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AN INVESTIGATION OF THE ORGANIZATION OF
THE CEREBRAL CORTEX IN THE RAT: THE USE OF
HORSERADISH PEROXIDASE AND FAST BLUE TO EVALUATE
THE COLUMNAR HYPOTHESIS

A Thesis Presented

By

ANTON BLAINE DODEK

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May 1984

Department of Psychology

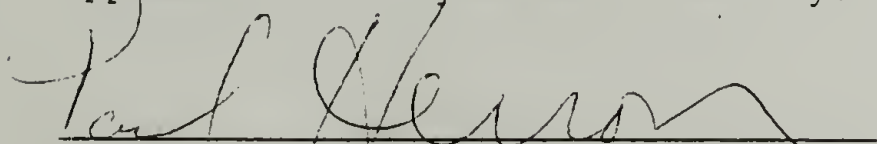
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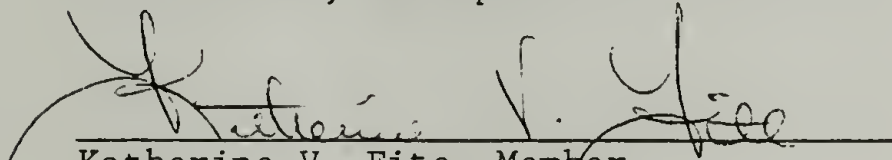
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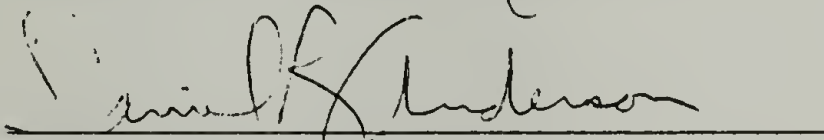
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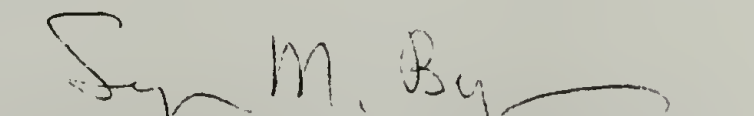
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ABSTRACT

An Investigation of the Organization of
the Cerebral Cortex in the Rat: The Use of
Horseradish Peroxidase and Fast Blue to Evaluate
the Columnar Hypothesis

May 1984

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The topographical organization of corticocortical and corticobulbar neurons in the sensory and motor areas in the rat were examined using the retrograde transport of horseradish peroxidase and of the fluorescent dye, fast blue (FB). HRP injections into the first motor area (MI) resulted in vertically-oriented clusters of labeled cells (250-400 μm wide) in Layers II, III, V and VI of the ipsilateral first somatic sensory area (SI). FB injections into the fifth motor nucleus (Mo5) of the brainstem demonstrated the distribution of corticobulbar cells in the sensorimotor cortex. These injections resulted in slabs of labeled cells concentrated in Layer V. In animals which received both injections, an overlap of the corticocortical and corticobulbar systems in the sensorimotor cortex was evident. Areas exist in MI and SI that contain both corticocortical and corticobulbar efferents. The

results of this study are taken as support of the columnar hypothesis of cortical organization. Because certain corticofugal cells are restricted to specific laminae, dual tracer studies may show the borders of these columns more distinctly.

TABLE OF CONTENTS

ABSTRACT	iii
LIST OF TABLES	
LIST OF FIGURES	
Chapter	
I. INTRODUCTION	1
Physiological Evidence Supporting the Columnar Hypothesis	3
Anatomical Evidence Supporting the Columnar Hypothesis	4
Evidence of Columnar Organization in All Cortical Areas	5
Somatosensory cortex	5
Visual cortex	6
Auditory cortex	6
Motor cortex	7
Connectivity Data Supporting the Columnar Hypothesis	8
Purpose of the Present Study	9
II. METHODS	12
Animals and surgical procedure	12
Neuroanatomical tracers	12
Injection techniques	13
HRP injection in MI	13
FB injection in Mo5	14
HRP injection in SI or MI with FB injection in Mo5	14
Histological procedure	14
III. RESULTS	17
Injection of HRP into MI	17
Injection placement	17
Corticocortical projections	20
Injection of FB into Mo5	20
Injection placement	20
Corticobulbar connections	34

Injection of HRP in SI and FB in Mo5	34
Injection placements	34
Interaction of corticobulbar and corticocortical systems	34
Injection of HRP in MI and FB in Mo5	41
Injection placements	41
Interaction of corticobulbar and corticocortical systems	41
IV. DISCUSSION	48
Methodological Considerations	48
Support for the Columnar Hypothesis in the Rat Sensorimotor Cortex	49
The Cortical Column Defined	50
Usefulness of Extensive Comparative Data	52
Conclusion	53
.	
REFERENCES	56

LIST OF TABLES

1. Summary of Injection Placements and Location of Labeled
cells 18

LIST OF FIGURES

1.	Representation of the Body surface of the Rat as Determined by Cytoarchitecture and Microstimulation Mapping	19
2.	Photomicrograph of a Coronal Section Depicting an HRP Injection site in MI	22
3.	Photomicrograph of a Coronal Section Illustrating the Dendritic Configuration of SI Pyramidal Cells (Layer III) labeled with HRP Following Injection in MI.	24
4.	Photomicrograph of a Coronal Section Showing HRP-labeled Cells Grouped into Vertically Oriented Clusters in SI Following Injection in MI	26
5.	Photomicrograph of a Coronal Section Showing HRP-labeled Cells Grouped in Vertically Oriented Clusters in MI Following Injection in a Homotopic Area of the Contralateral Hemisphere	28
6.	Montage Demonstrating the Extent of Labeling in Animal TD 1 Following HRP Injection in MI	29
7.	Montage Demonstrating the Extent of Labeling in Animal TD 2 Following HRP Injection in MI	30
8.	Montage Demonstrating the Extent of Labeling in Animal TD 3 Following HRP Injection in MI	31
9.	Montage Demonstrating the Extent of Labeling in Animal TD 4 Following HRP Injection in MI	32
10.	Montage Demonstrating the Extent of Labeling in Animal TD 5 Following HRP Injection in MI	33
11.	Photomicrograph of a Tangential Section Illustrating a Typical FB Injection Site in the Trigeminal Nuclei of the Brainstem	36
12.	Reconstruction of the distribution of FB-labeled Cells in the Sensorimotor Cortex Following Injection in Mo5	37
13.	Photomicrograph of FB-labeled fluorescent Cells in MI Following Injection in Mo5	39
14.	Reconstruction of the Distribution of HRP- and FB-labeled Cells in a Tangential Section of the Sensorimotor Cortex Following HRP Injection in SI and FB Injection in Mo5	40
15.	Reconstruction of the Distribution of HRP- and FB-labeled Cells in a Tangential Section of the Sensorimotor Cortex Following HRP Injection in MI and FB Injection in Mo5	42
16.	Double-labeling in SI Following an HRP Injection in MI and FB Injection in Mo5. Two identical Microphotographs: A: fluorescent Wavelength of 360 nm; B: normal light	45
17.	Schematic representation of the Functional Heterogeneity of the Sensorimotor Cortex as Determined by the Distribution of Corticocortical and Corticobulbar Cells	47

C H A P T E R I

INTRODUCTION

For many years extensive research efforts have focused on the role of the cerebral cortex in the elicitation and control of behavior. By describing the attributes of neuronal populations, a better understanding of the functional connectivity of the cortex has developed. This knowledge has aided in the elucidation of the physiology of behavior. Certain structural features of the cerebral cortex such as its horizontal lamination have long been established. In 1909 Brodmann described six cytoarchitecturally distinct layers within the cerebral cortex. The outermost layer, Layer I, contains few, relatively small cells and stains lightly in color. Layer II consists of a considerable number of small densely packed stellate cells and has a granular appearance. Layer III is a relatively wide lamina and contains pyramidal cells of varying sizes. The inner granular layer, Layer IV, is composed of densely packed stellate cells. Layer V is considered the ganglionic layer and is characterized by the presence of pyramidal cells. And, finally, Layer VI is a polymorphic layer consisting of cells of different forms.

Despite intensive efforts, certain aspects of the structural and functional organization of the cerebral cortex remain controversial. As Towe (1975) mentioned there are basically three

theories of cortical organization: topographic, columnar, and cytoarchitectonic. According to the topographic hypothesis, a small shift in the site of peripheral stimulation would result in a small shift of maximal cerebral activity. Similarly, a smaller shift in the site of stimulation causes a smaller shift in the site of maximal cerebral activity. In opposition to this theory is the columnar hypothesis which asserts that the cortex is divided into discrete units. Marked homogeneity exists within these units and there are definite distinctions between units. Therefore, a small shift in the site of peripheral stimulation would result in either no change in the site of maximal cerebral activity (same column) or in a marked shift (different column). And, thirdly, the cytoarchitectonic theory states that cytoarchitectonic differences underlie functional differences. Moreover, this hypothesis asserts that there is uniformity of function within a cytoarchitectonic field.

It is the columnar hypothesis, first proposed by Lorente de No (1938), that has generated the most rigorous research in this field. Lorente de No (1938) presented evidence that the vertical spread of certain dendritic and axonal arborizations were responsible for a vertically-oriented functional cortical unit:

"Studies on the fine structure of the cortex have revealed that, although in architectonic pictures the horizontal stratification seems to be the most important fact in cortical organization, the intracortical connections are established chiefly in vertical directions so that the whole vertical section of the cortex must be considered as a unitary system. The cortical cells are arranged in

vertical chains and the architectonic layers indicate only where the bodies of cells, which are similar links in the chains, are located. But these cells, by means of long dendrites, establish connections in other layers." (Lorente de No, 1938)

Physiological Evidence Supporting the Columnar Hypothesis

Many years later Mountcastle (1957) and Powell and Mountcastle (1959a, b) presented physiological evidence showing distinct transitions in response patterns in the horizontal plane of the cortex. In the 1957 study, Mountcastle performed 59 experiments on anesthetized cats involving the modality and topographic characteristics of 685 single neurons. These authors concluded that an elementary cortical unit of organization is comprised of neurons organized in vertical columns extending from Layer II to Layer VI. Cells within this unit were activated by stimuli applied to the same class of peripheral receptors from similar peripheral receptive fields and at latencies which were not significantly different for cells at various layers. Furthermore, Powell and Mountcastle (1959a, b) obtained similar data using macaque monkeys as subjects. All neurons, responsive to mechanical stimuli, were grouped into one of four categories according to their responses. The four categories included hair, pressure, joint, and fascia. Those cells responsive to hair and pressure receptors were termed cutaneous while joint and fascia responding neurons were called deep.

Anatomical Evidence Supporting the Columnar Hypothesis

In addition to the physiological evidence reported by Mountcastle et al., other researchers have found anatomical data supporting the existence of the cortical column (Jones and Powell, 1973; Jones, 1975a, b). This evidence is derived mainly from Golgi studies and demonstrates the existence of horizontal layers within the cortex which are unified by pyramidal and stellate cells whose axonal and dendritic patterns are essentially vertically oriented. Hence, interneurons with vertically distributed axonal and dendritic arborizations cross the cortical layers and connect constituent neurons (Jones and Powell, 1973).

Many of the morphological characteristics of these interneurons have been determined. Jones and Powell (1970) found that afferent input synapses mainly on stellate cells in Layers IV, IIIb, and upper II of the cortex in the monkey. As Jones (1975b) demonstrated in a later study using squirrel monkeys stellate cells are located in Layer IV at the termination of specific thalamic afferents. Moreover, Jones (1975b) has distinguished between several cell types in the cortex. The neurons he termed types 3 and 7 have the necessary distribution of axonal and dendritic processes to receive afferent terminals from the thalamus. Located mostly in Layers II and upper III, the type 3 cell axon may travel as far as Layer V and, therefore, is capable of exciting a large unit of cortex. Thus, the characteristics of type 3 stellate neurons are conducive to distributing afferent information entering the upper layers of the column to lower cortical

layers. In addition, the type 7 cell, located in Layer IV, has similar attributes.

Evidence of Columnar Organization in All Cortical Areas

The characteristics of columnar organization appear to be present throughout the cortex. Data supporting the columnar hypothesis has been reported in studies dealing with various cortical areas including somatic sensory, visual, auditory and motor.

Somatosensory cortex

In the somatic sensory cortex, studies determined the existence of columns electrophysiologically by oblique microelectrode penetrations. Evidence of columnar organization resulted when receptive fields shifted due to increased depth of the penetration. These studies were performed in a variety of species and under differing experimental conditions. For example, several investigators have reported columnar organization in the first somatosensory cortex (SI) of unanesthetized, neuromuscularly blocked macaque and squirrel monkeys (Werner and Whitsel, 1968; Mountcastle et al., 1969; Whitsel, Dreyer, and Ropollo, 1971; Dreyer et al., 1975). Also, evidence has been found in support of the columnar hypothesis in SI of waking, behaving macaque monkeys (Carli, Lamotte, and Mountcastle, 1971) and in anesthetized neonatal (Armstrong-James, 1975) and adult rats (Welker, 1971). Furthermore, columnar organization has been observed in the second somatic sensory cortex (SII) in anesthetized cats

(Carreras and Anderson, 1963) and in SI in neuromuscularly blocked macaque monkeys (Whitsel, Petrucelli, and Werner, 1969).

In the rat, a special anatomical cell distribution was found in SI which may provide a morphological basis for the columnar hypothesis. Woolsey and Van der Loos (1970) found that each sinus hair of the contralateral face is physiologically correlated with a particular column of cells that is shaped in a "barrel" form.

Visual cortex

Some of the most extensive evidence for the existence of cortical columns is derived from the visual cortex. The elegant work of Hubel and Wiesel (see 1977 review) has provided evidence for columnar processing in area 17 of the visual cortex. Using electrophysiological and anatomical methods, these researchers discovered vertical slabs of cells that respond alternately to left eye and then right eye stimuli. In horizontal sections, the visual cortex manifested alternating stripes about 400 μm thick. This pattern of ocular dominance columns has been supported by autoradiographic data (Wiesel et al., 1974) and by data obtained in studies of tangential microelectrode penetrations showing abrupt transitions (Albus, 1975).

Auditory cortex

Electrophysiological evidence exists for columnar organization in the primary auditory cortex. Abeles and Goldstein (1970) recorded from cells by passing a microelectrode down a vertical column. The cells they encountered responded to a nearly identical frequency.

Motor cortex

The motor cortex has also been shown to possess characteristics of the columnar hypothesis. Asanuma and Rosen (1972a, b) produced movements of distal joints in cells arranged in a vertical column 0.5-1.0 mm in diameter by using intracortical microstimulation. These researchers were also able to define afferent input to motor cortical cells in the direct surroundings by recording through the stimulating electrode. In a follow-up study, Asanuma and Rosen (1973) used two microelectrodes to record and stimulate simultaneously. They found that stimulation in the upper cortical layers produced excitation locally as well as in lower layers in cells vertically oriented towards each other. This column of neurons was approximately 1 mm in diameter and was bordered by a pericolumnar zone of inhibition. When deeper layers were stimulated local excitation and the same pericolumnar inhibition resulted.

Hence, it has been well established that there exists in the cerebrum a certain amount of vertical organization. However, the definition of such a cortical column is somewhat vague. The original hypothesis of Lorente de No (1938) was of a functional unit composed of a vertical chain of interconnecting neurons. On the other hand, Jones (1975b) defined two types of columns anatomically: one of a comparatively small diameter (250 μm) unified by the input of a common vertically-oriented stellate cell and the other of a larger diameter (1.0 mm) unified by the input from one thalamic afferent fiber. He concluded that the larger column was the functional

Concl:

cortical unit since its dimensions were better correlated with the physiological data. However, there are some criticisms of the columnar hypothesis. In Towe's opinion (1975), this theory lacks critical support to be considered valid. He argued that the following data are necessary to evaluate the columnar hypothesis more fully: the number of distinct modalities, the number of columns for each modality, and the exact sizes and shapes of the columns. At present, it is unclear what physiological characteristics determine the boundaries of a vertical unit and whether these boundaries are sharp or fuzzy. As Lemon and Porter (1976) demonstrated in the motor cortex of monkeys some cortical units overlap and may gradually fade into each other when studied physiologically. Clearly a better description of the attributes of a cortical column is needed in order to increase our understanding of the functional connectivity of the cortex and its role in behavior.

Connectivity Data Supporting the Columnar Hypothesis

One approach to this problem has been the use of anterograde and retrograde tracing methods. Much information has been gathered concerning cortical connections and the columnar hypothesis by employment of techniques such as horseradish peroxidase (HRP) histochemistry, autoradiography, and fluorescent tracing techniques. Jones, Burton, and Porter (1975), using tritiated amino acids (anterograde) and HRP (retrograde) in monkeys, found that commissural and corticocortical fibers terminate in discrete terminal groupings.

Also, these researchers reported that the cells of origin of these fibers are arranged in distinct clusters 0.5-1.0 mm in width. In a later study, Jones, Coulter and Wise (1979) found that the bundles of callosal fibers which terminate in column-like zones in SI originate from neurons in Layer IIIb and form reciprocal connections. Moreover, studies concerning corticotectal (Wise and Jones, 1977) and corticothalamic (Jones, Wise, and Coulter, 1979) connections add credence to the columnar hypothesis. Wise and Jones (1977) injected HRP into the rat superior colliculus and found labeled cells in SI that were clustered in columns 250 μm in the mediolateral plane and 1.0 mm in the anteroposterior dimension. In the monkey, Jones, Wise and Coulter (1979) found that aggregates of cells within the ventrobasal complex of the thalamus project to cortical zones less than 1.0 mm in width. Adjacent columns in SI were connected with clusters of cells situated in adjacent lamellae of the ventrobasal complex. This study employed both autoradiographic and HRP histochemical techniques.

Purpose of the Present Study

The characteristics of the cortical column as determined physiologically differ from those suggested by the anatomical evidence. The column diameter reported in anatomical studies is approximately 250-600 μm . This is in contrast to the physiologically determined column dimension of approximately 800-1000 μm (Mountcastle, 1974). Furthermore, the physiological evidence describes the column boundaries as more distinct than the anatomical data. Thus, the present

study was designed to elucidate some of the remaining questions concerning the organization of the cortex and the columnar hypothesis by utilizing the retrograde tracers HRP and the fluorescent dye, fast blue (FB). By evaluating the relevance of the columnar hypothesis to cortical organization a better understanding of the connectivity of the cortex and the physiology of behavior may be obtained. Much work has been completed concerning the connections of the somatosensory cortex. For example, Jones and Powell (1973) reported that SI and SII are reciprocally connected in the cat. ✓ Wise (1975) reported that in the rat neuronal clusters in SI project to homotopic clusters in contralateral SI and SII. Furthermore, Killackey (1973) found clustering of thalamocortical fibers from the VPL nucleus ending in Layer IV of each barrel of a barrel subfield in the mouse. In a more extensive study of cortical connections, Jones and Wise (1977) reported that specific corticofugal fibers are confined to certain layers of the cerebral cortex. These authors reported that corticopontine, corticorubral, and corticobulbar fibers arise from pyramidal cells in middle Layer V whereas corticocortical fibers originate from Layer III pyramidal cells.

In the present study, dual tracer injections were used to further research the connections between MI, SI, and the fifth motor nucleus of the brainstem (Mo5) to determine the validity of the columnar hypothesis and to refine the definition of a cortical column. Since it is known that corticocortical cells and corticobulbar cells are situated in different layers such a paradigm is

useful in studying both the vertical and horizontal organization of the cortex. By analyzing two different types of corticofugal connections in one area of the cortex, it should be possible to better evaluate the columnar hypothesis. In order to substantiate this hypothesis, the results of this study must meet certain criteria. Firstly, injection of a tracer must result in clusters of groupings of cells resembling a cortical column. Although it is not necessary for the dimensions of this clustering to be strictly defined, there must be definite zones of labeled neurons segregated from each other by areas devoid of labeled cells to support the columnar hypothesis. Random labeling of cells would clearly negate the columnar hypothesis. The patterning of the two types of label may take one of three forms. For example, following an injection of FB into Mo5 and HRP into SI there may be total overlap of areas containing the two labels in MI. Conversely, MI may contain a population of FB-labeled cells totally segregated from clusters of HRP-labeled cells. Or, perhaps, clusters of HRP-labeled cells may partially overlap FB-labeled neurons. If, however, there exists a random patterning of the two labels the validity of the columnar hypothesis would be questionable.

C H A P T E R I I

METHODS

Animals and surgical procedure

Ten albino male rats weighing 150-250 g were used as subjects and were anesthetized by an intraperitoneal injection of 2.5 ml/kg Equithesin. The animals were placed in a stereotaxic instrument and an incision made in the skin down the midline of the skull. After folding back the skin, the muscle and fascia were dissected away. The bone (approximately 4 mm in area) was then removed with a dental drill and rongeurs exposing the dura above the left somatosensory cortex (SI), the left side of the motor cortex (MI), or the cerebellum (for brain-stem injections) depending on the injections made. The MI-SI boundary was determined electrophysiologically in pilot studies and confirmed by histology. At this point, the dura was removed by opening a hole with a .22 gauge needle and then dissecting it away with forceps. The exposed cortex was covered with saline or agar at 42°C.

Neuroanatomical tracers

Neuroanatomical tracing methods take advantage of the neuronal properties of axonal transport. Through a network of microfibrils and microtubules the neuron is capable of transporting proteins and other essential molecules in either an anterograde or retrograde direction relative to the cell body. Exogenously administered neuroanatomical tracers can be taken up at axon terminals through

endocytosis and transported back to the cell body where they accumulate. Depending on the tracer used, specific methods are used to visualize the labeled cell bodies resulting from an injection. In this study, HRP, an enzyme which forms a blue reaction product following a histochemical reaction with tetramethylbenzidine was used as one tracer. The second tracer, fast blue, is visualized when exposed to a fluorescent excitation wavelength of 360 nm.

Injection techniques

The ten animals were divided into three groups. Five rats received only an HRP injection in MI. A FB injection in Mo5 was given to two animals. And three animals received both an HRP and a FB injection.

HRP injection in MI. A HRP micropipette was inserted into the left motor area normal to the surface of the cortex at a depth of approximately 1700 μm . The depth of the motor cortex which is slightly more than that of the sensory cortex was determined in pilot studies. HRP micropipettes were made from glass capillaries pulled to a tip diameter of approximately 100 μm (see Mori et al., 1981). The tip of the pipette was dipped in liquid paraffin (m.p. 57°C) allowing the paraffin to be drawn into the pipette by capillary action. The paraffin at the outer 250 μm of the tip was then dissolved in ether for 15 minutes. After a two hour waiting period to ensure that the ether had evaporated the newly exposed tips were gently packed with crystalline HRP. The micropipette remained in the cortex for 30 minutes during which time the crystalline HRP diffused

into the brain. During the diffusion, the micropipette was raised 300 μm every five minutes to ensure that all layers of the cortex would be labeled.

FB injection in Mo5. A pressure injection of FB (4% solution) was made using a 1 μl Hamilton syringe and a microdrive assembly attached to the stereotaxic apparatus. The coordinates of the trigeminal motor nucleus were determined stereotaxically (Paxinos and Watson, 1982). One microliter of the tracer was injected over a period of 30 minutes.

HRP injection in SI or MI with FB injection in Mo5. For these experiments, the HRP injection was performed as described above for MI injections. The somatosensory injection was placed in the barrel subfield of SI as described by Welker (1976). Three days prior to the HRP injection in SI or MI, FB was injected into the trigeminal motor nucleus of the pontine region of the brainstem. The protocol for the FB injection in Mo5 is as described above.

Histological procedure

The MI injections were followed by a 48 hour postoperative survival period. The animals were anesthetized with 1 cc Nembutal and perfused transcardially with three solutions: 50 ml saline, 500 ml fixative (1.25% glutaraldehyde, 1% paraformaldehyde in a 0.1 phosphate buffer at pH 7.4 at 21°C), and 500 ml of the same buffer at 4°C to which 10% sucrose was added. The brain was then excised and stored in the 10% sucrose buffer solution over night at 2-5°C. Coronal sections of 40 μm in thickness were cut serially using a

freezing microtome, and reacted according to the tetramethylbenzidine (TMB) protocol (Mesulam, 1978) for demonstrating the HRP reaction product. According to this procedure, the sections are briefly washed in three changes of distilled water. Free-floating sections are then immersed in a pre-reaction soak for 20 minutes at room temperature. The pre-reaction solution consists of two solutions which are mixed together immediately prior to introduction of the sections. Solution A contains 92.5 ml distilled water, 100 mg sodium nitroferrocyanide, and 5 ml of 0.0 acetate buffer at pH 3.3. Solution B contains 5 mg TMB in 2.5 ml absolute ethanol. Following the pre-soak, the tissue undergoes the enzymatic reaction for 20 minutes at 25°C. This is initiated by adding 2-4 ml of 0.3% H₂O per 100 ml of the medium. Following the reaction the sections are rinsed in six 5-minute changes of acetate buffer solution (pH 3.3) before mounting. The sections were mounted on chrom-alum subbed slides, air dried, and counterstained with neutral red.

Animals which received FB and HRP injections or a FB injection only were given a six day survival time following the FB injection. This allowed three days for the transport of HRP in the dual injection experiments. These animals were perfused as described above for the MI injection. However, after excising the brain the cortex was removed and flattened (see Welker, 1971). Three sets of 40 µm sections (2 cortical; 1 brainstem) were cut tangentially using a vibrotome immediately following the perfusion. One cortical set was reacted according to the TMB protocol (Mesulan, 1978) for

demonstrating the HRP reaction product, mounted on chrom-alum subbed slides, air dried, and counterstained with neutral red. The second set of cortical sections was immediately mounted on chrom-alum subbed slides after sectioning and air dried. Without cover-slipping these sections were analyzed using a fluorescent attachment to a light microscope. Under an excitation wavelength of 360 nm, FB fluoresces blue. Photomicrographs were taken recording the distribution of FB-labeled cells. Sections reacted with TMB were also analyzed for FB labeling prior to counterstaining. A third set of 40 μ m sections consisted of brainstem sections confirming the FB injection site in Mo5. Sections not reacted according to the TMB procedure were counterstained with thionin. The data was analyzed for the pattern and distribution of labeled cells in MI and SI. The number and distribution of labeling was recorded either by microphotographs (FB-labeled cells) or plotted under a light microscope (HRP-labeled cells).

C H A P T E R I I I

RESULTS

The results of this study are separated into four classifications: 1) the distribution of labeled cells in SI as determined by HRP injections in MI; 2) the distribution of corticofugal cells in MI and SI as determined by FB injections in Mo5; 3) the interaction of the distribution of FB and HRP labeled cells in MI following injections in SI and Mo5; 4) the interaction of the distribution of FB and HRP labeled cells in SI following injections in MI and Mo5. For each set of data, descriptions of the injection placements will be followed by an analysis of the distribution of labeled neurons. Table 1 summarizes these data.

Injection of HRP into MI

Injection placement. The first somatosensory cortex (SI) of the rat is heterogenous; consisting of granular cortex and agranular areas. The granular cortex, characterized by a densely packed layer of stellate cells (Layer IV), accounts for the majority of SI cortex. Agranular cortex contains relatively few Layer IV stellate cells and does not have a granular appearance. Within this granular layer there exists the cortical "barrel" field described by Welker (1976). Each cortical barrel is responsive to a single contralateral vibrissa. The motor area of the rat lies rostral and medial to SI and a second somatosensory area (SII) lies ventrolateral to SI (Figure 1).

TABLE 1
SUMMARY OF INJECTION PLACEMENTS AND LOCATION OF LABELED CELLS

ANIMAL	TRACER	INJECTION LOCATION	SIZE (μm)	LABELED-CELLS
TD 1	HRP	MI	1400 x 600	FL, TRIG, SII
TD 2	HRP	MI	1000 x 600	FL, TRIG, SII
TD 3	HRP	AG _m /MI	900 x 600	FL, TRIG, SII, TR
TD 4	HRP	AG _m /MI	1100 x 600	FL, TRIG, SII
TD 5	HRP	MI	900 x 750	FL, TRIG, SII, TR
TD II	FB	Mo5	1500 (in diam)	AG _m , MI, FL, TRIG
TD III	FB	Mo5	1350 (in diam)	AG _m , MI, FL, TRIG
TD IV	HRP FB	FL Mo5	480 x 600 1500 (in diam)	MI, SII AG _m , MI, FL, SII
TD VI	HRP FB	MI Mo5	440 x 540 1600 (in diam)	FL, SI AG _m , MI, FL, TRIG
TD VII	HRP FB	FL Mo5	480 x 600 1500 (in diam)	MI, SII AG _m , MI, FL, TRIG

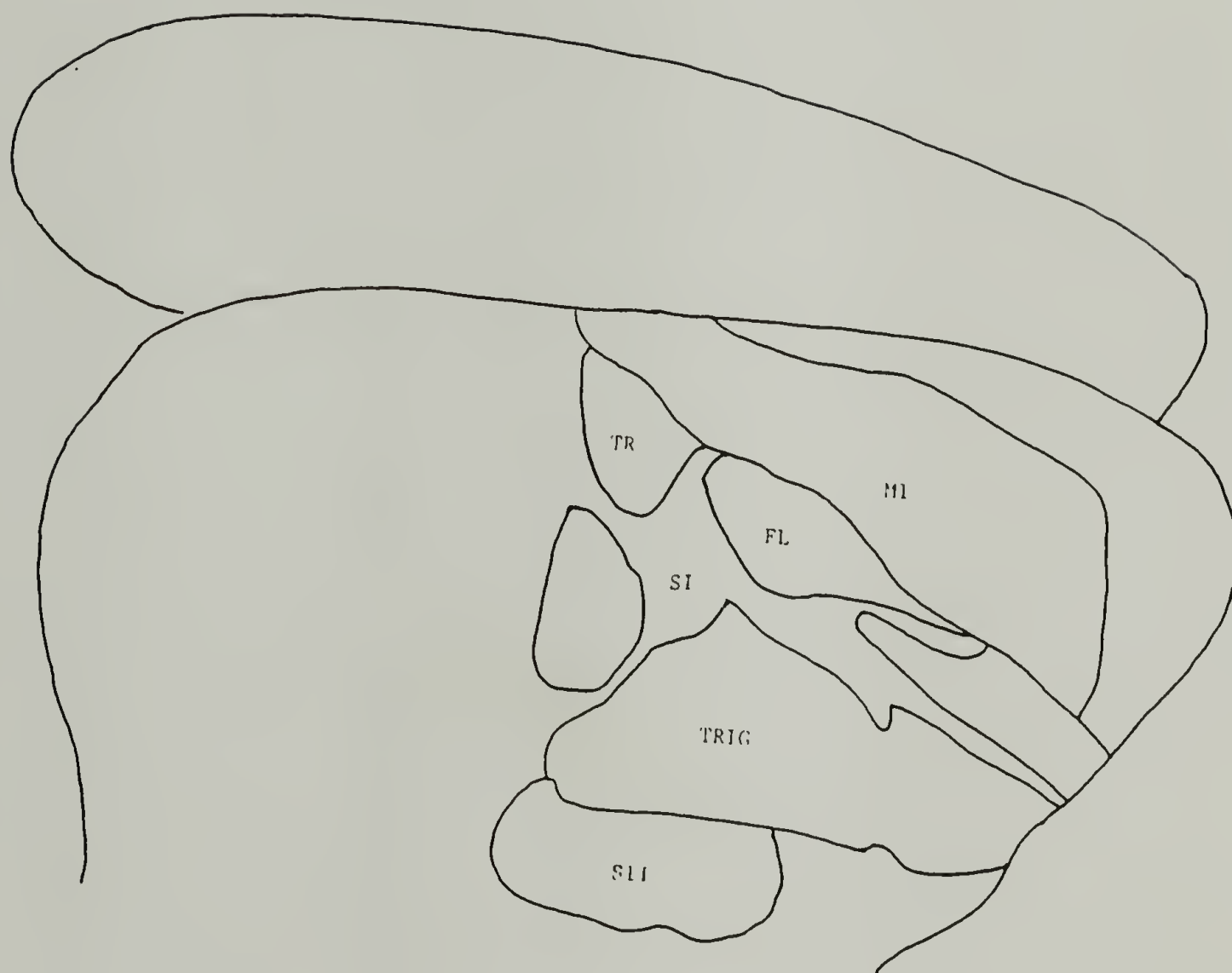


Fig. 1. Representation of the body surface of the rat as determined by cytoarchitecture and microstimulation mapping. (From Donoghue & Wise, 1982).

The MI injections were placed within the vibrissal representation region of MI. Injection by the packed micropipette method of Mori et al. (1981) ensures a strictly localized injection site. The injected regions corresponded to the region of rat frontal cortex identified as the vibrissal representation in MI (Hall and Lindholm, 1974). The extent of the dense HRP reaction product was approximately 600 μm wide and concentrated in layers III-VI (Figure 2). In two other animals, the injections extended into the white matter. However, any extraneous labeling due to this fact has no bearing on the results of this study which is concerned mainly with the ipsilateral corticocortical connections.

Corticocortical projections. HRP-labeled cells were observed in Layers II, III, V, and VI in SI. The majority of labeled cells appeared in Layers III and V. In some cases, the amount of retrogradely transported HRP was great enough to illustrate the dendritic configuration of Layer III pyramidal cells (Figure 3). In addition, labeled cells in SI were grouped into vertically oriented clusters 250-400 μm wide (Figure 4). These clusters were also observed in the contralateral homotopic areas of MI (Figure 5). The HRP labeled somata were observed throughout SI as far as 2.6 mm caudal to the injection site. Figures 6 through 10 illustrate the extent of labeling in the five animals which received the HRP injection in MI only.

Injection of FB into Mo5

Injection placement. Histology revealed that FB injections into the Mo5 infringed on other brainstem pathways. In these injections,

Figure 2. Photomicrograph of a coronal section depicting an HRP injection site in MI.



Figure 2

Figure 3. Photomicrograph of a coronal section illustrating the dendritic configuration of SI pyramidal cells (Layer III) labeled with HRP following injection in MI.



Figure 3

Figure 4. Photomicrograph of a coronal section showing HRP-labeled cells grouped into vertically oriented clusters in SI following injection in MI.

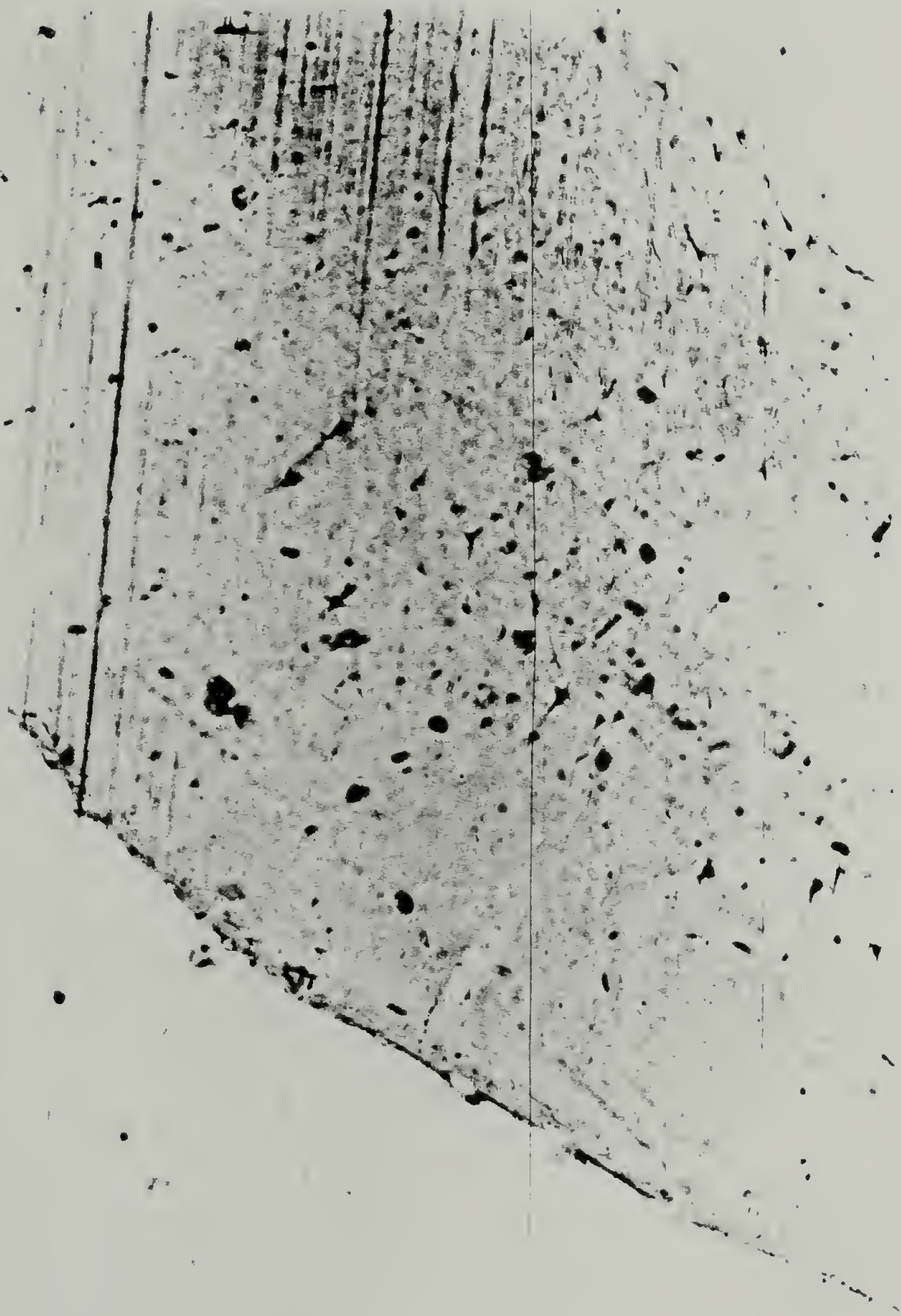


Figure 4

Figure 5. Photomicrograph of a coronal section showing HRP-labeled cells grouped in vertically oriented clusters in MI following injection in a homotopic area of the contralateral hemisphere.



Figure 5



Fig. 6. Montage demonstrating the extent of labeling in animal TD 1 following HRP injection in MI.

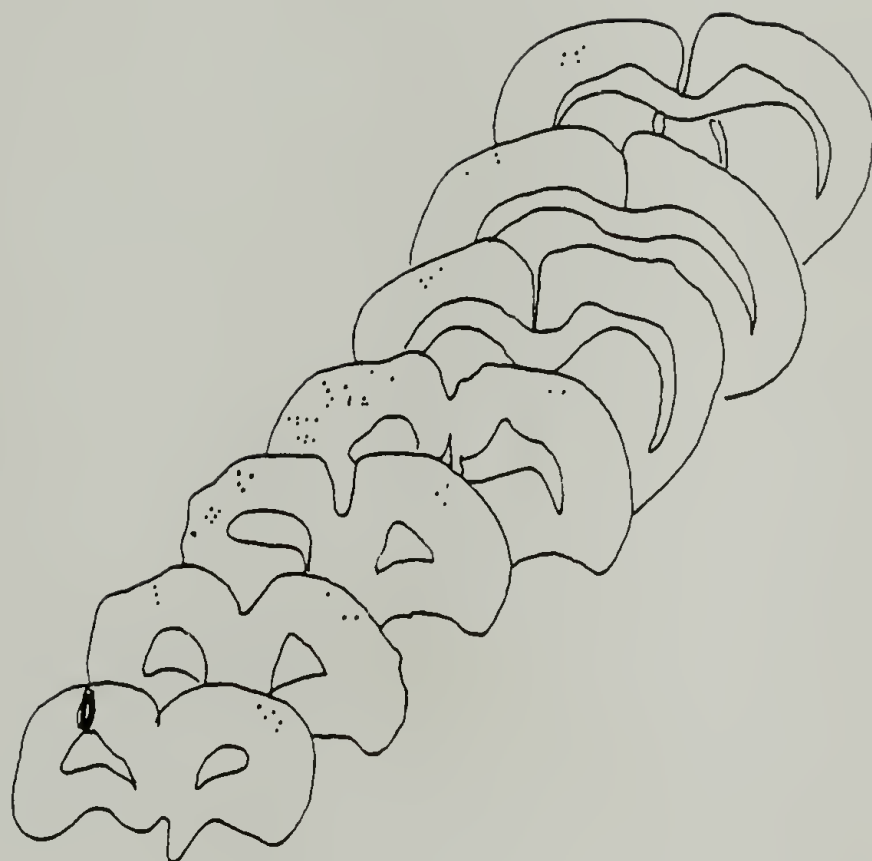


Fig. 7. Montage demonstrating the extent of labeling in animal TD 2 following HRP injection in MI.

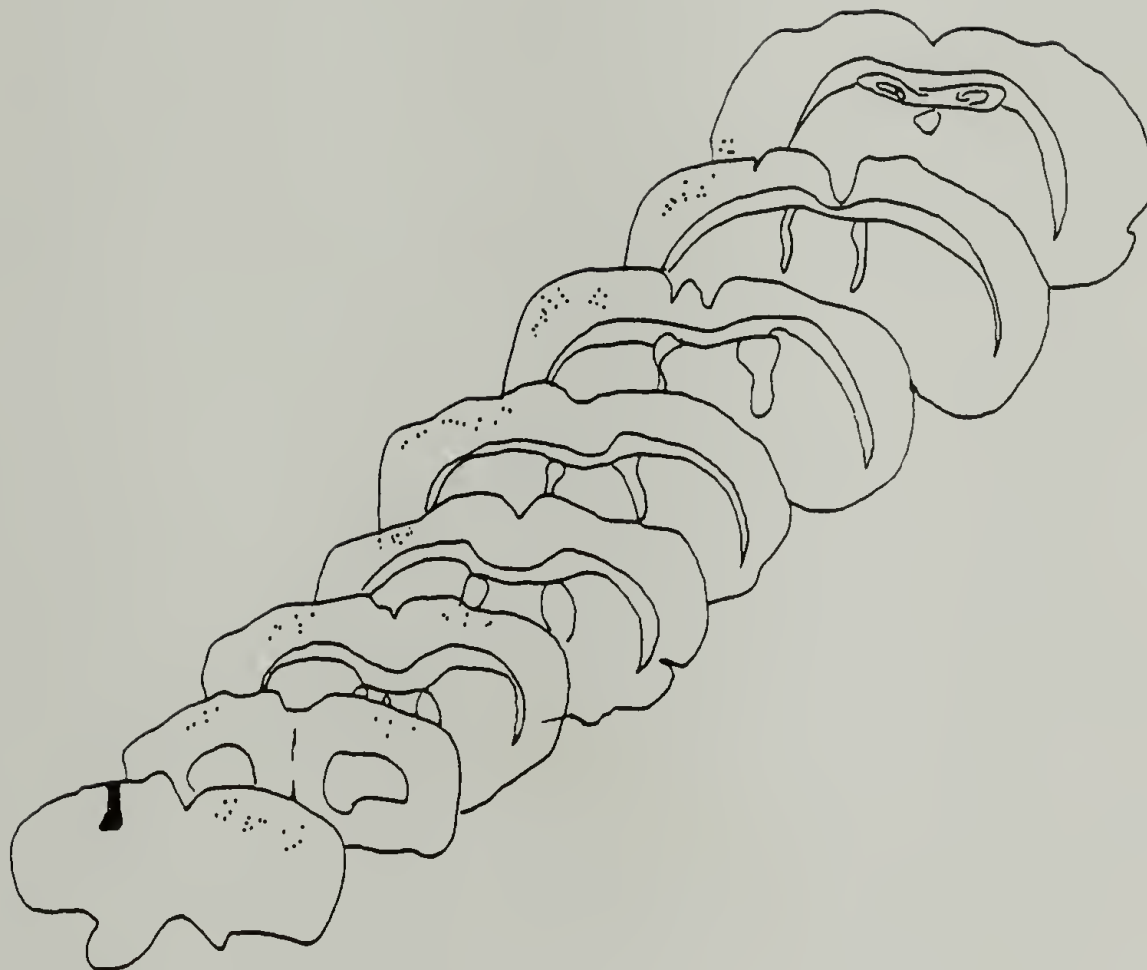


Fig. 8. Montage demonstrating the extent of labeling in animal TD 3 following HRP injection in MI.

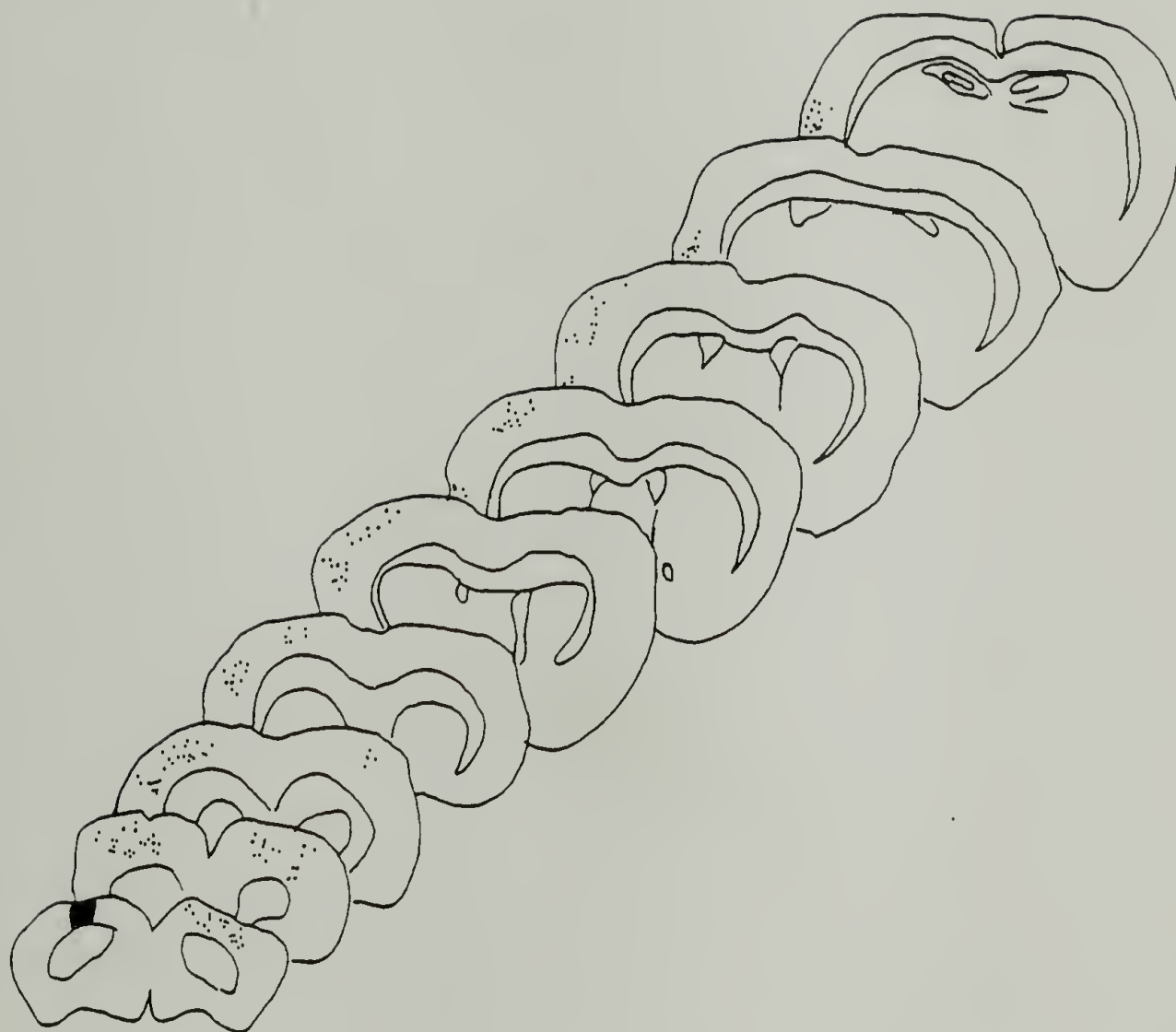


Fig. 9. Montage demonstrating the extent of labeling in animal TD 4 following HRP injection in MI.

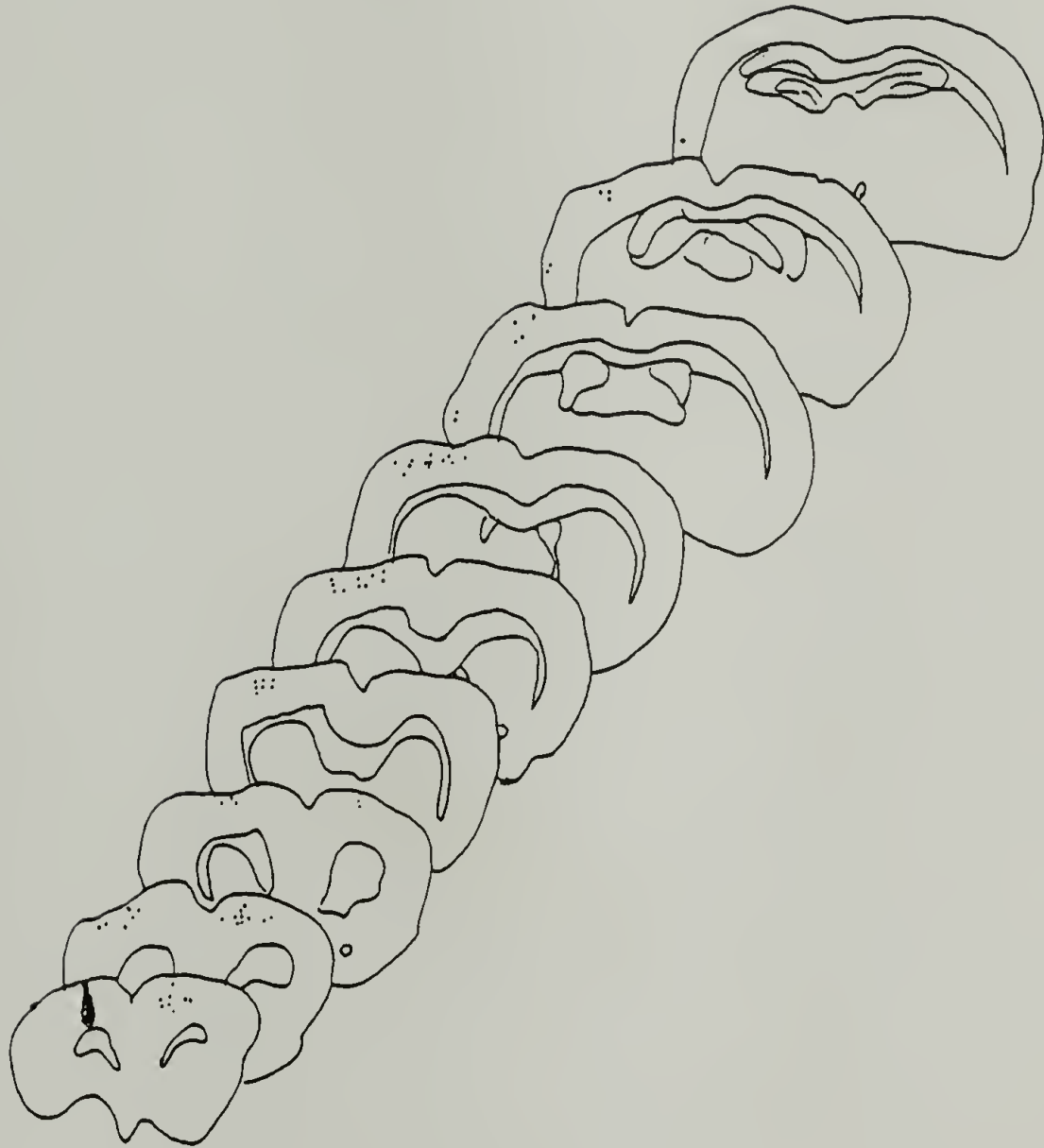


Fig. 10. Montage demonstrating the extent of labeling in animal TD 5 following HRP injection in MI.

the principal nuclei of the trigeminal nerve and the medial lemniscus were also injected with FB. Figure 11 illustrates a typical FB injection site.

Corticobulbar connections. Figure 12 demonstrates the distribution of FB-labeled cells in the sensorimotor cortex following injection into Mo5. As is apparent from the figure, the rat cerebral cortex is heterogenous with respect to the distribution of corticobulbar cells. These neurons appeared mostly in Layer V and were equally distributed in both the granular and agranular regions. The frontal pole of the cortex contained the brightest fluorescent cells and the highest density of these neurons (Figure 13). The slab of labeling extends laterally in the more caudal areas of the cortex.

Injection of HRP in SI and FB in Mo5

Injection placements. The HRP injection was placed in the forelimb representation area of SI (Donoghue and Wise, 1982). The injection site was localized and approximately 480 x 600 μm . The Mo5 injection site was relatively large and included other brainstem structures.

Interaction of corticobulbar and corticocortical systems. Figure 14 illustrates that the HRP labeled somata in the sensorimotor cortex of these animals lie in three distinct foci. One of these foci lie 1.5 mm rostral to the injection site in the motor cortex. Cells of this focus lie mainly in Layer III. The second focus of labeled somata resulting from this SI injection was located 7.5 mm lateral to the injection site within the trigeminal representation area of the SI cortex. The HRP labeled neurons were confined to Layers III and V. The third

Figure 11. Photomicrograph of a tangential section illustrating a typical FB injection site in the trigeminal nuclei of the brainstem.

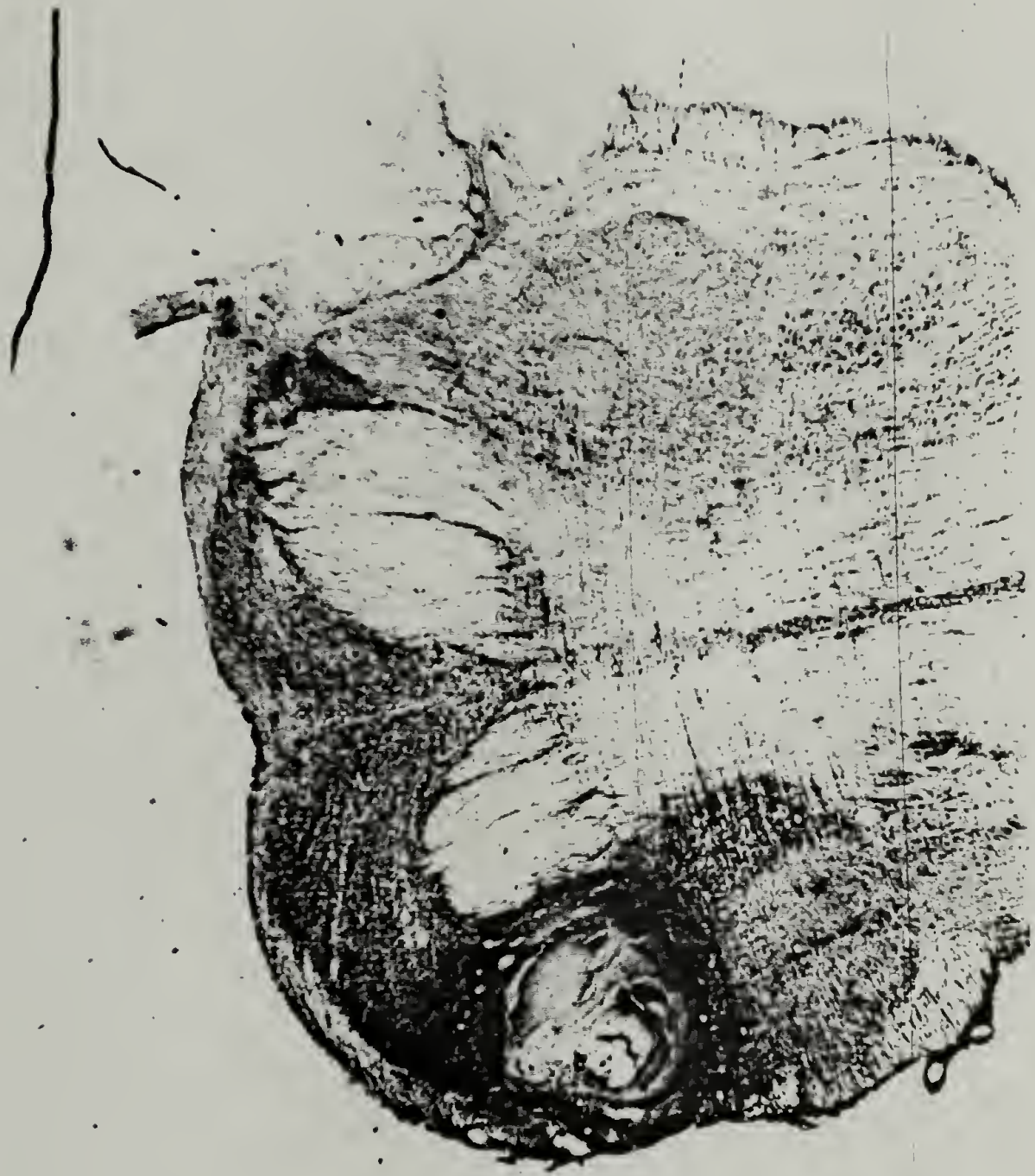


Figure 11



Fig. 12. Reconstruction of the distribution of FB-labeled cells in the sensorimotor cortex following injection in Mo5.

Figure 13. Photomicrograph of FB-labeled fluorescent cells in MI following injection in Mo5.

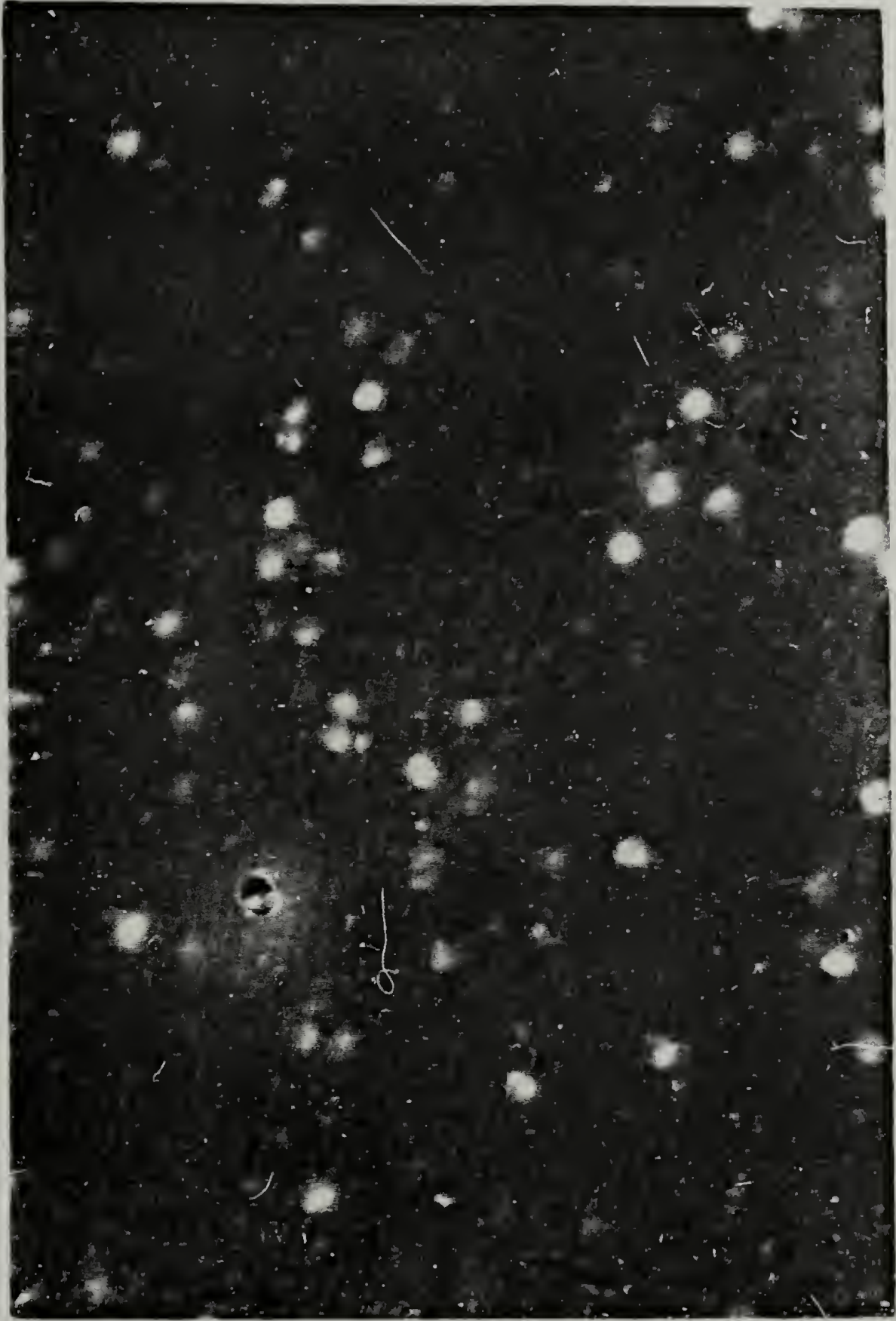


Figure 13

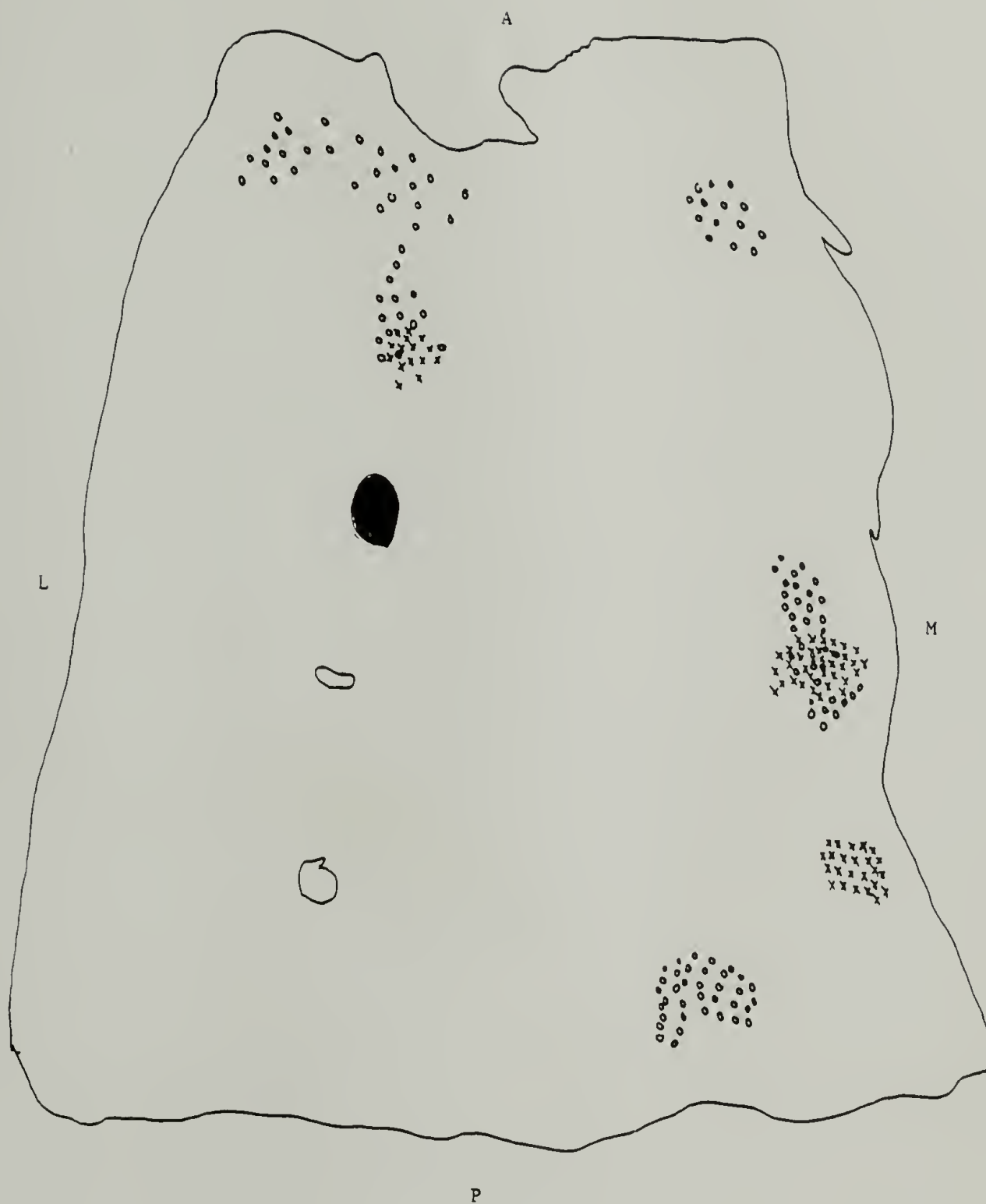


Fig. 14. Reconstruction of the distribution of HRP- and FB-labeled cells in a tangential section of the sensorimotor cortex following HRP injection in SI and FB injection in Mo5. A: Anterior; P: Posterior; L: Lateral; M: medial (x = HRP-labeled cells, o = FB-labeled cells).

cluster of HRP-labeled perikarya appeared in Layers III and V of the head representation region of SI and were situated lateral and caudal to the injection site in SII.

As can be seen from Figure 14, there is a definite overlap of FB-labeled slabs and HRP labeled clusters in SI. The FB-labeled cells are confined exclusively to Layer V in both MI and SI. These vertically oriented areas of double labeling, situated in granular cortex, indicate heterogeneity of functioning within the rat sensorimotor area. There appear to be areas in MI and SI that contain both corticocortical and corticobulbar efferents. Also, regions exist which lack both of these connections.

Injection of HRP in MI and FB in Mo5

Injection placements. The HRP injection was placed in the vibrissal region of MI as described by Hall and Lindholm (1974). The characteristics of the injection site are similar to those described above for MI injections. Also, the parameters of the FB injection in Mo5 are as described earlier.

Interaction of corticobulbar and corticocortical systems. As is apparent from Figure 15, HRP labeled somata lie in discrete clusters in the sensorimotor cortex following an MI injection. These clusters lie in SI directly caudal and slightly lateral to the injection site and are distinctly separated from each other by areas devoid of labeling. These HRP labeled cells are situated in Layers II, III, and V. Figure 15 also illustrates the extent of FB labeling following

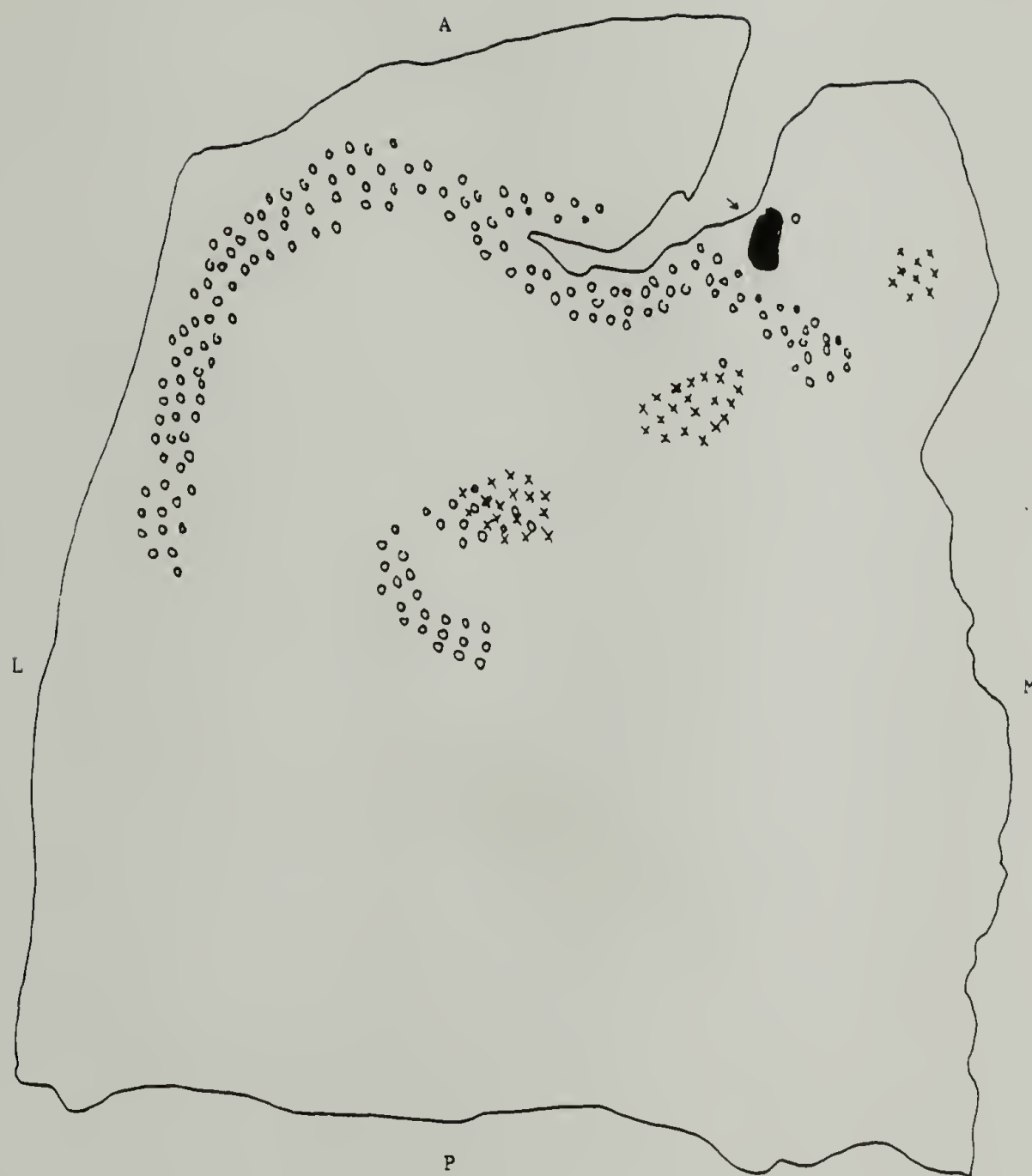


Fig. 15. Reconstruction of the distribution of HRP- and FB-labeled cells in a tangential section of the sensorimotor cortex following HRP injection in MI and FB injection in Mo5. (x = HRP-labeled cells, o = FB-labeled cells).

injection of that tracer in Mo5. Again, there is a specific topographical organization corresponding to this corticobulbar projection. A slab of FB labeling was observed stretching from the frontal pole to areas in SI situated in a lateral-caudal direction.

Figure 15 shows an overlap of FB labeled slabs and HRP labeled clusters in SI. The FB labeled somata are restricted to Layer V and areas of double-labeling are located in the granular region of SI (Figure 16). As indicated in Figure 17, the SI cortex of the rat is heterogenous in function. There are discrete vertically-oriented zones in SI that project to the brainstem and the motor cortex. Furthermore, there are zones in SI which project to neither the brainstem nor the motor cortex. There are also areas in SI from which corticobulbar but not corticocortical fibers originate. Finally, there are areas in SI from which corticocortical but not corticobulbar fibers originate.

Figure 16. Double-labeling in SI following an HRP injection in MI and FB injection in Mo5. Two identical microphotographs: A: fluorescent wavelength of 360 nm; B: normal light.



Figure 16

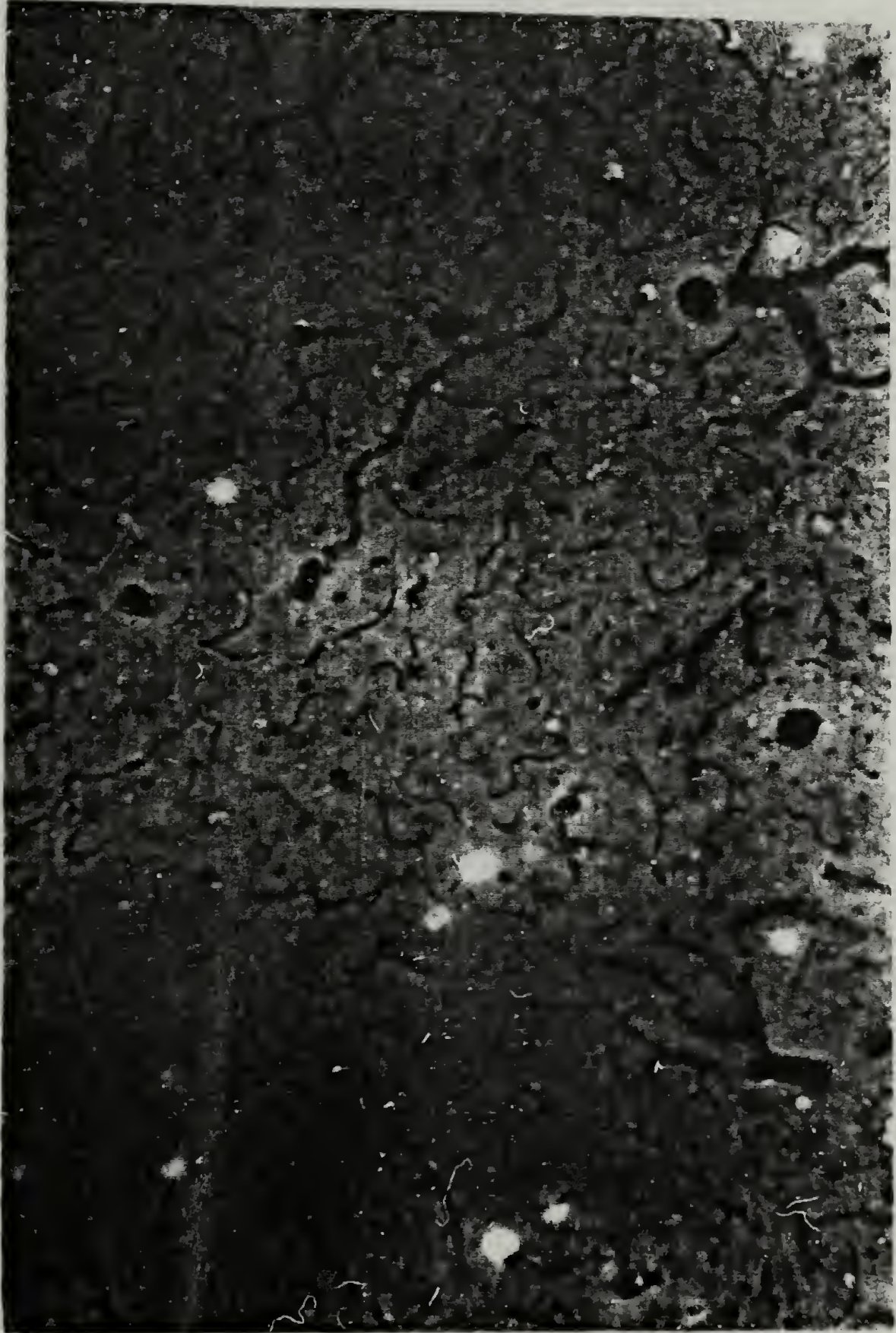


Figure 16 continued

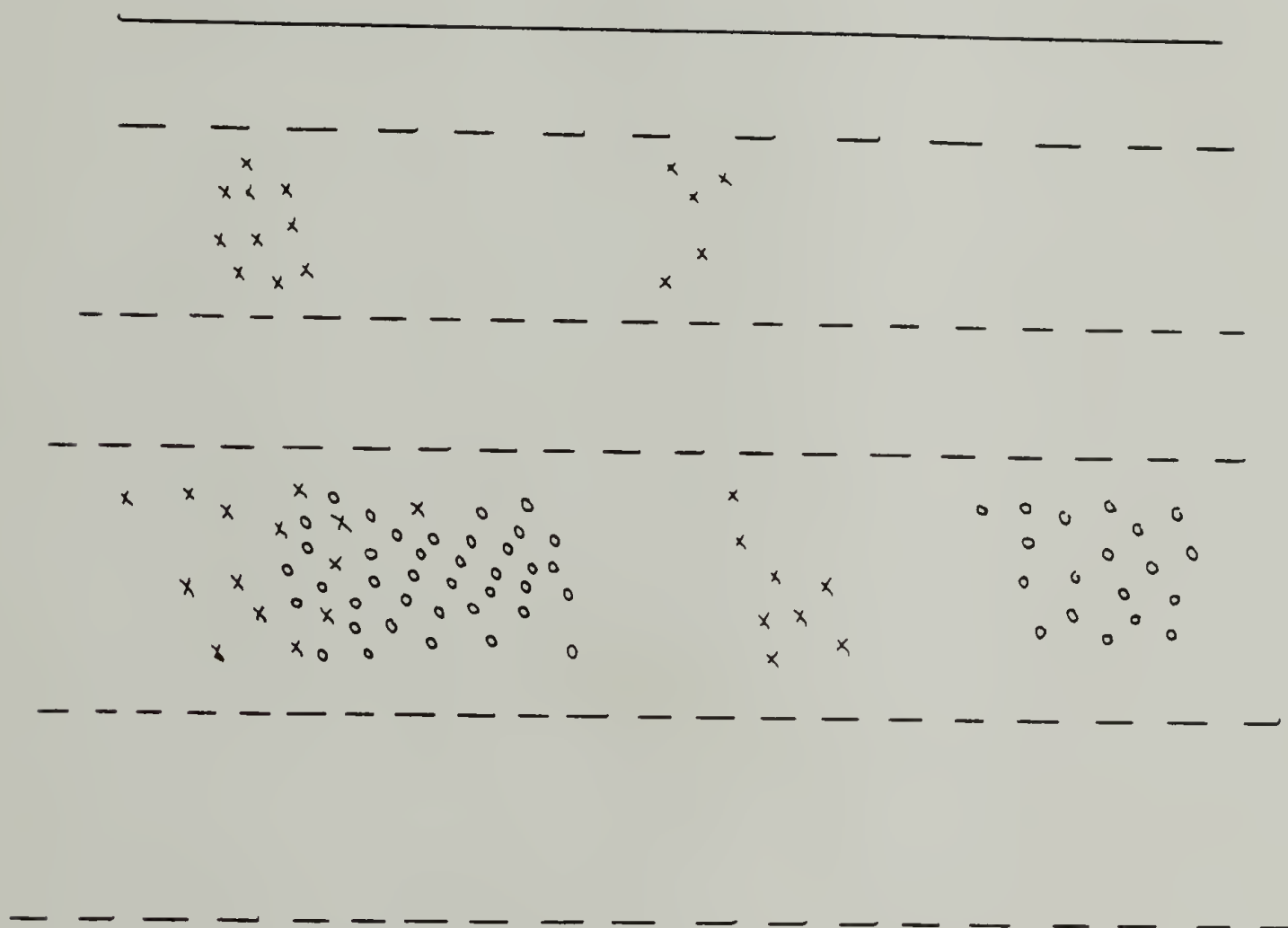


Fig. 17. Schematic representation of the functional heterogeneity of the sensorimotor cortex as determined by the distribution of corticocortical and corticobulbar cells. (x = HRP-labeled cells, o = FB-labeled cells).

C H A P T E R I V

DISCUSSION

Methodological Considerations

This study demonstrates that a double tracer paradigm is a useful tool in examining the organization of the cerebral cortex. However, when combining two retrograde tracing techniques certain methodological issues must be considered. These methods depend on the capacity of axon terminals to take up and transport to the cell body exogenous proteins. It is possible that one method may alter the neuronal properties on which the other depends. In some instances, it is difficult to discern the label of one tracer from that of another. For example, it is difficult to distinguish a histochemical reaction product in cells stained by the Golgi method. A great advantage of using HRP in conjunction with a fluorescent dye such as fast blue is that the two are easily distinguishable. Fluorescent tracing methods also have other advantages. In contrast to autographic techniques, fluorescent tracers are less time consuming. In addition, they are relatively simple to use and very sensitive. The sensitivity of a neuroanatomical tracing method is a crucial factor in the interpretation of the obtained results. The absence of labeled cells is as important to the conclusions of this study as are the observed location of such neurons. As in all neuroanatomical techniques there are

certain drawbacks with fluorescent tracers, however, These tracers are prone to fading after a relatively short period, particularly when illuminated. Also, some of these tracers (nuclear yellow, in particular) have a tendency to diffuse out of retrogradely labeled cells. Of all the fluorescent methods available fast blue was an optimum choice. It is less likely to diffuse and its fluorescent intensity increases with survival time (Steward, 1981).

Support for the Columnar Hypothesis in the Rat Sensorimotor Cortex

The results of this work are supportive of the columnar hypothesis of cortical organization. There exists a specific topographic pattern of labeling in the sensorimotor cortex as a result of SI, MI, and Mo5 injections. The corticocortical MI-SI connection illustrates a vertically-oriented cluster of cells. This finding confirms the data of previous reports (see Jones' review, 1981). Previous authors have reported vertical connectivity between the six laminae of the cortex. Evidence exists for a cell with a limited inhibitory horizontal spread that may demarcate the boundaries of these vertical clusters (Jones, 1975; Szentagothai, 1976). The basket cell which has horizontal branches extending for 1-2 mm is oriented as a flattened disk in a plane at right angles to the vertical column. The existence of the basket cell has implications for the observation that excitation of one place- and modality-specific column in somatosensory cortex is accompanied by inhibition in adjacent columns. This anatomical finding is supportive of the physiological evidence.

By analyzing the laminar location of corticobulbar cells in relation to corticocortical cells, the present study yields a new perspective to the columnar hypothesis. Making use of the fact that certain corticofugal cells are restricted to specific laminae, this work demonstrates the distinctions between columns more clearly. As can be seen from Figure 17, there are vertical zones in the sensorimotor cortex of the rat that are functionally different. There are areas with 1) corticocortical projections but no corticobulbar fibers; 2) corticobulbar projections but no corticocortical fibers; 3) both corticobulbar and corticocortical connections; and 4) neither type of corticofugal fiber. Subsequent studies using injections in other areas containing corticofugal fibers should further substantiate these findings. For example, it would be interesting to look at the distribution of corticothalamic cells (Layer VI) in relation to corticobulbar and corticocortical patterns. Another possibility would be to use a combination of anterograde tracers injected into the spinal cord and thalamus to analyze the distribution of cortical afferents. (The combination of different tracers and subcortical injection sites would be extensive.)

The Cortical Column Defined

According to Mountcastle (1978) the functional subdivisions of the neocortex (i.e., motor, somatosensory, visual, auditory) consist of replicated local neural circuits. These vertical modules or columns may vary in cell number and extrinsic connections but are similar

intrinsically. Each column processes incoming information and transforms it into output which is communicated via specific extrinsic connections. These segregated modules form precisely connected but distributed systems and it is the interaction among columns that constitute cortical functioning.

A model for the basic functional cortical module is represented by the data of Rockel, Hiorn, and Powell (1974). These authors reported that the number of neurons in a 30 μm diameter vertical cylinder across the cortex is 110 cells. This was a comparative study of five species: mouse, rat, cat, macaque, and man. The counts were consistent in each animal in the five areas studied: motor, somatosensory, frontal, parietal, and temporal. It seems that the enlargement of the neocortex in phylogeny has resulted in an expanse in surface area without changes in vertical organization.

As was discussed in the introduction, columnar organization appears throughout the cerebral cortex. However, the reported widths of these columns have varied from 400-1000 μm depending on the location in the cortex and the experimental method employed. For example, columns determined physiologically (800-1000 μm) tend to be larger than those demonstrated anatomically (400-600 μm). Mountcastle (1978) termed a column of 400-1000 μm a macrocolumn. A macrocolumn is composed of minicolumns and is defined by the static and dynamic properties of its neurons. In the case of the somatosensory cortex, place on the body surface and modality type are the two defining parameters of a macrocolumn. In this way, a multi-variable representation is inherent in each macrocolumn.

Usefulness of Extensive Comparative Data

Investigation of columnar organization in the cortex has been restricted mostly to traditional laboratory animals such as the mouse, rat, cat, and monkey. Although the consistent pattern of the minicolumn exists in these species, further research on the presence of columns in other species would be useful to determine the incidence and variation of the cortical column. To date, the literature lacks a comprehensive comparative approach to the columnar hypothesis. It would be interesting to investigate the existence of columns in animals representing an earlier stage of cortical development in phylogeny.

To this effect, Woolsey, Welker and Schwartz (1975) undertook a comparative anatomical study of the face area of SI with special reference to the occurrence of barrels. Barrels are multicellular units located in Layer IV best visualized in tangential sections. Each barrel has been shown to be physiologically related to a vibrissa of the contralateral face (Woolsey and VanderLoos, 1970). The barrel may provide a morphological basis for the columnar hypothesis.

Woolsey, Welker, and Schwartz (1975) examined seven mammalian orders: marsupialia (opposum), chiroptera (bat), primates (tree shrew and macaque), carnovora (dog, cat, raccoon), lagomorpha (rabbit), and rodentia (15 species). Barrels were found in the Australian bush-tailed opossum but not in two marsupials from the western hemisphere. Barrels were also demonstrated in the rabbit and in thirteen of the fifteen rodentia species. The results of this study present two possible conclusions. Barrels may represent a relatively primitive

pattern of cortical organization because they were found in marsupials and rodents but not in carnivores or primates. Conversely, they may represent a sophisticated anatomical solution to certain functional circumstances.

Similar comparative studies using electrophysical or neuroanatomical tracing techniques could further our understanding of columnar organization. It would be interesting to determine if more primitive species also exhibit the minicolumn. In addition, it would be enlightening to determine the existence of macrocolumns and their characteristics in these species.

Conclusion

The finding that there are functionally different, vertically-oriented zones in the sensorimotor area of the rat leads to the conclusion that the cerebral cortex is heterogenous. This heterogeneity has been demonstrated in various manners. For example, Wise, Murray, and Coulter (1979) reported that the corticotrigeminal system is organized somatotopically in the cortex of the rat. Using retrograde tracing methods, these authors found that there is little overlap between parts of the sensory cortex which project to one region of the spinal cord or trigeminal system and those which project to other spinal regions. Furthermore, in MI the hindlimb representation region projects to the lumbar enlargement while the forelimb representation region projects to the cervical enlargement. In another study, Jones, Burton, and Porter (1975) using tritiated amino acids and HRP found

that the cells of commissural and corticocortical fibers are segregated from each other and arranged in distinct clusters in monkeys. Other researchers have reported that in the rat cells of origin of callosal fibers are arranged in vertical arrays that are spatially separated from vertical clusters that project to the thalamus (Akers and Killackey, 1978; Wise and Jones, 1978). This disparity of function among vertical units of the cortex is also evident in physiological studies (e.g., Mountcastle, 1957; Welker, 1976; Asanuma, 1975). In the monkey, Dreyer et al. (1975) found that a single region in the periphery is multiply represented in several separate areas of SI. Thus, it appears that the vertically oriented clusters superimposed upon the horizontal laminae of the cortex provide a basis for a heterogeneous mosaic of local neural circuits. These circuits maintain a diversity of connections and, therefore, a variety of functions. In the cerebral cortex, such distinctions as sensory and motor would then lose much of their heuristic value.

In fact, this formulation is basic to the original principle concerning the columnar hypothesis as proposed by Mountcastle (1957). He contends that the brain is a complex of widely and reciprocally interconnected systems and it is the connections between systems that is the essence of neural activity. Mountcastle maintains that within the neocortex there are modules or local neural circuits that are grouped into cortical areas by a common extrinsic connection or the need to replicate a function over a topographic representation. The cortex,

then, is a composite of specifically connected but distributed systems performing different functions.

If valid, the columnar hypothesis of cortical organization offers the fundamental circuit elements that may be the basis of the functional connectivity within the neocortex. The usefulness of this study, in part, lies in the analysis of the anatomy of the vertical cluster. The significance of the morphological differences among these vertical groupings is essential for the understanding of the physiology of the nervous system and behavior.

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