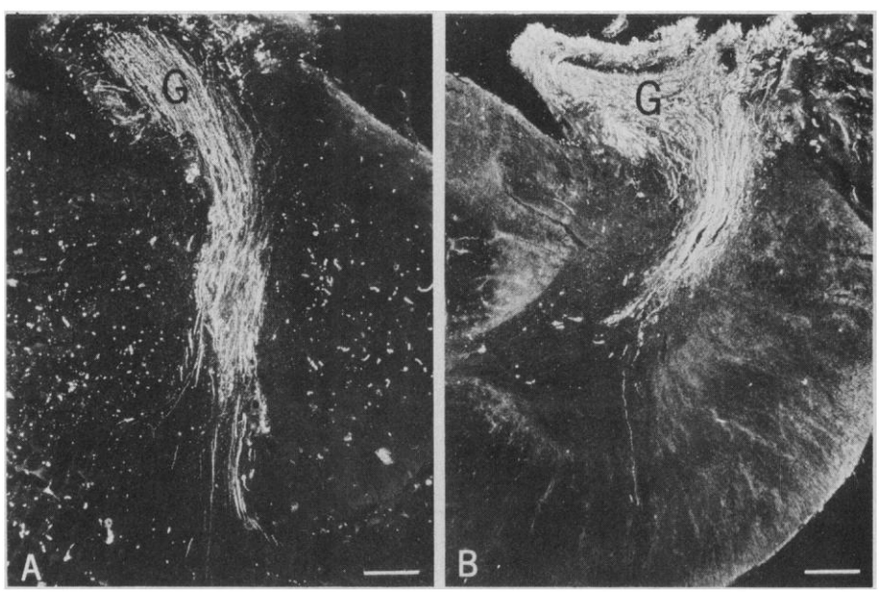


Fig. 3. Cross sections of (A) the medulla oblongata and (B) the spinal cord, illustrating the course of axons at the junction of the PNS graft (*G*) and the CNS tissue in two animals 26 and 30 weeks after grafting (dark-field micrographs, 48 hours after labeling with HRP). Bar, 200 μm.

In the seven animals used to demonstrate both retrograde and anterograde labeling, 450 neurons were labeled in the medulla (Figs. 1B and 2A) (Table 1) and 1022 in the gray matter of the spinal cord (Figs. 1B and 2B) (Table 1). Labeled neurons of various sizes were scattered in the neighborhood of the graft insertions at both these levels. Most of these cells were located ipsilaterally to the graft, along a territory that extended rostrocaudally for approximately 4 mm in the brainstem and 6.5 mm in the spinal cord (Fig. 1B). The following brainstem nuclei (*7*) contained labeled neurons: (i) nucleus intercalatus; (ii) nucleus reticularis lateralis; (iii) nucleus reticularis medullae oblongatae pars dorsalis; (iv) nucleus olivaris inferior; (v) nucleus accessorius olivaris dorsalis; (vi) nucleus raphe pallidus; (vii) nucleus tractus spinalis nervi trigemini; (viii) nucleus reticularis paramedianus; (ix) nucleus reticularis medullae oblongatae pars ventralis; and (x) nuclei gracilis and cuneatus. In the spinal cord the HRP-labeled neurons tended to be evenly distributed within the gray matter ipsilateral to the graft, but the superficial laminae of the dorsal horn contained few labeled cells. Labeled neurons were also found in dorsal root ganglia above and below the insertions of the graft.

Because only four labeled cells were demonstrated in the control animals (Table 1, group C), we conclude that the majority of neurons in the experimental groups were not labeled spuriously by diffusion along the graft or hematogenous extravasation.

In the nine rats in which anterograde transport of HRP was also investigated, the tracer was applied to the stump of the graft, approximately 5 mm from the neuraxis. Labeled axons from the graft were shown to have penetrated the spinal cord and medulla only for approximately 2 mm, a distance that represents

more than half the normal width of both these structures (Fig. 3, A and B). By light and electron microscope examination it was documented that, along their course within the spinal cord or medulla, many of these fibers were ensheathed by Schwann cells that had migrated into the CNS tissues; other axons were surrounded by glial processes. Many of the penetrating fibers terminated in close proximity to CNS neurons, but it is not known if they formed synapses because connectivity was not investigated.

The results of these studies indicate that some of the axons within the PNS "bridges" originate from neurons in the spinal cord and brainstem. Under the conditions of these experiments such axons have been shown to be capable of a growth that exceeds 30 mm, a distance that could be equal to or greater than the length of axons from some of these neurons in the intact rat.

This new experimental model has several advantages for studies of regeneration in the living animal. (i) By selectively positioning the graft, it is possible to direct the course of axons from and into specific regions of the CNS. (ii) The origin, length, and termination of axons within the graft can be documented. (iii) The long extraspinal course of these grafts should facilitate the electrophysiologic investigation of axons within the "bridges." (iv) Because these animals are not paralyzed and retain bowel and bladder control, in contrast to the case with animals grafted after complete transection (*4*), their care and survival is greatly facilitated. (v) If it is eventually demonstrated that axons from CNS neurons establish functional connections with cells in the target regions to which they have been directed, it may be possible to devise experimental strategies for selected populations of axons to bypass damaged CNS tissue and connect with specific groups of neurons at a distance.

Whether the central axons in the bridging grafts originate by regrowth of damaged CNS fibers or by sprouting from uninjured neurons in the proximity of the graft endings, or both, could not be decided in this study. Regardless of the mechanisms involved, the remarkable elongation of axons in these animals suggests that PNS tissues exert a striking facilitation of the growth of axons from central neurons after CNS injury. Even though the cells of origin varied in size and were distributed widely within the CNS areas neighboring the site of entry of the graft, it remains to be determined whether they constitute a special neuronal population or whether their responses are examples of a more general potential for regeneration. Our experiments also demonstrate that regenerating axons only penetrate the damaged CNS for short distances. It is possible that elongation fails in the CNS because the central neural environment lacks the growth-promoting properties of the PNS or because there are changes in the injured CNS that inhibit fiber growth (*8*). If the conclusion is corroborated that interactions between axons and their immediate environment play a determinant role in the success or failure of regeneration, the study of the molecular basis of these interdependencies may lead to better experimental approaches to promote CNS regeneration.

Table 1. Boldface numbers represent the numbers of spinal and medullary neurons labeled in each animal after the application of HRP to the caudal end of the graft, approximately 30 mm away from the medulla (group A) or to the rostral end of the graft at the same distance from the spinal cord (group B). Nonboldface numbers designate cells labeled by HRP applied to the shorter, 5-mm long, remaining stump of the bridging nerve. Group C represents findings in control rats in which the regenerated grafts were crushed approximately 30 minutes before HRP application.

| Labeled neurons | | Weeks after grafting |
|---|---|---|
| Spinal cord | Medulla | |
| | *Group A* | |
| 53 | **162** | 22 |
| 48 | **13** | 24 |
| 493 | **24** | 25 |
| 112 | **62** | 30 |
| | *Group B* | |
| **247** | 159 | 26 |
| **69** | 9 | 27 |
| **0** | 21 | 27 |
| | *Group C* | |
| 4 | 0 | 25 |
| 0 | 0 | 25 |
| 0 | 0 | 25 |


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## Spinal Motoneuron Recruitment in Man: Rank Deordering with Direction but Not with Speed of Voluntary Movement


Abstract. *Single motor units in human interosseous muscle are recruited in order from small to large in slow or brisk voluntary abduction of the index finger. When the same muscle acts as a synergist as opposed to a prime mover, about 8 percent of the unit pairs consistently reversed their recruitment order. Motor commands appear to be patterned in terms of movements rather than muscles and to involve different connectivities to the motoneuron pool of a muscle executing movements in different directions.*


The voluntary motor commands to a pool of spinal motoneurons can be analyzed in intact humans by recording the action potentials of single motor units with fine metal electrodes inserted through the skin (*1*). In graded voluntary contractions of a muscle, motor units are recruited in a stereotyped order at reproducible levels of muscle force (*2*). This is usually referred to as Henneman's size principle (*3*) because the recruitment sequence is correlated with several graded properties such as the size of the motoneuron and the diameter of its motor axon (*4*). The orderly recruitment of motor units that prevails when the muscle is used as a prime mover undergoes significant changes when the same muscle contracts as a synergist in another movement. In contrast to the concept of a fixed recruitment order, it has been occasionally reported that human subjects, when provided with visual or auditory feedback from their active motor units, can learn to voluntarily activate or suppress any arbitrarily chosen motor unit



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