An Anatomical, Electrophysiological, and Behavioral Study of the Effects of Partial Mid-Thoracic Spinal Cord Lesions on Neonatal and Adult Rats

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AN ANATOMICAL, ELECTROPHYSIOLOGICAL, AND BEHAVIORAL STUDY OF
THE EFFECTS OF PARTIAL MID-THORACIC SPINAL CORD LESIONS ON
NEONATAL AND ADULT RATS

BY

MICHAEL F. DAUZVARDIS

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

November
1988
DEDICATION

To Mary, Ricky, Alex, Buffy, Yankee, Merle, and Kugie.
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I wish to express my thanks to Dr. Anthony J. Castro. His guidance and patience were invaluable.

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I thank all of the faculty and staff for their input and technical assistance, especially in those hectic final weeks of compiling my work.

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Finally, I thank my family for postponing their lives on my behalf.
VITA

The author, Michael F. Dauzvardis, was born on April 14, 1954, in Chicago, Illinois.

In 1972 he graduated from Lockport Township High School in Lockport, Illinois, and in the fall of that year he entered Lewis University in Romeoville, Illinois. He graduated from Lewis in January, 1977, receiving a Bachelor of Arts degree with a double major in Chemistry and Biology. He began his graduate studies in the Department of Anatomy at Loyola University Stritch School of Medicine, Maywood, Illinois, in January, 1982. While at Loyola, he received a Basic Science Fellowship and taught in the gross anatomy, histology, and neuroscience courses. In 1984 he assisted in teaching gross anatomy to occupational therapists in the Department of Anatomy at Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois.

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The author is married to Mary Dauzvardis and they have two children, Alex and Ricky.
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INTRODUCTION

Numerous studies of axonal regeneration and functional recovery after spinal transection in mammals have generally led to inconclusive results (Clemente, 1964; Puchala, 1977; de la Torre, 1981). Several experiments which seemed to present convincing evidence of spinal regeneration (Feringa et al., 1975; Sugar and Gerard, 1940; Kao et al., 1977) were subsequently questioned or invalidated (Barnard and Carpenter, 1950; Feringa et al., 1976). Problems in verifying spinal cord regeneration stem not only from histological misinterpretation, since most early studies relied on undependable silver techniques, but also from the misinterpretation of behavioral results. For example, it has been demonstrated that if as few as 5% of the normal complement of fibers are spared, some form of locomotor activity may return (Eidelberg et al., 1977; Feringa et al., 1976; Windle, Smart, and Beers, 1958). Because of these inherent technical difficulties, literature reports on spinal regeneration and behavioral restitution after transection are often contradictory.

In contrast to the typically disputed findings of studies concerning axonal regeneration and recovery of function, reports of neuroanatomical remodelling (plasticity) after spinal cord injury are numerous and widely accepted (Goldberger and Murray, 1974; Prendergast and Stelzner, 1976; Murray and Goldberger, 1976; Schreyer and Jones,
Distinguished from the regeneration of severed axons across an area of lesion, plasticity is a rather general term used to refer to various forms of remodelling including axonal sprouting proximal to the site of injury (Goldberger and Murray, 1974; Prendergast and Misantone, 1980), increased collateral growth after injury (Prendergast et al., 1976), or a rerouting of later growing pathways (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983; Bregman and Goldberger, 1983; Dauzvardis and Castro, 1985). Although all of these phenomena have been documented following lesions to a wide variety of areas in the adult CNS (see Cotman et al., 1981 for reviews), plasticity in the mammalian spinal cord appears to be much more prevalent following neonatal injury (Goldberger, Gorio, and Murray, 1986). In addition, functional recovery appears to be greater following neonatal as compared to adult spinal cord injury (Goldberger, 1986).

Anatomical reports of the remodelling of cortical projections, specifically the corticospinal tract (CST), after partial spinal cord injury in neonatal rats are particularly relevant to this dissertation (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983; Dauzvardis and Castro, 1985). The CST represents a major pathway involved in the control of discreet forelimb and hindlimb movements (Castro, 1972; Kalil and Schneider, 1975; Reh and Kalil, 1982). The precise topography of the CST system, originating from specific areas of the cortex and projecting to specific levels of the spinal cord, reflects its involvement in the control of discreet limb and digital function.
Additionally, the CST system predominantly develops postnatally. At birth, axons of the rodent CST have only reached upper levels of the spinal cord; growth to lower lumbar levels is achieved the ninth postnatal day (Schreyer and Jones, 1982; Donatelle, 1977; Kort et al., 1985).

The well described anatomical, functional, and developmental characteristics of the rodent CST make it a particularly useful model to study neuronal growth and plasticity. Accordingly, the experiments of this dissertation are designed to examine, using anatomical, electrophysiological, and behavioral techniques, the response of CST axons to partial mid-thoracic spinal cord lesions made in rats of various ages. These lesions were intended to (1) sever the fully formed CST in adult rats; (2) sever the growing CST in six-day old rats; or (3) destroy the path of CST fibers prior to their arrival at thoracic levels in one day-old rats.

In experiment I, using anterograde and retrograde neuronal tracers, a rerouting of CST fibers around T-8 dorsal funicular spinal cord lesions was observed in rats sustaining such lesions at postnatal day (PND-) 1 and as late as PND-6. CST rerouting was not observed in adult lesion animals.

In experiment II, hindlimb movements evoked by intracortical microstimulation (ICMS) techniques suggested the apparent functional integrity of CST fibers that had rerouted and had grown caudal to mid-thoracic dorsal funicular spinal cord lesions sustained by neonatal animals (PND-1 and PND-6). These evoked movements were not observed in adult lesion animals.
In experiment III, a footprint analysis paradigm was used to determine whether age-related differences in CST remodelling would be reflected in a behavioral response. Demonstrating deficits in hindlimb placement while traversing a confined walkway, the adult animals were more affected by partial mid-thoracic spinal cord lesions than were the neonatal animals. These behavioral findings corresponded to the degree of neuroanatomical remodelling and electrophysiological activity observed across age groups in Experiments I and II.
REVIEW OF RELATED LITERATURE
INTRODUCTION

The rodent corticospinal tract (CST) has been used as the basis for many studies addressing axonal growth and response to injury. The CST system represents a useful experimental model for several reasons: (1) It is surgically accessible at its cortical origins as well as its course through the brainstem and spinal cord. (2) The protracted postnatal development of the CST facilitates the design of experiments based on transecting the immature pyramidal fibers or the tissue that the mature tract will eventually occupy. (3) The involvement of the corticospinal system in controlling individual, discreet limb movements and tactile placing is well described, as is its function, based on limb movements evoked by intracortical microstimulation.

THE CORTICOSPINAL SYSTEM: ANATOMY, DEVELOPMENT, AND FUNCTION

Anatomy of the CST

The rodent neocortex is divided into frontal, parietal, temporal and occipital regions (Zilles and Wree, 1985). In mature rats, the bulk of corticospinal projections arise from frontal and parietal somatosensory areas (Wise et al., 1979; Jones and Wise, 1977), specifically from primary and secondary motor and somatosensory cortex.
(Wise et al., 1979; Neafsey et al., 1986). Although corticospinal axons originating from other areas, such as visual cortex, are considered transient and regress in adulthood (Stanfield and O'Leary, 1985), a prominent spinal projection from the visual cortex has been discovered (Miller, 1987). The corticospinal tract arises exclusively from layer V of these cortical areas (Hicks and D'Amato, 1977; Wise and Jones, 1977; Ullan and Artieda, 1981).

The rat motor cortex demonstrates a somatotopic distribution of corticospinal projections. Anatomically (Wise et al., 1979) and electrophysiologically (Sievert and Neafsey, 1982) corticospinal neurons that project to the cervical (i.e., forelimb) areas of the spinal cord occupy a separate and distinct area in the motor cortex as opposed to those which project to lumbar (hindlimb) areas of the cord (see Fig. 1). Therefore, there appears to exist a definite somatotopic pattern with the face, forelimbs, trunk, and hindlimbs represented sequentially forming a rough outline of the body on the surface of the brain in the rat. In addition to the primary forelimb and hindlimb motor areas, a rostral forelimb area can be defined in the rat on the basis of forelimb movements evoked by low threshold intracortical microstimulation (ICMS) (Neafsey and Sievert, 1982) and also by anatomical studies demonstrating a second group of CST neurons projecting to the cervical enlargement (Hicks and D'Amato, 1977; Wise et al., 1979; Neafsey and Sievert, 1982; Donoghue and Wise, 1982). A recent study suggests that a modified version of the entire rat body similar to that found in the primary motor area may also be represented in the rostral frontal cortex (Sievert, 1985).
Projections and Somatotopy  The CST descends ipsilaterally through the brainstem forming a compact bundle along the ventral surface of the medulla (the medullary pyramid) and decussates at the spinal medullary junction. CST fibers are principally located contralaterally in the ventral apex of the dorsal funiculi (King, 1910; Brown, 1971) although a few fibers are found in the same position ipsilaterally (Sievert, 1985; Goodman et al., 1966). A small number of corticospinal fibers have been found bilaterally traversing the lateral funiculi (Sievert, 1985; Donatelle, 1977) and ipsilaterally traversing the ventral funiculi (Vahlsing and Feringa, 1980). A small group of axons coursing in the base of the dorsal horn in cervical segments has also been described (Schreyer and Jones, 1982). Additionally, Bernstein and Stelzner (1983) have observed small numbers of fibers crossing the midline just dorsal to the central canal and terminating in the ipsilateral gray matter. At the level of the pyramids the total unilateral axon count in the corticospinal tract has been estimated at 240,000, with 140,000 of these fibers being unmyelinated (Leenen et al., 1985). At mid-thoracic levels the population has been estimated at 75,000, with 45% of these unmyelinated (Schreyer and Jones, 1988). Fiber diameters ranged from 0.2 um to 5.0 um with unmyelinated fibers being the smallest (Harding and Towe, 1985; Leenen et al., 1985).

Terminations  In cervical, thoracic, and lumbar spinal cord levels, the CST projection is almost exclusively unilateral to lamina II-IV of the dorsal gray and more sparsely to the intermediate gray
(lamina VI and lamina VII) (Valverde, 1966; Brown, 1971; Donatelle, 1977; Schreyer and Jones, 1983; Bernstein and Stelzner, 1983). Limited terminals in the contralateral ventral (Goodman et al., 1966; Sievert, 1985) and in the ipsilateral dorsal, intermediate, and ventral gray have also been described (Goodman et al., 1966; Sievert, 1985; Reinoso and Casr tro, 1988). In sacral and coccygeal cord, bilateral terminations of CST fibers have been reported (Bernstein and Stelzner, 1983).

Growth and Development of the CST

The CST undergoes extensive postnatal growth and development (DeMeyer, 1967; Hicks and D'Amato, 1970, 1974; Donatelle, 1977). Recent studies employing anterograde WGA-HRP (Schreyer and Jones, 1982), retrograde fluorescent tracers (Schreyer and Jones, 1988), and transmission electron microscopy (Schreyer and Jones, 1988; de Kort et al., 1985) have described the temporal outgrowth of pyramidal tract axons in considerable detail. From these studies, it has been determined that corticospinal axons traverse the diencephalon by gestational day (E-) 17.5, reach the pontine nuclei by E-19.5, and arrive at the caudal limit of the medulla by E-20.5, i.e., just before birth. On the day after birth, postnatal day (PND-) 1, corticospinal axons have crossed in the pyramidal decussation and extended into the dorsal columns of the upper cervical spinal cord. The thoracic, lumbar, and sacral segments are reached on PND-3, 6, and 9, respectively. The lowest portion of the cord is reached only after PND-14.
Growth of the corticospinal axons into the spinal gray matter is delayed 2-3 days after the initial arrival of the tract at a given spinal segment (Schreyer and Jones, 1982). Growth of the CST down the dorsal columns is characterized by accelerated axonal growth spurts on PND-4 and PND-9. Myelination commences between postnatal days 10 and 12 and may continue as long as day 21.

The development of the CST also involves the regression of exuberant axonal projections. Retrograde fluorescent techniques have demonstrated the existence of occipital projections (Stanfield and O'Leary, 1985) and also widespread frontal and parietal projections (Schreyer and Jones, 1988) to the cervical cord via the CST in the neonatal but not in the adult rat. Although these axonal projections were shown to regress as the animal matured, the cells of origin were found to exist into adulthood apparently projecting to rostral regions of the brainstem.

Function of the CST

Studies based on cortical or medullary pyramid lesions in both rats (Castro, 1972; Price and Fowler, 1981) and hamsters (Kalil and Schneider, 1975; Reh and Kalil, 1982) demonstrate CST involvement in the control of the fine forelimb movements as used in handling food pellets or sunflower seeds. Also ambulatory skills, such as those required to traverse narrow runways or grids, appear to be dependent on the integrity of the CST (Whishaw and Kolb, 1988). Additionally, the tactile placing reflex in the rat (Donatelle, 1977) and in cats
(Bregman and Goldberger, 1983) was diminished or abolished by lesions placed in the corticospinal tract. Studies in monkeys (Lawrence and Kuypers, 1968; Beck and Chambers, 1970; Laursen, 1971; Woolsey et al., 1972) demonstrated diminished extensor muscle tone, diminished cutaneous reflexes, and loss of discrete usage of the digits after pyramidal section.

Further evidence for the role of the CST in mediating independent limb movements comes from studies employing intracortical microstimulation (ICMS) techniques in monkeys (Woolsey et al., 1972), in cats (Asanuma et al., 1981), in dogs (Gorska et al., 1980), and in rats (Kartje-Tillotson et al., 1985). In these studies, ICMS evoked contralateral movements, presumably mediated by the CST at low current thresholds. Furthermore, after medullary pyramidal tract lesions these low threshold movements were either diminished or no longer observed.

CST REMODELLING

Several projection systems have been observed to form new connections in response to neonatal CNS lesions. For example, anomalous axonal pathways have been described following monocular enucleations (Land and Lund, 1979; Rhoades and Dellacroce, 1980; O'Leary and Cowan, 1984; Grigonis et al., 1986; Olavarria et al., 1987), as well as after lesions of the superior colliculus (Schneider, 1973; Leong, 1976), cerebellum (Leong, 1980; Gramsbergen and Ij kema-Paassen, 1982; Molinari et al., 1986), and olfactory cortex.

The CST has been shown to project aberrantly after cortical, medullary and spinal cord injury (for review see Castro 1988). The formation of these anomalous connections is commonly what has been described as neuronal plasticity or remodelling (see Lund for Review). Plasticity, in general, is particularly prominent in the newborn (Goldberger, 1980). Axonal projections from undamaged normal tissue have been shown to project into the vicinity of synaptic sites made vacant by damaged afferents, maintain normally regressive projections in response to unilateral lesions, and reroute their growing projections around lesion-induced blockage or after deafferentation of a given target area. The following is a review of CST plasticity after cortical, medullary, and spinal cord injury.

**Cortical Lesion Studies**

**Anatomy** Anatomical studies of the plasticity of cortical efferent pathways have shown that following unilateral cortical lesions in newborn animals, the spared, unablated hemisphere forms anomalous connections with the spinal cord as well as with various brainstem regions. The corticospinal tract in rodents is primarily a crossed
projection, although a small ipsilateral component has been reported (Vahlsing and Feringa, 1980). However, after unilateral cerebral cortical lesions in newborn rats, a quantitative increase in ipsilateral CST fibers originating from the unablated hemisphere has been described in several papers (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). This expanded ipsilateral CST is not observed in response to cortical lesions made beyond PND-17 (Hicks and D'Amato, 1970; Leong, 1976). Initially, these fibers were observed traversing from the base of the ipsilateral dorsal funiculus, but recent work using sensitive anterograde WGA-HRP tracing techniques described an additional expanded CST projection within the ipsilateral ventral funiculus (Reinoso and Castro, 1986). These anomalous ipsilateral projections appear to terminate in the areas of the spinal cord gray matter that correspond to normal CST terminations. Electron microscopic studies further indicate that these projections make normal synaptic contacts (McClung and Castro, 1978; Leong, 1976).

**Electrophysiology** ICMS evokes low threshold contralateral limb movements that are presumably mediated by the CST. However, ICMS of the unablated cortex in the rats that sustained unilateral cortical lesions at birth evoked bilateral limb movements at low current thresholds (Kartje-Tillotson et al., 1985). Unilateral medullary pyramidotomy abolished the low threshold ipsilateral response, suggesting that the anomalous ipsilateral CST was responsible for mediating these evoked movements (Kartje-Tillotson et al., 1987). Furthermore, the neonatal administration of the neurotoxin 6-Hydroxydopamine, believed to block cortical plasticity (Kasamatsu et
al., 1976, 1979, 1981), abolished these abnormal ipsilateral limb movements evoked by intracortical microstimulation in adult rats that sustained unilateral cortical lesions at birth (Castro et al., 1986).

Behavior Frontal cortex lesions in mature rats were shown to disrupt manipulation (as tested by latch opening paradigm) but not locomotion (as tested by recording the time necessary to course a narrow runway), while damage of the medial parietal cortex was shown to disrupt locomotion but not manipulation (Gentile et al., 1978). Frontal cortical lesions have also been shown to adversely affect the rat's ability to retrieve food pellets with its forepaw (Castro, 1972) and with its tongue (Castro, 1975). Additionally, unilateral frontal motor cortical ablations were reported to produce permanent deficits, as measured by force and rate of response recorded by force transducers interfaced with a microcomputer, in the limb contralateral to the lesion (Price and Fowler, 1981).

Several studies demonstrating significant behavioral recovery or sparing after neonatal cortical injury as compared to similar injury sustained in adulthood suggest the functional integrity of the anomalous CST. Stride length was found to be shortened in rats sustaining motor sensory lesions as adults as compared to neonates (Hicks and D'Amato, 1970). Animals sustaining unilateral cortical ablations as adults consistently used the limb ipsilateral to the lesion to retrieve food pellets, while rats sustaining unilateral cortical ablations as neonates displayed contralateral or ambilateral limb preference (Castro, 1977). Initial studies by Whishaw and Kolb (1985, 1987) reported a poorer performance by rats sustaining neonatal
parietal cortical lesions as compared to adults sustaining similar lesions when tested on a battery of sensorimotor and maze learning tasks; but a latter study (Whishaw and Kolb, 1988) showed that rats receiving neonatally placed cortical lesions exhibited less impairment than an adult group when tested on a skill forelimb reaching task where they were required to reach between cage bars to grasp food pellets. Similarly, cats examined on an extensive series of complex motor and behavioral tests showed greater recovery after lesions in the neonatal period as compared to adult operates (Villablanca et al., 1986; Burgess and Villablanca 1986; Burgess et al., 1986; Leonard and Golberger, 1987).

Tactile placing reflexes may be mediated via the CST fibers (Laursen and Wiesendanger, 1966; Amassian, 1978) and, as a result, may provide another behavioral test for the presence and functionality of CST fibers. While analysis of the unilateral cortical lesions in newborn rats demonstrated normal forelimb reflexes contralateral to the intact cerebral cortex, corresponding to the normal crossed CST, no reflexes could be elicited on the side corresponding to the anomalous ipsilateral CST (Hicks and D'Amato, 1970, 1975; Brooks and Peck, 1940). Although these studies in rats were inconclusive in their attempt to couple a behavioral placing response with existence of the anomalous CST, sparing of tactile placing in the limb ipsilateral to a neonatal sensorimotor cortical lesion was observed in cats (Leonard and Goldberger, 1987).
Medullary Lesion Studies

Anatomy Unilateral pyramidotomy has been shown to result in the formation of an aberrant CST projection in the rat (Castro, 1978), hamster (Kalil and Reh, 1979), and cat (Tolbert and Der, 1987). In the hamster, developing CST axons were observed to grow around a medullary pyramid lesion made shortly after birth (Kalil and Reh, 1979, 1982; Kalil, 1984). These fibers grew caudally into the spinal cord and were interpreted to represent a regeneration of transected developing axons. However, a similar remodelling observed in kittens was attributed to the growth of later developing uncut axons as shown by injection of an anterograde fluorescent tracer (Tolbert and Der, 1987).

Electrophysiology ICMS-evoked ipsilateral limb movements were evoked at abnormally low thresholds in adult rats that sustained neonatal cortical lesions (Kartje-Tillotson et al., 1985). These ipsilateral low threshold movements were abolished by unilateral pyramidotomy performed contralateral to the original cortical ablation (Kartje-Tillotson et al., 1987), suggesting that the anomalous CST was mediating these movements.

Behavior Kalil and Schneider (1975) found a persistent deficit in the hamster's ability to manipulate sunflower seeds after unilateral pyramidal tract section in adulthood. Similar surgery in the neonate resulted in little or no deficit in the adult animal, and a second slightly more caudal lesion abolished the spared behavior suggesting that the behavior was mediated via a regrown or remodelled CST (Reh and Kalil, 1982; Kalil, 1984). A similar study (Kartje-Tillotson et al., 1982) based on limb preference after unilateral pyramidotomy in adult
and neonatal rats failed to find any behavioral difference across age groups.

Spinal Cord Lesion Studies

Anatomy Transection of CST axons in the adult rodent spinal cord causes the permanent loss of all pyramidal fibers distal to the injury (Fishman and Kelly, 1984). However, partial mid-thoracic spinal cord lesions, which bilaterally transected the dorsal funiculi in newborn (PND-1) rats, led to the growth of CST fibers around and caudal to the lesions (Schreyer and Jones, 1983; Dauzvardis et al., 1985).

 Autoradiographic analysis after cortical injections of tritiated proline demonstrated that spinal cord "overhemisection", i.e., a hemisection that extends across the spinal cord midline, in PND-1 rats led to a rerouting of CST fibers across the spinal cord midline (Bernstein and Stelzner, 1983). In addition to the ventral or lateral displacements of the major CST, an expansion of the small lateral or ventral ipsilateral corticospinal tracts has also been observed (Dauzvardis and Castro, 1986). The observed rerouting, however, does not represent CST regeneration since the CST had not yet reached the spinal level where the lesion was made.

 CST rerouting was also observed after partial spinal cord lesions in neonatal kittens but was not observed after similar lesions in adult cats (Bregman and Goldberger, 1983). Additionally, nitrocellulose filter paper impregnated with laminin and implanted into small lesion sites in the thoracic spinal cord of neonatal rats has been shown to support corticospinal growth (Schreyer and Jones, 1987).
While studies based on neonatal spinal cord injury do not demonstrate neuronal regeneration of severed axons, they do show that developing axons can grow around a lesion. These studies are considered particularly important in light of the traditional concept that the glial scar resulting from traumatic lesions forms a barrier to axonal regeneration (Guth et al., 1985; Matthews et al., 1979). Since this concept is primarily derived from studies on mature animals, perhaps scar formation after neonatal lesions presents less of a barrier to growing axons (Kalil and Reh, 1979; Prendergast and Stelzner, 1976; Ramon y Cajal, 1928; Tsukahara, 1978). Furthermore, recent studies derived from neuronal transplantation techniques suggest the presence of growth-promoting factors after lesions in neonates (Cotman and Nieto-Sampedro, 1984). The response of CST axons to partial spinal cord lesions in rats 6-9 days of age, an age at which the CST has approached its caudalmost extent but is still actively growing and myelinating, has not been extensively examined.

**Electrophysiology** Recent analysis of limb movements evoked by ICMS suggested the electrophysiological integrity of CST remodelling after partial mid-thoracic spinal cord lesions in infant rats (Dauzvardis et al., 1985; Dauzvardis and Castro, 1986). In this work, evoked hindlimb movements were observed after lesions that led to a rerouting of CST fibers. They were not observed in an adult lesion group that did not demonstrate CST remodelling.

**Behavior** Behavioral studies based on transection of the rat spinal cord (Weber and Stelzner, 1977) have been supportive of the 'Kennard Principle' of greater recovery following neonatal lesions
Spinal cord transection in the adult rat has been shown to produce more spinal shock, more spasticity, and greater deficits in hindlimb support, hopping, and tactile placing than transection in the neonate (Stelzner et al., 1981). Studies based on hemisection or partial spinal cord lesions in rats (Prendergast and Stelzner, 1982; Prendergast et al., 1982) have also demonstrated sparing of function or enhanced recovery after neonatal lesions as compared to adult, but, in addition, they have observed other motor patterns which appear to be more impaired by partial spinal cord lesions made in the neonatal time frame. Prendergast (1982) demonstrated that although neonatal lesion animals demonstrated greater sparing of hopping reflexes, adult lesion animals fared better at narrow runway and grid tests. Bregman (1983) showed that while tactile placing reflexes were spared after neonatal but not adult spinal cord hemisections, neonatal operates performed worse on tests of accurate locomotion. The gamma-aminobutyric acid (GABA) antagonist bicuculline (BCC) enhanced treadmill performance in cats spinalized as adults but hindered the performance of cats spinalized as neonates (Robinson and Goldberger, 1986).

Sparing of function in rats that sustained neonatal transections may be attributed to increased synaptogenesis both rostral and caudal to the lesion (Matthews et al., 1979; Weber and Stelzner, 1980; Cummings et al., 1981; Bernstein et al., 1981; Bryz-Gornia and Stelzner, 1986). Increased sprouting of ascending fibers in the isolated spinal cord stump may fill synaptic sites left vacant by the neonatal transection and thereby stabilize the caudal cord and enhance
its reflex performance (Weber and Stelzner, 1980). The greater sparing and enhanced recovery of some motor functions after neonatal hemisection may also be due to the increase collateral sprouting observed after neonatal but not adult lesions (Prendergast and Stelzner, 1976; Stelzner, 1979; Prendergast and Misantone, 1980). Also, as described above, the CST has been shown to remodel and grow around neonatally produced midthoracic hemisections or dorsal funiculotomies (Dauzvardis and Castro, 1986; Dauzvardis et al., 1985; Schreyer and Jones, 1983; Bregman and Goldberger, 1983).

The apparent better behavioral performance of rats and cats on some motor tasks that have sustained lesions as adults, compared to neonatal operates, may in part be due to the susceptibility of the neonatal neuron to axotomy. Many more neurons of the red nucleus die after neonatal as compared to adult axotomy (Prendergast and Stelzner, 1976, Bregman, 1986). In addition, Bregman (1983) and others have theorized that there exists a "spinal locomotor generator" that, when mature, can sustain a degree of locomotor activity when isolated from surasplinal input. In contrast, the locomotor generator in the neonate has limited supraspinal input, is not yet mature, and cannot function well when isolated by partial or total spinal cord injury. Brainstem centers are apparently more susceptible to degeneration after spinal cord lesions in the neonate than in the adult (Prendergast and Stelzner, 1976), and perhaps degeneration of the reticulospinal, rubrospinal, and vestibulospinal nuclei may have a greater effect on locomotion than does cortical damage (Eidelberg et al., 1981). Eidelberg (1981) in a review article on spinal cord lesions and
locomotor activity goes as far as stating "...from the clinical and experimental data on partial cord lesions we must abandon the idea of a significant role for the corticospinal system in locomotor activity." Perhaps this statement is somewhat strong, and what is lacking is a test that is sensitive enough to detect subtle changes in CST function as it pertains to locomotion.

In addition to some of the behavioral tests previously mentioned, studies of the rat gait have been used to assess the integrity of CNS projections. Pharmacological, radiological, and surgical lesions of the CNS and PNS have been shown to affect the locomotion of experimental animals in a manner detectable by changes in footprint patterns (Rushton et al., 1963; Mullenix et al., 1975; Schallert et al., 1978; Jolicoeur et al., 1979; Hruska et al., 1979; De Michele et al., 1982; De Medinacelli et al., 1982, 1984; De Medinacelli and Freed, 1983; Burgess and Villablanca, 1986). Midthoracic hemicordotomy in neonatal rats has been shown to shorten stride length and increase lateral rotation of the hindlimb as measured by footprint analysis techniques (Bregman, 1987). It is possible that corticospinal damage may account for these subtle changes in gait as measured by small alterations in footprint patterns.
CORTICOSPINAL TRACT REMODELLING IN ADULT RATS THAT SUSTAINED MID-THORACIC SPINAL CORD LESIONS AT POSTNATAL DAY ONE OR POSTNATAL DAY SIX
ABSTRACT

The corticospinal tract has been shown to remodel following cortical (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975) and medullary (Castro, 1978) lesions. Single injections of wheat-germ agglutinin horseradish peroxidase (WGA-HRP) were placed within the right sensorimotor cerebral cortex in normal adult rats and in rats that had sustained partial mid-thoracic spinal cord lesions at postnatal day (PND-) 1, at PND-6, or as adults. WGA-HRP label was observed in the CST caudal to the lesion site in rats that sustained lesions at PND-1 or at PND-6 but not in rats that had sustained similar lesions as adults. In the PND-1 and PND-6 animals, the CST was shown to by-pass the regions of spinal injury by deflecting laterally or ventrally. Below the lesion site the small lateral and dorsal ipsilateral CST tracts were often found to be expanded. Anomalous CST fibers were also observed in the ipsilateral and ventral funiculi. Injections of the retrograde fluorescent tracer (FB) into the spinal cord of several animals sustaining partial mid-thoracic spinal cord injury at PND-6 indicated that the remodelled CST was capable of projecting as far caudally as L1. In addition, GFAP staining indicated that reactive gliosis was greatest in animals sustaining partial mid-thoracic spinal cord lesions as adults.
INTRODUCTION

The ontogenetically late developing rodent corticospinal tract (CST), which does not reach thoracic spinal cord levels until three days after birth, has been shown to grow past partial mid-thoracic spinal cord lesions produced in the neonatal period (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983; Dauzvardis et al., 1985). In contrast, similar lesions made in weanling or adult rats prevented growth of CST fibers caudal to the site of injury (Schreyer and Jones, 1983). The ability of the CST to grow beyond lesions made 1-2 days after birth is explained by the observation that late growing CST fibers, which have not yet reached the thoracic dorsal funiculi, remain undamaged after dorsal funiculotomy (Schreyer and Jones, 1982). While these studies primarily examined the growth of CST fibers after spinal cord lesions made at 1-2 days of age, data from a few older animals indicated a limited (2-3mm) CST growth caudal to mid-thoracic spinal cord lesions made in rats at PND-6 (Schreyer and Jones, 1982; Bernstein and Stelzner, 1983). At this age, some growing CST fibers would have been severed by the lesion (Schreyer and Jones, 1982; Donatelle, 1977). The present study was designed to examine further the response of the CST to a midthoracic spinal cord lesion produced in rats at PND-1, PND-6, or at 9 weeks of age with special focus on the PND-6 lesion group.
METHODS

Subjects

Forty-one Long-Evans black hooded rats that sustained midthoracic dorsal funicular spinal cord lesions were used in this study. The surgically placed lesions were made at days PND-1 (n=17), PND-6 (n=13), or at nine weeks of age (n=11). At 35 to 40 days postoperatively, WGA-HRP was injected into the area of the hindlimb motor cortex as defined electrophysiologically in previous studies (Neafsey et al., 1986; Dauzvardis et al., 1985) of all animals except four of the PND-6 and three of the 9 week old group. The fluorescent tracer fast blue (FB) was injected on either side of the lumbar spinal cord (L1-L2) of the four remaining PND-6 animals. In addition, the lesion sites of five animals (PND-6, n=2; 9 week, n=3) were examined immunocytochemically for the presence of GFAP at the lesion sites. Five non-lesion control animals received cerebral cortical injections of WGA-HRP. Two other control rats received spinal injections of FB and were also examined for GFAP.

Surgical Lesion Methods

Newborn rats (PND=1 or PND=6) were anesthetized by hypothermia (crushed ice in a petri dish). A laminectomy exposed spinal cord segment T8, and a bilateral dorsal funicular lesion was made with a
number 11 scalpel blade, followed by the insertion of an electric cautery knife. The skin was then sutured, and each pup was warmed under an incandescent lamp and returned to its mother until weaning.

Adult rats were anesthetized with sodium pentobarbital (40mg/kg) and given single doses of atropine sulfate (0.05mg). Animals were placed in a stereotaxic frame, and vertebrae T10 was immobilized with a vertebral clamp. A laminectomy was performed at T7, and the dorsal funiculi at spinal cord level T8 were transected as in the neonates. The muscle tissue was sutured, and the skin wound was closed with auto-clips.

Anatomical Methods

Using a microsyringe affixed with a glass-micropipette, injections (0.01-0.02ul) of wheat-germ agglutinin conjugated with horseradish peroxidase (WGA-HRP, Sigma-1.0%) were placed stereotaxically 1.2mm deep into the hindlimb motor cortical area. Animals were sacrificed three days later by sodium pentabarbital overdose and perfusion with a 4% paraformaldehyde - 1.25% glutaraldehyde solution. Injection sites and spinal cords were frozen sectioned (50u) and reacted for HRP (Mesulam, 1978). Several segments above and below the lesion sites were horizontally sectioned while the lesion site was cut in cross-section. Alternate sections through the thoracic cord were stained with a Nissl stain to better characterize the extent of spinal cord lesions.

Four rats which had previously sustained midthoracic dorsal funicular lesions at PND=6 underwent laminectomy exposing the L1-L2 spinal cord segment. Using a Hamilton syringe mounted with a glass
pipette, four stereotaxic injections of 0.02ul of 2% Fast Blue (FB) were placed on the right side of the spinal cord of each animal. The injections were spaced approximately one mm apart in a rostro-caudal fashion. Animals were killed seven days later by anesthetic overdose and perfused transcardially with 10% formalin. Brains and spinal cords were removed and sectioned at 30 microns using a cryostat. Brains were cut sagitally, lesion sites were cut coronally, and fluorescent dye injection sites horizontally. Cells were counted using a Leitz epifluorescent microscope and a Houston Instruments digitizing pad coupled to an Apple IIe computer.

The lesion sites from two of the PND-6 animals injected with fluorescent dyes, three additional adults lesion animals and two non-lesion control animals were imbedded in paraffin, sectioned at five microns, and reacted for GFAP (Sternberger et al., 1979; Dako Pharmaceuticals).
RESULTS

Lesions Sites

All lesion sites were grossly visible upon spinal cord removal in both the adult and neonate lesion group. The spinal cord dorsal funiculi were typically not apparent for several spinal segments across the level of the lesion, and in several animals the anterolateral fissures appeared to fuse at the midline (Fig. 1).

Histologically the severity of tissue damage at the level of maximum injury in the newborn lesion groups (PND-1 and 6) ranged from mild (two animals) to apparent hemisection of the cord (four animals). Six of 17 rats of the PND-1 group and three of nine in the PND-6 group demonstrated lesions confined to the dorsal funiculi. In these animals the dorsal funiculi at the lesion site were absent and the dorsal horns merged at the midline (Fig. 2, Table I-III).

Apparent scar tissue formation which seemed continuous with the overlying dura mater was generally more prominent in the adult lesion group. Also, a greater distortion of normal morphology and syrinx formation were characteristically found associated with lesions in adult rats. GFAP staining was more intense in the adult lesion group than in the PND-1 and PND-6 lesion groups (Fig. 2).
WGA-HRP Anterograde Labeling of CST Projections

WGA-HRP cerebral cortical injection sites were approximately one mm in diameter encompassing all six layers of the cortex and occasionally involving the white matter. The resulting projections and terminations are summarized in table form (Table I-III).

Normal CST Projections At thoracic spinal cord levels, the majority of labeled corticospinal tract fibers were observed as a dense bundle within the base of the dorsal funiculus contralateral to the cortical injection site (Fig. 3). Emanating from this fasiculus, CST fibers appeared to terminate mainly in Rexed's laminae II-VII of the spinal cord gray matter (McClung and Castro, 1978). Sparse labeling was typically also seen traversing the contralateral lateral funiculus near the dorsal horn. Occasionally, several solitary fibers were observed coursing in the ipsilateral dorsal funiculus and the ipsilateral ventral funiculus above spinal cord level T8. These "accessory CST projections", including those fibers observed in the lateral funiculus, were not found caudal to T8 in control animals. Similarly, sparse labeling within the ipsilateral gray matter was only found in levels rostral to T8.

Adult Lesion Group CST projections to levels rostral to the lesion site of the adult lesion group were comparable to those found in corresponding sections of the control group. Label was found in the dorsal funiculus of all adult lesion animals above spinal cord level T8. At the level of the lesion (T8), WGA-HRP label was observed in only two of eight animals. Trace amounts of label were observed in longitudinal sections below the site of maximum injury in the same 2
animals. One of these animals, (ADFL-5), had sustained an apparently incomplete lesion of the dorsal funiculi.

Neonatal Lesion Group (PND=1) WGA-HRP labeling in sections rostral to the lesion site was comparable to that observed in the control group except that four of 17 animals of the PND-1 lesion group demonstrated an apparently denser distribution within the ipsilateral gray matter. Anterogradely labeled CST axons were observed in cross sections through the lesion site in thirteen of seventeen animals. In cases where the lesion spared some dorsal funicular tissue, label was found within or adjacent to this white matter. In cases of complete dorsal funiculotomy, defasciculated CST axons were found to course through the gray matter, the contralateral lateral funiculus, and/or through the ipsilateral lateral funiculus. When observed traversing the gray matter, labeled axons were found either confined to the dorsal horn adjacent to the lesion or widely dispersed throughout the gray matter.

Labeled CST axons were observed caudal to the lesion site in 16 of 17 animals, extending to T11-T12 in most cases. They appeared to return to their normal position in the contralateral dorsal funiculus in most cases although labeled fibers traversing the contralateral lateral funiculus, ipsilateral dorsal funiculus, ipsilateral lateral funiculus, and the gray matter remained expanded as compared to controls. In one animal the CST appeared to remain entirely within the dorsal horn gray matter caudal to the lesion. In addition, terminal labeling in these PND-1 lesion animals was found consistently bilateral as compared to unilateral in control animals.
PND-6 Lesion Group  Longitudinal sections of the T6-T7 spinal cord segment in animals that sustained midthoracic spinal cord lesions at PND-6 demonstrated CST labeling similar to that of the PND-1 group. The principal CST found in the contralateral dorsal funiculus was labeled above the lesion site in all nine animals of this group. Three of nine animals had clearly labeled tracts in the contralateral lateral funiculus and five rats demonstrated terminal labelling in the ipsilateral gray matter.

At the level of the lesion, spinal cord morphology typically showed more distortion than in the PND-1 group. WGA-HRP labelled axons were observed in seven of nine animals at the T8 lesion site. These fibers were found almost exclusively coursing through the dorsal horn gray matter, although two cases demonstrated considerable labeling in the contralateral lateral funiculus. One of these two animals (PND-6-50), showed a large projection in the ipsilateral ventral funiculus (Figs. 4, 5). Caudal to T8, all nine animals demonstrated varying amounts of WGA-HRP labeling that extended to T11-T12 in most cases, even though label was observed at the T-8 lesion site in only seven of nine. This can be explained by the fact that labeled axons cut in cross section are harder to detect than those cut longitudally, and, therefore, label could have been missed at the lesion site in two animals. In contrast to the PND-1 group in which labelled axons were found mostly in the contralateral dorsal funiculus, the majority of labelled fibers in this group were found in the contralateral lateral funiculus. Label was also slightly more dense in the ipsilateral funiculi in this group as compared to the PND-1 lesion group.
Retrograde Fluorescent Labelling

Retrogradely labeled cortical neurons were consistently found after FB injections placed in the spinal cord gray matter caudal to the lesion site in animals of the PND-6 lesion group and after similar placements in normal rats. Considerably more fluorescent labeled cortical neurons were found in normal animals in comparison to lesion animals (Table IV). In both groups labeled neurons were comparably distributed in lamina V extending approximately 6mm laterally from the dorsomedial convexity of the cortex. In normal rats, the percentage of ipsilateral labeling was much lower (1.5%) in comparison to lesion animals (16.4%)
DISCUSSION

CST remodelling after partial spinal cord lesions in newborn rats, as observed in this and previous studies (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983; Dauzvardis et al., 1985) is attributed to the relatively late ontogenetic development of the rodent CST (DeMeyer, 1967; Donatelle, 1977; Schreyer and Jones, 1982). Since CST projections do not reach mid-thoracic levels until PND-3 (Schreyer and Jones, 1982, 1987), the observed CST growth caudal to T-8 lesions made in the path of CST axons at PND-1 demonstrated that undamaged growing fibers can navigate through the area of damage. Similar CST growth observed caudal to T-8 lesions made on PND-6, as clearly demonstrated by our findings, raises the possibility that transected CST axons that were cut during their growth phase may have regenerated. Alternatively, findings in the PND-6 lesion group may be attributed to the growth of late developing CST fibers that were not damaged by the lesions. In related work, CST remodelling after medullary pyramidotomy in newborn hamsters has been attributed to a regeneration of cut axons (Kalil, 1984; Kalil and Reh, 1979, 1982), although recent work suggests that this remodelling may represent the growth of late developing, undamaged CST fibers (Tolbert and Der, 1987).

Previous studies of CST growth caudal to mid-thoracic spinal cord lesions made in rats at PND-6 indicated that this response only
occurred when the growing fibers coursed through the dorsal funiculi caudal to the lesion (Schreyer and Jones, 1983). Although based on the results of only two animals, these findings suggested that the growing CST fibers may necessarily depend upon axonal guidance cues located along the major CST projection pathways. In contrast, our findings based on similar lesions demonstrated CST fibers growing caudally within the lateral or ventral funiculi as well as the dorsal funiculi. While this response may simply reflect a random growth of CST fibers caudal to the lesion, the various trajectories observed corresponded to the major and minor projection patterns of normal CST fibers. These observations suggest that the CST fibers may be responding to axonal guidance cues for the various components of the normal CST system. Studies of CST remodelling after neonatal cortical lesions have similarly shown that anomalous CST projections originating from the unablated hemisphere may course to the spinal cord not in a random fashion but rather through projections corresponding to the normal course of the major and minor components of the CST (Reinoso and Castro, 1988).

Our results after partial spinal cord lesions suggest that if growing CST fibers that were disrupted by lesions can reach one of the normal CST projectories, they may then continue growing caudally by responding to normal axonal guidance cues. While individual fibers may reach the various possible pathways by a random growth process, those fibers may then serve as pioneer fibers leading the way for other growing CST fibers. In contrast, the failure to reach and follow normal CST projectories may result in a profusion of axonal growth
within the gray matter as was observed in some cases. The notion that growing axons can follow the course of growing pioneer fibers has been previously advanced in relation to the growth and development of collosal fibers in the mouse (Silver et al., 1982), of trigeminal ganglionic connections in the chick (Moody and Heaton, 1983), and of regenerated spinal connections in the newt (Singer et al., 1979).

GFAP labeling was found to be more intense at the lesion site in the adult lesion group than at similar spinal cord levels in the PND-1, PND-6, and control group, supporting previous work suggesting that glial scarring may play a role in inhibiting regrowth or remodelling in the adult animal (Guth et al., 1985).

Spinal injections of the retrograde tracer fast blue (FB) indicated that in four PND-6 animals the CST was capable of growing as far caudal as L1-L2. In addition, an increase in the number of ipsilaterally projecting neurons as compared to controls was also observed. This finding, plus the observation of ipsilateral projections as labelled by WGA-HRP, suggests that some CST fibers were rerouted across the midline when they encountered the mid-thoracic spinal cord lesion sites.

In summary CST axons were found to traverse partial mid-thoracic dorsal funicular spinal cord lesions made in PND-1, PND-6, but not in adult rats. Furthermore, the CST in lower thoracic levels was observed to project ipsilaterally as well as contralaterally and to terminate bilaterally with greater frequency than was observed in control animals. Intracortical microstimulation techniques have been used to study the functional efficacy of anomalous CST pathways created in
response to unilateral neonatal sensorimotor cortical ablation (Kartje-Tillotson et al., 1982, 1985). In the next chapter of this dissertation, these techniques were used as a tool for establishing the functional capabilities of CST fibers which have remodelled in response to mid-thoracic dorsal funicular lesions sustained by rats at various ages.
Fig. 1. Photograph of dorsal view of rat spinal cord demonstrating a mid-thoracic lesion site (LX). Note the absence of the paired dorsal funiculi at the lesion site.
Fig. 2. Photomicrograph of sections through the lesion site of an animal that sustained a partial mid-thoracic spinal cord lesion at PND-6 (col. 1, (a) and higher power (b)) and another animal sustaining a similar lesion as an adult (col. 2, (a) and higher power (b)). All sections were reacted for the presence of GFAP. Arrows indicate reactive astrocytes.
Fig. 3. Darkfield photomicrographs illustrating WGA-HRP labelling in spinal cord sections of a control animal (A-D) and an animal which sustained a partial mid-thoracic spinal cord lesion at PND-6 (Al-D1). Asterisks indicate the dorsal funiculus. Arrows are pointing to individual axons.
Fig. 4. Lower power (A) and higher power (B) darkfield photomicrographs of a section through the lesion at PND-6. Arrows indicate labelled fibers in the ipsilateral ventral funiculus. (C) is a camera lucida drawing of the same section.
Fig. 5. Darkfield photomicrographs of longitudinal sections through the T-10 spinal cord segment of the animal in figure 4. Note labelled fibers in the ipsilateral ventral funiculus (A, higher power - B), and also in the contralateral lateral funiculus (C, higher power - D). M = midline.
Fig. 6. Fluorescent photomicrograph of corticospinal neurons in a control animal labelled with the retrograde tracer fast blue (A). High power photomicrograph of a fast blue labelled neuron in the ipsilateral cortex of an animal sustaining a partial mid-thoracic spinal cord lesion at PND-6 (B).
Fig. 7. Camera lucida drawing of a section through the brain of a control animal (C) and a PND-6 animal (PND-6) demonstrating the location of retrogradely labelled neurons. Sections were approximately 0.5 mm lateral to the midline.
TABLES I-III. Tabulation of the extent of WGA-HRP labelling observed in sections rostral and caudal to mid-thoracic lesion sites (middle row) of animals in the PND-1, PND-6, and adult lesion groups. Abbreviations: DF = dorsal funiculus, LF = lateral funiculus, VF = ventral funiculus, BLTM = bilateral terminal labelling. Black dots indicate the presence of observable label.
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**TABLE II**

PND - 6  
ROSTRAL TO LESION  
LESION  
CAUDAL TO LESION  

<table>
<thead>
<tr>
<th>T8-T9</th>
<th>CONTR F</th>
<th>IPSI F</th>
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<tbody>
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<td>ANIMAL NUMBER</td>
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<td>A-12</td>
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<tr>
<td>Control</td>
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TABLE IV. FAST BLUE CORTICAL LABELLING

<table>
<thead>
<tr>
<th></th>
<th>Contralateral</th>
<th>Ipsilaterial</th>
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<tr>
<td>Normal n=3</td>
<td>1669</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>(1020 - 2240)</td>
<td>(22 - 30)</td>
</tr>
<tr>
<td>PND - 7 lesion</td>
<td>61</td>
<td>10</td>
</tr>
<tr>
<td>n=4</td>
<td>(25 - 77)</td>
<td>(0 - 17)</td>
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</table>

Counts represent the mean and range (in parenthesis) of labelled neurons estimated according to the method of Abercrombie. Contra and ipsilateral is in reference to the side of the spinal cord injections.
CORTICALLY-EVOKED HINDLimb MOVEMENTS IN ADULT RATS
THAT SUSTAINED SUSTAINED PARTIAL MID-THORACIC SPINAL
CORD LESIONS AT 1 OR 6 DAYS OF AGE
Corticospinal fibers have previously been reported to grow around partial mid-thoracic spinal cord lesions made in neonatal animals (Shreyer and Jones, 1983; Bernstein and Stelzner, 1983; Dauzvardis and Castro, 1985). Intracortical microstimulation (ICMS) techniques were employed in this study in an attempt to analyze the electrophysiological integrity of CST fibers that had grown around partial mid-thoracic spinal cord lesions. Evoked hindlimb movements were observed in animals that sustained dorsal funicular lesions at postnatal day one or six but were not observed in animals sustaining similar lesions as adults. In addition, low threshold ipsilateral hindlimb movements were observed in animals of both neonatal lesion groups. These findings suggest that corticospinal tract fibers that grow caudal to partial mid-thoracic spinal cord injuries, even those that lose their laterality, are functional.
INTRODUCTION

The corticospinal tract (CST) has been shown to grow past areas of partial mid-thoracic spinal cord lesions made in neonatal rats (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983) and kittens (Bregman and Goldberger, 1982, 1983). This trans-lesion growth, which was not found after comparable lesions in adult animals, has been postulated to contribute to the enhanced motor recovery of neonatal operates in comparison to adult operates (Goldberger, 1985).

In search of electrophysiological support for the suggested functional implications of the rerouted CST, the present study was undertaken. Using intracortical microstimulation (ICMS) methods, we measured current intensities needed to evoke hindlimb movements in adult rats that sustained partial midthoracic spinal cord lesions at postnatal day (PND-) 1 or PND-6 in comparison to animals receiving comparable lesions at maturity. In previous work, these ICMS techniques were used to demonstrate the electrophysiological efficacy of aberrant cortical pathways found after unilateral sensorimotor cortical ablation in the neonate (Kartje-Tillotson, 1982, 1987; Reinoso and Castro, in press). In the present study our findings consistently demonstrated that the threshold currents necessary to elicit contralateral hindlimb movements in animals that sustained partial midthoracic spinal cord lesions at PND-1 and PND-6 were significantly lower than the threshold currents found in adult lesion animals.
MATERIALS AND METHODS

Subjects

Fifty-two Long-Evans, black hooded rats sustained dorsal funicular spinal cord lesions at mid-thoracic levels on postnatal day one (PND-1; n=21), PND-6 (n=21), or at nine weeks of age (n=10). At 30-40 days postoperatively animals were tested for limb movements evoked by intracortical microstimulation. Subsequently, animals were perfused with 10% buffered formalin, and their fixed brains and spinal cords were removed, sectioned, and stained with Pyromin Y or Toluidine Blue. Eleven animals served as non-lesion controls.

Surgical Lesion Methods

Rat pups (PND-1 or PND-6) were anesthetized by hypothermia, and a vertebral laminectomy was performed exposing spinal cord segment T8. The dorsal funiculus was cut with a #11 scalpel blade followed by the insertion of an electric cautery knife. The skin was then sutured, and the pups were warmed under an incandescent lamp. They were then returned to their mothers until weaning and then housed individually until tested electrophysiologically.

Adult rats were anesthetized with sodium pentobarbital (40mg/kg) and given a single dose of atropine sulfate (0.05mg). Animals were placed in a stereotaxic headholder, and vertebra T10 was immobilized with a vertebral clamp. A laminectomy was performed at T7, and the
dorsal funiculus was transected as in the neonates. The muscle tissue was sutured, and the skin wound was closed with auto-clips.

Intracortical Microstimulation (ICMS)

Intracortical microstimulation was performed according to methods previously used in our laboratory (Kartje-Tillotson, 1985). Rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (100mg/kg), with supplemental doses given as needed to prevent spontaneous movements. Animals were secured in a stereotaxic headholder while resting on a narrow heating pad that was servocontrolled at 37°C. The hair on the hindlimbs and forelimbs was clipped and the limbs were allowed to hang free to facilitate the observation of evoked movements. Prior to opening the skull, the cisterna magna was opened and drained to reduce swelling of the cortex. The right cortex was exposed and the overlying dura left intact. A sharpened, glass insulated tungsten wire with a 100um tip served as the stimulating electrode (Neafsey, 1981). Using bregma as a reference point and utilizing recently published cortical maps (Neafsey et al., 1986), the cortical forelimb and hindlimb areas were explored in 0.5mm steps in both a mediolateral and rostrocaudal direction, with smaller or larger intervals used to avoid blood vessels on the cortical surface. The stimulation current (5-100uA) was applied as a 300msec train of 0.025msec pulses at 350Hz at a depth of 1.7mm below the cortical surface (Kartje-Tillotson and Castro, 1982). Recent studies utilizing microstimulation techniques in the rat reported that this depth resulted in limb movements at the lowest current intensities (Donoghue et al., 1982; Neafsey et al.,
Stimulus currents were monitored on an oscilloscope by measuring the voltage drop across a 10,000 Ohm resistor.

The number of points stimulated was approximately 25 per animal, with the majority of points being confined to the hindlimb motor area. Each point was stimulated several times in order to determine the lowest current needed to reliably produce a visible movement, i.e., the threshold current. Thresholds were recorded for contralateral and ipsilateral movements. Two investigators collaborated in observing the evoked movements, and for all points the type of movement was also recorded, i.e., elbow flexion, knee extension, etc.

Histology

Upon completion of electrophysiological testing, animals were sacrificed by sodium pentobarbitol overdose and perfusion with a 4% paraformaldehyde-1.25% glutaraldehyde solution. Cortical stimulation sites and spinal cords were frozen sectioned (50 microns) and stained with Pyromin Y or Toluidine Blue. Segments above and below the lesion were horizontally sectioned while the spinal cord lesion site was cut in cross-section.

Data analysis

Cortical stimulation points were plotted using bregma as a reference on outline drawings depicting the rostral portion of the right cerebral hemisphere. Mean threshold values were computed for hindlimb and forelimb movements for each rat. Values for the adult lesion group and the neonatal lesion group were statistically compared.
to each other and to control values using a Student t-test. Camera lucida drawings were made of sections of spinal cord through the lesion sites.
RESULTS

Gross Appearance of Lesions

Approximately 50% of all lesion sites were grossly visible upon spinal cord removal in both the adult and neonatal lesion group. In these cases the dorsal funiculus was typically not apparent for several spinal segments, and in several animals the anterolateral fissures had met at the midline.

Histologically, the severity of tissue damage at the level of maximum injury in the PND-1 neonatal lesion group ranged from undetectable (2 animals) to apparent hemisection of the cord (1 animal). Seven of 21 rats of this group presented with near focal dorsal funiculotomy. In these animals, the dorsal funiculi at the lesion site were almost completely missing, and the dorsal horns appeared to fuse at the midline (Fig.1). Tissue damage at the lesion site in the PND-6 lesion group was comparable.

Scar tissue and vacuoles or cysts were less apparent at the midthoracic lesion sites in the neonatal lesion groups as compared to the adult lesion group. Apparent scar tissue formation which seemed continuous with the overlying dura mater was generally more prominent at the site of maximum injury in the adult lesion group. Also, a greater distortion of normal morphology and syrinx formation were characteristically found associated with lesions in adult rats.
Intracortical Microstimulation (ICMS).

Contralateral forelimb movements were elicited in all animals (Fig. 2). The mean threshold current for these movements in the PND-1 and PND-6 lesion group were 42.2 ± 1.5 uamps and 31.2 ± 2 uamps respectively. The mean thresholds for the adult lesion and control groups were 53.8 ± 2.2 uamps and 37.1 ± 1.5 uamps respectively. Although the majority of evoked forelimb movements involved the contralateral forelimb, a few ipsilateral forelimb movements were observed at current levels above 50uamps in several animals.

Contralateral hindlimb movements were elicited at a mean threshold of 54.3 ± 1.4 uamps in animals that sustained partial mid-thoracic spinal cord lesions at PND-1 and at a mean threshold of 54.8 ± 4.7 uamps in animals that sustained spinal cord lesions at PND-6. There was no significant difference between the PND-1 hindlimb value and the control hindlimb value of 43.4 ± 2.1 uamps (p=0.052) obtained from non-lesion animals. The PND-6 hindlimb value was only slightly higher than the mean threshold current needed to evoke hindlimb movements in non-lesion control animals (p=0.033). In contrast, cortically-evoked movements could not be elicited at current values under 100uamps in 8 out of 10 animals receiving partial mid-thoracic lesions at maturity. The two remaining animals showed hindlimb movements at mean thresholds of 63.0 and 79.0 uamps.

ICMS in non-lesion control animals typically evoked contralateral hindlimb and forelimb movements at low threshold current levels although ipsilateral movements could be evoked with higher intensities. In animals that sustained spinal cord lesions at PND-1 or PND-6,
contralateral hindlimb movements were also generally found, but some ipsilateral movements were observed under 100uamps. One animal in the PND-1 group and one animal in the PND-6 group exhibited ipsilateral hindlimb movements below 25uamps, although the mean threshold current for contralateral hindlimb movement in non-lesion control animals was 43.4uamps.

Cortical stimulation points eliciting forelimb and hindlimb movements were topographically located. In non-lesion control animals, FL points extended from 2.5 mm rostral to bregma to approximately 0.5 mm caudal to bregma; the HL area characteristically extended from this FL zone to approximately 3.0mm caudal to bregma (Fig. 3, 4, 5). This same topographical distribution was generally found in animals that sustained spinal cord lesions at PND-1 as well as at maturity. However, three animals receiving lesions at PND-1 showed a positional shift of the FL area extending 1.5 mm posterior to bregma. The HL area was not similarly displaced. While stimulation generally did not elicit HL movements in animals receiving spinal cord lesions at nine weeks of age, the eight penetrations that did elicit HL movements in two animals were located within areas corresponding to the topography of normal animals.

Hindlimb movement consisted of extension or flexion at the hip, knee, or ankle, with hip flexion being the most commonly observed. Two of three animals in the PND-1 lesion group that demonstrated a positional shift of the FL area also displayed simultaneous or "yoked" hindlimb and forelimb movements. Stimulation at the same point was capable of eliciting a concurrent hindlimb and forelimb movement. This
occurred at three stimulation points in the one animal and one point in
the other. The remaining animal that exhibited a caudally shifted
forelimb map did not present with yoked movements but did display
bilateral hindlimb movements evoked by stimulation at six different
points. Since no hindlimb movements under 100uAmps could be elicited
in the adult lesion group, except for the two animals mentioned, no
hindlimb topographical maps could be plotted for this group.
DISCUSSION

This study has demonstrated a difference in threshold current values needed to evoke hindlimb movements in adult rats sustaining neonatal (PND-1 or -6) midthoracic dorsal funicular lesions in comparison to rats sustaining similar lesions as adults. Cortically evoked hindlimb movements were absent (except for 2 animals) at current values less than 100uA in the adult lesion group. Hindlimb movements were elicited in the neonatal lesion groups with a mean threshold value not statistically different from that of controls.

ICMS has been used to generate a composite motor output map of the rat frontal and parietal cortex (Neafsey et al., 1986). The borders of this map correspond to the neurons of origin of the CST based on anatomical studies employing the use of spinally injected retrograde tracers (Miller, 1987; Ullan and Artieda, 1981; Wise et al., 1979; Hicks and D'Amato, 1977). This implies that movements evoked by ICMS are mediated through the CST. Additional evidence for the involvement of the CST in mediating cortically evoked movements is derived from studies that indicate an elevation in current thresholds necessary to evoke limb movements after pyramidal tract section in cats (Asanuma et al., 1981), dogs (Gorska et al., 1980), monkeys (Wise et al., 1979), and rats (Kartje-Tillotson et al., 1987).

In the present study, ICMS evoked low threshold contralateral FL and HL movements (presumably mediated by the CST) in adult control
animals and in adult animals that had sustained mid-thoracic dorsal funicular lesions at PND-1 or PND-6. In contrast, low threshold FL movements could be evoked in animals sustaining similar surgery as adults, but low threshold HL movements were absent. These results correspond to previous reports of greater CST remodelling in response to neonatal as compared to adult spinal cord lesions (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983; Bregman and Goldberger 1983; Dauzvardis and Castro, 1985). These studies demonstrated the growth of CST projections caudal to the site of mid-thoracic lesions made within several days of birth. This remodelling was not found in animals sustaining similar lesions as young adults.

In other work on CST plasticity, unilateral sensorimotor cerebral cortical ablation in newborn rats caused an increase in ipsilaterally projecting CST fibers originating from the unablated hemisphere (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). ICMS has been used to study the functional efficacy of these anomalous CST projections produced by neonatal unilateral sensorimotor cortical ablation (Kartje-Tillotson et al., 1985). In control animals, only contralateral low threshold movements were evoked by ICMS whereas stimulation of the unablated hemisphere in mature rats that sustained unilateral cortical lesions at birth evoked bilateral limb movements at low current thresholds. Furthermore, disruption of the bilaterally evoked movements by transection of the medullary pyramid ipsilateral to the spared hemisphere indicated that the responses were in part mediated by an anomalous or remodelled CST projection (Kartje-Tillotson
et al., 1987). Low threshold, ipsilateral movements were not observed in rats sustaining sensorimotor cortical ablation as adults.

In our study, ICMS evoked HL movements under 100uA in only 2 out of 10 animals sustaining mid-thoracic dorsal funicular lesions as adults, and one of these sustained only a partial lesion of the dorsal funiculi. This absence of evoked HL movements in the adult lesion group concurs with anatomical findings showing the absence of CST projections caudal to lesions made in the mid-thoracic spinal cords of adult rats (Dauzvardis and Castro, 1985). The presence of evoked HL movements in rats sustaining T7-T8 dorsal funicular spinal cord lesions as neonates corresponds to the remodelling CST observed using anterograde tracing methods in rats sustaining mid-thoracic dorsal funicular lesions as neonates (PND-1 or PND-6) (Dauzvardis and Castro, 1985). These electrophysiological and anatomical findings lend support to the work of Kennard (1944) and others (Schneider, 1970; Goldberger, 1985) that reported on greater anatomical and behavioral recovery after neonatal as compared to adult CNS injury.

"Yoked" movements were observed in 3 of the neonatal operates. These findings suggest the presence of lesion-induced axonal collaterals at cervical spinal cord levels of CST neurons that typically only project to more caudal levels of the spinal cord. While normally characterized by projections to precise spinal cord levels (Wise et al., 1979; Ullan and Artieda, 1981; Miller, 1987), the possibility of axonal collateralization is supported by studies demonstrating the formation of rubrospinal collaterals rostral to thoracic spinal cord lesions (Prendergast, 1976). Axonal
collateralization could also explain the caudal shift in forelimb
cortical topography observed in three animals. By failing to reach
lumbar spinal levels but yet forming abnormal axonal collaterals in
cervical regions, CST neurons that normally mediate hindlimb movements
could now affect forelimb responses. In fact, a slight increase in
terminal labelling was observed in sections rostral to the lesion in
the neonatal groups.

The normal CST is predominately a crossed projection, but our
anatomical findings demonstrate the presence of bilateral projections
caudal to mid-thoracic lesions made at PND-1 and PND-6. This
bilaterality thus provides the anatomical framework for the bilateral
hindlimb responses evoked by ICMS in several of our neonatal operates.

It should be noted that our control FL threshold values were
somewhat higher than those reported in previous studies employing ICMS
techniques (Kartje-Tillotson, 1985; O'Donoghue et al., 1986). These
studies reported mean FL threshold values in the low 20 uA range in
comparison to our 37.1 uA value. This discrepancy is most likely due
to the difference in the ceiling or cutoff current considered. We
recorded all responses under 100 uAs while these authors considered
only contralateral FL movements evoked under 30 uAs.

In summary, the present study has provided electrophysiological
evidence for motor cortical output distal to the site of partial spinal
cord ablation in the neonatal rat. Additionally, these findings lend
support to the possible role of cortical efferent remodelling in the
recovery of motor function following damage to the developing spinal
cord.
Fig. 1. Camera lucida drawings of representative lesion sites of several animals that sustained partial mid-thoracic spinal cord lesions at PND-6 (a-i). Note how the dorsal hours have merged in c, e, f, and g.
Fig. 2. Histogram depicting contralateral mean threshold values for forelimb (FL) and hindlimb (HL) movements in control and experimental groups (PND-1, PND-6, and adult).
SpC-X - Spinal Cord Lesion

CONTROL (11)
ADULT SpC-X (10)
PND 1 SpC-X (21)
PND 7 SpC-X (21)

FL
HL

*
Fig. 3. Schematic diagram of the brain of an adult rat illustrating the location of cortical stimulation points and resultant evoked movements in an animal sustaining an partial midthoracic spinal cord lesion as an adult.
A. WRIST EXTENSION
B. NO RESPONSE
C. NO RESPONSE
D. NO RESPONSE
E. NO RESPONSE
F. NO RESPONSE
G. NO RESPONSE
H. NO RESPONSE
I. NO RESPONSE
J. NO RESPONSE
K. NO RESPONSE
L. WRIST EXTENSION
M. ELBOW FLEXION
N. ELBOW FLEXION
O. NO RESPONSE
P. NO RESPONSE
Q. NECK
R. ELBOW FLEXION
S. WRIST EXTENSION
T. ELBOW FLEXION
U. ELBOW FLEXION
V. WRIST EXTENSION
Fig. 4. Schematic diagram of the brain of an adult rat illustrating the location of cortical stimulation points and resultant evoked movements in a control animal.
A. ELBOW FLEXION
B. WRIST EXTENSION
C. ELBOW FLEXION
D. DIGIT FLEXION
E. NO RESPONSE
F. NO RESPONSE
G. KNEE FLEXION
H. NO RESPONSE
I. NO RESPONSE
J. ANKLE FLEXION
K. ANKLE FLEXION
L. NO RESPONSE
Fig. 5. Schematic diagram of the brain of an adult rat illustrating the location of cortical stimulation points and resultant evoked movements in an animal sustaining a mid-thoracic spinal cord lesion at PND-1.
A. NO RESPONSE
B. ELBOW FLEXION
C. WRIST EXTENSION
D. WRIST EXTENSION
E. NO RESPONSE
F. HIP FLEXION
G. NO RESPONSE
H. ANKLE FLEXION
I. NO RESPONSE
J. NO RESPONSE
K. ANKLE FLEXION
L. NO RESPONSE
M. NO RESPONSE
N. HIP FLEXION
O. NO RESPONSE
P. NO RESPONSE
Q. ELBOW EXTENSION
R. NO RESPONSE
S. KNEE FLEXION
T. NO RESPONSE
U. NO RESPONSE
Fig. 6. Schematic diagram illustrating the location of all cortical stimulation points in all four groups resulting in evoked hindlimb movements. B denotes bregma.
Fig. 7. Schematic diagram of one hemisphere of an adult rat brain indicating the location of every cortical penetration point that evoked a yoked (Y) or ipsilateral hindlimb movement (I) in 5 PND-1 and 6 PND-6 lesion animals.
A BEHAVIORAL STUDY OF RATS THAT SUSTAINED PARTIAL MID-THORACIC SPINAL CORD LESIONS AT VARIOUS AGES USING FOOTPRINT ANALYSIS TECHNIQUES
ABSTRACT

As previously reported, bilateral mid-thoracic destruction of the dorsal funiculi in newborn and six-day old rats does not prevent the caudal growth of the corticospinal tract (CST), although this growth may be aberrant in terms of projection course or laterality. In the present study, an analysis of gait based on footprint patterns was used to compare hindlimb function in rats sustaining partial mid-thoracic spinal cord lesions in adulthood, at one day, or at six days of age. The adult lesion group demonstrated an increase in heel contact and slipping as reflected in an overall increase in footprint length (FPL). This deficit was not observed in the day one and day six lesion groups and, therefore, correlated with previous reports of corticospinal remodelling after partial mid-thoracic spinal cord injury in neonatal rats.
INTRODUCTION

Corticospinal tract (CST) fibers have been observed to grow around partial mid-thoracic spinal cord lesions made in newborn rats (Bernstein and Stelzner, 1983; Schreyer and Jones, 1983; Dauzvardis and Castro, 1986). These rerouted fibers, which were not found after similar lesions in older rats, typically extended to lumbar levels and distributed within the spinal cord gray matter. The apparent electrophysiological integrity of these fibers was suggested by studies demonstrating hindlimb movements evoked by electrical microstimulation of the cerebral cortex (Dauzvardis et al., 1985; Dauzvardis and Castro, 1986). Corresponding to the absence of CST rerouting after spinal cord lesions in older rats, these cortically-evoked movements which are believed to be mediated by CST fibers (Asanuma, 1981; Kartje-Tillotson et al., 1985) were not observed after partial spinal cord lesions in older rats.

CST rerouting has been similarly observed after partial spinal cord lesions in newborn kittens (Bregman and Goldberger, 1983a,b). This remodelling has been suggested to provide the anatomical basis for the CST-mediated tactile reflexes that were spared after lesions in the newborn but were abolished by lesions created at maturity (Goldberger, 1986). In order to examine further the possible behavioral significance of CST rerouting, the present study was initiated to examine the effects of partial mid-thoracic spinal cord lesions made in
newborn postnatal day (PND-) 1 and PND-6 and adult rats on gait patterns as measured by footprint analysis techniques (Rushton et al., 1963; Mullenix et al., 1975; Schallert et al., 1978; Hruska et al., 1979; Jolicoeur et al., 1979; De Michele et al., 1982; De Medinacelli et al., 1982, 1984; De Medinacelli and Freed, 1983).
MATERIALS AND METHODS

Subjects

Sixty-four Long Evans black-hooded rats were used in this study. Twenty-six animals received dorsal funicular spinal cord lesions at PND-1 (n=8), PND-6 (n=7), or at 9 weeks of age (n=11). Twenty-seven served as age matched surgical controls, and eleven were used as unoperated controls.

Surgical Lesion Methods

Rat pups (PND-1 and -6) were anesthetized by hypothermia and a vertebral laminectomy was performed exposing spinal cord segment T8. The dorsal funiculus was cut with a #11 scalpel blade followed by the insertion of an electric cautery knife. The skin incision was then sutured, and the pups were warmed under an incandescent lamp. They were then returned to their mothers until weaning and subsequently housed individually.

Adult rats were anesthetized with sodium pentobarbital (40mg/kg) and given a single dose of atropine sulfate (0.05mg). Animals were secured in a stereotaxic frame, and vertebra T10 was immobilized with a vertebral clamp. A laminectomy was performed at T7, and the dorsal funiculus was transected as in the neonates. The muscle tissue was sutured, and the skin wound was closed with auto-clips.
Behavioral Testing

Analysis of gait based on rat footprint patterns was used to assess the effects of mid-thoracic spinal cord lesions using methods first described by Hruska (1979) and modified by De Medinaceli (1982). Testing was initiated at 30-35 days after the placement of lesions at various ages. Animals were required to cross a confined walkway measuring 10 cm wide by 50 cm long. After two or three conditioning trials, the rats walked readily toward a dark shelter placed at the end of the walkway. The floor of the walkway was then covered with unexposed x-ray film, and, prior to the next run, an animal's hindpaws were dipped in a flat dish containing photographic developer (Kodak R.P. X-OMAT). Traversing the walkway they immediately demonstrated hindlimb paw prints which became permanent after air drying. Each rat was tested again until four sets of prints were collected in a single testing period. This procedure was repeated two days later such that 8 sets of tracks were collected for each animal. Animals receiving lesions as adults (9wks) were similarly tested before and after surgery.

Histology

After behavioral testing, all animals were injected with an overdose of sodium pentobarbital and perfused through the heart with saline followed by 10% formalin. The spinal cord lesion sites were frozen sectioned at 40 um using a Leitz cryostat. Sections were stained with toluidine blue, and the site of maximum spinal damage was drawn by camera lucida.
Data Analysis

The second and third interdigital pads on the soles of the rat's hindfeet show clearly in a normal print (Fig. 1). The small space between these was the point from which measurements were made. With the use of an Apple II Plus computer, Biometrics Bioquant II software package, and a Houston Instruments Hipad digitizing tablet, three distances were measured for each stride: the distance between successive placements of the same hindfoot (stride length, L), the distance between one hindprint and the interposed contralateral hindprint (step length, l), and the base of support (w,) indicated by a line drawn perpendicular from L to the interposed contralateral hindfoot. From these three parameters the step angle (theta) and the stride angle (gamma) were calculated. Approximately 50 of these "footprint triangles" were examined for each animal. Additionally, the overall length of each footprint (FPL), including heel marks and toe slips when present, was also measured. The mean values were calculated for each of the six parameters measured (L, l, w, theta, gamma, and FPL). Using the RS1 statistical analysis software program, multiple comparisons were made using the Bechhofer-Dunnett-Krishnaiah-Armitage or Bonferoni test for multiple comparisons between groups of unequal number (Wilcox et al., 1987). All testing used the 0.05 level of significance. For ease of comparison the mean footprint triangle was drawn for each animal, and these were superimposed according to experimental group (Fig. 2).
RESULTS

Lesion Morphology

The spinal cord lesion sites were generally readily visible upon spinal cord removal. The characteristic superficial appearance of the paired dorsal funiculi was typically not apparent for 1-2 spinal segments at the level of the lesion, and in several animals, the anterolateral fissures converged at the midline.

Histologically, the severity of spinal cord damage in the area of maximum injury appeared comparable in the PND-1 and PND-6 lesion groups (Fig. 3). The lesions ranged from the focal ablation of the dorsal funiculus to hemisection of the cord with loss of all gray matter at that level. The scar tissue and vacuoles or cysts which were prominent in the adult lesion group (Fig. 5) were less pronounced at the mid-thoracic lesion sites in the neonatal lesion groups. In all animals of the PND-1 and PND-6 lesion groups, the normal path of the major CST which traverses the ventral portion of the dorsal funiculus was destroyed. In one animal of the PND-1 lesion group (D1-7) all gray matter at the level of the lesion was destroyed. The adult lesion group typically demonstrated less reduction in overall spinal cord diameter at the level of the lesion site than that of the neonatal lesion animals. The apparent scar tissue formation which seemed continuous with the overlying dura mater was generally more prominent at the site of maximum injury in the adult lesion group. Also, a greater
distortion of normal morphology was characteristically found associated with lesions in adult rats. In animal A-8, all gray matter was lacking at the lesion site. Only one animal from the adult lesion group sustained a lesion that apparently spared the corticospinal tract (A-13). The spinal cords of the sham and unoperated control animals appeared normal both grossly and histologically.

Behavior

Thirty days post operatively all animals appeared to walk normally upon casual inspection and demonstrated no apparent signs of discomfort or disability.

Footprint Triangles

The mean values for the footprint parameters (except for FPL) obtained for the animals in each of the experimental groups are presented in Table I. These data are graphically demonstrated in a computer generated drawing presenting an overlay of mean footprint triangles for unoperated control, spinal cord lesion, and sham surgery animals in each age group (Fig. 2). The various measures derived from these triangle patterns showed no statistical differences between spinal cord lesion and unoperated control animals in each of the groups. Only the sham operated animals in the PND-6 groups showed statistical variation which was expressed in all measures (stride length, step length, angle theta, and angle gamma) except base of support.
The adult lesion group exhibited the least amount of difference in footprint patterns between unoperated control, sham, and lesion animals. Even lesions as large as those found in animals A-6 and A-8 (Fig. 4) failed to significantly alter gait patterns.

Footprint Length (FPL)

The mean values for FPL obtained for each experimental group are presented in Table I and Figure 5. This measure showed significantly larger footprints between adult lesion animals and age-matched surgical and unoperated controls. FPL in the adult lesion group were also larger than those observed in the neonatal group.
DISCUSSION

Analysis of footprint patterns using testing methods based on several previous reports (Rushton et al., 1963; Mullenix et al., 1975; Schallert et al., 1978; Hruska et al., 1979; Jolicoeur et al., 1979; De Michele et al., 1982; De Medinacelli et al., 1982, 1984; De Medinacelli and Freed, 1983) demonstrated no abnormalities in stride length, step length, or base of support in animals sustaining partial mid-thoracic spinal cord lesions at neonatal or adult ages. These findings, which were particularly surprising in regard to animals receiving large spinal cord lesions, differ from recent work which used similar testing methods to reveal a decrease in slip length and an increase in lateral rotation of the hindlimb after spinal cord hemisection in 1-2 day old rats (Kunkel-Bagden and Bregman, 1987). These differences from our findings may be attributed to their placement of lesions that typically appeared larger than those sustained by our animals, as well as to their use of a treadmill to obtain footprints. Our findings also differ from previous studies which used footprint measurements to reveal gait alteration after central nervous system or peripheral nervous system injury in rats (Kunkel-Bagden and Bregman, 1987; De Medinacelli et al., 1982, 1984; De Medinacelli and Freed, 1983; Mullenix et al., 1975) and cats (Burgess and Villablanca, 1986).
However, in agreement with previous work (Kunkel-Bagden and Bregman, 1987), measurement of footprint lengths demonstrated a larger footprint in animals sustaining spinal cord lesions at maturity. This measurement of footprint length includes any slipping or dragging of the developer-moistened hindfoot when placed on the smooth film surface covering an elevated walkway. Indicating a decrease in gait dexterity after lesions in adult rats, these findings concur with previous reports of a more pronounced spinal shock and spasticity as well as greater deficits in hindlimb support, hopping, locomotion, and tactile placing after spinal cord injury in the adult as compared to the neonate (Stelzner et al., 1975; Weber and Stelzner, 1977, 1980; Cummings et al., 1981; Prendergast et al., 1982; Bregman and Goldberger, 1983).

Although the lesions showed considerable variability in their cross-sectional extent, particularly regarding the neonatal operates, the dorsal funiculi were transected at mid-thoracic levels in all but one of the experimental animals. Accordingly, the course of the CST, which primarily traverses the dorsal funiculus (King, 1910; Brown, 1971; Vahlsing and Feringa, 1980), was consistently transected. In previous work CST fibers were observed to circumnavigate and extend caudal to mid-thoracic lesions made at PND-1 or PND-6 (Dauzvardis et al., 1985). This response, which was not observed after spinal cord lesions in older animals, is primarily attributed to the protracted postnatal development of the CST system (Schreyer and Jones, 1982;
Donatelle, 1977). The remodelling of CST fibers around partial spinal cord lesions is therefore proposed to account for the better gait dexterity seen in our neonatal lesion group.

Further support for the functional viability of CST projections that grow caudal to spinal cord lesions made in the newborn is derived from electrophysiological analysis (Dauzvardis and Castro, 1986). In this work, intracortical microstimulation evoked low-threshold hindlimb movements in adult rats that sustained partial mid-thoracic spinal cord lesions at birth. Other reports indicate that such low-threshold movements, which were not found in animals sustaining spinal cord lesions at maturity, are mediated by CST fibers (Asanuma et al., 1981; Kartje-Tillotson et al., 1985).

The lack of any significant change after dorsal funicular lesions in the adult group is explained by Bregman (1983) and others who have theorized that there exists a "spinal locomotor generator" that, when mature, can sustain a degree of locomotor activity when isolated from supraspinal input. In contrast, the locomotor generator in the neonate has limited supraspinal input, is not yet mature, and cannot function well when isolated by partial or total spinal cord injury. Brainstem centers are apparently more susceptible to degeneration after spinal cord lesions in the neonate than in the adult (Prendergast and Stelzner, 1976), and perhaps degeneration of the reticulospinal, rubrospinal, and vestibulospinal nuclei may have a greater effect on locomotion than does cortical damage (Eidelberg et al., 1981). Eidelberg (1981) in a review article on spinal cord lesions and locomotor activity goes as far as stating "...from the clinical and
experimental data on partial cord lesions we must abandon the idea of a significant role for the corticospinal system in locomotor activity."

Perhaps this statement is somewhat strong, and what is lacking is a test that is sensitive enough to detect subtle changes in CST function as it pertains to locomotion.

In summary, the present findings demonstrate that mid-thoracic lesions which transect the course of the CST have a greater effect on locomotion in the rat when such lesions are created in the adult versus the perinatal time frame. These results combined with our previous anatomical and electrophysiological studies indicate that remodelling or rerouting of the CST may contribute to these age related differences. More recent work indicates that a further sparing of deficits may be found by grafting embryonic spinal cord into the gap produced by a neonatal spinal cord hemisection (Kunkel-Bagden and Bregman, 1987).
Fig. 1. Schematic diagram of the hindpaw footprints of an adult rat illustrating the footprint triangle and the method of establishing step length \( L \), stride length \( l \), base of support \( w \), angle gamma \( \gamma \), angle theta \( \Theta \), and footprint length \( FPL \).
Fig. 2. Computer generated overlay of the mean footprint triangle for each animal arranged according to experimental sub-groups (A). The mean footprint triangle for each sub-group (B). The overlay of these sub-group mean triangles arranged according to their group age at time of surgery (C). Abbreviations: Dle = day one, experimental; Dlc = day one, unoperated controls; Dls = day surgical control (sham); D6e = day six, experimental; D6c = day six, unoperated control; D6s = surgical control (sham); Ae = adult experimental; Ac = adult, unoperated control; As = adult, surgical control (sham).
Fig. 3. Camera lucida drawings through thoracic lesion sites of spinal cords of rats sustaining dorsal funicular ablation at postnatal ages one (D1) and six (D6). Cross-hatching indicates scar tissue.
Fig. 4. Camera lucida drawings through thoracic lesion sites of spinal cords of rats sustaining dorsal funicular ablation as young adults (A). Asterisks denote presence of vacuoles or large cysts and cross-hatching indicates apparent scar tissue.
Fig. 5. Histogram demonstrating mean footprint length (FPL) of control, lesion, and sham animals sustaining surgery at postnatal day 1, 6, and as young adults (nine weeks). Note the increase of FPL in the adult lesion group compared to age matched unoperated controls and sham animals. Asterisk indicates significant difference from control and sham groups ($P < 0.05$)
ANALYSIS OF FOOTPRINT LENGTH AFTER MID-THORACIC SPINAL CORD INJURY

![Bar graph showing footprint length comparison between control, lesion, and sham groups on Day 1, Day 6, and Adult phases.](image-url)
Table V  Mean values of each experimental sub-group for stride length (L), step length (l), base of support (w), angle theta, angle gamma, and footprint length (FPL).
<table>
<thead>
<tr>
<th></th>
<th>1 DIE</th>
<th>2 DIC</th>
<th>3 DIS</th>
<th>4 D6E</th>
<th>5 D6C</th>
<th>6 D6S</th>
<th>7 AE</th>
<th>8 AC</th>
<th>9 AS</th>
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<tr>
<td>1 L</td>
<td>9.1 (0.6)</td>
<td>10.8 (0.6)</td>
<td>9.4 (0.7)</td>
<td>8.6 (0.3)</td>
<td>9.2 (0.5)</td>
<td>12.0 (0.2)</td>
<td>11.2 (0.5)</td>
<td>10.5 (0.2)</td>
<td>11.0 (0.4)</td>
</tr>
<tr>
<td>11</td>
<td>4.6 (0.3)</td>
<td>5.3 (0.3)</td>
<td>4.7 (0.2)</td>
<td>4.3 (0.2)</td>
<td>4.6 (0.3)</td>
<td>6.0 (0.1)</td>
<td>5.8 (0.2)</td>
<td>5.4 (0.1)</td>
<td>5.7 (0.3)</td>
</tr>
<tr>
<td>3 theta</td>
<td>34 (2)</td>
<td>24 (2)</td>
<td>34 (1)</td>
<td>35 (2)</td>
<td>32 (2)</td>
<td>28 (1)</td>
<td>33 (2)</td>
<td>31 (3)</td>
<td>28 (2)</td>
</tr>
<tr>
<td>4 gamma</td>
<td>113 (5)</td>
<td>134 (3)</td>
<td>113 (3)</td>
<td>111 (4.0)</td>
<td>118 (3)</td>
<td>124 (2)</td>
<td>115 (3)</td>
<td>118 (2)</td>
<td>124 (4)</td>
</tr>
<tr>
<td>5 w</td>
<td>2.8 (0.2)</td>
<td>2.3 (0.2)</td>
<td>2.9 (0.2)</td>
<td>3.0 (0.2)</td>
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<td>3.3 (0.1)</td>
<td>3.0 (0.1)</td>
<td>2.8 (0.2)</td>
</tr>
<tr>
<td>6 FPL</td>
<td>25.72 (2.2)</td>
<td>24.55 (1.0)</td>
<td>25.05 (0.6)</td>
<td>25.05 (13)</td>
<td>28.46 (1.1)</td>
<td>25.90 (0.5)</td>
<td>39.35 (2.8)</td>
<td>28.46 (0.7)</td>
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GENERAL DISCUSSION

Neuroanatomical plasticity in response to damage of the CNS has been clearly demonstrated in numerous studies over the past two decades. Several of these studies focused on the CST as a model system to study neuroplasticity. The popularity of the CST as an experimental model stems from its well defined anatomy and its surgical accessibility, and its protracted growth and development. Unilateral cortical ablation in newborn rats has been shown to result in the formation of an enlarged ipsilateral CST projection (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). Comparable remodelling of the unablated CST was also observed after unilateral medullary pyramidotomy in the newborn rat (Castro, 1978), while regeneration or a rerouting of the transected CST was observed following similar surgery in the hamster (Kalil and Reh, 1979, 1984, 1982). Further down the neuraxis, partial mid-thoracic lesions of the spinal cord in newborn rats resulted in the remodelling of CST projections as they grew caudally (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983; Dauzvardis and Castro, 1985). While a positive correlation between CST remodelling and functional recovery after neonatal cortical and pyramidal lesions in the rodent has been made electrophysiologically (Kartje-Tillotson et al., 1982, 1985, 1987) and behaviorally (Castro, 1972; Reh and Kalil, 1982), such a correlation has not been made after
neonatal spinal cord lesions.

Previous accounts concerned with CST remodelling following partial mid-thoracic spinal cord lesions indicated that, although such remodelling was prevalent when lesions were created on or near PND-1, no such growth was observed when lesions were made as late as PND-6 (Schreyer and Jones, 1983; Bernstein and Stelzner, 1983). The first study in this dissertation was undertaken to determine if lesion induced CST remodelling could occur after mid-thoracic spinal cord injury in six day old rats, an age at which growing CST axons would be cut at mid-thoracic lesion levels.

In this study, CST fibers labelled by cortical injections of WGA-HRP was observed caudal to spinal cord level T8 in rats sustaining partial mid-thoracic spinal cord lesions at PND-1 and PND-6. The labelled CST fibers were observed below T8 in the contralateral and ipsilateral dorsal and lateral funiculi, the spinal gray, and, in addition, the ipsilateral ventral funiculus. Ipsilateral CST projections within the ventral funiculis in normal animals are few and are generally restricted to cervical levels (Vahlsing and Feringa, 1980; Reinoso and Castro, 1988). Their presence within the lower thoracic cord of neonatal lesion animals further attests to the plastic nature of the growing CST. Virtually no labelling was observed caudal to T8 in the spinal cords of the adult lesion group.

Spinal injections of the retrograde fluorescent tracer fast blue (FB) reinforce the WGA-HRP findings. In previous work (Reinoso and Castro, 1988) spinal injections of fluorescent tracers into rats which had sustained unilateral sensorimotor cortical lesions at birth
labelled cells in the spared cortex ipsilateral to the injection sites. As reported in experiment one of this dissertation, injection of FB into the L1-L2 spinal cord segment of rats sustaining dorsal funicular lesions at PND-6 labelled cells in the contralateral and ipsilateral cortices. These findings further demonstrate that CST fibers are capable of circumnavigating mid-thoracic lesions produced as late as PND-6 and can do so by crossing over to aberrant ipsilateral positions.

The proliferation of glial elements after CNS injury (glial scarring) may be more extensive after injury to the adult CNS as compared to the neonatal CNS (Windle et al., 1956; Guth, Bright, and Donati, 1978) and, therefore, may contribute to the limited remodelling observed after injury to the adult CNS. Findings in experiment one supported this theory since GFAP immunocytochemical techniques demonstrated the greatest reactivity in the lesion sites of rats sustaining partial spinal cord lesions as adults.

Intracortical microstimulation of the unablated hemisphere in mature rats that sustained unilateral cortical lesions at birth evoked bilateral limb movements at low current thresholds (Kartje-Tillotson et al., 1985). Disruption of the evoked movements by medullary pyramidotomy indicated that these movements were mediated in part by anomalous corticospinal fibers (Kartje-Tillotson et al., 1987). In the second experiment of this dissertation, hindlimb movements evoked by ICMS were observed after partial mid-thoracic spinal cord lesions that led to a remodelling or rerouting of CST fibers but were not present in an adult lesion group in which CST remodelling was absent. These results suggest the electrophysiological integrity of CST fibers that
grew around mid-thoracic spinal cord lesions. The additional observation of cortically-evoked, low threshold ipsilateral hindlimb movements and simultaneous forelimb and hindlimb movements supports findings of the first experiment which indicate that the remodelled CST may lose its laterality and topographic distribution patterns.

Possible behavioral correlations to CST remodelling after partial spinal cord lesions at birth are derived from studies in rats and cats. Testing for postural reflexes and locomotor tasks after mid-thoracic hemisection made in rats at birth or at maturity demonstrated deficits which were task-dependent but generally less pronounced in animals receiving lesions at birth (Prendergast, Shusterman, and Philips, 1982). Like the rat, the late developing feline CST (Goldberger, 1986; Tolbert and Der, 1987) was also observed to grow caudally beyond partial spinal cord lesions made on the day of birth (Bregman and Goldberger, 1983). Also similar to results obtained using rats, extensive behavioral analysis comparing the effects of spinal cord lesion in newborn and adult cats demonstrated that the degree of recovery exhibited between the newborn and adult lesion groups varied according to the particular test (Bregman and Goldberger, 1983). However, the reported sparing of CST-mediated tactile-placing responses in neonatal as compared to adult operates corresponded to the CST remodelling only observed in the neonatal lesion group (Goldberger, 1986). Similarly, in hamsters that sustained medullary pyramidotomy at birth, the ability to manipulate sunflower seeds digitally, a skill particularly associated with an intact CST (Reh and Kalil, 1982), corresponded to CST plasticity.
The third part of this dissertation consisted of a behavioral investigation undertaken in an attempt to correlate functional sparing with the anomalous cortico-efferent pathways generated by ablation of the mid-thoracic dorsal funicular area in neonatal and adult rats. The results demonstrated that the experimental groups did not differ in terms of the relevant placement of their hindpaws while traversing an enclosed runway. However, a significant difference in the degree of slipping, dragging, and heel contact was observed between adult and neonatal lesion groups, as reflected in an overall increase in footprint length in the adult lesion group. This increase of footprint length may be considered a behavioral deficit corresponding to a lack of observed remodelling in the adult lesion group.

In conclusion, the experiments in this dissertation report the possibility that remodelled cortico-efferent pathways play an important functional role in the rat and perhaps in the human with spinal cord damage. Recent success in post-mortem WGA-HRP techniques (Beach and McGeer, 1986) may provide evidence of plasticity in the human spinal cord following neonatal damage. Accordingly, basic science research utilizing animal models to study the mechanisms of CNS repair may someday benefit patients suffering from spinal cord damage. Recent advances in neural transplant techniques and neuroimmunological research have already proved hopeful in this regard.
SUMMARY

The ability of the CNS to recover after injury is greater when such damage is sustained at birth than at maturity. It was the intent of the present study to anatomically, electrophysiologically, and behaviorally examine the course and development of remodelled CST pathways established in response to mid-thoracic dorsal funicular lesions.

Anatomical Findings:

Single injections of wheat-germ agglutinin horseradish peroxidase (WGA-HRP) were placed within the right sensorimotor cortical area to study the course and termination of remodelled CST projections. Results obtained from this study agreed with those from previous studies demonstrating considerable CST remodelling after partial mid-thoracic spinal cord lesions sustained at birth. In addition, considerable remodelling was also observed in rats using WGA-HRP or the retrograde fluorescent dye fast blue on lesions sustained as late as PND-6. Also glial fibrillary acid protein (GFAP) reactivity, an index of glial proliferation or scarring, was shown to be reduced in animals sustaining surgery at birth or PND-6 compared to adults sustaining similar surgery.
Electrophysiological Findings:

Intracortical microstimulation of the motor cortex in rats sustaining partial mid-thoracic spinal cord lesions at birth, at PND-6, or at maturity correlated with the anatomical findings in that evoked low threshold hindlimb movements were present in the animals sustaining surgery at birth or at PND-6 but were absent in animals sustaining surgery as adults. These findings suggest that the CST projections that have remodelled or rerouted in response to neonatal spinal injury are electrophysiologically functional.

Behavioral Findings:

Using the behavioral measurement of footprint length, the possibility of recovery of function in animals sustaining partial mid-thoracic spinal cord injury at birth or PND-6 as compared to animals sustaining similar lesions as adults was evaluated. The results from this study indicated that the animals with neonatal lesions (birth or PND-6) exhibited no increase of overall footprint length due to slipping or dragging and heel contact. The animals with adult lesions did show an increase in overall footprint length. These findings suggest that the remodelling CST projections may indeed be capable of mediating a behavioral recovery of function.
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Dec 6, 1988
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