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A Study of Corticospinal Tract Remodelling in Adult Rats That Sustained Unilateral Cortical Ablation at Birth

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A STUDY OF CORTICOSPINAL TRACT REMODELLING IN ADULT RATS THAT SUSTAINED UNILATERAL CORTICAL ABLATION AT BIRTH

By

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DEDICATION

To Dad, Mom, Cherry, Margie, J.C. and Paolo

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VITA

The author, Blesilda S. Reinoso, is the oldest daughter of Julio Cesar and Maria Reinoso. She was born on March 27, 1957 in Manila, Philippines.

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INTRODUCTION

Neuronal plasticity pertains to "the capability of synapses to modify their function, to increase or decrease in number, or to be replaced in response to internal or external perturbations" (Cotman and Nieto-Sampedro, 1984). That such plasticity may persist throughout the life span of higher animals is commonly regarded to reflect the behavioral modifications that occur in response to environmental stimuli which may induce alterations at the synaptic level. This neuronal malleability provides the basis for learning and implies a uniqueness for individual central nervous systems (CNS) which vary according to life's experiences (Purves and Lichtman, 1985). Additionally, the modifiable characteristic of nervous tissue can account for the numerous examples of neuronal remodelling observed after CNS lesions. This remodelling is often advanced in explanation for the variable degree of functional recovery which is often observed after damage to neural tissue, yet a clear relationship between plasticity and recovery has been difficult to establish.

While several obstacles may be encountered by studies in search of a causal relationship between plasticity and recovery of function after lesions in mature animals, the identification of typically subtle lesion-induced changes in normal morphology or physiology as well as the formulation of accurate behavioral measures are generally the most problematic (Goldberger, 1986). However, after lesions in newborn

animals, comparatively striking anatomical remodelling has been commonly reported in numerous studies since initial reports involving visual (Schneider, 1970; Land and Lund, 1979) and motor (Hicks and D'Amato, 1970) pathways. Similarly, functional recovery has often been found to be more prominent after neonatal lesions as first described by Kennard (1940, 1942). Because of these factors, i.e., increased anatomical plasticity and the "Kennard principle", also referred to as the "infant lesion effect" (Bregman and Goldberger, 1983), the newborn appears more amenable than the adult in the study of plasticity.

The rodent corticospinal tract (CST) is considered to be an excellent model for studying neuronal plasticity. Its normal anatomy (Brown, 1970; Wise et.al., 1979; Schreyer and Jones, 1982), developmental sequence (DeMyer, 1967; Donatelle, 1977; Hicks and D'Amato, 1970; Schreyer and Jones, 1982; De Kort et.al., 1985) and participation in the control of limb movements are well established (Castro, 1972; Phillips and Porte, 1977; Heffner and Masterton, 1983; Kartje-Tillotson et al., 1985). Additionally, its capacity for remodelling has been reported in several studies demonstrating the formation of anomalous CST projections after neonatal lesions of the cerebral cortex (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975), medullary pyramid (Castro, 1978; Kalil and Reh, 1979; Tolbert and Der, 1987) or spinal cord (Bernstein and Stelzner, 1983; Bregman and Goldberger, 1983). Furthermore, the correlation of normal and anomalous CST fibers with limb movements evoked by intracortical microstimulation (Kartje-Tillotson et al., 1985, 1987)

provides an experimental test for the physiological efficacy of CST fibers.

Attempts to correlate lesion-induced remodelling with possible functional roles requires a precise description of the course and distribution of the anomalous pathways. Because previous anterograde tracing studies of CST remodelling after neonatal cortical lesions were primarily based on axonal degeneration staining methods (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975), analysis of CST remodelling using the more sensitive WGA-HRP staining method was undertaken in Experiment I. In addition to anomalous CST fibers traversing the ipsilateral dorsal funiculus, as described in earlier work (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975), a previously undescribed projection coursing within the ipsilateral ventral funiculus was found.

The precise, topographic distribution within the cerebral cortex of corticospinal-projecting neurons (Wise et al., 1979; Sievert, 1985) corresponds to the CST's role in the control of individual limb movements as demonstrated by intracortical microstimulation (Neafsey et al., 1986). In Experiment II, using retrogradely transported fluorescent tracers, a similar topographic distribution was found for neurons giving rise to anomalous ipsilateral CST projections. The ipsilateral fibers were additionally found to arise from a separate population of cells and do not represent axonal collaterals from normal crossed CST fibers. The apparently precise origin and distribution patterns of the anomalous CST

projections lend further support to their possible involvement in recovery after neonatal cortical lesions.

Several factors have been implicated to influence neuronal plasticity after damage to the newborn CNS. For example, studies using the neurotoxin 6-hydroxydopamine (6-OHDA) have suggested that the noradrenergic (NA) system may regulate visual cortical plasticity (Kasamatsu et al., 1976, 1979, 1981). While these findings have been disputed (Bear and Daniels, 1983; Daw et al., 1984; Adrien et al., 1985), additional work has indicated that 6-OHDA treatment may also disrupt motor cortical plasticity as measured electrophysiologically (Castro et al., 1986). However, contrary to these latter findings, Experiment III demonstrated that 6-OHDA treatment did not disrupt motor cortical plasticity as observed using anatomical tracing methods. Instead of disrupting the formation of anomalous CST in response to neonatal cortical lesion, 6-OHDA pretreatment appeared to promote CST plasticity.

BACKGROUND

The Corticospinal Tract

The rodent isocortex is divided into frontal, parietal, temporal and occipital regions (Zilles and Wree, 1985). In mature rats, corticospinal projections arise mainly from motor and somatosensory areas (Wise et al., 1979; Jones and Wise, 1977), specifically from primary (MI) and secondary motor cortex and from primary and secondary (SI and SII) somatosensory cortex (Wise et al.,1979; Neafsey et al.,1986). The corticospinal tract (CST) descends ipsilaterally through the brainstem forming a compact bundle along the ventral surface of the medulla oblongata (Doetsch and Towe, 1981) and crosses at the spino-medullary junction (DeMyer, 1967; Hicks and d'Amato, 1970, 1974; Donatelle, 1977; Schreyer and Jones, 1982).

The course and termination of the corticospinal tract (CST) in the spinal cord varies among mammals (Armand, 1984). In rats, CST fibers are principally located contralaterally in the ventralmost portion of the dorsal funiculus (King, 1910; Brown, 1971; Schreyer and Jones, 1982) with few fibers in the same position ipsilaterally (Sievert, 1985). A small number of corticospinal fibers have been found bilaterally traversing the lateral funiculi (Sievert, 1985) and a few CST fibers course through the ipsilateral ventral funiculus (Vahlsing and Feringa, 1980). In addition, an intermediate fasciculus of axons coursing through the base of the dorsal horn in cervical segments has been reported (Schreyer and Jones,

1982). Contralateral CST terminations are dense in the dorsal horn and sparse in the intermediate and ventral horn. The distribution of the ipsilateral CST are of lesser density and are localized in the same regions of the gray matter as those of the contralateral CST (Goodman, 1966; Sievert, 1985).

Developmentally, corticospinal axonal elongation primarily occurs postnatally preceding completion of the growth and differentiation of its cortical origins (D'Amato and Hicks, 1978; Reh and Kalil, 1981). At birth the rat CST has grown only to the level of the spino-medullary junction with most fibers crossing and extending into the upper cervical spinal cord (DeMyer, 1967; Hicks and d'Amato, 1970, 1974; Donatelle, 1977; Schreyer and Jones, 1982). CST fibers are reported initially to project to a given spinal cord level, and after an observed 'waiting period' within the white matter the axons subsequently project into the gray matter and form synapses (Distel and Hollander, 1980; Schreyer and Jones, 1982). The immature state of CST development at birth in rats makes it amenable to experimental manipulations for studies on motor cortical plasticity (Kalil, 1984). Functionally, a close temporal relationship has been observed between the appearance of CST axons within the spinal cord gray matter and the appearance of forelimb or hindlimb placing responses (Donatelle, 1977).

CST fibers control movement of distal extremity musculature (Kuypers, 1964), specifically fine limb movements (Castro, 1972; Phillips and Porter, 1977; Heffner and Masterton, 1983). Although the capability

for digital flexion is retained, rats that sustained bilateral frontal cortical lesions performed poorly in tests requiring them to extend their forelimbs toward food pellets and then grasp and bring them to their mouth (Castro, 1972). Overextension in attempts to grasp food pellets after cortical lesions suggested proprioceptive deficits (Castro, 1972) which are expected considering the overlap of somatosensory and motor areas within the frontal cortex (Hall and Lindholm, 1973; Hayes and Rustioni, 1981; Neafsey et al., 1986).

Cerebral Cortical Plasticity.

The rat cerebral cortex demonstrates a rapid growth period within the first postnatal week (Hicks and D'Amato, 1968; Raedler et al., 1980). Brain injury during this period commonly produces widespread reduction in brain size accompanied by a variety of major structural changes (Kolb et al., 1983). CST remodelling, in the sense of the formation of anomalous projections, is more prominent in rats with lesions made within three days of age in comparison to lesions at later ages (Leong, 1976). This differential anomalous growth response to cortical lesions early versus late in the perinatal period is in agreement with the concept that lesions sustained in infancy may cause less severe functional deficits than similar lesions sustained in adulthood (Kennard, 1940; LeVere, 1983).

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 enucleation (Land

and Lund, 1979; Leong, 1980; Rhoades, 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 Ijkema-Paasen, 1982; Molinari, 1986), and olfactory cortex (Friedman and Price, 1986). The formation of anomalous corticofugal projection following neonatal cortical ablation have also been described (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975; Goldman, 1978; Leong, 1980; Caminiti and Innocenti, 1981; Neumann et al., 1982; Castro and Mihailoff, 1983; Lent, 1984; Sharp and Gonzalez, 1986; Naus et al., 1986; Leonard and Goldberger, 1987; Tillablanca and Gomez-Padilla, 1987).

Unilateral frontoparietal cortical destruction in neonates results in the formation of an enlarged uncrossed CST (Hicks and d'Amato, 1970; Leong, 1973; Castro, 1975). Although quantitatively smaller than normal contralateral projections, the anomalous ipsilateral CST is appropriately located primarily within the ventral portion of the dorsal funiculus (Castro, 1975; Hicks and d'Amato, 1974; Leong, 1976).

While the mechanisms involved in the formation of normal and anomalous projections are not understood, several reports on plasticity suggest that lesion-induced growth may produce synapses similar in appearance, number, and termination to the normal axonal projections (Cotman et al., 1981; Kalil and Reh, 1982). Three phenomena have been cited to explain the formation of anomalous pathways in response to CNS injury (Finger and Almli, 1985). Firstly, <u>reactive synaptogenesis</u>

suggests a process in which intact axons form new branches or terminals to occupy synaptic sites in response to some stimulus that is not part of the normal developmental process (Cotman et al., 1981; Finger and Almli, 1985). For example, when the principal terminal sites of retinofugal axons are ablated or blocked in newborn rodents, anomalous retinofugal projections are established in other visual structures with alternative terminal space (Schneider, 1970; Devor et al., 1976; Baisinger et al., 1977). Specifically, in hamsters, ectopic retinal projections to the ventrobasal nucleus or the medial geniculate nucleus are reliably induced by lesions which ablate two principal targets of retinofugal axons (i.e., lateral geniculate body and superior colliculus) and which thereby liberate terminal space in ventrobasal or medial geniculate nuclei (Frost, 1982). Secondly, there may be a persistence of neural pathways that normally retract in the course of development. Several reports demonstrate the normal retraction of projections that are exuberantly present in early development (Land and Lund, 1979; Caminiti and Innocenti, 1981). The persistence of these otherwise transient fibers after neonatally placed lesions has been suggested to account for increase in an uncrossed retinotectal projection observed after neonatal monocular enucleation (Land and Lund, 1979; Laemle and Labriola, 1982; Thanos and Bonhoeffer,1984) and also for the anomalous callosal projection following partial lesions of somatosensory cortical areas at birth (Caminiti and Innocenti, 1981; Lent, 1984). In contrast, developmental studies using axonal degeneration staining methods,

electron microscopy and autoradiography (Nah, 1980) demonstrated that lesion-induced CST remodelling is not due to the abnormal persistence of otherwise transient fibers. Thirdly, a rerouting of axons may occur by lesion-induced deafferentation of a target area (D'Amato and Hicks, 1978) or if the normal locus of termination is damaged before developing axons reach it (Finger and Almli, 1985). Unilateral cortical ablation in newborn rats results in the formation of a small uncrossed CST which is believed to be formed by the rerouting of later growing CST fibers that normally cross (D'Amato and Hicks, 1978). The quantitative imbalance between CST projections descending from both hemispheres has been proposed to disrupt fiber interactions at the spinomedullary junction leading to the failure of later growing CST fibers arising from the unablated cortex to cross (Verhaart and Kramer, 1952; Leong and Lund, 1973).

Effects of Noradrenaline Depletion on Cortical Development and Plasticity.

Noradrenergic (NA) neurons are primarily located in the locus coeruleus and the lateral ventral tegmental fields. The widespread coerulear NA efferents primarily innervate the: (1) cerebral cortex, (2) specific thalamic and hypothalamic nuclei, (3) olfactory bulb, (4) cerebellar cortex, and (5) mesencephalon and spinal cord (Cooper et al., 1982). In general, NA fibers arising from the lateral ventral tegmental fields intermingle with those arising from the locus coeruleus. NA fibers from posterior tegmental levels contr[Dute mainly to descending

fibers within the mesencephalon and spinal cord (Cooper et al., 1982).

The coeruleo-cortical projection in the rat is bilateral with a predominantly ipsilateral component (Morrison et al., 1981). Developmentally, NA innervation in the cerebral cortex proceeds in a rostro-caudal and latero-medial gradient (Schlumpf et al., 1980; Morrison et al., 1981; Verney et al., 1984). Morphological and biochemical evidence indicates that ascending noradrenaline-containing axons innervate the rat neocortex by embryonic day 16-17 (Lauder and Bloom, 1974; Coyle and Molliver, 1977; Levitt and Moore, 1979; Schlumpf et al., 1980; Krisst et al., 1980; Verney et al., 1984). By the first postnatal week, approximately 30% of all synapses in the somatosensory cortex are noradrenergic with the greatest concentration of synapses occurring in the area that corresponds to layer IV (Molliver and Krisst, 1975). However, the relatively dense monoaminergic innervation in this layer is probably transient since monoaminergic synapses in mature cortex are reported to be sparse (Coyle and Molliver, 1977). The arrival of coerulo-cortical projections prior to the completion of cortical differentiation (Wise et al., 1979) suggests that noradrenergic projections may have neurotrophic (Lauder and Bloom, 1974; Felten et al., 1982; Brenner et al., 1985) and inductive functions (Lidov et al., 1978; Schlumpf et al., 1980; Kolb and Whishaw, 1985) in cortical development. Cytological analysis subsequent to early deprivation of locus coeruleus projections have shown alterations in cellular morphology within the neocortex (Maeda et.al., 1974; Onteniente et al., 1980; Imamoto et al.,

1982; Felten et al., 1982). Because endogenous noradrenaline levels are low early in development, these biogenic amines may act as a neuromodulator (Descarries et al., 1977; Jonsson et al., 1979; Kasamatsu et al., 1979) rather than as excitatory or inhibitory transmitters.

The use of neurotoxins to disrupt specific neurotransmitter pathways has been widely used to determine the role of these systems in normal development and plasticity. The catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) is a strong reducing agent that undergoes rapid oxidation in neutral aqueous solution (Tranzer and Thoenen, 1968; Suzuki et al., 1984). Upon administration, it is transported into the adrenergic neuron by a 'membrane pump' uptake mechanism which is localized at the axonal membrane (Malmfors and Sachs, 1968; Jonsson, 1971). Inside the adrenergic neuron, 6-OHDA can be taken up, concentrated and stored by the amine-storage granules, probably via ATP-Mg++ dependent mechanisms (Tranzer, 1971; Malmfors, 1971; Jonsson and Sachs, 1975). In vitro studies using adrenergic neurons from mouse atria showed that there is considerable leveling off of uptake between 30-60 minutes (Jonsson, 1971). A certain concentration of 6-OHDA has to be reached in the extragranular cytoplasm in order to initiate degeneration and destroy the amine uptake-storage mechanism (Jonsson, 1971, 1975; Malmfors, 1971; Trombley et al.,1986). Terminal degeneration results from the transition of the oxidation product, 6-OHDA quinone, to 6-OHDA in the extraneuronal compartment where a rapid equilibrium between the two is reached (Jonsson and Sachs, 1975; Saner and Thoenen, 1971). The

terminal projections of the locus coeruleus are preferentially affected by 6-OHDA treatment (Malmfors and Sachs, 1968; Tranzer and Thoenen, 1968; Sachs and Jonsson, 1975), thereby inducing degeneration of NA terminals that project to the neocortex (Kostrzewa and Harper, 1975).

In addition to being transported into neurons, 6-OHDA may also accumulate within extraneuronal elements such as smooth muscles and meningeal cells (Bonisch, 1980; Pehlemann, 1985; Sievers, 1985). The neurotoxic effects of 6-OHDA on NA fibers has been demonstrated by desmethylimipramine (DMI) pretreatment. DMI prevents 6-OHDA uptake into the neurons by blocking the membrane pump (Jonsson et al.,1975; Malmfors and Sachs, 1968; Breese and Traylor, 1971; Cooper et al., 1975; Sachs and Jonsson, 1975).

The ocular dominance shift observed in response to monocular deprivation (Wiesel and Hubel, 1965) has been widely used as a model to study the effects of 6-OHDA on cortical plasticity. In kittens, the critical period of visual cortical plasticity induced by monocular deprivation ranges from 2 weeks to several months with peak sensitivity at 4-5 weeks (Daw, 1983). Monocular deprivation during the critical period results in a strong bias toward cortical neurons driven exclusively by the undeprived eye (loss of binocularity) as measured electrophysiologically. In contrast, this ocular dominance shift response is absent in 6-OHDA-treated animals subjected to monocular deprivation (Kasamatsu and Pettigrew, 1976; Paradiso et al., 1983; Nelson et al., 1985). Local microperfusion of noradrenaline in the visual

cortex accelerated the recovery of cortical cells from the effects of brief monocular deprivation, leading to faster restoration of binocularity and suggesting that normally functioning catecholamine fiber pathways are necessary in maintaining visual cortical plasticity (Kasamatsu et al., 1981). While the specificity of 6-OHDA for catecholamines (Malmfors and Sachs, 1968; Jonsson et al., 1975) has recently been challenged (Sillito, 1986), Bear and Singer (1986) suggest that 6-OHDA disrupts visual cortical plasticity by blocking both noradrenergic and cholinergic modulation of cortical activity.

Additional studies involving 6-OHDA-induced NA depletion in kittens have failed to disrupt visual cortical plasticity (Bear and Daniels, 1983; Daw et al.,1984; Adrien et al.,1985). The failure of NA depletion in neonates to prevent the ocular dominance shift response to monocular deprivation suggests that remarkably adaptive mechanisms exist early in development that can compensate rapidly for the depletion of NA (Bear and Daniels, 1983). Following neonatal administration of 6-OHDA, the major manifestation of the destruction of normal NA input is a supersensitivity in the adrenergic receptor/adenylate cyclase system wherein there is a 45-70% increase in density of these receptors in the cortex (Harden et al., 1977).

The role of NA on development and plasticity remains a controversial issue. The conflicting results obtained in studies of the effect of NA depletion on visual cortical plasticity may be attributed to the variety of paradigms used to ablate NA fibers (Daw, 1985).

Noradrenaline has been depleted by neonatal injection of 6-OHDA (Bear and Daniels, 1983), electrolytic lesions of the dorsal NA bundle (Daw et al., 1984), stereotaxic injections of 6-OHDA into the coeruleus complex (Adrien et al., 1985), intraventricular injections of 6-OHDA (Daw et al., 1984; Kasamatsu, 1979), intracortical microperfusion (Kasamatsu 1979, 1981), systemic and intraventricular injection of a NA neurotoxin DSP4 (Daw et al., 1985; Jonsson 1981, 1982), and intraperitoneal injections of clonidine (Nelson et al.,1985).

The effect of 6-OHDA on motor cortical plasticity has also been examined. Neonatal administration of 6-OHDA in rats results in poor performance in motor tests (Jonsson et al., 1979). Whereas low-threshold ipsilateral forelimb movements were evoked electrophysiologically in animals that sustained neonatal cortical lesions, this response was not seen in similarly ablated 6-OHDA-treated animals. This suggests that noradrenaline may influence corticospinal plasticity (Castro et al., 1986). In addition, the depletion of cortical NA completely blocks sparing of behavioral function typically observed after neonatal frontal cortex damage (Sutherland et al., 1982).

ANOMALOUS VENTRAL CORTICOSPINAL PROJECTIONS AFTER UNILATERAL CEREBRAL CORTICAL LESIONS IN NEWBORN RATS

ABSTRACT

Multiple injections of wheat-germ agglutinin horseradish peroxidase (WGA-HRP) were placed within left sensorimotor cerebral cortical areas in normal adult rats and in adult rats that sustained right frontoparietal cortical lesions at birth. Analysis of anterograde labeling within the spinal cord of normal rats demonstrated the presence of CST projections mainly within the base of the contralateral dorsal funiculus with considerably fewer CST projections observed bilaterally within the lateral funiculi and ipsilaterally within the dorsal and ventral funiculi. CST distribution within the gray matter in normal rats was mainly contralateral. Similar CST projections were found in adult rats that sustained unilateral cortical ablation at birth with the addition of anomalous CST projections within the ipsilateral dorsal and ventral funiculi and the ipsilateral gray matter. While the anomalous CST fibers observed within the ipsilateral dorsal funiculus have been previously described in several reports, anomalous projections within the ventral funiculus have not been observed in these earlier reports which used axonal degeneration staining techniques.

INTRODUCTION

The corticospinal tract (CST) in rats is primarily a crossed projection descending within the ventral aspect of the spinal cord dorsal funiculus (King, 1910; Brown, 1971; Donatelle, 1977; Schreyer and Jones, 1982). In addition to this major component, sparse CST fibers have been found bilaterally within the lateral funiculi of the spinal cord (Shreyer and Jones, 1982) and ipsilaterally in the dorsal and ventral funiculi (Goodman et al., 1966; Vahlsing and Feringa, 1980). The CST system has also been found to be an ontogenetically late developing system (Donatelle, 1977; Shreyer and Jones, 1982) and this may account for its capacity to form anomalous projections in response to various central nervous system lesions as described in several reports. For example, after cortical (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975) or medullary pyramid (Castro, 1978) lesions, which destroyed CST projections on one side in newborn rats, the spared, primarily crossed CST system developed anomalous projections to the ipsilateral side of the spinal cord. In further work, developing CST fibers were found to grow around medullary pyramid lesions in newborn hamsters (Kalil and Reh,1982) and around partial spinal cord lesions in newborn rats (Bernstein and Stelzner, 1983; Bregman and Goldberger, 1983; Dauzvardis and Castro, 1986).

Since previous anterograde tracing studies of CST remodelling after neonatal cortical lesions were primarily based on axonal degeneration methods, the present study was undertaken to reexamine cortical lesion-induced CST remodelling using the WGA-HRP tracing method (Mesulam, 1978). In comparison to axonal degeneration methods, anterograde transport of HRP from the injection site facilitates visualization of the axons and axonal terminals regardless of fiber diameter. Additionally, background labeling is minimal and labeled projections may be studied within a few days of HRP injection (De Olmos and Heimer, 1981).

The application of HRP technique to the study of normal CST projections (Sievert, 1985; Schreyer and Jones, 1982; O'Donoghue et.al., 1987) revealed a more complete description of CST pathways and terminations than described by axonal degeneration methods. Similarly, a previously undescribed anomalous ventral CST projection was observed in addition to previously reported anomalous projections in adult rats that sustained unilateral cortical lesions at birth. This study has appeared in abstract form (Reinoso and Castro, 1986).

MATERIALS AND METHODS

Twenty nine Long Evans black-hooded rats were used in this study. Newborn to 2 day old rats (n=20) were anesthesized by hypothermia and the right fronto-parietal cortex was aspirated using a glass pipette with a 0.5 mm tip (Fig. 1). Postoperatively, the pups were warmed under an incandescent lamp and returned to their mothers until weaning.

At 2-4 months of age and under Ketamine anesthesia (50mg/kg), multiple injections of 1% WGA-HRP were placed into the left cerebral cortex of animals that sustained right cortical lesions at birth. Nine normal adult control rats received comparable injections. The WGA-HRP injections (0.04 ul/injection) were made in two clusters at a depth of 1.2mm from the cortical surface. Rostrally, 4 injections were located in an area 4mm rostral to bregma and 2mm from the midline (Fig. 1), i.e., in the frontal cortical area corresponding to the second forelimb motor cortex (Neafsey and Sievert, 1982). Caudally, 6-9 injections were placed within a $3mm^2$ area located approximately 0.5mm rostral to bregma and 3mm from the midline (Fig. 1), which corresponds to portions of the caudal forelimb and hindlimb sensorimotor area (Neafsey et al.,1986). Some control animals received larger WGA-HRP injections.

After a 2-day survival, animals were perfused intracardially with a 1.0% paraformaldehyde-1.25% glutaraldehyde fixative followed by 10% buffered sucrose. Fifty micron coronal sections of the brain and 50um

coronal and horizontal sections of the cervical, thoracic and lumbar cord were cut with a freezing microtome and gathered in trays of 0.1M phosphate buffer. Spinal cord and alternate brain sections were reacted with tetramethyl benzidine (TMB) (Mesulam, 1978) and mounted on subbed slides and stained with 0.025% Toluidine blue. All slides were dehydrated, defatted and coverslipped with Depex. The WGA-HRP stained sections were viewed using a Leitz polarized light microscope. The course and terminal distribution of crossed and uncrossed CST projections was qualitatively evaluated.

RESULTS

Lesion and injection sites

Large cortical lesions included the right frontoparietal area and occasionally involved dorsal portions of the caudate-putamen and thalamus (Fig.1B). Gross inspection of the ventral medulla demonstrated a substantial reduction of the pyramid ipsilateral to the cortical lesion.

Toluidine blue-stained coronal sections through levels containing the injection sites showed that the micropipette tracks extended from superficial cortical layers to lamina IV and V. Reference to cytoarchitectural maps of the rat sensorimotor cortex (Donoghue and Wise, 1982; Neafsey, et.al., 1986) indicated that injections were located in rostral and caudal portions of the lateral agranular field and medial portions of the granular cortex of the primary somatosensory area. Adjacent sections reacted with TMB demonstrated a confluence of labeling such that individual injection sites were indistinguishable. The injections were confined to the neocortex with some diffusion into the corpus callosum.

Normal Adult Rats

WGA-HRP labeled corticospinal fibers were found traversing several pathways in the spinal cord with a generally decreasing labeling density at cervical, thoracic and lumbar levels. The most dense, primary

CST projection was seen within the ventral portion of the contralateral dorsal funiculus and spreading along its lateral border adjacent to the base of the dorsal horn (Fig. 2A). Considerably less labeling was seen within the contralateral lateral funiculi, and a trace of labeling could occasionally be found within the ipsilateral lateral funiculus. Sparse labeling was also found within the ipsilateral dorsal and ventral funiculi (Fig.2A-C). Ventral funicular fibers were only seen at cervical levels (Fig.2. A,C). In the spinal cord gray matter, terminal labeling was heaviest contralaterally within the medial borders of the base of the dorsal horn and the intermediate gray (Fig.3A). Few labeled fibers were seen within the ventral horn at cervical, thoracic and lumbar levels. No labeled fibers were seen in the apex of the dorsal horn corresponding to Rexed's lamina I-II (McClung and Castro, 1978). Labeling within the ipsilateral gray matter was very sparse.

Adult Rats with Neonatal Cortical Lesions

The contralateral course and distribution of WGA-HRP labeled fibers originating from the unablated hemisphere in rats that received unilateral cortical lesion at birth was comparable to control animals. These fibers descended primarily within the contralateral dorsal funiculus and distributed most heavily within the dorsal and intermediate gray matter (Fig.3B), and also like control animals, sparse bilateral labeling within the lateral funiculi was observed. In contrast to

control animals, more prominent CST projections were observed traversing the ipsilateral dorsal and ventral funiculi (Fig.2A'-C'; Fig.4). The increased projection within the dorsal funiculus was found in cervical, thoracic and lumbar levels, but the increased ventral funicular projection was only observed in cervical levels. Corresponding to these anomalous projections, increased labeling within the ipsilateral gray matter was also found in all levels (Fig.3B; Fig.4). The distribution of this labeling was similar but less dense than contralateral labeling. The density of labeled fibers within cervical spinal cord gray matter was heavier than in lumbar gray matter.

DISCUSSION

The present study concurs with previous findings that the CST in normal rats is mainly a crossed projection traversing the ventral aspect of the dorsal funiculus (King, 1910; Brown, 1971; Schreyer and Jones, 1982) with a few fibers coursing within the contralateral lateral funiculus (Schreyer and Jones, 1982; Sievert, 1985). Sparse labeling within the ipsilateral dorsal, lateral and ventral funiculi also corroborates previous work (Goodman et.al., 1966; Vahlsing and Feringa, 1980). The terminal distribution of CST fibers, observed primarily within the contralateral spinal cord gray matter, supports previous reports using HRP tracing methods (Shreyer and Jones, 1982; Sievert, 1985; O'Donoghue et.al., 1987). Unlike earlier studies based on silver degeneration methods which showed CST terminations primarily within the dorsal horn (King, 1910; Brown, 1971), terminations were additionally found within the intermediate gray with a few fibers coursing into the ventral horn.

The observed increase of CST fibers traversing the base of the ipsilateral dorsal funiculus after unilateral cortical ablation at birth supports initial reports of CST plasticity (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). However, the anomalous uncrossed CST projections found within the ventral funiculus have not been previously described in rats although a similar projection has been

described in humans born with a unilateral encephalocele (Verhaart and Kramer, 1952). While the density of CST fibers coursing within the ipsilateral dorsal funiculus of our animals appeared comparable to earlier studies (Castro, 1975) the appearance of anomalous fibers within the ipsilateral ventral funiculus is attributed to the increased sensitivity of the WGA-HRP method. The anomalous ventral CST projection may therefore be composed of thin, unmyelinated fibers which were not detected by axonal degeneration stains and which have been found in the normal rat CST (Leenen et.al., 1985).

The mechanisms involved in the growth of the anomalous CST fibers are largely unknown although analysis using retrograde fluorescent tracers demonstrate that they arise from neurons in topographically appropriate regions within the sensorimotor areas of the unablated cortical hemisphere (Reinoso and Castro, 1988). This same study also found that the cortical lesion-induced ipsilateral CST fibers do not form as collaterals from normal, crossed CST axons. Earlier work showed that they do not represent an abnormal persistence of developmentally transient projections (Nah et.al., 1980) despite evidence of postnatal reduction of axonal numbers within the dorsal funiculus (Chung and Coggeshall, 1987) and the occurrence of transient occipitospinal projections (Stanfield and O'Leary, 1982).

The neonatal lesion-induced formation of anomalous CST projections is correlated with the immature status of the rodent CST

system at birth (DeMyer, 1967; Hicks and D'Amato, 1970, 1974; Donatelle, 1977; Shreyer and Jones, 1982). Possibly, the anomalous CST projections arise from developmentally later growing CST fibers that normally decussate within the medulla but which fail to do so after lesions that destroy one corticospinal system early in development (Hicks and D'Amato,1970). In this regard, the quantitative imbalance of CST fibers growing through the spinomedullary junction after unilateral lesions at birth has been proposed to disrupt an interaction between the two systems which normally leads to their decussation (Verhaart and Kramer, 1952; Leong and Lund, 1973). The reported decreasing density of anomalous cortical efferent projections found after cortical lesions made at progressively older ages up to postnatal day 20 (Leong,1976) conforms to the general principle derived from numerous studies that neuronal remodelling is typically more prominent after lesions at an early critical age. Accordingly, the absence of CST remodelling after neonatal cortical lesions in cats (Leonard and Goldberger, 1987) and monkeys (Sloper et.al., 1983) is attributed to the more mature status of the feline and primate CST systems at birth (Purpura et.al., 1964; Passingham et.al., 1983) in comparison to rodents. However, the development of anomalous corticostriate projections after prenatal cortical lesions in rhesus monkeys (Goldman, 1978) demonstrates the capacity for primate remodelling when lesions occur at an early developmental stage.

The course and topographic distribution of anomalous CST fibers

suggests that they are capable of innervating synaptic sites deprived of their normal inputs by neonatal cortical lesions. While it is difficult to envision how cortical neurons can send axons long distances to innervate targets in the spinal cord, the course taken by the anomalous projections would appear to provide a partial explanation. These projections are found to follow routes that normally contain at least a few normal CST fibers rather than following entirely novel trajectories. Accordingly, once growing CST axons fail to decussate in the medulla, they may respond to guidance mechanisms ordinarily followed by normal CST axons and thus increase the normally sparse ipsilateral CST pathways to innervate available synaptic sites.

Fig. 1. Schematic diagram of the brain of an adult rat illustrating the extent of right fronto-parietal cortical aspiration lesions sustained at birth. The distribution of multiple WGA-HRP injections placed into the unablated ocrtex at maturity is shown.

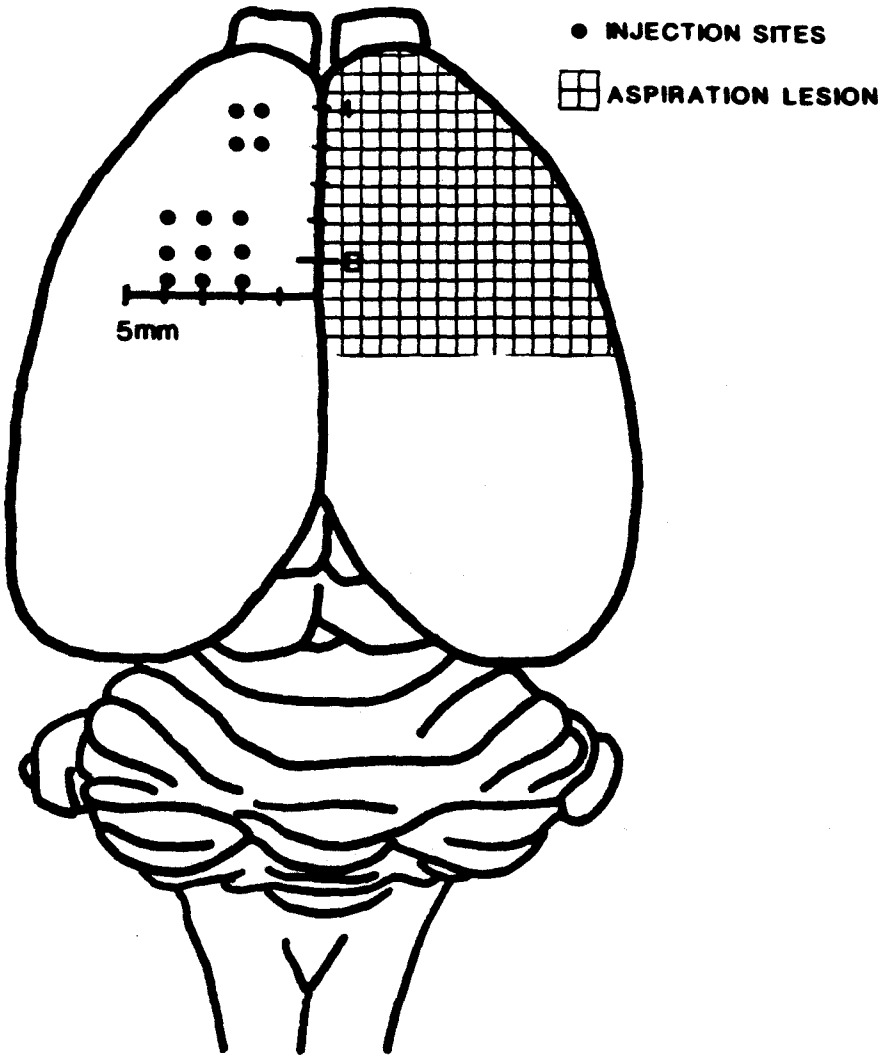

INJECTION SITES
ASPIRATION LESION
5mm

Fig. 2. Darkfield photomicrographs of spinal cord sections demonstrating WGA-HRP labeled CST fibers in normal adult rats (A-C) and adult rats that sustained unilateral cortical ablation at birth (A^1-C^1). Representative coronal (A,A^1) and horizontal (B,B^1,C,C^1) sections through the cervical spinal cord show the normal crossed CST projections (*) and ipsilateral uncrossed CST fibers traversing the dorsal ($dCST_i$; arrows) and ventral funiculus (vCST). Note how the ipsilateral dCST and the vCST appear enlarged in A^1-C^1.

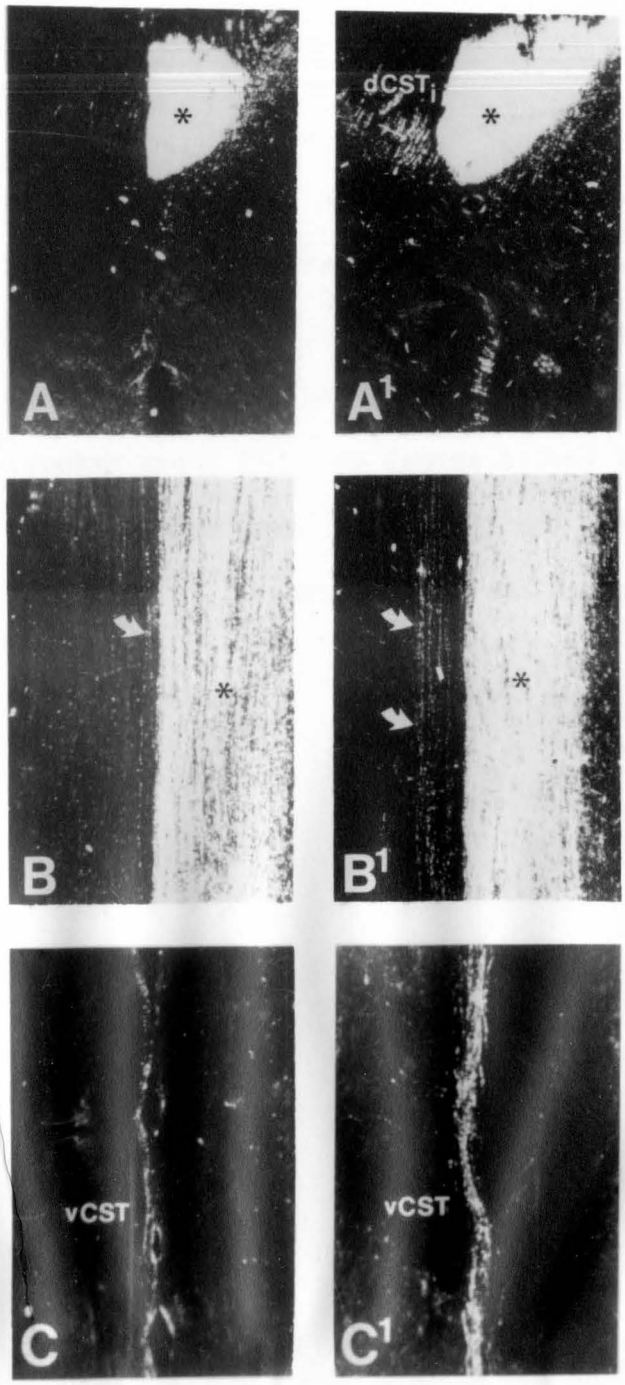

A
A1
dCST
B
B1
vCST
C
vCST
C1

Fig. 3. Camera lucida drawings through cervical spinal cord levels demonstrating the distribution of WGA-HRP labeled CST fibers in normal adult rats (A) and adult rats that sustained unilateral cortical lesions at birth (B). The ipsilateral dCST (solid arrow) and vCST (open arrows) and terminations within the ipsilateral gray matter appear more dense after neonatal cortical lesion.

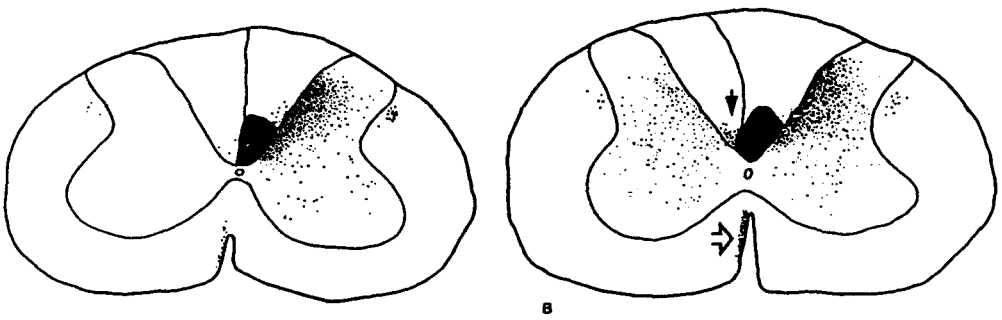
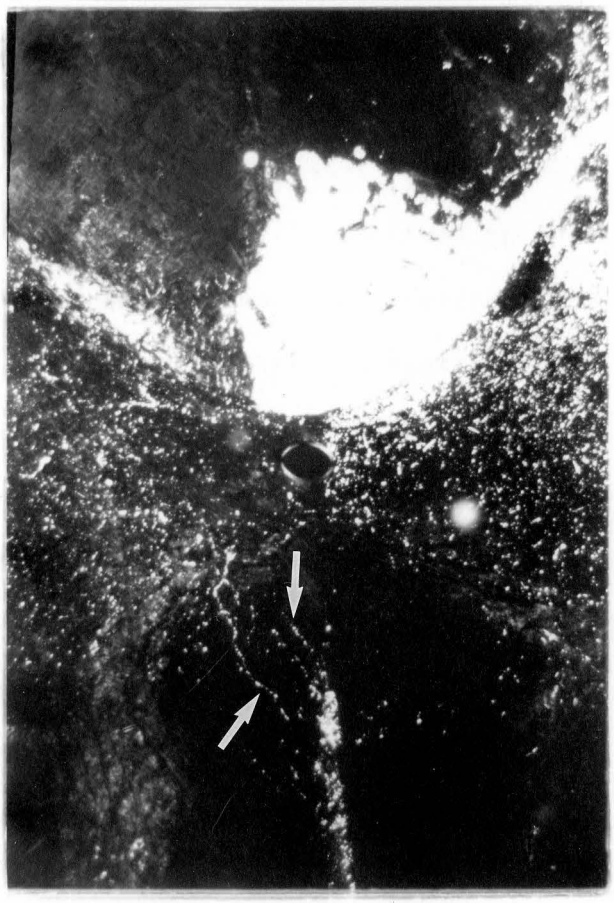


Fig. 4. Darkfield photomicrograph of a cervical spinal cord cross-section showing bilateral CST distribution within the spinal cord gray matter of adult rats that sustained unilateral cortical lesions at birth. Few CST fibers from the ipsilateral ventral funiculus (arrows) are seen coursing into the medial aspect of the ventral horn.



A STUDY OF CORTICOSPINAL REMODELLING USING RETROGRADE FLUORESCENT TRACERS IN RATS

ABSTRACT

The retrogradely transported fluorescent tracers fast blue (FB) and diamidino yellow (DY) were injected into the spinal cord of adult rats that sustained unilateral frontoparietal cortical lesions at birth. Analysis of the resulting cortical labeling pattern in comparison to comparably injected control animals demonstrated an increase of retrogradely-labeled neurons within the unablated cerebral hemisphere ipsilateral to spinal cord injections. These ipsilateral labeled cells corresponded to previous descriptions, based on anterograde tracing techniques, of anomalous uncrossed CST (corticospinal tract) fibers. Additional findings indicated that the ipsilateral CST fibers are not axonal collaterals of normal, crossed CST fibers. Fluorescent tracer injections into cervical and lumbar spinal cord levels demonstrated a somatotopic distribution pattern of labeled cells within the ipsilateral cortex that was similar to the topographic pattern found contralateral to spinal cord injections in normal animals. That the somatotopic distribution pattern of the anomalous uncrossed CST neurons is consistent with the topographic pattern of cells that elicit abnormal forelimb movements (as determined by intracortical microstimulation), suggests that the neonatal cortical lesion-induced increase of ipsilateral CST fibers may be functional.

INTRODUCTION

Previous studies on the course and distribution of normal (Brown, 1971; Schreyer and Jones, 1982) and anomalous rodent corticospinal tract (CST) projections (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975) have used various anterograde tracing techniques. From these investigations, an increased ipsilateral CST projection was found in adult rats that sustained unilateral cerebral cortical lesions at birth (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). These projections, which originated from the unablated hemisphere, traversed the ipsilateral dorsal funiculus of the spinal cord and were considerably less dense than normal crossed projections primarily located in the contralateral dorsal funiculus (Brown, 1971; Schreyer and Jones, 1982) More recently, a neonatal cortical lesion-induced increase of ipsilateral CST fibers traversing the ventral funiculus has also been reported (Reinoso and Castro, 1986). While distribution patterns of normal rodent CST neurons have been studied using retrograde tracing techniques, these methods have not been used to study CST remodelling after neonatal cortical lesions. The use of fluorescent tracers which are retrogradely transported at comparable rates is appropriate for studying the somatotopy of a pathway (e.g., CST) which projects to various levels of the neuraxis. Additionally, because of the capacity of different

fluorescent dyes to label separate aspects of a neuron, the presence of double-labeled cells retrogradely labeled from separate injection sites confirms the occurrence of collateralization of neuronal pathways (Kuypers et al., 1980; Bentivoglio et al., 1980).

Injections of horseradish peroxidase into various levels of the rat spinal cord of normal rats have shown a somatotopic topographic distribution of retrogradely-labeled neurons within the cerebral cortex (Hicks and D'Amato, 1977; Wise et al., 1979; Miller et al., 1987; Wise and Jones, 1977; Leong, 1983). Further work based on the retrograde transport of fluorescent tracers injected into spinal and subcortical areas demonstrated that CST fibers extended axonal collaterals to various brainstem structures (Catsman-Berroevoets and Kuypers, 1979, 1981; Ugolini and Kuypers, 1986; Keizer and Kuypers, 1984; Bentivoglio and Rustioni, 1986). In the present study, retrograde fluorescent labeling techniques were used to examine the possible somatotopic topographic distribution pattern of ipsilateral CST neurons in rats that sustained neonatal cortical lesions and to examine whether ipsilateral CST fibers arise from an independent population of cells or as collateral branches of crossed CST projections. Preliminary results have been previously reported (Reinoso and Castro, 1985; Reinoso and Castro, 1986).

MATERIALS AND METHODS

Long Evans black-hooded rats were used in this study. Twenty rats (0-3 days old) were anesthesized by hypothermia, and the right fronto-parietal cortex was aspirated using a glass pipette with a 0.5 mm tip. Postoperatively, pups were warmed under an incandescent lamp and returned to their mothers until weaning.

At maturity (2-8 months of age) and under anesthesia induced by sodium pentobarbital (42 mg/kg), laminectomy at vertebral levels C5-C7 was performed on 12 of the rats that sustained neonatal cortical lesions and five normal adults. Using a Hamilton syringe fitted with a 100um glass tip, fast blue (FB) and diamidino yellow (DY) were injected (Bentivoglio, 1980; Keizer, 1983) and into the gray matter of the left and right sides of the cervical spinal cord, respectively. The dyes were introduced into the cord at a 15° vertical angle to avoid labelling descending CST fibers within the dorsal funiculus. Each side of the spinal cord received five injections of one dye (either FB or DY) spaced 1mm apart at a depth of 1-1.5mm. Each 0.02 ul injection of 2% FB was placed on the left side of the cord and the five 0.06 ul injections of 2% DY on the right side. In three animals with cortical lesions, the placement of dyes was reversed.

Similar to the above procedure, the remaining eight animals with neonatal cortical lesions received dye injections at cervical (C5-7) and

lumbar (L2-4) levels. Five 0.02ul injections of 2% DY were placed into the left cervical cord and five 0.06ul injections of 2% FB were placed into the same side at lumbar levels. Three normal adults received similar injections.

At six days after dye injections, animals were overdosed with sodium pentobarbital and perfused intracardially with 10% buffered formalin and buffered sucrose. After 24-48 hours immersion in 30% buffered sucrose, 50um horizontal sections through the spinal cord injection sites were cut with a cryostat, mounted, coverslipped and viewed under a Leitz epifluorescent microscope to check for spreading of dyes across the midline of the spinal cord.

The brains from animals with dyes confined unilaterally within the spinal cord were cut sagittally at 40 um. Every fourth section was mounted on subbed slides and coverslipped with Entellan following standard histological dehydrating and defatting procedures. Adjacent sections were stained with pyronin Y and coverslipped with Depex. Retrogradely labeled fluorescent neurons observed within the cerebral cortex in unstained sections were plotted and counted using an X-Y digitizing system (Minnesota Datametrics). The mean number of labeled neurons retrogradely labeled from injection sites within the contralateral and ipsilateral cervical spinal cord were determined in lateral, medial and mediolateral portions of the left cerebral cortex.

The laterality of retrograde labeling within the red nuclei was

also examined. Because the rubrospinal tract is mainly a crossed pathway (Massion, 1967; Flumerfelt and Gwyn, 1974), the presence of only one dye within neurons of the red nucleus provided further indication of the laterality of injection sites.

RESULTS

Injection sites

Injections were principally located in the spinal cord gray, and the path of the pipette invariably did not damage the dorsal funiculus, i.e., the principal course of CST fibers. Injection sites appeared not to spread across the midline in 13 of the 17 animals receiving bilateral injections at cervical levels. This was further confirmed by the laterality of labeling within the brainstem red nucleus. Similarly, injections were confined to the left side of the spinal cord in all eleven animals receiving dye injections at cervical and lumbar levels.

Normal Adult Rats

Sagittal sections through the cerebral hemispheres of animals that received bilateral cervical spinal cord injections demonstrated numerous (Appendix) single-labeled neurons contralateral to the injection sites (Figs.1,A-G and 2A). No double-labeled neurons were found and only an occasional labeled cortical neuron corresponding to an ipsilateral injection was observed in two animals. Labeled cells were localized within lamina V and were widely distributed within motor and somatosensory cortical areas. An area with sparse cortical labeling

intervened between somatosensory areas I and II (Figs. 1B; 3B). Medially, labeled cells typically formed a continuous band of neurons with a separated cluster located rostrally near the frontal pole (Fig. 1, E-G).

Numerous retrogradely labeled cortical neurons were also found contralaterally in animals receiving dye injections at cervical and lumbar levels (Fig. 3,A-H). While some intermingling of the distribution patterns were found, neurons corresponding to cervical injections were generally located rostral and lateral to neurons labeled by lumbar injections. An isolated cluster of neurons within the frontal pole corresponded to cervical injections (Fig. 3I). No double-labeled neurons were found after cervical and lumbar injections.

Adult Rats with Neonatal Cortical Lesion: Bilateral Cervical Injections

Right hemisphere lesions included the frontoparietal cortex and generally involved the subcortical white matter with slight involvement of the dorsal portion of the caudate-putamen. Gross inspection of the ventral surface of the brainstem showed the absence or reduction of the medullary pyramid corresponding to the ipsilateral cortical lesion.

Sagittal sections through the unablated (left) cerebral cortex following bilateral cervical spinal cord injections demonstrated both FB and DY single-labeled neurons. Retrograde labeling within the unablated hemisphere was typically very dense with respect to the tracer injected

into the contralateral side of the spinal cord. Considerably less labeling corresponded to the ipsilaterally placed spinal cord injections. Labeled cell counts (Abercrombie, 1946) in four animals showed an approximate 3:1 ratio of contra- to ipsilateral labeling. No double-labeled cells were found. Topographically, both DY- and FB-labeled cells were widely distributed within lamina V of the motor and somatosensory cortex (Figs. 1 A_1-H_1 and 2B). A slight rostral and lateral shift of labeled neurons corresponding to ipsilateral cervical spinal cord injections was observed. In these cases, the gap between SI and SII was smaller than that found in normal animals. Similarly, the discontinuous labeling pattern with an isolated cluster of labeled cells in the frontal pole as seen in control animals, was not found in animals with neonatal cortical lesions. Instead, they typically demonstrated a continuous band of cells extending rostrally toward this frontal pole. Labeled neurons within this area as well as those found between SI and SII corresponded to ipsilateral cervical injections. No labeled neurons were found within occipital regions of both hemispheres (Fig.1 F_1-G_1). Similar results were obtained in 3 animals wherein placement of dyes was reversed.

Adult Rats with Neonatal Cortical Lesion: Unilateral Cervical and Lumbar Injections

Unilateral tracer injections into cervical (DY) and lumbar (FB)

spinal cord levels ipsilateral to the unablated hemisphere demonstrated single- labeled neurons within the unablated cortex. The distribution pattern of this ipsilateral labeling was similar to the contralateral labeling patterns seen in normal animals, i.e., cells projecting to cervical levels were generally rostral and lateral to neurons projecting to lumbar levels (Fig.3, A_1-I_1).

DISCUSSION

The observed distribution of retrogradely labeled neurons within the contralateral cerebral cortex after FB and DY injections into the cervical spinal cord of normal rats concurs with several previous reports based on the retrograde transport of HRP (Hicks and D'Amato, 1977; Wise et al., 1979; Donoghue and Wise et al., 1982; Leong, 1983; Miller, 1987). Reference to published cytoarchitectural maps of the rodent cerebral cortex (Hall and Lindholm, 1974; Wise et al., 1979; Donoghue and Wise, 1982; Zilles and Wree, 1985; Neafsey et al., 1986) indicates that this labeling is primarily within motor and somatosensory (SI and SII) cortical areas. In accordance with anterograde tracing methods showing a sparse ipsilateral CST projections in normal rats, the presence of only occasional ipsilateral cortical labeling further demonstrated that the normal CST is predominantly a crossed projection. Additionally, the topographic distribution of labeled cortical neurons found after cervical and lumbar injections in control animals corresponds to previous work (Wise et al., 1979; Hicks and D'Amato, 1977; Leong, 1983; Miller, 1987). The differential labeling according to the level of the injection sites as well as the absence of double-labeling, indicating a lack of axonal branching to upper and lower spinal cord levels, concurs with the cortical somatotopy demonstrated by studies using electrical stimulation mapping techniques (Welker, 1971; Hall and Lindholm, 1974; Donoghue and

Wise, 1982; Sievert, 1985; Neafsey et.al., 1986).

In contrast to the predominantly contralateral cortical labeling found in control animals, numerous retrogradely-labeled cortical neurons were found ipsilateral to spinal injections in adult animals that sustained unilateral cortical lesions at birth. The presence of these labeled neurons within the unablated hemisphere corroborates several reports based on anterograde tracing techniques demonstrating anomalous ipsilateral CST fibers after neonatal cortical lesions (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). The distribution of ipsilateral labeled neurons within motor and somatosensory cortical areas generally overlapped the normal contralateral distribution and thus indicates that the ipsilateral CST neurons display a somatotopic organization resembling normal crossed CST neurons. Related work on corticopontine remodelling after neonatal cortical lesions similarly demonstrated a topographic distribution of anomalous crossed corticopontine fibers which resembled normal ipsilateral corticopontine projection patterns (Kartje-Tillotson et al., 1986).

The absence of double-labeled cortical neurons after FB and DY injections into the left and right sides of the cervical spinal cord in animals sustaining neonatal lesions indicates that the lesion-induced increase of ipsilateral CST fibers is due to the existence of an independent population of ipsilaterally projecting neurons and is not a result of abnormal axonal collaterals branching from normal crossed CST

projections. This is consistent with the finding that a neonatal cortical lesion-induced increase of contralateral corticorubral fibers was not due to the formation of axonal collaterals from normal uncrossed projections (Naus et al., 1986). The intermingled distribution of ipsi- and contralateral corticorubral projecting neurons as found after neonatal cortical lesions (Naus et al., 1986) resembled our findings concerning ipsi- and contralateral CST projecting neurons. Both of these examples, as well as studies of corticopontine remodelling (Castro and Mihailoff, 1983; Kartje-Tillotson et al., 1986; Leong, 1976) demonstrate the formation of anomalous connections to areas deprived of normal inputs by a cortical lesion. However, corticorubral and corticopontine remodelling differs from CST remodelling in that the former demonstrate an increase of crossed projections whereas CST fibers exhibit an increase of uncrossed projections after neonatal cortical lesions. While remodelling observed in these studies may represent an increased collateralization of the small number of axons that normally terminate in the areas showing an increased input, previous data (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975; Reinoso and Castro, 1986) indicate an actual increase of ipsilateral CST descending within the spinal cord white matter. Similarly, corticopontine remodelling appears to reflect an increase of crossed projections and not just an enhanced terminal collateralization of normal crossing fibers (Castro and Mihailoff, 1983).

The mechanisms that prompt growing axons to cross or not cross the midline, even in normal situations, are not established. The question of fiber decussation in relation to remodelling becomes more intriguing in view of double labeling studies of normal corticofugal projections. In this work, crossed CST projections were found to send axonal collaterals to the ipsilateral red nucleus (Catsman-Berrevoets and Kuypers, 1981) or to the ipsilateral pontine gray (Ugolini and Kuypers, 1986). These findings therefore demonstrated that single cortical neurons with ipsi- and contralateral axonal branches is not an uncommon feature in cortical efferent organization. The question is therefore raised as to whether the lesion-induced, crossed corticorubral or corticopontine projections arise as axonal collaterals from anomalous uncrossed CST fibers. This possibility, which could be tested by the use of retrograde fluorescent tracers, implies that in response to neonatal lesions individual cortical projection neurons would have reversed the laterality of their axonal branches. However, the final distribution of projections would be in accordance with the normal developmental patterns of corticorubral and corticopontine terminations being opposite CST projections.

The reversed laterality of cortical efferents, even though we do not know whether this may be reflected in a reversed distribution of collaterals, would seem likely to have dire behavioral consequences. However, recent electrophysiological data, based on limb movements evoked by low-threshold intracortical microstimulation, indicates that the

anomalous ipsilateral CST is functional (Kartje-Tillotson et al., 1985, 1987). The topographic distribution of stimulation points which evoked ipsilateral forelimb and hindlimb movements corresponded to normal CST topography. This topographic pattern also corresponds to the distribution of single-labeled cortical neurons as observed in the present report after dye injections into cervical and lumbar spinal cord levels. While movements evoked by cortical stimulation correlate with the topography of ipsilateral CST fibers, the possible contributions of remodelled corticorubral and corticopontine projections requires further consideration.

Limb movements evoked by low-threshold currents are generally considered to be mediated by CST fibers (Kartje-Tillotson et.al., 1987) but the possible contribution of other simultaneously stimulated pathways such as the corticorubral and corticopontine fibers has not been described even in normal animals. However, tracing the course of pathways activated by cortical stimulation clearly indicates involvement of several systems in the cortical control of movement. For example, the opposite side of the spinal cord can be influenced by cortical stimulation via crossed CST and cortico- rubrospinal tract systems. Collaterals from CST axons to the red nucleus could further provide an integrating coactivation of the spinal cord and red nucleus. In the case of adult animals that sustained neonatal cortical lesions, cortical stimulation could affect the ipsilateral side of the spinal cord via

ipsilateral CST fibers as well as anomalous crossed corticorubral fibers. Accordingly, the topography of ipsilateral CST fibers, as evident from our data, and the possibility of these fibers sending collaterals to the contralateral red nucleus could provide the appropriate anatomical framework for functional recovery after neonatal cortical lesions.

Table 1. Mean Number of CST Neurons Within the Left Hemisphere Retrogradely Labeled with Fluorescent Tracers from the Contralateral and Ipsilateral Cervical Spinal Cord.

	CONTRALATERAL		IPSILATERAL		
	$\bar{x}$	S.E.M.	$\bar{x}$	S.E.M.	Ratio contra/ipsi
CONTROL					
lateral	5.23	0.36	0	0	
ML	24.87	5.33	0.30	0.19	83:1
medial	16.12	4.40	0.42	0.23	38:1
LESION					
lateral	7.51	1.87	2.08	0.64	4:1
ML	20.77	4.17	4.14	0.70	5:1
medial	10.00	4.86	5.30	1.10	2:1

ML - mediolateral

Fig. 1. Camera lucida drawings of sagittal sections through the left hemisphere of normal adult rats (A-G) and adult rats that sustained right frontoparietal ablations at birth (A_1-H_1). The drawings illustrate the distribution of retrogradely labeled cortical neurons found after bilateral cervical spinal cord injections of FB(circles) and DY (dots) as diagrammed by the right inset.

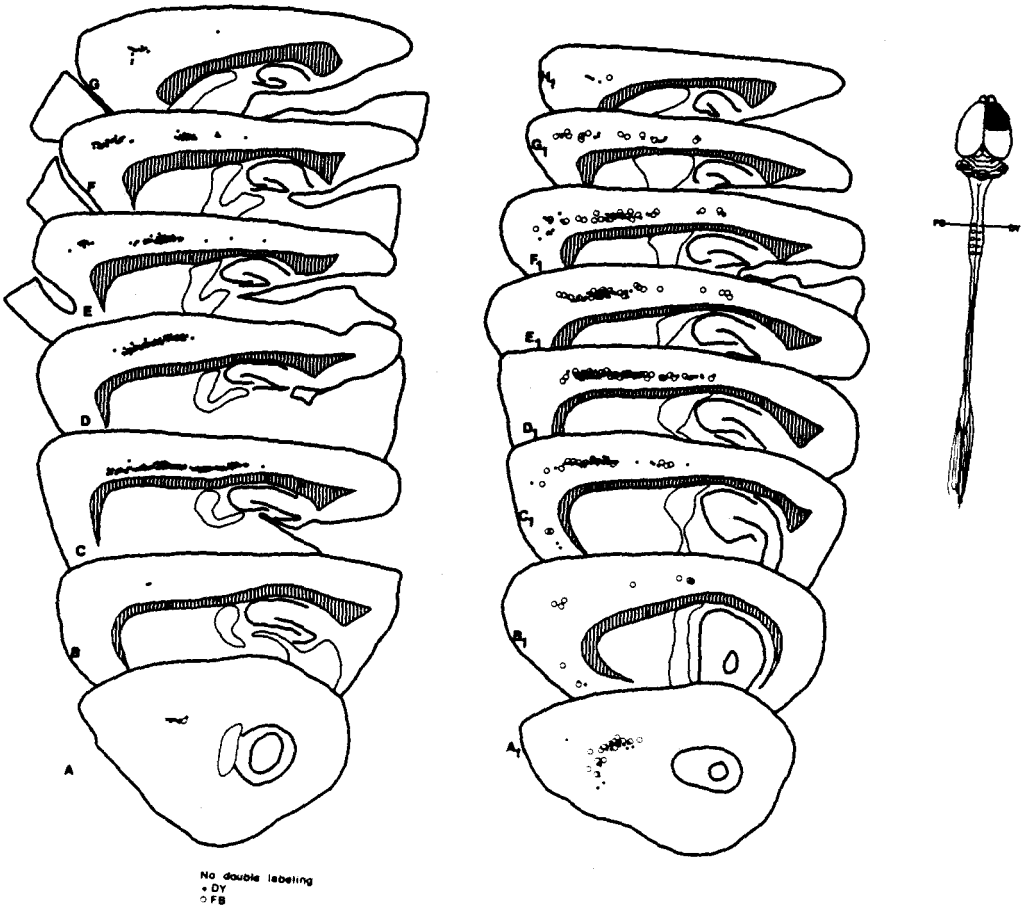

G
F
E
D
C
B
A
No double labeling
• DY
○ FB

Fig. 2. Fluorescent photomicrographs showing retrogradely labeled cortical neurons found in the left cerebral cortex after bilateral cervical spinal cord injections with FB and DY in normal rats (A) and in an adult rat that sustained a neonatal right frontal cortical lesion (B). The primarily spherical shaped nuclear labeling in A corresponds to contralateral DY injections while the cell body labeling (arrows) in B corresponds to ipsilateral FB injections. Scale = 100um.

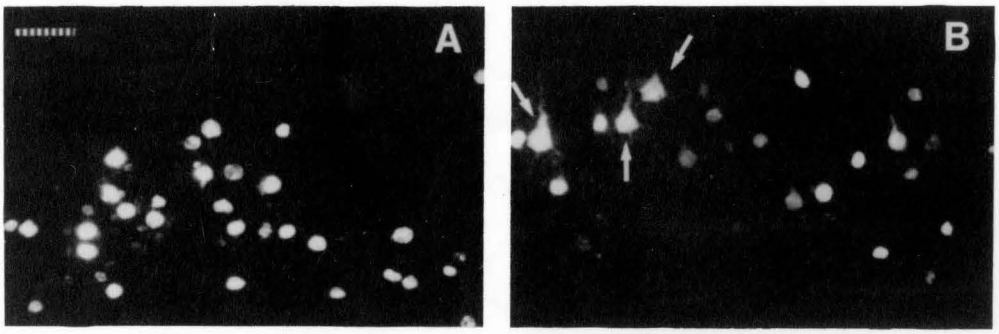
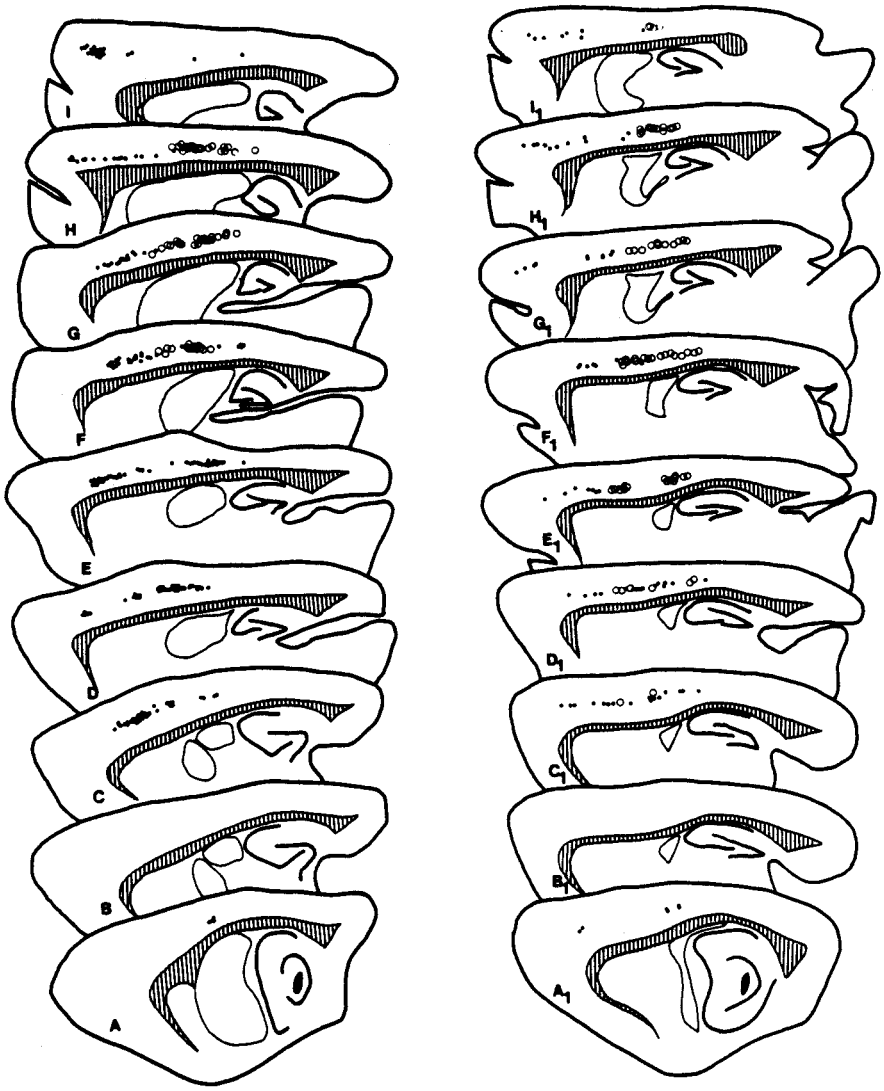

A
B

Fig. 3. Camera lucida drawings of sagittal sections through the left hemisphere of normal adult rats (A-I) and adult rats that sustained right frontoparietal ablation at birth (A_1-I_1). The drawings illustrate the distribution of retrogradely labeled cortical neurons found after unilateral (left) cervical and lumbar spinal cord injections of FB (circles) and DY (dots) as diagrammed by the right inset.



No double labeling
• DY
○ FB

EFFECTS OF NEONATAL 6-HYDROXYDOPAMINE TREATMENT ON CORTICOSPINAL PLASTICITY

ABSTRACT

Intracortical microstimulation (ICMS) of the left sensorimotor cortex in adult rats that sustained unilateral cortical aspiration lesions at birth demonstrated normal contralateral as well as ipsilateral forelimb movements evoked by low current intensities (mean current threshold = 33.98 uamps). In previous work, these abnormal forelimb movements were correlated with the anomalous ipsilateral corticospinal tract (CST) projections which develop in response to neonatal cortical lesions (Kartje-Tillotson, 1985, 1987). In adult rats that were pretreated with 6-hydroxydopamine (6-OHDA) prior to unilateral cortical aspiration at two days of age, ipsilateral forelimb movements could only be elicited by high current intensities (mean threshold value = 55.6 uamps) similar to control animals. Multiple injections of wheat-germ agglutinin horseradish peroxidase were placed into forelimb sensorimotor areas. Normal control animals demonstrated predominantly contralateral CST distribution within the spinal cord. All 6-OHDA-treated animals showed bilateral CST terminations which were denser in comparison to non-6-OHDA-treated animals. Among animals treated with 6-OHDA, those that sustained unilateral cortical ablation demonstrated an enlarged uncrossed CST projection within the dorsal and ventral funiculi as seen in non-treated animals that sustained similar cortical aspiration lesions. In contrast, CST projections within the ipsilateral funiculi were sparse in

6-OHDA-treated animals without cortical aspiration lesions. The apparent lack of correlation between abnormal limb movements evoked by low current thresholds and anomalous uncrossed CST projections in rats treated with 6-OHDA prior to cortical aspiration at birth indicates that the presence of ipsilateral CST fibers is not of itself sufficient to evoke ipsilateral limb movement by cortical microstimulation. Additionally, the positive correlation between the extent of lesion-induced spinal cord deafferentation and CST remodelling suggests that growth of the anomalous CST pathway is dependent upon the availability of terminal sites.

INTRODUCTION

Cerebral cortical noradrenergic (NA) afferents from the locus coeruleus have been described in many studies (Lauder and Bloom, 1974; Coyle and Molliver, 1977; Lidov et al. 1978; Schlumpf et al., 1980; Loughlin et al., 1982; Cooper et al., 1982; Lindvall and Bjorklund, 1984). These NA corticopetal projections are present within the neocortex prior to maturation of cortical dendritic arborizations (Wise et al., 1979). The early growth of NA afferents into the neocortex (Lauder and Bloom, 1974; Coyle and Molliver, 1977; Levitt and Moore, 1979; Schlumpf et al., 1980; Berger and Verney, 1984) and the reported alterations in cortical structure following perinatal ablations of the NA system (Maeda, 1974; Imamoto et al., 1980; Onteniente et al., 1980; Felten, 1982) suggest a neurotrophic or neuromodulatory role for noradrenaline in cortical development (Lauder and Bloom, 1974; Lidov et al., 1978; Schlumpf et al., 1980; Reader et al., 1979; Waterhouse and Woodward, 1980).

A possible influence of noradrenaline on cortical plasticity has also been suggested from studies on the visual system of kittens. In this work, visual cortical plasticity as measured electrophysiologically after monocular deprivation (Wiesel and Hubel, 1965) was suppressed by the administration of the neurotoxin 6-hydroxydopamine (6-OHDA) and accelerated by local microperfusion of noradrenaline (Kasamatsu et

al.,1976, 1979a,b, 1981). In other work, neonatal administration of 6-OHDA disrupted motor cortical plasticity as measured electrophysiologically (Castro et al., 1986). The abnormal ipsilateral limb movements which can be evoked by intracortical microstimulation in adult rats that sustained unilateral cortical lesions at birth (Kartje-Tillotson et al., 1985, 1987; Nation et al., 1985) were abolished in similarly ablated rats pretreated with 6-OHDA (Castro et al., 1986).

Since previous work provided only electrophysiological evidence for the loss of cortical plasticity in animals treated with 6-OHDA, the present study was initiated to determine if 6-OHDA treatment would disrupt the formation of anomalous CST projections found in rats sustaining neonatal cortical lesions at birth (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). Because these anomalous ipsilateral CST fibers are believed to mediate the stimulation-evoked ipsilateral limb movements found in animals sustaining neonatal cortical lesions (Kartje-Tillotson et.al., 1985,1987), the failure of these fibers to form in animals treated with 6-OHDA prior to lesion-placement would correspond to the absence of ipsilateral evoked movements in 6-OHDA-treated animals (Castro et al., 1986). The

Portions of this study have been reported in abstract form (Reinoso and Castro, 1986).

MATERIALS AND METHODS

Twenty-eight Long-Evans black-hooded rats were used in this study. Subcutaneous injections of 6-OHDA (100 mg/kg dissolved in 0.2% ascorbic acid-physiological saline solution) or vehicle solution were administered to 15 of these animals at postnatal days 0 and 1. At postnatal day 2 and using hypothermic anesthesia, 6-OHDA-treated (n=7), vehicle-treated (n=2) and untreated (n=7) animals sustained right frontoparietal lesions by aspiration with glass pipettes. The remaining animals (n=12) did not receive aspiration lesions. The various treatment groups are summarized in Table 1. Pups were returned to their mothers and inspected for possible infection until weaning.

At maturity, the animals were anesthesized with Ketamine (100mg/kg) with supplemental doses (20 mg/kg) given as needed to prevent spontaneous movements. The left cortex was exposed by craniotomy and the forelimb area (Neafsey, et.al., 1986) was explored by intracortical microstimulation for points that evoked limb movements. The glass insulated tungsten wire stimulating electrode (100um tip) was lowered to a depth of 1.7 mm with stimulation current (5-100uA) applied as a 300 ms train of 0.25 ms pulses at 350 Hz (Kartje-Tillotson, et. al.,1985). Each chosen forelimb point was multiply-stimulated with decreasing current intensities to determine the lowest current needed to reliably produce a

Table 2. Experimental Procedures.

Group Number	Number of animals	Vehicle solution	6-hydroxydopamine	Neonatal Lesion
1	6	-	-	-
2	2	+	-	-
3	4	+	+	-
4	7	-	-	+
5	2	+	-	+
6	7	+	+	+

visible movement, i.e., the threshold current. The threshold levels for both contralateral and ipsilateral forelimb movements were determined. Typically, observation of these limb movements was done by at least 2 individuals to prevent bias.

Stimulation points used in statistical analysis were selected on the basis of two criteria (Kartje-Tillotson et.al., 1985): firstly, the contralateral forelimb threshold should be equal to or below 30 uA; secondly, the ipsilateral threshold should not exceed 100 uA. Previous studies (Kartje-Tillotson et.al., 1985; Nation et.al., 1985) have shown that in control animals higher currents are often needed to evoke ipsilateral limb movements in comparison to similar movements on the contralateral side. The upper limit of 100 uamps was set to minimize tissue damage resulting from excessive currents. Only stimulation points that evoked bilateral forelimb movements and met the set criteria were included in statistical analysis. Typically, 14 points were obtained per animal and the mean difference in thresholds was determined between the six groups (Fig. 1). Linear comparisons between independent groups with equal variances were determined using the Bechhofer-Dunnett multiple comparison procedure (Wilcox, 1987).

Subsequent to intracortical microstimulation (ICMS) multiple injections of 1% WGA-HRP were placed into the left cerebral cortex in all groups. These WGA-HRP injections (0.04 ul/injection) were made in two clusters at a depth of 1.2mm. Rostrally, 4 injections were located in an

area 4mm rostral to bregma and 2mm from the midline (Fig. 1A), i.e., in the frontal cortical area corresponding to the second forelimb motor cortex (Neafsey and Sievert, 1982). Caudally, 6-9 injections were placed at points within the caudal forelimb sensorimotor area (Neafsey et al.,1986) as determined by prior microstimulation.

After a 2-day survival, animals were perfused intracardially with a 4% paraformaldehyde-glutaraldehyde fixative followed by 10% buffered sucrose. Fifty micron coronal sections of the brain and 50um coronal and horizontal sections of the cervical spinal cord were cut with a freezing microtome and gathered in trays of 0.1M phosphate buffer. Spinal cord and alternate brain sections were reacted with tetramethyl benzidine (TMB) (Mesulam,1978) and mounted on subbed slides and stained with 0.025% Toluidine blue. All slides were dehydrated, defatted and coverslipped with Depex. WGA-HRP stained sections were viewed using a Leitz polarized light microscope. The course and terminal distribution of crossed and uncrossed CST projections was qualitatively evaluated.

RESULTS

Lesion analysis

Lesions invariably included the rostral half of the right cerebral cortex and underlying corpus callosum. Lateral portions of the parietal cortex were spared in some animals while the entire mediolateral extent of the parietal area and occasionally portions of the caudate-putamen were ablated in others. Gross inspection of the ventral medulla demonstrated a substantial reduction of the pyramid ipsilateral to the cortical lesion.

Forelimb responses evoked by ICMS

The current thresholds needed to evoke contralateral and ipsilateral forelimb movements are presented in figure 1. Mean current thresholds (20-23 uamps) needed to evoke contralateral forelimb movements were comparable in all groups. Ipsilateral forelimb movements were evoked at relatively high-threshold currents (above 57 uamps) in all rats without cortical lesions. However, ipsilateral forelimb movements were evoked at relatively low threshold currents (30-35 uamps) in animals sustaining neonatal cortical lesions but not pretreated with 6-OHDA (at a significance level of 0.05). In contrast, cortical lesion animals pretreated with 6-OHDA did not demonstrate low-threshold ipsilateral forelimb movements. The high stimulation currents needed to evoke ipsilateral limb movements in these animals were comparable to

ipsilateral threshold values found in all nonlesion groups.

WGA-HRP Injection Sites

Nissl-stained coronal sections through levels containing WGA-HRP injection sites showed that the micropipette tracks extended from superficial cortical layers to lamina IV and V. Reference to cytoarchitectural maps of the rat sensorimotor cortex (Donoghue and Wise, 1982; Neafsey, et.al., 1986) indicated that injections were located in rostral portions of the lateral agranular field and medial portions of the granular cortex of the primary somatosensory area. Adjacent sections reacted with TMB demonstrated a confluence of labeling such that individual injection sites were indistinguishable. The injections were confined to the neocortex with some diffusion into the corpus callosum.

Spinal cord labeling

Analysis of anterograde axonal labeling within the spinal cord was restricted to cervical levels because cortical injections of WGA-HRP were placed into forelimb sensorimotor areas. Corticospinal tract labeling for all groups of animals was most dense contralaterally, principally within the ventral portion of the dorsal funiculus. Additionally, sparse corticospinal labeling was consistently seen within the lateral funiculus although contralateral labeling was more prominent than ipsilateral labeling (Fig. 2A-D).

A. Animals without neonatal cortical aspiration lesions

CST labeling within the spinal cord white matter was comparable in all animals that did not sustain neonatal cortical aspiration lesions. Labeling was heaviest within the contralateral dorsal funiculus while sparse labeling was seen ipsilaterally within the dorsal and ventral funiculi (Fig. 2A,B). In animals not treated with 6-OHDA, labeling within the spinal cord gray matter was mainly within the contralateral base of the dorsal horn and intermediate gray. In comparison, animals treated with 6-OHDA but not sustaining cortical aspiration lesions, demonstrated more dense bilateral labeling within the spinal cord gray matter. In these animals, labeling was heaviest contralaterally, within the base of the dorsal horn and intermediate gray while an apparently less dense labeling seemed to be more uniformly distributed thoughout the ipsilateral gray matter (Fig.2B). The increased CST labeling in animals treated with 6-OHDA was not accompanied by obvious alterations in the projection patterns of CST pathways. (Fig. 2B).

B. Animals with unilateral cortical aspiration lesions

An anomalous increase of CST fibers traversing the ipsilateral dorsal and ventral funiculi was observed in all animals sustaining neonatal cortical aspiration lesions (Fig. 2C,D). These projections corresponded to the observed bilateral distribution of CST terminations

found in these animals and contrasted with the predominantly contralateral distribution found in animals not sustaining cortical aspiration lesions. An increase in CST terminations was again observed in animals pretreated with 6-OHDA. Labeling within the ipsilateral gray matter was less dense within the base of the dorsal horn although ipsilateral labeling was typically less dense than contralateral labeling.(Fig. 2D; Fig.3B). The labeling density within the ipsilateral dorsal and ventral funiculi was comparable in non-treated and 6-OHDA-treated animals with cortical lesions (Fig 2C,D).

The dense labeling within the spinal cord of 6-OHDA-treated animals is not likely due to large amounts of WGA-HRP injection. Separate control animals receiving larger quantities of WGA-HRP lacked a corresponding increase in labeling within the spinal cord white and gray matter.

DISCUSSION

Intracortical microstimulation (ICMS) of the motor cortex of normal rats demonstrated low-threshold contralateral and high-threshold ipsilateral forelimb movements. In adult rats that sustained unilateral cortical ablation at birth, ICMS of the unablated cortex resulted in bilateral low-threshold forelimb responses. These findings concur with previous work on motor cortical plasticity after neonatal cortical lesion (Kartje-Tillotson et al., 1985; Nation et al., 1985). However,in adult rats pretreated with 6-OHDA before sustaining neonatal cortical lesions, ipsilateral forelimb responses were only elicited at high current thresholds similar to normal controls. These results are in agreement with a previous work indicating that 6-OHDA disrupts lesion-induced motor cortical plasticity (Sutherland et al., 1982; Castro et al., 1986).

Anterograde cortical injections of WGA-HRP have shown bilateral CST terminations within the spinal cord gray matter of all 6-OHDA-treated animals. Additionally, animals pretreated with 6-OHDA prior to unilateral cortical lesion placement at birth demonstrated an anomalous uncrossed CST projection within the dorsal and ventral funiculi as seen in animals with similar lesions but not treated with 6-OHDA. These findings suggest that neonatal 6-OHDA treatment prior to lesion placement did not prevent the growth of the anomalous CST projection traversing the

ipsilateral dorsal and ventral funiculi. Additionally, the presence of bilateral CST terminations within the gray matter of all 6-OHDA-treated animals indicates that neonatal 6-OHDA treatment induces CST remodelling. Similarly, increased synaptogenesis within the visual cortex (Blue and Parnavelas, 1982) and axonal sprouting of serotonergic fibers within the cerebral cortex have been described following neonatal 6-OHDA treatment in rats. In parallel to the present results, neonatal administration of 6-OHDA did not prevent the characteristic sprouting of commissural fibers following neonatal deafferentation of entorhinal projections to the dentate gyrus (Amaral et al., 1980). In addition, the degree of lesion-induced sprouting seen in 6-OHDA-treated animals was greater in comparison to non-treated animals (Amaral et al., 1980).

In the present study, it is unknown whether the increased density of CST projections observed in 6-OHDA-treated animals is due to noradrenergic depletion or nonspecific effects of 6-OHDA (Sporn et al., 1976; Jonsson and Hallman, 1978; Harik et al., 1981). In other work, the disruption of visual cortical plasticity as measured electrophysiologically after monocular deprivation (Wiesel and Hubel, 1965) in 6-OHDA-treated kittens was attributed to noradrenergic depletion (Kasamatsu et al., 1976, 1979, 1981). However, similar studies (Bear and Daniels, 1983; Daw et al., 1985; Adrien et al., 1985; Trombley et al., 1986) have shown that doses sufficient to deplete cortical levels of noradrenaline do not affect visual cortical plasticity. The conflicting

reports on the effect of 6-OHDA on visual cortical plasticity (see review by Sillito, 1986) may be attributed to the varying paradigms used (Daw et al., 1985). Specifically, the timing of 6-OHDA administration is a critical factor affecting visual cortical plasticity (Allen et al., 1987).

In the present study, it is unlikely that prenatal administration of 6-OHDA prior to cortical aspiration would have prevented the growth of the anomalous CST. Although 6-OHDA was administered postnatally, 6-OHDA-treatment preceded the induction of CST remodelling. In other work, prenatal administration of 6-OHDA failed to show any significant alteration in pyramidal cell differentiation (Lidov and Molliver, 1982). However, as in studies on the effect of 6-OHDA on visual cortical plasticity, reports on the effect of neonatal administration of 6-OHDA on cortical development have yielded conflicting results. While several studies reported that neonatal 6-OHDA treatment significantly alters cortical morphology (Maeda, 1974; Imamoto et al., 1980; Onteniente et al., 1980; Felten, 1982), there have been reports to the contrary (Wendlant et al., 1977; Wagner et al., 1982).

In 6-OHDA-treated rats without cortical aspiration lesions, the presence of dense ipsilateral CST terminations within the spinal cord gray matter despite the paucity of uncrossed CST fibers traversing the dorsal and ventral funiculi suggest that 6-OHDA-induced formation of anomalous CST projections may be due to collateralization of the normal

crossed CST projection. The mechanisms involved in 6-OHDA-induced sprouting of axonal fibers is unknown. The increase in synaptogenesis within the visual cortex of rats treated with 6-OHDA at birth suggests that the NA system exerts an inhibitory influence on synapse formation (Blue and Parnavelas, 1982). A similar phenomenon may explain the sprouting of CST projections following neonatal 6-OHDA treatment. Alternatively, reactive synaptogenesis of CST axons occurred in response to the availability of terminal sites resulting from 6-OHDA-induced disruption of NA projections (Ungerstedt, 1968; Breese and Traylor, 1971; Sachs and Jonsson, 1975; Jonsson et al., 1979; Schmidt and Bhatnagar, 1979) into the spinal cord. The widespread distribution of CST projections within the spinal cord gray matter of 6-OHDA-treated animals which corresponds to noradrenergic terminations (Westlund et al., 1983) supports this possibility. Sprouting into terminal sites normally occupied by axonal projections with a different neurotransmitter has previously been described (Raisman and Field, 1973; Snyder et al., 1981; Stachowiak et al, 1984).

The lack of correlation between anomalous CST projections and abnormal forelimb movement in rats that were treated with 6-OHDA prior to neonatal lesion placement may be due to 6-OHDA-induced alterations on anomalous CST function. Neonatal administration of 6-OHDA may disrupt the neuromodulatory (Reader et al., 1979; Waterhouse and Woodward, 1980) role of noradrenaline. Possibly 6-OHDA-induced noradrenergic depletion

disrupts the 'balance of coexistence' between a peptide and neurotransmitter (Schultzberg and Hokfelt, 1982; Morrison and Magistretti, 1983; Rostene et al., 1987). Vasointestinal peptide (VIP) coexists with noradrenaline in the cerebral cortex (Morrison and Magistretti, 1983; Bloom, 1987). Possibly, a similar colocalization between VIP and NA occurs in the spinal cord. The functional interaction between VIP and NA may be analogous to that of somatostatin and acetylcholine. Somatostatin (SS-14), a neuropeptide widely distributed in the central and peripheral nervous system, causes a dose-dependent enhancement of ACh-induced facilitations (Bloom, 1987). However, by itself, SS-14 depresses spontaneous discharge rate (Bloom, 1987).

The selective effect of neonatal 6-OHDA treatment on ipsilateral forelimb movements in rats that sustained unilateral cortical ablation is perplexing. In view of this dichotomous effect of 6-OHDA on forelimb movements, the experimental paradigm used in the present investigation may be a good model for analyzing various aspects of synaptogenesis and plasticity.

Fig. 1. Histogram demonstrating mean current thresholds ± S.E.M. which elicit ipsilateral forelimb responses by intracortical microstimulation of the unablated cortex of experimental groups listed in Table 1.

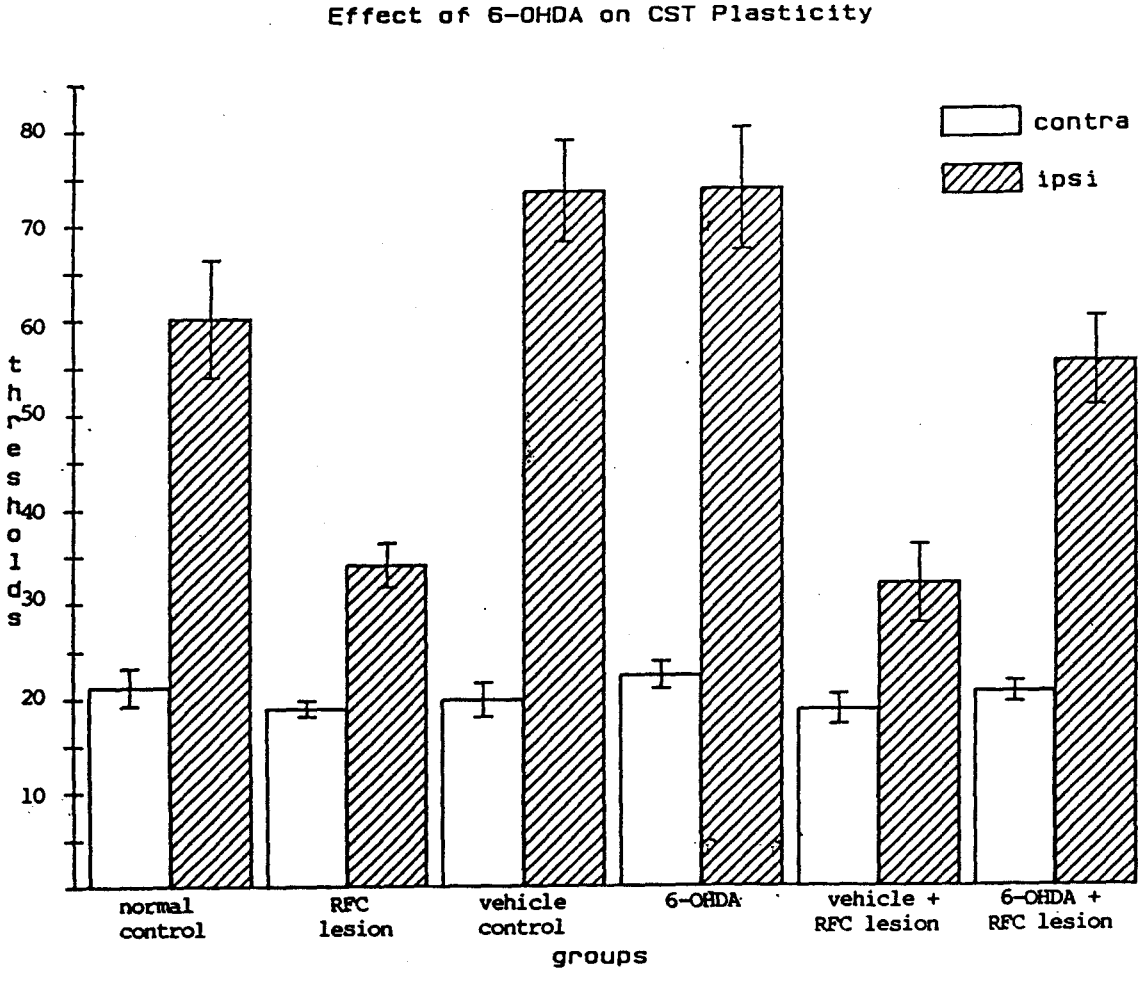

Effect of 6-OHDA on CST Plasticity
contra
ipsi
thresholds
80
70
60
50
40
30
20
10
normal control
RFC lesion
vehicle control
6-OHDA
vehicle + RFC lesion
6-OHDA + RFC lesion
groups

Table 3. All Pairwise Comparisons of Mean Current Thresholds Eliciting Ipsilateral Forelimb Movement:

	RFC Lesion	Vehicle Control	6-OHDA	Vehicle + RFC Lesion	6-OHDA + RFC Lesion
1 Normal Control	25.9±23.8[a]	-15.7±41.2	-25.9±32.5	19.8±41.1	-2.7±28.0
2 RFC Lesion		-41.6±40.4*	-51.8±31.6*	-6.1±40.4	-28.6±26.9*
3 Vehicle Control			-10.2±43.6	35.5±50.4	13.0±40.4
4 6-OHDA				45.8±43.6*	23.2±31.6
5 Vehicle + RFC Lesion					-22.5±40.4

* $p < .05$, Tukey-Kramer test, all pairs (Wilcox, 1987)

[a] $p < .05$, one-way Bechhofer-Dunnett tst, 6 linear comparisons (Wilcox, 1987)

Fig. 2. Camera lucida drawings of representative coronal sections through the cervical spinal cord showing the distribution of WGA-HRP labeled CST fibers in normal controls (A), 6-OHDA-treated animals without cortical aspiration lesions (B), non-treated animals that sustained unilateral cortical aspiration lesions at birth (C), and animals treated with 6-OHDA prior to unilateral cortical aspiration at birth (D). Location of the lesion (RFC) and HRP injections (dots) are indicated by the inset drawing.

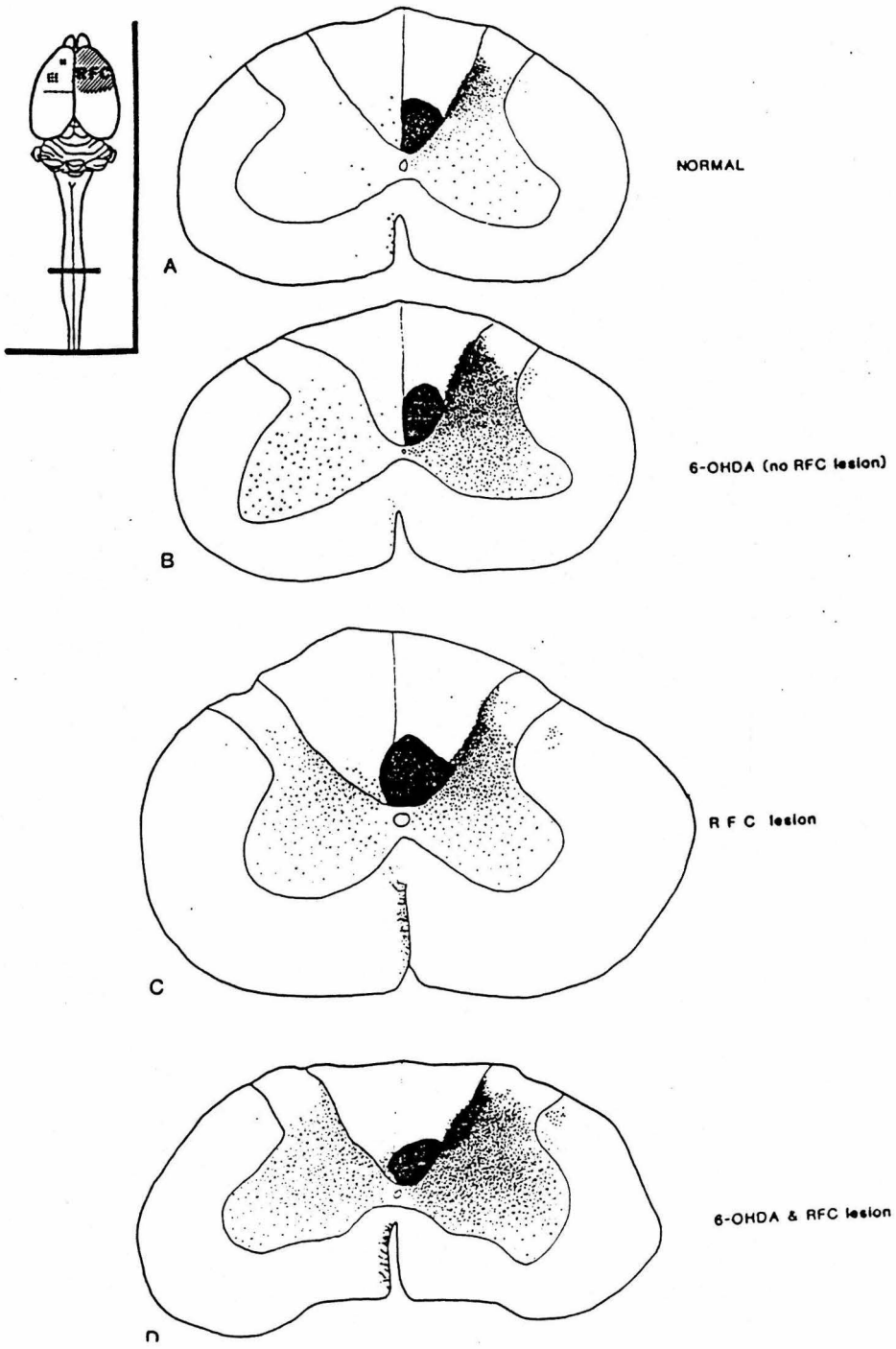
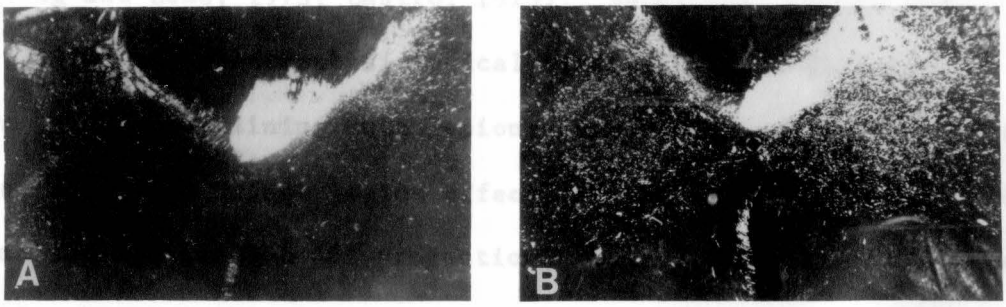

RFC
A
NORMAL
B
6-OHDA (no RFC lesion)
C
R F C lesion
D
6-OHDA & RFC lesion

Fig. 3. Darkfield photomicrographs of cervical spinal cord cross-sections showing the distribution of WGA-HRP labeled CST projections in animals that sustained unilateral cortical aspiration lesions at birth (A) and animals treated with 6-OHDA prior to similar cortical aspiration at birth (B).


A
B

GENERAL DISCUSSION

Unilateral cortical ablation in newborn rats results in the formation of an enlarged ipsilateral CST projection (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). Behavioral (Hicks and D'Amato, 1970) and electrophysiological studies (Kartje-Tillotson et al., 1985) in animals sustaining such lesions demonstrate sparing of motor function, i.e., the infant lesion effect, of the limb corresponding to the anomalous ipsilateral CST projection. However, despite an apparent correlation between the presence of anomalous CST projections and the sparing of function, a causal relationship between the two phenomena has yet to be established. A strong argument in favor of the notion that neural reorganization following neonatal CNS lesions underlies recovery of function is lacking. While a positive correlation between neural organization and behavioral recovery has been proposed (Hicks and D'Amato, 1970; Loesche and Stewart, 1977; Kartje-Tillotson et al., 1987), other investigations have associated neural remodelling with maladaptive behavior (Schneider et al., 1979; Gramsbergen, 1981).

Previous accounts of anomalous CST projections were based on axonal degeneration methods (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). The first study in this dissertation was undertaken to re-examine cortical lesion-induced CST remodelling using wheat-germ agglutinin horseradish peroxidase (WGA-HRP). In this study, the terminal

distribution of normal and anomalous CST projections concurred with previous reports (Schreyer and Jones, 1982; Sievert, 1985; Leong and Lund, 1973; Castro, 1975). However, an enlarged uncrossed ventral CST projection was seen in addition to previously reported anomalous ipsilateral CST fibers within the dorsal funiculus (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975). A large uncrossed CST projection within the ventral funiculus has also been reported in humans with unilateral encephalocele (Verhaart and Kramer, 1952). Ipsilateral CST projections within the ventral funiculus (Vahlsing and Feringa, 1980; Chambers and Liu, 1957; Liu and Chambers, 1964), which are generally restricted to cervical spinal cord levels, have been associated with proximal motor neurons (Liu and Chambers, 1964). Accordingly, the presence of few anomalous CST fibers coursing into the medial aspect of the ventral horn from the ipsilateral ventral funiculus suggests that ventral uncrossed CST fibers mediate movement of proximal, i.e., cervical musculature. In view of the pivotal role of the cervical area in maintaining equilibrium (Bach-y-Rita, 1978), the lesion-induced formation of anomalous ventral CST projections may contribute to the execution of effective body control by maintaining stability within the cervical area.

The mechanisms underlying the infant lesion effect remain unknown. The lack of ipsilateral CST fibers in newborn rats suggest that the enlarged uncrossed CST projection present after unilateral cortical lesions at birth is not due to a persistence of normally transient

projections (Nah et.al., 1980; Schreyer and Jones, 1982). In the second study, bilateral cervical spinal cord injections of FB and DY revealed that anomalous uncrossed CST fibers do not arise from neurons that give rise to normal crossed CST projections. Additionally, the numerous retrogradely labeled neurons of the uncrossed CST projections in rats with neonatal cortical lesions indicates that, in comparison to similarly injected normal controls, the anomalous CST fibers are not collaterals of normally sparse uncrossed CST projections.

The demonstration of motor patterns that have not yet developed at the time of CNS lesion has led to the notion of functional sparing being a compensatory strategy occurring early in development but not at maturity (Goldberger and Murray, 1986). In this regard, the presence of the anomalous CST pathway is generally attributed to the immature status of the CST system at birth (DeMyer, 1967; Hicks and D'Amato, 1970, 1974; Donatelle, 1977; Shreyer and Jones, 1982). Possibly, the anomalous CST projections arise from developmentally later growing CST fibers within the unablated cortex that normally decussate within the medulla but fail to do so after lesions which destroy one corticospinal system at birth (Hicks and D'Amato, 1970).

The course of anomalous CST fibers caudal to the spinomedullary junction indicates that these projections follow routes that normally contain at least a few normal CST fibers rather than follow entirely novel trajectories. This finding suggests that, once growing CST axons

fail to decussate in the medulla, they may respond to guidance mechanisms ordinarily followed by normal CST axons and thus increase the normally sparse ipsilateral CST pathways to innervate available synaptic sites. Various physical and/or chemical cues arising from non-neuronal elements within the nervous system and/or potential target areas have been proposed to affect neuronal pathfinding and axonal guidance (Purves and Lichtman, 1986; Hankin and Silver, 1986). Recent studies (Kalil and Skene, 1986; Schreyer and Jones, 1987) suggest that local factors secreted into the extracellular matrix following neonatal CNS lesions may be beneficial in CST remodelling by influencing axonal growth patterns.

Numerous studies have shown that anomalous corticofugal projections resulting from neonatal cortical lesions mirror the topographic organization of normal pathways (Nah and Leong, 1976; Leong, 1980; Castro and Mihailoff, 1983; Kartje-Tillotson et al., 1986; Naus et al., 1986). For example, anomalous contralateral corticorubral (Naus et al., 1986) and corticopontine (Kartje-Tillotson et al., 1985) projections present in rats that sustained unilateral cortical ablation were distributed in a topographic pattern similar to normal ipsilateral corticorubral and corticopontine projections. Similarly unilateral cervical/lumbar placement of fluorescent tracers in the present study showed that the distribution of ipsilaterally labeled CST neurons within motor and somatosensory cortical areas was generally comparable to the normal contralateral distribution. These results indicate that the

anomalous uncrossed CST projection displays a somatotopic projection resembling normal crossed fibers.

In view of findings demonstrating that single cortical neurons send ipsi- and contralateral efferents (Phillips and Porter, 1977; Catsman-Berrevoets and Kuypers, 1981; Keizer and Kuypers, 1984; Ugolini and Kuypers, 1986; Bentivoglio and Rustioni, 1986) anomalous crossed corticorubral (Leong and Lund, 1973; Nah and Leong, 1976; Naus et al., 1986) or corticopontine (Leong and Lund, 1973, 1976; Castro and Mihailoff, 1983; Kartje-Tillotson et al., 1986) projections may arise as axonal collaterals from anomalous ipsilateral CST fibers in rats that sustained unilateral cortical ablation at birth. This possibility implies that in response to neonatal lesions individual cortical projection neurons would have reversed the laterality of their axonal branches. However, the final distribution of projections would be in accordance with the normal developmental patterns of corticorubral and corticopontine terminations being opposite CST projections. This hypothesis is consistent with the notion that anomalous pathways course along specific routes albeit along the opposite side of the neuraxis.

The somatotopic distribution of anomalous CST neurons observed in the present study using retrograde fluorescent tracers corroborates previous electrophysiological findings in rats with similar cortical lesions (Kartje-Tillotson et al., 1985). In this study, the topographic arrangement of cortically evoked anomalous ipsilateral limb movements

were similar to normal control animals (Kartje-Tillotson et al., 1985). The absence of abnormal limb movements following transection of the pyramid corresponding to the anomalous uncrossed CST pathway is in agreement with the notion that limb movements evoked by low-threshold currents are mediated by the anomalous CST projections (Kartje-Tillotson et al., 1985, 1987).

In recent work, anomalous ipsilateral forelimb movements evoked by low current cortical microstimulation were lacking in rats that were treated with 6-hydroxydopamine (6-OHDA) prior to unilateral cortical ablation at birth (Castro et al., 1986). These results support previous findings on the kitten visual system suggesting that 6-OHDA treatment prevents cortical plasticity (Kasamatsu et al.,1979). In view of the correlation between CST projections and limb movement evoked by low-threshold current, the third study in this dissertation was undertaken to verify (using WGA-HRP) whether the absence of anomalous forelimb movements in rats pretreated with 6-OHDA prior to lesion placement is associated with a lack of the anomalous CST projection.

In rats treated with 6-OHDA prior to unilateral cortical aspiration at birth, the absence of abnormal ipsilateral forelimb movements evoked by low current thresholds concurred with previous findings suggesting that neonatal 6-OHDA treatment disrupted motor cortical plasticity (Castro et al., 1986). Analysis of WGA-HRP labeled CST projections showed that 6-OHDA treated animals demonstrated denser

bilateral CST terminations within the gray matter in comparison to non-6-OHDA-treated rats. Anomalous ipsilateral CST projections were present within the dorsal and ventral funiculi of all animals with cortical aspiration lesions. These results suggest that 6-OHDA treatment enhances rather than disrupts corticospinal plasticity.

Sprouting in response to neonatal 6-OHDA treatment has been observed in the cerebral cortex (Blue and Parnavelas, 1982; Blue and Molliver, 1987) and the hippocampus (Amaral et.al., 1980). In 6-OHDA-treated rats without cortical aspiration lesions, the presence of dense ipsilateral CST terminations within the spinal cord gray matter despite the paucity of uncrossed CST fibers traversing the dorsal and ventral funiculi suggest that 6-OHDA-induced formation of anomalous CST projections may be due to collateralization of the normal crossed CST projection. The mechanisms involved in sprouting of axonal fibers following neonatal administration of 6-OHDA is unknown. The increase in synaptogenesis within the visual cortex of rats treated with 6-OHDA at birth suggests that the NA system exerts an inhibitory influence on synapse formation (Blue and Parnavelas, 1982). The same may be true for the observed sprouting of CST projections following neonatal 6-OHDA treatment. Alternatively, reactive synaptogenesis of CST projections occurred in response to the availability of terminal sites resulting from the 6-OHDA-induced lesion of NA afferents to the spinal cord.

The absence of low current threshold ipsilateral forelimb

movements despite the presence of anomalous CST projections in 6-OHDA treated animals with cortical aspiration lesions does not necessarily contradict the notion that abnormal forelimb movements in rats that sustained unilateral cortical ablation at birth are mediated by the anomalous uncrossed CST projection (Kartje-Tillotson et al., 1985, 1987). Possibly, neonatal 6-OHDA administration resulted in a loss of function of the anomalous CST pathway.

In conclusion, the experiments in this dissertation demonstrate that the cerebral cortex of young animals have a large capacity to undergo remodelling after tissue damage. The similarity in trajectory and topographical distribution of normal and anomalous CST projections suggest that injury-induced neuronal remodelling is a process regulated by developmental rules (Finger and Almli, 1985). Additionally, the positive correlation between the extent of lesion-induced deafferentation of synaptic sites within the spinal cord and that of CST remodelling concurs with the notion that the formation of anomalous connections is dependent on the availability of terminal space (Schneider, 1973). More work has to be done toward the elucidation of mechanisms involved in lesion-induced axonal pathfinding and remodelling. It is hoped that the rapid accumulation of information regarding central nervous system plasticity obtained from animals models will serve as invaluable tools toward the search for methods to ameliorate of deficits resulting from neurological damage in humans.

SUMMARY

The capacity of the central nervous system to modify neuronal pathways and synaptic connections after injury is more prominent when such lesions are sustained at birth than at maturity. Following unilateral cortical ablation in newborn rats, an anomalous uncrossed corticospinal tract (CST) projection is present in addition to the normal crossed CST pathway. In contrast, anomalous CST fibers are lacking in rats that sustained similar lesions at maturity. The present study investigated the origin, course and terminations of the anomalous CST pathway using retrograde and anterograde neuroanatomical tracers.

Multiple injections of wheat-germ agglutinin horseradish peroxidase (WGA-HRP) were placed within the left sensorimotor cortical area to study the course and termination of the anomalous CST projection. Results obtained from this study concurred with previous reports (based on axonal degeneration techniques) of an enlarged uncrossed CST projection within the dorsal funiculus. In addition, a previously undescribed anomalous uncrossed ventral CST projection was observed.

Fluorescent tracers fast blue and diamidino yellow were injected into the spinal cord of adult rats that sustained unilateral cortical ablation at birth. The distribution of retrogradely labeled CST neurons within the unablated cortex was examined. The lack of double labeled cells indicated that anomalous CST projections are not collaterals of the normal crossed CST pathway. Additionally, anomalous CST neurons were

distributed in topographically appropriate areas within the unablated cortex. These findings support previous electrophysiological findings indicating that the neonatal cortical lesion-induced increase of ipsilateral CST fibers may be functional.

In adult rats that sustained unilateral cortical aspiration lesions at birth, intracortical microstimulation (ICMS) of the motor cortex elicited low threshold ipsilateral forelimb movements. These abnormal limb movements were not observed in adult rats treated with 6-OHDA prior to unilateral cortical aspiration at birth. Anterograde WGA-HRP labeling of CST fibers in 6-OHDA treated rats with cortical aspiration lesions demonstrated the presence of anomalous uncrossed CST fibers traversing the dorsal and ventral funiculi as seen in non-treated animals with similar cortical aspiration lesions. However, 6-OHDA-treated animals showed denser bilateral CST terminations in comparison to non-treated animals. These results suggest that neonatal 6-OHDA administration enhances rather than disrupts CST remodelling. The lack of correlation between low-threshold limb movements and anomalous CST projections in rats treated with 6-OHDA prior to cortical aspiration at birth may be due to 6-OHDA-induced alterations on anomalous CST function. Possibly, neonatal administration of the catecholamine neurotoxin, 6-OHDA, disrupts the neuromodulatory role of noradrenaline.

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APPENDIX A

The material in Appendix A supplements Experiment II. The tables show raw numbers of labeled neurons obtained from representative sagittal sections through the left cerebral cortex (lateral to medial) of 4 normal adult rats. These neurons were retrogradely labeled with fast blue and diamidino yellow injected into the ipsilateral and contralateral cervical spinal cord, respectively.

CONTROL 1

	Contralateral	Ipsilateral
	6.00	0.00
	8.00	0.00
	7.00	0.00
	11.00	0.00
	53.00	0.00
	56.00	1.00
	54.00	0.00
	45.00	1.00
	16.00	0.00
	32.00	1.00
	18.00	0.00
	10.00	1.00
Mean:	26.33	0.33
S.E.M.:	5.39	0.13

CONTROL 2

	Contralateral	Ipsilateral
	6.00	0.00
	17.00	0.00
	4.00	0.00
	6.00	0.00
	8.00	0.00
	13.00	0.00
	7.00	0.00
	6.00	0.00
	9.00	0.00
	12.00	0.00
	4.00	0.00
	4.00	0.00
Mean:	8.00	0.00
S.E.M.	1.09	0.00

CONTROL 3

	Contralateral	Ipsilateral
	7.00	0.00
	1.00	0.00
	10.00	0.00
	40.00	0.00
	69.00	1.00
	15.00	1.00
	23.00	4.00
	22.00	1.00
	19.00	1.00
	29.00	2.00
	10.00	0.00
	18.00	1.00
	15.00	1.00
	3.00	1.00
Mean:	18.87	0.93
S.E.M.:	4.35	0.27

CONTROL 4

	Contralateral	Ipsilateral
	6.00	0.00
	8.00	0.00
	7.00	0.00
	11.00	0.00
	10.00	0.00
	8.00	0.00
	19.00	0.00
	42.00	0.00
	85.00	0.00
	64.00	0.00
	65.00	0.00
	58.00	0.00
	50.00	0.00
	64.00	0.00
	62.00	0.00
	55.00	0.00
	59.00	0.00
	37.00	0.00
	26.00	0.00
	13.00	0.00
Mean:	37.45	0.00
S.E.M.:	5.47	0.00

APPENDIX B

The material in Appendix B supplements Experiment II. The tables show raw numbers of labeled neurons obtained from representative sagittal sectins through the left unablated cerebral cortex (from lateral to medial) of 4 adult rats that sustained right fronto-parietal cortical ablation at birth. These neurons were retrogradely labeled with fast blue and diamidino yellow injected into the ipsilateral and contralateral cervical spinal cord, respectively.

LESION 1

	Contralateral	Ipsilateral
	12.00	3.00
	9.00	1.00
	5.00	0.00
	13.00	1.00
	57.00	2.00
	32.00	6.00
	16.00	6.00
	13.00	9.00
	12.00	10.00
	8.00	6.00
	17.00	7.00
	16.00	14.00
	6.00	5.00
	11.00	7.00
	14.00	3.00
Mean:	16.07	5.27
S.E.M.	3.13	0.92

LESION 2

	Contralateral	Ipsilateral
	20.00	0.00
	23.00	0.00
	11.00	2.00
	22.00	4.00
	9.00	2.00
	12.00	1.00
	18.00	2.00
	24.00	1.00
	1.00	3.00
	4.00	2.00
	6.00	3.00
	1.00	6.00
	0.00	0.00
	5.00	1.00
	0.00	2.00
	4.00	1.00
	0.00	2.00
Mean:	9.00	1.88
S.E.M.	2.02	0.35

LESION 3

Contralateral	Ipsilateral
2.00	13.00
0.00	6.00
6.00	2.00
1.00	0.00
5.00	7.00
26.00	9.00
87.00	8.00
15.00	5.00
2.00	5.00
6.00	10.00
7.00	17.00
5.00	9.00
0.00	11.00
1.00	12.00
1.00	19.00
0.00	14.00
0.00	3.00
0.00	4.00
Mean: 9.11	8.55
S.E.M. 4.57	1.15

LESION 4

Contralateral	Ipsilateral
27.00	2.00
32.00	7.00
40.00	8.00
7.00	6.00
0.00	4.00
2.00	4.00
45.00	10.00
63.00	10.00
82.00	11.00
63.00	7.00
66.00	15.00
82.00	8.00
80.00	9.00
49.00	10.00
66.00	12.00
53.00	4.00
55.00	9.00
63.00	11.00
37.00	17.00
51.00	16.00
47.00	13.00
44.00	15.00
31.00	11.00
8.00	11.00
3.00	14.00
Mean: 43.84	9.76
S.E.M.: 4.84	0.77

APPROVAL SHEET

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The final copies have been examined by the director of the thesis/dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis/dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March 24, 1988
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