1984

An Anatomical, Electrophysiological, and Behavioral Comparison of the Primary and Supplementary Motor Areas of the Rat

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AN ANATOMICAL, ELECTROPHYSIOLOGICAL, AND BEHAVIORAL COMPARISON OF THE PRIMARY AND SUPPLEMENTARY MOTOR AREAS OF THE RAT

BY

Carl F. Sievert

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

November

1984

DEDICATION

To Linda and Mark

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. E.J. Neafsey, for his support, expert advice, and most of all for his friendship. His continual enthusiasm made this work possible.

I would also like to thank the members of my committee for their comments and criticisms, especially Dr. LaVelle for her willingness to listen.

Many other members of the Department of Anatomy provided me with technical assistance or moral support, and I thank them for their efforts.

Finally, I would like to extend a special thanks to my family for their understanding when I couldn't be with them. My wife Linda was always supportive, especially when times were rough. My brother Mark was good company during the late night episodes, and my son Mark was a constant source of joy.

VITA

The author, Carl F. Sievert, was born on December 30, 1954 in Elmhurst, Illinois.

In 1973 he graduated from Oak Park River Forest High School in Oak Park, Illinois, and that fall he entered St. Olaf College in Northfield, Minnesota. He graduated from St. Olaf in May, 1977 with a Bachelor of Arts degree with a major in Biology. He began his graduate studies in the Department of Anatomy at Loyola University Stritch School of Medicine, Maywood, Illinois, in October, 1978. While at Loyola, he received a Basic Science Fellowship and taught in the gross anatomy, histology and neurosciences courses. In 1981 he was elected into the Society of Sigma Xi. He was awarded a University Dissertation Fellowship in 1982, and in 1983 he became a member of the Society for Neuroscience.

After completion of his doctorate, he will begin a post-doctoral fellowship in the Department of Physiology at Loyola University Stritch School of Medicine under the supervision of Dr. R.D. Wurster.

The author is married to Linda V. Sievert and they have one son, Mark Sievert.

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CHAPTER I

INTRODUCTION

The results of three recent experiments have demonstrated significant differences between the primary motor and the supplementary motor areas of the cerebral cortex. First, Deecke and Kornhuber (1978) demonstrated a "readiness potential" on the surface of the scalp which precedes voluntary movement and is largest over the SMA. Secondly, Brinkman and Porter (1979), testing the response properties of single units in the awake monkey found that the SMA neurons received much less peripheral sensory input than the primary motor area, and that the activity of SMA neurons increased prior to the onset of the movement. These findings imply that the SMA may be a movement initiator which requires little knowledge about the present state of the targeted body parts. Finally, further support for the theory of SMA as movement initiator came from cerebral blood flow studies which demonstrated that the SMA is active during thinking of a movement, even if the movement itself is not carried out (Roland et al., 1980). These interesting findings led Sir John Eccles (1984) to state: "Thus there is strong support for the hypothesis that the SMA is the sole recipient area of the brain for mental intentions that lead to voluntary movements." Eccles may have exaggerated the overall

importance of the SMA in controlling movement, but it is clear thatthis area deserves further study from both an anatomical and physiological point of view.

Supplementary motor areas have been found in rabbits, raccoons, porcupines, primates and man (Woolsey, 1958) but, as yet, no such area has been identified in the rat. Recently, a second forelimb motor area has been identified in the rat motor cortex (Neafsey and Sievert, 1982), and it has been proposed that this area may be a part of the rat's SMA. With many investigators turning to the rat as a model for motor control, it is important to learn if this animal has a supplementary motor area, and, if present, how it compares anatomically and physiologically with the primary motor area. The purpose of this dissertation was to characterize the anatomical and physiological properties of the second or rostral forelimb area, in order to compare it with the primary motor area. It was hoped that the results of this research would provide sufficient information to identify the rostral forelimb area as either primary or supplementary motor cortex.

Many experiments have already delineated some of the similarities and differences between the primary and supplementary motor areas in the monkey. For example, in the monkey both primary and supplementary motor areas contain a somatotopic representation of the contralateral forelimb and hindlimb, determined by both electrical stimulation experiments and by anatomical demonstration of projections

from each area to the cervical and lumbar enlargements (cf. review by Tanji, 1984). Although the two areas are similar in terms of somatotopy, they have markedly different levels of responsiveness to peripheral sensory input. Numerous studies have shown that the SMA receives considerably less peripheral sensory input than the primary motor cortex (Brinkman and Porter, 1979; Wise and Tanji, 1981; Tanji and Kurata, 1982). This lack of sensory input might be expected from an area that is concerned with initiating a movement as opposed to carrying it out and is consistent with the finding that the dorsal column nuclei do not receive a projection from the SMA (Jurgens, 1984), whereas they do receive a projection from the primary motor area (Kuypers, 1964). It is not surprising that an area without significant sensory input would not be concerned with regulating transmission of incoming sensory input. A fourth point of comparison between the two motor areas is provided by lesion studies which have demonstrated that lesions of the SMA produce only transient effects on an animal's ability to perform discrete digital movements, but lasting effects on an animal's ability to perform bimanual coordination tasks (Brinkman, 1984). These results are clearly different from those of lesion studies on the primary motor area which demonstrate long lasting deficits in an animal's ability to perform discrete digital movements (Fulton and Kennard, 1934; Denny-Brown and Botterall, 1948). A final potential area of comparison between the SMA and the primary motor area is the exact location of spinal cord terminations from the two areas. While the areas of terminations from the primary motor

area are clearly defined (cf. review by Kuypers, 1981), no studies have been done on the spinal terminations of the SMA in the monkey.

In order to make a similar series of comparisons between the rostral forelimb area and the primary forelimb motor area of the rat cerebral cortex five separate studies were planned to determine:

1. the effects of small lesions of the two forelimb areas on a forelimb digital task.
2. the origins of corticospinal neurons in the two areas and their relation to physiological mapping studies and cortical cytoarchitecture.
3. the course and terminations of the corticospinal tract in the spinal cord.
4. the terminations of the two cortical areas in the dorsal column nuclei.
5. the amount and type of peripheral sensory input reaching the two forelimb areas.

It was hoped that the data collected from these five studies would allow us to suggest classification of the second forelimb area as either supplementary or primary motor cortex. The results of these studies should add considerable information to the growing body of knowledge concerning the sensorimotor cortex of the rat.

CHAPTER II

REVIEW OF LITERATURE

Historical Perspective

Although a central motor area in the brain was hypothesized by Jackson in 1860 based on his observations of epileptic seizures in humans (Jackson, 1932), it was not until the discovery of an area of cortex in animals where electrical stimulation produced movements (Fritsch and Hitsig, 1870; Ferrier, 1875) that the concept of a "motor cortex" became widely accepted. In 1917 Leyton and Sherrington determined that in primates the central sulcus was the caudal boundary of the motor cortex; but, much later, Woolsey et al. (1958) also in primates included part of the postcentral cortex because it was responsive to electrical stimulation, although at higher thresholds. They termed this postcentral area sensory-motor and the precentral excitable cortex as motor-sensory, indicating the predominant characteristics of each area first. Presently, in common usage, the caudal border of the motor cortex is identified as the central sulcus in the monkey (Powell and Mountcastle, 1959; Phillips et al., 1971; Jones and Porter, 1980). Cytoarchitectural studies have demonstrated that the primary motor area in man is an area where layer V is made up of large pyramidal cells and layer IV is absent (Brodmann, 1903; Vogt

and Vogt, 1919). For comparison, somatic sensory cortex has a well developed layer IV with numerous axon terminations due to the incoming thalamic input (Kievit and Kuypers, 1977). This differential organization of layers IV and V has prompted the use of the descriptive terms agranular cortex for primary motor (MI) and granular for primary sensory (SI). A similar cytoarchitectural scheme seems to be present in most mammals (Krieg, 1946; Zilles et al., 1980; Donoghue and Wise, 1982).

Even though the boundaries of the motor cortex were generally well accepted, debate has continued over the question raised by Jackson as to whether individual muscles or movements were represented in the motor cortex. In an elegant pioneering study, Chang et al., (1947) showed that although some muscles appeared to be represented in a mosaic pattern of nonoverlapping zones, the representations of most individual muscles were in general partially overlapping. The general consensus on the question of muscles versus movements has shifted every three to five years (Landgren et al., 1962; Asanuma and Sakata, 1967; Anderson et al., 1975; Jankowska, 1975; Asanuma et al., 1976; Kwan et al., 1978). At present, the studies by Fetz and Cheney, (1978); Neafsey, (1981); Humphrey et al., (1982); and Schmidt and McIntosh, (1984) seem to indicate that movements and not muscles are represented within the motor cortex.

Penfield and Rasmussen (1950) were the first to describe in man's precentral motor cortex a complete somatotopic body representation. This "homunculus" (in man) had unequal

representations of the body parts, with the face and hands covering a much larger area than the trunk and legs. Some of the most complete maps of somatotopic localization were generated by Woolsey et al. (1952), and these maps complimented those of Penfield and Rasmussen by including a number of different species. All animals seemed to have each body part represented at least once in both the primary motor and the primary sensory cortical areas. Lately, investigators have described multiple representations of the body parts within motor (Strick and Preston, 1978, 1982a, 1982b; Kwan et al., 1978) and sensory cortical areas (Kaas et al., 1979). In addition to multiple representations within an individual motor or sensory area, there seem to be secondary motor (MII or SMA) and sensory (SII) areas which in turn possess a somatotopic body representation (Adrian, 1941; Penfield and Rasmussen, 1950; Woolsey et al., 1952; Whitsel et al., 1969; Robinson and Burton, 1980) Recently, the anatomical and physiological characteristics of the supplementary motor area (SMA) have been compared with those of the primary motor area (MI). A a comparison of current data about these two cortical zones will constitute the remainder of this literature review.

Lesions of SMA and MI and Related Areas

The amount of movement-related deficit produced from lesions of the precentral motor cortex varies considerably from one study to another but seems to be clearly related to the task the animal is asked to perform (Castro, 1972). In the monkey, lesions of the motor

cortex (areas 4 and 6) resulted in a number of short term motor deficits including: paresis and spasticity in the contralateral limbs (Fulton and Kennard, 1934; Denny-Brown and Botterell, 1948); initial hypotonia which progressed to hypertonia (Gilman et al., 1974); and deficits in coordinated movements of the distal extremities (Passingham et al., 1983). Although there appears to be widely different results from these lesion studies, it is generally well accepted that the long term effect of motor cortex lesions is an inability to orient the hand in space, weakness, and a loss of ability to perform discrete digital movements (Denny-Brown, 1960). In view of the massive corticospinal projections from motor cortex (Crevel and Verhaart, 1963) it is not surprising that lesions of the pyramidal tract in monkeys produce similar results (Tower, 1940; Lawrence and Kuypers, 1968; Gilman et al., 1971). The inability to perform discrete digital movements and the altered states of reflexes seen after lesions of the pyramidal tract are due to losses of direct inputs to alpha and gamma motor neurons, and loss of inputs to incoming sensory input (Gilman et al., 1971).

Results of lesions of the supplementary motor area in man and primates produce more diverse results than those involving the primary motor area. Consequently, it is difficult to draw conclusions as to the function of the SMA based solely on behavioral deficits observed after lesions. In monkeys, a transient grasp reflex has been one of the more consistent effects of SMA lesions (Penfield and Welch, 1949; Travis, 1955; Smith et al., 1981), but some investigators report no

deficits from SMA lesions (Devito and Smith, 1959; Coxe and Landau, 1965). In man, Laplane et al., (1977) have demonstrated a deficit in a patient's ability to perform different tasks simultaneously with each hand. More recently, bimanual coordination deficits and a transient inability to perform discrete digital movements have been found in monkeys with SMA lesions (Brinkman, 1984). The differences in deficits resulting from SMA lesions are difficult to reconcile; but, as is always the case with behavioral testing, the deficit only shows up if the testing regimen tasks the motor system. In general, it can be said that lesions of the SMA result in less pronounced motor deficits than similar lesions in the primary motor area (see review by Wiesendanger, 1981).

In rats, lesions of the sensorimotor cortices result in lasting deficits of an animal's ability to perform discrete digital movements (Castro, 1972; Price and Fowler, 1981; Kolb and Holmes, 1983). It appears that the motor cortex of the rat has a similar role to that of the primate, in that it seems to impart speed and dexterity to the digits (Castro, 1972). In addition to causing deficits in fine motor control, lesions of small areas of motor cortex are also capable of producing a transfer of handedness (Peterson and Barnett, 1961; Peterson and Devine, 1963). Lesions of the sensory portion of the rat cortex cause deficits in the animals ability to perceive its environment (Finger et al., 1972). As yet no supplementary motor area has been localized in the rat, and consequently lesions confined to the SMA have not been performed in this animal.

Corticospinal Projections: Origin and Terminations

The origin of corticospinal fibers has been studied in a number species through the use of retrograde tracing techniques. Cell bodies of corticospinal fibers are located in the primary motor cortex, Brodmann's area 4 (MI); the premotor cortex, area 6; and the primary sensory cortex, areas 3, 1, and 2 (SI) (Kuypers, 1958a,b,c, 1960; Nyberg-Hansen and Brodal, 1963; Liu and Chambers, 1964; Jones and Wise, 1977; Wise and Jones, 1977; Hicks and D'Amato, 1977; Wise et al., 1979; Murray and Coulter, 1981; Hayes and Rustioni, 1981). Small portions of the corticospinal tract also arise from the second somatosensory area (SII) in cat (Nyberg-Hansen, 1969b), monkey (Murray and Coulter, 1981), and rat (Wise et al., 1979; Neafsey and Sievert, 1982; Donoghue and Wise, 1982); the supplementary motor area in monkey (Murray and Coulter, 1981); the sensory association area 5 of the parietal cortex (Coulter et al., 1976). Within the patches of corticospinal neurons, there is a definite somatotopic pattern with the face, forelimbs, trunk and hindlimbs represented sequentially forming a rough outline of the body on the surface of the brain in the rat (Wise et al., 1979; Neafsey and Sievert, 1982), cat (Coulter et al., 1976; Groos et al., 1978) and monkey (Coulter et al., 1976; Jones and Wise, 1977). Corticospinal neurons are located solely in cortical layer V in rats (Hicks and D'Amato, 1977; Wise and Jones, 1977; Ullan and Artieda, 1981), cats (Coulter et al., 1976), and monkeys (Coulter et al., 1976; Jones and Wise, 1977; Biber et al., 1978; Murray and Coulter, 1981).

Between species, the corticospinal pathway varies in trajectory and terminations. Generally, axons descend from the cortex into the ipsilateral cerebral peduncle, where they occupy the middle two thirds of this large fiber bundle. The fibers continue through the brainstem in a ventral position and split up into bundles in the pons where they are surrounded by the pontine nuclei. At the lower border of the pons the fibers reunite to form the prominent medullary pyramid, located ventrally. Near the caudal end of the medulla, the pyramidal tract decussates in all species and takes up residence in virtually any one of the funiculi of the spinal cord. A small bundle of fibers known as the Henle-Pick bundle ascends and terminates in the dorsal column nuclei and the spinal trigeminal nucleus (Valverde, 1966).

The location of the corticospinal fibers in the cord varies among species (see review by Kuypers, 1981). In monotremes, insectivores, and elephants the major component of this tract is located within the ventral funiculus, whereas in ungulates, carnivores, and primates the major tract is found in the lateral funiculus. In marsupials, edentates, and rodents the major tract is located within the the ventral part of the dorsal funiculus. Minor components of the tract may be found in any of the three funiculi (Schoen, 1964), and ipsilaterally located fibers have also been described for a number of species (Glees, 1961; Nyberg-Hansen and Rinvik, 1963; Armand and Kuypers, 1977).

Terminations of the corticospinal tract vary in region and

extent in different species (see Kuypers, 1981, for an extensive review). Animals, such as the cat, which are unable to perform discrete digital movements generally possess corticospinal terminations limited to the dorsal horn and intermediate gray (Chambers and Liu, 1957; Nyberg-Hansen and Brodal, 1963). The monkey corticospinal tract, on the other hand, terminates in the ventral as well as the dorsal horn and in the intermediate gray of the spinal cord (Kuypers, 1958b, 1960; Liu and Chambers, 1964; Kuypers and Brinkman, 1970; Coulter and Jones, 1977). These results imply that in the cat alpha motor neurons are activated via internuncials, whereas in the monkey motor neurons may be directly activated by corticospinal fibers (Phillips and Porter, 1977). These direct corticomotoneuronal projections are thought to control fine, independent digital movements characteristic of primates (Kuypers, 1958b). The raccoon is also capable of performing discrete digital movements and, as might be expected, has direct corticospinal connections with motor neurons (Petras and Lehman, 1966; Buxton and Goodman, 1967; Wirth et al., 1974).

Previously, it was thought that the rat corticospinal tract only terminated in the dorsal horn and intermediate gray (Torvik, 1956; Valverde, 1966; Brown, 1971; Donatelle, 1977). Recent physiological studies have indicated direct monosynaptic connections to the alpha motor neurons of the spinal cord in the rat (Elger et al., 1977). Anatomical contact could be made via dendrites of alpha motor neurons in the intermediate gray (Scheibel and Scheibel, 1966)

or there may be direct projections to the ventral horn in the rat. Goodman et al., (1966), in an abstract, described such direct ventral horn terminations in the rat, but no study done since has been able to repeat their results.

The corticospinal terminations of the supplementary motor area have not been well documented. In the cat, corticospinal fibers from a medial cortical area thought to be the supplementary motor area terminate in the dorsal horn and intermediate gray (Nyberg-Hansen, 1969a). No study has been done on the spinal cord terminations of the SMA in the monkey. A supplementary motor area has not been described for the rat.

Motor Cortex Microstimulation Maps

As has already been stated, mapping of the movement zones of the motor cortex has undergone considerable change since the early mapping studies of Penfield and Rasmussen (1950). The preferred technique at present is intracortical microstimulation (Asanuma and Sakata, 1967). Recent studies using this technique have demonstrated multiple representations of one body part within the MI representation (Strick and Preston, 1978; Kwan et al., 1978) thus breaking the strict somatotopic pattern described in earlier studies (Woolsey et al., 1952). In the rat motor cortex, a similar second representation of the forelimb has been described (Neafsey and Sievert, 1982), but it is not known whether this second forelimb is part of the MI representation or possibly a part of the heretofore undescribed

supplementary motor area of the rat (Wise et al., 1979; Donoghue and Wise, 1982). Microstimulation mapping in the supplementary motor area of the monkey has produced varying results including very high threshold complex synergistic movements (Penfield and Welsh, 1951; Penfield and Jasper, 1954) and low threshold individual limb movements similar to those seen in the primary motor area (Macpherson et al., 1982). It is generally agreed that a second whole body representation is present in the SMA of the monkey (Woolsey et al., 1952).

Sensory Input to the Motor Cortex

In the past 30 years, studies on the motor cortex have increasingly emphasized the nature and function of sensory input to motor areas. The impetus for these studies came from the discovery that neurons in the motor cortex are responsive to peripheral sensory input (Adrian and Morruzzi, 1939). This finding led to a number of hypotheses concerning the function of such sensory input, the most widely accepted of which held that the input was a part of a closed loop feedback mechanism for changing motor output in response to an unforseen change in the load imposed on the system during a motor command (Phillips, 1969; Marsden et al., 1972; Evarts and Tanji, 1976). Since that time, researchers have been frustated in their attempts to supply evidence to confirm this hypothesis. Further, a direct anatomical pathway from the periphery to the cortex has never been conclusively demonstrated. Malis et al., 1953, demonstrated that

the peripheral input did not reach the motor cortex through the sensory cortex, but until recently an alternate path could not be demonstrated. Presently, there is evidence to indicate that the peripheral sensory input does reach the motor cortex via the sensory cortex, as well as from a direct lemniscal thalamic route (Asanuma et al., 1979; Lemon and Burg, 1979; Horn and Tracey, 1979). The function of the sensory input is still problematic, but a recent study by Asanuma and Arissian (1984) gives evidence that the input is not involved in adjusting motor control in response to perturbations, but instead, the sensory input is part of a corticoperipheral loop which sets up the excitability levels of cortical efferent zones.

The study of peripheral sensory input has provided valuable information concerning the input-output relations of the motor cortex. In general, the motor cortex receives less peripheral input than the sensory cortex. The peripheral input that it does receive is related more often to deep structures instead of cutaneous (Rosen and Asanuma, 1972; Lemon et al., 1976; Wong et al., 1978; Fetz et al., 1980). Two representations of a body part exist within the motor cortex, one receiving predominantly cutaneous input, the other receiving mostly deep input (Strick and Preston, 1978, 1982b; Tanji and Wise, 1981). Besides the obvious difference in the type of input to separate areas of the motor cortex, little predictable correlation has been seen between the direction of passive joint movements which cells responded to and the direction of active joint movement produced by

intracortical microstimulation (Fetz and Baker, 1969; Lemon et al., 1976; Murphy et al., 1978; Fetz et al., 1980). However, some studies have claimed that the sensory input is from passive joint movement in the same direction as that resulting from active contraction of the target muscle (Asanuma et al., 1968; Rosen and Asanuma, 1972). The correlation which was consistently found was that the sensory input was generally at or near the site of the microstimulation-evoked movement (Fetz and Baker, 1969; Murphy et al., 1978; Rosen and Asanuma, 1972).

Smith (1979) has recently examined the peripheral sensory input to the supplementary motor area in monkeys. He found that the SMA receives complex, polymodal, sensory input which is often weak and at times includes the whole limb. SMA neurons thus appear to be less tightly coupled to incoming sensory input than those in the primary motor cortex (see review by Wiesendanger, 1981). More precise information about the sensory input to the SMA has come from several recent studies which examined SMA neurons during passive and active movements (Brinkman and Porter, 1979; Wise and Tanji, 1981). These studies demonstrated that the percent of cells responsive to sensory input in the SMA was approximately ten times less than that in the primary motor area. In an attempt to formulate a hypothesis as to the function of the SMA, Tanji (1984) has proposed that the SMA is involved in the programming or planning of voluntary movements.

Sensory input to the rat motor cortex has only been examined by Sapienza et al., 1981. They did not quantify the amount of sensory input, nor did they describe the location of the responsive cells in terms of cytoarchitecture, so that correlation with the monkey data is difficult. They did however, state that there was only a rough correlation between input and output within the rat motor cortex. Clearly, additional studies involving the rat are necessary before any comparison can be made to results obtained in the monkey.

CHAPTER III

DEFICITS IN A FORELIMB MOTOR TASK FOLLOWING LESIONS OF THE ROSTRAL OR CAUDAL FORELIMB AREA OF RAT MOTOR CORTEX

Introduction

Large lesions of the rat sensorimotor cortex produce lasting deficits in the animal's ability to perform various motor tasks, including those involving digital control (Peterson and Barnett, 1961; Peterson and Devine, 1963; Castro, 1972; Price and Fowler, 1981; Misantone and Schaffer, 1982; Kolb and Holmes, 1983). Recently, a second rostral forelimb motor region has been described in the frontal cortex of the rat where intracortical microstimulation (ICMS) most often evokes wrist and digit movements at threshold currents as low as those found in the primary forelimb area (Sanderson et al, 1981; Neafsey and Sievert, 1982). These digit and wrist movements are not well represented in the more caudal primary forelimb motor area, suggesting that behavioral deficits in digital usage seen after large sensorimotor cortex lesions may be due to damage to this rostral area.

The current study was undertaken to test this hypothesis by comparing the effects of lesions of rostral forelimb, caudal forelimb, and hindlimb motor cortex on performance of a digital usage task (Castro, 1972). The results of this study indicate that rostral forelimb lesions cause only a short term deficit in the animal's ability to perform a task involving discrete digital movements. Lesions of the caudal forelimb produce a longer lasting deficit than those of the rostral forelimb area, and lesions of the hindlimb area do not cause any deficit.

Materials and Methods

Training

Eleven adult, male, black-hooded, Long-Evans rats weighing 250-350 grams were used in this study. The animals were put on a reduced intake diet to lower their body weight 10-15 grams and then kept on a diet that maintained this body weight. Each animal was trained for a maximum of 2 weeks or until they reached at least 70% success on a task which tested for digital usage (Castro, 1972). The task requires that the animal extend one forelimb through a slot (1.5 cm wide) in the front of the cage to retrieve a food pellet. There are ten such slots next to each other in the testing cage, and there is an 8 mm gap between the floor of the slot and the cage (for a picture of the testing apparatus see Castro, 1972). If the animal attempts to drag the pellet across the slot, the food will drop irretrievably through the gap. Thus, in order to make a successful attempt, the animal must grasp the food pellet with its paw. During a testing session each animal had three trials of ten seconds each in which to grasp as many pellets (up to ten) as possible. An attempt was recorded each time the animal touched a pellet and was considered successful when the animal was able to bring the pellet to his mouth. After the training period, each animal's performance was recorded for 12 additional days to establish a preoperative baseline or control value of per cent success. Paw preference was recorded with each animal. Data were recorded as the number of attempts and the number

of successes, and plotted on a graph as percent success. After the animals had reached a success rate of 70% or higher, they received cortical lesions and were then tested for their postoperative level of success. Postoperative testing was continued on a daily basis (five days a week) until there was no further change in the animals success rate.

Surgery

The three groups of animals in this experiment were rostral forelimb lesions, caudal forelimb lesions or hindlimb lesions (control group). five animals received bilateral rostral forelimb lesions. Two animals received bilateral caudal forelimb lesions. Finally, the control group consisted of 4 animals which received bilateral hindlimb lesions.

Surgery was performed under ketamine HCl (100 mg/kg) anesthesia. Animals were placed in a stereotaxic apparatus (rounded ear bars were used to avoid breaking the tympanic membrane), and a craniotomy was made over the limb sensorimotor areas of cortex bilaterally. The motor cortex was mapped using intracortical microstimulation (ICMS) (see Appendix I) to identify the hindlimb, forelimb or rostral forelimb region, depending on where the lesion was to be placed. Once the boundaries of the area to be lesioned were determined, a small lesion was made using a suction pipette and/or a surgical cautery tool. Following the surgery, each animal was allowed 2 days for recovery before testing was resumed. Postoperatively,

animals were not neurologically tested, but were watched for signs of infection or weakness. Postoperative testing was continued on a daily basis (five days a week) until there was no change in the animal's success rate.

Prior to sacrifice, some of the animals in each of the two forelimb lesion groups underwent an additional surgery for either remapping of the cortex by microstimulation or an injection of wheat germ agglutinin HRP (WGA-HRP) into the cervical spinal enlargement. These two experiments were done to check for completeness of the lesion and to be sure that the unlesioned cortical areas still made functional connections with the spinal cord. In the caudal forelimb lesion group, 2 animals were remapped in the cortex opposite the preferred paw, and then injected with WGA-HRP in the cervical spinal enlargement on the same side as the preferred paw. Of the 5 animals in the rostral forelimb group, 4 underwent cortical remapping, and 1 received an injection of WGA-HRP in the cervical enlargement.

All animals were killed with an overdose of sodium pentobarbital, and perfused through the heart with 10% buffered formalin, or in the case of the HRP injected animals, a buffered gluteraldehyde-paraformaldehyde fixative. The brains were removed and cut at 50 micron sections on a freezing stage microtome. The HRP brains were processed according to the technique of Mesulam (1978). Sections were stained with a Nissl stain and examined for the extent of the lesion. The lesions were reconstructed from coronal sections and plotted on Lashley (1921) brain diagrams.

Results

Postoperative observations

All forelimb lesioned animals showed little sign of motor deficit during ambulation as early as one day postoperatively. By the second day postoperatively, forelimb lesioned animals appeared normal when compared to an unoperated animal. The hindlimb lesioned animals had more difficulty using their hindlegs for walking, but were perfectly capable of performing the digital usage task. Other than these effects, the animals did not exhibit any unusual symptoms and appeared normal in all respects.

Lesions

The lesions in this study varied in size. The largest lesion was 3.5 mm long by 2.5 mm wide, while the smallest was approximately 1.5 mm x 1.5 mm. The average lesion was 2.0 mm x 3.0 mm at the surface, but much smaller in the depth of the cortex. The lesion drawings presented with the graphs illustrate the size of the lesion on the surface of the cortex. Since most of the lesions taper in the depth of the cortex, the actual loss of layer 5 pyramidal cells may be less than what is shown on the lesion drawings. A Nissl stain of each type of lesion, rostral forelimb, forelimb,and hindlimb, is shown in figure 1A-C. As is seen in the pictures, the underlying white matter was usually not involved in the lesion.

Testing Results

Test results for all lesion groups are presented as graphs which display 12 days of preoperative baseline percent success followed by post-operative percent success for up to 40 testing days. The mean percent success of the 12 preoperative test sessions is displayed, as is the percent success value two standard deviations below the mean. This latter value (-2 SD) was used in this study as the border between normal and subnormal performance. Percent success scores consistently below this value were considered to indicate a deficit. Test scores which fell within 2 SD of the mean were considered to be normal, thus, when an animal returned to within 2 SD of the mean recovery was assumed to have taken place.

Hindlimb Lesions: (n=4)

All four hindlimb lesioned animals attained preoperative success rates within one day of postoperative testing. Two graphs from animals BHL62, and BHL101 are shown in figures 2A+B. It is obvious from these two graphs that lesions of the hindlimb area of motor cortex do not significantly affect a rat's ability to perform a task specific for forelimb digital usage.

Caudal forelimb Motor Lesions: (n=2)

Both animals in this group showed some deficit in their ability to perform the task. The decrease in percent success, as well as the duration of the deficit seemed to increase with the size of the

lesion. Results of testing for both caudal forelimb lesioned animals are shown in figure 3A+B. There was a considerable difference between the duration of the deficit for the two animals. The average deficit was 63 days S.E.=27. The average relative amount of deficit based on the decrease in percent success from preoperative levels to the first day postoperative for both animals in this group was 53.5% (S.D.=6.3).

Two of the caudal forelimb lesioned animals were remapped by ICMS prior to sacrifice. Hindlimb movements were always seen caudal to the lesion, and forelimb movements were always seen rostral to the lesion in the rostral forelimb area verifying that the lesion was restricted to the caudal forelimb area. The other consistent finding was that forelimb motor points were found on the periphery of the lesioned cortex at thresholds of 70ua or less. These points were not responsive (100ua) during the initial prelesion mapping experiment.

As a final test for completeness of the lesion and sparing of the rostral forelimb, both animals received injections of wheat germ agglutinin HRP in the cervical enlargement prior to sacrifice. These animals exhibited a pattern of labeling consistent with the remapping results, that is, they had many cells in the rostral forelimb area even if the brain in this region was deformed, and there was a patch of cells lateral to the lesion in forelimb sensory cortex which extended some distance behind bregma, lateral to the hindlimb area of sensorimotor cortex. The pattern of retrogradely labeled cells was reconstructed from coronal sections and plotted on a dorsal view of

the rat brain. The results of one of these plots is shown in figure 5A.

Rostral Forelimb Lesions: (n=5)

Animals receiving lesions of the rostral forelimb area exhibited deficits lasting from 1 to 21 days. The average deficit for 5 animals was 10 days (SE=3.2). Although the exact size of the lesions is difficult to determine, it did not appear that lesion size was important to the amount of the impairment. Two cases are presented in figure 4A+B, illustrating the shortest and longest lasting deficit. The relative amount of deficit on the first day postoperatively varied among animals from 30% to 76%. The average decrease in percent success was 48% (S.D.=18.6).

Four of the five animals in this group were remapped by ICMS prior to sacrifice. We were always able to find low threshold (20ua) caudal forelimb points, but only saw much higher threshold (100ua) rostral forelimb responses near the periphery of the lesion. One animal in this group received multiple injections of WGA-HRP in the cervical enlargement prior to sacrifice. The location of retrogradely labeled cells is plotted on a dorsal view of the rat brain in figure 5B, and correlates well with the results of the remapping experiments in that there are functional connections remaining at the periphery of the lesioned area, and the caudal forelimb seems to be undamaged.

Statistical Analysis:

The percent decrease from the mean on each postoperative test day and the average length of the deficit were compared for the two experimental groups. The mean percent decrease in success on the first postoperative test day is 54% (SD=6.3) for the caudal forelimb group and 48% (SD=18.6) for the rostral forelimb group. This difference is not significant when a t-test is used. The average duration of the deficit was computed by counting the postoperative test sessions until the animal was consistently above the -2SD mark. The average deficit in days (not including weekends) is 63 days (SE=27) for the caudal forelimb and 9.6 days (SE=3.2) for the rostral forelimb. The difference in the time to recovery is significant ($p<.05$) when compared with a t-test.

Discussion

The results of the present study indicate that lesions of the rostral forelimb area of the rat motor cortex cause only a short term deficit in the animal's ability to perform a task requiring discrete digital movement while lesions of the caudal forelimb area produce a significantly longer lasting deficit. The duration of the deficits (9.6 days for the rostral forelimb and 63 days for the caudal) are significantly different (two tailed unpaired T-test, $p=.02$). Lesions of a similar size in the hindlimb representation do not cause any deficit of the animals' abilities in performing the task. The fact that the hindlimb area lesioned animals appeared to be impaired in walking but were still able to perform the task confirms the specificity of the task for testing forelimb digital usage (Castro, 1972). Deficits from caudal forelimb lesions in the present study were nearly as long lasting as those seen by Castro (1972).

Although the entire area of cortex where ICMS up to 100 ua evoked forelimb movements was removed, functional connections are still made between the cortex lateral and caudal to the lesion and the cervical cord. This is verified by the identification of retrogradely labeled cells and the presence of ICMS evoked forelimb movements as early as three weeks after the lesion. Glees and Cole (1950) showed a similar expansion of the electrically excitable cortex following lesions in the monkey M1, and attributed it to isolated colonies of Betz cells in the adjacent sensory cortex. The reason for the

expansion we have seen is not clear, but it may be the unmasking of synaptic connections from the remaining cortex to the cervical cord which are present but not normally used for the particular function under study. These pathways can be called upon when the ordinarily dominant system fails (Wall, 1980). The caudal strip of retrogradely labeled cells seen in figures 5A+B is present in normal animals (Sievert and Neafsey, 1982), but is not an area where forelimb movements can be evoked in normal animals during low threshold ICMS (Sanderson et al., 1981; Neafsey and Sievert, 1982; Donoghue and Wise, 1982; Sanderson et al., 1984). This strip of cells appears to be part of the S1 forelimb representation (Hall and Lindholm, 1974; Welker, 1976; Sanderson et al., 1984) which may be partially responsible for the recovery from the lesion since its threshold for evoking forelimb movements had decreased when recovery was complete.

A recent study on the monkey following SMA lesions demonstrated a short term deficit of the animal's ability to perform discrete movements, and a lasting impairment of bimanual co-ordination skills (Brinkman, 1984). Early studies on lesions of SMA in the monkey have shown forced grasping to be a consistent sign (Travis, 1956; Woolsey et al., 1974). Case studies on humans have shown that lesions of the SMA cause little paresis but they do result in a transient paucity of movements and speech (Laplane et al., 1977). The relatively short duration of the deficit following the rostral forelimb lesions is evidence that this region may be a portion of the rat's supplementary

motor area (SMA), as has been suggested by several studies (Wise et al., 1977; Neafsey and Sievert, 1982; Donoghue and Wise, 1982). Although the present study did not test for bimanual co-ordination or forced grasping, the transient nature of the deficits is consistent with the results in primates.

In summary, lesions of the rostral forelimb area result in transient deficits in digital usage as compared to deficits seen following caudal forelimb lesions. Although the rostral forelimb lesioned animals achieved preoperative success levels sooner than the caudal forelimb lesions, both groups did show almost complete recovery. This recovery may be at least partially attributed to the remaining functional connections which were demonstrated in the adjacent S1 forelimb representation. Since similar transient deficits are seen in primates following SMA lesions, it is more likely that the rat rostral forelimb area is a part of the supplementary motor area than a subdivision of the primary motor area.

Figure 1. Nissl stain of each type of lesion.

A. Rostral forelimb lesion. Bar=1mm.

B. Caudal forelimb motor lesion. Bar=2mm.

C. Hindlimb sensorimotor lesion. Bar=2mm.

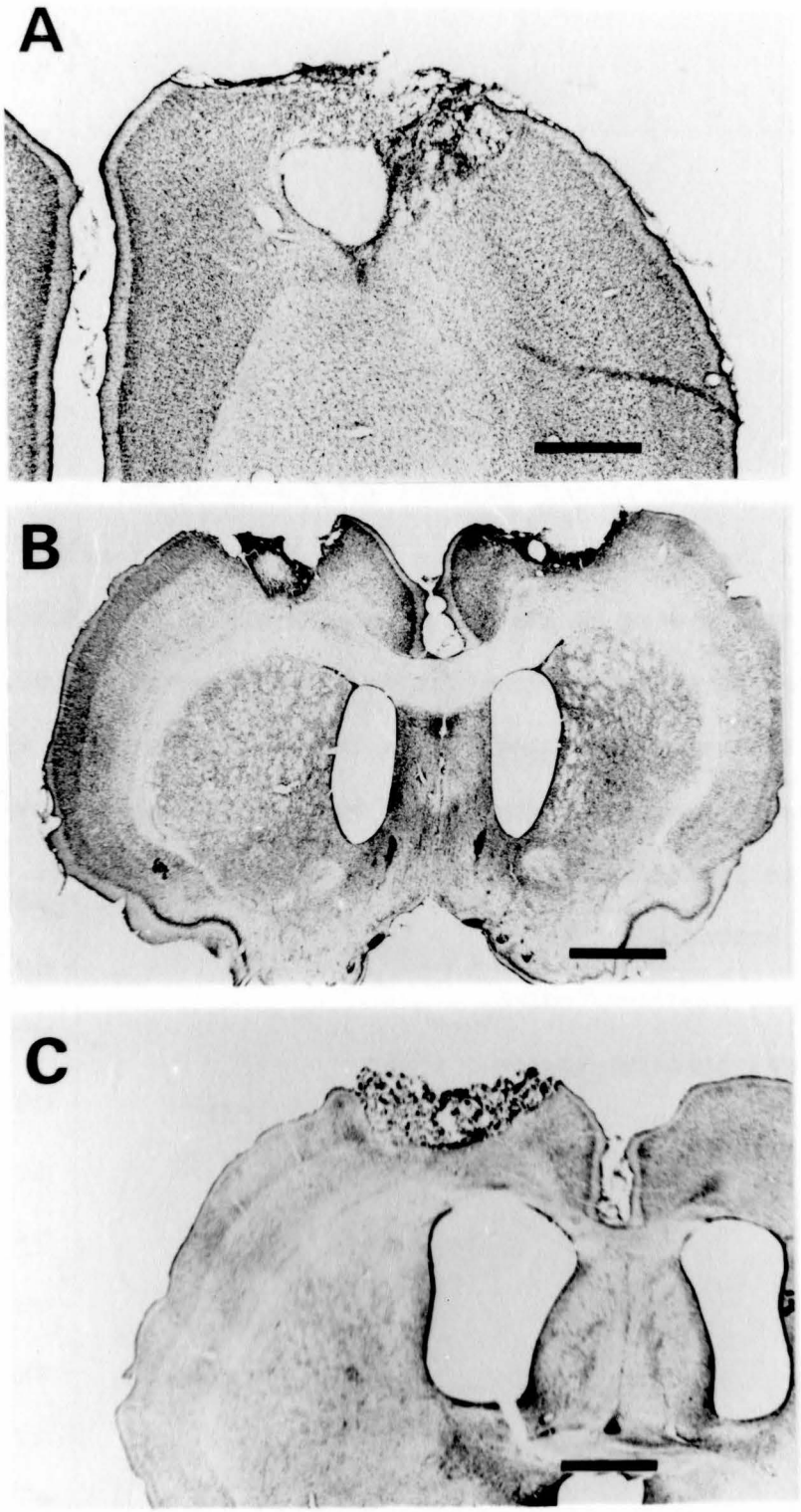
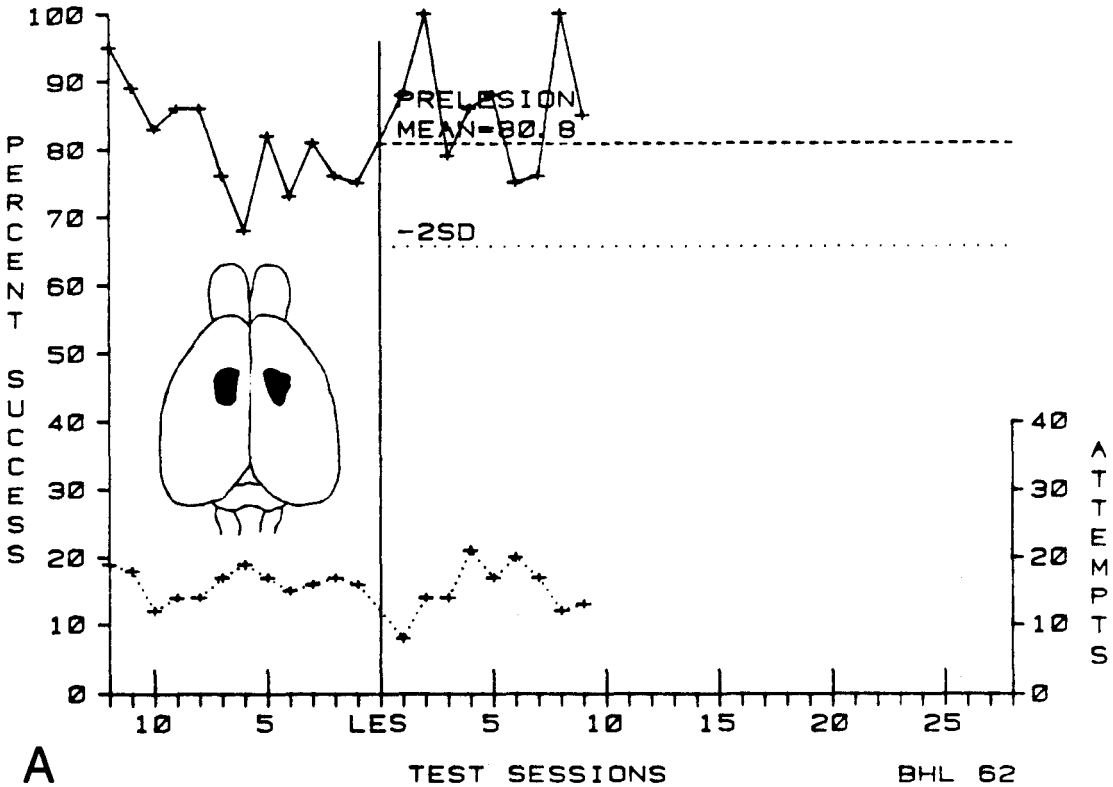

A
B
C

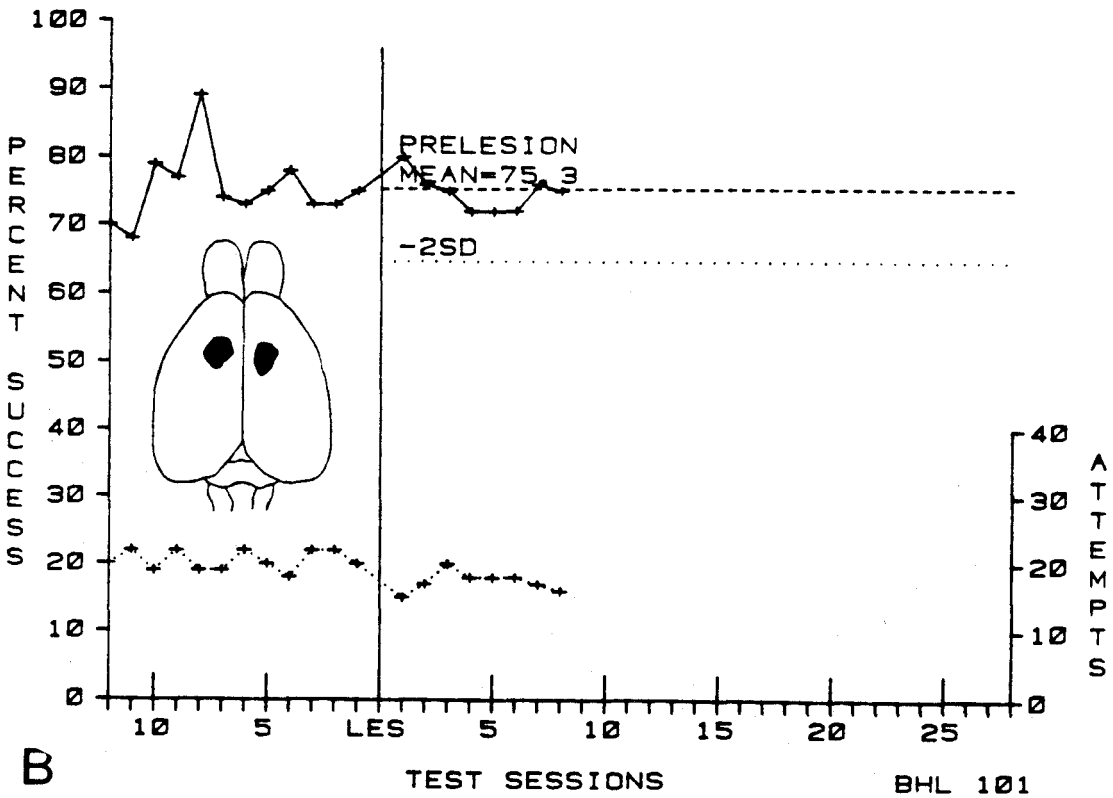
Figure 2. Hindlimb lesion plots. Percent success is on the left Y-axis, and the number of attempts is on the right Y-axis. Test sessions in days is plotted on the X-axis with 12 sessions of preoperative testing before the vertical line marked LES. The prelesion mean is plotted, as is the level of success which corresponds to 2 standard deviations below the prelesion mean. The size and location of the lesion is plotted on a dorsal view of the rat brain.

A. Bilateral hindlimb lesion.

B. Bilateral hindlimb lesion.


PERCENT SUCCESS
ATTEMPTS
PRELESION
MEAN=80.8
-2SD
LES
TEST SESSIONS
BHL 62

A

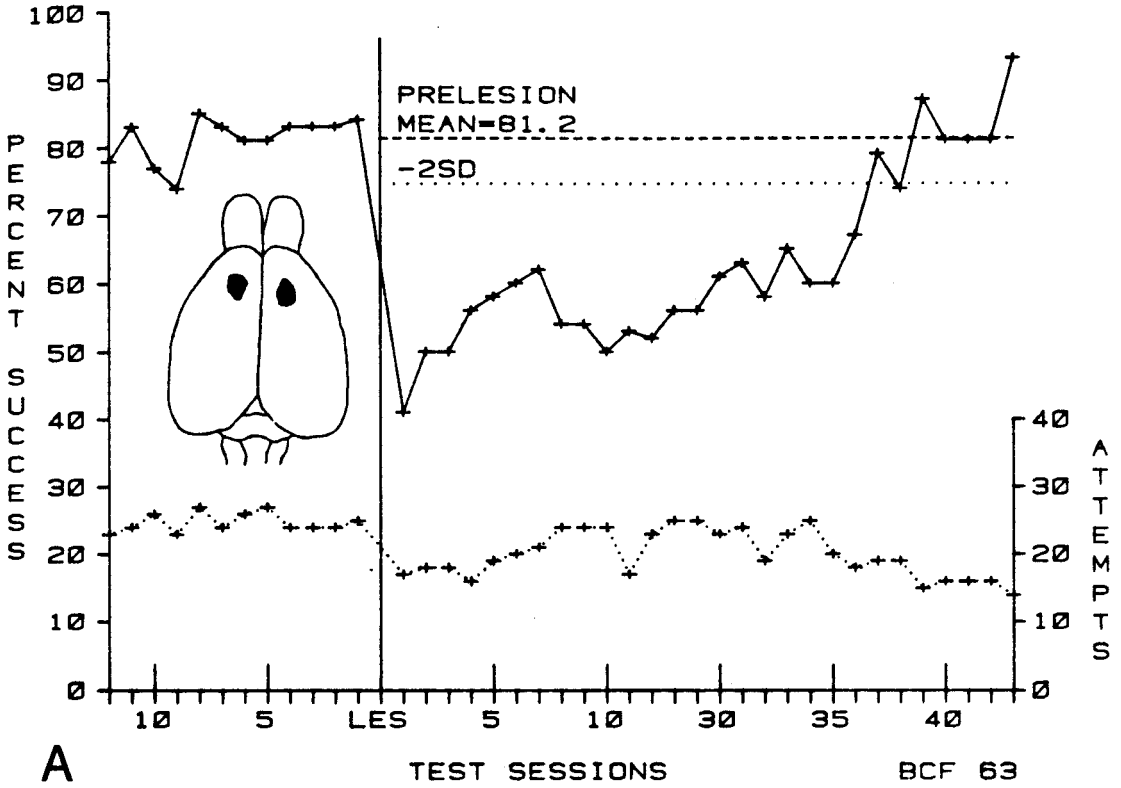

PERCENT SUCCESS
ATTEMPTS
PRELESION
MEAN=75.3
-2SD
LES
TEST SESSIONS
BHL 101

B

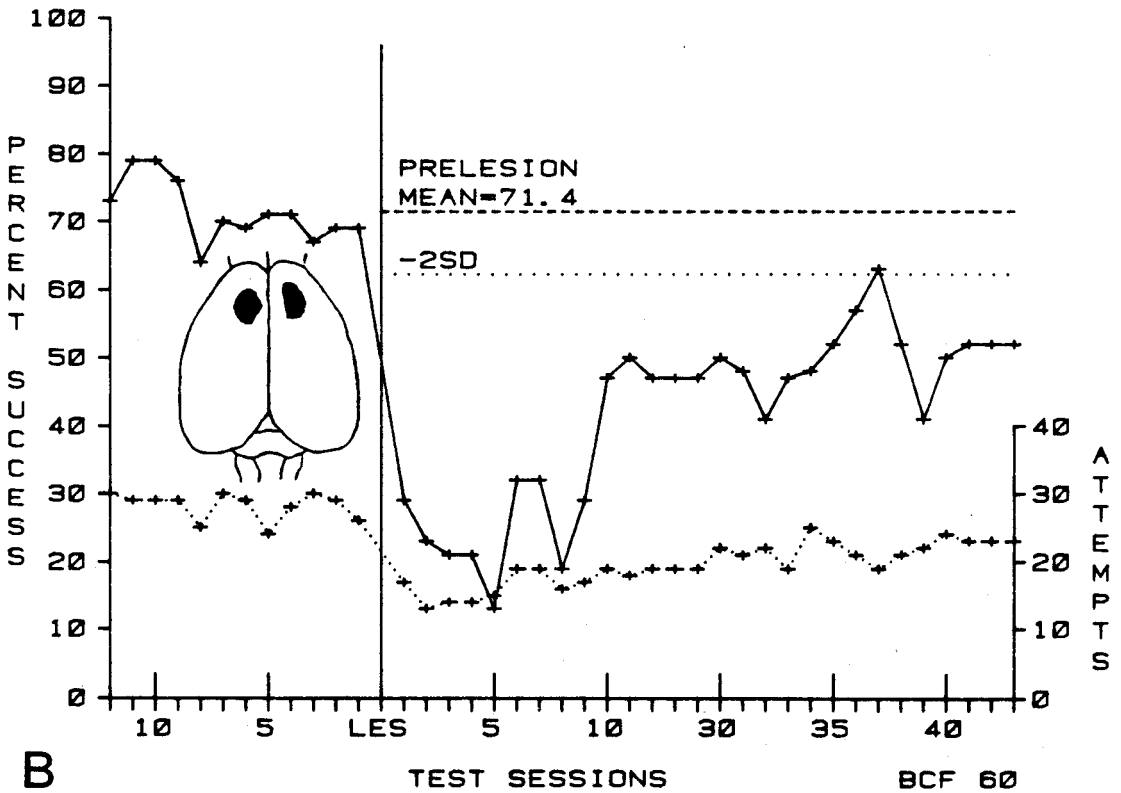
Figure 3. Caudal forelimb lesion plots (See figure 1 for a description of the graph).

A. Bilateral caudal forelimb lesion (BCF63). Note the increase in test sessions (points not plotted are from session 14 to session 30).

B. Bilateral caudal forelimb lesion (BCF60). The number of test sessions is the same as it was for figure 3A.


PRELESION
MEAN=81.2
-2SD
PERCENT SUCCESS
ATTEMPTS
100
90
80
70
60
50
40
30
20
10
0
40
30
20
10
0
10
5
LES
5
10
30
35
40
TEST SESSIONS
BCF 63

A

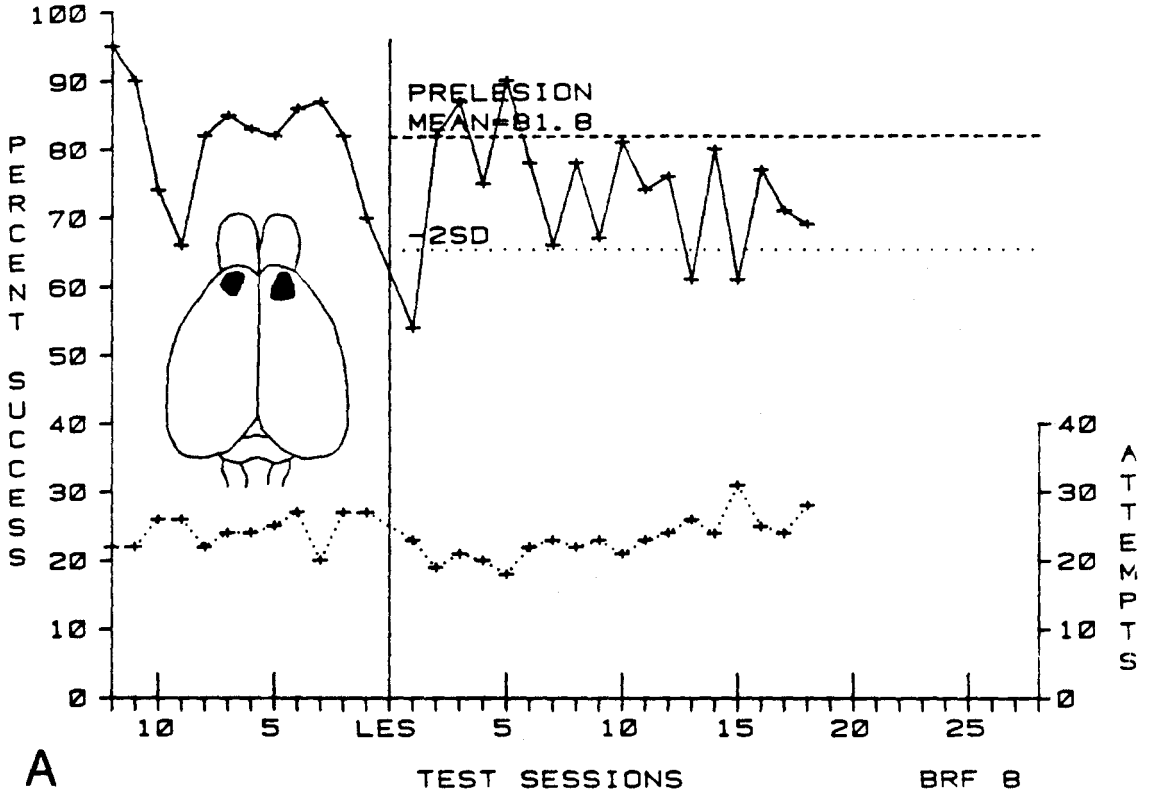

PRELESION
MEAN=71.4
-2SD
PERCENT SUCCESS
ATTEMPTS
100
90
80
70
60
50
40
30
20
10
0
40
30
20
10
0
10
5
LES
5
10
30
35
40
TEST SESSIONS
BCF 60

B

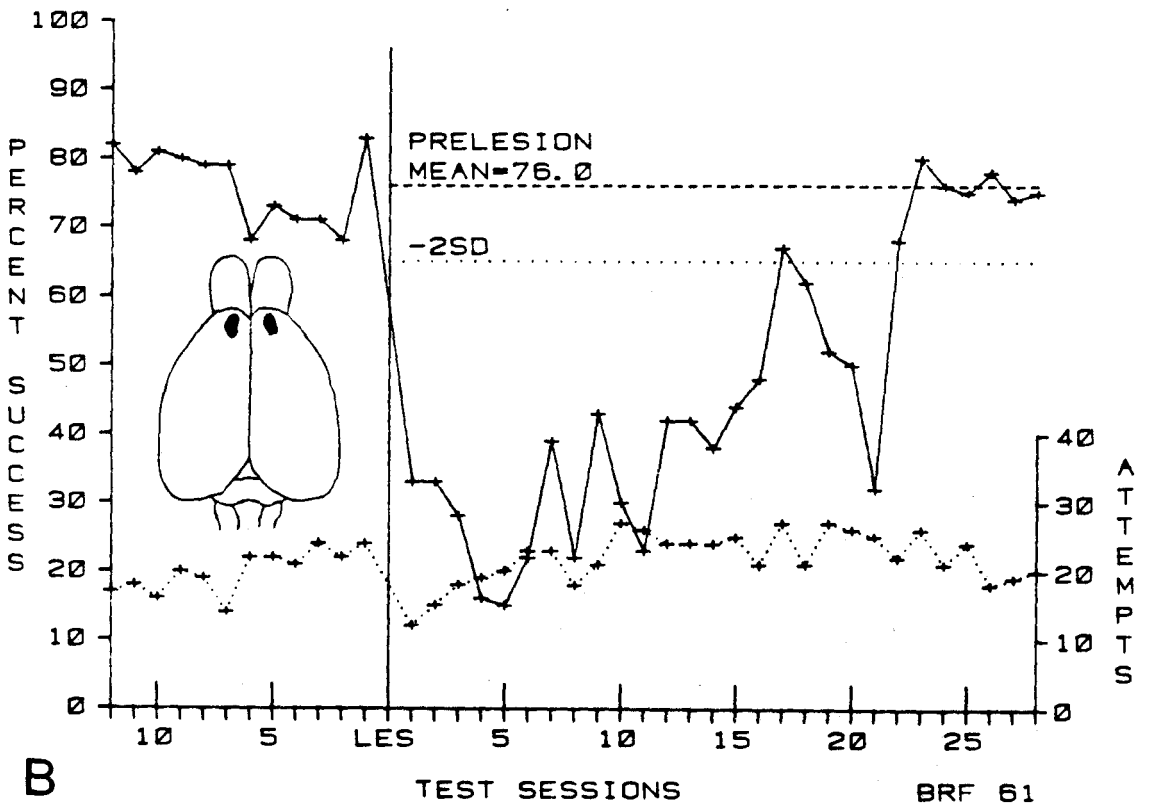
Figure 4. Rostral forelimb lesion plots (See figure 1 for a description of the graph).

A. Bilateral rostral forelimb lesion (BRF8) with the shortest duration of motor deficit.

B. Bilateral rostral forelimb lesion (BRF61) with the longest lasting motor deficit.


PRELESION
MEAN=81.8
-2SD
PERCENT SUCCESS
ATTEMPTS
100
90
80
70
60
50
40
30
20
10
0
40
30
20
10
0
10 5 LES 5 10 15 20 25
TEST SESSIONS
BRF 8

A

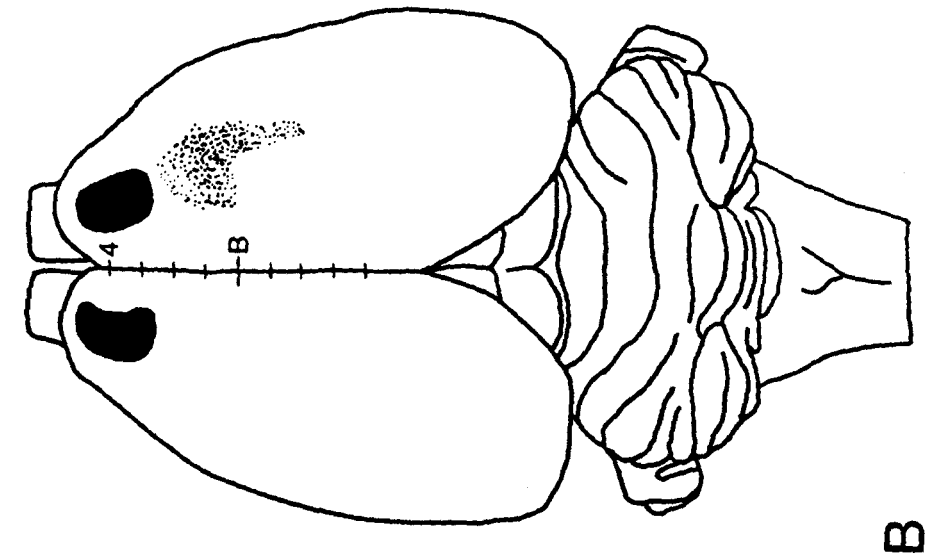

PRELESION
MEAN=76.0
-2SD
PERCENT SUCCESS
ATTEMPTS
100
90
80
70
60
50
40
30
20
10
0
40
30
20
10
0
10 5 LES 5 10 15 20 25
TEST SESSIONS
BRF 61

B

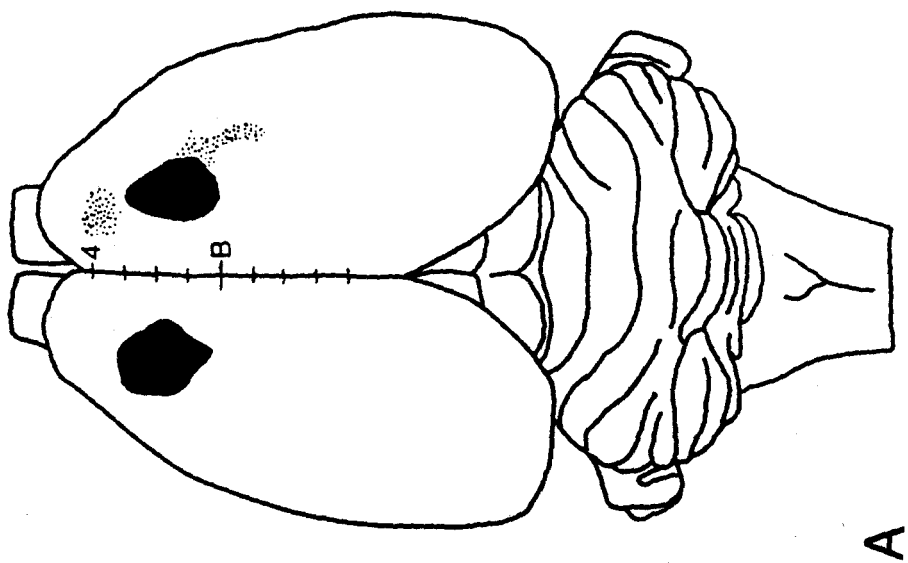
Figure 5. Plot of labeled cells from a cervical enlargement injection of HRP on a dorsal view of the rat brain.

A. Area of label seen in an animal which had sustained a bilateral lesion of the caudal forelimb motor area. The blackened area represents the extent of the lesion. The stippled area represents the area where retrogradely labeled cells were seen. The rostral patch of cells is in the location of the rostral forelimb area, and there is a strip of labeled cells extending lateral and caudal from the lesion which is in the location of part of the S1 forelimb representation. B=Bregma, Divisions are in mm.

B. Area of label seen in an animal which had sustained a bilateral lesion of the rostral forelimb area. In this animal no retrogradely labeled cells were found in the region of the lesion, but a large patch of labeled cells was seen in the area corresponding to the caudal forelimb sensorimotor area.



B



A

CHAPTER IV

ORGANIZATION OF CORTICOSPINAL NEURONS IN FORELIMB, TRUNK AND HINDLIMB SENSORIMOTOR AREAS

Introduction

The somatotopic organization of corticospinal projection neurons in rat primary motor and sensory cortex has been clearly demonstrated (Wise, Murray and Coulter, 1979; Ullan and Artieda, 1981; Neafsey and Sievert, 1982), but several questions concerning the relation of corticospinal neuron topography to physiological maps of sensorimotor cortex and to cortical cytoarchitecture remain unresolved; and thus, prompted the present study.

The first question is whether there is a somatotopic organization of corticospinal neurons in the region of the second rostral forelimb motor area in rat frontal cortex (Neafsey and Sievert, 1982). The rostral forelimb area is a separate area of cortex, distinct from the primary forelimb motor area, where forelimb movements can be evoked by low threshold intra-cortical microstimulation (ICMS) (Neafsey and Sievert, 1982) and where corticospinal neurons projecting to the cervical enlargement have been found (Hicks and D'Amato, 1977; Wise et al., 1979; Neafsey and Sievert, 1982; Donoghue and Wise, 1982). It has been suggested that the rostral forelimb area could be a second representation of the forelimb within the primary motor area (Neafsey and Sievert, 1982), similar to what has been shown in the monkey (Strick and Preston, 1978, 1982a). It has also been suggested that it may be a part of the supplementary motor area (SMA) of the rat (Wise and Jones, 1977; Wise et al., 1979; Neafsey and Sievert, 1982; Donoghue and Wise, 1982). We

have recently evoked hindlimb movements with ICMS within this rostral motor area (Neafsey et al., in preparation), a finding which suggests that there may be a whole body representation in this region. Since there appears to be a whole body representation in the monkey SMA (Woolsey et al., 1952; Murray and Coulter, 1981; Macpherson et al., 1982a; Tanji and Kurata, 1982), it seemed important to reexamine neurons projecting to cervical, thoracic and lumbar cord levels from this rostral motor area in the rat.

The second question concerns the amount of overlap between primary motor (MI) and primary sensory (SI) cortex in the rat. At present it is thought that MI and SI forelimb areas partially overlap while MI and SI hindlimb areas completely overlap (Hall and Lindholm, 1974; Wise et al., 1979; Donoghue et al., 1979; Donoghue and Wise, 1982; Sanderson et al., 1984). However, close inspection of Figure 6 in Donoghue and Wise (1982) illustrates an agranular portion of rat hindlimb motor cortex containing corticospinal neurons and located medial to granular hindlimb sensorimotor cortex which suggests that overlap of hindlimb SI and MI may not be complete. In addition, distinct hindlimb sensory and motor regions in the rat have been found in a series of cortical mapping experiments using the combined techniques of multiunit recording and ICMS through the same electrode (R. Kosinski, personal communication).

A third open question concerning the organization of the rat motor cortex is the extent of collateralization and/or overlap of

corticospinal neurons projecting to widely separated levels of the spinal cord. Corticospinal neuron collaterals to both cervical and lumbar spinal cord have been demonstrated physiologically in the cat (Shinoda et al., 1976) and monkey (Shinoda et al., 1979) but were not seen in a double label anatomical study in the hamster (Kassel and Kalil, 1982). As yet, no similar study has been performed in the rat.

Materials and Methods

Twenty-three male Long-Evans hooded rats (300-450g) were used in this study. Two retrograde tracing methods were employed in this study and the methods for each are presented separately.

WGA-HRP Experiments:

The first procedure utilizes wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) as a retrograde tracer (Mesulam, 1978). Eleven animals were initially anesthetized with ketamine HCL (100 mg/kg) and placed in a stereotaxic apparatus. All animals in this group received a craniotomy over the right cortex from 3 mm caudal to bregma to 4 mm rostral to bregma, and from 1.5 mm lateral to the midline to 4.5 mm lateral. In addition to the craniotomy, the cisterna magna was opened to prevent cortical swelling. Two types of physiological mapping were performed in this group. The first type consisted of intracortical microstimulation (ICMS) at a depth of 1.7 mm below the cortical surface with a glass-insulated tungsten microelectrode (tip exposed 100 mu) (Neafsey, 1980). Stimulation parameters were a 300 msec train of negative, 0.25 ms pulses at 350 hz. Current strength was kept below 100 microamps. For a detailed description of the stimulating and recording procedures see appendix 1. In seven of the eleven animals in this group, the motor forelimb and hindlimb areas were defined by ICMS and small lesions (10 ua, 10 sec) were made in some of the electrode tracks to aid in

histological reconstruction. After ICMS mapping, the animals were deeply anesthetized with sodium pentobarbital, and a laminectomy was performed over the cervical or lumbar enlargement. The animals received multiple injections (0.02 ul/injection) of WGA-HRP in the gray matter of the spinal cord. In the cervical enlargement, injection penetrations were made in the region of C5-T1; care was taken to avoid the corticospinal tract which runs beneath the dorsal columns in the rat. In the lumbar enlargement, injection penetrations were made at T13, L1 and L2. The L2 penetration was made through the dorsal funiculus and into the corticospinal tract in order to damage and label any CST fibers which extended below this level. The wounds were closed, and the animals allowed to survive for two to three days.

The remaining four animals in this group underwent more extensive cortical mapping which included recording of evoked multiunit activity by peripheral cutaneous stimulation (Welker, 1976). Rows of electrode tracks 0.5 mm apart were made from medial to lateral, and ICMS and sensory mapping were performed in each track. The evoked multiunit activity was studied at a depth of 0.5 mm below the surface while the ICMS was delivered at a depth of 1.7 mm. Electrolytic marking lesions (10 ua/10 sec.) were made laterally at the point where movement thresholds rose above 100 uamps and medially at the border of the sensory evoked multiunit activity. After the physiological mapping was complete, these animals were deeply anesthetized with sodium pentobarbital (40 mg/kg) and a laminectomy made over the cervical (2 animals) or lumbar (2 animals) enlargements

to expose the cord. These animals received injections of HRP in the gray matter just as the first group did, and were allowed to survive for two (cervical injection) or three (lumbar injection) days.

After the survival period, all animals in both mapping groups were deeply anesthetized with sodium pentobarbital and perfused through the heart with 0.9% saline (500 ml), followed by a solution of 1.0% paraformaldehyde, 1.25% glutaraldehyde in 0.1 M phosphate buffer (1000 ml) and finally with 10% sucrose in 0.1 M phosphate buffer (1000 ml) (cf. Rosene and Mesulam, 1978). The brains were removed and 50 um sections cut on a freezing stage microtome. Sections were cut in either the coronal or horizontal plane. Prior to sectioning horizontally, brains were flattened dorso-ventrally to remove some of the curvature (cf. C. Welker, 1976). This procedure facilitates sectioning the majority of any one cortical layer within a few adjacent sections. Most of the sections were reacted for HRP histochemistry according to the TMB technique of Mesulam (1978), but sections from three brains were reacted according to the modified TMB technique of Gibson et al., (1984). The latter technique was found to be equally sensitive to Mesulam's but with less artifact. Reacted sections were mounted on chrome-alum subbed slides, coverslipped, examined and plotted under polarized light microscopy for the location of cell bodies. In the coronally sectioned brains alternate sections were stained for cell bodies with a Nissl stain.

Double Label (DY-FB) Experiments:

The second experimental technique utilized the retrograde tracing properties of two flourescent dyes, Diamidino yellow (DY) and Fast blue (FB), to examine the possibility of corticospinal tract collaterals to widely separated levels of the spinal cord. This group consisted of twelve animals which were anesthetized with sodium-pentobarbitol (40 mg/kg IP) and placed in a stereotaxic apparatus. All animals received a laminectomy over two areas of the spinal cord for injection of the two different dyes. Four animals received injections of DY and FB in the cervical enlargement and thoracic cord (T7) respectively. Four animals received injections of FB and DY in the thoracic cord (T7) and lumbar enlargement, respectively, and four animals received injections of DY and FB in the cervical and lumbar enlargements, respectively. Injections were made at three depths along a penetration, 1.7 mm, 1.2 mm and 0.75 mm deep, and 0.04 ul of dye was injected at each depth. In the cervical and lumbar enlargements, the number and level of penetrations were identical to the HRP procedure. In the thoracic cord, only two penetrations were made. Care was taken to avoid the corticospinal tract in the cervical and thoracic injections. After the injections, the wounds were closed and the animals allowed to survive for five days. The animals were then deeply anesthetized with sodium pentobarbital and perfused through the heart with 0.9% saline (500 ml), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (1000 ml) and finally 10% sucrose in 0.1 M phosphate buffer. The brains

were removed and allowed to sink in a 30% sucrose solution for two to five days and then cut in the coronal plane on a freezing microtome at 50 um thick. Sections were mounted out of 0.01 M sodium acetate buffer onto chrome-alum subbed slides. The sections were viewed on an Olympus microscope under epi-flourescence illumination (360 nm), and the retrogradely labeled cells were plotted on line drawings of every fourth section. Once cell plotting was complete, the sections were stained with cresyl violet, coverslipped, and examined for cytoarchitectonic boundaries.

In order to visualize the overall pattern of cortical cell labeling with respect to cytoarchitectonic boundaries, the labeling from both coronal and horizontal sections was plotted on a three dimensional view of the rat brain. This drawing was generated from a model of the rat brain (scale: 19 mm=1 mm) constructed out of styrene fiberglass foam using the Nissl plates from the atlas of Paxinos and Watson (1982). Briefly, plates 1 mm apart were traced onto pieces of foam 19 mm thick. These tracings were then cut out on a band saw and glued together to form a large scale model of the rat brain. Since the curvature and size of adjacent sections were not the same, the model had to be shaped with a surform to make a continuous smooth surface. Once completed, the entire surface was marked off in 1 mm divisions (scale 19 mm=1 mm) from the midline laterally and from bregma rostrally and caudally, and the points connected by lines drawn on the surface of the brain model. The finished model was then

photographed and line drawings were made from the photograph. The perspective lines seen on the final drawing (Fig. 1) are accurate depictions of the 1 mm grid distance from the midline and bregma as seen in a slightly rostral, dorsolateral view of the surface of the rat brain. The cytoarchitectonic boundaries of the rat brain are shown on the same drawing in heavy lines. The boundaries were obtained from the Nissl plates of Paxinos and Watson (1982) and from a paper by Zilles et al (1980).

Results

Cervical Enlargement Injections (WGA-HRP)

Injections in the cervical enlargement filled the dorsal horn, intermediate gray and ventral horn, and did not damage the corticospinal tract (Fig. 2A). Three patches of retrogradely labeled cells were found in the contralateral hemisphere. The largest patch corresponds with the primary motor and sensory forelimb areas and includes the agranular lateral (AgL) cytoarchitectonic subdivision, the granular subdivision (Gr), and patches of dysgranular (Dys) cortex interspersed between the two. The AgL cortex is an area where lowest threshold movements can be elicited during ICMS, whereas the granular cortex is responsive to cutaneous peripheral inputs in the anesthetized animal (Fig. 4B). The large patch of labeled cells extends caudally, lateral to the hindlimb representation as defined by ICMS and is located in part, underneath a layer IV granular patch which is responsive to forelimb peripheral sensory input (Fig. 4B, sections 3-8). In none of the cervical cord injections were labeled cells of the large caudal patch found medially in the medial agranular subdivision (AgM). The second largest patch of retrogradely labeled cells is rostral to the first and corresponds with the rostral forelimb area defined by microstimulation (Neafsey and Sievert, 1982). The majority of the labeled cells in this area were located in the agranular lateral field (AgL), but some were found in the medial agranular (AgM), anterior cingulate (AC), and prelimbic (PL)

cytoarchitectonic areas (Fig. 4B, sections 1+2 and Fig. 7C). The rostral patch of cells was more extensive than the area where ICMS evoked forelimb movements. Most of the cervical enlargement injection animals had complete separation of the rostral and caudal patches of labeled cells, but in one animal the two patches of cells were linked by a string of five cells from the lateral border of the rostral region. The third patch of retrogradely labeled cells was found far laterally, just above the rhinal sulcus, and appeared to be located within SII, the second somatosensory area (Welker and Sinnha, 1972). The rostral-caudal location of this patch of cells was generally located between bregma and 2.5 mm caudal to bregma (Fig. 4B, section 8). The overall pattern of retrograde labeling from a cervical enlargement injection is plotted on a dorsal view of the rat brain in figure 3.

Lumbar Enlargement Injections (WGA-HRP)

Following lumbar enlargement injections (Fig. 2B), only two patches of retrogradely labeled cells could be found in the contralateral hemisphere. The first patch was located in the hindlimb primary sensorimotor representation as determined by ICMS (Fig. 5). Cells in this area were found in the agranular lateral (AgL) and granular (Gr) cytoarchitectonic areas (Fig. 5). Both low threshold ICMS evoked movements and sensory responses can be found through much of the hindlimb area. However, the most medial portion of the hindlimb representation is not responsive to peripheral sensory input, but does

show low threshold ICMS responses and retrogradely labeled cells (Fig. 6B, sections 2-4). As was the case in the forelimb cortex, the labeled cells did not extend across the border between medial and lateral agranular cortex (Figs. 6B, sections 3+4 and Fig. 7A). The second patch of retrogradely labeled cells was located medial to the rostral forelimb area in an area where higher threshold (50ua current) trunk and hindlimb movements were evoked (Figs. 5, and 6B section 1). Usually only a few labeled cells can be seen in this area, and they are located in the medial agranular and anterior cingulate cytoarchitectonic fields (Fig. 6B section 1 and Fig. 7B). No labeled cells were ever seen in the second somatosensory area following injections into the lumbar enlargement.

Double label injections (DY and FB)

Photomicrographs of the three types of injection sites (cervical, thoracic and lumbar) can be seen in figure 8. Although some tissue destruction was visible at the injection site, the tracer substance did not appear to reach the corticospinal tract. This observation was confirmed by the different patterns of retrograde labeling seen in the cortex for each type of injection site and by the lack of double labeled cells.

The results of injections of (DY) into the cervical enlargement and (FB) into the lumbar enlargement are plotted on a three dimensional view of the rat brain in figure 9. The same pattern of labeling is seen here as was seen in the HRP injection animals except

the rostral, lateral border of labeled cells does not extend as far lateral for the main patch of cervical enlargement projection neurons. There is very little overlap of cells projecting to the two areas except in the border zone between the two patches near bregma (Fig. 12B), and in the medial portion of the main hindlimb patch (Figs. 9 and 13). The caudolateral tail of the DY patch is separated from the FB patch by a gap (Figs. 9 and 12C). No double labeled cells were seen in any of the four animals in this group. The rostral patch of labeled cells had numerous cervical projection neurons, but only a few lumbar projection neurons (Fig. 12A). As was seen in the HRP animals, only cells projecting to the cervical enlargement were found in the second somatosensory area.

Injections of DY into the cervical enlargement and FB into the thoracic cord produced the pattern of cortical labeling illustrated in Figure 10. The DY labeling was identical to the pattern seen after a cervical HRP injection, but there were fewer cells labeled in all three of the patches. FB labeled cells were found in three patches. The first patch was seen at the caudal border of the forelimb motor representation, and extended into both AgL and Gr cortices (Fig. 10). The second patch was seen entirely in the granular cortex, medial to the caudal limb of forelimb labeled cells in the granular cortex (Figs. 10 and 13). There was some overlap in the agranular cortex between the DY and FB cell populations caudomedially, but no overlap was seen between the two populations laterally in the granular cortex. The last patch of FB labeled cells was found medial to the

rostral forelimb area and appeared to cross the Agl and Agm boundaries (Fig. 10). There were no FB labeled cells in the second sensory area. No double labeled cells were seen in any of the animals in this group.

The third type of experiment involved injections into the thoracic cord (FB) and the lumbar enlargement (DY) and produced a pattern of retrograde labeling which was consistent with the description for thoracic and lumbar injections in the previous two experiments. The only new information which was gained from this experiment concerned the degree of overlap between the cells projecting to the two areas. In the rostral patch of cells, the FB and DY cells were entirely overlapping except for some thoracic projection neurons found in AgL (Fig. 11). In the caudal patch of cells, the largest area of overlap was found in the Agl hindlimb representation (Fig. 11). No labeled cells of either type were seen in SII, and no double labeled cells were seen.

Discussion

The results of the present study confirm the somatotopy in M1 and S1 described by other investigators (Wise et al., 1977; Hall and Lindholm, 1974; Ullan and Artieda, 1981; Neafsey and Sievert, 1982; Donoghue and Wise, 1982) in that the forelimb sensorimotor cortex projects to the cervical cord and the hindlimb sensorimotor cortex projects to the lumbar cord. In addition to somatotopy, this study has demonstrated a second representation of the forelimb, trunk and hindlimb located near the frontal pole. Previously, only a forelimb representation had been described in this region (Hicks and D'Amato, 1977; Wise et al., 1979; Neafsey and Sievert, 1982; Donoghue and Wise, 1982). The representation of much of the rat body in the rostral frontal cortex supports the proposal that the rostral motor area is the supplementary motor area of the rat (Wise et al., 1979; Donoghue and Wise, 1982; Sievert and Neafsey, 1983) since a whole body representation has been described in the SMA of the monkey (Woolsey et al., 1952; Brinkman and Porter, 1979; Murray and Coulter, 1981; Macpherson et al., 1982a; Tanji and Kurata, 1982). The fact that the labeled neurons in the rostral area of cortex are found in several cytoarchitectonic areas (AgL, AgM, AC and PL) confirms the results of Donoghue and Wise (1982). The significance of the cells in AC and PL is not clear since no limb movements are evoked by microstimulation in these regions.

Although the medial agranular cortex (AgM) has been considered a part of the limb motor cortex (Donoghue and Wise, 1982; Donoghue and Parham, 1983; Sanderson et al., 1984), the absence of retrogradely labeled neurons in this area following lumbar, thoracic or cervical cord injections, except in the rostral zone, suggests that AgM is not primarily involved in direct control of any of these body parts. Those investigations which did report ICMS evoked limb or trunk movements in the agranular medial zone (Donoghue and Wise, 1982; Sanderson et al., 1984) also reported that responsive points in AgM were infrequently found and had higher thresholds. Furthermore, AgM is an area where vibrissae, eye and head orienting movements are evoked during low threshold ICMS (Hall and Lindholm, 1974; Neafsey and Sievert, 1982; Sinnamon and Galer, 1984). Agranular medial cortex receives input from the visual cortex (Miller and Vogt, 1984), and has also been shown to project heavily to the superior colliculus (Hardy and Leichnetz, 1981). A recent stimulation study in the rat reported that both eye and vibrissae movements are elicited from wide areas of the superior colliculus at low current intensities (McHaffie and Stein, 1982). This suggests that AgM is a cortical region primarily involved in coordinating head, eye and vibrissae movements via its projection to the superior colliculus.

In the present study, as in past investigations (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Sanderson et al., 1984), some movements could be evoked by ICMS in the granular sensory cortex. This finding has led to the proposal that sensory and motor cortex

overlap for part of the forelimb representations and for most, if not all, of the hindlimb representation (Hall and Lindholm, 1974; Donoghue et al., 1979). However, determination of the extent of overlap based entirely on the presence of ICMS evoked movements is unwarranted since it is difficult to estimate the actual effective spread of ICMS (Jankowska et al., 1975). Furthermore, stimulation in the monkey (Woolsey, 1958) and human (Woolsey et al., 1979) sensory cortex also evokes movements. Donoghue and his coworkers, aware of these difficulties, have offered anatomical evidence that the hindlimb area of rat sensorimotor cortex receives thalamic input from both the ventrobasal (VB) and ventrolateral (VL) thalamic nuclei (Donoghue et al., 1979). This dual projection is consistent with the "hindlimb overlap" hypothesis. However, their HRP injections appear to have been made into only the granular portion of hindlimb area which would confirm overlap, but could not determine if there was also a non-overlapping portion of hindlimb motor cortex. In the present study following a lumbar enlargement injection of HRP, there was a cluster of labeled cells in an area of agranular cortex which yielded low threshold ICMS hindlimb movements and which did not respond to peripheral sensory stimulation in the anesthetized animal. This area, which appears to be only motor, was as large as 1.0 mm wide rostrally, and as long as 2.5 mm. On the basis of these results, the overlap of hindlimb sensory and motor cortices in the rat which has been previously described (Hall and Lindholm, 1974; Donoghue et al., 1979; Sanderson et al., 1984) appears to have been overestimated. A truly

accurate estimate of the amount of sensory-motor overlap would come from a determination of thalamic inputs to both agranular and granular regions of hindlimb cortex.

We were unable to demonstrate any double labeled neurons following injections of different dyes into separate levels of the spinal cord. This is consistent with findings using a similar technique in the hamster (Kassel and Kalil, 1982) and also with earlier HRP findings by Wise et al, (1977). It appears that the collateralization of corticospinal fibers demonstrated physiologically in cats and monkeys (Shinoda et al., 1977, 1979) is not present in the rat. In the present study, populations of labeled cells projecting to different cord levels were for the the most part separate, but did appear to overlap in the medial area of the hindlimb representation. The functional significance of this is unknown, but it may represent a means for coordinated control of limb and trunk movements during locomotion.

In summary, a composite figure depicting the results of both HRP and double label studies (Fig. 13) shows the overall pattern of retrograde cell labeling from the cervical, thoracic and lumbar cord. First, there is a second representation of the limbs and trunk near the frontal pole. Cells in this area cross a number of cytoarchitectonic areas (AgL, AgM, Ac and Pl), and are partially overlapping. That is, the digits are represented laterally, the trunk medial to this and the hindlimb most medially, but there is some overlap of all three areas within AgM. Second, the caudally located

forelimb sensory representation is continuous with the motor forelimb, and the hindlimb sensory is continuous with the hindlimb motor. The trunk motor area appears to be completely separate from the laterally located trunk sensory area. Third, a portion of the hindlimb motor representation appears to be separate from the hindlimb sensory area, and projects to cervical thoracic and trunk levels of the spinal cord. Collaterals of corticospinal neurons to widely separated levels of the spinal cord were not demonstrated in these studies. Finally, regarding the SII representation, it appears that cells in this area do not directly project to cord levels below upper thoracic.

Figure 1. Dorsolateral view of the rat brain with perspective lines and cytoarchitectonic boundaries. The grid lines are 1 mm apart. Cytoarchitectonic boundaries were drawn from the Nissl plates of the rat atlas (Paxinos and Watson 1982), as well as from the dorsal and lateral views shown in the paper by Zilles et al (1980). Our interpretation of the boundaries agrees closely with Zilles. The heavy lines indicate the boundaries between adjacent cytoarchitectonic areas. AgM=agranular medial, AgL=agranular lateral, Gr=granular sensory cortex, SII=second somatosensory cortex, Te=temporal, Cl=anterior cingulate dorsalis, Rag=retrosplenialis agranularis, Oc=occipital, Cli=claustro isocortical, Rf=rhinal fissure.

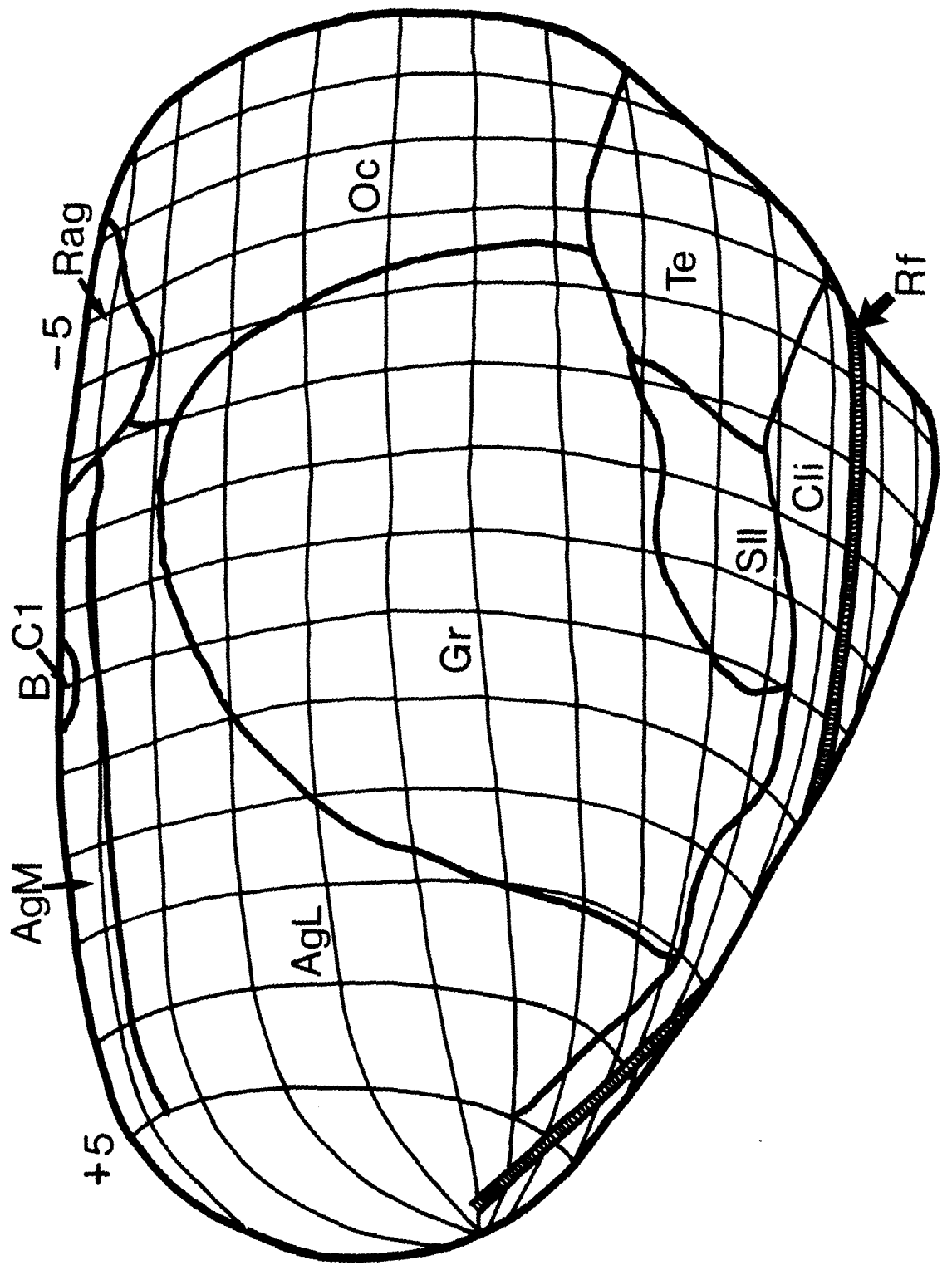
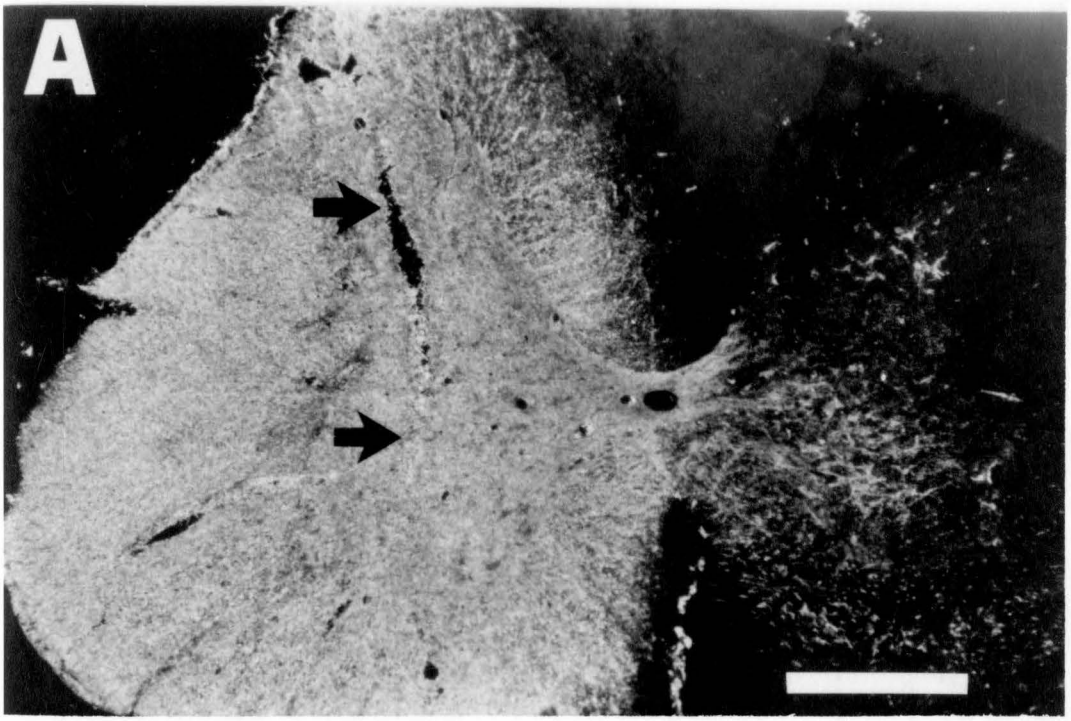

AgM
B_C1
-5
Rag
+5
AgL
Gr
Oc
Te
SII
CIi
Rf

Figure 2. WGA-HRP injection sites in the spinal cord.

A. Dark field photomicrograph of a cervical enlargement injection (approximately C6). The pipette track, marked with arrows, is located in the dorsal horn, intermediate gray and ventral horn. There was no damage to the CST which courses in the dorsal funiculus of the spinal cord in the rat. Scale bar=500 um.

B. Dark field photomicrograph of a lumbar enlargement injection (approximately L1). Here again, the pipette track is indicated with arrows and is clearly located in the spinal gray matter. Scale bar=500 mm.


A

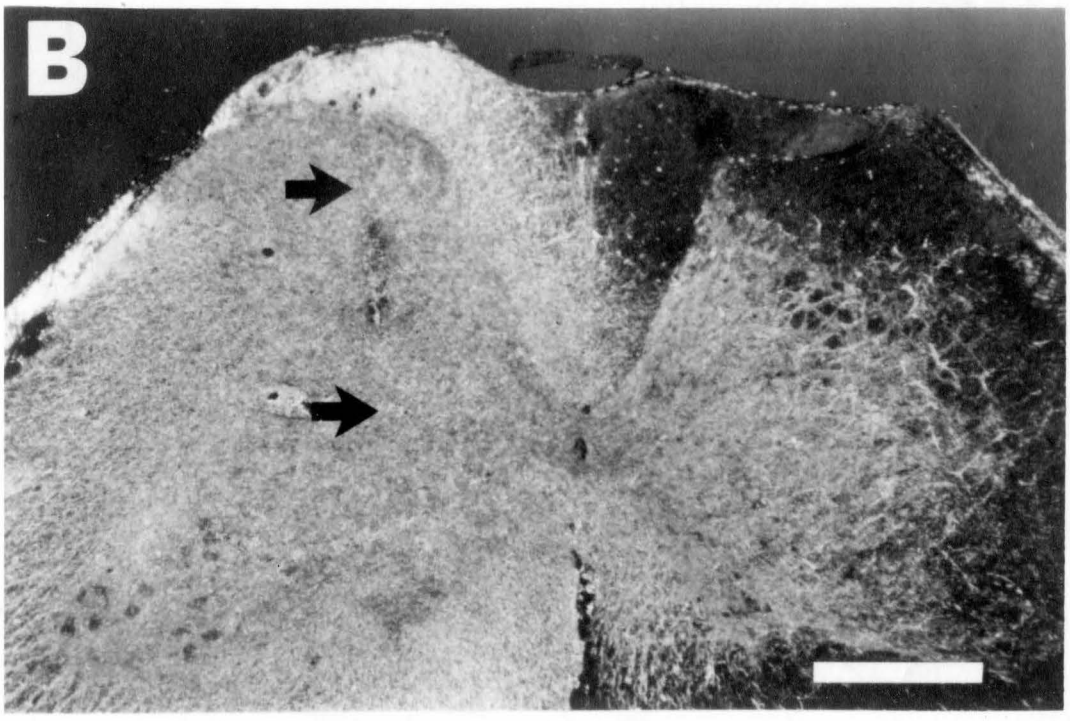

B

Figure 3. Plot of retrogradely labeled cells found in the cortex following a cervical enlargement injection of WGA-HRP. Three patches of cells were found. The most rostral patch surrounds an electrode track where forelimb movements were evoked during ICMS. The large caudal patch also corresponds with an area where forelimb movements could be evoked during ICMS and does not include an area where hindlimb movements were evoked by ICMS. The third patch is located laterally near the rhinal fissure and corresponds with the second somatosensory area SII. The boundaries between AgM, AgL and Gr cortices are indicated with dark lines. Electrode penetrations were made at each letter and movements evoked were as follows; F=forelimb, V=vibrissae, H=hindlimb, N=no response.

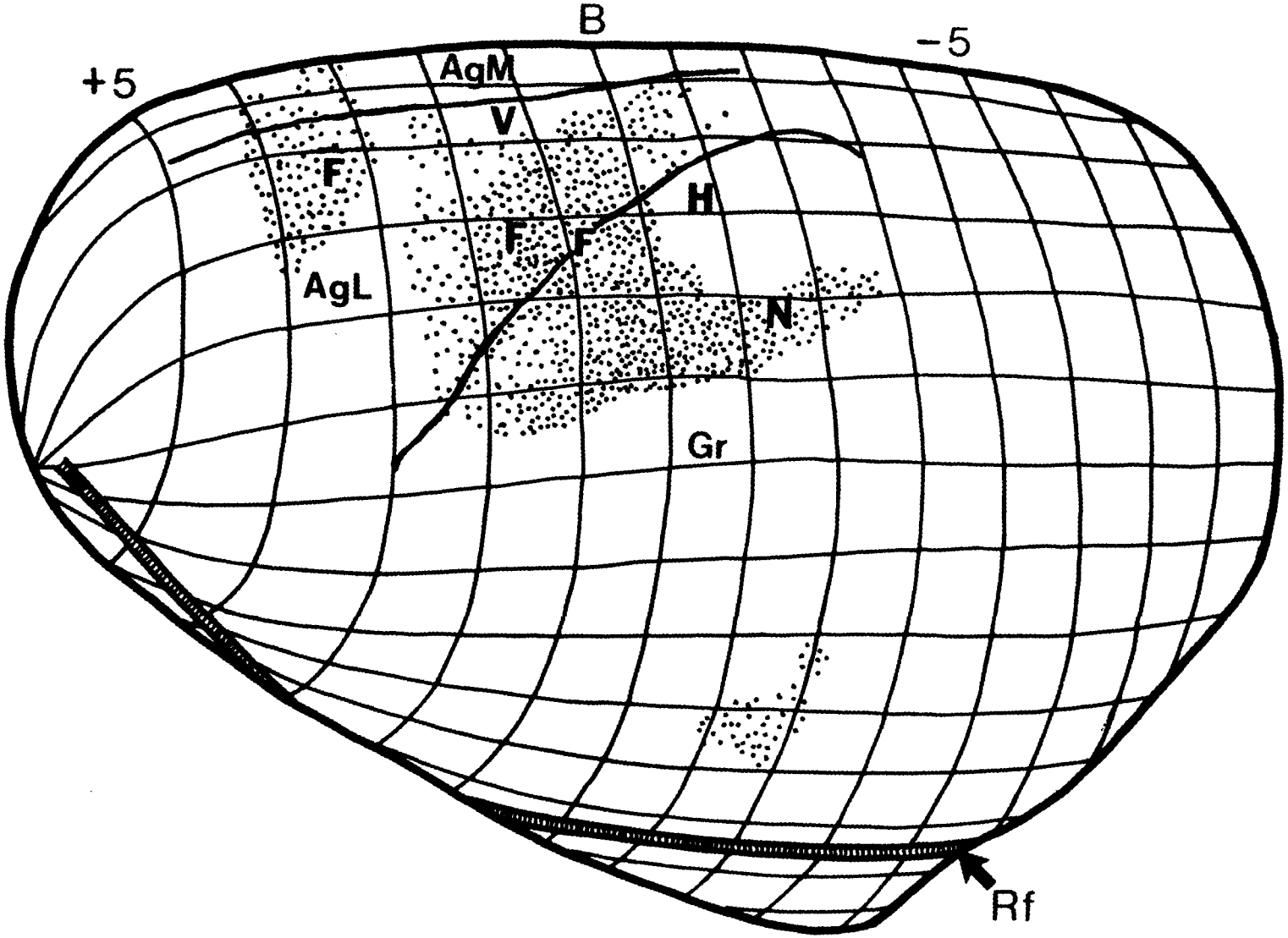

B
+5
-5
AgM
V
F
H
F
F
AgL
N
Gr
Rf

Figure 4A. Line drawing of a horizontal view of the rat brain depicting the level of section of the eight coronal sections seen in Figure 4B. B=Bregma, division markers along midline are in mm.

Figure 4B. Results of ICMS, sensory recording, retrograde cell labeling from cervical enlargement and cortical cytoarchitecture plotted on line drawings of coronal sections. Electrode tracks are indicated by vertical lines through the cortex. The ICMS evoked movement is indicated by the abbreviation at the bottom of the electrode track, and the threshold current in uamps is indicated below in the white matter. T=trunk, Df=digit flexion, V=vibrissae, We=wrist extension, Ef=elbow flexion, Hf=hip flexion, Se=shoulder extension, N=no response. Abbreviations at the top of each electrode track indicate body parts where peripheral stimulation evoked multiunit activity. N=no response, P=paw, D=digits, Fa=forearm, H=hindlimb, Sh=shoulder. Granule cell patches are outlined in layer IV. Retrogradely labeled cells are indicated by dots. The border between AgM and AgL is marked on each section with an arrow on the cortical surface. Asterisk=electrolytic lesion. The scale bar at the lower right equals 2 mm.

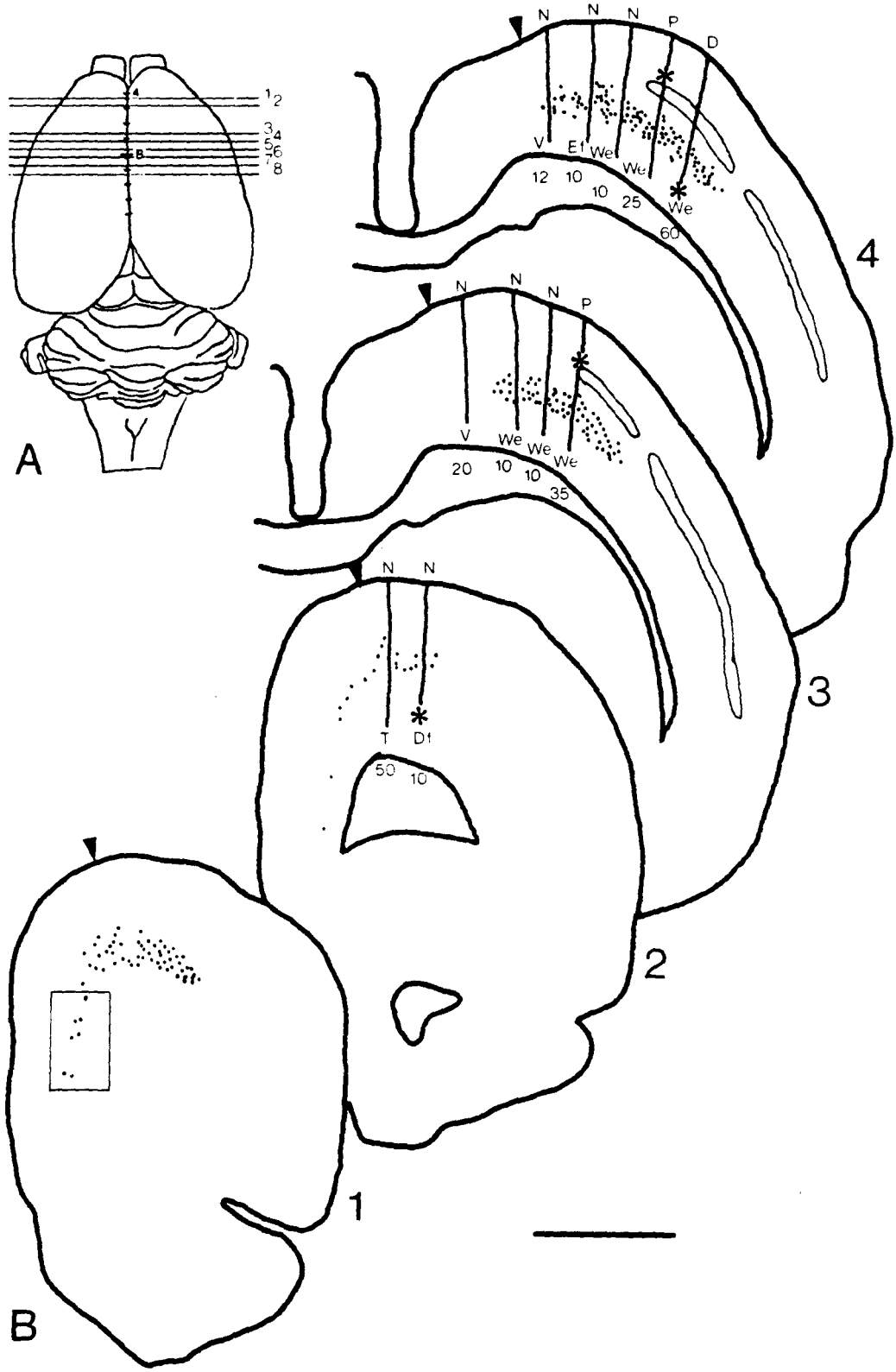

A
B
1
2
3
4
N N N P D
V E1 We We We
12 10 10 25 60
N N N P
V We We We
20 10 10 35
N N
T D1
50 10

Figure 4. continued:

Sections 1+2 show retrograde labeling in the rostral forelimb area, as indicated by the ICMS response in section 2. Note the higher threshold required to elicit a trunk response medial to the digit representation. Note also the retrograde cell labeling extending over the convexity and down the midline. A dark field photomicrograph of the area in the box is shown in figure 7C.

Sections 3-6 demonstrate the large caudal patch of retrogradely labeled cells and correlation of the same with ICMS movements and evoked sensory responses. Note that the thresholds of ICMS movements increase as the electrode is moved from agranular to granular cortex, and that evoked sensory responses can only be elicited from granular cortex. Also note that the labeled cells do not extend into the agranular medial zone.

Sections 7+8 depict the laterally located tail of the forelimb representation, with the hindlimb representation medial to the labeled cells seen in these sections. Note the small patch of retrogradely labeled cells in SII marked by the arrow in section 8.

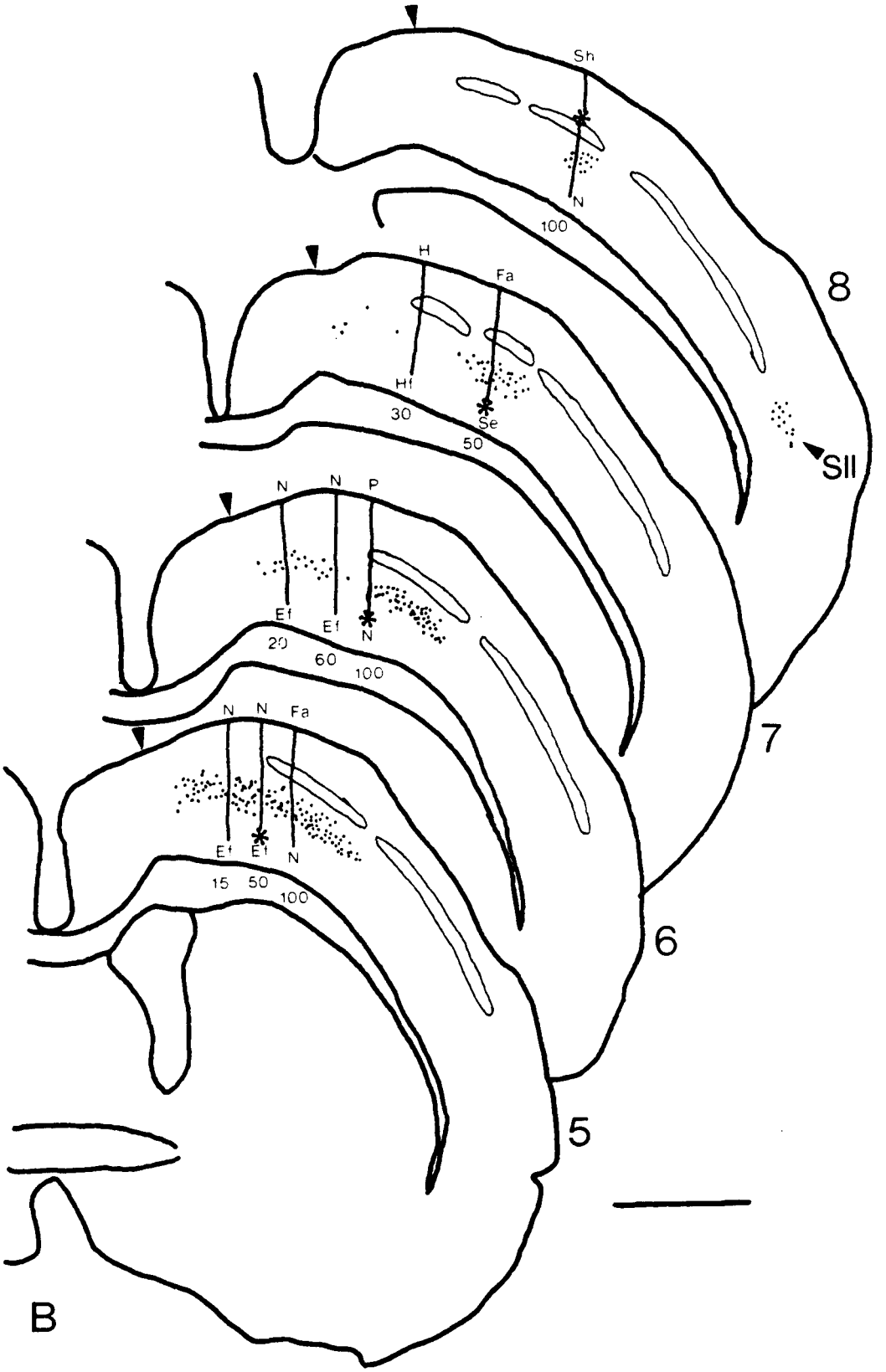

Sh
N
100
H
Fa
Hf
Se
30
50
SII
8
N
N
P
Ef
Ef
N
20
60
100
7
N
N
Fa
Ef
Ef
N
15
50
100
6
5
B

Figure 5. Plot of retrogradely labeled cells found in the cortex following a lumbar enlargement injection of WGA-HRP. Two patches of cells were found. The rostral patch surrounds an electrode track where a hindlimb movement was evoked by ICMS, and is medial to an electrode track where a forelimb movement was evoked by ICMS. The caudal patch surrounds three electrode tracks where ICMS yielded hindlimb movements. Note that the caudal patch crosses the granular-agranular border, but does not extend into the medial agranular zone. Note also that many cells in the caudal patch are found medial to the granular cortex indicating a zone of non-overlapping motor cortex. SII was not labeled from a lumbar injection. The boundaries between AgM, AgL and Gr cortical areas are indicated with dark lines. Electrode penetrations were made at each letter and movements evoked were as follows; F=forelimb, N=neck, H=hindlimb.

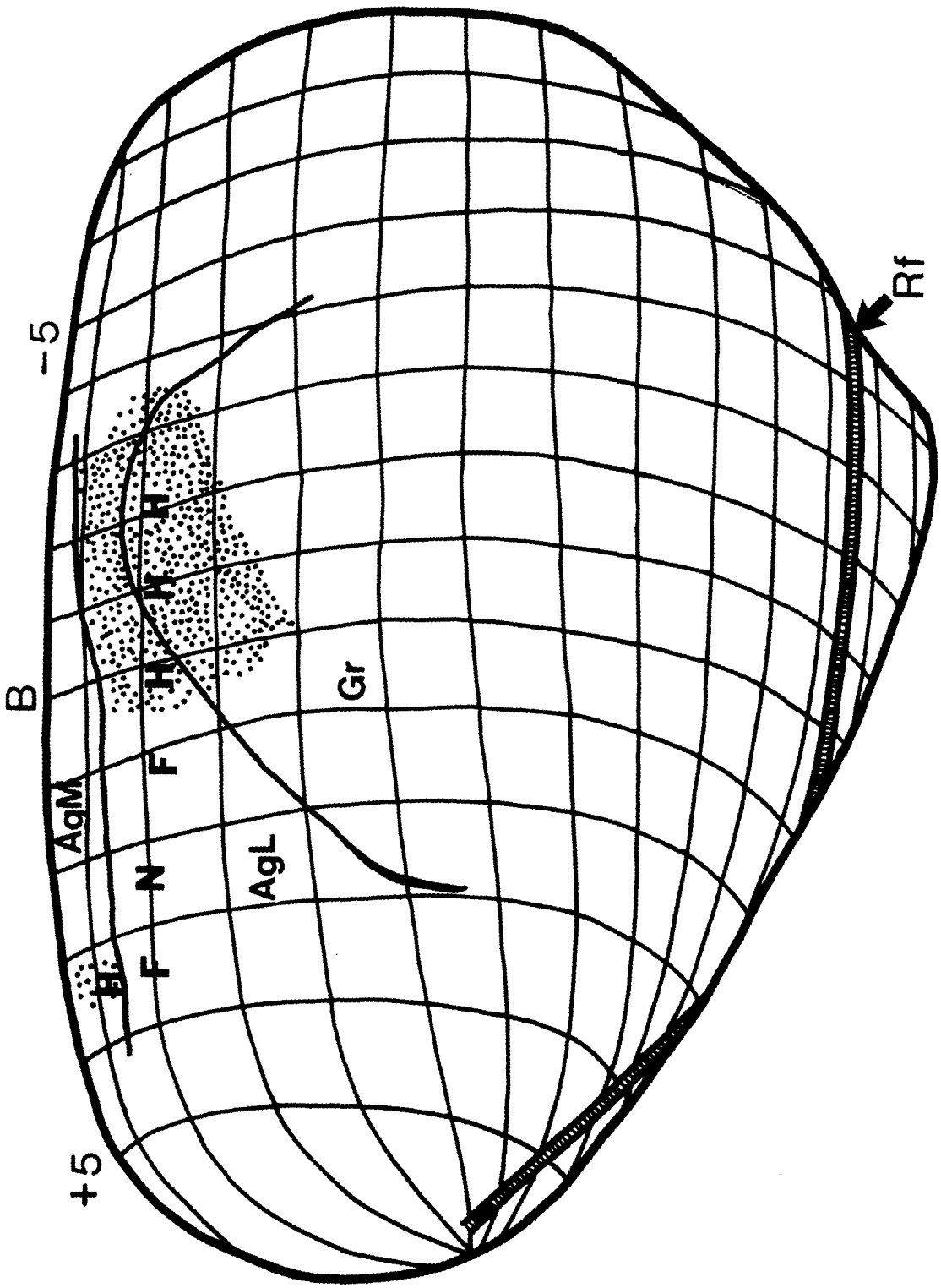

B
-5
+5
AgM
AgL
Gr
Rf

Figure 6A. Line drawing of a horizontal view of the rat brain depicting the level of section of the four coronal sections seen in figure 6B. B=Bregma, division markers along the midline are in mm.

Figure 6B. Results of ICMS, sensory recording, retrograde cell labeling from lumbar enlargement and cortical cytoarchitecture plotted on line drawings of coronal sections. Electrode tracks are indicated by vertical lines through the cortex. The ICMS evoked movement is indicated by the abbreviation at the bottom of the electrode track, and the threshold current in uamps is indicated below the movement abbreviation in the white matter. Hf=hip flexion, Df=digit flexion, Af=ankle flexion, Te=toe extension, Se=shoulder extension, Kf=knee flexion, N=no response. Abbreviations at the top of each electrode indicate body part where peripheral stimulation evoked multiunit activity. N=no response, F=foot, Fa=forearm, S=shoulder, A=ankle. Granule cell patches are outlined in layer IV. Retrogradely labeled cells are indicated by dots. The border between AgM and AgL is marked on each section with an arrow on the cortical surface. The scale bar at the lower right equals 2 mm.

Section 1. Low threshold digit movements were evoked by ICMS laterally, and higher threshold hip movements were evoked medially. The retrogradely labeled cells seen in this section were found in three adjacent sections. A photomicrograph of the boxed area from one of three sections is shown in figure 7B.

Section 2-4. The large caudal patch of labeled cells can be seen in all three sections. Hindlimb stimulation points are always located within the labeled area, and hindlimb sensory responses are only seen in the granular cortex. Note the rise in stimulation threshold as the electrode moves laterally into granular cortex. Also note that there is an area of labeled cells medial to the granular patches where low threshold ICMS movements are evoked, and sensory responses are not found. Labeled cells only extend up to the border between AgM and AgL as is demonstrated in figure 7A.

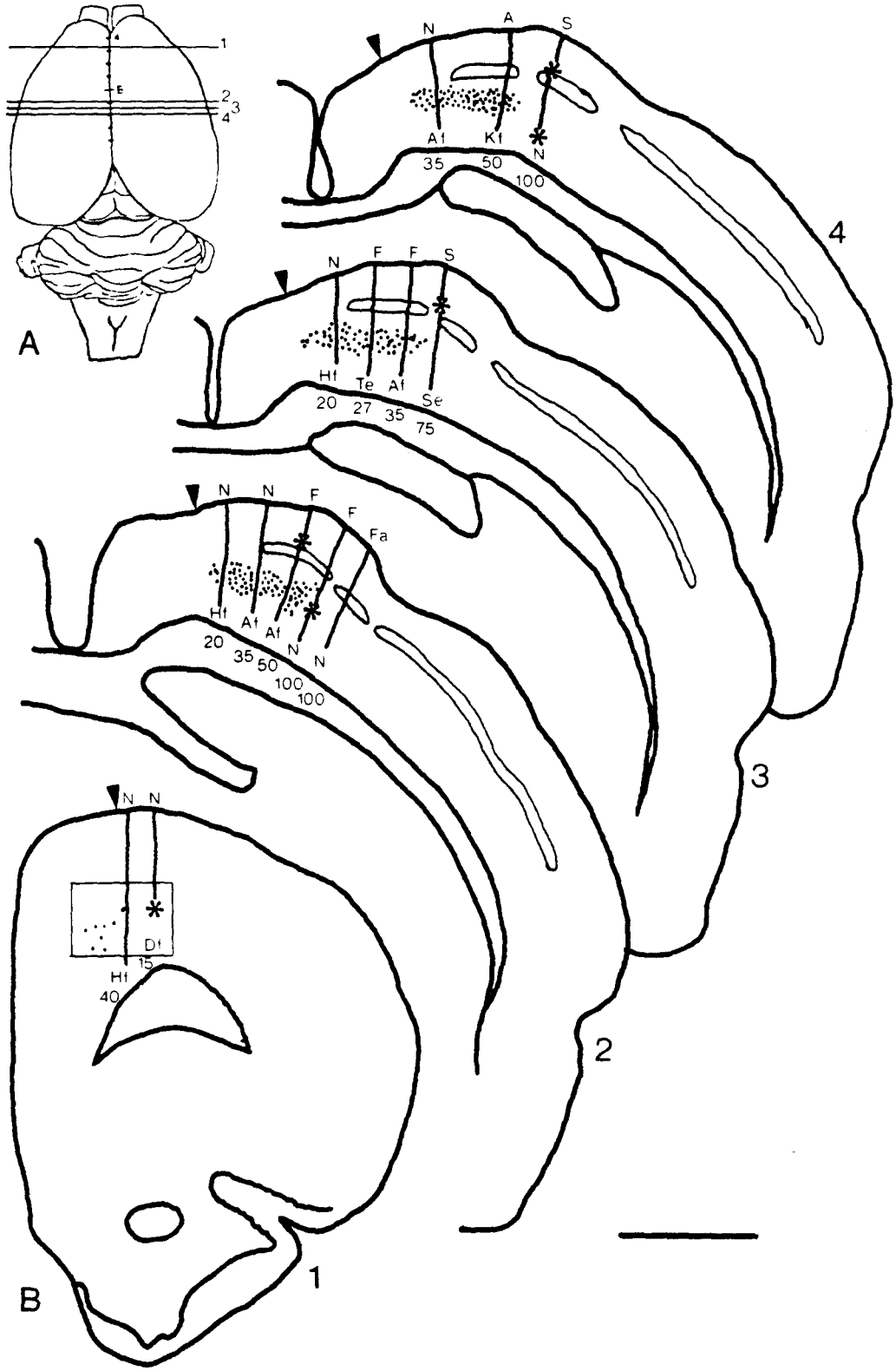

A
1
E
2
3
4
B
N
A
S
Af
Kf
N
35
50
100
4
N
F
F
S
Hf
Te
Af
Se
20
27
35
75
N
N
F
F
Fa
Hf
Af
Af
N
N
20
35
50
100
100
3
N
N
Df
Hf
15
40
2
1

Figure 7. Photomicrographs of retrogradely labeled cells from cervical and lumbar injections of WGA-HRP.

A. Coronal section taken approximately at the level of section 4 in figure 6B to demonstrate the cell labeling medial to the granular cortex. Also apparent in this picture is the absence of labeled cells in the medial agranular zone. AgM=agranular medial, AgL=agranular lateral, Gr=granular. Arrows on cortical surface indicate the cytoarchitectonic borders. Scale bar=2 mm.

B. Dark field photomicrograph of boxed area seen in figure 6B section 1. Asterisk marks the lesion in both sections for orientation. Large arrows mark the electrode tracks, and small arrows point to three labeled cells. Scale bar=500 um.

C. Dark field photomicrograph of the boxed area seen in figure 4B section 1. Arrows point to labeled cells in the prelimbic area. Scale bar=250 um.

AgM
AgL
Gr
A
B
C

Figure 8. Dark field photomicrographs of horizontal sections of the spinal cord at cervical, thoracic and lumbar levels. DY and FB injection sites are shown. In all three pictures, the border between gray matter below and the dorsal funiculus above is indicated by the arrows. The sections are purposely taken at the level of the corticospinal tract to demonstrate that the pipette did not damage the corticospinal fibers. Scale bars for all three are equal to 500 um.

A. DY injection sites in the cervical cord. These injection sites are small at this level, but they are larger as they reach the intermediate gray and ventral horn.

B. FB injection sites in the thoracic cord. Some tissue damage is seen in the lateral white matter, but the CST appears undamaged.

C. DY injection sites in the lumbar enlargement of the spinal cord. The two injection sites are centered in the gray matter of the dorsal horn with no apparent damage to the CST.

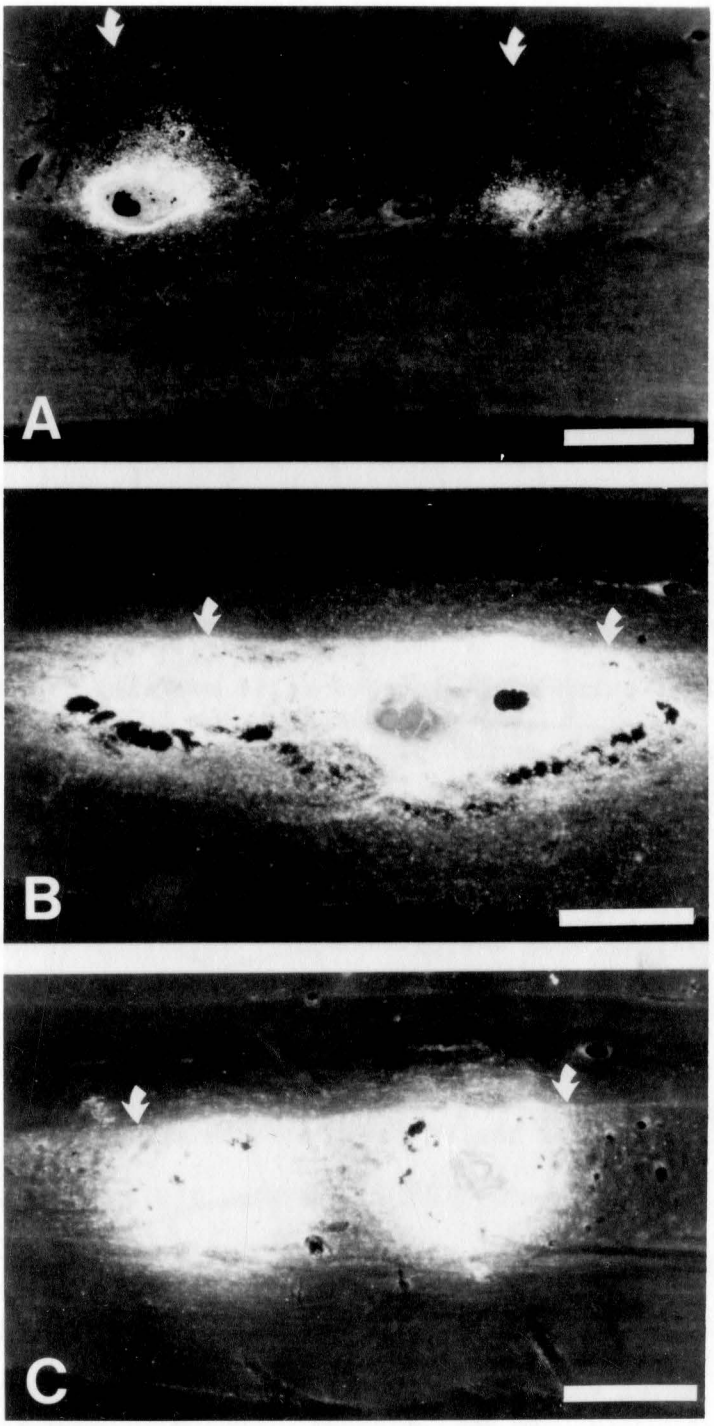

A
B
C

Figure 9. Results of DY (cervical) and FB (lumbar) injections plotted on a dorsolateral perspective view of the rat brain. Small dots represent DY labeled cells, and large dots represent FB labeled cells. Double labeled cells were not seen. Note the small patch of FB cells at the front of the brain located in AgM. These cells were mixed with some DY cells also found rostrally, but the majority of the rostral patch of DY labeled cells was located in AgL. The two types of labeled cells in the rostral pole are shown in a photomicrograph in figure 12a. The caudal patch of DY labeled cells was also located in AgL, as was the large caudal patch of FB labeled cells. Caudally, neither group of labeled cells crossed into AgM, but both the DY and FB patches crossed into the granular zone. Near the border between the two patches (DY) and (FB), there is some mixing of the two cell populations (see Fig. 12b). Laterally, there is a gap where no labeled cells were found between the lateral border of the FB patch and the medial border of the caudal tail of the DY patch (see Fig. 12c). This gap appears to be part of the trunk representation. DY labeled cells were the only kind found in SII. The borders between AgM, AgL and Gr are shown with heavy lines. The lower case letters a,b and c indicate the rostrocaudal level of the three sections seen in figure 12.

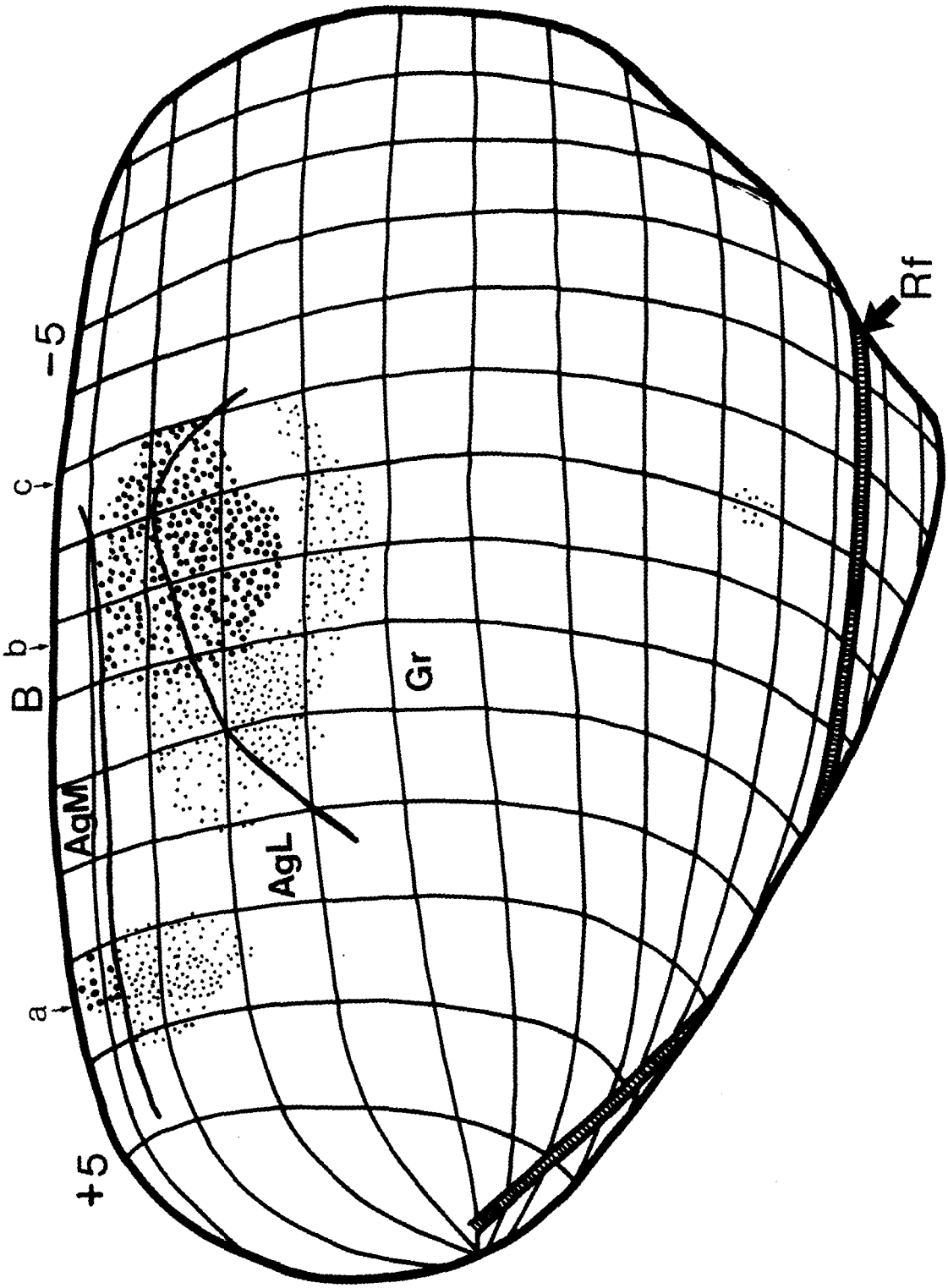

Rf
-5
+5
a
B b
c
AgM
AgL
Gr

Figure 10. Results of DY (cervical) and FB (thoracic) injections plotted on a dorsolateral perspective view of the rat brain. Small dots represent DY labeled cells and large dots represent FB labeled cells. The borders between AgM, AgL and Gr cortex are indicated with heavy lines. Here again, both DY and FB labeled cells were found in the rostral pole. The DY patch was described in the previous figure legend, and is identical in this experiment. The FB patch is more extensive than that seen from a lumbar injection of FB, but still smaller than the DY patch. Some FB cells extend out into AgL, but most are within AgM. The caudal patch of FB labeled cells is divided into two areas, one area of label is medial in AgL, and the other is lateral in Gr. The medial area seems to be almost entirely overlapping with the AgL portion of label from a lumbar injection (see Fig. 13). The lateral area on the other hand, seems to fill in the gap between forelimb labeled cells and hindlimb labeled cells seen in figure 9. The second somatosensory area (SII) contained only DY labeled cells.

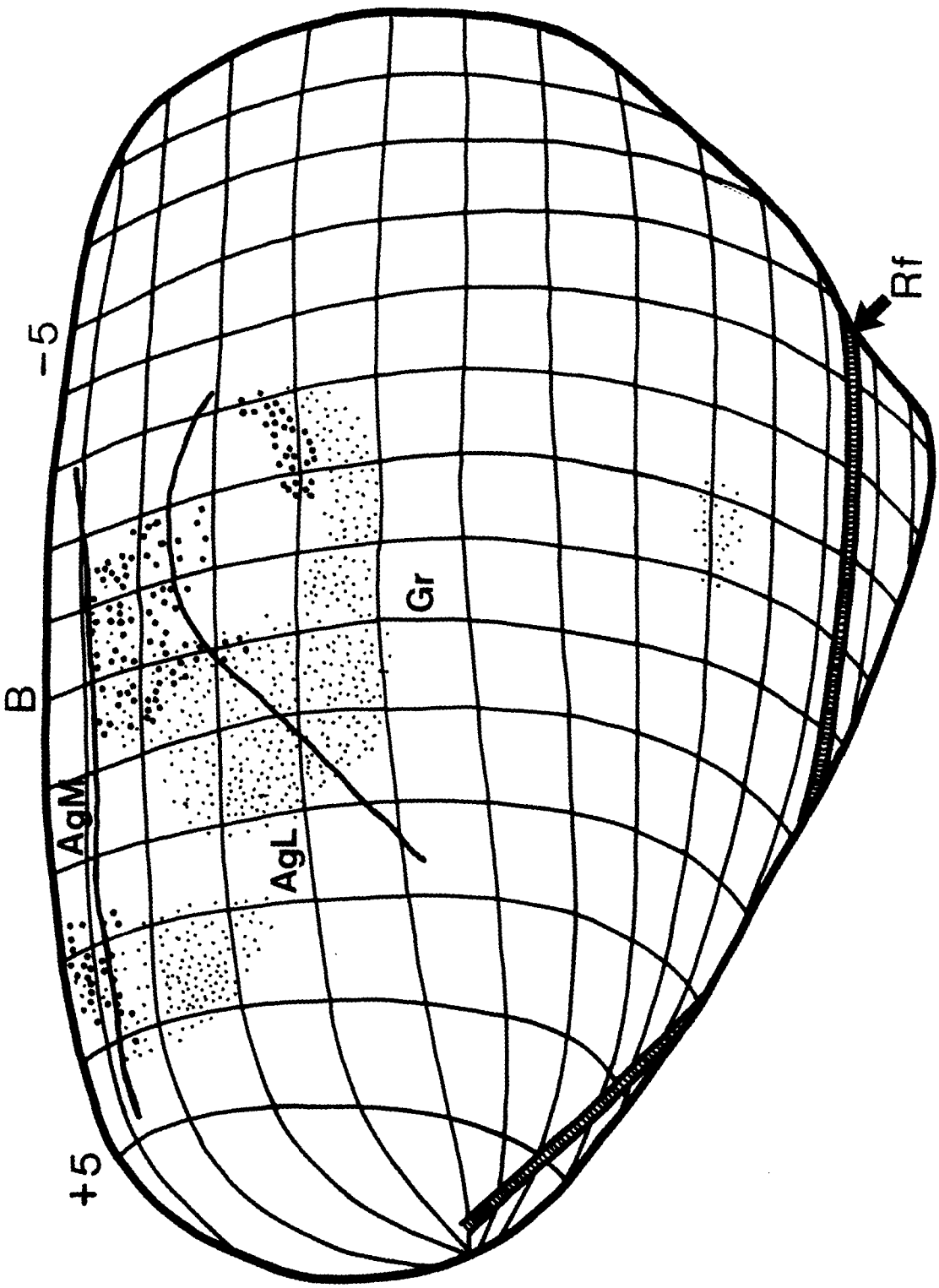

B
-5
+5
AgM
AgL
Gr
Rf

Figure 11. Results of FB (thoracic) and DY (lumbar) injections plotted on a dorsolateral perspective view of the rat brain. The boundaries between AgM, AgL and Gr are marked with heavy lines. Small dots represent FB labeled cells and large dots represent DY labeled cells. The labeling pattern is the same as that seen for a similar type of injection in figures 9 and 10. Note the preponderance of FB labeled cells in the frontal pole as compared to DY cells. Note also the separate patch of FB labeled cells just rostral to bregma (motor trunk representation), and the overlap of FB and DY labeled cells in AgL just caudal to bregma. The lateral patch of FB labeled cells seen in the granular cortex do not overlap with the DY labeled cells, but instead seem to fill the gap which was seen in figure 9. SII did not contain any labeled cells.

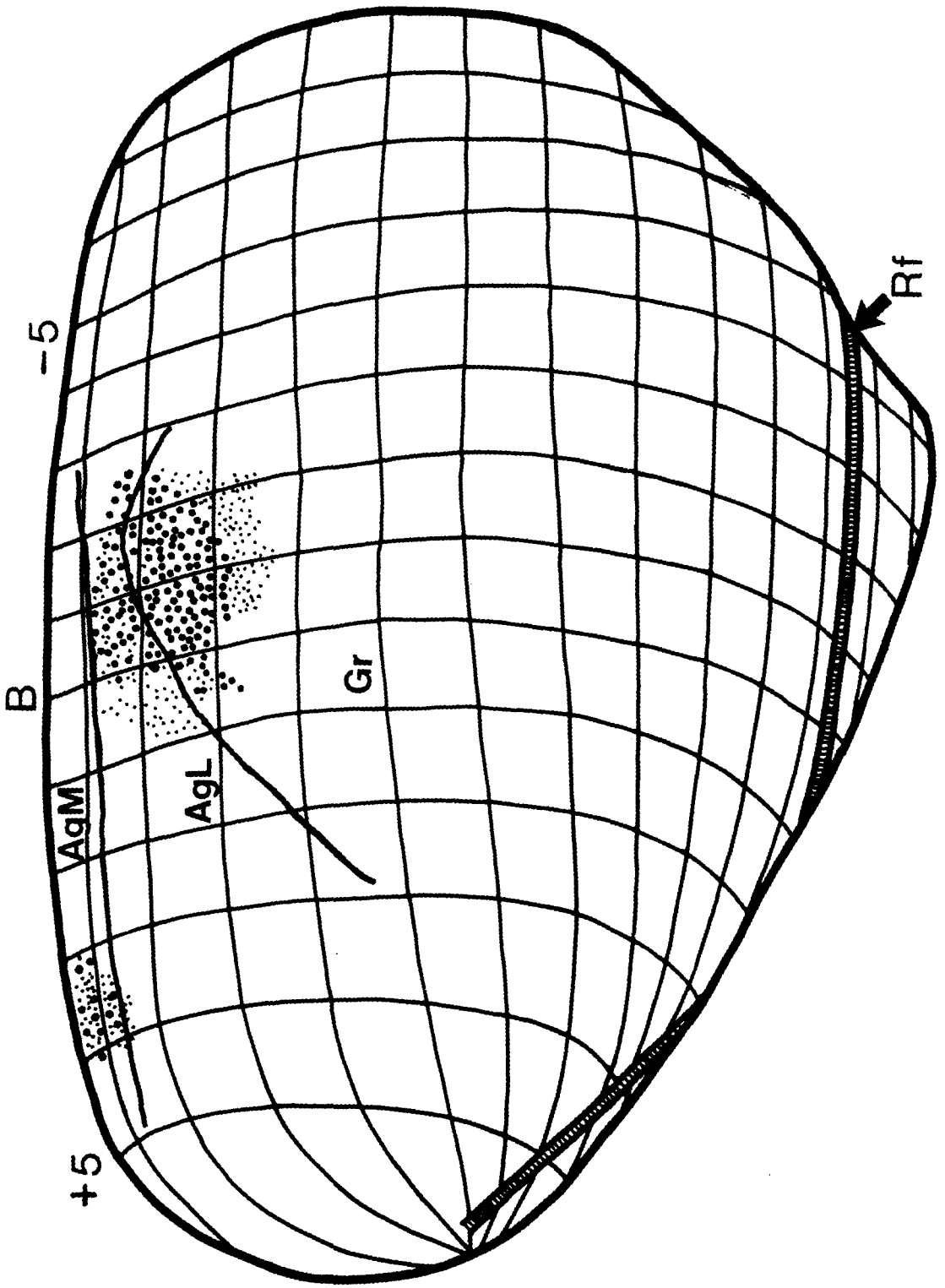

B
-5
+5
AqM
AgL
Gr
Rf

Figure 12. Flourescence photomicrographs of labeled cells from DY (cervical) and FB (lumbar) injections.

A. Coronal section taken at the level (a) indicated in figure 9. The midline is to the left and the scale bar=200um. The small arrows point to three FB labeled cells, and the large arrow points to a patch of DY labeled cells. Other DY labeled cells are visible in the micrograph.

B. Coronal section taken at the level (b) indicated in figure 9. The midline is to the left and the scale bar=200 um. Many FB labeled cells are visible in the photo, but only a few DY labeled cells are seen in this section (arrows).

C. Coronal section taken at the level (c) indicated figure 9. The midline is to the left and the scale bar=400 um. Note the two patches of labeled cells (DY) and (FB) with an unlabeled area separating the patches.

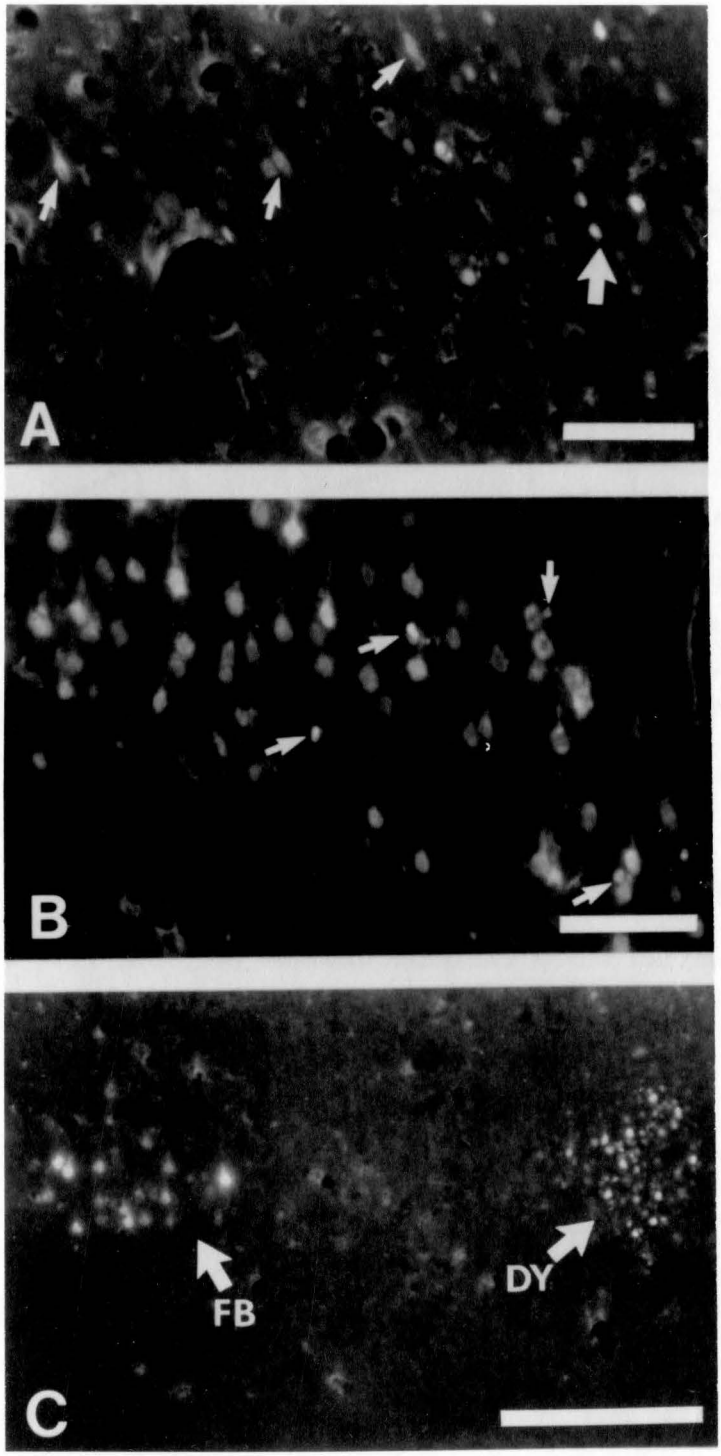
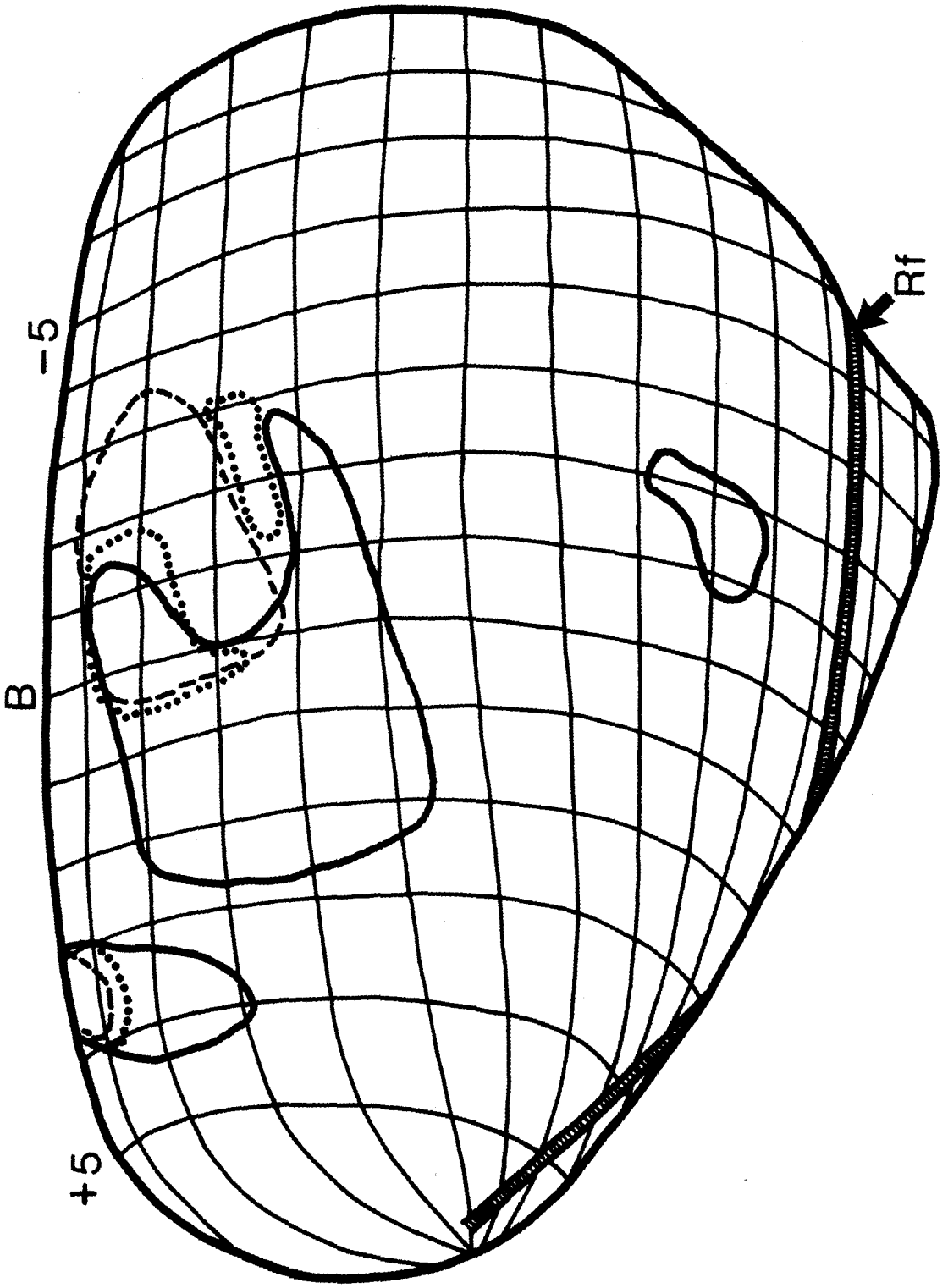

A
B
FB
DY
C

Figure 13. Summary drawing showing extent of retrograde labeling from all three types of injections (cervical, thoracic and lumbar) superimposed on one brain drawing. Solid lines represent area of label following a cervical injection. Dotted lines represent area of label following a thoracic cord injection. Dashed lines represent area of label following a lumbar cord injection. Note the whole body representation (with the exception of head) near the frontal pole. In the primary sensorimotor area (rostral and caudal to bregma), there is an area of overlap which includes neurons projecting to all three levels of the spinal cord. Lateral and caudal to this overlap zone, the lumbar, thoracic and cervical cord projection neurons do not overlap. Neurons in the second somatosensory area do not project below the lower cervical or upper thoracic levels of the spinal cord.


B
-5
+5
Rf

CHAPTER V

THE RAT CORTICOSPINAL TRACTS. COURSE AND TERMINATIONS IN THE SPINAL CORD: AN HRP STUDY

Introduction

There is currently disagreement on the course of the rodent corticospinal tract (CST). Early reports which used the Marchi technique for degenerating fibers demonstrated only one completely crossed corticospinal pathway in the dorsal funiculus (King 1910, Revely 1915). Later, using the Nauta technique for degenerating fibers, five CST pathways were found (Goodman et al, 1966), running in the dorsal funiculus bilaterally, the lateral funiculi bilaterally and the ventral funiculus on the ipsilateral side. Valverde (1966) used the rapid Golgi technique and described the rat CST as the most versatile of any descending pathway, capable of traveling in any funiculus; but he did not actually describe the location and size of each respective path. A subsequent degeneration study reported that the rat CST is only found in the dorsal funiculus and is completely crossed (Brown, 1971), while another study utilizing autoradiography demonstrated one major tract in the dorsal funiculus and one minor tract in the ipsilateral ventral funiculus (Vahlsing and Feringa, 1980).

In addition to the controversy over the course of the rat CST, there is also disagreement concerning its area of terminations in the spinal cord. An early degeneration study described terminations exclusively to the contralateral dorsal horn and intermediate gray (Torvik, 1956), and Valverde's (1966) Golgi study confirmed these obsevations. In contrast, Goodman et al (1966) described bilateral

terminations in the dorsal horn, intermediate gray and ventral horn. Most recently, Brown (1971) was only able to demonstrate terminations in the dorsal horn. Finally, a recent physiological study in the rat by Elger et al (1977) has demonstrated monosynaptic CST connections to both contralateral and ipsilateral cervical enlargement alpha motor neurons, implying ventral horn terminations. The development of a more sensitive anatomical tracing method utilizing anterograde transport of Wheat germ agglutinin conjugated with HRP (WGA-HRP) (Mesulam and Mufson, 1980) suggested that a conclusive determination of the normal course and terminations of the rat CST might now be made. The results of such a study would be important not only in themselves but also for their significance to studies on the plasticity of the corticospinal tract (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975, 1978; Kartje-Tillotson et al, in press).

Further motivation for undertaking such a study comes from the more detailed maps of the rat sensorimotor cortex provided by recent studies using intracortical microstimulation (ICMS) (Hall and Lindholm, 1974; Neafsey and Sievert, 1982; Donoghue and Wise, 1982; Sanderson et al, 1984). One of these studies demonstrated a second forelimb area rostral to the first (Neafsey and Sievert, 1982) which may be a part of the rat's supplementary motor area. In order to further define the course and terminations of the CST from these different cortical areas, the present study utilized the techniques of ICMS and multiunit recording to identify various areas of sensory and motor cortex for subsequent injection WGA-HRP.

The results of this study confirm those found by Goodman et al (1966) for the location of the CST, finding five separate CSTs that run in both dorsal funiculi, both lateral funiculi, and the ipsilateral ventral funiculus. Furthermore, this study has shown that there are terminations from the CST into the dorsal horn, intermediate gray and ventral horn on both sides of the cord, and that the predominant area of terminations depends on the site of injection of tracer.

Materials and Methods

Summary of Experiments

Twenty-eight male Long-Evans hooded rats 300-500 grams were used for this study. The animals were divided into six groups which received injections of WGA-HRP into physiologically identified areas of cortex. Seven animals received injections of WGA-HRP in the rostral forelimb area, 8 animals received injections in the caudal forelimb motor area, 6 animals received injections in the hindlimb motor-sensory area, 4 animals received injections in the sensory forelimb area and 2 animals received multiple injections in a strip of cortex which covered the whole forelimb and hindlimb motor area. In addition to these groups, the spinal cord from an animal which received an injection of HRP in the second somatosensory area was available for this study.

Physiological Mapping and Cortical Injections

Animals were anesthetized with ketamine HCL (100 mg/kg IM) and placed in a stereotaxic frame. A craniotomy was made over the left cortex to expose either the forelimb sensory-motor or the hindlimb sensory-motor area. The cisterna magna was opened to prevent cortical swelling. Motor injections were made on the basis of ICMS maps generated in the following manner. An iron coated tungsten electrode, tip exposed 100 um (Neafsey, 1980), was driven 1.7 mm into the cortex. The electrode was connected to a stimulus isolation unit which relayed

the output of a Grass stimulator. Currents were monitored by noting the voltage drop across a 10 kOhm resistor inserted in the return path. Stimulation parameters were 0.25 msec pulses, 350 hz, and 300 msec trains. Stimulation currents were started at 50 uamps and lowered to threshold, defined as the lowest current which could reliably evoke visible movements. After the appropriate area had been mapped, a single injection (0.02-0.04 ul) of 1% WGA-HRP was made (1.2 mm deep) in the rostral forelimb, caudal forelimb or hindlimb motor area. The rostral forelimb and hindlimb motor area injections were made in the middle of the rostral-caudal extent of each area to avoid spread to adjacent areas. Forelimb motor injections were made as medial as possible (usually 2.0 mm lateral to the midline) to avoid spread of WGA-HRP to the laterally situated sensory cortex. Two animals received multiple injections along the entire extent of the limb motor areas.

Limb sensory cortex injections were only be made in the forelimb sensory area because of the large amount of overlap between sensory and motor in the hindlimb area (Hall and Lindholm, 1974; Donoghue et al., 1979). The forelimb sensory area and the second somatosensory area were delineated using the sensory mapping technique described by Welker (1971). Briefly, the same electrode that was used for ICMS was inserted to a depth of 0.5 mm and the extracellular multi-unit recording signal was amplified and monitored on a loudspeaker during brushing, bending or tapping of the forelimb. The cortex was mapped in a grid pattern, with points 0.5 mm apart. Once the boundaries of

the forelimb sensory cortex had been established, a single injection 0.02 ul of WGA-HRP was made (1.2 mm deep) in the middle of the rostral to caudal extent and as far lateral (usually 4.0 mm lateral to the midline) as possible. Two animals received two injections (0.02 ul each) within forelimb sensory cortex, with the second injection 1.5 mm rostral to the first. The second somatosensory area (SII) injection of WGA-HRP was also made at a depth of 1.2 mm.

After injection, the incisions were closed and the animals allowed to survive for 2-3 days, at which time they were reanesthetized and perfused through the heart according to the technique of Rosene and Mesulam (1978). The brains and spinal cords were removed and cut on a freezing stage microtome at 50 um thick. The cortex was cut either horizontally to note the amount of spread of HRP, or coronally to demonstrate the cytoarchitecture at the injection site. Spinal cords were cut in horizontal and coronal planes in the following manner; C6 coronal, C7-T1 horizontal, T6 coronal, T7-T9 horizontal, L1 coronal, L2-S1 horizontal. Most of the tissue was processed for TMB histochemistry according to the technique of Mesulam (1978), but at least one experiment in each group was processed according to the modified Mesulam technique described by Gibson et al. (1984). Processed tissue was examined on an Olympus microscope under bright field and polarized light for the extent of the injection site, the locations of the corticospinal fibers, and the terminations in the spinal cord. Drawings were made using a camera lucida attachment.

Results

Cortical Injection Sites

All injection sites had a visible spread of not more than 1 mm in any direction from the center. It is important to note that our injection sites were reacted in TMB which results in a larger appearing injection site than those reacted in DAB (Mesulam, 1982). It is still unclear whether the halo area seen around the injection site is an area of effective uptake, but one study by Horton et al. (1979) suggests that it is not. Nonetheless, when analyzing an injection site it is important to show the largest visible area of uptake. With the exception of two sensory injections which had some spread into the forelimb motor area, and one rostral forelimb injection which spread into caudal forelimb, all other injections did not extend into adjacent physiologically identified areas. A drawing of the locations of each type of injection can be seen in figure 1A, and a line drawing from the center of each type of injection site is shown in figures 1B-E. Note that on the dorsal view of the brain (Fig. 1A), the injection sites do not overlap with each other. The total extent of a caudal forelimb injection, including electrode tracks from stimulation in adjacent areas, is depicted in a line drawing in figure 2. A corresponding Nissl stained section is shown in figure 3.

Location of Corticospinal Tracts

The rat sensorimotor cortex projects to the spinal cord via five corticospinal tracts. The two animals which received multiple injections were used as standards for categorizing the numbers of fibers found in each location. It is important to note that some of these pathways are very small and can only be seen in horizontal sections of the spinal cord. The locations of the CSTs are shown in a cut away diagram of the spinal cord (Fig. 4). The largest projection to the spinal cord is found in the contralateral dorsal funiculus below the dorsal columns (DCSTc) (Figs. 4 and 5B). On the ipsilateral side, also below the dorsal columns, is a smaller tract (DCSTi), where a maximum of 30 fibers have been visualized in any one animal (Figs. 4 and 5B). The second largest tract consisted of as many as 40 fibers and was found in the contralateral lateral funiculus next to Rexed lamina II-V in the dorsal horn (LCSTc) (Figs. 4 and 5A). There were also a few fibers in the ipsilateral lateral funiculus (LCSTi) (Figs. 4 and 5C). The LCSTi was the smallest of all the pathways (5 fibers found) and was only seen in animals which received forelimb sensory or hindlimb injections. The last pathway was found along the medial border of the ventral medial fissure on the ipsilateral side (VCSTi), never contained more than 15 labeled fibers, and was not found in all animals (seen in 20 of 27) (Figs. 4 and 5D). All five paths extended as low as the lumbar enlargement in animals which received hindlimb area injections.

Identification of Terminations

In this report, anterograde label seen in the gray matter in the form of dots or strings of dots was considered to be indicative of terminal or preterminal endings (Mesulam, 1982). The location of terminations in this study is described according to the spinal cord lamination pattern seen in the cat by Rexed (1954). These lamina have been identified in the rat spinal cord (McClung and Castro, 1978), and are depicted in Figure 4.

Rostral Forelimb Injection

Seven animals received injections in the rostral forelimb area (Figs. 1A and B), one of which had some spread of the injection site into the caudal forelimb area. All the CST pathways were present in every animal except for the LCSTi which was not seen in these animals. Generally, no fibers or terminations were seen below T9, except in animal RF106 which received two injections of HRP, one in the RF as described and the other 0.5 mm medial to the first in an area where ICMS evoked hindlimb movements. This animal had fibers and terminations in the lumbar enlargement. All the RF injected animals had a similar pattern of terminations (Figs. 6A+B and 8B) with the heaviest terminations in contralateral lamina VIII, X, and medial lamina VI and VII. There were some terminations found in lamina V, the lateral portion of lamina IV and the medial motor neuron group of lamina IX. Ipsilateral terminations were seen in the same areas but were much lighter. The pattern of terminal labeling was similar but

diminished in the thoracic cord and was absent in the lumbar cord except in RF106.

Caudal Forelimb Injection

The eight animals which received caudal forelimb motor injections (Figs. 1A,C,2 and 3) contained the same four pathways as the RF injection animals (DCSTc, DCSTi, LCSTc and VCSTi). The LCSTi was not seen in these animals. Generally, the CST paths ended in low thoracic levels, but three of the animals had some continuation of the DCSTc into the lumbar enlargement with labeling present in the gray matter.

Terminations of the CSTs were present contralaterally in lamina V-VII. The ventro-medial portion of lamina IV contained light labeling and the midline lamina X and the ventral lamina VIII contained only sparse terminations (Figs. 6C,D and 8A). Some fibers were viewed extending into the motor nuclei, lamina IX of the ventral horn (Fig. 6C,D and 7A). On the ipsilateral side, terminations were seen in the same areas but were much less dense (Figs. 7B and 8B).

Forelimb Sensory Injections

Injections of HRP into forelimb sensory cortex (Fig. 1A+D) resulted in all 5 CSTs carrying fibers to the spinal cord, including the LCSTi. None of the paths were seen below the mid thoracic (T6) level of the spinal cord.

The terminations were very heavy contralaterally in the dorsal horn, especially to the medial portion of lamina III-VI (Figs. 6E and 8C). Lamina VII, VIII and IX received sparse terminations. The two animals which had injection sites that spread into the forelimb motor area had more terminations in lamina VII and IX than the two animals that had well isolated sensory forelimb injections. Ipsilateral terminations appeared in similar areas to those seen on the contralateral side and seemed slightly more dense than those found after forelimb motor injections (Figs. 6F and 8C).

The one animal which received a single injection of WGA-HRP in the second somatosensory area (Fig. 1A+E) had labeled corticospinal fibers in the DCSTc. Terminal labeling was heaviest medially in the dorsal horn of the cervical enlargement on the contralateral side in lamina III-VI and sparse terminations were seen in the lateral part of lamina V, VI and VII (Figs. 6G and 8D). No label was seen on the ipsilateral side or below the cervical enlargement on either side.

Hindlimb Injection

Injections of HRP into the hindlimb sensorimotor cortex (Fig. 1A+E) labeled the same five pathways as were seen for the forelimb sensory cortex, including the LCSTi which was present in 3 of 6 animals. All five paths extended through the lumbar enlargement in two animals, but the smaller tracts (DCSTi, LCSTi and VCSTi) ended at low thoracic (T9) levels in the other four animals.

Terminations in the spinal cord above mid-thoracic levels were sparse in some animals and absent in others (3 of 6). In the lumbar enlargement, terminations were heaviest on the contralateral side in lamina II-VI, but some labeling was present in lamina VII (Figs. 6H and 9). The overall picture looked much like that seen in the cervical cord after a sensory forelimb injection except the label did not extend ventrally beyond lamina VII. Ipsilateral terminations were present in similar areas to those seen contralaterally but were much lighter (Fig. 9).

Discussion

The present study describes five separate corticospinal tracts in the rat, located bilaterally in the dorsal and lateral funiculi, and ipsilaterally in the ventral funiculus. These results are in agreement with Goodman's abstract (1966), except that the ipsilateral lateral tract (LCSTi) he described was not always found. Other studies (Dunkerly and Duncan, 1962; Brown, 1971; Vahlsing and Feringa, 1981; and Schreyer and Jones, 1982) were unable to identify all the pathways we have seen, a difference which can probably be accounted for on the basis of the greater sensitivity of the WGA-HRP technique and the type of sectioning. In this study and the previous study by Goodman et al (1966), horizontal sections were cut for viewing the CST. We found that the smaller pathways could only reliably be seen in these horizontal sections. Additionally, we used the TMB reaction for WGA-HRP, a technique which is probably the most sensitive available for demonstrating efferent fibers (Mesulam 1982). Our results supply conclusive evidence that the rat pyramidal tract is not a completely crossed fiber pathway, but rather is a predominately crossed pathway with a variety of routes corticospinal fibers may take to the cord. This pattern exists in a number of other species (Glees, 1961; Armand and Kuypers, 1977) including man (Verhaart, 1952; Nathan and Smith, 1955; Nyberg-Hansen and Rinvik, 1963).

The present study demonstrated bilateral terminations to the spinal cord, with sensory areas projecting heavily to the dorsal horn

and motor areas projecting to the intermediate gray and ventral horn in agreement with Goodman et al., (1966). Fibers terminating in the intermediate gray and ventral horn can make monosynaptic contacts with alpha motor neurons via dendrites (Cajal, 1909; Scheibel and Scheibel, 1969), thus supporting the physiological findings in the rat of bilateral monosynaptic connections with cervical alpha motoneurons (Elger et al., 1977). The hindlimb area of motor cortex does not appear to have terminations to the ventral horn, a finding that concurs with a physiological study demonstrating polysynaptic corticospinal connections to alpha motor neurons in the rat lumbar enlargement (Janzen et al., 1977). From these results it seems that the rat CST, at least in the cervical enlargement, has dual functions of regulating sensory transmission (Fetz, 1968) and of controlling motor neurons more or less directly (Elger et al, 1977). This type of differential projection from sensory and motor cortical areas has been reported in other animals (see Kuypers for an extensive review 1981) including primates (Liu and Chambers, 1964; Coulter and Jones, 1977).

Injections of WGA-HRP tracer into the forelimb motor cortex resulted in terminations as far caudal as the lumbar cord in three animals, and injections of WGA-HRP into the hindlimb area of motor cortex resulted in terminations as far rostral as the cervical enlargement. There are three possible explanations for this finding: First, there could have been leakage of HRP into the adjacent hindlimb area. This is unlikely because the injection sites did not spread into areas where ICMS had evoked hindlimb movements. Second, there

could be collaterals to the lumbar enlargement from cervical enlargement projecting cells. This has been shown to occur in cats and monkeys (Shinoda et al, 1976, 1979), but we failed to find double labeled neurons in the rat motor cortex following injections of different dyes into the cervical and lumbar enlargements (Sievert cf chapter 4). Finally, there could be a mixing of CST neurons projecting to the cervical and lumbar enlargements. This seems the most likely explanation since we have seen such overlap of CST neurons in the border zones of hindlimb and forelimb motor areas, following injection of different dyes into the cervical and lumbar enlargement (Sievert, cf chapter 4).

The rostral forelimb area was the only region that had extensive projections to lamina 8 of the spinal cord. Lamina 8 is the origin of the long descending propriospinal tract and is contacted mainly by vestibulospinal and some tectospinal fibers (see Kuypers 1981 for review). In some primates, however, the corticospinal fibers also reach lamina 8 (Kuypers, 1960; Kuypers and Brinkman, 1970; Liu and Chambers, 1964; Petras, 1969). The finding of terminations in lamina 8 only from the rostral forelimb area suggests that this area might have a different role in movement than the caudal forelimb, but at present it is not clear what this might be. It has been suggested that the rostral forelimb area may be a part of the supplementary motor area of the rat (Donoghue and Wise, 1982), and recent findings by our lab give support to this hypothesis (Sievert cf chapters 3,4 and 7). Very little is known about the corticospinal terminations

from the SMA in other animals. In the cat terminations from an area thought to be the SMA approximate those of M1 and do not enter lamina 8 (Nyberg-Hansen, 1969). However, one of the lesions from a study by Kuypers and Brinkman (1970) on the Rhesus monkey appeared to include the SMA and had terminations to Lamina 8.

In this study, ipsilateral pathways and terminations were seen throughout the rostro-caudal extent of the spinal cord. Although the ipsilateral terminations were present in greatly diminished numbers compared to the contralateral side, their significance is highlighted by the results of several recent studies including the demonstration of bilateral monosynaptic connections with cervical alpha motoneurons (Elger et al., 1977) and the finding of ipsilateral deficits of forelimb motor control following unilateral cortical lesions in the rat (Price and Fowler, 1981). Furthermore, the demonstration of these ipsilateral pathways is also important for the interpretation of the many studies which have demonstrated the formation of an aberrant ipsilateral CST after neonatal pyramidotomy (Castro 1978), or neonatal cortical lesions (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975; Kartje-Tillotson et al., in press). Our results indicate that the abnormal tracts described in these studies are not newly formed pathways, but instead they are expansions of normally occurring small pathways.

In summary, the rat CST reaches the spinal cord via five pathways which are located on both sides of spinal cord (DCSTc, DCSTi, LCSTc, LCSTi, VCSTi). Furthermore, terminations to both sides of the

cord have been demonstrated, with the majority on the contralateral side. The results of this study have also shown that there is a differential projection to the spinal cord from sensory, motor and the rostral forelimb areas of cortex. The major differences are that sensory cortex projects most heavily to the dorsal horn, whereas, motor cortex projects to the intermediate gray and the rostral forelimb area projects to the intermediate gray and lamina 8. The ventral horn in the cervical cord is contacted by a few fibers from all three areas, whereas the ventral horn in the lumbar cord does not receive any fibers from any cortical area studied.

Figure 1. Location of injection sites.

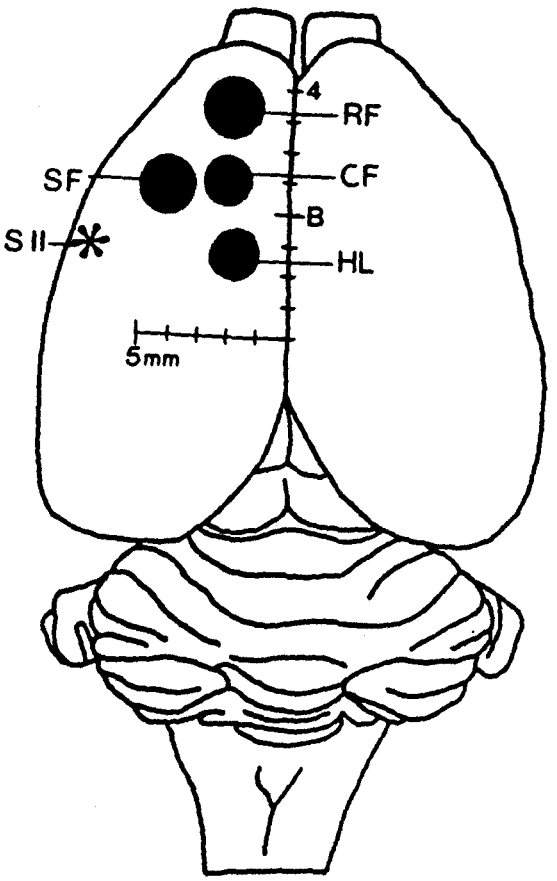
A. Injection sites from a rostral forelimb (RF), caudal forelimb (CF), sensory forelimb (SF), hindlimb (HL) and second somatosensory area (*). All injection sites were reconstructed from coronal sections and plotted on a dorsal view of the rat brain. B=Bregma, Divisions are in mm.

B. Drawing of a coronal section from the center of a rostral forelimb area injection site depicting the widest area of visible HRP. The dots surrounding the dark area indicate the "halo" area of the injection site. The millimeter bar seen in section E applies to all coronal sections shown in this figure.

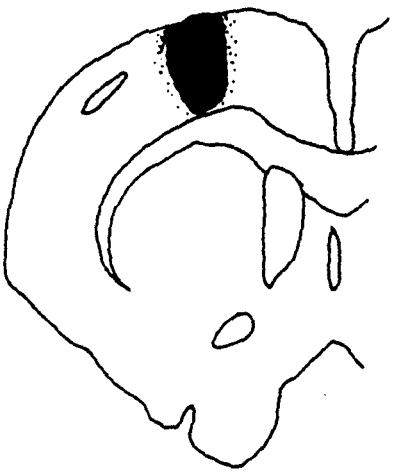
C. Drawing of a coronal section from the center of a caudal forelimb area injection. The granule cell layer of sensory cortex is also outlined to the left of the injection.

D. Drawing of a coronal section from the center of a sensory forelimb cortex injection.

E. Drawing of a coronal section from the center of a hindlimb motor area injection site. The size and location of the second somatosensory area (SII) injection site is also shown on this section.



A



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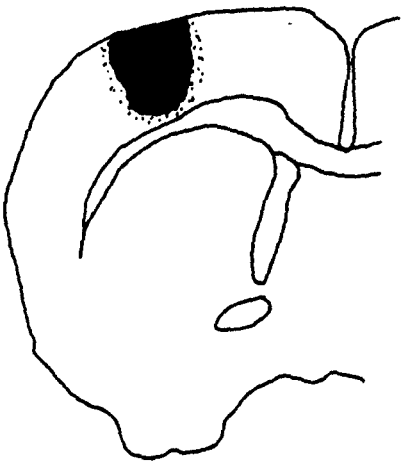
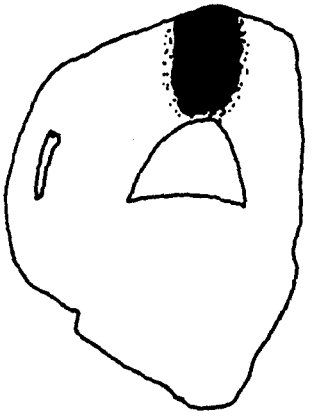
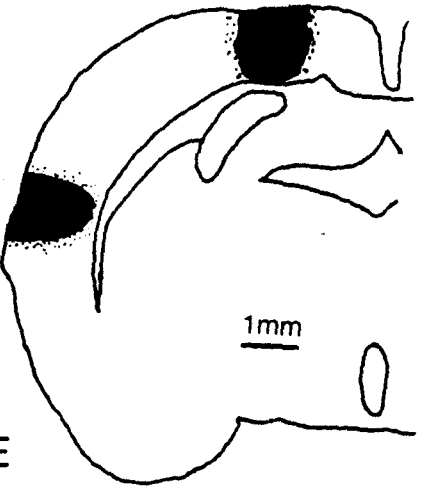


D



B



E

Figure 2. Drawing of the extent of a caudal forelimb motor area injection site.

A. Map of responsive points from ICMS performed prior to the injection. B=bregma, divisions are in mm, 1=wrist extension (threshold=12 uamps), 2=neck (25 uamps), 3=neck (25 uamps), 4=elbow flexion (10 uamps), 5=elbow flexion (20 uamps), 6=wrist extension (20 uamps).

B. Drawings of coronal sections taken at each of the six electrode tracks shown in Figure 1A. Dark stipled area indicates the area of visible injection site. The sensory cortex granule cell patches are outlined just beneath the cortical surface. The stipled area seen in sections 1,2 and 6 is from anterograde label (dots), and retrogradely labeled cells (short lines). Note that the entire extent of the injection site is not in the granule cell patches.

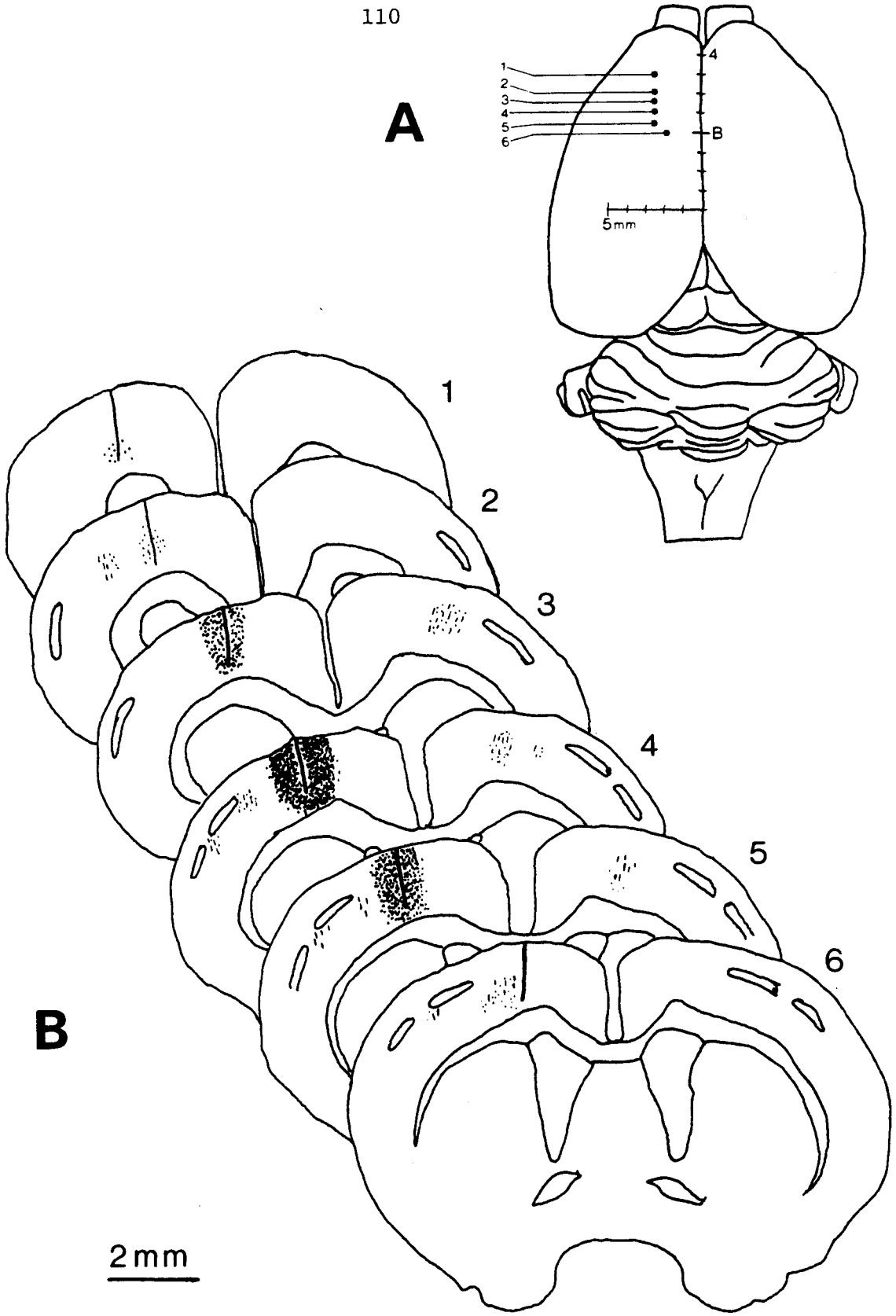

A
1
2
3
4
5
6
4
B
5mm
B
1
2
3
4
5
6
2mm

Figure 3. Nissl stained coronal section taken from the center of the caudal forelimb injection site shown in figure 2 (section 4). The medial border of the forelimb sensory cortex granule cell patch is indicated on the surface (arrow). The injection site is clearly contained within agranular cortex and it avoids the underlying white matter. Bar=2 mm.

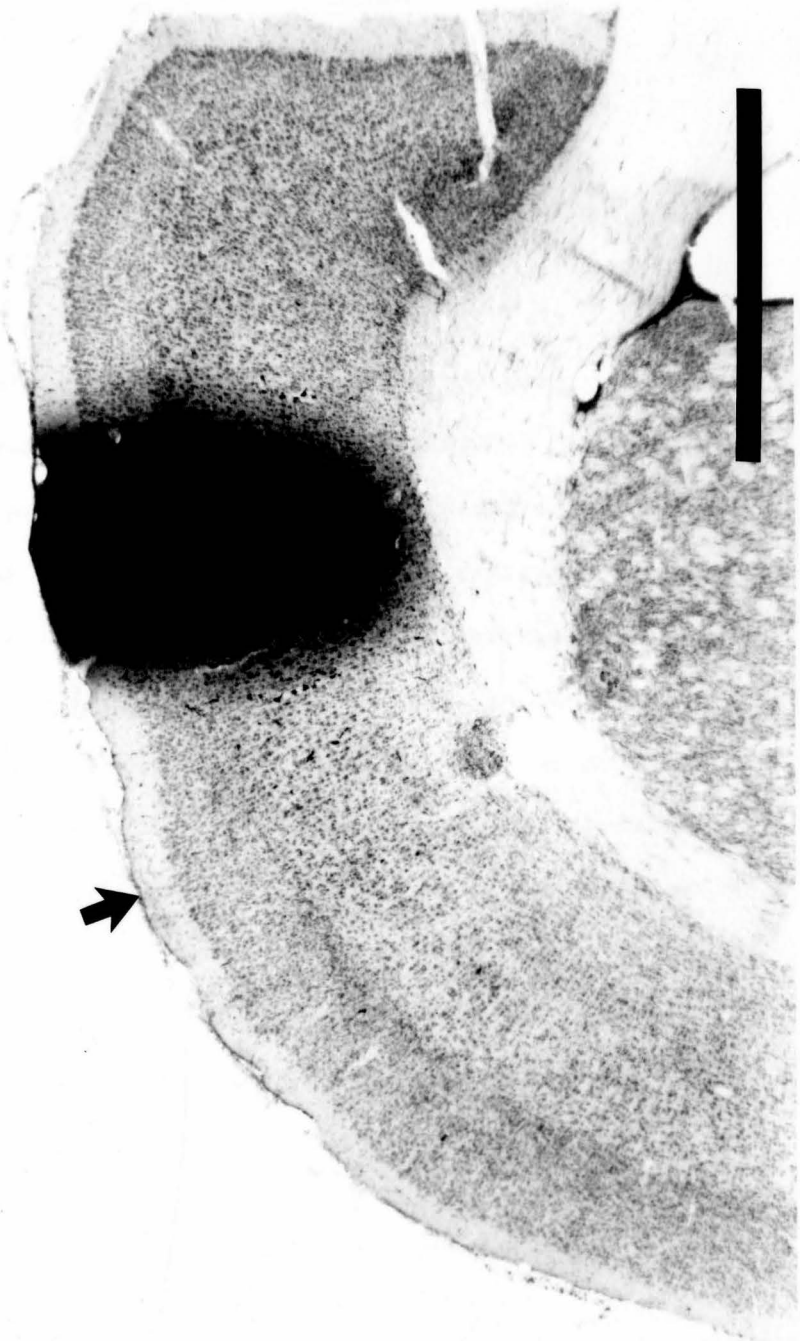


Figure 4. Summary diagram depicting the five paths where corticospinal fibers could be found in this study. The coronal view shows the location of the paths (stipled areas) and the approximate pattern of spinal cord lamination taken from Rexed (1954) and modified for the rat (McClung and Castro 1978). The five corticospinal tracts (CST) are; dorsal CST contralateral (DCSTc), dorsal CST ipsilateral (DCSTi), lateral CST contralateral (LCSTc), lateral CST ipsilateral (LCSTi) and ventral CST ipsilateral (VCSTi). Some of the areas where fibers left the tract and entered the gray matter are indicated. The lines marked A-D indicate the levels of horizontal sections shown in figures 5 and 7B.

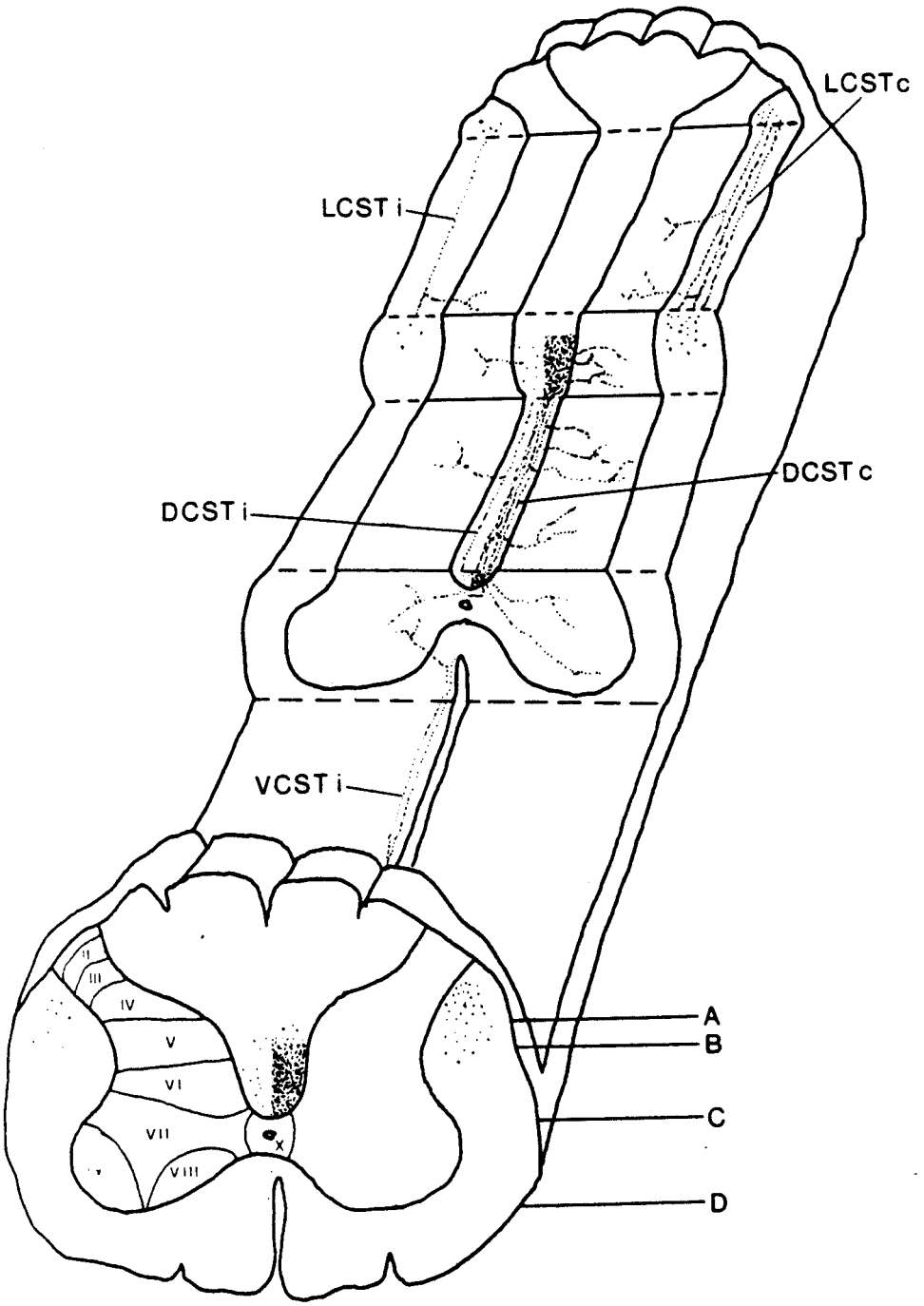

LCSTc
LCST i
DCSTc
DCST i
VCST i
II
III
IV
V
VI
VII
VIII
X
A
B
C
D

Figure 5. Dark field photomicrographs of the five corticospinal paths as seen in horizontal section.

A. Section taken through level A (Fig. 4). Bar=250 um, DH=dorsal horn. The white line coursing from top to bottom on the left side of the section indicates the midline. The small arrows point to a few fibers of the DCSTc which is just beginning to appear at this level. The large arrows point to two fibers of the LCSTc which is in the white matter just lateral to the dorsal horn.

B. Section taken through level B on Fig. 4. Bar=250 um, DH=dorsal horn, asterisk=DCSTc. The small arrows point to two DCSTi fibers coursing alongside the large DCSTc. The large arrow points to one fiber from the LCSTc which is not seen much below this dorsal-ventral level.

C. Section taken just below level A on Fig. 4. Bar=500 um, asterisk=DCSTc. Small arrows indicate two DCSTi fibers adjacent the large DCSTc. The large arrows indicate two fibers from the LCSTi. The dorsal horn is not labeled in this section.

D. Section taken through level D on Fig. 4. Bar=100 um, VMF=ventral median fissure. Small arrows indicate the location of three VCSTi fibers.

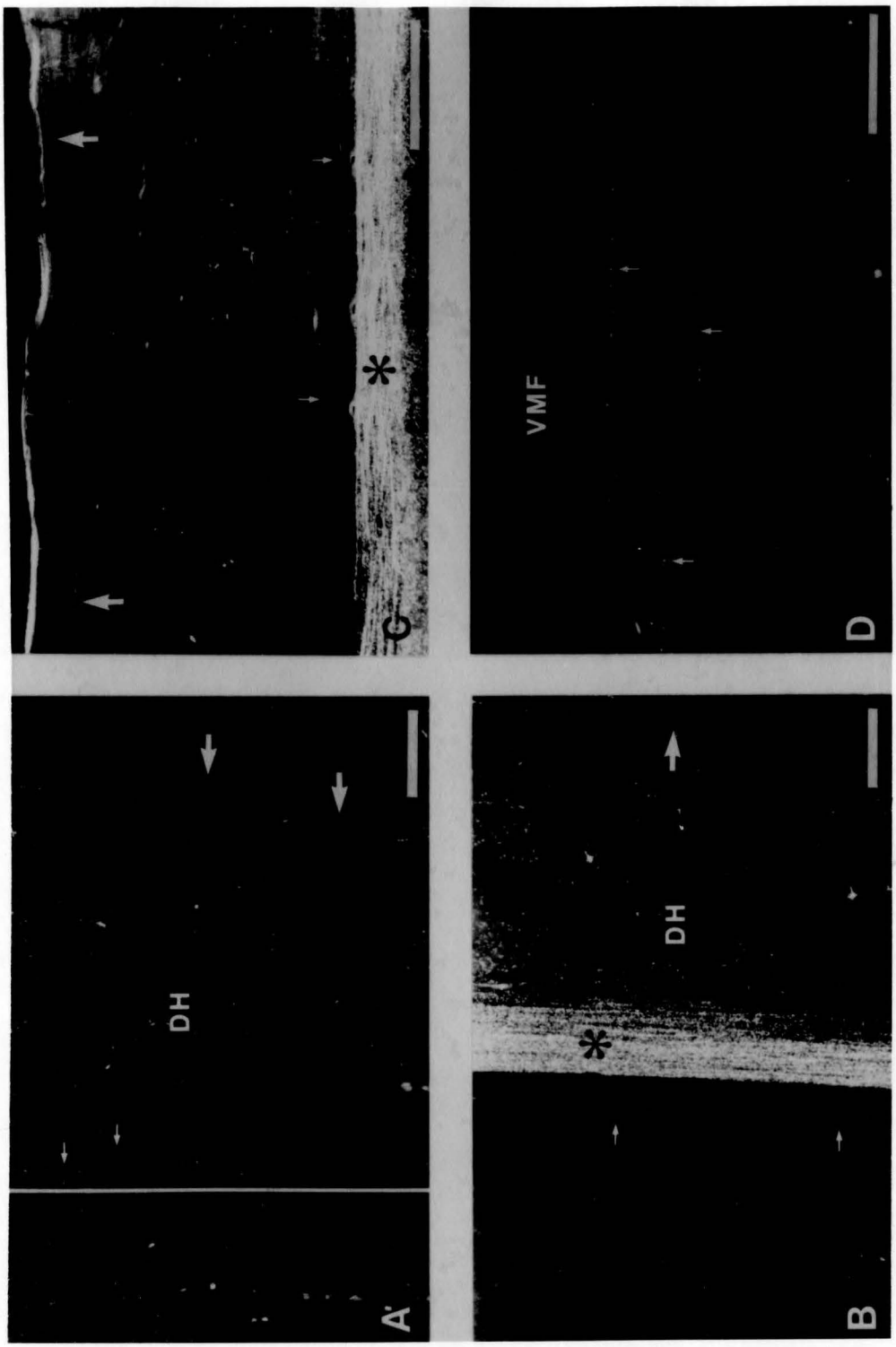

A
DH
B
DH
C
D
VMF

Figure 6. Dark field photomicrographs of coronal sections through cervical and lumbar enlargements after five differently located injections of WGA-HRP.

A. Labeling seen in the cervical enlargement after a rostral forelimb injection. Bar=500 um, C=central canal. Note the label in lamina VIII.

B. Higher magnification of the right ventral horn and intermediate gray from the section shown in 6A. Bar=250 um.

C. Labeling seen in the cervical enlargement after a caudal forelimb motor injection. Bar=500 um, C=central canal, arrow indicates artifact for orientation in the adjacent section D. Note: lamina VIII sparing and ventral horn terminations.

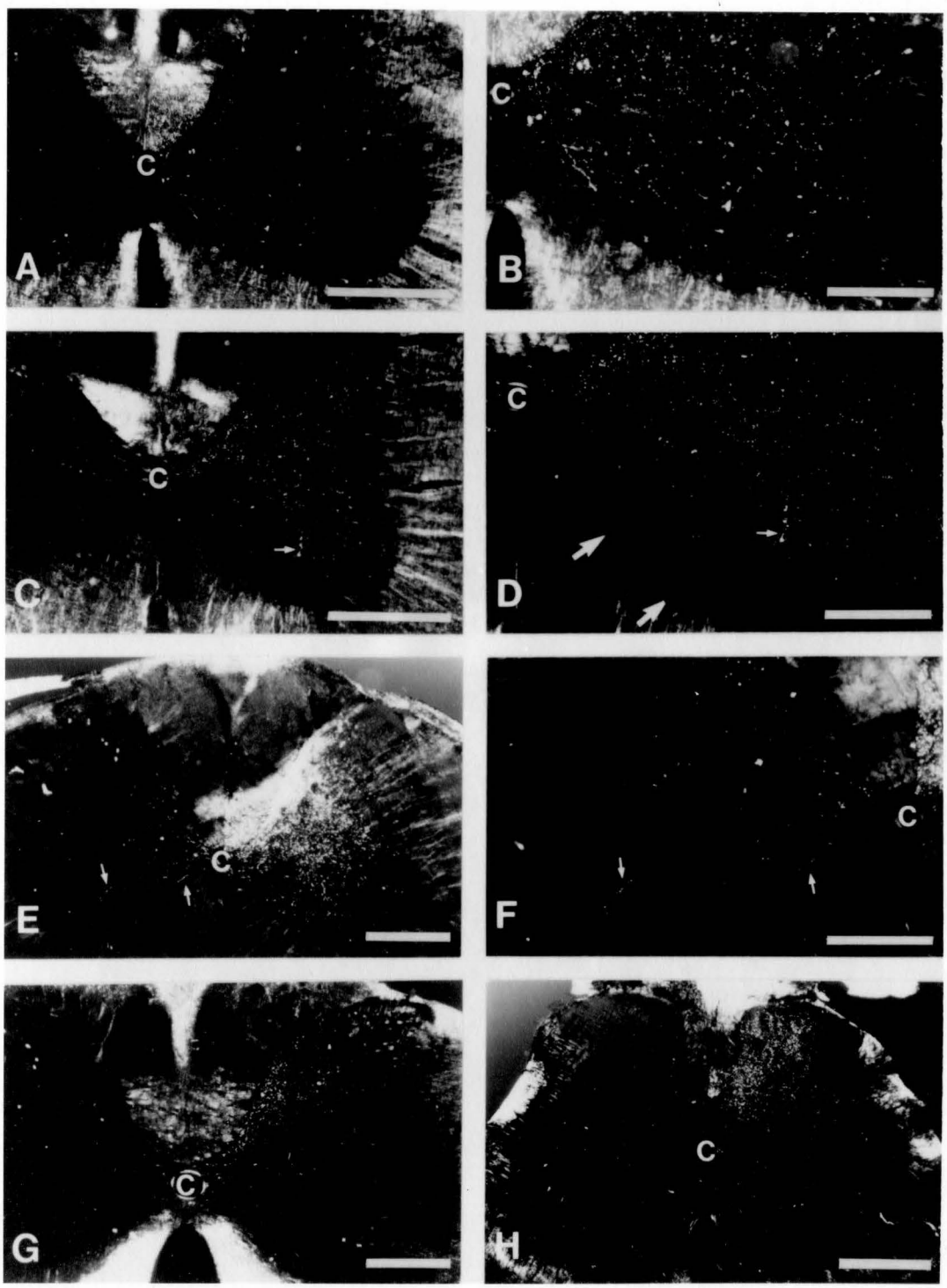
D. Higher magnification of section seen in C. Bar=250 um. The small arrow indicates the artifact seen in C for orientation, and the large arrows indicate the border of the ventral horn.

E. Labeling in cervical cord after a sensory cortex injection. Bar=500 um. Arrows indicate ipsilateral terminations for orientation in figure 6F.

F. Higher magnification of ipsilateral side from E. Bar=250 um. Note ipsilateral terminations.

G. Labeling in cervical cord after an SII injection. Bar=500 um.

H. Labeling in lumbar cord after a hindlimb sensorimotor injection. Bar=500 um.


A
C
B
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C

Figure 7. Dark field photomicrographs of corticospinal terminations.

A. Cervical enlargement ventral horn terminations after a motor forelimb injection. Arrows indicate some of the terminations. C=central canal, CST=corticospinal tract (DCSTc).

B. Horizontal section at level C in figure 4. The white line indicates the midline, and arrows indicate ipsilateral terminations. The arrow at the top near the midline points to a fiber that is coming from the opposite side.

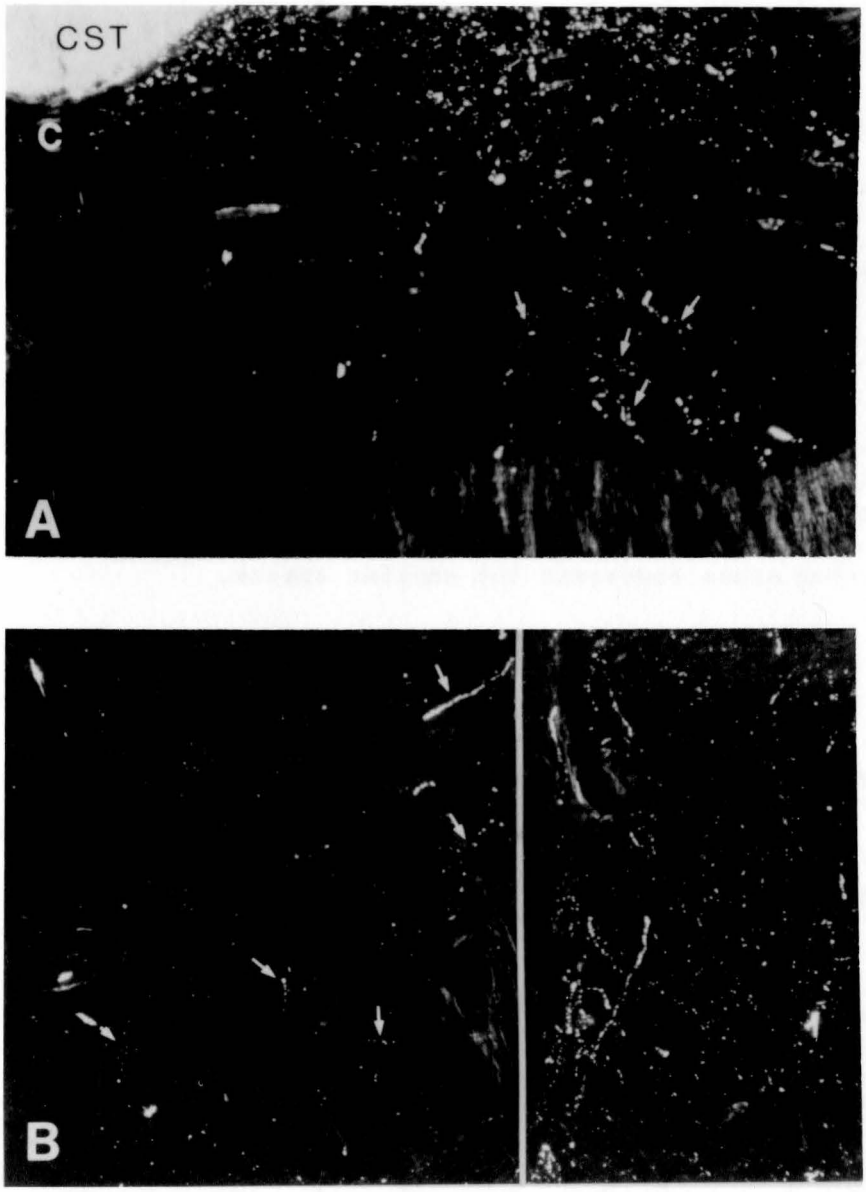

CST
C
A
B

Figure 8. Camera lucida drawings of cervical enlargement terminations from four differently located cortical injection sites. The dark area represents the major corticospinal tract and the hatched areas represent the smaller tracts.

A. Labeling seen after a caudal forelimb motor injection.

B. Labeling seen after a rostral forelimb injection.

C. Labeling seen after a sensory forelimb injection.

D. Labeling seen after a second somatosensory area (SII) injection.

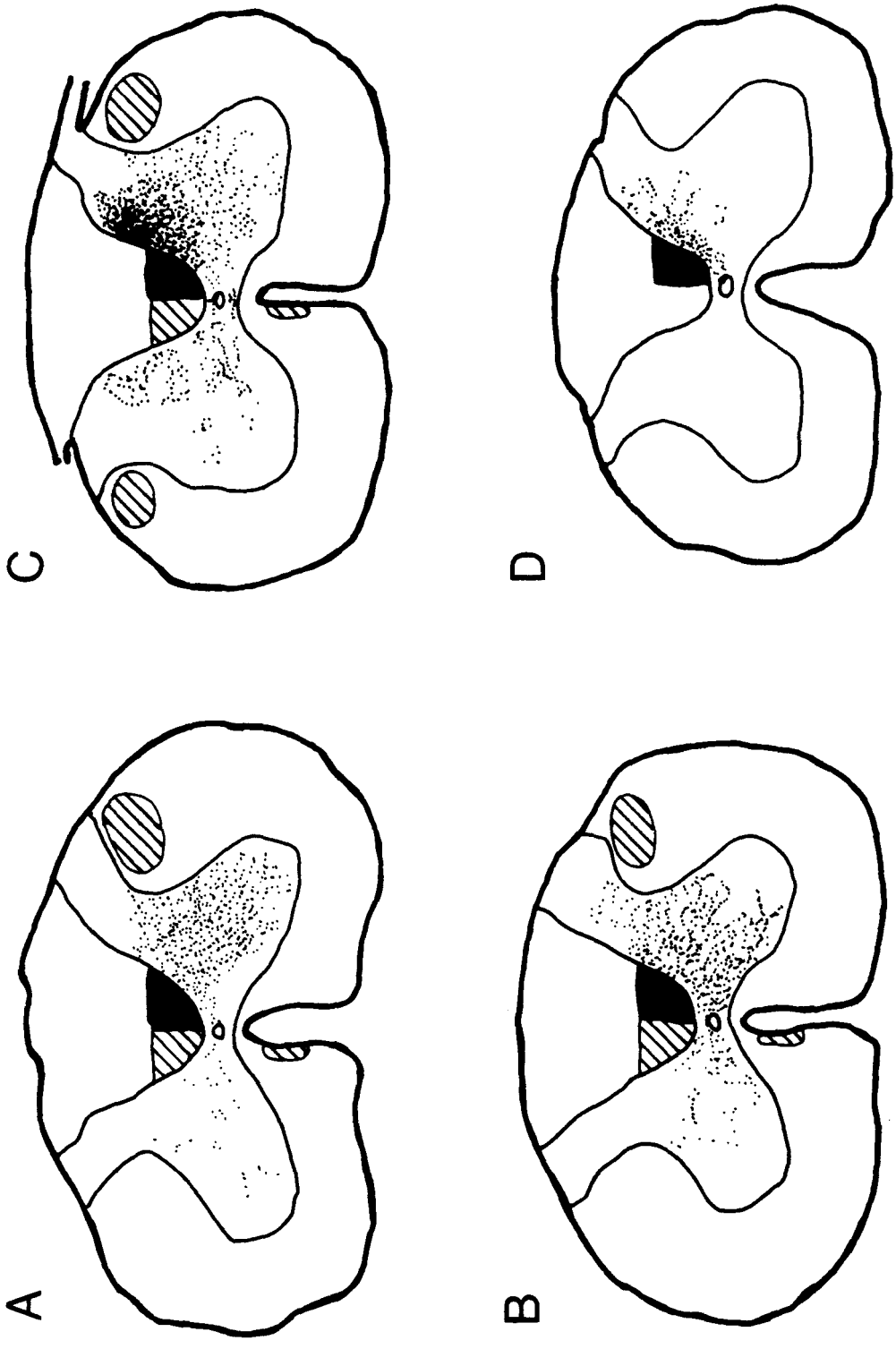
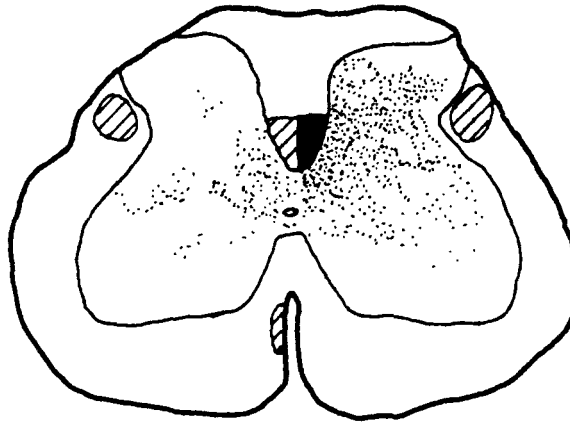

C
D
A
B

Figure 9. Camera lucida drawing of the labeling seen in the lumbar enlargement after a hindlimb sensorimotor injection.



CHAPTER VI

DIFFERENTIAL PROJECTIONS OF THE RAT SENSORY AND MOTOR CORTICES TO THE DORSAL COLUMN NUCLEI: AN HRP STUDY

Introduction

The somatotopic projections of the primary sensorimotor cortices to the dorsal column nuclei (DCN) have been demonstrated in rats (Zimmerman et al., 1964), cats (Kuypers and Tuerk, 1964; Weisberg and Rustioni, 1979), and monkeys (Kuypers, 1958b; Liu and Chambers, 1964). Generally, these studies have shown that forelimb sensorimotor cortex projects to the rostral and ventral portions of nucleus cuneatus, whereas hindlimb sensorimotor cortex projects throughout most of nucleus gracilis. Although the projections from various cytoarchitectonic divisions of the primary motor and sensory cortical areas to the DCN have been examined in cats (Weisberg and Rustioni, 1979) and monkeys (Kuypers and Tuerk, 1964), the rat cortico-DCN projection has only been studied after relatively large lesions of cortex (Zimmerman et al., 1964).

Since the time of these studies, the rat sensorimotor cortex has been electrophysiologically mapped in greater detail by many investigators (Hall and Lindholm, 1974; Welker, 1976; Sanderson et al., 1982; Neafsey and Sievert, 1982; Donoghue and Wise, 1982; Sanderson et al., 1984). One result of these studies has been the finding that the forelimb area of motor cortex is almost entirely separate from that of the forelimb sensory cortical area, whereas the hindlimb motor and sensory areas almost entirely overlap (Hall and Lindholm, 1974; Donoghue et al., 1979; Donoghue and Wise, 1982; and Sanderson et al, 1984). Another observation is that the rat cortex

contains two separate areas where forelimb movements can be evoked during intracortical microstimulation (Sanderson et al., 1982; Neafsey and Sievert, 1982). The identity of the second forelimb area is not known, but it has been speculated that it may be part of the rat supplementary motor area (Wise et al., 1979; Donoghue and Wise, 1982; Neafsey and Sievert, 1982). In light of these results, the present study was undertaken to compare the cortical projections to the DCN from electrophysiologically defined sensory and motor cortical areas, including the two forelimb motor cortical areas.

Materials and Methods

Seventeen male Long-Evans Hooded rats (300-500 grams) were used for this study. The animals were divided into four groups, each of which received injections of wheat germ agglutinin HRP (WGA-HRP) in various electrophysiologically identified areas of sensory and motor cortex. The groups were as follows: 4 animals received rostral forelimb motor cortex injections, 5 received caudal forelimb motor cortex injections, 4 received hindlimb sensorimotor cortex injections, and 4 received forelimb sensory cortex injections. For a complete description of the mapping, injection and histological processing procedures refer to chapter 4 of this dissertation. Briefly, each animal's cortex was mapped by intracortical microstimulation or extracellular multi-unit recording while under the influence of Ketamine HCl anesthesia (100 mg/kg, IP) and subsequently injected with 0.02-0.04 ul of 1% WGA-HRP. After a suitable survival time (two to three days), the animals were sacrificed and the brains removed and sectioned in the coronal plane at 50 microns. The tissue was processed for HRP histochemistry according to the TMB technique of Mesulam (1978). At least one animal in each group was processed according to the modified TMB technique of Gibson et al. (1984), which was found to be equally sensitive to Mesulam's (1978) but produced less artifact. Sections containing the dorsal column nuclei (DCN) were examined for the presence and extent of anterograde label,

defined as the small refractile dots seen under polarized light which are considered to be evidence of terminals (Mesulam, 1982). The distribution of label was plotted on line drawings of the sections using a camera lucida drawing tube.

Results

Injection Sites

Most of the injections were well localized with visible spread not more than 1 mm in any direction. The injection sites were reacted with TMB which results in a larger appearing injection site than those reacted in DAB (Mesulam, 1982). With the exception of two sensory injections which had some spread into the forelimb motor area, and one rostral forelimb injection which spread into caudal forelimb, all other injections did not appear to extend into adjacent physiologically identified areas. The location of each type of injection is depicted on the dorsal surface of the rat brain in figure 1A. Note that the various types of injection sites do not appear to overlap. A line drawing through the center of each type of injection site is depicted in figure 1B-E, and a stacked line drawing through the entire extent of a caudal forelimb motor injection is depicted in figure 2B. A photomicrograph of a Nissl stained section taken from the center of the caudal forelimb injection site is shown in figure 3. It is clear that the injection does not spread into the adjacent granular cortex (arrow in Fig. 3).

Rostral Forelimb Injections

Injections centered in the rostral forelimb motor cortex resulted in only trace amounts of label in the DCN on the contralateral side. Figure 4A is a stacked line drawing of the lower

brainstem taken from a representative animal. While sparse labeling was seen in reticular areas just ventral to the cuneate nucleus, only a few terminal fibers appeared to enter the contralateral middle third of the nucleus, particularly in its ventral aspect (Fig. 4A sections 2+3, Fig. 6D). No additional label was seen in either cuneate, external cuneate, or gracile nuclei.

Hindlimb Injections

All the animals which received hindlimb injections had a similar pattern of labeling in the DCN, which is illustrated in figure 4B. Both external cuneate and gracilis nuclei were labeled bilaterally, but much heavier contralaterally. Nucleus gracilis contained dense terminations throughout its rostrocaudal extent with slightly lighter labeling in its most rostral-ventral portion (Fig. 4B, section 3 and Fig. 6C). The cuneate nuclei were labeled bilaterally through most of their rostral caudal extent. Ipsilateral cuneate received sparse labeling, whereas contralateral cuneate received a moderate to heavy projection in the ventro-medial aspects. (Fig. 4B, sections 1-4). In addition, sparse projections from the hindlimb sensorimotor cortex were also seen within the medullary reticular formation contralateral to the cortical injection site.

Caudal Forelimb Motor Cortex Injections

The five animals which received injections of HRP in the caudal forelimb motor cortex had the pattern of terminations in the DCN

illustrated in figure 5A. Moderate amounts of terminations were seen bilaterally in the external cuneate nuclei. The contralateral rostral levels of the main cuneate nucleus revealed a diffuse pattern of cortical terminations which were heavier ventrally (see section 4 of Fig. 5A and Figs. 7A+B). Sparse ipsilateral label was also seen at this level. The middle portion of the contralateral cuneate nucleus exhibited moderate labeling which was heaviest along its ventral aspect, while the caudal portions of this nucleus exhibited label in the ventromedial aspect contralaterally, and a small amount of label dispersed throughout the nucleus bilaterally (see Figs. 5A, sections 1-3 and 7C-F). A small amount of label was also seen bilaterally within middle portions of nucleus gracilis (Fig. 5A section 2). The medullary reticular formation contained diffuse labeling contralateral to the injection site.

Sensory Forelimb Injections

The pattern of labeling in the DCN after a forelimb sensory cortex injection can be seen in figure 5B. The external cuneate nuclei were labeled bilaterally, heavier contralaterally. The contralateral nucleus cuneatus contained heavy labeling throughout its rostrocaudal extent, except for a slight sparing of the ventromedial aspect of the middle third of the nucleus (see section 2 of Fig. 5B, and Figs. 6A+B). No label was seen in the ipsilateral cuneate nucleus. The medullary reticular formation revealed diffuse terminations contralateral to the injection site.

Discussion

The present study confirms the basic somatotopic distribution pattern of sensorimotor cortex projections to the DCN seen by other investigators (Kuypers, 1958b, 1960; Kuypers and Tuerk, 1964; Liu and Chambers, 1964; Zimmerman et al, 1964; Weisberg and Rustioni, 1979; and Albright and Friedenbach, 1982) in that hindlimb cortical areas project to the gracile nuclei and forelimb cortical areas project to the cuneate nuclei. This study has also shown that there is a differential projection to the DCN from sensory and motor areas of cortex. In particular, forelimb motor cortex projected primarily to the ventral and rostral aspects of nucleus cuneatus, whereas forelimb sensory cortex terminated throughout the entire cuneate nucleus, primarily within the dorsal portions. Previous studies in the rat by Zimmerman et al. (1964) and Valverde (1966) demonstrated a slight cortical projection to the dorsal and a heavy projection to the ventral part of the cuneate nucleus. These previous studies, however, utilized either degeneration techniques with lesions that involved both sensory and motor cortical regions (Zimmerman et al., 1964) or Golgi techniques (Valverde, 1966), and consequently were unable to make a distinction between sensory and motor cortical terminations. The heavier projection seen in the present study could possibly be accounted for on the basis of the more sensitive anterograde HRP technique as opposed to the degeneration and Golgi techniques used in these earlier studies. Our study did not find such a distinction in

projections from hindlimb sensorimotor cortex to the DCN, a result which is not surprising since sensory and motor hindlimb representations are either entirely overlapping (Hall and Lindholm, 1974; Donoghue et al., 1979) or too small and close together to inject separately (see chapter 4 of this dissertation).

The differential organization of forelimb sensory and motor cortical to DCN projections reported here may be related to the internal functional and cytological organization of the DCN. This internal pattern has been best studied in the cat (Dykes et al., 1982). In general, the rostral, caudal and ventral regions of the DCN (reticular areas) have been associated with the processing of deep, proprioceptive types of input, whereas the more central and dorsal aspects of these nuclei (cell nests) are related to the processing of cutaneous afferents with a high degree of place and modality specificity. Previous physiological mapping studies of the rat DCN (McComas, 1963; Nord, 1967) have not been sufficiently detailed to localize the regions of the DCN that are devoted to deep inputs. Anatomical studies of the rat DCN have demostrated that they are similar to the cat DCN, containing cellular bricks which correspond to the cat's cell clusters region (Basbaum and Hand, 1973; Odutola, 1977). Further similarities to the cat are indicated from the results of a physiological study which demonstrated small, modality specific receptive fields in the central (cellular bricks) regions, and large, non-specific receptive fields in the rostral and ventral (reticular) regions (McComas, 1963). As is the case in the cat (see Towe for

review, 1973), the centrally located cells with small receptive fields exhibited the phenomenon of surround inhibition (McComas, 1963). The results of our study combined with these data suggest that, in the rat DCN, forelimb sensory cortex projections are primarily concerned with modulating well localized, modality specific cutaneous input to the cell bricks (clusters) region, while the forelimb motor cortex is primarily concerned with modulating deep, proprioceptive inputs to the reticular region. However, this hypothesis could only be confirmed by a conclusive physiological study in the rat DCN, similar to that done by Dykes and coworkers in the cat (1982).

The almost total lack of DCN terminations from the rostral forelimb area seen in this study points toward a different role for this area from that of the primary motor cortex. A recent study on the efferents of the supplementary motor area (SMA) of the squirrel monkey has shown that the DCN do not receive a projection from the SMA, whereas the primary motor area does terminate in the DCN (Jurgens, 1984). In contrast to Jurgens findings, a study on the Rhesus monkey demonstrated labeled cells in the SMA following an injection of HRP into the DCN (Weisberg and Rustioni, 1977). The difference in results between the two monkey studies may be due to spread of the DCN HRP injection site into the neighboring reticular formation which does receive projections from the SMA (Kunzle, 1978; Jurgens, 1984). In light of these findings, the results of the present study suggest that the rostral forelimb area is a part of the supplementary motor area of the rat.

Figure 1. Location of injection sites.

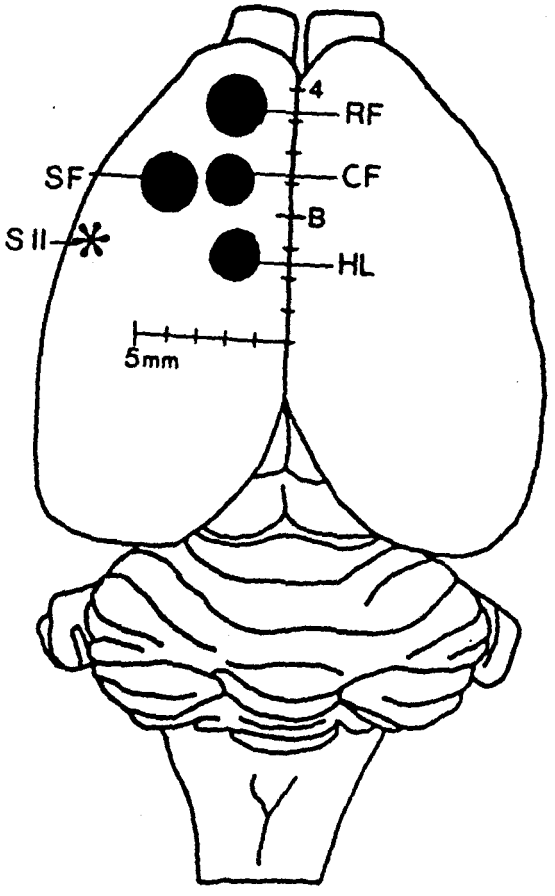
A. Injection sites from rostral forelimb (RF), caudal forelimb (CF), sensory forelimb (SF), and hindlimb (HL). All injection sites were reconstructed from coronal sections and plotted on a dorsal view of the rat brain. B=Bregma, Divisions are in mm.

B. Drawing of a coronal section from the center of a rostral forelimb area injection site depicting the widest area of visible HRP. The dots surrounding the dark area indicate the "halo" area of the injection site. The millimeter bar seen in section E applies to all coronal sections shown in this figure.

C. Drawing of a coronal section from the center of a caudal forelimb area injection. The granule cell layer of forelimb sensory cortex is is also outlined to the left of the injection.

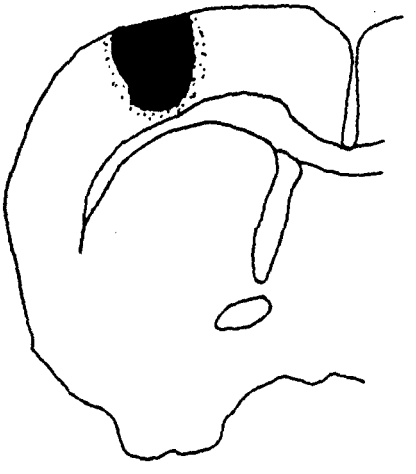
D. Drawing of a coronal section from the center of a sensory forelimb cortex injection.

E. Drawing of a coronal section from the center of a hindlimb sensorimotor area injection.

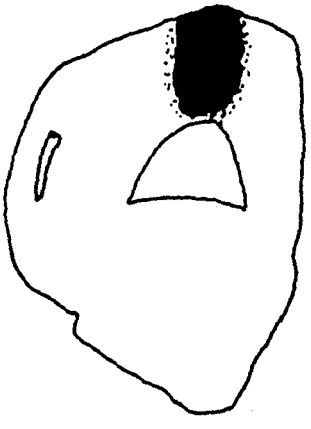


A

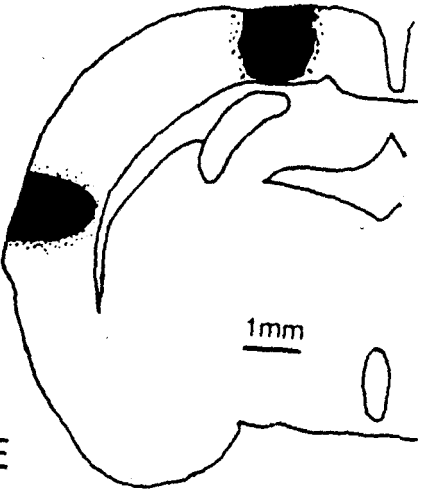
C



D



B



E

Figure 2. Drawing of the extent of a caudal forelimb motor area injection site.

A. Map of responsive points from ICMS performed prior to the injection. B=bregma, divisions are in mm, 1=wrist extension (threshold=12 uamps), 2=neck (25 uamps), 3=neck (25 uamps), 4=elbow flexion (10 uamps), 5=elbow flexion (20 uamps), 6=wrist extension (20 uamps).

B. Drawings of coronal sections taken at each of the six electrode shown in figure 1A. Dark stipled area indicates the area of visible injection site. The sensory cortex granule cell patches are outlined beneath the cortical surface. The stipled area seen in sections 1,2 and 6 is from anterograde label (dots), and retrogradely labeled cells (short lines). Note that the injection site does not infringe at all on the granule cell patches.

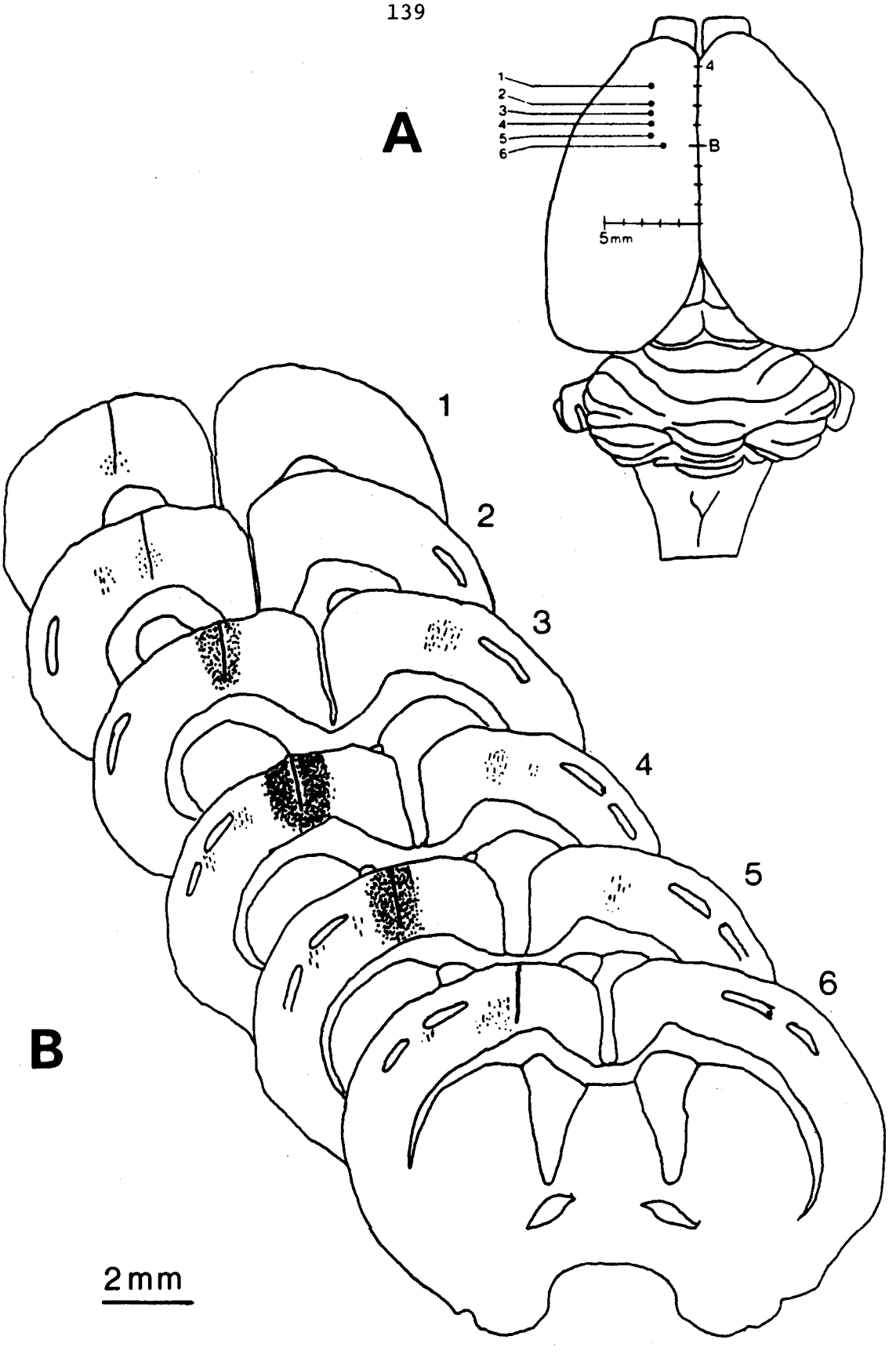

A
1
2
3
4
5
6
4
B
5mm
1
2
3
4
5
6
B
2mm

Figure 3. Nissl stained section taken from the center of the caudal forelimb injection site shown in figure 2 (section 4). The medial border of the forelimb sensory cortex granule cell patch is indicated on the surface (arrow). The injection site is clearly contained within agranular cortex, and it avoids the underlying white matter. Bar=2 mm.

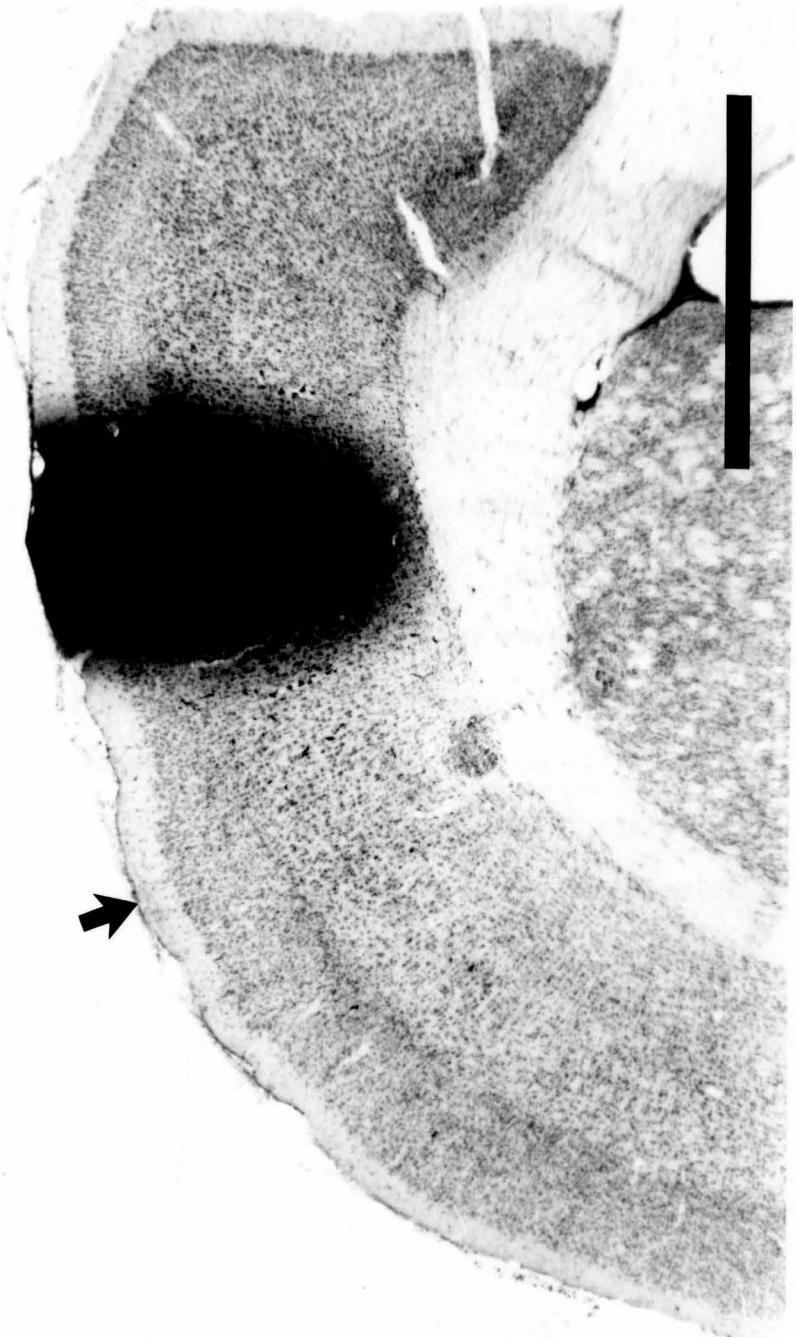
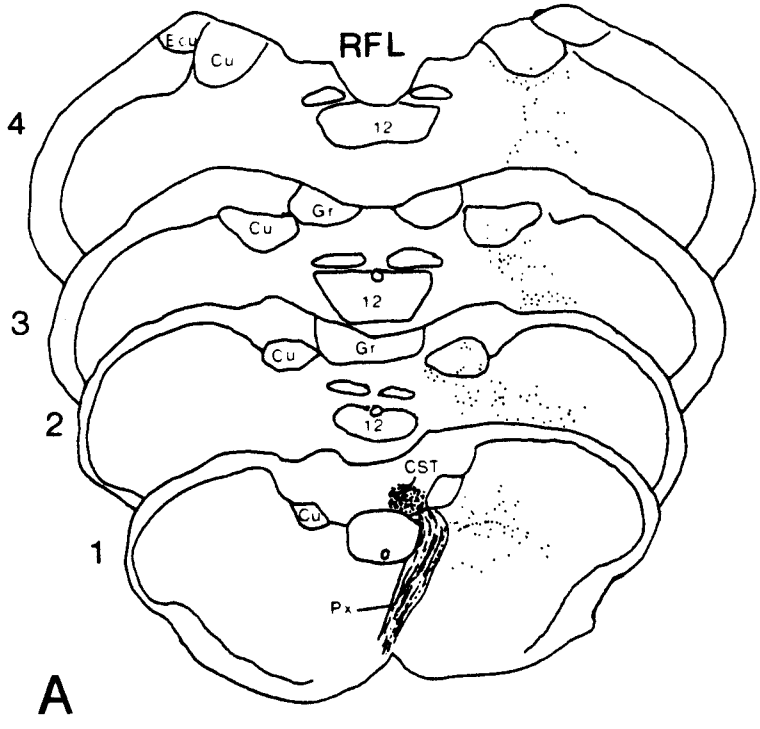


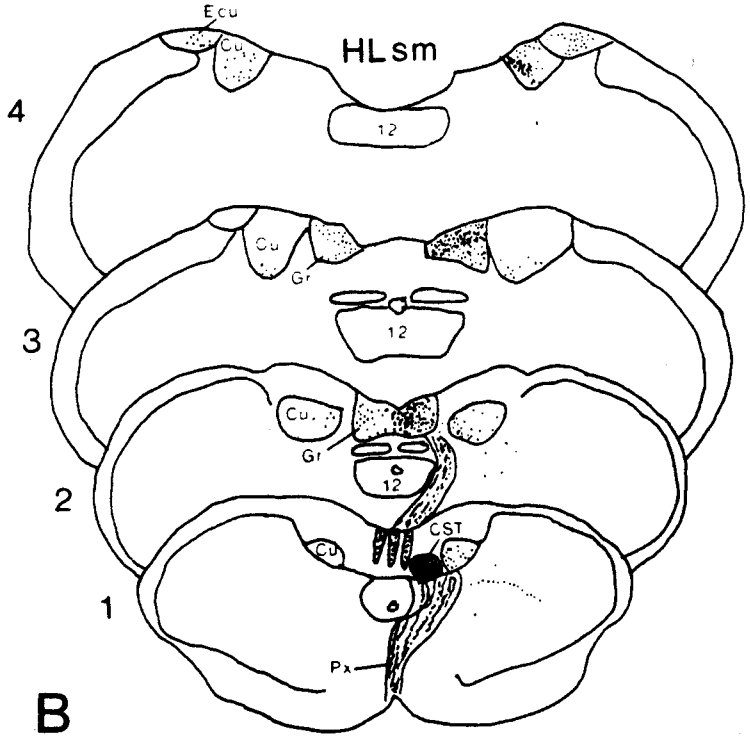
Figure 4. Line drawings of anterograde label in DCN. Cu=cuneatus, ECu=external cuneate, Gr=gracilis, Px=pyramidal decussation, CST=corticospinal tract.

A. Terminal labeling seen in the DCN (sections 1-4 correspond to caudal, middle, and rostral levels respectively) following a rostral forelimb injection (RFL). Note the absence of labeling in the gracilis and external cuneate nuclei, and the sparse label in the contralateral nucleus cuneatus (sections 2 and 3).

B. Terminal labeling seen in the DCN following a hindlimb sensorimotor injection (HLsm). Note the labeling in nucleus gracilis (sections 1-3), heaviest contralaterally, and labeling in nucleus cuneatus (section 1-4).


RFL
Ecu
Cu
4
12
Gr
Cu
3
12
Cu
Gr
2
12
CST
Cu
1
Px

A

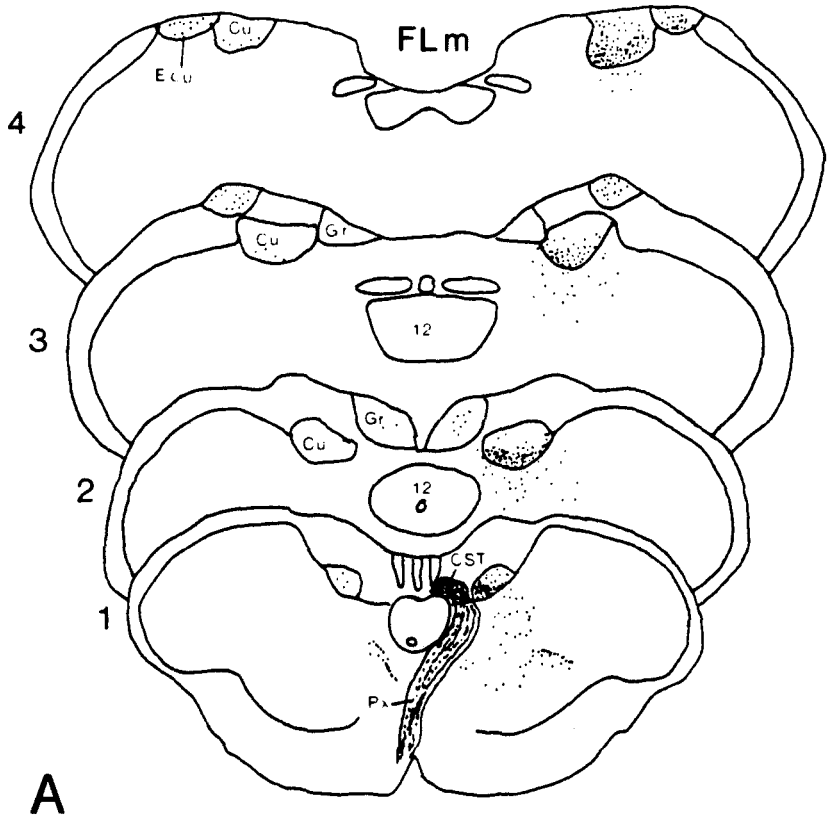

Ecu
Cu
HLsm
4
12
Cu
Gr
3
12
Cu.
Gr
12
2
CST
Cu
1
Px

B

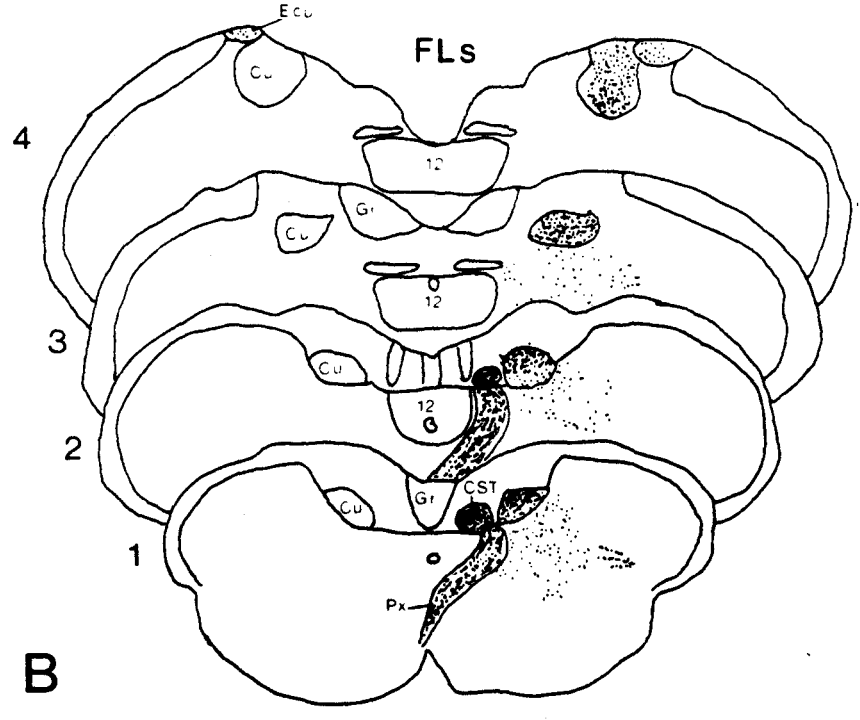
Figure 5. Line drawings of anterograde label in DCN. Abbreviations as in figure 4.

A. Terminal labeling seen in the DCN (sections 1-4 correspond to caudal, middle, and rostral levels respectively) following a caudal forelimb motor injection (FLm). There is labeling bilaterally in external cuneate (sections 3 and 4), bilaterally in cuneate (heaviest contralaterally) (sections 1-4), and a small amount of label in nucleus gracilis (section 2).

B. Terminal labeling seen in the DCN following a sensory forelimb injection (FLs). Note the bilateral labeling in external cuneate (section 4), and heavy contralateral labeling through all of nucleus cuneatus (sections 1-4).



A



B

Figure 6. Polarized light photomicrographs of terminal labeling in DCN.

A. Coronal section taken at level 3 in figure 5B (sensory forelimb injection). C=central canal, arrows indicate the borders of the cuneate nucleus, Bar=500 um. Note heavy labeling in nucleus cuneatus.

B. Higher magnification of nucleus cuneatus seen in section A. Arrows indicate the border of the cuneate nucleus, Bar=250 um.

C. Coronal section taken at level 3 in figure 4B (hindlimb injection). C=central canal, G=nucleus gracilis, Bar=500 um.

D. High power photomicrograph of a coronal section taken at level 2 in figure 4A (rostral forelimb injection). Arrows indicate the borders of the cuneate nucleus. Note the lack of terminal labeling, Bar=250 um.

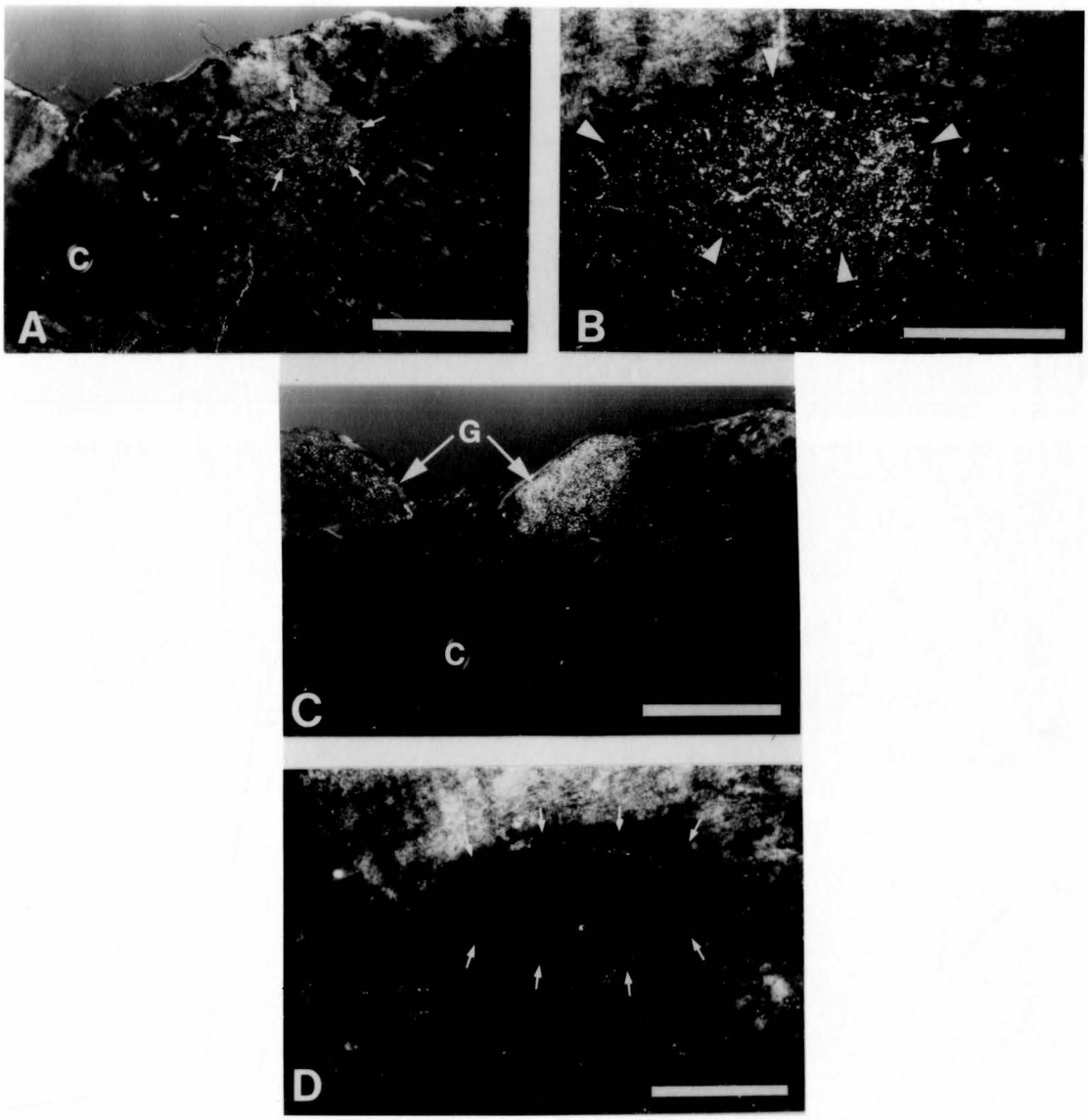

A
C
B
C
G
C
D

Figure 7. Polarized light photomicrographs of coronal sections through three levels of nucleus cuneatus following a caudal forelimb motor injection.

A. Section through the rostral portion of nucleus cuneatus. Arrows indicate the borders of the nucleus, Bar=500 um.

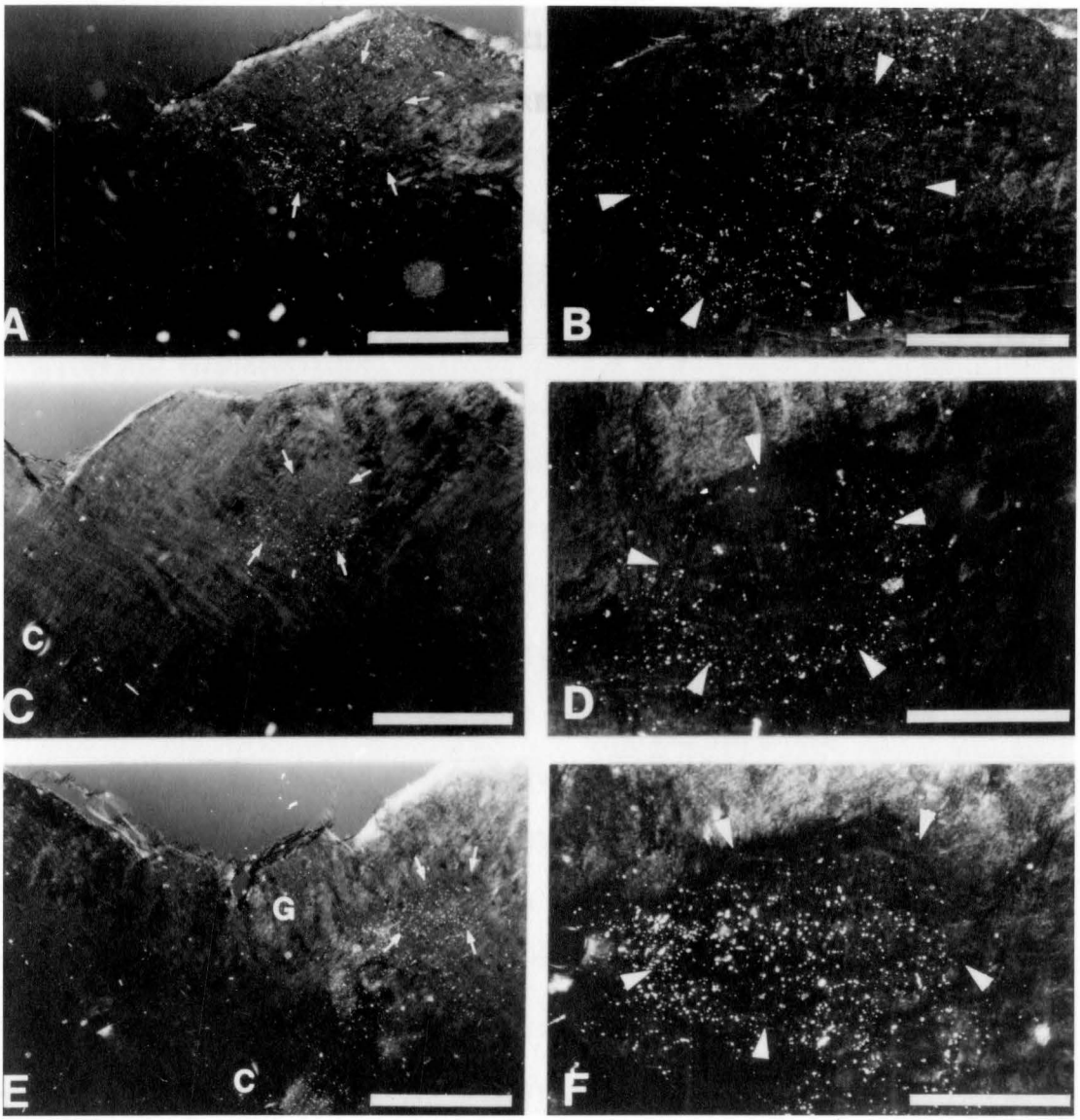
B. Greater magnification of the section shown in A. Arrows indicate the borders of nucleus cuneatus, Bar=250 um.

C. Section through the middle portion of nucleus cuneatus. Arrows indicate the borders of nucleus cuneatus, Bar=500 um, C=central canal.

D. Greater magnification of the section shown in C. Arrows circumscribe nucleus cuneatus, Bar=250hum.

E. Section through the caudal portion of the nucleus cuneatus. Arrows indicate the borders of the nucleus, C=central canal, G=gracilis nucleus, and Bar=500 um.

F. Greater magnification of the section shown in E. Arrows indicate the borders of the cuneate nucleus, Bar=250 um.


A
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CHAPTER VII

SENSORY PROPERTIES OF FORELIMB SENSORIMOTOR NEURONS IN THE AWAKE RESTRAINED RAT

Introduction

Within the rat motor cortex two separate areas are found where forelimb movements can be evoked by intracortical microstimulation (Neafsey and Sievert, 1982). Anatomical studies have shown that both of these areas project heavily to the cervical spinal cord (Hicks and D'Amato, 1977; Wise et al., 1979; Neafsey and Sievert, 1982; Donoghue and Wise, 1982). The large, caudal forelimb motor area appears to be clearly a part of the primary motor cortex (Hall and Lindholm, 1974; Woolsey et al., 1952) and is located primarily in the agranular lateral (AgL) cytoarchitectonic subdivision (Donoghue and Wise, 1982). The functional identity of the rostral forelimb area is as yet unclear, but two possibilities are likely. First, it could be a second representation of the forelimb within the primary motor area (M1), as has been seen for the hand in the monkey (Strick and Preston, 1978, 1982a). The other alternative is that it may be a part of the supplementary motor area (SMA) of the rat, although as yet no SMA has been reported in this species. One of the major differences between M1 and SMA is the amount and type of peripheral sensory input arriving in each region. For example, both distal limb representations in monkey M1 receive peripheral sensory input, with deep inputs going to the rostral distal limb area and cutaneous inputs going to the caudal distal limb area (Tanji and Wise, 1981; Strick and Preston, 1982b). In the SMA, however, there is much less sensory input (15% of SMA neurons responsive compared to 60% of M1 neurons); and the input may

be more complex in nature, with many cells activated by multiple joints, as well as cutaneous and ipsilateral inputs (J. Brinkman and Porter, 1978; C. Brinkman and Porter, 1979; Wise and Tanji, 1981).

In light of these differences between M1 and SMA, the present study comparing sensory inputs to both forelimb motor areas in the rat was undertaken. The results of this study show that the rostral forelimb area of the rat is probably a part of the supplementary motor area, and that the rat primary motor cortex is similar to the monkey in terms of quality of inputs and the relationship between input and output.

Materials and Methods

Ten male Long-Evans hooded rats (350-450g) were adapted to handling and trained to sit on a rodent harness (Alice King Chatham, Pasadena, California). After the adaptation period of 3-5 days, each animal was anesthetized with ketamine HCL (100 mg/kg IM) and placed in a stereotaxic apparatus. Rounded ear bars were used to avoid breaking the tympanic membrane. Two 2-56 screws with their heads ground to a rectangle were inserted into slots made in the skull and rotated ninety degrees. One screw was placed over the left parietal area and was long enough to attach to an L shaped bracket attached to the base of a stereotaxic electrode carrier (Kopf 1760/1761) which also held a miniature hydraulic microdrive (Haer). The other screw was placed over the cerebellum in the midline. Two additional screws were glued (cyanoacrylate) into tapped holes in the skull. A craniotomy was made over the limb sensorimotor areas as defined by electrophysiological studies (Neafsey and Sievert, 1982; Donoghue and Wise, 1982; Neafsey et al., in preparation). The area exposed extended from 1 mm caudal to bregma to 4.5 mm rostral, and from 1 mm lateral to 4.5 mm lateral. This area includes the rostral and caudal forelimb areas, most of the sensory forelimb area and part of the hindlimb sensorimotor area. A plastic "beem" capsule, for embedding tissue in electron microscopy, was fitted and cemented to the skull over the craniotomy and was used as the recording chamber. Dental acrylic was used to fix the chamber to the skull and to hold two additional mounting screws to the

anchoring system. One mounting screw was placed over the right parietal area and the other placed over the olfactory bulb area. This system is illustrated in figures 1B and C. The wounds were closed and the animals allowed to recover for two days. During the recovery period the animals were slowly adapted to having their heads held, and eventually tolerated head fixation for 2-3 hours at a time without apparent discomfort (Fig. 1A). During the recording session the animals periodically took applesauce from a stick. Two days after surgery the animals were usually ready for a short recording session.

Unit recording and intracortical microstimulation (ICMS) were performed using a glass insulated, tungsten microelectrode which had 15 um of tip exposed (Neafsey, 1980). The signal was amplified conventionally and sent to a window discriminator, spike signal enhancer and stereo amplifier for audio monitoring. A recording session lasted several hours, and consisted of one electrode penetration from the cortical surface to the white matter. Intracortical microstimulation (300 ms trains of negative 0.25 ms pulses at 350 hz) was performed at a depth of 1.7 mm in each penetration. Currents were monitored across a 10 kOhm resistor inserted in the return path, and no currents greater than 25 ua were used. During the session, each well isolated cell encountered was tested for a receptive field by peripheral manipulation. Inputs were categorized as cutaneous or deep. Cutaneous input included hair bending and light touch, whereas deep input included pressure, tapping and joint manipulation. The depths of the cells were noted relative

to the onset of activity at the surface and the change in the background noise of the unit recording pattern as the electrode entered the white matter. Small electrolytic lesions, maximum 150 um (10 ua, 10 sec), were made at varying depths in several electrode tracks to aid in histological reconstruction. Five to fifteen penetrations, one penetration per day, were made in each animal prior to sacrifice. At the time of sacrifice each animal was reanesthetized with sodium pentobarbital, perfused thru the heart with 10% buffered formalin, followed by 10% sucrose in buffered formalin; and the brains were sectioned on a freezing microtome at 50 um. Sections were stained with a Nissl stain and examined for the laminar and cyto-architectural location of each cell or stimulation point on the electrode tracks.

Results

Summary

Sixty five electrode penetrations were made in ten rats, and 398 cells were tested for receptive fields. Of these, 117 cells (14 tracks) were located in the rostral forelimb area while 114 cells (23 tracks) were located in the caudal forelimb area. A total of 82 cells (11 tracks) were located in the sensory forelimb area. Finally, 86 cells (17 tracks) were located in motor-sensory areas other than the forelimb. One penetration into the hindlimb area was made, but our restraint system made it impossible to test for receptive fields in this area so no further attempts were made. Most cells were characterized by an initially negative going extracellular action potential. The units were commonly held without evidence of injury for 15 minutes while the sensory stimulation was delivered.

Depths

In order to place responsive cells in different cytoarchitectonic areas it was necessary to estimate the precision of our cell depth measurements. The amount of error in our depth measurement for individual cells was calculated on the basis of the difference between the observed and expected depths for 22 electrode penetrations where lesions were made. The calculated mean error was $\pm$ 0.22 mm (S.D.=0.14). Thus, in any one electrode penetration the depths of responsive cells could be 0.22 mm above or below the

recorded depth. A depth histogram of all the responsive cells in three areas is shown in figure 3A.

Rostral Forelimb Area

Fourteen penetrations were located in the RFL on the basis of stereotaxic coordinates, ICMS-evoked forelimb movements, and the presence of neck or vibrissae points caudal to the penetration. All 14 tracks were located in AgL (Fig. 3A). Cells in this area were active during active movements, but of the 117 cells tested, only one cell responded to peripheral mechanical stimulation. This cell responded to passive flexion of the contralateral elbow, and the movement evoked during ICMS was also elbow flexion (Fig. 3B). Movements evoked in the rostral forelimb area by ICMS were usually digit and wrist, but some elbow and shoulder movements were also seen.

Caudal Forelimb Motor Area

Penetrations located in AgL behind the neck region, and having ICMS evoked forelimb movements or forelimb receptive fields were classified as forelimb primary motor. There were 23 such penetrations in caudal forelimb motor cortex and 114 cells were tested. Thirty-six of the 114 cells (32%) had peripheral receptive fields. The majority (83%) of these 36 responsive cells were related to deep input, usually manipulation at a single joint. However, 17% of the cells appeared to respond to cutaneous inputs (Fig. 2B). Peripheral input was typically excitatory to cells in Agl. In 4 units, however, a reciprocal

response was seen in which the cells were activated by a passive movement in one direction and inhibited by a passive movement in the opposite direction. All responses except one were phasic, occurring only during the movement, and thus could not be considered position sensitive. One responsive cell was tonically active as long as a specific joint position was maintained.

When the relationship between sensory inputs and microstimulation evoked movements were analyzed for each penetration in the caudal forelimb area two types of relationship were found. The first type was seen in 15 electrode tracks. Ten of the tracks had one responsive cell, and the receptive field was at the same joint as the movement produced by ICMS. The remaining five penetrations had more than one responsive cell, but all the receptive fields were identical and the ICMS evoked movement was also at the same joint. Together, these 15 penetrations are typical of motor cortex penetrations in the rat, that is, usually a deep receptive field around a joint, and a microstimulation evoked movement at the same joint (Figs. 3C+D). The second type of relationship was seen in six tracks where two or more responsive cells with different receptive fields were seen along the track. Five of these tracks had all receptive fields pertaining to the same limb, and one track had fields relating to forelimb and vibrissae. Histological examination of these penetrations showed that they were obliquely oriented and consequently, may have traversed a number of cortical columns.

In 17 penetrations the receptive field and the ICMS-evoked movement were at the same joint and could be analyzed for the nature of input-output coupling. These 17 penetrations were evenly divided into 9 tracks where the ICMS-evoked movement (e.g. elbow flexion) was opposite the direction of the receptive field (e.g. passive elbow extension), and 8 tracks where the direction of the ICMS evoked movement and the receptive field's passive movement were identical.

Sensory Forelimb Cortex

Eleven penetrations were located in sensory cortex and were identified on the basis of forelimb receptive fields and location in granular or dysgranular cytoarchitectonic areas. Of the 82 cells tested in sensory cortex, 57 cells (70%) were responsive, especially to cutaneous inputs (Fig. 3). All penetrations in sensory cortex were oblique to the cortical columns (Figs. 4A+C), which probably accounts for the number of different body parts represented in any one penetration (Figs. 4B+D). Since all the electrode tracks were oblique, and many crossed from one cytoarchitectonic area into another, it was difficult to accurately place the location of responsive cells. Nonetheless, using marker lesions for depth reference and our error estimate for those tracks without lesions, it appeared that both granular and dysgranular areas contain cells responsive to both cutaneous and deep inputs (Figs. 4B+D). There is, however, a marked difference in the relative amounts of deep and cutaneous inputs to both areas with the dysgranular zone receiving a

much higher percentage (74% of responsive cells in dysgranular area) of deep inputs (Fig. 2B).

Other Sensory and Motor Areas

Seventeen electrode penetrations were located in areas other than forelimb e.g. face, neck, trunk and vibrissae. Twenty of the 82 cells (25%) found in these penetrations were responsive to peripheral stimulation. This number is probably low due to the difficulty in distinguishing cell activity related to active vibrissae and face movements from that evoked by passive vibrissae or face movements in the awake rat. One clear finding was the correlation between receptive field and ICMS evoked movement in those penetrations which were parallel to the cortical columns. For example, in one penetration there was a cell responsive to light touch on one side of the nasal opening. Intracortical microstimulation at 1.7 mm deep in the same penetration produced a bilateral flaring of the nostrils.

Discussion

The most obvious finding from this study is the difference in the amount of sensory input to the rostral forelimb area as compared to the caudal forelimb area. A summary drawing of all of the forelimb sensori-motor electrode tracks drawn on a dorsal view of the rat brain is depicted in figure 5. It is evident from this drawing that the caudal forelimb motor area has at least one responsive cell per electrode track whereas, sensory input to the rostral forelimb area is almost non-existent. Recording studies on the monkey have shown that the supplementary motor area receives much less sensory input than the primary motor area (J. Brinkman and Porter, 1978; C. Brinkman and Porter, 1979; Wise and Tanji, 1981). Additionally, the input that it does receive is often bilateral, across multiple joints, or coming from a large area, clearly different from sensory input to M1 (Brinkman and Porter, 1979). In the present study we did not see receptive fields of this type in the rostral forelimb area. It is possible that this reflects a species difference between rat and monkey SMA. It is also possible that some cells with fields of this type exist in rat rostral forelimb area, but we classified them as nonresponsive because of the lack of brisk, well localized responses. Whatever the case, the rostral forelimb area does not appear to be a part of MI. Its lack of sensory input, although much more complete than that seen in the monkey SMA, makes it seem plausible to consider the rostral forelimb area as a part of the SMA in the rat.

There is additional evidence, besides the lack of sensory input, to support this proposal. In the rat, we have found that ICMS medial to the forelimb representation can elicit hindlimb and trunk movements (Neafsey et al., in prepartion), and that there are direct projections from this rostral hindlimb area to the thoracic and lumbar cord (see chapter 4 of this dissertation). These results suggest that there may be a whole body representation in the rostral motor area of the rat cortex, similar to the whole body representation within the monkey SMA (Woolsey et al., 1952; Murray and Coulter, 1976; Coulter et al., 1979; Brinkman and Porter, 1979; Macpherson 1982a, 1982b).

More evidence to support this proposal comes from a lesion study by our laboratory which tested digital usage in rats with caudal or rostral forelimb lesions (see chapter 3 of this dissertation). In this study, the animals had difficulty performing a grasping task for a short period (Average 10 days) following a small lesion of the rostral forelimb area. These results are in agreement with a recent lesion study on the SMA of the monkey where the animals demonstrated transient difficulty performing digital usage tasks (Brinkman, 1984). The combination of all these pieces of information concerning the rostral forelimb area leave little doubt that it is a part of the SMA of the rat.

The present study and that by Donoghue and Wise (1982) have placed the rostral forelimb in the lateral agranular field (AgL). However, it appears that the more medially located rostral hindlimb

and trunk areas are located in the medial agranular field (AgM) (see chapter 3). In addition, retrograde labeling studies have shown that some of the labeled cells following a cervical cord injection extend into AgM, anterior cingulate, and prelimbic cytoarchitectonic areas (Sievert and Neafsey, 1982; Donoghue and Wise, 1982). These data indicate that the rat SMA crosses cytoarchitectonic boundaries, at least that between AgL and AgM. This situation also appears to exist for MI since Sanderson et al (1984) have suggested that the rat MI extends from Agl into AgM, and a number of studies (e.g. Kwan et al., 1978) have shown that the monkey Ml extends from area 4 into area 6.

The second contribution of the present study is the detailed description it provides of the sensory properties of neurons in the primary motor area Ml of the rat. The only previous study in the awake rat (Sapienza et al., 1981) made no mention of the numbers of responsive cells or the differences in receptive field properties seen in cells in different cytoarchitectonic areas. In addition, Sapienza and coworkers stated that there was only a rough correlation between input and output and that comparison with the monkey was difficult. Our results however, indicate that the input-output organization of the rat Ml correlates well with what has been shown in the monkey. For example, we found in 50% of the penetrations the stimulation evoked movement was in the same direction as the passive movement the cells responded to, and in the other half the cases the ICMS evoked movement was in the opposite direction of the passive movement activating the cells. These results are almost identical to those of

earlier studies on the monkey (Fetz and Baker, 1969; Rosen and Asanuma, 1972; Lemon et al., 1976; Murphy et al., 1978; Fetz et al., 1980). In addition, although the quantities of responsive cells are much lower for the rat 32% as compared to 60% or higher for M1 of the monkey (Rosen and Asanuma, 1972; Wong et al., 1978), the quality of the input seems to be similar to that found in the monkey, that is, predominately single joint receptive fields (Rosen and Asanuma, 1972; Lemon and Porter, 1976; Wong et al., 1978; Fetz et al., 1980).

Finally, although this study was not focused on the granular sensory cortex, it does add to existing knowledge about S1 organization. In S1, cutaneous and deep inputs were found in dysgranular and granular areas, but in different proportions. A recent study by Welker et al (1984) on the anesthetized animal has shown only cutaneous input to the granular cortex, but he specifically notes that deep inputs were not tested. Chapin and Woodward (1982) have reported finding only cutaneous inputs in granular cortex with deep and cutaneous inputs reaching dysgranular cortex. The differences between their findings and ours may be due to the definition of and testing regimen for deep and cutaneous inputs. However, several studies on area 3b of the monkey, the homolog of the rat granular cortex (Wise and Jones, 1977), have shown that as many as 20% of the area 3b cells receive deep input (Heath et al., 1976; Hyvarinen and Poranen, 1978), an observation which supports our findings .

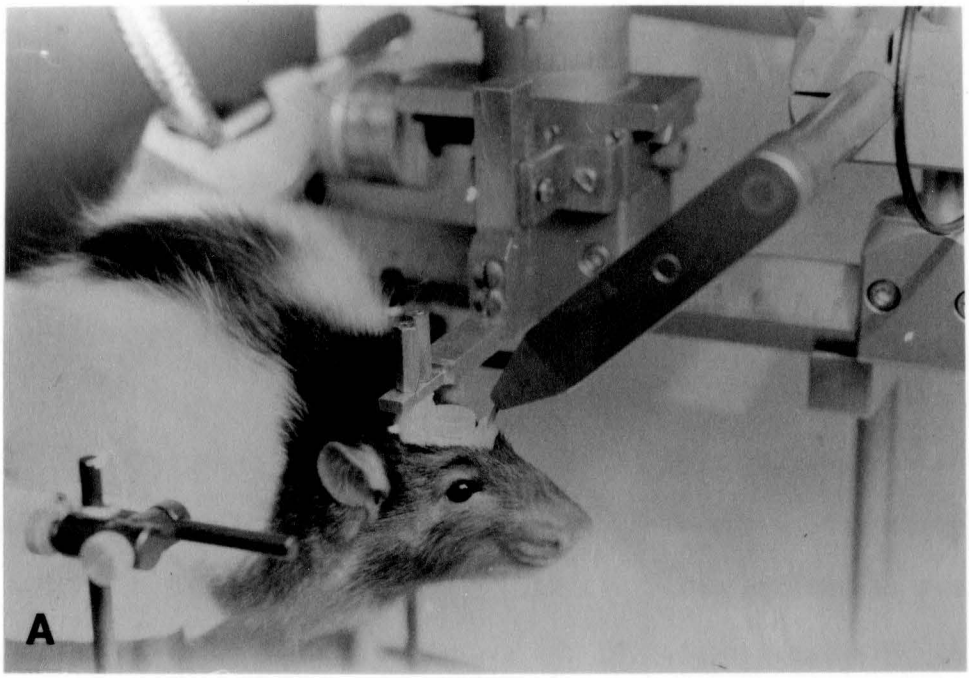
In summary, we have shown that the rostral forelimb area of the rat is probably a part of the SMA of the rat. We have also demonstrated that the M1 representation of the rat is similar to that of the monkey in terms of types of sensory inputs and their relation to motor outputs. Finally, this study contributes additional information about the type of sensory input to the granular and dysgranular regions of S1 in the rat.

Figure 1. Method of head fixation.

A. Picture of rat in head fixation apparatus. Note the three point mounting system and the recording chamber.

B. Dorsal view of rat skull (Paxinos and Watson, 1982) depicting the method of head fixation. Shaded areas represent places where the skull has been removed. Asterisk indicates position of third mounting screw to be imbedded in cement. Dotted lines represent the heads of the two mounting screws which were inserted under the bone and rotated 90 degrees.

C. Lateral view of rat skull showing the location of screws for head fixation. 1=(2-56) screw for attaching to front mounting apparatus, 2 and 5=(1-72) screws for threading into the skull, 3=plastic "beem" capsule, 4 and 6=(2-56) screws with heads ground and inserted under the skull. Line indicates dental acrylic. Drawing reprinted from Paxinos and Watson (1982).


A

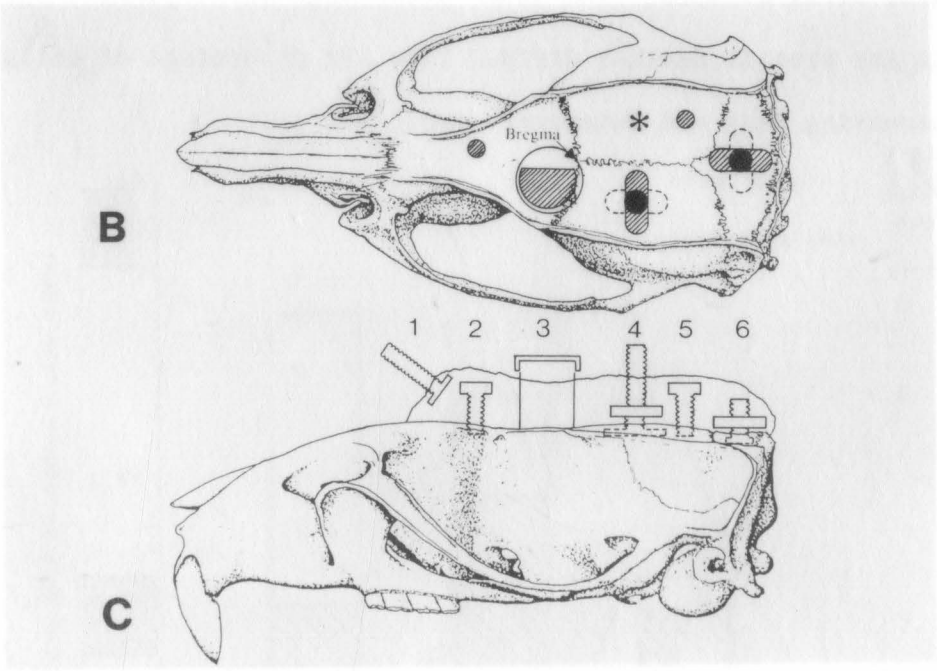
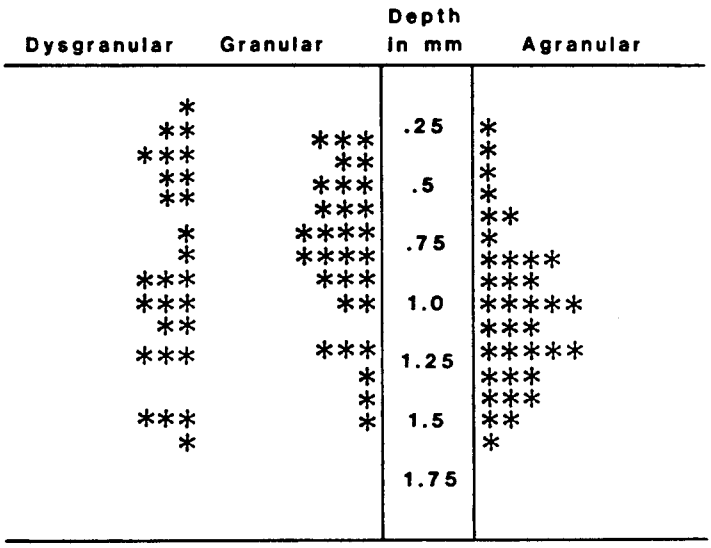

B
Bregma
1 2 3 4 5 6
C

Figure 2. Depth and receptive field characteristics of responsive cells in AgL.

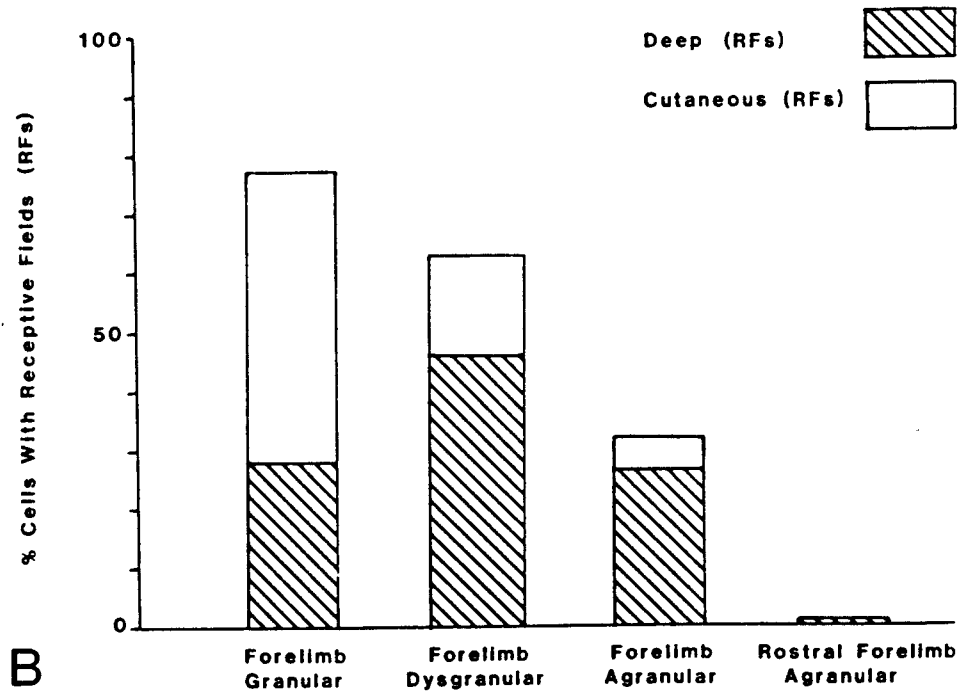
A. Depth histogram of responsive cells for each area, dysgranular, granular and agranular. Each asterisk represents one responsive cell.

B. Histogram depicting the percentages of responsive cells in each cortical area. The total percentage of responsive cells in any area is further divided into the percentage of cells receiving deep and cutaneous input.

Cortical Depths of Responsive Cells



A



B

Figure 3. Electrode penetrations and extracellular recordings in agranular lateral.

A. Nissl stained coronal section through the rostral forelimb area of the left hemisphere. The only responsive cell in this area was found 1.4 mm beneath the surface and was activated during passive elbow flexion of the contralateral forelimb. Lesion marks the depth of the responsive cell and is indicated by the arrow. Vertical line on surface indicates the boundary between AgM and AgL. Bar=1 mm.

B. Extracellular recording trace of cell in Figure 3A. Two bursts of activity occurred during passive elbow flexion. The lower trace is the instantaneous frequency of the cells action potentials. Scale=50 hz on the vertical and 0.5 sec. on the horizontal.

C. Nissl stained section through the caudal forelimb area. A lesion marking the depth of a responsive cell (1.0 mm) is indicated by the arrow. The surface boundaries between cytoarchitectonic areas are marked with vertical lines. Bar=1 mm.

D. Extracellular recording trace of cell in figure 3C. The three bursts of activity occurred during passive wrist extension of the contralateral forelimb. The lower trace is the instantaneous frequency of the cells action potentials. Scale=100 hz on the vertical and 0.5 sec. on the horizontal.

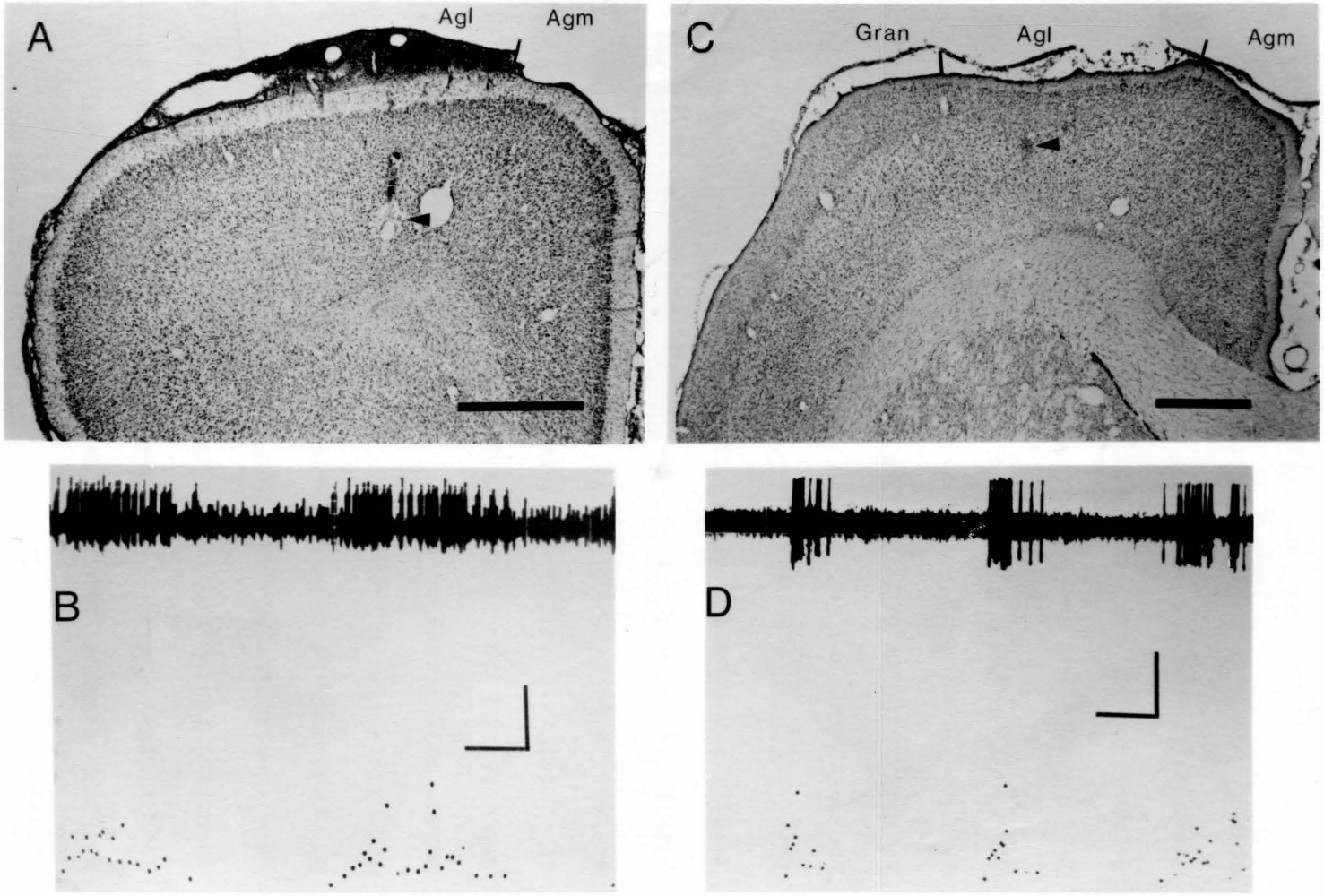

A
Agl
Agm
C
Gran
Agl
Agm
B
D

Figure 4. Sensory cortex penetrations.

A. Nissl stained coronal section containing a penetration through the granular area of the left sensory cortex. Dark arrow indicates lesion at 1.2 mm deep. Surface arrow indicates point of entry of electrode. Cytoarchitectonic areas are delimited by the vertical lines on the surface. Bar=1 mm for A-D.

B. Line drawing of penetration seen in figure A showing receptive fields of cells found along the track. Open circle indicates the lesion. Arrow indicates point of non-responsive ICMS. Cutaneous receptive fields are to the left and deep receptive fields are to the right. Wabd=wrist abduction, D5t=tip of fifth digit, D1-5p=pads of digits 1-5, Palm=ventral surface of hand.

C. Nissl stained section containing a penetration in granular and a penetration in dysgranular cortical areas. Surface arrows mark points of entry. Vertical lines delimit the cytoarchitectonic boundaries. Larger arrows indicate lesions.

D. Line drawing of penetration seen in figure C showing receptive fields of cells found along the track. Cutaneous receptive fields are to the left and deep receptive fields are to the right. Arrows indicate location of ICMS. Farm=forearm, Ef=elbow flexion, Sext=shoulder extension, Sflex=shoulder flexion.

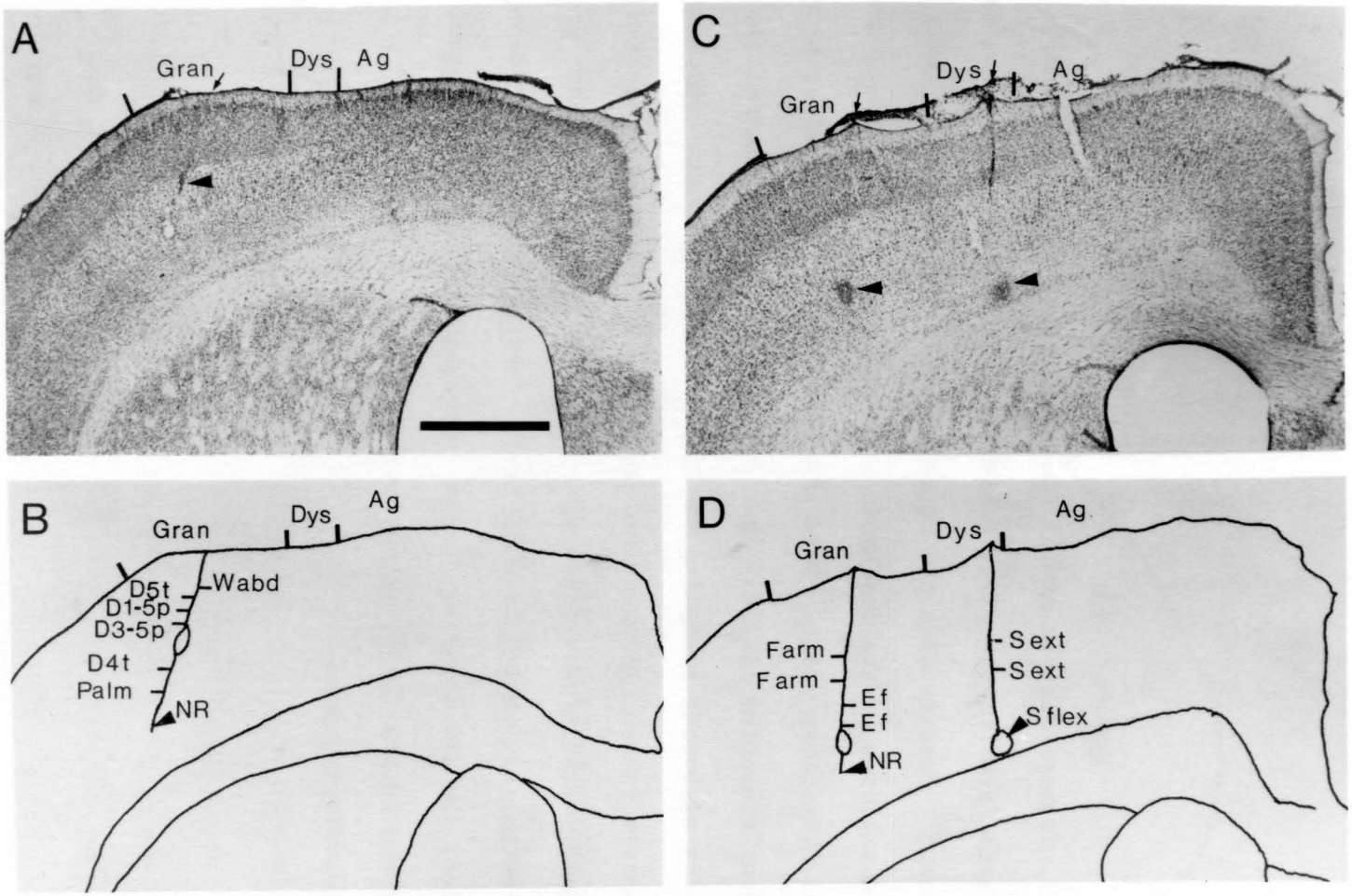
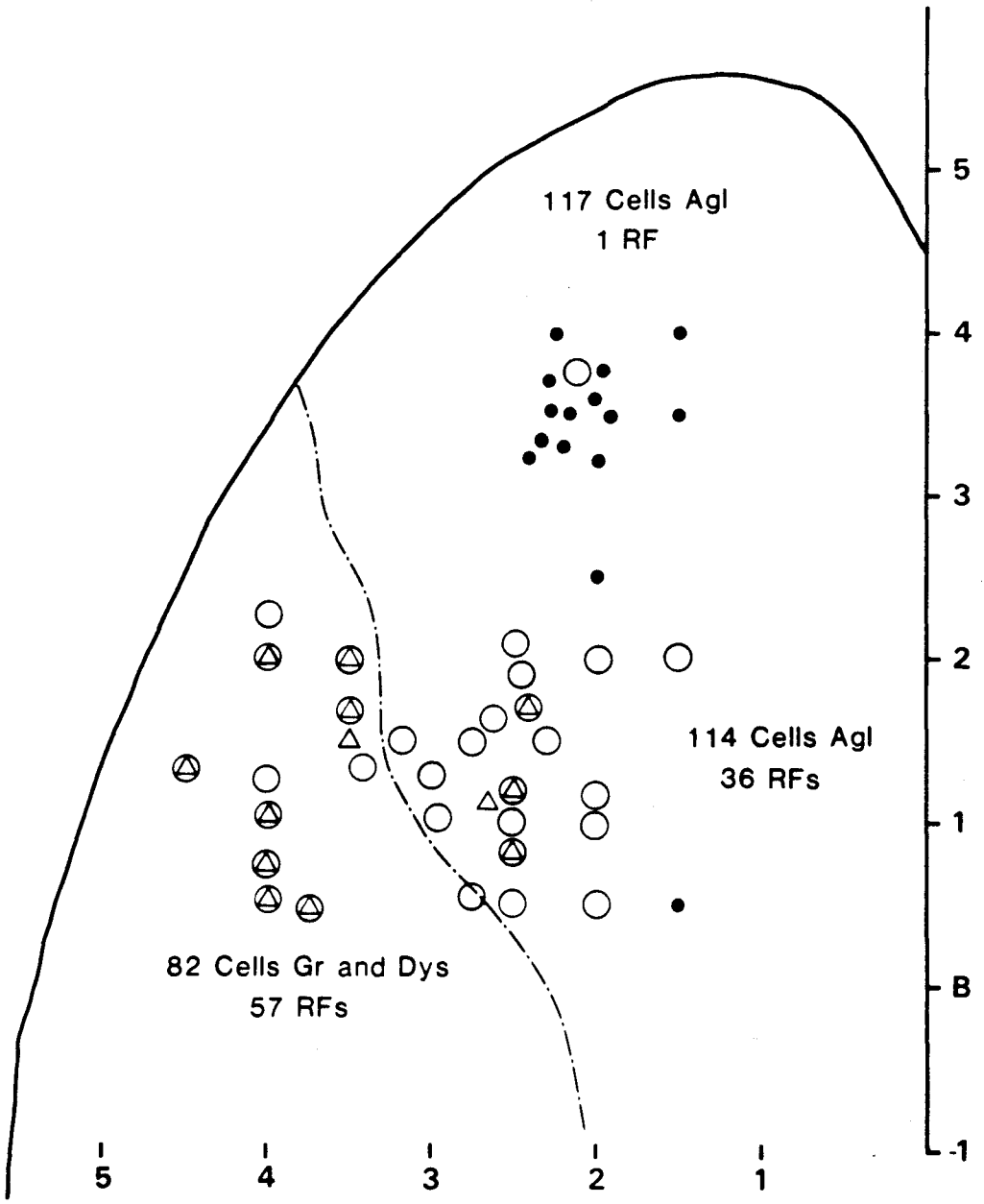

A
Gran
Dys
Ag
C
Gran
Dys
Ag
B
Gran
Dys
Ag
D5t
Wabd
D1-5p
D3-5p
D4t
Palm
NR
D
Gran
Dys
Ag
Farm
Farm
Ef
Ef
NR
Sext
Sext
Sflex

Figure 5. Summary diagram of all the penetrations from ten animals where forelimb receptive fields or ICMS evoked movements were found. Penetrations are depicted on an outline drawing of a dorsal view of the rat brain. Dots indicate penetrations with no responsive cells. Open circles indicate penetrations with deep receptive fields. Open triangles indicate penetrations with cutaneous receptive fields. Dotted line marks approximate border between granular and agranular cytoarchitectonic areas. The cluster of non-responsive electrode tracks at 3.5 mm rostral is located in the rostral forelimb area. B indicates bony surface landmark Bregma. Divisions are in millimeters. RF=receptive field, AgL=agranular lateral, Gr=granular, Dys=dysgranular.


117 Cells Agl
1 RF
114 Cells Agl
36 RFs
82 Cells Gr and Dys
57 RFs
5
4
3
2
1
B
-1
5
4
3
2
1

CHAPTER VIII

DISCUSSION AND CONCLUSIONS

The anatomical connections, sensory input properties, and function in motor behavior of the rat sensorimotor cortex have been studied in the five projects of this dissertation. The primary goal of these projects was to compare the primary motor area (MI) to the rostral forelimb area in an attempt to identify the latter as part of the primary motor area or as a supplementary motor area. Four of the five experiments involved in this dissertation project have also yielded information regarding the anatomical and physiological properties of the primary and secondary somatosensory areas.

Results of behavioral lesion studies on the primary and supplementary motor areas of the primate have indicated that the primary motor area is involved in the control of fine coordinated hand movements, whereas the supplementary motor area is not directly involved in carrying out the movement but instead may play a more important role in initiating the movement (Eccles and Robinson, 1984). The first study was specifically designed to test a rat's ability to perform discrete digital movements before and after small lesions of the rostral forelimb and primary motor areas. Compared to similar size lesions in an area of cortex which is not involved in the chosen

task, both rostral forelimb and MI lesions caused a reduced ability to perform the task. However, the animals with lesions of the rostral forelimb area recovered (within two standard deviations of preoperative mean levels) sooner than the animals with lesions of Ml. In fact, one of the MI lesioned animals never reached recovery in the 90 days of postoperative testing. The control lesioned group showed absolutely no deficits in performing the task when compared to their own preoperative means. The shorter duration of the deficit seen following rostral forelimb lesions is consistent with the results of recent lesion studies on the monkey SMA (Brinkman, 1984). It was concluded from this experiment that the rostral forelimb area is most likely a part of the SMA.

The anatomical studies of this dissertation addressed a number of unanswered questions concerning the topography of rat corticospinal neurons. First, are neurons projecting to the cervical enlargement the only ones present in the rostral forelimb area, or is there a complete somatotopic arrangement within this region of cortex? Do corticospinal neurons send collaterals to widely varying levels of the spinal cord? How does the topography of corticospinal neurons in the limb areas of motor cortex relate to physiological maps generated by ICMS and multiunit sensory evoked response, and what is the correlation between these results and the results of other investigators studying cortical cytoarchitecture? Finally, is the medial agranular cortex part of the limb motor area? The anatomical experiments were designed in an attempt to answer these questions and

consisted of a series of retrograde single and double label experiments, some of which were combined with physiological techniques. The results of these studies have shown that the rostral forelimb area of the rat does contain neurons which project to cervical, thoracic and lumbar levels of the spinal cord, indicating that there is a large part of the rat's body represented within this area of the rat's cortex. This finding suggests the existence of a supplementary motor area in the rat since supplementary motor areas in other species also contain trunk and limb representations separate from the primary motor area (see review by Tanji, 1984). Moreover, this study has shown that the rat sensorimotor areas, unlike those of the monkey, do not possess neurons which have projections to widely spaced levels of the spinal cord. It has also been demonstrated by these experiments that neurons projecting to the cervical and lumbar enlargements are coextensive with MI and SI forelimb and hindlimb areas, as defined by ICMS and sensory evoked multiunit recordings. Concerning the medial border of the limb motor areas, it was found that there were no retrogradely labeled cells in the medial agranular zone. In addition, labeled cells in SII were only seen after a cervical enlargement injection of retrograde tracer. In the rostral motor area neurons were found crossing into AgM, as well as anterior cingulate and prelimbic areas. The significance of this remains unclear. The lack of cervical or lumbar projecting neurons in AgM and the presence of strong projections to the superior colliculus suggests that AgM is a motor area involved in eye and head orienting behavior

instead of limb control as was previously thought. Finally, we have described an area of hindlimb MI cortex which contained labeled cells from a lumbar enlargement injection of WGA-HRP, was responsive to low threshold ICMS, did not respond to peripheral evoked sensory stimulation, and was located medial to the hindlimb granular patch of cortex. This suggests that overlap between hindlimb sensory and motor cortex is not complete. The following four conclusions are drawn from these anatomical findings: The rostral forelimb area is probably the SMA of the rat. The second somatosensory area SII does not contain neurons which project to the mid-thoracic or lumbar levels of the spinal cord. There is a separate MI hindlimb representation which does not overlap with the SI hindlimb representation. AgM is not part of the MI limb representation.

The third study examined the course and terminations of the rat corticospinal tracts, topics of considerable disagreement among various investigators and studies. Using anterograde transport of WGA-HRP, it was found that in addition to the commonly described large contralateral dorsal corticospinal tract, four other smaller corticospinal tracts are present in the rat spinal cord, all of which may reach lumbar levels. Terminations of the corticospinal tracts reached different areas of the spinal gray matter depending on whether the sensory, second somatosensory, motor or rostral forelimb motor area was injected. Although considerable overlap was found between the terminations from any one of the aforementioned areas, a general area of terminations could be found for each specific cortical area

injected. The SI and SII forelimb injections terminated heavily in the dorsal horn of the cervical cord, but SII terminations were limited to the medial part of the dorsal horn. The motor forelimb area injection spared the dorsal horn and lamina VIII, and terminated heavily in the intermediate gray with some terminations in lamina IX of the ventral horn. The rostral forelimb area injection terminated in the same areas as the motor injection with additional heavy terminations in lamina VIII. The rostral forelimb area also had terminations to the lumbar enlargement, confirming the results of the previous retrograde labeling study, which demonstrated a hindlimb and trunk representation in the rostral forelimb area. The hindlimb sensorimotor area terminated in the dorsal horn and intermediate gray, but not in lamina VIII and IX. Finally, ipsilateral corticospinal terminations were present from all areas except the second somatosensory area. Conclusions drawn from this study are that the rat corticospinal tract can reach the spinal cord via a number of pathways. Second, both sides of the spinal cord may be influenced from one side of the sensorimotor cortex via ipsilateral connections. Third, sensory and motor cortical areas influence movement differently by virtue of their strikingly different areas of termination within the spinal cord. Fourth, the rat sensorimotor cortex may have direct corticomotoneuronal connections. Finally, the rostral forelimb area of motor cortex has a different area of termination within the spinal cord than the primary forelimb motor cortex. No comparable data are at present available on the spinal terminations of the monkey SMA.

Sensory, motor and rostral forelimb area cortical projections to the dorsal column nuclei (DCN) were assessed in the fourth study. It was found that the cortex projects somatotopically upon the DCN, in that hindlimb areas of cortex project to the gracilis nucleus and forelimb areas project to the cuneatus nucleus. Moreover, there is a differential projection to nucleus cuneatus from the forelimb sensory cortex as compared to the forelimb motor cortex. The motor area projects to the ventral, rostral and caudal portions of the nucleus, while the sensory cortex projects heavily to the dorsal portions of the nucleus and has a lighter projection to those areas which receive input from the motor cortex. The rostral forelimb area of motor cortex has an extremely light projection to the DCN. It appears that the sensory cortex is involved in modulating well localized, modality specific cutaneous input to the cell bricks area, while the motor cortex seems to be primarily involved in modulating deep, proprioceptive inputs to the reticular zones of the DCN. In addition, the lack of rostral forelimb area terminations to the DCN is in agreement with recent studies on the DCN terminations of the SMA in the monkey. This supplies further evidence that the rostral forelimb area of the rat should be considered as a part of the SMA.

The final study of this dissertation project was designed to assess the sensory response properties of neurons in the rostral forelimb as compared to the primary motor caudal forelimb area. In the course of this study, the SI granular cortex was also investigated. Within the three areas studied, the sensory cortex

received the largest amount of input (70% of cells responsive), the primary motor area was second (30% of cells responsive) and the rostral forelimb area received the least amount of peripheral input (less than 1% of cells responsive). There was also a difference in the modality of input to the two responsive areas, in that the sensory cortex received mostly cutaneous input whereas the motor cortex received mostly deep input. Input-output correlations were also assessed, and it was found that when the electrode was inserted perpendicular to the cortical surface the inputs were near the area which moved when ICMS was performed in the deep layers of the cortex. It was concluded from the results of this study that the rat sensory and motor cortices are similar to the monkey's in terms of input-output correlations. The other major conclusion made is that the rostral forelimb area's lack of sensory responsiveness makes it likely that this region is a part of the supplementary motor area of the rat.

Figure 1. Summary figure depicting the results of studies on the supplementary motor area of the monkey for comparison with the results of these dissertation projects in the rat.

	Monkey SMA	Rat Rostral Forelimb
1. SOMATOTOPY OF CST	A. PROJECTIONS TO CERVICAL, THORACIC AND LUMBAR SPINAL CORD B. FORELIMB, TRUNK AND HINDLIMB MOVEMENTS DURING ICMS	A. PROJECTIONS TO CERVICAL, THORACIC AND LUMBAR SPINAL CORD B. FORELIMB, TRUNK AND HINDLIMB MOVEMENTS DURING ICMS
2. SPINAL PROJECTIONS	UNKNOWN	A. TERMINATIONS TO INTERMEDIATE GRAY AND VENTRAL HORN B. TERMINATIONS TO LAMINA VIII
3. DCN PROJECTIONS	NO TERMINATIONS TO DCN	EXTREMELY LIGHT TERMINATIONS TO DCN
4. LESION DEFICITS	A. BIMANUAL COORDINATION DEFICITS B. FORCED GRASPING C. SHORT DURATION OF SKILLED MOVEMENT DEFICIT COMPARED TO M1	A. BIMANUAL COORDINATION NOT TESTED B. NO FORCED GRASPING C. SHORT DURATION OF SKILLED MOVEMENT DEFICIT COMPARED TO M1
5. SENSORY INPUTS	MUCH LESS PERIPHERAL SENSORY INPUT THAN FOUND IN M1 (14% COMPARED TO 60% IN M1)	MUCH LESS PERIPHERAL SENSORY INPUT THAN FOUND IN M1 (1% COMPARED TO 30%)

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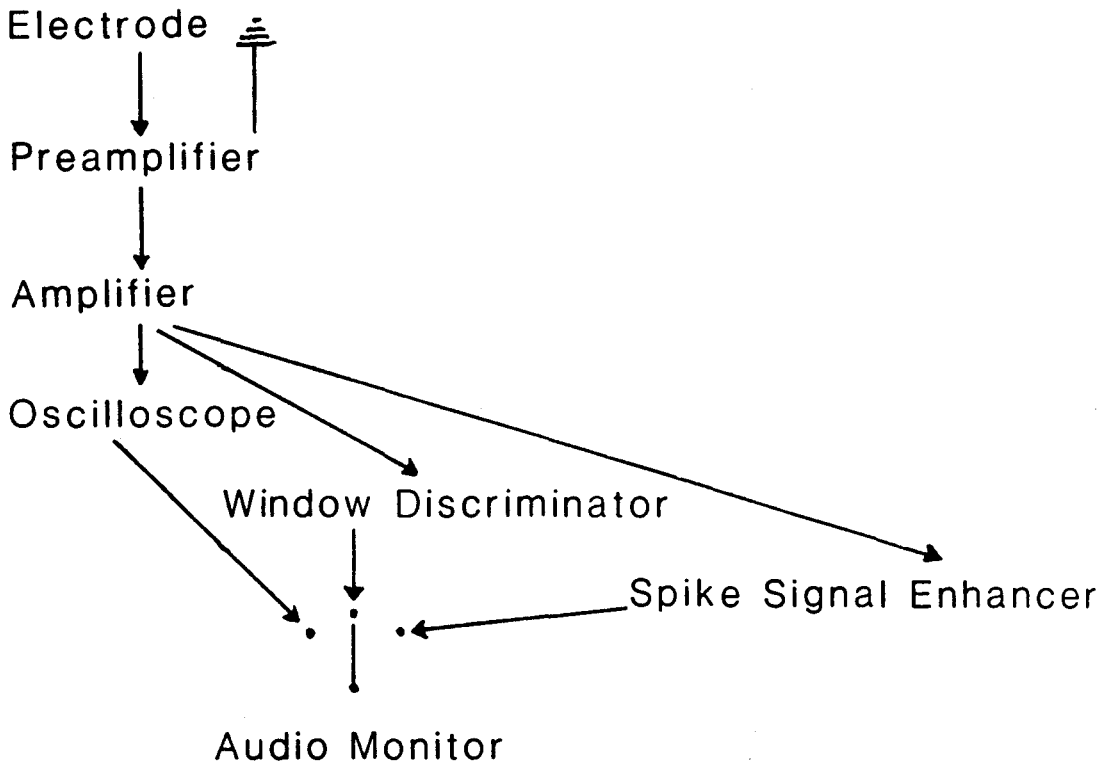
Appendix A

Appendix A

A. The evoked sensory response recording technique employed in this dissertation was performed in the following manner: The electrode was inserted into the cortex to a depth of approximately 0.5 mm, and evoked multiunit activity was examined during manipulation of body parts. Single unit recording utilized a smaller electrode tip and was performed through the entire depth of the cortical gray matter. The recording circuit is diagrammed in figure A.

B. The micromapping technique employed in this dissertation utilized the following parameters: 350 hz, 300 msec trains, and 0.25 msec pulses. The depth of the electrode in the rat brain cortex was approximately 1.7 mm. A diagram depicting the stimulation circuit is shown in figure B. In this circuit the dual function constant current stimulus isolation unit (SIU) was used in order to obtain a constant current. The accurate measurement of stimulation current was obtained by utilizing the relationship of Ohm's law. Voltage (V)=Current (I) x Resistance (R). Since we are using a known resistance (10,000 ohms) and reading the voltage directly from the oscilloscope screen, we can determine the value of the stimulus current.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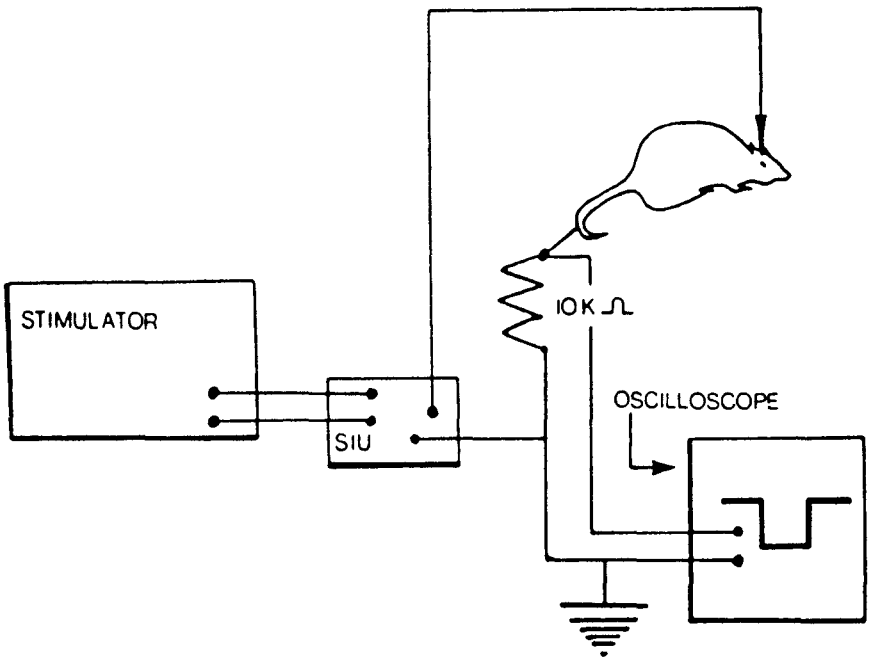
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B

APPROVAL SHEET

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the 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.

Dec. 5, 1984
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