Anatomical Plasticity and Sparing of Function After Spinal Cord Damage in Neonatal Cats

Abstract. *Spinal cord damage in neonatal cats has different effects on different spinal pathways. Corticospinal projections exhibit anatomical plasticity, forming an aberrant pathway that bypasses the lesion. In contrast, brainstem-spinal pathways undergo massive retrograde degeneration. Neither of these responses occurs in adult cats. Sparing of motor function is found in cats operated on as neonates but not in cats operated on as adults, and appears to depend on the plasticity of the corticospinal tract.*

Brain damage in infants often results in less severe neurological impairments than those which follow brain damage in adults (*1*). There may be sparing of function, greater recovery of function, or both. The biological basis for this "infant lesion effect" is uncertain. Growth of undamaged axons is one mechanism suspected of mediating recovery. Such growth is greater after lesions of the young nervous system (*2*) than of the adult (*3*). On the other hand, brain damage in infants results in the death of many more nerve cell bodies than in adults (*4*), and this marked loss might be expected to result in less complete recovery. In the present report we show (i) that the effects of neonatal spinal hemisection on different spinal pathways differ dramatically; (ii) that neonatal but not adult spinal lesions are followed by anatomical sparing and considerable plasticity of at least one major pathway in the central nervous system; and (iii) that this anatomical plasticity results in greater recovery and sparing of motor function than when the same lesion is made in adulthood.

Partial hemisections sparing both dorsal columns were made in 20 kittens on the day of birth and in ten adult cats at the first lumbar or cervical segment. Motor development was studied in the kittens with lesions and in normal littermates. Motor recovery was studied in the adult cats. Long-term recovery of motor function (at least 1 year after surgery) was studied in both groups and compared. We evaluated monopedal hopping, proprioceptive placing, tactile placing, vestibular placing, and other postural reflexes (*5*). Accuracy of limb placement during locomotion was tested on a series of complex runways (a grid of rungs 2 cm in diameter, narrow parallel bars, and an obstacle course). A 30-cm-wide runway was used as a control to test locomotion without the requirement of accuracy. Speed of crossing was measured and errors in limb placement were counted. Kinematics of locomotion and reflexes were studied by analyzing stick figures drawn from high-speed films with a planimeter interfaced with a PDP/11 computer. Joint angles were measured and the excursion of each joint and the duration of movements were calculated.

Most postural reflexes developed in the animals lesioned neonatally and recovered in the animals lesioned as adults. Both groups displayed accurate limb placement during locomotion but were slightly deficient in this respect compared to control animals. The most striking difference between the two groups was the sparing of tactile placing in all the animals lesioned at birth and a permanent loss of this reflex in the animals lesioned as adults (Fig. 1a). Tactile placing was spared in forward, backward, medial, and lateral directions in the forelimbs and hind limbs corresponding to the side of the lesion in animals with cervical lesions and in the hind limbs of animals with thoracic lesions. The responses (Fig. 1b) were hypermetric and slow, however, and therefore lacked the speed and economy of the normal response. Hypermetria and slowness are characteristic of hind limb tactile placing in 3- to 6-week-old kittens (*6*). Thus, although there is a sparing of function when the spinal cord is hemisected neonatally, the spared response never matures completely.

Tactile placing in normal cats is mediated by the contralateral sensorimotor cortex (through the crossed corticospinal tract), red nucleus, cerebellum, and vestibular nuclei (*7*). Spinal hemisection in adults destroys the corticospinal, rubrospinal, vestibulospinal, and other brainstem-spinal tracts. Thus it is not surprising that although considerable recovery of motor function occurs in cats lesioned as adults tactile placing is permanently abolished. Since sparing of tactile placing in animals lesioned at birth might reflect a reorganization of motor pathways descending to the cord, we investigated the origins of these pathways by injecting horseradish peroxidase (HRP) (1 to 5 μl of a 30 to 50 percent solution) into the spinal cord several segments caudal to the hemisection. Seven cats operated on as adults were injected with HRP 3 to 18 months after

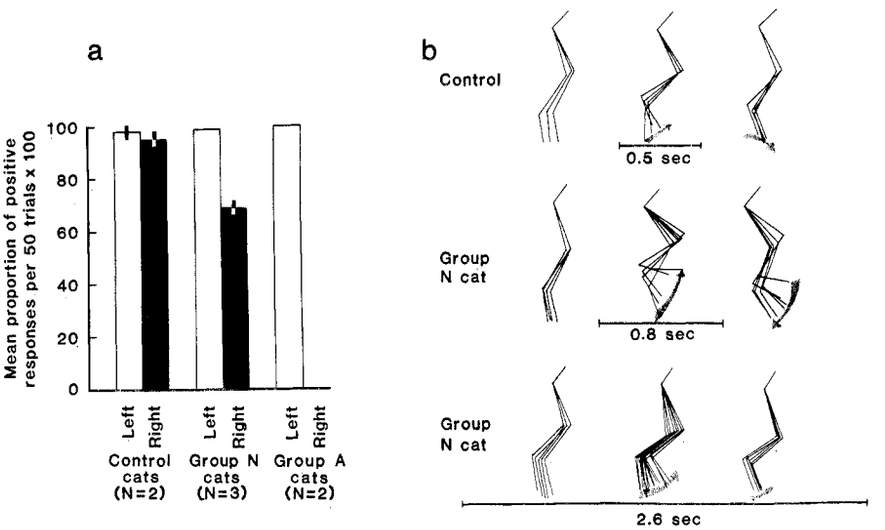

Fig. 1 (a). Frequency of tactile placing of hind limbs. Control animals responded 100 percent or almost 100 percent of the time, with no statistically significant differences between limbs. Group A cats, even 1 year after surgery, never responded with the limb ipsilateral to the lesion (the right limb), but responded in 100 percent of the trials when the control (left) limb was stimulated. Group N cats responded with both limbs, thus displaying sparing of function. (b) Kinematics of tactile placing of hind limbs. The stick figures are drawn directly from high-speed film (64 frames per second). The limbs were shaved and five bony prominences were marked. The position of the limb in every other frame was drawn by connecting the five marks. Three phases of tactile placing are shown: stimulus (premovement), lifting (flexion), and descent (extension) to gain support. The number of tracings in each phase reflects its duration. Bars beneath each series of tracings indicate the total time required for tactile placing. The middle series of tracings is an example of a hypermetric response of the hind limb. The bottom series, from another animal, displays prolongation of the response without hypermetria.

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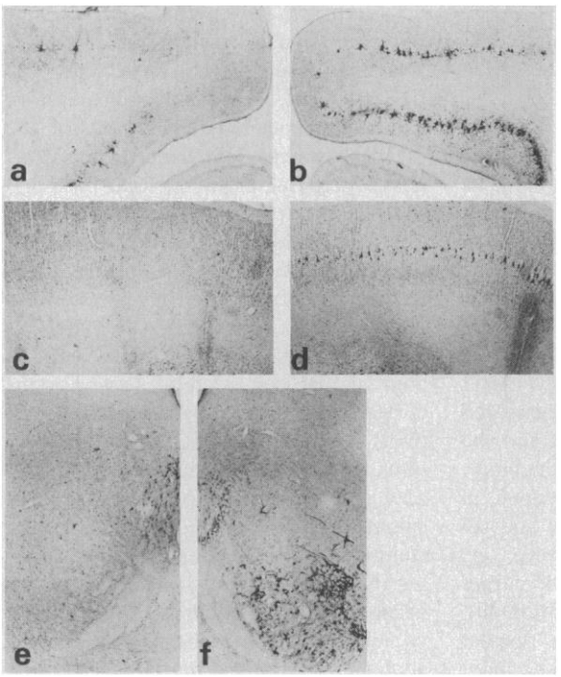

Fig. 2. Sections of motor cortex and brainstem from cats with hemisected spinal cords. Horseradish peroxidase was injected into spinal segments caudal to the lesion. (a and b) Group N cat motor cortex projecting to the lesion side (a) and to the normal side (b). (c and d) Group A cat motor cortex projecting to the lesion side (no cells are labeled) (c) and to the normal side (d). (e and f) Brainstem from the same group N cat represented in (a) and (b), showing the red nucleus projecting to the cut side of the cord (e) and to the normal side (f).

surgery (group A), and ten cats operated on as neonates and displaying spared tactile placing were injected 12 to 18 months after surgery (group N). Horseradish peroxidase is transported retrogradely to the cell bodies of axons extending into or through the injected area of spinal cord. We allowed 72 hours for retrograde transport of the HRP and then anesthetized the animals and perfused them intracardially. The brains were removed and sectioned and tissue was reacted for HRP by the TMB method (*8*). The labeled cells in the brainstem and cortex were mapped and the lesions were confirmed histologically.

The distribution of labeled cells in Deiters' nucleus (major origin of the uncrossed vestibulospinal tract) and the red nucleus (origin of the crossed rubrospinal tract) was the same in both groups. On the side of the brainstem projecting to the intact side of the cord, labeled neurons were distributed normally. On the side corresponding to the lesion, no labeled cells were seen (Fig. 2e). Furthermore, in group N animals there was a massive loss of cell bodies in the red nucleus and Deiters' nucleus, presumably due to retrograde degeneration. Thus, sparing of function was not associated with anatomical reorganization of the vestibulospinal or rubrospinal tracts. In the cortex, however, 700 to 1000 labeled cells were found contralateral to the lesion in each group N animal (Fig. 2a). In contrast, almost no labeling was found in the contralateral cortices of group A animals (Fig. 2c) (*9*). The labeled cells were primarily in area 4 (motor cortex), with a small number in areas 3, 1, 2, and 5, corresponding to the normal distribution; all were in lamina V and were morphologically indistinguishable from normal cells. Since the lesions were comparable in the two groups, this finding indicates that some corticospinal projections survived in group N but not group A cats.

To determine whether the sparing of cortical projections was responsible for the sparing of tactile placing, we removed the sensorimotor cortex contralateral to the lesion in four group N animals (with spared tactile placing) after they reached maturity. This also allowed us to distinguish the spared tactile placing response from related spinal reflexes not dependent on the cortex (*10*). The cortical ablation abolished the spared tactile placing in all four animals, as it always does in normal adult cats (*11*). Other reflex responses, such as proprioceptive placing (which is not dependent on the cortex), could still be elicited easily. This suggests that the sparing of function was dependent on the cortex containing the spared cells and also that it did not represent a spinal reflex.

Preliminary studies show that, in cats subjected to spinal cord damage as neonates, the presence of a corticospinal projection (which was abolished by the same lesion made in adults) is not attributable to regeneration (*12*). Rather, the corticospinal fibers reached the lumbar cord by taking an aberrant path around the lesion site. Thus, sparing of function was dependent on the anatomical plasticity of the corticospinal tract. The failure of limb placing responses to mature to an adult level may be related to the lack of plasticity displayed by brainstem-spinal pathways. These motor tracts failed to survive neonatal hemisection altogether. The different responses of these pathways cannot be explained by the age of the animal. Rather, differences in the relative maturity of the corticospinal and brainstem-spinal pathways at the time the lesion was made may explain the differences in their anatomical response. Many brainstem-spinal tracts develop early and their axons are present in the spinal cord at birth. The corticospinal tract develops late. Red nucleus and Deiters' nucleus cells were therefore axotomized by the lesion while corticospinal cells were not (*12*). Thus, sparing of function is not a ubiquitous feature of the nervous system following neonatal damage. Such sparing may be dependent on the survival of pathways that develop late and thereby escape the effects of direct damage.

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5. The stimulus for monopedal hopping is displacement of the animal's center of gravity from its base of support. In response, the animal lifts the appropriate limb and replaces it beneath the new center of gravity so that support is regained. The placing response consists of flexion of the limb to clear the edge of a table, and subsequent extension and placement of the paw on the surface. The stimulus for tactile placing is hair bending only. The stimulus for proprioceptive placing includes hair bending, skin deformation, joint bending, and muscle stretching. The stimulus for vestibular placing is lowering the animal rapidly toward a horizontal surface; the normal response is extension of the limbs for support.
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9. In the cat the uncrossed ventral component of the corticospinal tract does not descend beyond thoracic levels. This partly explains the lack of retrograde transport through uncrossed pathways to the cortex contralateral to the lesion in group A cats. There is an uncrossed lateral component, however, and it is probably responsible for the occasional labeling of cells contralateral to the lesion in group A cats (< 40 cells per animal). The possibility exists, therefore, that the uncrossed corticospinal tract expands (or fails to retract) in response to neonatal hemisection. This possibility has not yet been explored. Preliminary degeneration studies demonstrate, however, that the crossed corticospinal tract grows around and descends caudal to the lesion site.
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Presynaptic Location and Axonal Transport of β_1-Adrenoreceptors in the Rat Brain

Abstract. *Interruption of the ascending noradrenergic neurons of the locus coeruleus in the rat forebrain with 6-hydroxydopamine produced a progressive accumulation, proximal to the lesion, of tritiated dihydroalprenolol binding activity over 2 days. This accumulation could be blocked by interrupting the neurons closer to their cell bodies. Competitive binding studies with the β_2 agonist Zinterol suggested that the accumulated β-receptors were primarily of the β_1 subtype. These results suggest that, in the rat brain, some β_1-adrenoreceptors are located in presynaptic, noradrenergic locus coeruleus neurons and are transported in their axons.*

The development of techniques for defining subtypes of various adrenoreceptors has led to a search for the cellular location and function of these receptors. For example, most studies point to a predominantly postsynaptic location of β_1-adrenoreceptors in the rat cerebral cortex, although it has been difficult to delineate the types of cells on which such receptors are located (*1*). Because cholinergic (muscarinic and nicotinic), cholecystokinin, and opioid receptors undergo axonal transport in presynaptic nerves (*2*), it seems that adrenoreceptors might also be transported in presynaptic noradrenergic neurons. Norepinephrine, its synthetic enzymes tyrosine hydroxylase and dopamine β-hydroxylase, and other proteins are transported in the ascending noradrenergic neurons of the locus coeruleus, which innervate the rat cerebral cortex (*3*). It is reported here that binding sites for the β-adrenoreceptor antagonist dihydroalprenolol (DHA) also move in these neurons, suggesting that β-adrenoreceptors are located in presynaptic neurons and undergo axonal transport in rat brain.

Adult male Sprague-Dawley rats (200 to 300 g) were injected stereotactically in the left forebrain (9.0 mm anterior to the intra-aural line, 2.0 mm left of the midline, and 8.0 mm below the skull's surface) (*4*) with 2 μl of 6-hydroxydopamine (6-OHDA) (4 μg/μl in distilled water containing 0.1 μg of ascorbic acid per microliter). Such injections produce a relatively selective interruption of the ascending noradrenergic neurons of the locus coeruleus and cause an accumulation of tyrosine hydroxylase and dopamine β-hydroxylase activities proximal to the lesion (*5*). The rats were decapitated at various times after the injections, and 2-mm-thick sections of anterior hypothalamus (AH) proximal to the lesion site (5.0 to 7.0 mm anterior to the intra-aural line) were removed and bisected into injected (left) and uninjected (right) halves (*5*).

In three preliminary experiments membrane fractions were prepared from AH sections pooled from uninjected rats and assayed for β-adrenoreceptor binding by the method of Torda *et al.* (*6*). The AH membranes (2 to 3 mg/ml, final concentration) were suspended in 75 m*M* tris-HCl (*p*H 7.7) containing 25 m*M* $MgCl_2$, and seven different concentrations (0.5 to 40 n*M*, final concentration) of [^{3}H]DHA (45.0 Ci/mmole, New England Nuclear) were added to a total volume of 150 μl. Incubation of triplicate samples was carried to equilibrium at 35°C for 15 minutes and terminated by vacuum filtration on Whatman GF/C filters with four washes of ice-cold 0.9 percent saline. The filters were dried and the radio activity was counted by liquid scintillation spectrometry with 33 percent efficiency. Specific binding, determined with 10 μ*M* (−)propanolol, ranged from 45 to 60 percent.

In these control rats (eight to ten per experiment), [^{3}H]DHA bound to AH membranes with an affinity (K_d) of 7.2 ± 1.5 n*M* and a maximal density (B_{max}) of 120 ± 10 fmole per milligram of protein, as determined by Scatchard analysis of the binding data (*7*). Two days after the 6-OHDA injections, maximal [^{3}H]DHA binding proximal to the left forebrain injection sites was 35 percent higher ($P < .05$) than in comparable, uninjected right AH (Fig. 1). This increase in binding was primarily attributable to an increase in the density of β-receptors, since there was a decrease in the apparent binding affinity on the lesioned side. Thus the increased receptor density proximal to the lesions appeared to be due to the accumulation of mobile β-adrenoreceptors, that is, to anterograde axonal transport. This increase in binding proximal to the lesion site was linear over a maximum of 2 days (the increase was 25 percent at 24 hours), after which the difference from the controls remained constant until 5 days.

The apparent accumulation of β-receptor binding activity could have been attributable to increased numbers of receptors formed in postsynaptic hypothalamic cells as a result of denervation caused by the lesions. To evaluate this possibility, ten rats were injected simultaneously with 6-OHDA in the left forebrain and the left median forebrain bundle in the posterior hypothalamus (4.0 mm anterior to the intra-aural line, 2.0 mm left of the midline, and 8.0 mm below the skull's surface). Two days later the rats were decapitated. Such injections interrupt ascending noradrenergic neurons from brainstem nuclei and block axonal transport of norepinephrine, its synthetic enzymes, and other proteins in ascending locus coeruleus neurons (*3, 5*). The injections prevented the accumulation of [^{3}H]DHA binding activity proximal to the more distal forebrain injection site (B_{max} for

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