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The Neuroanatomical Connections and Somatotopic Organization of the Posterior Nuclear Complex of the Rat Thalamus

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THE NEUROANATOMICAL CONNECTIONS AND SOMATOTOPIC ORGANIZATION OF THE POSTERIOR NUCLEAR COMPLEX OF THE RAT THALAMUS

by

E. LUKE/BOLD

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DEDICATION

TO MY FAMILY

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VITA

The author, E. Luke Bold, was born on March 29, 1955 in Hammond, Indiana.

His secondary education was obtained at Chaminade College Prep in St. Louis, Missouri and Loyola Academy in Wilmette, Illinois, which he graduated from in June of 1973.

In September of 1973 he entered Lawrence University in Appleton, Wisconsin and graduated magna cum laude with a Bachelor of Arts degree in Biology and Chemistry in June of 1977. In July of 1977, he took a position as a research assistant at Children's Memorial Hospital in Chicago, Illinois and worked in the Department of Neurology.

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The author is married to Margaret Dwyer Bold.

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Anatomical Abbreviations

Cortical Areas

AC - anterior cingulate cortex
Agl - agranular lateral frontal cortex
AgM - agranular medial frontal cortex
AI - primary auditory cortex
CLi - isocortical claustral cortex
CLpc - periallocaortical claustral cortex
CLpri- proisocortical claustral cortex
IL - infralimbic cortex
PL - prelimbic cortex
Prpi - prepiriform cortex
Rsag - retrospenial agranular cortex
Rsg - retrosplenial granular cortex
SI - primary somatosensory cortex
SII - secondary somatosensory cortex
17 - primary visual cortex
18 - secondary visual cortex

Diencephalic Areas

AD - anterior dorsal thalamic nucleus
AM - anterior medial thalamic nucleus
AV - anterior ventral thalamic nucleus
BSC - brachium of superior colliculus
Cem - central medial thalamic nucleus
CL - central lateral thalamic nucleus
CP - cerebral peduncle
F - fornix
FR - fasciculus retroflexus
GP - globus pallidus
HC - habenular commissure
IC - internal capsule
ILN - intralaminar nuclei
IV - interventicular nucleus
LD - lateral dorsal thalamic nucleus
LGD - dorsal nucleus of lateral geniculate
LGV - ventral nucleus of lateral geniculate
LH - lateral habenular nucleus
LP - lateral posterior thalamic nucleus
MD - mediodorsal thalamic nucleus
MDpl- paralamellar part of mediodorsal nucleus
ml - medial lemniscus
mtt - mammilothalamic tract

MV - medioventral thalamic nucleus
OT - optic tract
P - pineal
pa - anterior pulvinar
Pc - paracentral thalamic nucleus
Pf - parafascicular thalamic nucleus
PO - posterior thalamic nucleus
PRT - anterior pretectal nucleus
PT - paratenial thalamic nucleus
PV - paraventricular thalamic nucleus
R - reticular thalamic nucleus
Rh - rhomboid nucleus
SG - supragenículate thalamic nucleus
Sm - submedial nucleus
SM - stria medullaris
sPf - subparafascicular nucleus
t - gustatory (taste) thalamic nucleus
VB - ventrobasal thalamic nucleus
VL - ventrolateral thalamic nucleus
VM - ventromedial thalamic nucleus
VPL - ventroposterior lateral nucleus
VPLc- caudal division of VPL
VPLo- oralis division of VPL
VPS - superior division of VPL
ZI - zona incerta

Brainstem Areas
Cnf - cuneiform nucleus
DMN - deep mesencephalic nucleus
DN - dentate cerebellar nucleus
DPB - dorsal parabrachial nucleus
DR - dorsal raphe
ECN - external cuneate nucleus
FC - fasciculus cuneatus
FG - fasciculus gracilis
GRF - gigantocellular reticular formation
IC - inferior colliculus
INC - interstitial nucleus of Cajal
INT - interpositus cerebellar nucleus
LC - locus coeruleus
LDTg- lateral dorsal tegmental nucleus
LVN - lateral vestibular nucleus
MGD - dorsal nucleus of medial geniculate
MGM - magnocellular nucleus of medial geniculate
MGP - principal nucleus of medial geniculate
mV - trigeminal motor nucleus

MVN - medial vestibular nucleus
NC - nucleus cuneatus
NDk - nucleus of Darkschewitsch
NPC - nucleus of posterior commissure
NG - nucleus gracilis
NLL - nucleus of lateral lemniscus
NTS - nucleus and tract of solitarius
PAG - periacqueductal gray
Pbg - parabigeminal nucleus
pc - posterior commissure
PPTN- pedunculo-pontine tegmental nucleus
PRF - pontine reicular formation
RMg - raphe magnus nucleus
RN - red nucleus
RRF - retrorubral field
RRN - retrorubral nucleus
SC - superior colliculus
SCP - superior cerebellar peduncle
Snc - substantia nigra pars compacta
SNr - substantia nigra pars reticulata
spV - spinal trigeminal nucleus
SVN - superior vestibular nucleus
VII - facial motor nucleus
Vp - principal trigeminal nucleus
Vtr - spinal tract of the trigeminal nucleus
VPB - ventral parabrachial nucleus
I-IX- Rexed's laminae of the spinal cord

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CHAPTER I

INTRODUCTION

The dorsal thalamus is located in the diencephalon of mammals and is composed of many nuclei, many of which transmit some type of sensory information to the cerebral cortex. While many thalamic nuclei are associated with one sensory modality, the posterior nuclear complex (PO) receives several types of sensory inputs, including somatosensory, visual, and auditory (Lund and Webster, 1967; Blum et al., 1979; Feldman and Kruger, 1980). Because of the multiplicity of sensory inputs and PO's widespread cortical projections, PO has been classified as one of the "non-specific" thalamic nuclei (Rose and Woolsey, 1949; Herkenham, 1980; Macchi, 1983). An understanding of PO's function is further complicated by the inconsistent terminology used in studies of this structure which are due in part to the sometimes striking phylogenetic variation in the appearance of PO (cf. Berman and Jones, 1982). To help resolve these problems and in an effort to understand the function of PO, this dissertation was undertaken to determine the afferents to PO, the precise pattern of PO's interconnections with various subdivisons of sensorimotor cortex, and to identify the relationship between the previously described afferents to PO from the dorsal column nuclei and PO neurons projecting to motor and somatosensory cortex (Lund and Webster, 1967,

Feldman and Kruger, 1980).

This dissertation is divided into five separate studies.

The first study examines the cytoarchitecture of the dorsal thalamus of the rat. Nissl stained photomicrographs are presented to establish a thalamic parcellation that is consistent with previous terminology used in the rodent. The derived atlas is used in defining the thalamic nuclei in subsequent studies in this dissertation.

The second study investigates the afferent input to the posterior nuclear complex in the rat by stereotaxically injecting small, well localized quantities of flourescent dyes (diamidino yellow or fast blue) into the PO complex and surrounding nuclei so that the afferent projections to PO could be determined. Special attention is directed to verifying the existence of dorsal column nuclei projections to PO.

The third study compares the thalamic afferents from PO and other nuclei to the primary (MI) and supplementary (SMA) motor areas of the rat using the multiple flourescent retrograde tracing technique. Different combinations of fluorescent dyes were injected into the forelimb, hindlimb, and vibrissae areas of primary motor and supplementary motor areas as identified using intracortical microstimulation techniques. The possible topographical distribution as well as the extent of collateralization of cortical projecting thalamic neurons is examined.

The fourth study compares the thalamic projections from PO and other nuclei to physiologically identified regions of the primary (SI)

and secondary (SII) somatosensory coritcal areas. Using multiunit recording techniques the forelimb, hindlimb, and face regions are mapped in both SI and SII and then one, two, or three flourescent dyes are injected into as many different areas of either SI or SII. These experiments provide information concerning the differential pattern of thalamic afferents to SI and SII and also help to determine whether thalamic neurons send collaterals to both SI and SII. In addition, the topographical organization of thalamic neurons projecting to SI and SII is examined so that the potential somatotopic organization of such projections could be assessed.

The fifth and final study combines the retrograde properties of fluorescent dyes with the anterograde labeling properties of wheat germ agglutinated horseradish peroxidase to answer the question: Do dorsal column nuclear teminations overlap in PO with motor cortex projecting thalamic neurons? Fluorescent dyes are injected into physiologically identified regions of motor and/or sensory cortex, and WGA-HRP is injected into either the nucleus gracilis or the nucleus cuneatus in the same animal. The overlap of the dorsal column nuclear terminations with retrogradely labeled cells in PO is examined following different combinations of cortical injections. In this way, anatomical evidence is provided which is relevant to the question of whether PO processes peripheral sensory information to the motor cortex in the rat. This question is one of the major unresolved issues in motor cortical research, and one major hypothesis states

that such peripheral sensory input is carried via a dorsal column-thalamic relay pathway directly to the motor cortex (Rosen and Asanuma, 1972; Tracey et al., 1980).

The final chapter in this disseratation briefly summarizes the results from all the above-mentioned studies and discusses their implications for understanding PO's function, especially in relation to its classification as a "non-specific" thalamic nucleus.

CHAPTER II

REVIEW OF RELATED LITERATURE

1. Classification of the thalamus

The earliest detailed studies on the thalamus were conducted by Gudden (1881) and subsequently by Ramon y Cajal (1911) who systematically analyzed the nuclear configurations and fiber connections of different divisions of the diencephalon. Several other pioneers of neuroanatomy (von Monakow, 1909; Nissl, 1913) using retrograde degeneration techniques recognized that the thalamus was made up of discrete regions with definite borders and that the neurons within these nuclear regions were directly connected with the cortex. These early investigators associated specific thalamic nuclei with specific regions of the cerebral cortex by lesioning specific areas of the cortex and identifying degeneration in specific thalamic regions. By 1920, the major ascending connections to the thalamus had been described, and the thalamus was conceptualized as the final link in transmitting afferents to the cortex. Subsequent studies using cyto- and myleoarchitecture methods (Gurdjian, 1927; Le Gros Clark, 1933; Walker, 1938; Olszewski, 1952) found that the egg-shaped thalamus was divided by a lamina of white matter, the internal medullary lamina (IML), into 3 gray masses or nuclear groups: anterior, medial, and lateral thalamic groups. Within each group, various subdivisions were

distinguished according to cytoarchitectural criteria. Further study revealed cell groups within the IML, referred to collectively as the intralaminar nuclei. Cell groups near the midline were designated the midline nuclei. An irregularly shaped shell of gray matter surrounded the thalamus rostrally and laterally, called the thalamic reticular nucleus, and was separated from the rest of the thalamus by a thin lamina of white matter known as the external medullary lamina (EML). This basic pattern has been found in all mammals studied (cf. Macchi, 1983).

Nissl's (1913) observation that the thalamus was dependent on the cortex, which was based on the finding that if the cortex is destroyed part of the thalamus degenerates, led several investigators to study the fiber connections between the thalamus and the cortex (Le Gros Clark, 1933; Walker, 1938). The parcellation scheme suggested by Walker (1938) based on studies of the thalamocortical relations of the monkey and chimpanzee thalamus is still widely accepted today.

Rose and Woolsey (1949) modified Walker's concept and divided the thalamic nuclei into extrinsic and intrinsic nuclei, the former receiving afferents from outside the thalamus and the latter receiving afferents exclusively from within the thalamus (e.g., the intralaminar nuclei). The reticular nucleus was not categorized because its connections were unknown.

Morrison and Dempsey (1942), using electrical stimulation and recording cortical evoked responses, were the first to describe a

thalamocortical system. This system was characterized by a wide distribution over the cortex of long latency negative waves (recruiting responses) after stimulation of a region they termed the "nonspecific" nuclei. Based on the type of response evoked in the cortex, Jasper (1949) subdivided the thalamus into two groups: the specific nuclei group with restricted cortical projections and the nonspecific nuclei with more diffuse cortical projections. Although this concept of thalamic organization still is cited, it appears to be an oversimplification. A more recent version of Jasper's notion was provided by Hassler (1964) who divided the thalamus into cortex-dependent and cortex-independent nuclei. Nuclei in the former category include the ventral tier nuclei, the lateral nuclear complex and the anterior nuclei while the latter group included the midline and intralaminar nuclei.

The thalamus also has been subdivided based on results of retrograde and anterograde degeneration studies (Macchi and Rinvik, 1976). Accordingly, the thalamus was divided into 3 regions: 1. nuclei with basal ganglia and rhinencephalic connections which included the anterior intralaminar and midline nuclei; 2. nuclei with cortical connections, and; 3. nuclei with unknown connections which included the posterior intralaminar nuclei and the reticular nucleus. Actually, Golgi studies (Scheibel and Scheibel, 1966) established that the thalamic reticular nucleus projected not to the cortex but to other thalamic nuclei and the brainstem. A later study reported that

the thalamic reticular nucleus and the ventral division of the lateral geniculate (LGV) were the only thalamic nuclei that did not send projections to the cortex (Jones, 1981). In the most recent classification (Macchi, 1983), the concept of diffuse and restricted projections was revised into four groups of projections: (1) nuclei which project to one "anato-functional homogenous" cortical region and which therefore includes not only the classical "relay" nuclei but also the mediodorsal and reuniens nuclei; (2) nuclei that project heavily to one cortical region and less heavily to other cortical regions; the lateral posterior- pulvinar complex is the only member of this group; (3) nuclei that project heavily to more than one cortical area and less to other regions, these nuclei include the intralaminar, ventromedial and ventroanterior nuclei; and (4) a region called the posterior nuclear complex that projects to widespread fields belonging to sensory and perisensory areas of the cortex (Macchi, 1983).

Another classification scheme based on the major cortical layers of termination of thalamocortical axons (Herkenham, 1980) also divides thalamic nuclei into four groups: (1) the thalamic relay nuclei for vision, audition and somesthesis which project to layer IV and/or III; (2) the intralaminar nuclei which project to layer VI and/or V; (3) the ventromedial nucleus and magnocellular division of the medial geniculate, both of which are characterized by a widespread distribution of thalamocortical terminations to layer I; and (4) those nuclei typified by terminations in both layer I and in additional

layers that depend on the cortical area in which the terminations are found. This fourth group includes the posterior nucleus which projects to layers I and IV of the second somatosensory area, to layers I and V of the immediately adjacent primary somatosensory area and to layers I and III of motor cortex.

2. Identification of PO

A posterior nuclear complex was first described by M. Rose (1935). J.E. Rose (1942), in his study of the ontogenetic development of the diencephalon of the rabbit, referred to an ill-defined cellular region in the caudal thalamus that was differentiated from the primary somatosensory receiving nucleus, the ventrobasal complex. Rose and Woolsey (1958) were the first to describe anatomically a posterior group of thalamic nuclei in the cat. This nuclear group included the magnocellular medial geniculate (MGM), the suprageniculate nucleus (SG), and the posterior nucleus (PO) described as a rather large region adjacent to and with rather indistinct boundaries with the ventral part of the lateral posterior nucleus, the medial part of VB and the medial geniculate (Rose and Woolsey, 1958). They suggested that PO may project diffusely upon auditory and adjoining cortical fields.

A pivotal physiological study in the cat (Poggio and Mountcastle, 1960) identified PO neurons that were responsive to painful stimulation, an observation that focused considerable attention on

this thalamic region. A subsequent anatomical study (Moore and Goldberg, 1963) subdivided the feline PO nucleus into three parts based on afferent connections: (1) a medial division (POm) which received afferents from the classical pain pathway, the spinothalamic tract (Boivie, 1971; Jones and Burton, 1974); (2) a lateral part (POl) receiving input from the inferior colliculus; and (3) an intermediate division (POi) receiving mixed auditory, vestibular, and somatosensory afferents (Blum et al. 1979). Thus, the PO complex in the cat has five components: MGM, SG, and the three PO subdivions, POl, POm, and POi.

In view of distinct afferent inputs to the different components of the PO nucleus, subsequent work examined the cortical projections from these individual components. POl was found to project exclusively to auditory regions of the cortex, whereas POi and MGM were found to have more diffuse projections to widely spaced areas of cortex (Burton and Jones, 1976). Thalamocortical fibers from POm, the spinal or somatosensory part of the PO complex, distributed to a region just posterior to the secondary somatosensory area (SII), which in monkeys is termed the retroinsular area (Burton and Jones, 1976; Robinson and Burton, 1980).

Analysis of POm projections have reported terminations to areas surrounding but not within SII (Jones and Leavitt, 1973; Heath and Jones, 1971). However, studies using more sensitive neuroanatomical tracing techniques describe feline POm efferents directly to SI and

SII (Spreafico et al., 1981; Bentivoglio, 1983; Burton and Kopf, 1984). Since PO in the rat has similar cortical connections (Bold and Neafsey, 1984), it is likely to be the homolog of POm in the cat (Bold et al., 1984). Lund and Webster (1967a) were the first to identify the rodent PO (called POm) and described afferent inputs from the dorsal column nuclei and the trigeminal system. In an investigation of the thalamocortical projections, several studies found rodent PO projections to the primary somatosensory cortex (SI, Jones and Leavitt, 1984; Donoghue et al, 1979). Subsequent rodent work described PO projections to SII (Lin and Chapin, 1981). The studies cited above which used the term POm to describe the posterior thalamic nucleus in the rat did so for conceptual convience, perhaps to more easily facilitate comparisons across species. This, however, confuses the issue because it raises questions concerning the other components found in the cat, some of which (POl and POi) are not readily distinguishable in the rat. In this dissertation the term PO is used in reference to the small nuclear region in the caudal part of the rodent diencephalon. This region was observed to have somatosensory as well as motor cortical projections. Only after thorough analysis of the afferent input as well as the pattern of cortical projections of this region will ti be possible to subdivide it. Studies directed toward this aim are presented in this dissertation.

3. Afferent Input to PO. The medial lemniscus was initially described as terminating exclusively and somatotopically in the ventroposterior lateral (VPL) nucleus of the thalamus (Le Gros Clark, 1936; Walker, 1938; Bowsher, 1958). Subsequent studies using more sensitive anterograde tracing techniques provided evidence that in rats, cats, and primates, parts of the posterior nuclear complex also received medial lemniscus fiber terminations (Lund and Webster, 1967a; Boivie, 1971; Jones and Burton, 1974; Boivie, 1978). However, this termination within PO was rather sparse and did not appear to be somatotopically organized. As with the medial lemniscus, the spinothalamic tract was also previously thought to project exclusively to VPL (LeGros Clark, 1936; Walker, 1938; Bowsher, 1958), but more recent studies have shown that it is also an input to PO (Mehler et al., 1960; Bowsher, 1961; Boivie, 1970; Burton and Jones, 1974). Furthermore, the feline lateral PO nucleus (PO1) also receives afferents from the lateral cervical nucleus (Metherate et al., 1984) as does VPL (Boivie, 1970).

Dorsal column nuclear projections have been described as fibers "peeling off" from the medial lemniscus and terminating in PO (Lund and Webster, 1967a) as well as in the intermediate geniculate nucleus (Feldman and Kruger, 1980). More recent studies show that PO receives input from the rostral parts of both nucleus gracilis and nucleus cuneatus, the former contributing the denser projection (Bold et al, 1983). Other inputs to the rodent PO have been observed from the deep

cerebellar nuclei (Faull and Carman, 1978) although these were not identified in a later study (Haroian et al, 1981). However, careful analysis of the figures in the latter study indicates that descriptions of cerebellar afferents to the lateral posterior (LP) nucleus may actually have been to dorsal PO. The trigeminal complex, particularly the principal sensory nucleus (Vp), has also been reported to project to PO in the rat (Lund and Webster, 1967b; Smith, 1973). Spinal cord projections to PO have also been found in the rat (Geisler, 1979) and in the cat (Metherate et al., 1984).

4. Thalamocortical Connections of Rat Sensorimotor Cortex. Several laboratories have used axonally transported substances to study thalamocortical connections in the rodent (e.g. Jones and Leavitt, 1974; Faull and Carman, 1978). However, Donoghue and Parham (1983) have best described the thalamic nuclei projecting to the agranular lateral field, which appears to coincide with the bulk of motor cortex (MI). The thalamic nuclei observed projecting to MI include ventromedial nucleus (VM), VL, all of the intralaminar nuclei (ILN), and PO. Although cortical injection sites were examined cytoarchitecturally, a detailed analysis of these connections to different somatotopic areas of MI was not made.

Studies of inputs from the thalamus to rat sensory cortex indicate that VB is the major source of input to SI (Killackey and Leshin, 1975) with sparser projections coming from the intralaminar nculei and

VM (Wise and Jones, 1978). The posterior nuclear complex (PO) has been recently found to project to both SI and MI (Bold et al, 1983) and to SII (Bold and Neafsey, 1984) in the rat but the possible detailed internal organization of these projection neurons is unknown.

5. Cerebral Cortex Somatotopy. Thalamic organization and function are generally described in terms of the distribution patterns of thalamocortical fibers. Since one major objective of the proposed work is to determine whether there is a somatotopic organization within PO, the somatotopic organization within the motor and sensory cortices, which receive afferents from PO, will be reviewed.

A. Somatotopy in Motor Cortex. Both early cortical surface stimulation studies (Settlage et al, 1948; Woolsey et al, 1952) and subsequent studies using more precise intracortical microstimulation methods (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Neafsey and Sievert, 1982; Sanderson et al, 1984) have demonstrated that the motor cortex is situated medial to the primary sensory (S1) representation in the region of agranular frontal cortex. The motor cortex appears to be organized somatotopically and this pattern is illustrated in Figure 1 (taken from the study of Neafsey et al. (1985)). In this illustration, note the presence of a second more rostral motor representation of the forelimb, trunk and hindlimb that has recently been described (Neafsey and Sievert, 1982) and which probably corresponds to the supplementary motor area seen in the primate.

Anatomical studies have corroborated the electrical stimulation studies by demonstrating a somatotopic distribution of cortical efferents, with distinct patches of cells within MI projecting to cervical, thoracic, or lumbar levels of the spinal cord (Hicks and D'Amato, 1977; Wise et al, 1979; Sievert and Neafsey, 1985).

B. Ml Cytoarchitecture. Due to the absence of a prominent internal granular layer (lamina IV), MI is classified as agranular cortex (Zilles et al, 1980) and can be further divided into lateral (AGl) and medial (AGm) components (Donoghue and Wise, 1980, 1982). Distinct and well defined lamina II and III are found in AGl in comparison to their more homogeneous appearance in AGm. Although most of MI appears to be located within the AGl, the hindlimb and forelimb regions of MI determined by microstimulation appear to overlap somewhat with granular sensory cortex (SI).

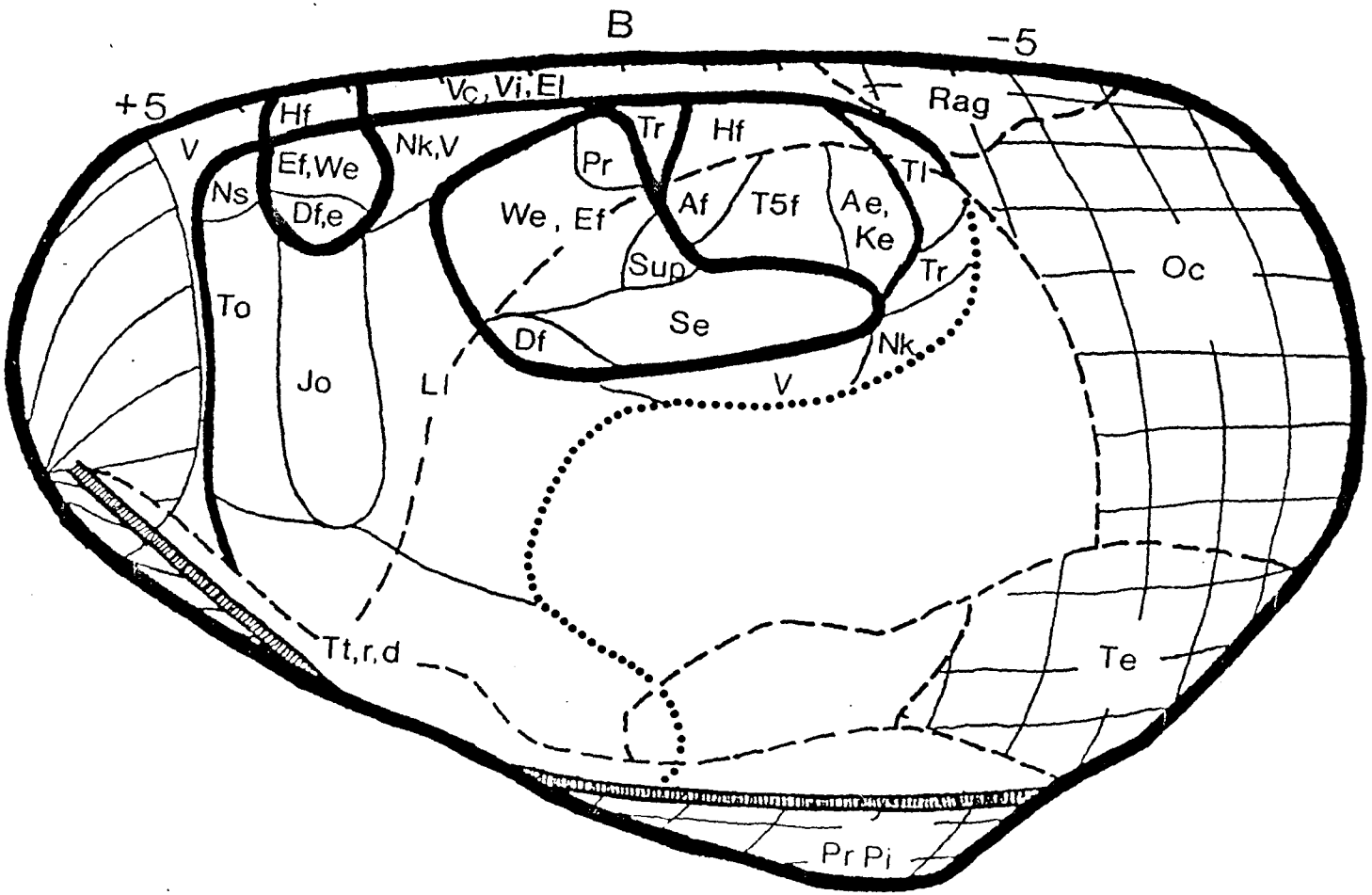
C.Somatotopy in Sensory Cortex. The primary sensory cortex (SI) has been defined using both physiological mapping techniques (Welker, 1971;1976; Hall and Lindholm, 1974; Donoghue et al, 1979; Welker et al, 1984) and anatomical methods (Wise and Jones, 1978). SI is located lateral to MI along the lateral aspect of the frontal and parietal cortex, and dorsal to SII. Receptive fields of neurons in SI have been mapped following peripheral stimulation, and a very distinct somatotopy within SI has been found (Figure 2). Within the face representation a particularly detailed somatotopy has been described corresponding to individual vibrissae (Welker, 1971). A second

somatosensory area (SII) located lateral to SI but dorsal to the rhinal sulcus, also has a distinct somatotopy with the head most rostral and then the forelimb, hindlimb and tail more caudally (Welker and Sinha, 1972).

6. Overlap between Motor and Sensory Cortical Areas. The degree of overlap between the motor and sensory cortices of the rat has been determined by combining intracortical microstimulation and sensory mapping techniques in the same animal (Hall and Lindholm, 1974; Donoghue et al, 1979; Sanderson et al, 1984). The hindlimb cortical representation shows almost complete overlap of sensory and motor representation whereas the forelimb sensory and motor representations show only a partial overlap along the caudolateral one-third of their border. The vibrissae, neck, pinnae, and face areas of MI are completely separate from the sensory representations of these body areas. The cytoarchitecture of the overlapped areas shows characteristic features of both MI and SI. For example, in the hindlimb overlap zone there is a distinct granular layer IV, typical of SI, and large pyramidal cells in layer Vb, typical of MI. In addition, the overlapped regions of SI and MI receive input from both the ventrobasal (VB) and ventrolateral (VL) nuclei (Donoghue et al, 1979).

Figure 1

Topography of rat motor cortex (Neafsey et al. 1985). A dorsolateral view of the left hemisphere of the rat brain. The dashed lines represent the extent of the primary somatosensory area (SI). The solid lines indicate the location and relative size of the components of the primary motor area, MI. The dotted line represents the caudal border of MI which varies from animal to animal. Note two forelimb representations within MI, one rostral and one caudal, a caudal hindlimb representation and a medially located vibrissae representation. B denotes bregma, the scale is in milimeters. Abbreviations: To = tongue out; Ns = nose; Hf = hip flexion; Ef = elbow flexion; We = wrist extension; Df = digit flexion; De = digit extension; Nk = neck; V = vibrissae; Jo = jaw open; Ll = lower lip; Pr = pronation; Tr = trunk; Sup = supination; Se = shoulder extension; Af = ankle flexion; T5f = toe 5 flexion; Ae = ankle extension; Ke = knee extension; Tl = tail; Rag = retrosplenial agranular cortex; Oc = visual cortex; Te = auditory cortex; PrPi = prepiriform cortex.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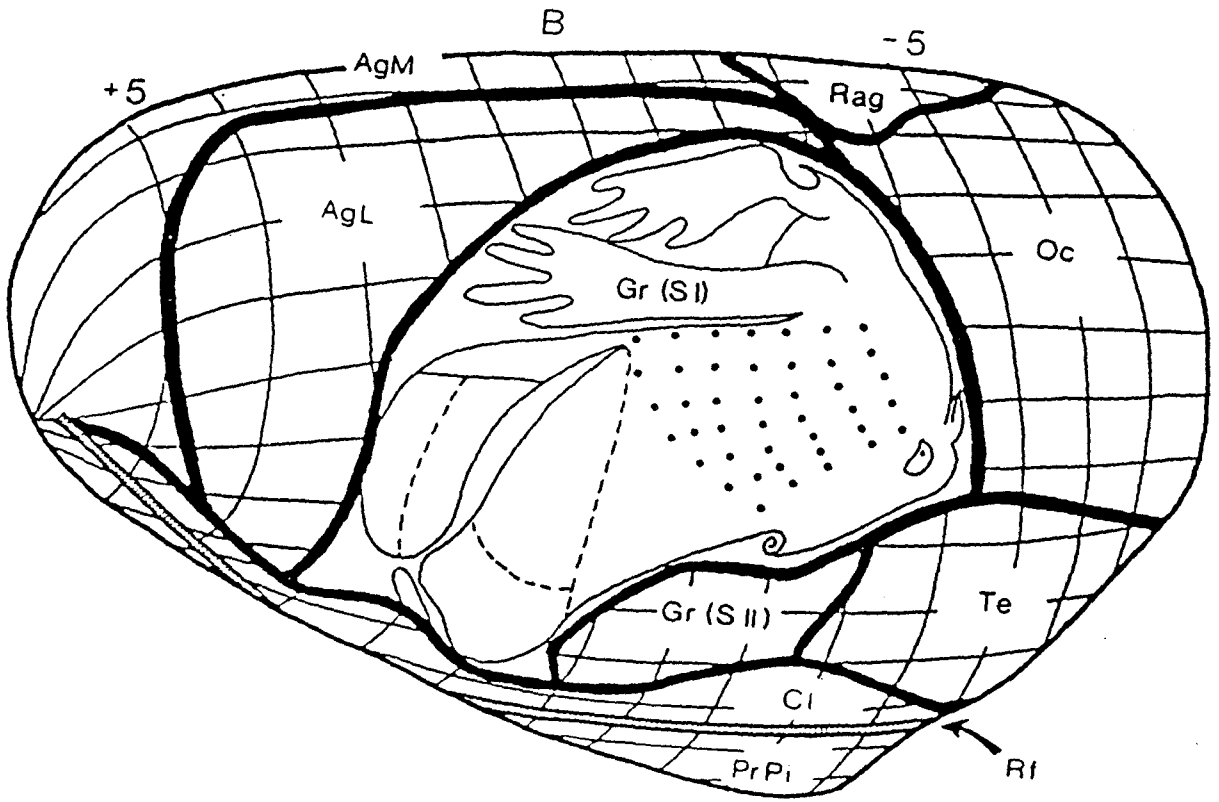
Pr Pi

Figure 2

Cytoarchitectonic boundaries of rat cerebral cortex. Schematic dorsolateral view of rat brain indicating the cytoarchitectonic divisions seen within the rat cortex. Heavy lines mark the borders of each area. B denotes bregma, scale is in mm. AgM = agranular medial cortex; AgL = agranular lateral cortex; Gr(SI) = granular cortex, primary somatosensory cortex; Gr(SII) = granular cortex, secondary somatosensory cortex; Rag = retrosplenial agranular cortex; Oc = visual cortex; Te = auditory cortex; Cl = claustrum; PrPi = prepiriform cortex; Rf = rhinal fissure. Ratunculus seen within Gr(SI) modified from Welker (1971).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CHAPTER III

EXPERIMENTS

A. CYTOARCHITECTURE OF THE DORSAL THALAMUS OF THE RAT

INTRODUCTION

This study was undertaken to provide a description of the cytoarchitecture of the rat dorsal thalamus accompanied by photomicrographs of Nissl-stained sections. The early description of the rat thalamus by Gurdjian (1927), although useful and detailed, is illustrated only with line drawings. A number of recent studies (e.g., Faull and Carman, 1968; 1978; Jones and Leavitt, 1974) have described the cytoarchitecture of various subregions of the normal rat thalamus but they do not describe the entire thalamus. The most thorough Nissl stained, light microscopic photographic atlas available is provided by Paxinos and Watson (1982). However, the relatively large one millimeter spacing between Nissl-stained plates, which is adequate for the intended stereotaxic applications of the atlas, is insufficient for a detailed analysis of thalamic nuclei. Accordingly, the normal cytoarchitecture of the rat thalamus is presented using a rostral to caudal series of photomicrographs (400 um intervals) of celloidin embedded, 33 um coronal sections stained with cresyl violet. The terminology is derived principally from Faull and Carman (1978) and Jones and Leavitt (1974).

MATERIALS AND METHODS

Three Long-Evans hooded rats were used in this study. The animals were deeply anesthetized with sodium pentobarbitol (40mg/kg, IP) and perfused transcardially with 0.9% saline followed by 10% formalin. The brains were removed and post-fixed for 3-5 days in the same 10% formalin fixative. The brains were then embedded in celloidin and cut in the coronal plane at 33 um. The sections were mounted and stained with cresyl violet. Sections were photographed at a magnification of 2.5X which was the optimal magnification both for delineating the cytoarchitecture within individual nuclei and for distinguishing between nuclei. The nuclear parcellation was done directly on the photographic prints with constant referral to the original Nissl-stained sections. The description of the cells within each cytoarchitectonic subdivision was based on analysis of the sections at higher magnifications than those illustrated.

RESULTS

Ventral Nuclear Complex: This complex is composed of three separate components: the ventromedial (VM), ventrolateral (VL) and ventrobasal (VB) subdivisions. VM is a longitudinally elongated nucleus containing densely packed medium to large-sized neurons. It is located within the ventromedial portion of the dorsal thalamus, positioned ventral and lateral to the mammillothalamic tract (MTT; designated by * in Figs. 4-7). At successively more caudal levels, VM

remains in about the same position while the MTT moves more ventrally relative to VM (Figs. 4-6). Most caudally the MTT lies within VM (Fig.7), (Herkenham, 1979). VM is bounded laterally at all levels by VL and VB and bounded dorsally at intermediate levels by the paracentral nucleus (Pc, Fig. 4) and more caudally by the central medial nucleus (Cem, Fig.6). At still further caudal levels, VM disappears and is replaced by the prerubral Field H of Forel (H, Fig. 8) which is located just lateral to the fasciculus retroflexus (FR), (Herkenham, 1979). A constant landmark medial to both VM and the MTT is the submedial nucleus (Sm, Figs. 3-5), (Jones and Leavitt, 1974). Sm is a nearly spherical nucleus which lies just medial to the MTT and is characterized by a paucity of both cells and fibers. Herkenham (1979) and Paxinos and Watson (1982) termed Sm the nucleus gelatinosus.

The ventrolateral nucleus (VL) consists of a rather diffuse collection of medium and more numerous large-sized cells. Rostrally, VL forms a large complete cap over the anterior pole of the ventrobasal complex (Fig. 2) and thus forms the most rostral component of the ventral nuclear complex (Faull and Carman, 1978). More caudally, VL extends dorsally over VB forming a lateral wing (Fig. 3) while at still further caudal levels VL extends over VM forming a medial wing (Figs. 4-5). Caudally, VL gradually diappears and its position becomes occupied by the posterior nuclear complex (PO, Fig 6).

The ventrobasal complex (VB) is composed of large and medium-sized deeply stained neurons with an abundance of fibers coursing throughout this nucleus. The cells in the dorsomedial aspect of VB, referred to as VPM (McAllister and Wells, 1981) appear to be more densely packed than ventrolaterally located cells designated VPL (Paxinos and Watson, 1982). VB is a relatively large, rounded nucleus that lies lateral to VM and medial to the reticular nucleus (R, Fig. 5). At mid-thalamic levels, VB is bordered dorsomedially by VL, PO, and the lateral posterior nucleus (LP, Figs. 5-6). The medial lemniscus (ml) which terminates within VB can be observed in Figures 7-9. The most medial extension of the ventrobasal complex, the gustatory nucleus (t, Fig. 7), (Norgren and Leonard, 1973; Herkenham, 1979) is observed between VM and the parafascicular nucleus (Pf) at caudal thalamic levels. Paxinos and Watson (1982) refer to this region as the parvocellular division of the ventroposterior thalamic nucleus.

Intralaminar Nuclei: Located between the ventral nuclear complex and the mediodorsal nucleus (MD), the intralaminar nuclei include the central medial (Cem), central lateral (CL), paracentral (Pc), and parafascicular (Pf) nuclei. The Cem and the Pc nuclei contain small, densely packed cells. The cells in Cem, which are slightly smaller than those in Pc, form a compact well defined nucleus located on the midline between the paracentral nuclei (Fig. 4). The somewhat elongated cells in Pc appear oriented transversely in the direction of the fibers of the internal medullary lamina (Figs. 4-6), (Jones and

Leavitt, 1974). The CL nucleus contains neurons similar in size to Pc but which tend to be more rounded with similar packing density. Medially, the junction between CL and the paralamellar division of the mediodorsal nucleus (pl) is not very distinct (Figs. 4-5) in contrast to the very distinct lateral border of CL adjacent to the smaller cells of the lateral posterior nucleus (LP, Figs. 3-5). Caudally, CL merges with the parafascicular nucleus (Jones and Leavitt, 1974). The Pf nucleus is made up of closely packed, medium-sized round neurons and appears to surround the fasciculus retroflexus (Figs. 6-7). Jones and Leavitt (1974) have described tongue-like ventral protrusions of Pf just posterior to the mediodorsal nucleus which have been designated the subparafascicular (sPf) nucleus in the present study (Figs. 7-8). Paxinos and Watson (1982) also refer to this region as the subparafasciculus nucleus.

Midline Nuclei: The midline nuclei include the medioventral nucleus (MV, also termed the reuniens nucleus), (Herkenham, 1978; Paxinos and Watson, 1982) the paratenial (PT), rhomboid (Rh), paraventricular (PV), and submedial (Sm) nuclei (Jones and Leavitt, 1974). The Cem nucleus is also usually considered a midline nucleus but in the present account was described with the intralaminar nuclei. The MV nucleus is located ventrally along the midline at rostral thalamic levels and is bordered laterally by VM and Sm and dorsally by the rhomboid nucleus (Figs. 2-6). MV is composed of loosely packed small to medium-sized cells and is characterized by fibers coursing

through it which give it a lightened appearance. It extends caudally to the level of the fasciculus retroflexus (Fig. 6). The small, oval-shaped PT nucleus is located at extreme rostral thalamic levels (Fig. 1) and contains loosely packed medium-sized cells. PT is bordered medially by the paraventricular nucleus (PV), dorsally by the stria medullaris (SM), laterally by the anterodorsal (AD) and anteroventral (AV) nuclei, and ventrally by the anteromedial nucleus (AM). The Rh nucleus is positioned between Cem dorsally and MV ventrally at rostral thalamic levels (Fig. 3). Rh contains medium-sized densely stained neurons which are very tightly packed. Rostrally, Rh is T-shaped (Fig. 3) but assumes its rhomboid shape at more caudal levels (Fig. 4). The PV nucleus is situated adjacent to the third ventricle and extends from rostral thalamic levels to the posterior commissure (PC, Figs. 1-6). This moderately packed nucleus contains many large and medium-sized densely stained neurons. The submedial nucleus (Sm) was described previously with the ventral nuclear complex.

Anterior Nuclear Group: This group consists of the anterodorsal (AD), anteroventral (AV), and anteromedial (AM) nuclei. The AD nucleus comprises a small but dense band of large, densely stained neurons in the dorsolateral aspect of the rostral thalamus (Fig. 1). It is bordered medially by the stria medullaris (SM) and PT nucleus and laterally by the AV nucleus. The larger AV nucleus demonstrates a similar band-like arrangement of loosely packed medium-sized cells

(Figs. 1-2). AV is bounded dorsomedially by AD, laterally by the reticular nucleus (R) and ventromedially by AM. AM is composed of very loosely packed and lightly stained cells and is further characterized by the fibers of the MTT which enter ventrolaterally (Fig. 1).

Mediodorsal Nucleus: The mediodorsal nucleus (MD) is an oval-shaped nucleus positioned dorsally in the thalamus lateral to the midline paraventricular nucleus. MD is characterized by an abundance of fibers, especially laterally which appear as pale areas, and contains many small, lightly stained and loosely packed neurons. It appears at rostral thalamic levels (Figs. 2-3) and extends throughout the thalamus, becoming wider more caudally (Figs. 4-5). MD is bounded medially by PV, laterally by CL, dorsally by the lateral habenular nucleus (LH), and ventrally by Pc and Cem. This nucleus has often been subdivided into three components (Leonard, 1969; 1972; Krettek and Preice, 1977), but in Nissl preparations only the paralamellar portion (pl) is easily differentiated within the lateral aspect of MD (Figs. 4-5). Paxinos and Watson (1982) have called this fiber-rich lateral segment of MD the lateral mediodorsal thalamic nucleus. In addition, they also designate an intermediodorsal thalamic nucleus along the midline which in the present study was included as part of the PV nucleus.

Lateral Nuclear Complex: The lateral nuclear complex includes the lateral dorsal (LD) and lateral posterior (LP) nuclei, both made up of

small lightly stained cells that are moderately packed. The LD nucleus is apparent at rostral thalamic levels just dorsal to the rostral cap of VL (Fig. 2) and extends caudally in its dorsolateral thalamic position to the level of the fasciculus retroflexus (Fig. 6) where it is replaced by the dorsal nucleus of the lateral geniculate body (LGD, Fig.7). The LP nucleus is found just medial to the ventrobasal complex, lateral to CL, and dorsal to PO (Figs. 4-5). It merges with the pretectum dorsomedially and with PO ventrally at the level of the fasciculus retroflexus (Figs. 6-7).

Posterior Nuclear Complex: This complex contains many fibers and is less densely populated with neurons than the more medially located intralaminar nuclei (Fig. 5). The small-celled PO nucleus has a distinct lateral border with the more deeply stained VB and is bounded ventrally by the remnants of VL (Figs. 5-6). At more caudal thalamic levels, PO lies just ventral to the pretectal nuclei (PRT) and forms the caudalmost boundary of the diencephalon where it gradually merges into the mesencephalic reticular core (Figs. 8-9). The rostral portion of PO has also been termed the dorsomedial division of the ventral nuclear group (Gurdjian, 1927; Konig and Klippel, 1963).

Reticular Nucleus: The thalamic reticular nucleus (R) forms a capsule around the lateral and rostral aspect of the dorsal thalamus, with a dorsal extension approaching LD rostrally and LP and LGD more caudally. The reticular nucleus also extends ventrally to form the ventralmost border of the thalamus where it blends into the zona

incerta (ZI) at mid-thalamic levels (Fig. 6). The reticular nucleus separates the internal capsule (IC) from the thalamus and contains large densely stained neurons which are moderately packed and oriented in the direction of the fibers of the external medullary lamina.

Diencephalic-Mesencephalic Junction: Several nuclear groups lie around or within the periaqueductal gray (PAG) in close association with the fasciculus retroflexus. These nuclei include the nucleus of Darkschewitsch (NDk, Fig. 9) which contains medium-sized, darkly stained neurons located within the ventrolateral PAG and the more caudally located interstitial nucleus of Cajal (not illustrated) which also contains large densely stained neurons which are fewer in number and are not found within the PAG proper. The posterior nuclear complex (PO) is the caudalmost structure of the dorsal thalamus where it merges with the deep mesencephalic nucleus. The nucleus of the posterior commissure (NPC) and the pretectal area (PRT, Fig. 9) are located dorsal to PO. Lateral to PO are found the magnocellular division of the medial geniculate body (MGM) and the suprageniculate nucleus (SG). The medial lemniscus (ml) and the zona incerta (ZI) are ventral to PO, and the PAG with its associated nuclei lies medial to PO (Fig. 9).

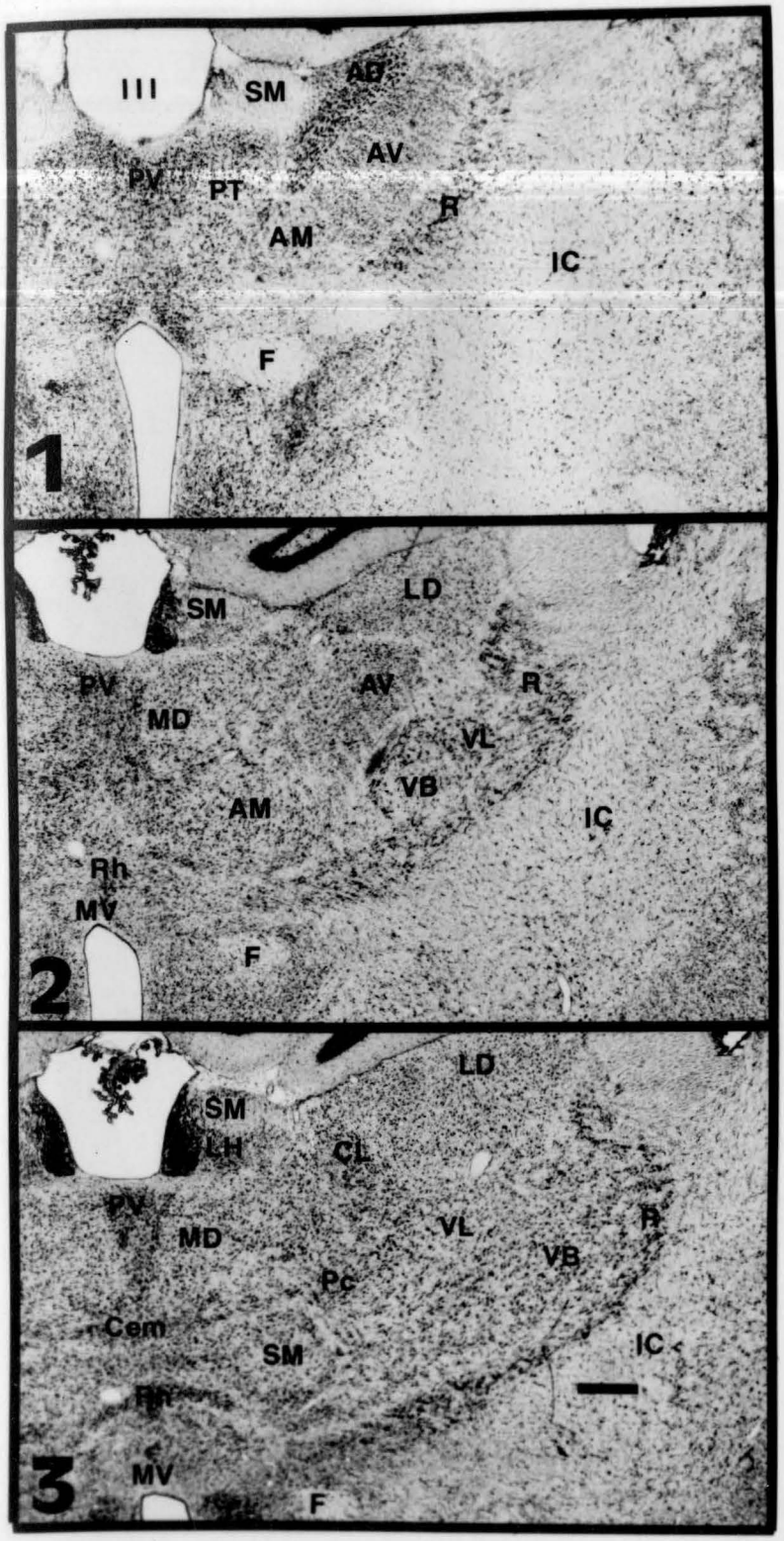
DISCUSSION

The subdivison of the rat thalamus described here is based on cytoarchitectonic characteristics including packing densities of

cells, relative size of neurons within specific nuclei and the presence or absence of fibers within specific nuclei. This study replicates and extends previous observations (Gurdjian, 1927; Konig and Klippel, 1963; Faull and Carman, 1968; 1978; Paxinos and Watson, 1982), and, although it does not actualy create any new terminology or descriptions, it does provide a complete descriptive atlas of the rodent thalamus with accompanying photomicrographs. This atlas should prove useful to other investigators as an aid in describing the results of anatomical and physiological experiments where the identification of a specific thalamic nucleus is needed.

Figures 1-3

Rostral-caudal sequence of photomicrographs at 400 um intervals through the rostral third of the normal rat thalamus, stained for cells with cresyl violet. Each section is 33 um in thickness and each section is oriented with dorsal at the top and lateral to the right. Calibration bar = 500 um. See text for abbreviations.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1
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MV
F
2
LD
SM
LH
CL
PV
VL
R
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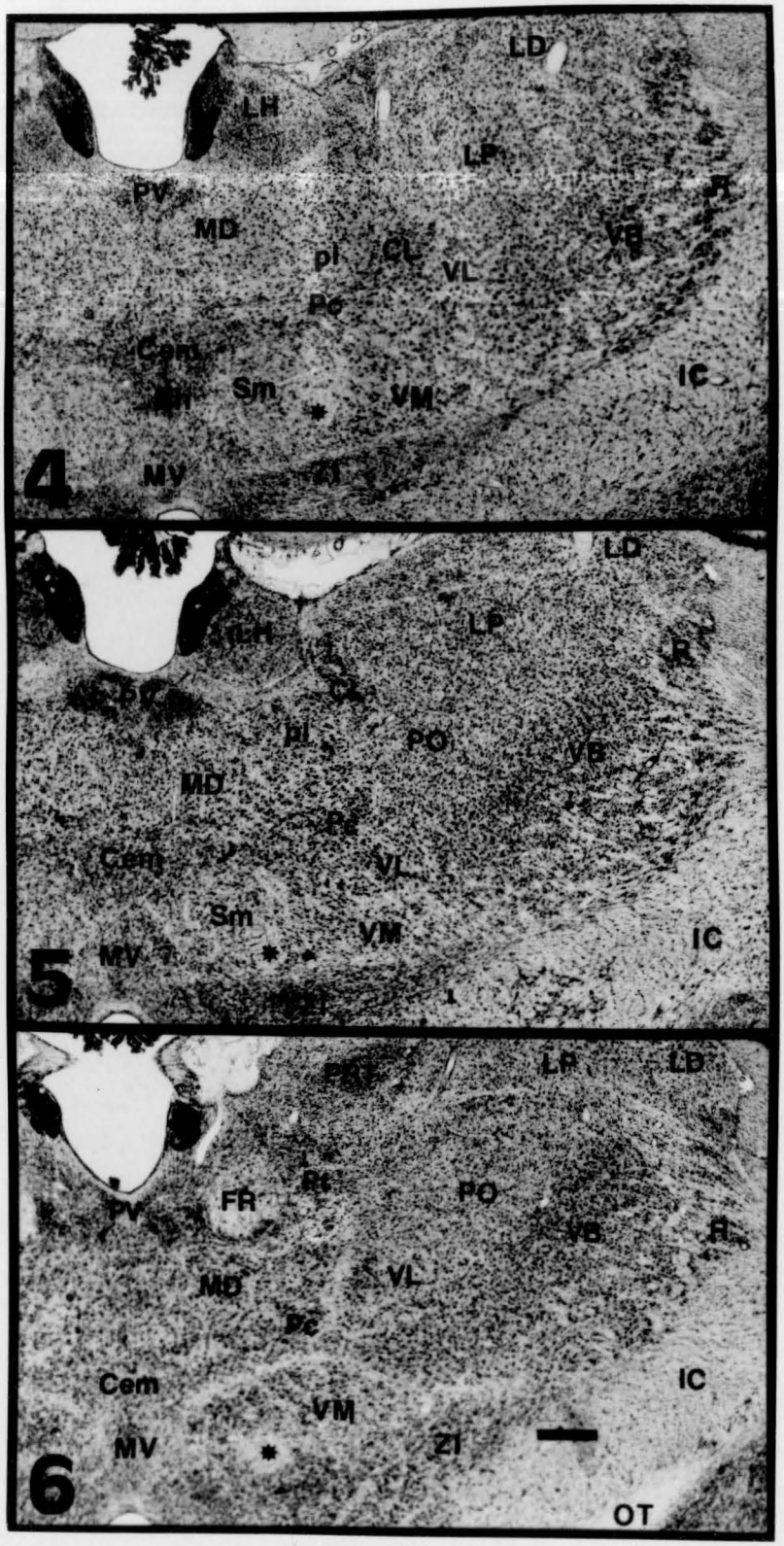
Pc
Cem
SM
IC
Rh
MV
F
3

Figures 4-6

Rostral-caudal sequence of photomicrographs at 400 um intervals through the middle third of the normal rat thalamus. Calibration bar = 500 um. See text for abbreviations.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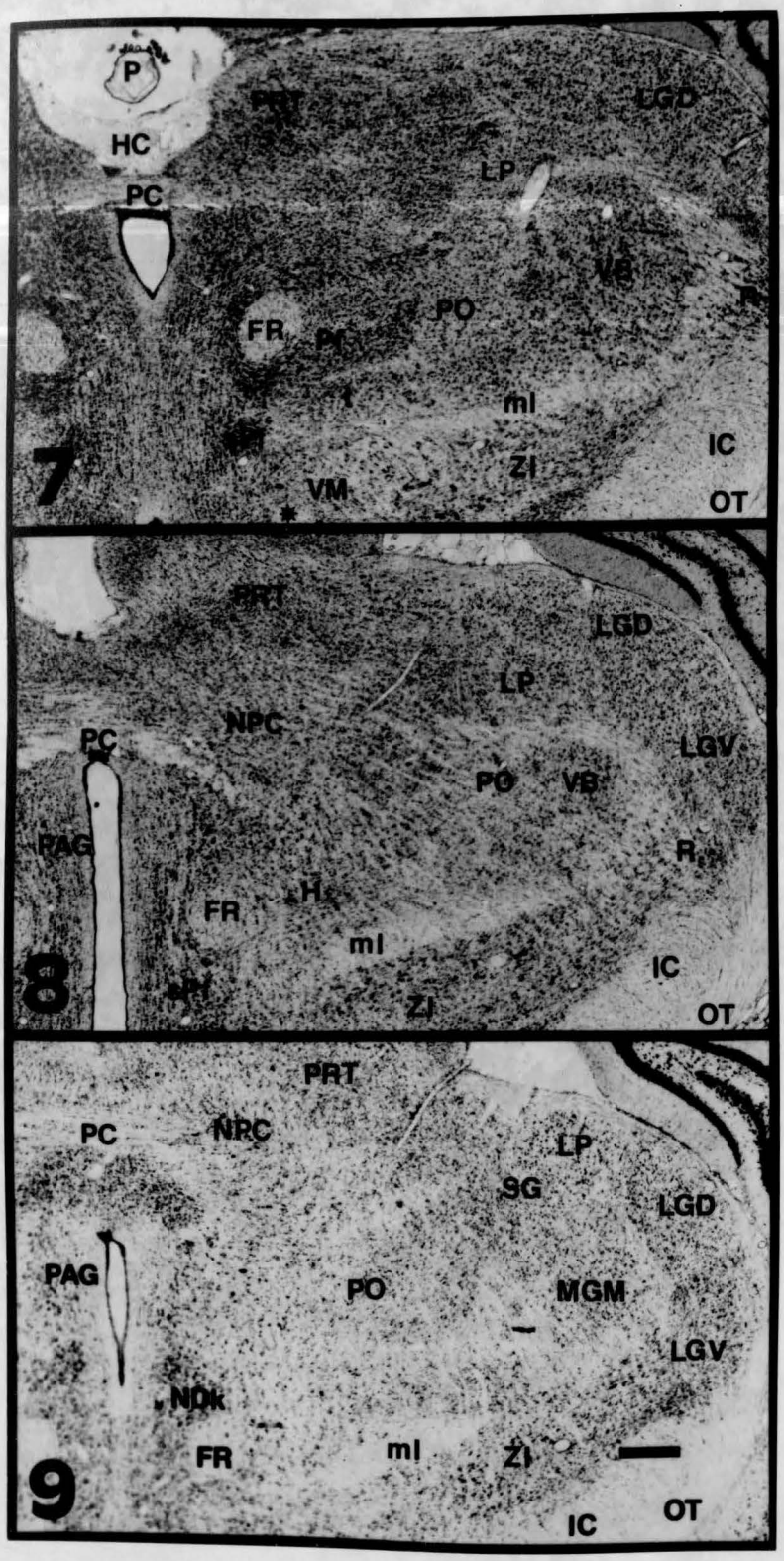
Figures 7-9

Rostral-caudal sequence of photomicrographs at 400 um intervals through the caudal third of the normal rat thalamus. Calibration bar = 500 um. See text for abbreviations.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NRC
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SG
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Chapter III

EXPERIMENTS

B. THE AFFERENT INPUT TO THE THALAMIC POSTERIOR COMPLEX IN THE RAT.

A Study Using Retrograde Fluorescent Tracing Techniques

INTRODUCTION

The precise location, anatomical connections, functions, and comparative homologies of the posterior nuclear complex (PO) of the mammalian thalamus are not clearly understood. In the cat, several cytoarchitectonically distinct nuclei, such as the suprageniculate (SG) and magnocellular medial geniculate (MGM) nuclei (Berman and Jones, 1982) and a posterior nucleus (PO) have been included in this thalamic region. The feline posterior nucleus has been further subdivided into three separate nuclei referred to as medial (POm), lateral (POl), and intermediate (POi, Berman and Jones, 1982). From the large number of anatomical and physiological studies that have focused on the thalamus, it appears that, at least in the cat and the monkey, the different posterior nuclear components subserve different functions. For example, POm relays somatosensory information, POi and SG relay visual information, and MGM and POl relay auditory information (Bentivoglio, et al., 1983). In the rat the nuclear region which has been called PO can be clearly differentiated from surrounding thalamic nuclei based on both cytoarchitectural and connectional data (Bold, et al., 1984; Bold et al., 1983). However, the rodent PO cannot be subdivided because of its rather homogeneous appearance when examined with Nissl stained material. An analysis of afferent and efferent connections to this region may help to clarify whether functional cytoarchitectonic subdivisions exist within the

rodent PO, and whether it is correctly classified as a nonspecific thalamic nucleus (Rose and Woolsey, 1948; Herkenham, 1980; Macchi, 1983).

The present study was undertaken to determine the inputs to PO using the retrograde transport of the fluorescent dye diamidino yellow. This tracer was chosen because of its minimal visible spread from the injection site compared to fast blue or horseradish peroxidase, a property which enabled us to confine the boundaries of the injection to PO.

MATERIALS AND METHODS

Sixteen adult Long Evans black hooded rats weighing between 250-350 grams were used in this study. Animals were anesthetized with sodium pentobarbitol (40 mg/kg, IP) and placed in a stereotaxic frame. A midline incision was made and the skin and soft tissues reflected, exposing the dorsal convexity of the skull and the atlanto-occipital membrane. The cisterna magna was opened to drain the cerebrospinal fluid in order to minimize cortical swelling. The coordinates for the injections of retrograde tracers into the PO nuclear complex were established using the atlas of Paxinos and Watson (1982). Bregma was used as the surface landmark for the rostrocaudal reference point, and the midsagittal suture was used as the midline reference point. Following a small craniotomy over the level of PO, a 1 ul Hamilton syringe fitted with a glass micropipette (tip diameter 40-50 um) mounted on a Kopf microdrive was lowered through the cortex, hippocampus, and lateral posterior nucleus into PO. The standard coordinates for a "typical" PO injection was 4.0mm caudal to bregma, 2.25mm lateral to the midline and 6.5mm below the surface of the skull. Each injection placement was corrected for the standardization used by Paxinos and Watson (1982) by measuring the distance between bregma and lambda. This established good accuracy in terms of rostrocaudal and mediolateral placement within PO. However, in the

dorsoventral axis below the skull surface, their coordinates produced injections that were consistently too dorsal to the intended location. This problem was corrected by using the brain surface as the starting point instead of the skull surface.

Six animals received injections of 0.02- 0.04 ul of 1% diamidino yellow diacetate (DY) aimed at PO, and two animals received injections of 0.02- 0.04 ul of 1% DY aimed at VB ventral to PO. Of the six injections aimed at PO, three were successfully placed within PO and three were inadvertently placed in the more dorsal lateral posterior nucleus (LP). The two injections intended for VB were on target. The fluorescent tracer fast blue was also used in several animals (n=8), and, while the results generally concurred with those observed using DY, the spread of dye to adjacent areas made interpetation of the results difficult and these experiments are not included in the present report.

Following a 3-4 day survival period animals were perfused with 4% buffered paraformaldehyde followed by 10% buffered sucrose. The brains were blocked and placed in 20-30% buffered sucrose until the brains sank to the bottom of the specimen jar, which took about 3-4 days. Fifty micron frozen coronal sections were cut and placed in 0.005M acetate buffer (pH 3.3, Spatz and Grabig, 1983). The sections were mounted on gelatinized slides and allowed to dry at room temperature since heat increases autofluorescence (Kuypers et al, 1979). Outline drawings were made of sections at 200 um intervals,

and the uncoverslipped tissue visualized using an epifluorescent filter with excitation wavelength of 330 nm. Retrogradely labeled cells were plotted onto the outline drawings using a camera lucida attachment. Once cells were plotted at 200um intervals, the cells were photographed with both color and black and white film. All of this was completed within two days of cutting the tissue, at which point one set was counterstained using Pyronin Y and the other set dehydrated and coverslipped without counterstaining. The parcellation of the thalamic and brainstem nuclei was done using the counterstained material.

Cells labeled by the injections into PO, LP and VB were all plotted in this manner. These data were compared in order to determine labeling that might be due to spread from PO injections both dorsally into LP and ventrally into VB. Other control injections were placed in the posterior parietal cortex and the hippocampus along the course of the pipette track, and the results of these experiments were also compared to the PO injection results. Different tracers other than DY (Fast Blue and wheat germ agglutinated-HRP) were used in these cortical and hippocampal injections and are not illustrated in this report since there did not appear to be significant spread of DY up the injection track. Cortical labeling is described using the cytoarchitectonic nomenclature of Zilles et al. (1980), Krettek and Price (1977), and Donoghue and Wise (1982). Thalamic and brainstem labeling is described using the terminology and criteria of Bold et

al. (1984) and of Paxinos and Watson (1982).

RESULTS

Analysis of injection sites revealed that three animals received DY injections that appeared confined to PO, and three additional animals had injections confined to LP or VB (Figure 1). Eight animals received FB injections centered in PO but which appeared to spread into adjacent areas. The results of these animals generally concurred with DY injections but did show slightly more extensive labeling patterns which may be attributed to the spread of the injections. Accordingly, the data are presented from cases receiving DY injections.

PO Injection

A small injection (0.02 ul) of 1% diamidino yellow was placed into the caudal aspect of the posterior nuclear complex (Figure 2A). Labeling resulting from this injection is illustrated in figure 3; this pattern was also seen in the other two DY injections localized within PO and also agrees substantially with the results seen following FB injections into PO.

Cortical Labeling: Retrogradely labeled cells in the cortex were found from the frontal pole to the visual cortex. Rostrally (Figure 3A-E), labeled cells were found in the lateral agranular field (AgL), the sensory granular areas (SI and SII), and the claustral cortex

(Cli). No labeled cells were seen in the medial agranular field (AgM). Within AgL (Figure 6A), labeled cells were clustered in lamina V-VI. In SI, a bilaminar pattern of labeling was evident, with a dense band of labeled cells in lamina V-VI and a second less dense band of labeled cells in lamina II-III. Labeled cells within SII were clustered in laminae III and V-VI. No labeled cells were found in either the anterior cingulate (AC) or retrospenial fields (Rsg or Rsag). Labeled cells were found, however, in the posterior parietal cortex, and in the auditory and visual cortical fields (Figure 3F-I). Labeling in the posterior parietal cortex was bilaminar, in lamina III and VI, whereas labeling in the visual and auditory areas was confined to a single band of cells occupying the infragranular laminae, V-VI. Labeling in the visual cortex was sparser than that seen more rostrally in somatic sensorimotor cortex.

Diencephalic Labeling: Retrogradely labeled cells were found in the thalamic reticular nucleus occupying only the dorsal one-third of the nucleus (Figure 3 E-F and Figure 6B). Labeling was evident in the zona incerta (ZI) but was found at only one level (Figure 3F). Labeled cells were also found the anterior pretectal region (PRT, Figure 3H). A distinct cluster of labeled cells was found in the ventral aspect of the magnocellular medial geniculate (MGM) and the mesencephalic reticular formation medial to the MGM (Figure 3I-J).

Brainstem Labeling: Within the midbrain (Fig. 3I-J) several small clusters of labeled neurons were found in the middle laminae of the superior colliculus (Fig. 3J). These clusters extended from the midline to the lateral border of the superior colliculus. No labeled cells were found in the red nucleus, but a cluster of cells was found in the retrorubral nucleus (RRN, Figure 3K). At the level of the pons, labeled cells were found in the inferior colliculus in four distinct clusters (Figure 3L). Labeled cells were also located in the lateral part of the pontine reticular formation (PRF) as well as in the ventral aspect of the nucleus of the lateral lemniscus (NLL, Figure 3L). Further caudally, labeled cells were found in the dorsal and ventral nuclei of the parabrachial complex (DPB, VPB), as well as bilaterally in the locus coeruleus (LC, Figure 3M). A few labeled cells were found in spV (Figures 3N and 6C) and in the cerebellar interpositus (INT) and dentate nuclei (DN, Figure 3O). At still further caudal levels, labeled neurons were located in the nucleus gracilis (NG) and nucleus cuneatus (NC), the superior vestibular nucleus (SVN) and a few cells were localized in the medial vestibular nucleus (MVN, Figure 3O). A few cells were found in the principal trigeminal nucleus (Vp, Figure 3O). At the first cervical (C1) spinal level (Figures 3S and 6D), a small number of cells were found in the dorsal horn in lamina IV and in the region of the fasciculus gracilis (FG).

Lateral Posterior Injection

A small injection (0.04 ul) of DY was placed in the lateral posterior nucleus in order to identify the source of afferents to this nucleus and to determine whether the afferents to LP are different than than afferents to PO (Figure 1B and 2B). This injection involved most of LP as well as the anterior portion of PRT but did not involve the posterior nuclear complex or lateral geniculate nucleus. The results of this injection are illustrated in figure 4 and are similar to those found in the other two LP injections.

Cortical Labeling: No labeled cells were found near the rostral pole, and very few cells were seen in the dorsal convexity of frontal cortex. However, retrogradely labeled cells in the frontal cortex were found in AgM, anterior cingulate (AC) and prelimbic (PL) cortical fields in lamina V-VI (Figure 4B). Caudally, labeled cells were found in the retrospenial granular (Rsg) and agranular fields (Rsag, Figure 4E-H); most caudally labeled cells were found in in the auditory cortex (Figure 4F-H) as a dense band of cells occupying the infragranular laminae V-VI. Retrogradely labeled cells filled lamina V-VI of the primary and secondary visual fields (Area 17 and 18). This differs from the small PO injection described above where extensive labeling was found in AgL, SI and SII, no cells were found in AgM, and many fewer labeled cells were found in the visual cortical

areas.

Diencephalic Labeling: Labeled cells in the thalamic reticular nucleus were confined to the dorsal aspect of this nucleus (Figure 4D-F). Unlike the previously described PO injection, no labeled cells were found in the ZI. The location and size of the injection site was such that any labeling in the pretectal area (Figure 4H) appeared to be the result of spread of the injected material into PRT rather than being the result of retrograde transport. A few labeled cells were found in the nucleus of Darkschewitsch (NDk), and similar labeling was seen in the interstitial nucleus of Cajal (INC, Figure 4H). Further caudally, labeling in the medial geniculate complex was confined to a cluster in the ventral part of MGM with a few cells extending more dorsally within this nucleus (Figure 4J).

Brainstem Labeling: At the level of the midbrain, heavy labeling was apparent in all laminae of the superior colliculus. Labeled cells filled almost the entire extent of this structure at its rostral extent (Figure 4I) and appear to be more medially located further caudally (Figure 4J). A few labeled cells were found within the PAG and in the dorsal raphe (DR) nucleus. Labeling within the inferior colliculus was seen as three distinct clusters, one located near the midline in the middle lamina, one cluster further lateral but extending ventrally, and a third cluster at the lateral edge of the

inferior colliculus, extending even further ventrally (Figure 4K). All of these cell clusters were located at the rostral level of the inferior colliculus, and no label was seen in the caudal portion of this structure (Figure 4L). Labeled cells were seen in the lateral dorsal tegmental nucleus (LDTN), the cuneiform nucleus (Cnf), the ventral part of the nucleus of the lateral lemniscus (NLL), the retrorubral nucleus (RRN), and the prerubral field (PRF, Figure 4K-L). At the level of the rostral medulla only a few labeled cells are seen in the contralateral ventral parabrachial nucleus (VPB, Figure 4M) No labeled cells were seen in the locus coeruleus nor in any of the trigeminal nuclei. A few labeled cells were found in the medullary reticular formation (PRF, Figure 4N), medial vestibular nucleus (MVN, Figure 4O) and the paragigantocellular reticular nucleus. No labeled cells were found in the nucleus gracilis (NG), nucleus cuneatus (NC), external cuneate nucleus (ECN) or upper cervical spinal cord (Figure 4P-S).

VB Injection

A larger amount (0.04 ul) of 1% DY was deposited in the thalamus in a position ventral to the PO complex (Figures 1C and 2C). The injection site was centered in the medial part of VB and also included the lateral parts of VM and only the most ventral part of PO. The midline nuclei, the ILN and the reticular nucleus were all spared,

while the dorsalmost part of the zona incerta was involved in this injection. The results of this injection are illustrated in figure 5, and are similar to that seen following the other VB injection.

Cortical Labeling: Retrogradely labeled cortical neurons were found only in the rostral two-thirds of the cortex. Most rostrally (Figure 5A-B), labeled cells filled the lateral agranular field and extended laterally into the granular somatosensory cortex. Like the small PO injection labeled cells were not found within the medial agranular field. Further caudally (Figure 5C-D), labeled cells were found in the deeper lamina of SI and in a similar position in SII. A significant number of cells extended ventrally into the dorsal agranular claustral cortex (Cli). At still further caudal levels (Figure 5E), only a few labeled cells were found in primary auditory cortex (AI), again in lamina V-VI. No cortical labeling was found caudal to this level.

Diencephalic Labeling: A few labeled cells were found in the ventral striatum (Figure 5D), a structure not labeled with PO or LP injections. Labeling in the thalamic reticular nucleus was also rather sparse, located in its most central part and only at extreme rostral thalamic levels (Figure 5D). The zona incerta was filled with retrogradely labeled neurons from mid-thalamic levels to the mesencephalic junction (Figure 5F-H). It is difficult to assess the

labeling in ZI because it is possible the dendrites of ZI neurons extended into the injection site. No labeled cells were found in any part of the medial geniculate complex, nor in the NDk or INC (Figure 5H). The deep mesencephalic nucleus (DMN), however, was densely populated with retrogradely labeled neurons (Figure 5H-I).

Brainstem Labeling: At the level of the midbrain, labeled cells in the deep mesencephalic nucleus (DMN) extended into the red nucleus and up to the border of the PAG. Only a few cells were actually seen in the PAG and these were located ventrolaterally (Figure 5J). No obvious labeling was found in the superior colliculus except for a rather large cluster of cells at its ventrolateral border where it merges with the medial geniculate complex (Figure 5I-J). A dense cluster of labeled cells were found in the substantia nigra, pars reticulata (Figure 5J). At the level of the pons (Figure 5K-L), the only labeled cells were found in the dorsal raphe (DR), the prerubral field (PPF), and the nucleus of the lateral lemniscus (NLL). Further caudally (Figure 5M), labeled cells were seen bilaterally in the principal trigeminal nucleus, heaviest contralaterally, and contralaterally in the ventral parabrachial (VPB) nucleus. At the level of the seventh cranial nerve (Figure 5O), labeled cells were found bilaterally in the dentate (DN) and interpositus (INT) nuclei, but the labeling was heaviest contralaterally. Also at this level, labeled cells were found in the spinal trigeminal complex, again

bilaterally and heaviest contralaterally, and in the contralateral medial vestibular nucleus (MVN). At more caudal brainstem levels (Figure 5P-Q), labeling in the spinal trigeminal complex is not as plentiful but remains bilateral, heaviest ipsilaterally, while a few cells were still labeled in the contralateral MVN. At the level of the obex, the contralateral dorsal column nuclei are filled with retrogradely labeled cells (Figure 5R) and a few cells are seen in the nucleus tractus solitarius (NTS, Figure 5Q) but no labeled cells were found in the external cuneate nucleus (ECN). Finally at the spinal level of C1, a large number of retrogradely labeled cells were found in lamina V-VII of the central gray.

DISCUSSION

The use of fluorescent dyes in tracing neuroanatomical connections was introduced by Kuypers et al (1980), who demonstrated that different dyes could be injected into spatially separated areas of the cortex and by retrograde transport could reliably identify populations of thalamic neurons projecting to one or both injection sites. Such fluorescent dyes can also be used singly, especially when small well localized injections are necessary. In studies based on the retrograde transport of fluorescent dyes, however, at least two sources of misinterpetation should be taken into account. First, the spread of the injected dye into neighboring structures may lead to false conclusions. This possibility was most apparent in cases receiving FB injections which were therefore not included in the data presentation although they generally agree with DY injected animals. Spread of the injection site was further controlled for by placing injections in two adjacent structures (LP and VB) which were likely to be involved in the spread of the injected material from PO. This allowed us to determine the afferents to these surrounding structures and thus attain a more accurate appraisal of the actual afferents to PO. A second source of possible misinterpetation considers the reported uptake of DY by damaged and undamaged fibers of passage (Swanson and Sawchenko, 1984), an observation which clearly suggests that caution must be used in interpeting data from studies using these tracers. Two fiber bundles can be traced through the rat PO, the

tectothalamic and corticotectal tracts (Perry, 1980). The tectothalamic tract arises in the superficial layers of the superior colliculus and traverses the dorsolateral part of PO in route to terminations in LP and several other nuclei (Perry, 1980). In our experiments no labeled cells were found in the superficial laminae of the superior colliculus following injections involving PO, but these laminae were labeled after injections involving LP. The corticotectal tract arises from the primary visual cortex (Area 17) and part of it passes through the dorsolateral part of PO (Olavarria and Van Slutyers, 1982). Very few retrogradely labeled cells were found in area 17 following the PO injections. These two observations suggest that uptake by fibers of passage was not a major element contributing to the labeling seen after PO injections.

The observed inputs to PO from virtually all levels of the neuraxis from cerebral cortex to spinal cord underscores the numerous functions that have been associated with this thalamic complex (Blum et al., 1979). While the techniques used in this study do not permit a detailed analysis of where within PO the afferents terminate, other studies have shown that various inputs to PO terminate in specific subregions of this nucleus (Lund and Webster, 1967; Geisler et al., 1979; Feldman and Kruger, 1980). Further anterograde tracing studies are needed to verify if this specific distribution of terminations within PO is characteristic of PO's other sources of afferent input. Our findings are presented under headings related to the major sensory

modalities that terminate in PO, beginning with somatosensory afferents.

Somatosensory Afferents: Inputs from areas likely to relay somatosensory-related information arise from both the primary and secondary somatosensory cortices, the principal sensory and spinal tract nuclei of the trigeminal nerve, the dorsal column nuclei and the dorsal horn of the cervical spinal cord. This supports previous anterograde degeneration studies describing dorsal column (Lund and Webster, 1976a) and trigeminal nuclear (Lund and Webster, 1967b; Smith, 1973) terminations in PO, as well as autoradiographic studies describing similar terminations from the dorsal column nuclei (Feldman and Kruger, 1980). Although projections from PO to sensorimotor cortex are well documented in the rat (Jones and Leavitt, 1974; Donoghue et al, 1979; Donoghue and Parham, 1982), mouse (White and DiAmicis, 1977) and cat (Jones and Burton, 1974; Burton and Kopf, 1984; Spreafico et al., 1982; Bentivoglio et al., 1983), our data additionally demonstrates reciprocal connections from cortex to PO. Although previous anterograde studies did not report spinal cord projections to the rodent PO (Geisler et al., 1979), studies in the cat have described terminations in POl from the lateral cervical nucleus (Metherate et al., 1985) and from the rostral spinal cord (Burton and Craig, 1984). Since we failed to find retrogradely labeled cells in the cord following the LP injections and since the PO

injections did not appear to spread to VB, where injections labeled a much larger number of cells, it appears that some spinal somatosensory afferents do terminate in PO, perhaps relaying nocioceptive information (Casey, 1966).

Auditory Afferents: Several auditory-related structures including MGM, IC, NLL, and AI projected to PO. Although MGM projections to the auditory cortex in the cat (Diamond et al., 1969), dog (Morest, 1964) and rat (Bold, 1977) have been reported, MGM projections to PO have not been previously described. This input may be part of the pathway for the auditory evoked responses that have been recorded in the feline PO (Blum et al., 1979). Although not previously found, inferior collicular inputs to PO were consistently seen in all cases of DY injections in PO. This labeling is not attributed to spread of the injection into LP since the labeling observed in the inferior colliculus after LP injections was more dorsal and rostral to the more caudal distribution found after LP injections.

Visual Afferents: The observed visual cortical afferents to PO are in agreement with previous autoradiographic work (Benzinger and Massopust, 1983) that described afferents from Area 18 to the caudal posterior thalamic region. Tectal afferents to PO have previously been reported to arise from the deeper laminae of the superior colliculus (Donnelly et al., 1983), which precisely corresponds to the

location of retrogradely labeled cells found after small PO injections. These projections, which appear distinct from LP afferents from the superior colliculus (Perry, 1980), suggest an extrageniculate source of visual input to PO.

Vestibular Afferents: A few labeled cells were consistently found in the superior and medial vestibular nuclei following PO injections. Labelled cells were not found in these areas after LP injections. Although the ascending efferent connections of the vestibular nuclei have not been studied using anatomical tracers in any species, physiological methods have demonstrated vestibular inputs to PO in the cat (Blum et al., 1979).

Visceral Afferents: The parabrachial nuclear complex is well documented to project heavily to the gustatory or taste thalamic nucleus (VBm, Norgren and Leonard, 1973). In addition, more recent studies (Saper and Loewy, 1980) have found sparse terminations in PO following lateral parabrachial injections of tritiated amino acids. Consistent with this finding, labelled cells were found within the parabrachial complex as well as within the claustral or taste cortex (Yamamoto et al., 1980) after PO injections.

Motor Afferents: Motor system related afferents appear to arise from the motor cortex as well as from the cerebellum and the zona incerta.

Labeled cells in the motor cortex were found only after PO and VB injections and suggest a reciprocal connection between the thalamus and this part of the cortex. Retrogradely labeled cells were consistently found in the contralateral dentate and interpositus nuclei following PO injections but not following LP or VB injections. The existence of cerebellothalamic projections to PO in the rat has not been found in previous axonal degeneration (Faull and Carman, 1978) and autoradiographic studies (Haroian et al., 1981). However, the autoradiographic study described these projections from the posterior aspects of DN and INT to an area they labeled LP, but which appears to actually be the dorsal part of PO (Bold et al., 1984). Concerning incertal afferents, only small LP injections failed to label cells in the ZI. Previous autoradiographic work on the incertal efferents reported projections to the intralaminar nuclei, Pf and CL, and to VM, but did not report projections from the ZI to PO (Ricardo, 1981). Since even our smallest PO injection labeled cells in the ZI, it is not likely that the labeling is due to spread of the injection to the intralaminar nuclei or VM. Since the ZI has been reported to receive strong cerebellar (Faull and Carman, 1978, Castro, 1978) and frontal cortex projections (Ricardo, 1981), its connection to PO may be related to integrating motor as well as other multimodal sensory information.

Additional Afferents: Labelled cells were found in several areas that

are not readily included within one of the preceding functional groups. For example, labeled cells were consistently found within the thalamic reticular nucleus, especially in the dorsal part of this nucleus at midthalamic levels when only PO was involved in the injection. More caudal labeling in this nucleus was found following VB injections. These findings are consistent with previous descriptions of intra- or thalamothalamic interconnections involving the thalamic reticular nucleus (Scheibel and Scheibel, 1966; Jones, 1975). Our data also suggest that PO also receives afferents from the deep mesencephalic nucleus and from the retrorubral nucleus. Bilateral labeling within the locus coeruleus concurs with previous descriptions of sparse noradrenergic projections to PO (Lindvall et al., 1971).

Functional Considerations

In addition to receiving diverse kinds of afferents, PO has been found to project to widely distributed areas of the cortex in the opossum (Neylon and Haight, 1983) and to motor (Donoghue and Parham, 1982) and somatosensory areas SI and SII in the rat (Bold, 1984a) and cat (Burton and Kopf, 1984; Spreafico et al., 1982; Bentivoglio et al., 1983). It has been suggested that PO plays a role in modulating the sensitivity of the cortical response to direct somatosensory inputs (Neylon and Haight, 1983). Other investigators have considered

PO as part of the LP-pulvinar complex, associated with visual inputs. In order to determine if the multisensory inputs and widepread projections of this nucleus to cortex are concerned with "nonspecific" modulation of the cortex, a study of its projections to these specific cortical targets is underway. Furthermore, since PO receives direct projections from the thalamic reticular nucleus and the zona incerta, it is possible that these three structures are involved in some internal thalamic circuitry whose eventual output is relayed to cortex by PO.

Comparison With a Recent Similar Study

A recent study of afferent inputs to the nucleus posterior thalami in the rat (Roger and Cadusseau, 1984) concurs with many of our findings, but significant differences also exist. These apparent differences appear related to different interpetations of the position and extent of PO. From their photomicrograph of a representative injection site (their figure 3A) and their reference to the stereotaxic atlas of Pellegrino et al. (1979), they identify PO as considerably more caudal than found in recent cytoarchitectural studies based on thionin (Bold et al, 1984) and acetylcholinesterase (Paxinos and Watson, 1982) staining methods. Their observed afferents to PO from sensorimotor cortex, zona incerta, superior colliculs, deep mesencephalic nucleus, superior vestibular nucleus, dentate, principal

trigeminal, and spinal trigeminal nulcei agree with our results. However, they did not identify inputs from the thalamic reticular nucleus and the dorsal column nuclei which we consistently observed. According to the coordinates they used in the Pellegrino et al. (1979) atlas, what they appeared to inject was the anterior pretectum of Paxinos and Watson (1982), and probably only the most caudal part of what we term PO was involved in their injections.

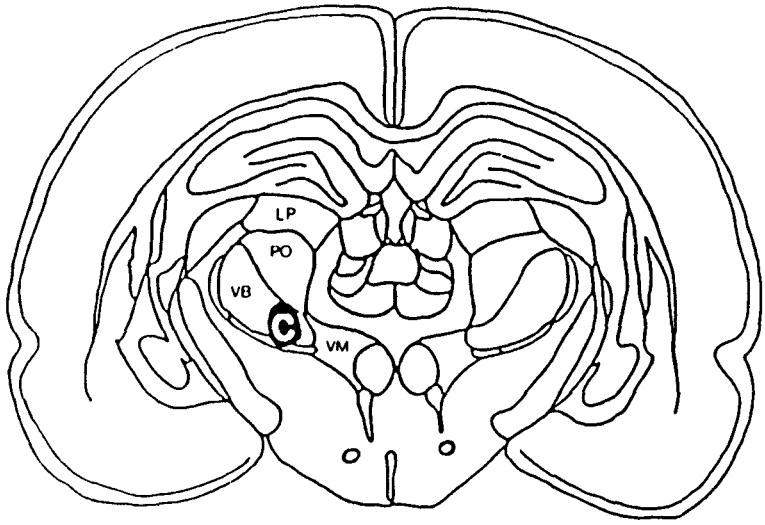
Evolutionary Considerations

Because PO appears to be a multisensory thalamic recipent zone and because PO neurons are among the first neurons to differentiate embryologically (Altman and Bayer, 1979), it has been suggested that PO represents a primitive archetypical sensory thalamus (Erickson et al., 1964) from which the traditional specific relay nuclei have differentiated. Consistent with this idea is that in both reptiles and birds there is no ventral tier of thalamic nuclei (Northcutt, 1984), but instead there is a nuclear region referred to as the posterior medialis or simply the medialis nucleus (Ulinski, 1984a). In amphibians, the DCN have been found to project exclusively to this nucleus (Northcutt, 1984), making posterior medialis the earliest known somatosensory receiving thalamic nucleus. Interestingly enough, however, posterior medialis does not project directly to the neocortex but rather to the anterior thalamus which then projects to the cortex.

Nevertheless, posterior medialis may very well be homologous to PO since these regions receive somatosensory input in both reptiles and rodents. Primitive mammals such as the egg laying Echnida appear to have a ventral tier, which raises the question of the derivation of these nuclei (Ulinski, 1984b). Since PO is one of the earliest regions of the thalamus to develop embryologically in the rat (Altman and Bayer, 1979), it is possible that PO in turn gives rise to the later developing ventral tier nuclei. However, there are problems with this proposal. First, PO has not been described in all mammalian thalami studied. Secondly, it is not known whether PO is the precursor or PO is the composite from which highly specialized thalamic nuclei have differentiated. And finally, since the transitional forms are extinct we can only look at modern day reptiles and attempt to homologize structures. Although not conclusive, our data appears consistent with the concept of PO as a multimodal "primitive" thalamus from which the highly specialized somatosensory thalamus emerged, as originally proposed by Erickson et al. (1964).

Figure 1

Outline drawings through the rostral (A) and caudal (B) levels of the posterior nuclear complex showing the relative placement of injection sites. The white letters A-C correspond to the actual injection sites illustrated in A-C of figure 2.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VB
C
VM

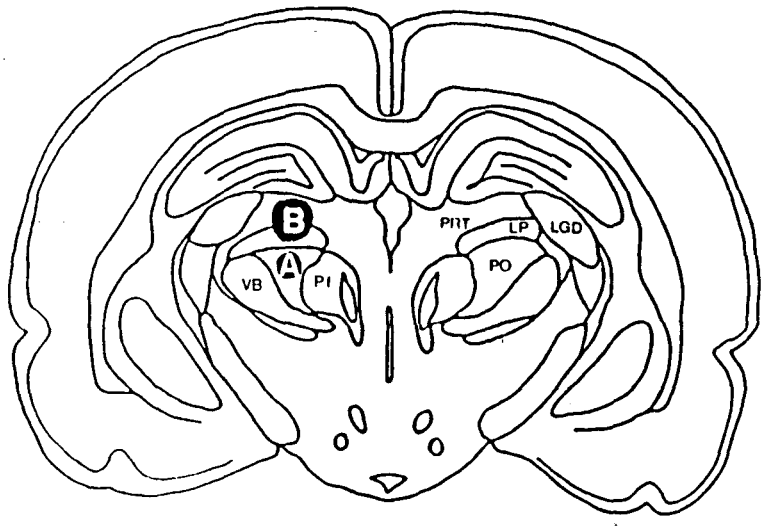
B
A
VB
PI
PIT
LP
LGD
PO

Figure 2

Photomicrographs of Nissl stained sections through each of the injection sites described in this report. The letters A-C correspond to the letters in Figure 1. Each section is a 50 um frozen section counterstained with Pyronin Y. A. Diamidino yellow in PO. B. Diamidino yellow injection in LP. C. Diamidino yellow injection in VB.

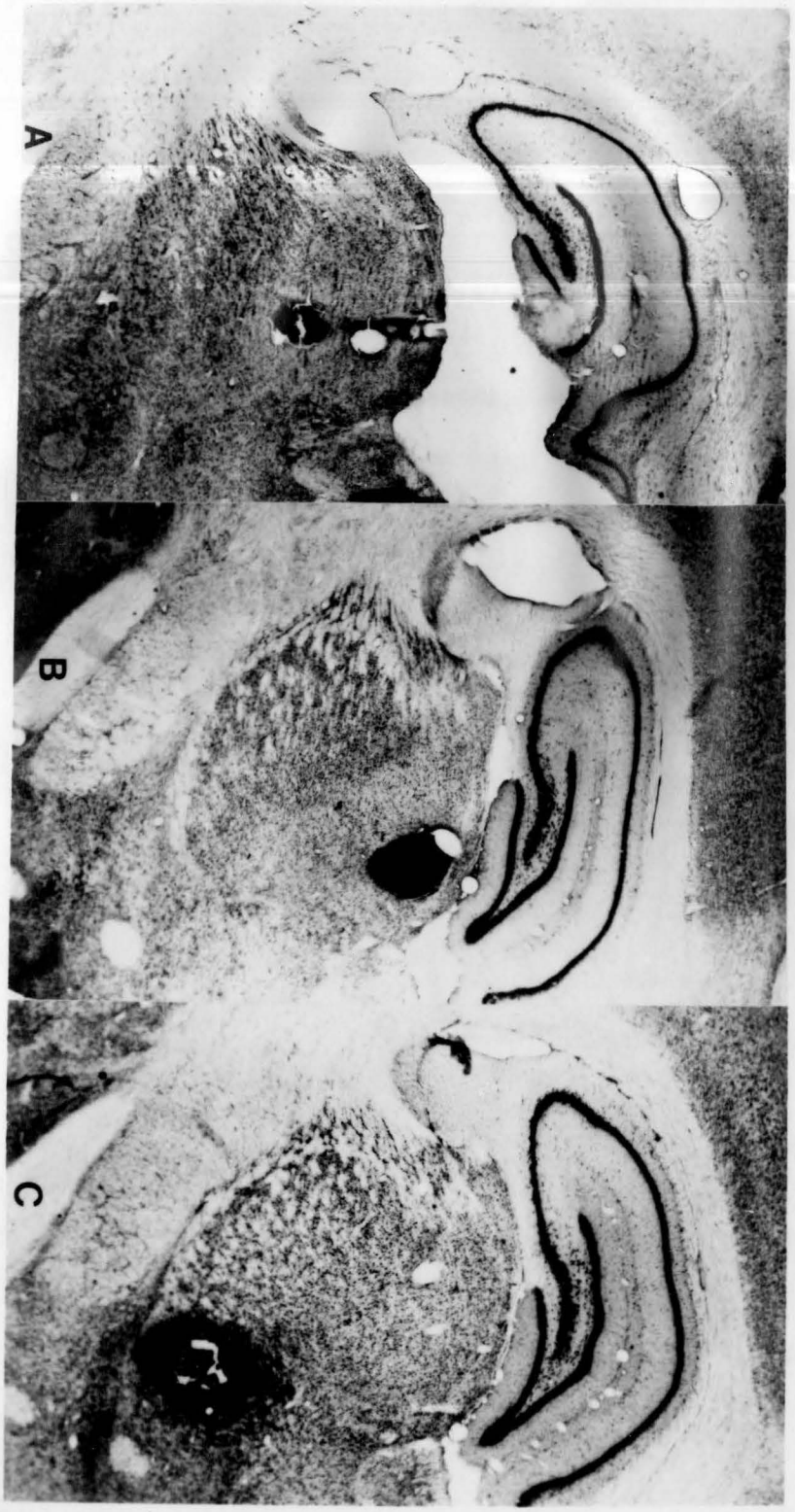
A
B
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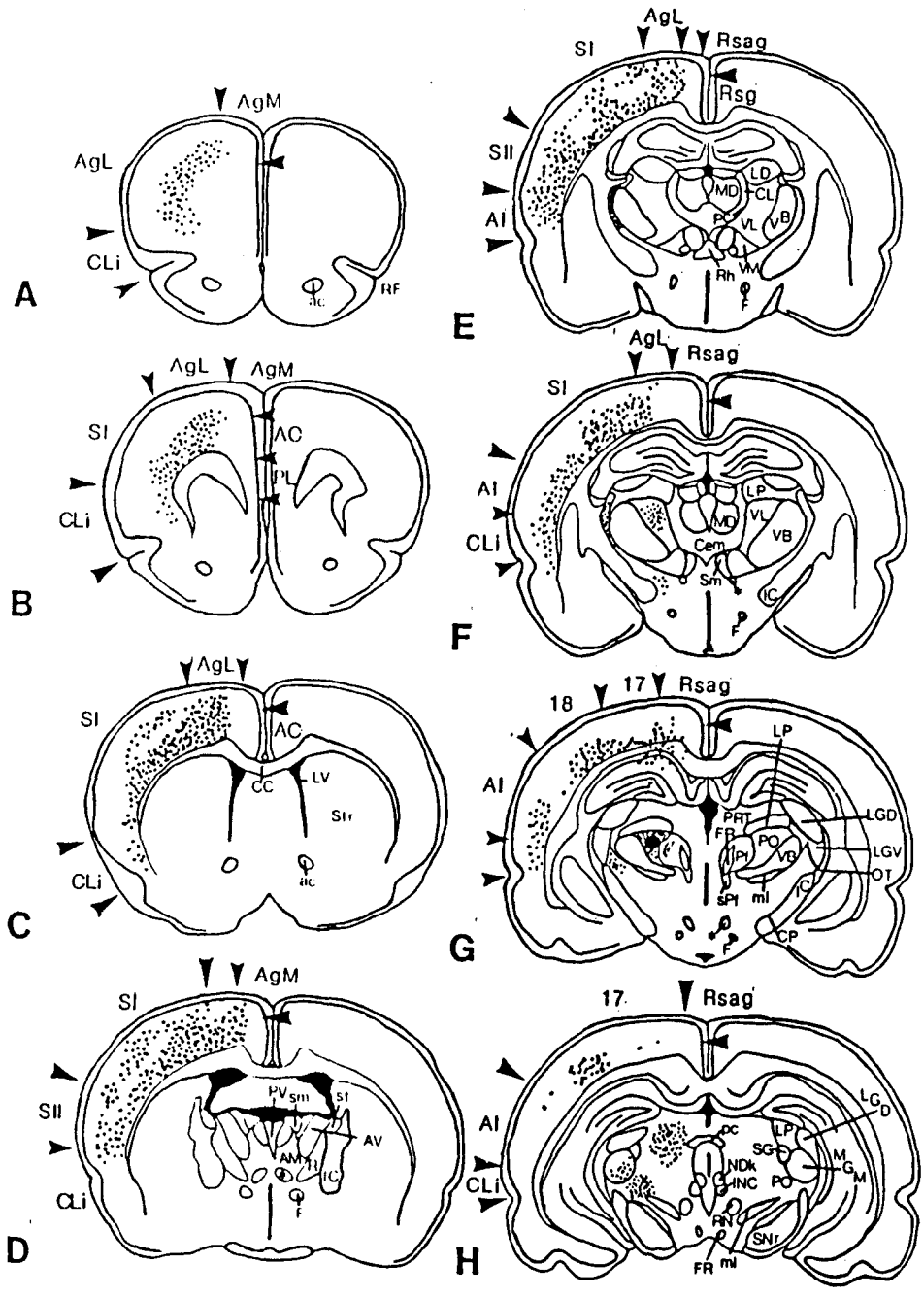
Figure 3

Drawings in rostral-caudal sequence at 1 mm intervals demonstrating labeled cells found after a DY injection in PO. Each dot represents one retrogradely labeled neuron.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AC
PL
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CC
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Str
CLi
ic
D
AgM
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SII
CLi
PVsm
st
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IC
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AgL
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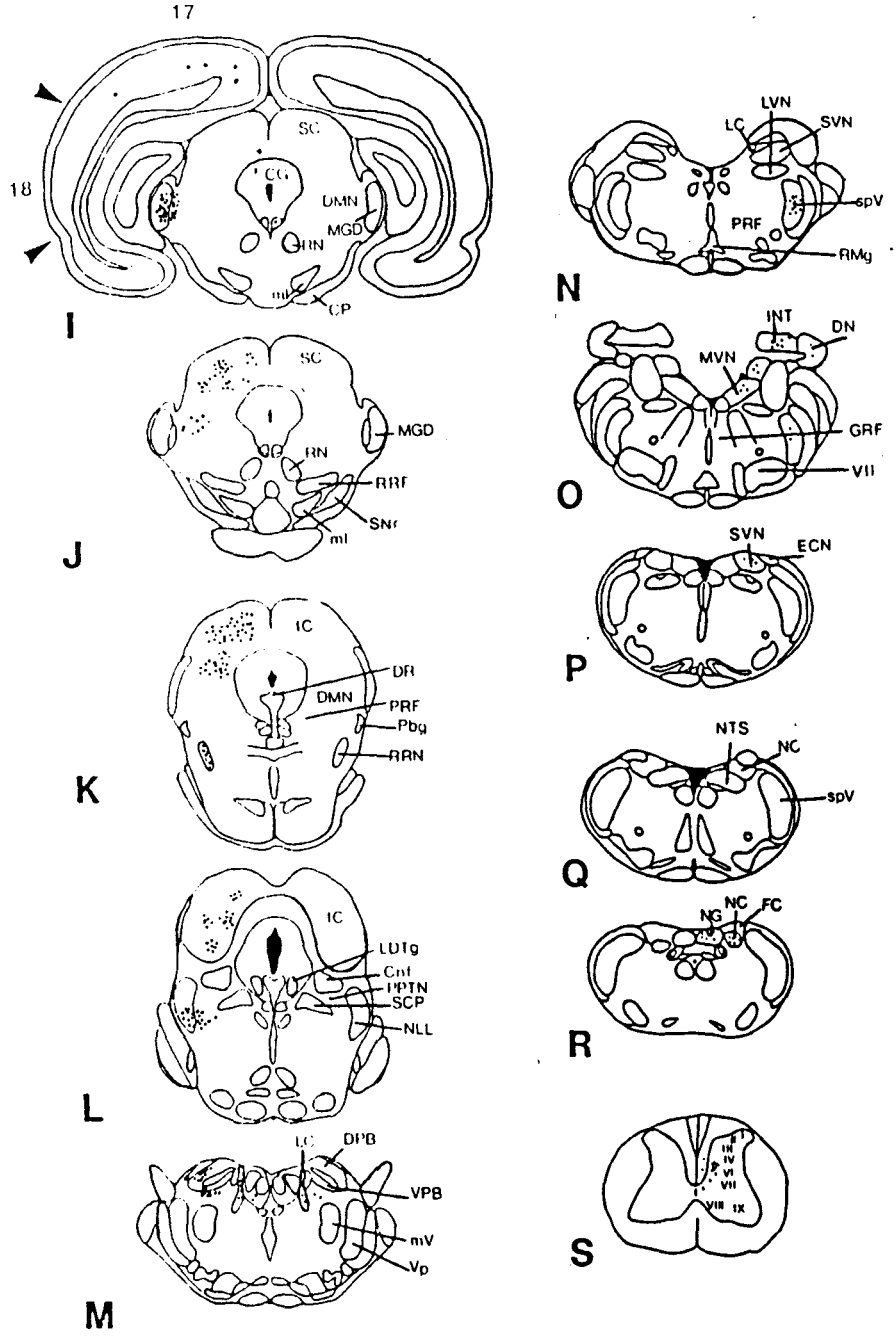
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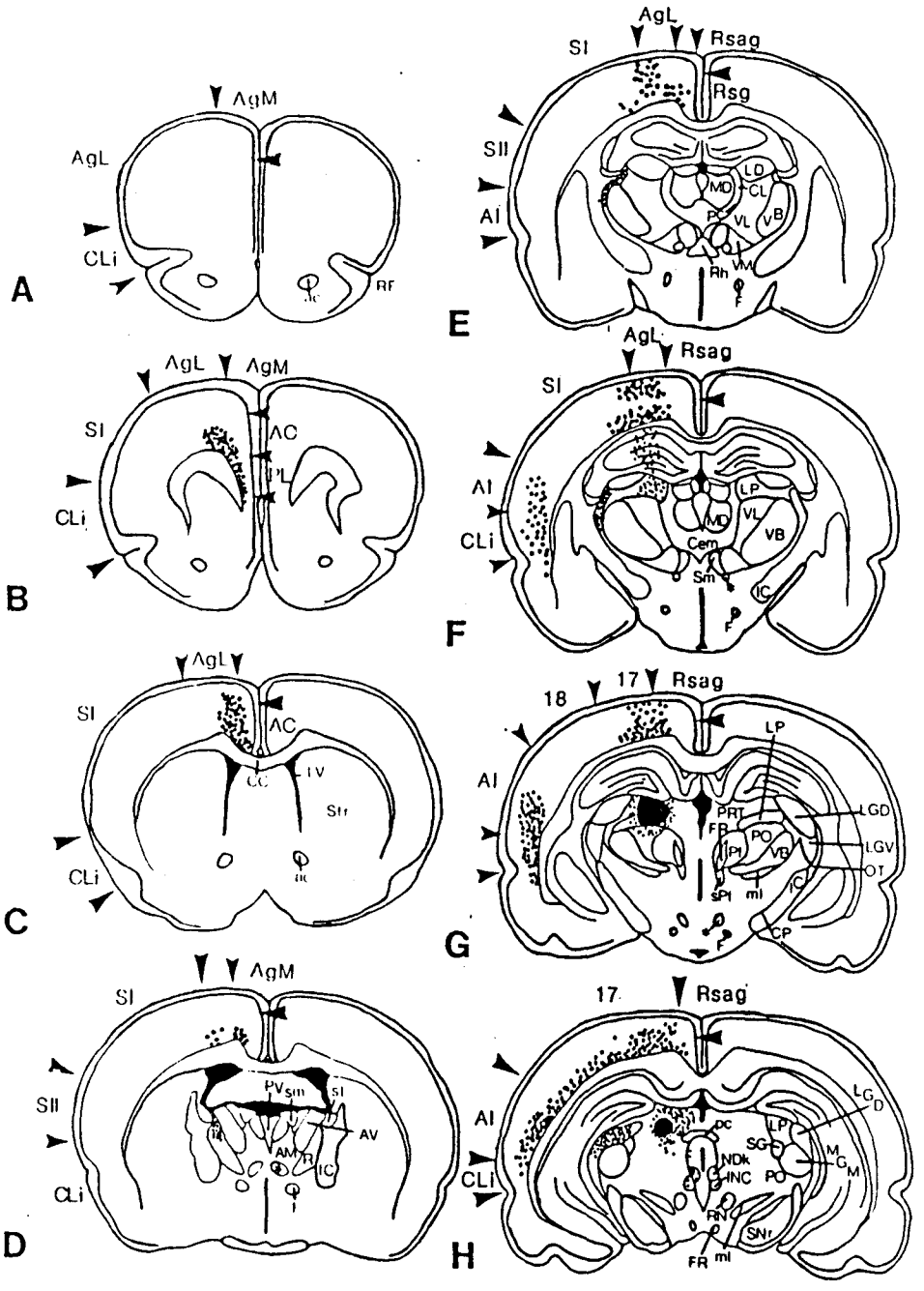
Figure 4

Drawings in rostral-caudal sequence at 1 mm intervals demonstrating labeled cells found after a DY injection in LP.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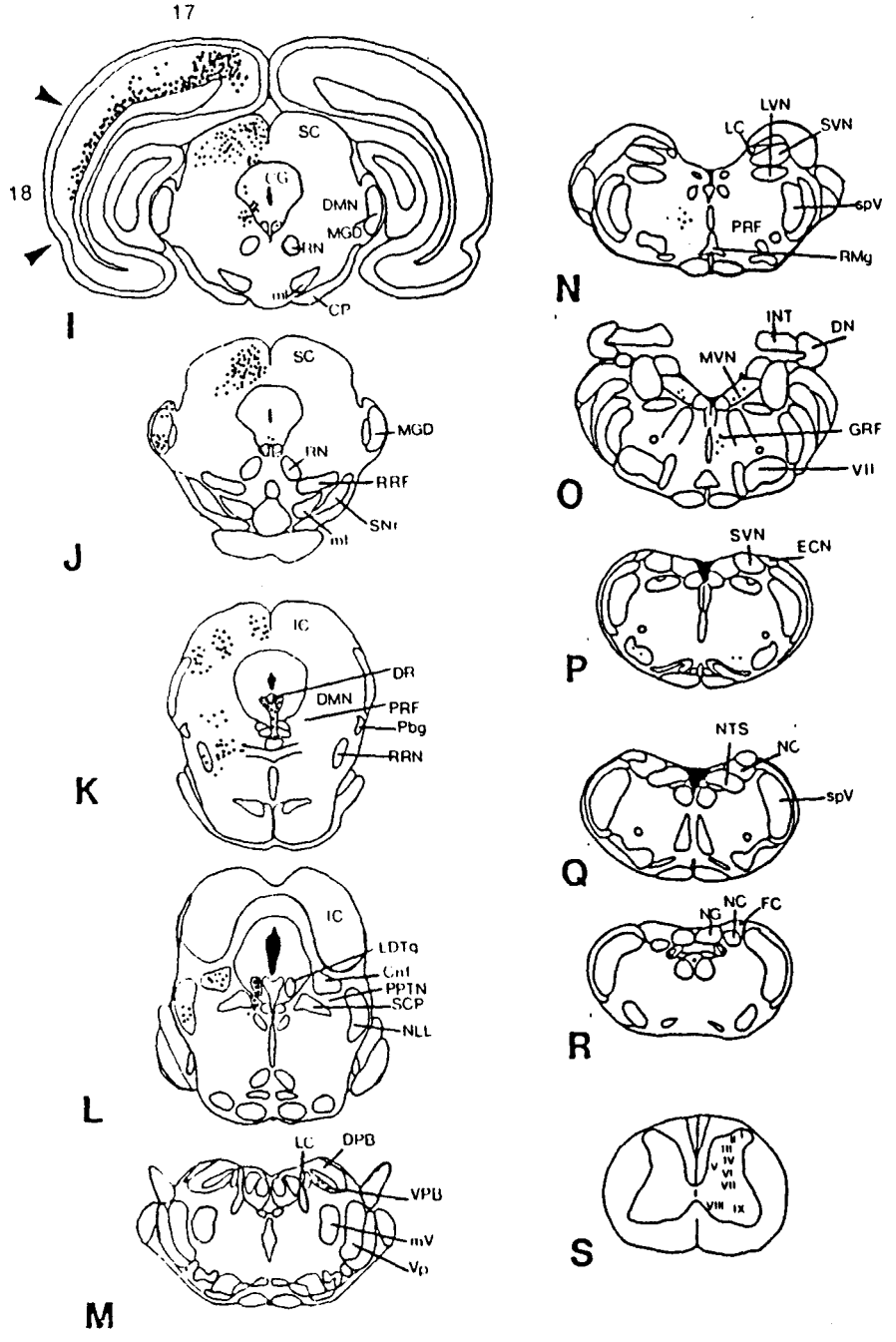
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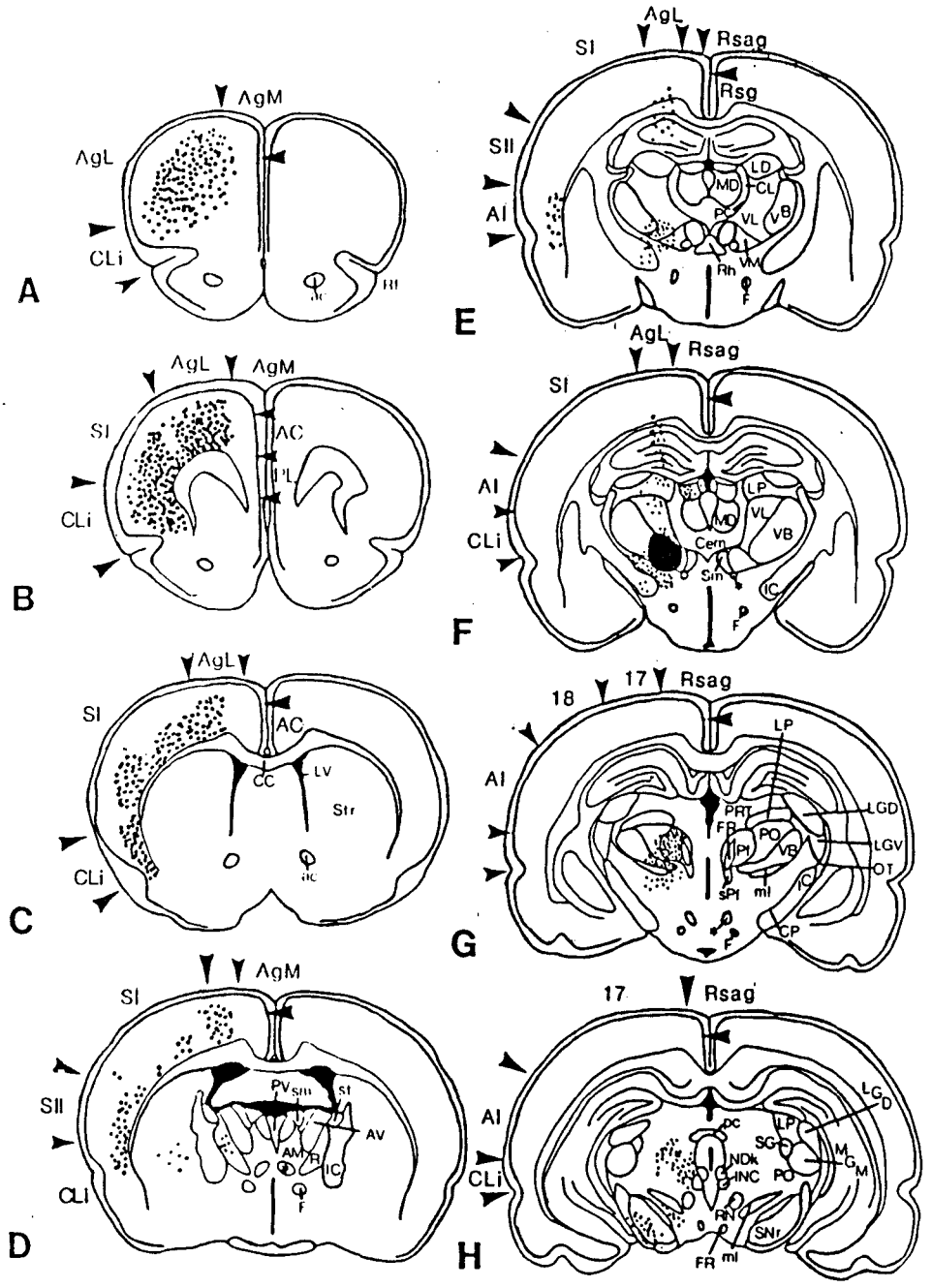
Figure 5

Drawings in rostral-caudal sequence at 1 mm intervals demonstrating labeled cells found after a DY injection in VB.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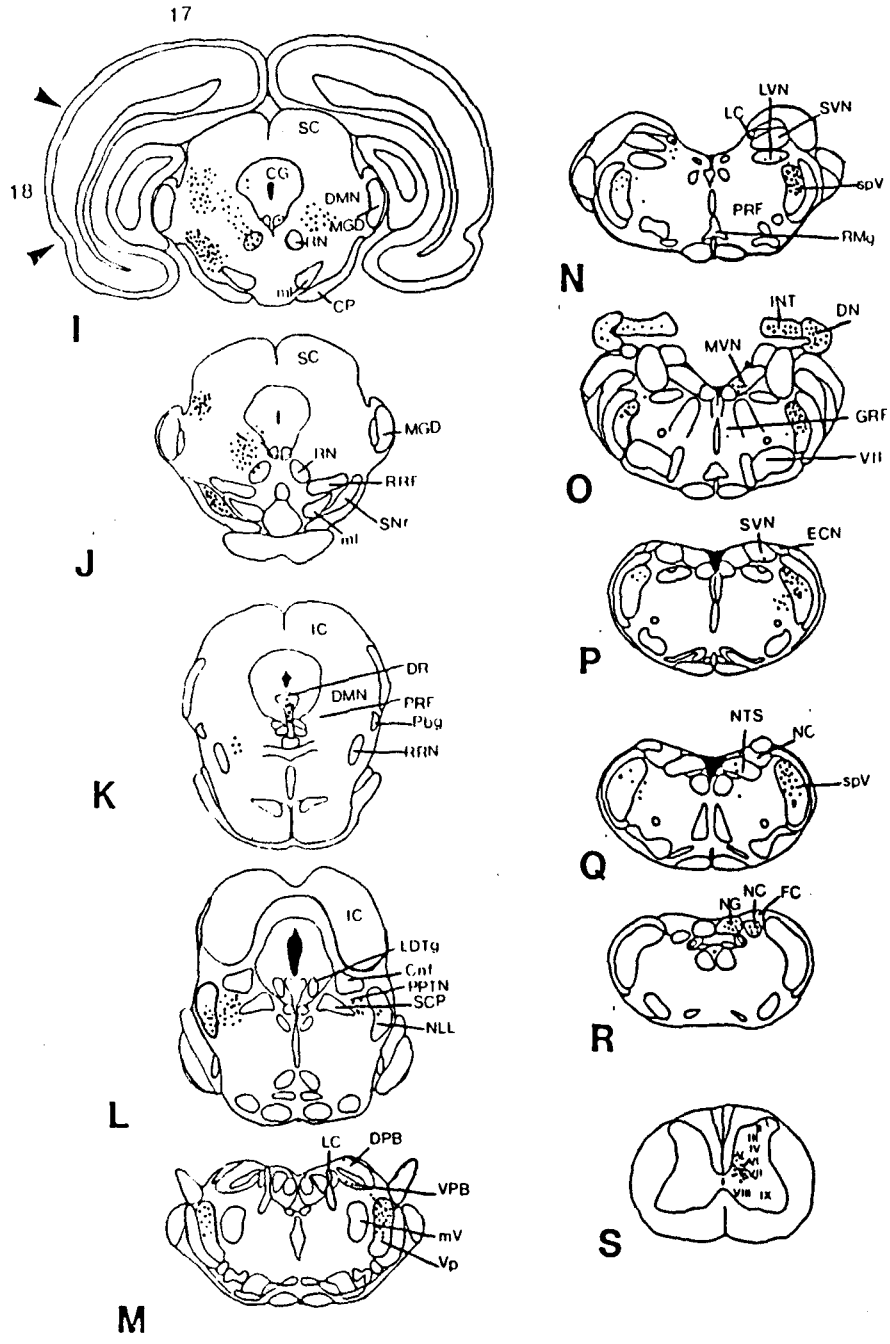
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GRF
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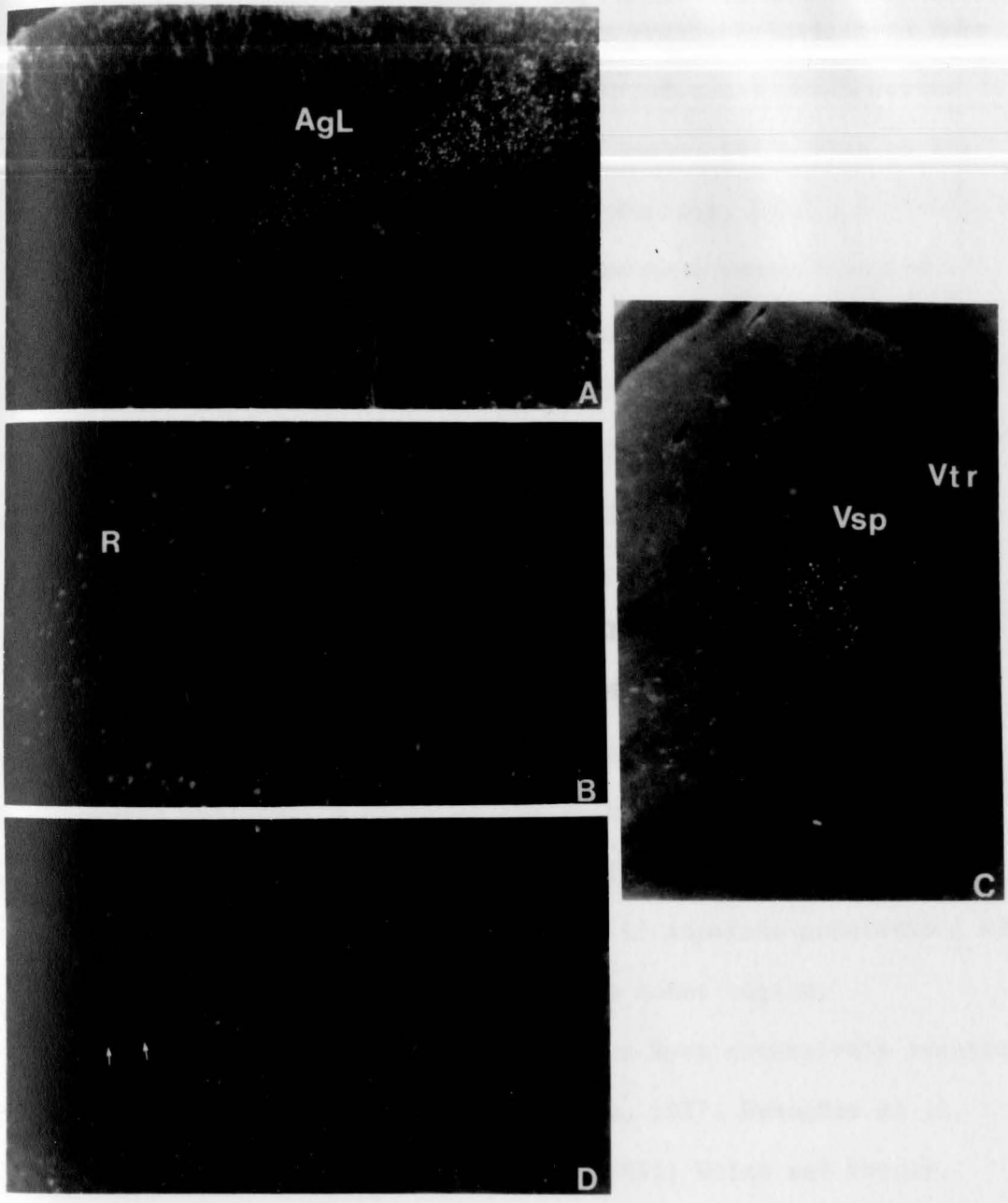
Figure 6

Photomicrographs of retrogradely labeled fluorescent cells in various areas. A. Retrogradely labeled cells in the deep lamina of the somatosensory cortex following a DY injection into PO (6X). B. Diamidino yellow retrogradely labeled cells in the thalamic reticular nucleus (R) following a small injection in PO (15X). C. Diamidino yellow retrogradely labeled cells in the spinal tract trigeminal nucleus (Vsp) following a small injection in PO (6X). D. Diamidino yellow labeled cells in the spinal cord following the small injection into PO (6X).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CHAPTER III

EXPERIMENTS

C. A COMPARISON OF THALAMIC AFFERENTS TO THE PRIMARY AND ROSTRAL MOTOR AREAS OF THE RAT

INTRODUCTION

Recent studies on the origin of the rat corticospinal tract have reported two regions on the dorsal convexity of the frontal cortex which project to the cervical (Hicks and D'Amato, 1977; Wise et al, 1979) and lumbar enlargements (Sievert and Neafsey, 1982). Intracortical microstimulation (ICMS) of the more rostral region evokes contralateral forelimb movements, especially digit flexion and wrist extension (Neafsey and Sievert, 1982). Termed the rostral forelimb region (RFL) in order to distinguish it from the previously described more caudal forelimb region (CFL), the RFL has been suggested based on anatomical and physiological studies (Wise et al, 1979; Sievert and Neafsey, 1983; Sievert, 1984) to be the rat's supplementary motor area (SMA). To further characterize these spatially separated motor regions and to study their relationship to the posterior thalamus, a study of the thalamocortical afferents was undertaken to determine the distribution of neurons projecting from different thalamic nuclei and to determine if separate populations of neurons within these nuclei project to each motor region.

Although frontal cortical afferents have been extensively examined (Jones and Leavitt, 1974; Krettek and Price, 1977; Donoghue et al, 1979; Herkenham, 1979; Bentivoglio et al, 1981; White and Porter, 1982; and Donoghue and Parham, 1982), previous studies did not employ combined electrophysiological and cytoarchitectural criteria to

describe definitively the cortical regions receiving thalamic projections. Since thalamocortical projections are exclusively ipsilateral to frontal cortex (Jones and Leavitt, 1974), our initial studies using wheat germ agluttinin conjugated with horseradish peroxidase (WGA-HRP) compared the thalamic projections to the RFL of one hemisphere with the thalamic projections to the CFL of the other hemisphere. In further work designed to examine axonal branching of thalamocortical fibers, the fluorescent double retrograde tracers diamidino yellow (DY), fast blue (FB), and/or Evans blue (EB) were injected into different subdivisions of the frontal motor cortex of the same hemisphere. Similar methods were used to compare these thalamic projections with those to two other areas of primary motor cortex, the vibrissae and hindlimb representations.

MATERIALS AND METHODS

Male Long Evans black-hooded rats weighing between 300-400 grams were anesthetized with Ketamine HCl (100mg/kg, IP, supplemented as needed with doses of 10-15 mg, IP) and placed in a stereotaxic frame. The cisterna magna was opened to prevent cortical swelling. In eight animals, a small piece of bone (5 mm X 2 mm) was removed just rostral to bregma on both sides, leaving a small strip of bone intact over the midline. The rostral and caudal forelimb regions were identified by locating the region where intracortical microstimulation (0.25 msec. pulses, 50 uamps or less, 300 msec trains at 350 Hz) evoked digit or wrist movements rostral or caudal to an area where neck movements were elicited (Neafsey and Sievert, 1982). In this manner, a physiological map of the cortex of each hemisphere studied was obtained before cortical injections were made, providing data on the "functional" nature of the injection site. Subsequently, WGA-HRP (0.02 ul of 1% WGA-HRP in saline) was injected into a previously stimulated electrode track in the RFL on one side and the CFL on the other side with a 1 ul Hamilton syringe fitted with a 40 um diameter pipette tip. In six additional animals, only one hemisphere was exposed and mapped; once the motor cortex boundaries were determined, 0.02 ul of 1% DY was injected into the RFL, 0.02 ul of 2% FB was injected into the CFL, and 0.02 ul of 1% Evans blue was injected into the hindlimb motor cortex (HL). In five other animals the vibrissae motor region was located on

one hemisphere, the hindlimb area was defined on the opposite hemisphere, and 0.02 ul of 1% DY was injected into the vibrissae region and 0.02 ul of 2% FB was injected into the hindlimb region.

Following survival periods of two days for the WGA-HRP treated animals and 3 days for the fluorescent dye treated animals, the animals were anesthetized with sodium pentobarbitol (40 mg/kg,IP). Those animals receiving WGA-HRP injections were transcardially perfused with 1.25% glutaraldehyde and 1% paraformaldehyde, and the brains removed and stored overnight in 10% buffered sucrose at 4 C. Those animals receiving fluorescent dye injections were transcardially perfused with 4% buffered paraformaldehyde, followed by 10% buffered sucrose. The fluorescent dye injected brains were removed and placed in 30% buffered sucrose for 4 days or until the brains sank to the bottom of the specimen jar. Frozen sections at 50 um intervals were cut in the transverse plane from the frontal pole to the level of the pons in all brains. The WGA-HRP injected tissue was processed for HRP histochemistry according to the tetramethylbenzidine (TMB) procedure of Mesulam (1978), mounted onto gelatinized slides and coverslipped. The fluorescent dye injected tissue was mounted onto gelatinized slides from 0.005 M acetate buffer (Spatz and Grabig, 1983) and were not coverslipped. For each brain, an alternate set of 50 um sections were counterstained with either Pyronin Y or with the Gallyas silver stain for cell bodies (Merker, 1983). Retrogradely labeled HRP neurons were identified under both dark field illumination and

polarized light and plotted using a camera lucida attachment. The fluorescent retrogradely labeled cells were visualized with epifluorescent filters providing an excitation wavelength of 330 nm. Fluorescent cells were also plotted using a camera lucida. In all animals, thalamic nuclei and cytoarchitectonic cortical fields were identified on the Nissl counterstained material using a projection microscope. Parcellation of the thalamic nuclei was based on criteria described in a previous report (Bold et al, 1984).

Since the locations of retrogradely labeled neurons within the thalamus were consistent no matter which tracer was employed, comparisons between experiments using different tracers were made. These comparisons only refer to the location of the labeled neurons but not to their numbers which varied depending on the tracer used.

RESULTS

RFL Analysis

A representative ICMS motor response map is illustrated in Figure 1A. All injections were made into the center of the RFL as determined by identifying the boundaries of both the RFL and the CFL. Of the fifteen animals receiving injections into the RFL area (6 WGA-HRP, 6 DY, 3 FB) only five were considered (<1.5mm in diameter) to be confined to the RFL area (1 WGA-HRP, 3 DY, 1 FB), and one typical case is presented to illustrate the basic pattern of labeling (Figure 1). This case utilized WGA-HRP as the tracer and the injection site is illustrated in Figures 1B,C. This injection appeared to involve portions of both the lateral and medial agranular fields (Donoghue and Wise, 1982). Retrogradely backfilled cells were found in several thalamic nuclei (left side of Figures 1E-L), including ventrolateral (VL), ventromedial (VM), the intralaminar nuclei (central lateral (CL), paracentral (Pc), and central medial (Cem)), the paralemellar (lateral) mediodorsal (MDpl), lateral posterior (LP), and the posterior complex (PO). In addition, a few cells were found scattered in the zona incerta (ZI), the medioventral nucleus (MV), nucleus basalis (BF), and cells scattered in the internal capsule (IC) which represent cells in the entopeduncular nucleus. At rostral thalamic levels (Figure 1F-G), labeling was most evident in VL where labeled cells are found throughout most of this nucleus. Clusters of labeled

cells were typically found in the central medial (Cem) and paracentral (Pc) intralaminar nuclei, as well as in the nucleus basalis. At midthalamic levels (Figure 1H), VL remains heavily populated with positively labeled cells, but the label is not confined to VL, especially dorsally where a cluster of cells are found within LP at the LP-VL border. Ventral to VL, labeled cells extend into the dorsalmost part of VM. As VL is replaced caudally by PO, labeled cells in VL diminish in number and more and more cells are labeled in central lateral (CL), Pc, and the dorsal aspect of VM. The overall pattern of labeling at this level of the thalamus changes from a single group of labeled cells confined mostly to VL to several clusters of labeled cells. A dorsomedial cluster of labeled cells extends from the medial half of PO to the CL nucleus. A ventral group of labeled neurons begins in the dorsolateral aspect of VM where VM borders VB and extends dorsomedially through the ventral aspect of caudal VL to the CL and Pc nuclei where the two clusters are joined. The third and fourth clusters of labeled cells are seen as small patches of cells within central PO at the LP/PO border and another patch of cells in the ZI.

More caudally within the thalamus (Figure 1J), the labeling within the dorsal and ventral groups within the intralaminar nuclei is now separated. The dorsal cell group now extends from the medial border of VB across the dorsal aspect of PO and across CL into the lateral part of MD (MDpl). Two distinct clusters are found within this dorsal

group of labeled cells, one lying within the dorsomedial PO and the other in the entire mediolateral extent of central CL. A few scattered cells are also seen labeled in Pc and dorsomedial VL (Figure 1J). The ventral group at this level extends across VM and along the border of Sm into Cem all the way to the midline. Within this ventral group, VM labeled cells are arranged in a band where all are dorsal to the mammillothalamic tract (mmt). The labeled cluster within Cem is very prominent with densely labeled cells, whereas the cells in the ZI are more scattered and appear to be less densely labeled (Figure 1J).

At the level of fasciculus retroflexus (FR, Figure 1K) the dorsal cell group extends across the dorsal aspect of PO from the LP border dorsally into the parafascicular nucleus (Pf) medially. A large cluster of labeled cells is seen within PO, and a separate cluster occupies the central aspect of Pf. A few cells can be seen more ventrally within Pf as well. The ventral cell group at this level is confined almost exclusively to VM although a few cells are found near the midline in MV. Cells are still present in the ZI as well as in the lateral hypothalamus dorsal to the fornix. Even further caudally at the mesodiencephalic junction (not illustrated in Figure 1 but presented in Figure 4), a few labeled cells are seen dorsal and medial to the FR in the nucleus of Darkschewitsch (NDk) and again in the caudalmost part of PO.

CFL Analysis

Eighteen animals received injections into the center of the physiologically defined CFL area (6 WGA-HRP, 6 FB, 6 DY). Only one WGA-HRP injection, all six DY and 3 FB injections were considered small enough to be confined exclusively to the primary forelimb motor area. The basic pattern of labeling seen in these ten animals was similar and the case involving the WGA-HRP injection is presented. The labeled cells are found in several ipsilateral thalamic nuclei including VL, VM, the intralaminar nuclei (CL, Pc, Cem), LP and PO (Figure 1E-L, right side). Rostrally (Figure 1E-H), labeling is most obvious in VL where cells extend dorsally into LP and medially into both CL and Pc. The label in VL is similar to the label seen in VL following RFL injections. Also at this rostral level, labeled cells are found in dorsomedial VM where it borders the intralaminar nuclei and the cluster of labeled cells in Cem extend from the midline laterally to the MTT. Only a few labeled cells are found in the rhomboid nucleus (Rh) and in the internal capsule (IC) but no labeled cells are present within the rostral ZI.

At midthalamic levels (Figure 1H-J) the distinct dorsal and ventral clusters of labeling that are seen with RFL injections become apparent but there is no union between the two cell groups within the intralaminar nuclei as seen with RFL injections. The dorsal cell group extends across VL and across the medial part of PO into CL. Cells from this group also extend ventrally into the dorsalmost part

of VL. The heaviest clusters of cells lie at the CL/PO border and within the central aspect of PO. A few labeled cells are seen extending dorsally from PO towards and into the dorsally contiguous LP. Scattered labeled cells are also seen in two intralaminar nuclei, Cem and Pc. The ventral cell group at this midthalamic level extends across the more central part of VM from the ventromedial corner of VB medially to the MTT. The largest and most dense cluster of cells in this ventral group is located in the ventrolateral aspect of VM where it borders VB and VL. A few scattered cells are seen in the ZI, again dorsal to the fornix as well as a few cells in the internal capsule.

More caudally (Figure 1J) the dorsal cell group is still present and remains separated from the ventral labeled cell group. A large cluster of labeled cells are found within central PO and extend medially into CL and dorsally into LP. Cells within CL do not continue into MD medially but occupy a large part of the mediolateral extent of CL. The ventral cell group is confined mostly to the ventral and medial aspects of VM extending from its lateral border with VB medially to the mammilothalamic tract. A distinct cluster of cells is found within Cem, and a few cells are found scattered in the ZI, especially just ventral to the mammilothalamic tract.

At the level of the fasciculus retroflexus (FR, Figure 1K), the two group pattern is still evident with the dorsal group occupying the dorsal half of PO extending medially in ventrolateral Pf, dorsally into LP and laterally towards VB but not extending into it. The

ventral cell group fills the central and ventral parts of VM and labeled cells extend ventrolaterally into the ZI. Cells within MV are grouped within a distinct cluster while the number of cells in the ZI, although sparsely labeled, is increased. Further caudally at the mesodiencephalic junction labeled cells are again obvious within NDk as well as in the most caudal parts of PO (Figure 4). A larger injection of the CFL which spread into the granular sensory cortex of SI, in addition to the labeling just described, also labeled a few cells in the forelimb representation of VB (Figure 5).

Vibrissae Motor Cortex Analysis

Six animals received injections into the center of the physiologically identified vibrisse representation of the motor cortex (2 WGA-HRP,3 DY,1 EB). The vibrissae region is located medial to the RFL and CFL representations, and thus retrograde thalamic labeling following vibrissae motor injections provides data on the connections to more medial parts of motor cortex (AgM). The basic pattern of labeling seen in these six cases was similar. Figure 2 illustrates an Evans blue injection into the vibrissae motor area of the left hemisphere and an injection of diamidino yellow in the center of the hindlimb representation on the right. Following the vibrissae injection, retrogradely labeled cells were found in a set of thalamic nuclei similar to those labeled after a caudal forelimb injection (Figure 2 F-M, left side). Cells are obvious in VL, VM, CL, Pc, Cem,

PO, the ZI, and the basal forebrain. However, in contrast to the CFL and RFL injections, labeled cells in VL were confined almost exclusively to the most ventromedial part of VL (Figure 2F-G). Labeled cells in VM were also found to be confined to the ventralmost part rostrally, but embraced the mamillothalamic tract more caudally as labeled cells in VM moved slightly more dorsal. Labeling in the intralaminar nuclei was similar to the RFL and CFL labeling, with cells found in Pc and Cem, especially at rostral levels. Like the RFL injections, vibrissae motor injections resulted in a few labeled cells in the paralamellar or lateral MD nucleus. Labeled cells in the posterior nucleus were located in its medial part at midthalamic levels (Figure 2J-K), but the cells are more dispersed in the dorsal aspect of PO at more caudal thalamic levels (Figure 2L-M). More caudally the label in CL appears as a distinct halo of labeling surrounding an area of CL that is not labeled (Figure 2J). Further caudally there is no label in Pf as was observed with more rostral and more lateral motor cortex injections. Cells in the zona incerta are not present at rostral levels but appear caudally. The separate dorsal and ventral labeled cell thalamic groups are again obvious, particularly from midthalamic levels to the level of the fasciculus retroflexus. The dorsal cell group occupies the medial part of VL and extends into CL and Pc. The ventral cell group is confined to VM.

Hindlimb Motor Cortex Analysis

Twelve animals received injections into the center of the physiologically identified hindlimb representation (4 WGA-HRP, 6DY, 2 FB). The basic pattern and location of labeled neurons was similar for all these cases except that labeling was not always present in VB and VL but was sometimes found in either VB or VL. The injection illustrated on the right side of Figure 2 is the smallest injection made into this area. As seen in the photomicrograph of the injection site, the extent of spread was minimal and the overall diameter of the injection site was less than 0.75mm. This injection is located at the border betwen granular and agranular portions of the hindlimb more cortex and provides information on the connections to the reported area of overlap of the motor and sensory fields in the hindlimb representation (cf. Donoghue et al., 1979). Labeled cells following this small hindlimb injection were observed in the ventrobasal complex (VB) and VM in the rostral half of the thalamus (Figure 2F-I, right side). Labeled cells were found in the basal forebrain and the internal capsule but no labeled cells were located in any of the intralaminar nuclei and only a few were seen in VL. At more caudal thalamic levels (Figure 2K-M) a few labeled cells were identified in the dorsal aspect of PO, and these became more numerous further caudally. No labeled cells were seen in the parafascicular nucleus. A few cells were located in the ZI and only at caudal levels. The two separated groups of labeled cells that were characteristic of other

motor injections were not evident following this small hindlimb injection. The few labeled cells seen in VL were located ventrally (Figure 2G), and this pattern of labeling differs from that seen following larger injections in this area (Figure 3 I-K). Also note that the label in VB was in the most lateral portion where "hindlimb" gracile nucleus terminations are located (Lund and Webster, 1967a; Feldman and Kruger, 1980).

RFL, CFL, HL Injection (DY,FB,EB)

In eight animals, three different retrograde fluorescent tracers were injected into different areas of the motor cortex of the same hemisphere to determine the extent of collateralization of thalamic projection neurons. In one group of animals (n=3) diamidino yellow was injected into the RFL, fast blue into the CFL, and Evans blue into the hindlimb representation. The results of one animal from this group are illustrated in Figure 3.

The location of single labeled cells generally agrees with previous groups using tracers not intended to examine double labeling, although there was some inter-animal variability in the detail of the labeling pattern. It is important to point out that no double-labeled cells were found in the thalamus in any of these experiments. The hindlimb injection resulted in labeled cells in the lateral part of the ventrobasal complex throughout its rostrocaudal extent (Figure 3H-O). Label in ventral VM was very slight and labeled cells were seen in the

dorsalmost part of VL with some cells extending dorsally into the lateral posterior nucleus and medially into the lateral part of CL. At more caudal levels (Figure 3L-O) the label in VL is replaced by label in PO. The label in PO is also dorsally located and is contiguous with labeling confined to the ventral part of LP. At the level of the fasciculus retroflexus (Figure 3N-O), the label in PO remains most dorsal and no labeling is seen in Pf as compared to the other motor area injections. Again, no labeled cells were seen in the intralaminar nuclei following HL injections.

After CFL injections, thalamic labeling was found in VB, VL, VM, Pc, and Cem rostrally, and in PO, VM, Pf, and the zona incerta caudally. The few cells found in the ventromedial part of VB (Figure 3I) were only seen after rather large CFL injections such as this one where the injection invaded the laterally adjacent granular sensory cortex (See Figure 3C). The labeling in VL occupies the middle and ventral parts and the cluster of labeled cells extends into the intralaminar nuclei, Pc and Cem (Figures 3I-K and 6A). Labeled cells within in VM were found laterally at rostral levels but within dorsal regions more caudally. PO shows labeled cells medially in its central portion and this group of cells is continous medially with labeled cells in CL. The labeled cells in PO after the CFL injection were found ventral and medial to those found after the HL injection. At the level of the fasciculus retroflexus (Figure 3N-O), CFL labeled cells are clustered in the ventromedial part of PO along the PO-Pf

border. Some labeled cells are positioned at the lateral border of Pf with a few found more medially in the more ventral part of Pf. There are no labeled cells in the rostral part of the zona incerta but distinct clusters are found at more caudal levels (Figure 3L-O). In addition, a few labeled cells were found medial to the fasciculus retroflexus in the nucleus of Darkschewitsch (not illustrated).

The pattern of retrogradely labeled cells seen after the RFL injection was very similar to that seen after the CFL injection, and labeled cells were found in the basal forebrain, VL, VM, and Pc rostrally, and in PO, CL, Pc, VM, Pf, and the zona incerta caudally. The labeling in VL is located in the more lateral and ventral parts and extended further caudally than the labeled cells in VL seen following the other two injections (Figure 3I-L). A distinct band of cells were found in the caudalmost part of VL at the border of the VM nucleus (Figure 3L). However, this band extends medially only to the border of the Cem nucleus. More rostrally labled cells did extend across VL medially into the Pc and ventral part of CL. The labeling in VM was more medially located than observed after the CFL injection; however, at more caudal levels (Figure 3L-M) the RFL labeling in VM is surrounded both dorsally and ventrally by labeled cells resulting from the CFL injection. In the intralaminar nuclei, a distinct cluster of labeled cells was confined to the paracentral nucleus rostrally but extends medially into the paralamellar part of the mediodorsal nucleus at more caudal levels. A few labeled cells were found in CL and

labeling in MD (Figure 3L-M) was always found after RFL injections but never after CFL or HL injections. At more caudal thalamic levels (Figure 3N) labeling in the posterior complex was in two distinct clusters. One cluster of labeled cells was found in lateral part of PO and another cluster was found in the ventromedial part of PO. CFL projecting neurons were identified between these clusters. At the level of the fasciculus retroflexus (Figure 3O), label in PO is confined to one large aggregation in the central part of the nucleus with CFL projecting cells positioned most medial and hindlimb projecting cells most dorsal within PO. Labeling in the parafascicular nucleus is very similar to that found following CFL injections where labeled cells are found in a band like arrangement along the PO-Pf border with a few labeled cells more medially located in Pf. Label in the zona incerta is much less than similar label following CFL injections. In fact, only a few cells are found in the zona incerta at caudal thalamic levels.

Considering the overall pattern of labeling seen following these four "types" of injections (RFL, CFL, VMI, HL), the only double labeled cells were found in the basal forebrain and the internal capsule and are designated by stars in those areas (Figure 6B). No double labeled cells were found in the thalamus, indicating an apparent lack of axonal branching projections to the rodent motor cortex. Since collaterals to different motor representations were not found and since the patterns of the labeled cells varied according to

injection locus but not according to the tracer used, the topography of the cells in PO projecting to various subdivisions of motor cortex was studied. PO was selected for this analysis because it has traditionally been considered as a "non-specific" thalamic nucleus without any obvious internal organization. The other nuclei with prominent projections to motor cortex, namely VL and VM, have been shown to have internal organization of their cortical projections (Strick, 1976; Herkenham, 1978).

In order to determine whether PO possesses a consistent internal topographical organization of its projections to cortex, a somewhat involved analysis of the data was performed. Data from five comparably placed DY injections in the RFL, five cases of FB injections in the CFL, five DY injections in HLMI, and three animals with DY injections in VMI were used. The area of labeling within PO from each animal in each separate group was outlined onto three coronal sections through PO, where it was superimposed on the outlines of the other animals in the same group. This is illustrated in Figure 7A-C for RFL injections, in Figure 7D-F for CFL injections, in Figure 7G-I for HLMI injections and in Figure 7J-L for VMI injections. The areas labeled that were common to all injections within a group were identified by the solid black area in Figure 7A-L. Looking at the overall pattern of labeling based on all comparable injections and individual experiments a distinct topography can be identified (Figure 7 M-O).

The topography thus established within PO appears to be somatotopic. Hindlimb projecting cells are always most dorsal, CFL projecting cells are centrally located and ventral to the HLMI cells, RFL projecting cells are found as two separate areas, one lateral and one medial to the CFL cells, and VMI cells are always dorsomedial, located medial to the HLMI cells. At most caudal levels the RFL cells only occupy a position lateral to the CFL cells. The VMI cells are found in the dorsomedial part of PO at all levels. The somatotopy is thus organized with HLMI cells most dorsal, CFL cells more ventrally located and the VMI cells dorsomedial.

DISCUSSION

The results of this study demonstrate that a similar but not identical set of thalamic nuclei project to the RFL, CFL, vibrissae, and hindlimb areas of the rat motor cortex. All four of these motor areas receive thalamic input from VM, VL, PO, the zona incerta, and the basal forebrain. Except for the hindlimb cortex, these areas also receive afferents from the intralaminar nuclei, CL,Pc, and Cem. The paralamellar part of the mediodorsal nucleus (MDpl) projects only to the RFL and the medially located vibrissae motor area, and the ventrobasal complex (VB) sends projections only to the hindlimb representation and to the area immediately lateral to the caudal forelimb area. In addition, an apparent topographical organization of the thalamic projecting neurons can be found in VM, VL, and PO.

The extensive labeling of cells within VL corroborates previous work demonstrating its projections to the dorsal frontal cortex in rats (Jones and Leavitt, 1974). The absence of labeled cells in caudal VL following either RFL or CFL injections also agrees with anterograde tracing studies based on the injections of labeled amino acid into caudal VL (Herkenham, 1980).

Labeling within VM was expected since VM has known connections with the dorsal frontal motor cortex (Herkenham, 1979). While the amount of labeling within VM following the two forelimb cortical

injections was comparable, the pattern of labeling within VM was different according to the injection site. RFL injections labeled cells in the medial aspect of VM whereas CFL injections labeled cells more laterally within VM. Hindlimb injections in general labeled the most lateral and ventral part of VM when present. Since VM and VL both receive afferent input from the cerebellum (Faull and Carman, 1978) and VM receives afferents from the substania nigra (Faull and Carman, 1968), it has been suggested that these nuclei together are homologous to the primate VA-VL complex (Faull and Carman, 1978).

Labeled cells were located throughout the rostral-caudal extent of the lateral part of VB (nucleus gracilis termination region; Lund and Webster, 1974) after an injection centered in HL cortex. Labeling in VB after CFL injections was only found when the injection spread to the granular cytoarchitectonic field. Studies using anterograde tracing methods have shown this part of VB receives afferents from nucleus cuneatus (Lund and Webster, 1967a; Feldman and Kruger, 1980), and thus represents the VB somatosensory forelimb area. The reported partial overlap of rodent sensory (S1) and motor (M1) forelimb cortex (Hall and Lindholm, 1974) suggests a corresponding convergence of VB-VL afferents to this area of overlap (Donoghue et al, 1979). However, our findings of labeled neurons in VB only after relatively large cortical injections indicate that the extent of overlap between cortical sensory and motor forelimb areas is rather small. In fact, our small HL injection located in AgL but at the border with granular

cortex failed to label cells in VL (Figure 2). Additionally, our findings clearly demonstrate no overlap between the RFL motor area and the SI forelimb area.

The observed labeling within the intralaminar nuclei after HRP injections into frontal cortex has been previously described (Jones and Leavitt, 1974). The pattern of intralaminar nuclear labeling was similar following either RFL or CFL injections although differences can be identified. Very little intralaminar nuclear labeling was seen following hindlimb injections, and the intralaminar labeling following vibrissae motor injections was very similar to that seen with RFL injections. The intralaminar nuclear projections to vibrissae motor cortex may be related to the participation of the intralaminar nuclei in the motor control of head and eye movements (Schlag-Rey and Schlag, 1977; Maldonado and Schlag, 1981). This concept is supported by the fact that the vibrissae motor area may coincide to some extent with the frontal eye fields which are primarily located within AgM and which sends heavy projections to the superior colliculus (Hardy and Leichnetz, 1981). The relationship of the intralaminar nuclei to the cerebral cortical control of forelimb movements is not understood.

Labeled cells in the paralamellar mediodorsal (MDpl) nucleus were seen only after RFL and vibrissae injections and were found only at midthalamic levels. Krettek and Price (1977) reported that this region of MD in the rat projects to layers I and III of the medial precentral cortical field, which they locate on the dorsomedial aspect

of the hemisphere where the cortex curves into the sagittal fissure. This region appears to correspond with the medial agranular field in rat frontal cortex (Donoghue and Wise, 1982). These latter authors report that microstimulation thresholds were higher in this region than in the adjacent lateral agranular field, and they do not include the medial agranular field in rat primary motor cortex (MI). The RFL area appears to be located primarily in the lateral agranular field (Donoghue and Wise, 1982) and thus the labeling seen in MD may be due to spread of the injection site into the adjacent medial agranular region.

The labeling within the posterior nuclear complex (PO) was quite extensive after all injections. RFL and CFL injections labeled cells within the dorsomedial aspect of PO, although the labeling following CFL injections was heavier than that after RFL injections. Both injection sites resulted in a few labeled cells extending from PO dorsally into the lateral posterior (LP) nucleus. Labeling in PO following HL injections was most dorsal while vibrissae injections labeled cells in PO dorsomedially. This finding of a fairly detailed topographical organization of PO's output to cortex, as depicted in Figure 7M-O, suggests that PO is a much more "specific" thalamic nucleus than previusly thought (Herkenham, 1980). Projections from PO to the rat sensorimotor cortex have been described previously (Donoghue et al., 1979). Dorsal column nuclei and spinothalamic tract projections to the rodent PO (Lund and Webster, 1967a,b) suggest that

it may be involved in transmitting peripheral sensory input to the motor cortex. This question will be considered in the last experimental study in this dissertation.

The retrogradely labeled neurons and anterograde label in NDk may have resulted from spread of the injection site to the medially located frontal eye fields (Hall and Lindholm, 1974), which lie within AGm. Projections from both the nucleus of Darkschewitsch and the interstitial nucleus of Cajal to the frontal eye field in cats have been reported (Pascuzzo and Skeen, 1982). Corticofugal connections to NDk (Kunzle, 1978) as well as to the nucleus of Bechterew (Leichnetz, 1982), which is contiguous ventrally with NDk, have also been described in the monkey. While the precise topography of these connections in the rat is not clear, these previous findings as well as our own data clearly indicate a reciprocal connection between the frontal cortex and NDk.

The data reported in our study on the thalamocortical projections to vibrissae motor cortex are not in complete accord with the findings of a strictly anatomical study of inputs to the vibrissae motor region of the mouse (White and Porter, 1983). In agreement with our data, thalamic projections to the VMI were found to emanate from VL, CL, and ZI in the mouse, but unlike our findings, they found no afferents to VMI from the posterior complex, any of the intralaminar nuclei, the basal forebrain, or the ventromedial nucleus. These differences may be species related because another recent study of afferents to the

lateral agranular motor field of the rat reported thalamic projections very similar to those reported here (Donoghue and Parham, 1983). The major difference between the present study and the recent report of Donoghue and Parham (1983) is the considerably greater label found in the ILN following caudal and rostral forelimb and vibrissae motor cortical injections in the present study.

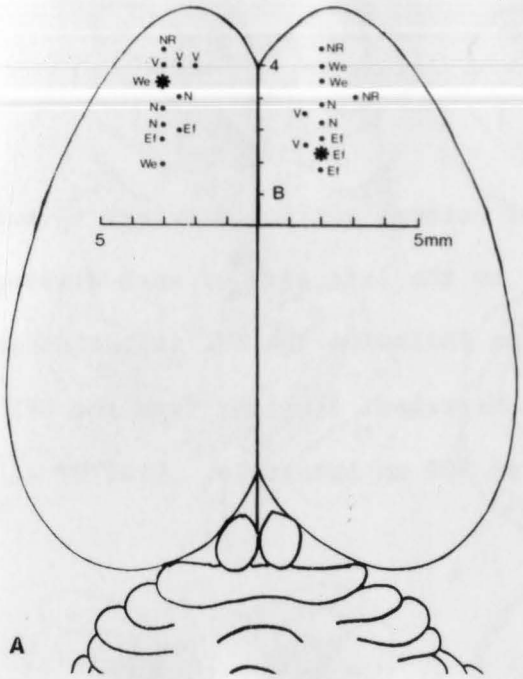
A recent study in the monkey has demonstrated distinct, non-overlapping thalamic regions projecting to primary (MI) and supplementary (SMA) motor cortical areas (Schell and Strick, 1984). Afferents to MI and SMA arise from VPLo and VLo respectively, and these two thalamic nuclei in turn receive inputs from the cerebellum and substania nigra, respectively. This resulting circuitry suggests that activity in these two primate motor cortical areas may be differentially influenced by cerebellar or basal ganglia systems. However, in the rat, the situation appears quite different since VL and VM both receive afferent input from the cerebellum and VM receives afferents from the substania nigra (Faull and Carman, 1968). These connections suggest that the rodent VM-VL complex is roughly homologous to the primate VA-VL complex but also demonstrate nigrothalamic and cerebellothalamic convergence within VM (Faull and Carman, 1978). Based on our findings, this convergent input could then be relayed to both MI and SMA cortical regions in rats. Accordingly, the rodent appears not to have distinct, parallel pathways providing cerebellar and nigral information to primary and

supplementary motor cortical areas as is seen in primates and suggests the SMA of the rat and primate are different in terms of their thalamocortical connections. The functional significance of this apparent species difference is not clear.

In summary, the overall pattern of labeling seen in the thalamus after rostral or caudal forelimb, hindlimb or vibrissae motor cortex injections consists of a single large rostral group of cells that splits caudally into two (dorsal and ventral) longitudinal columns of neurons which extend across several thalamic nuclear boundaries to the junction of the midbrain and thalamus. A similar pattern of thalamocortical projections was described by Kievet and Kuypers (1977) in monkeys. Although in our study the same basic set of thalamic nuclei were labeled following either RFL, CFL, HL or VMI injections, the location of labeled cells within each thalamic nuclei was different depending on the site of the cortical injection. In fact, the triple label experiment shows that separate populations of cells are projecting to three separate cortical areas. PO appears to have a much more detailed internal organization of its projections to cortex than previously thought.

Figure 1

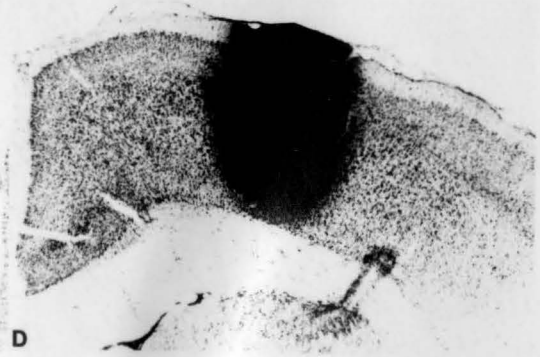
Results of a WGA-HRP injection into the RFL on the left side and an injection into the CFL on the right side. A. Drawing depicts the dorsal view of the rat brain showing the intracortical microstimulation points. Stars represent injection sites. The letters above each point indicate the movement evoked with 30 uA or less. NR, no response; V, vibrissae; Ef, elbow flexion; We, wrist extension; N,neck. B. Stacked drawings of sections through the two injection sites to show the actual spread (dark regions) of the injected material. Distance between sections is 200 um. C and D. Photomicrographs of the actual injection sites into RFL (C) and CFL (D) reacted for HRP histochemistry with the TMB chromagen. Both injection sites are within the agranular lateral cytoarchitectonic field.



A

B

C



D

Figure 1 Continued

E-L. Rostrocaudal series of coronal outline drawings through the thalamus. Labeled cells (dots) on the left side of each drawing represent the pattern of labeling following the RFL injection and labeled cells on the right side represent labeling from the CFL injection. These sections are at 300 um intervals. List of abbreviations are on page vii.

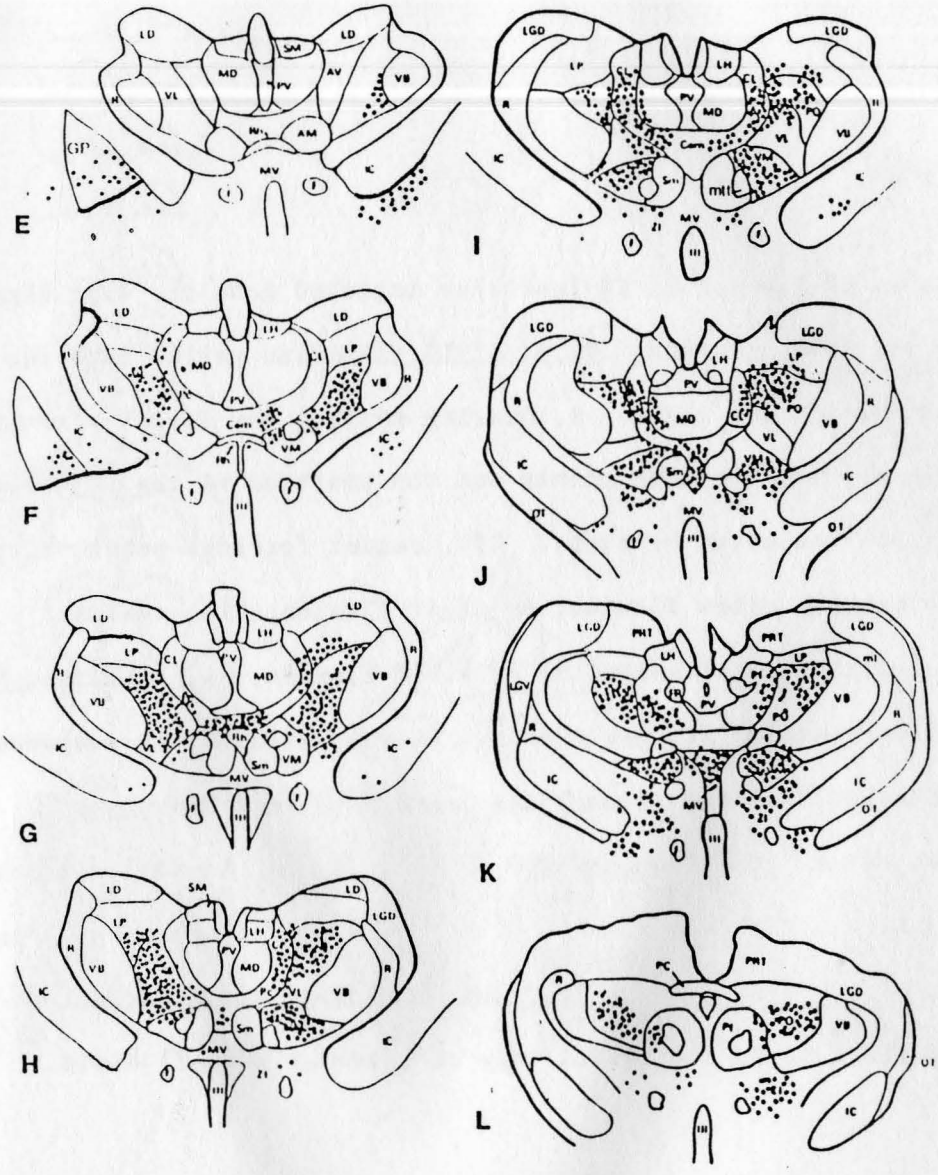

E
LD
SM
MD
AV
VB
VL
AM
MV
IC
F
LH
CL
LP
Com
VM
G
H
SM
I
LGD
PV
Sm
mtl
J
K
PRT
PC
L

Figure 2

Results of 0.04 ul of 2% fast blue injected into the vibrissae area on the left side and 0.01 ul of 1% diamidino yellow into the hindlimb area on the right. A. Drawing depicts the dorsal view of the rat brain showing the ICMS points and the position of the injection sites, again indicated by stars. CFL, caudal forelimb point, either wrist extension, elbow flexion, or digit flexion; RFL, rostral forelimb, either wrist extension or elbow flexion; HL, hindlimb; SR, shoulder retraction; JO, jaw opening; V, vibrissae;NR, no response. B and C. Stacked drawings through the respective left and right injection sites. Sections are 200 um apart. D+E. Photomicrographs of the actual injection sites. The vibrissae motor injection site is in the agranular medial cytoarchitectonic field and the hindlimb injection site is in the medial edge of granular sensory field.

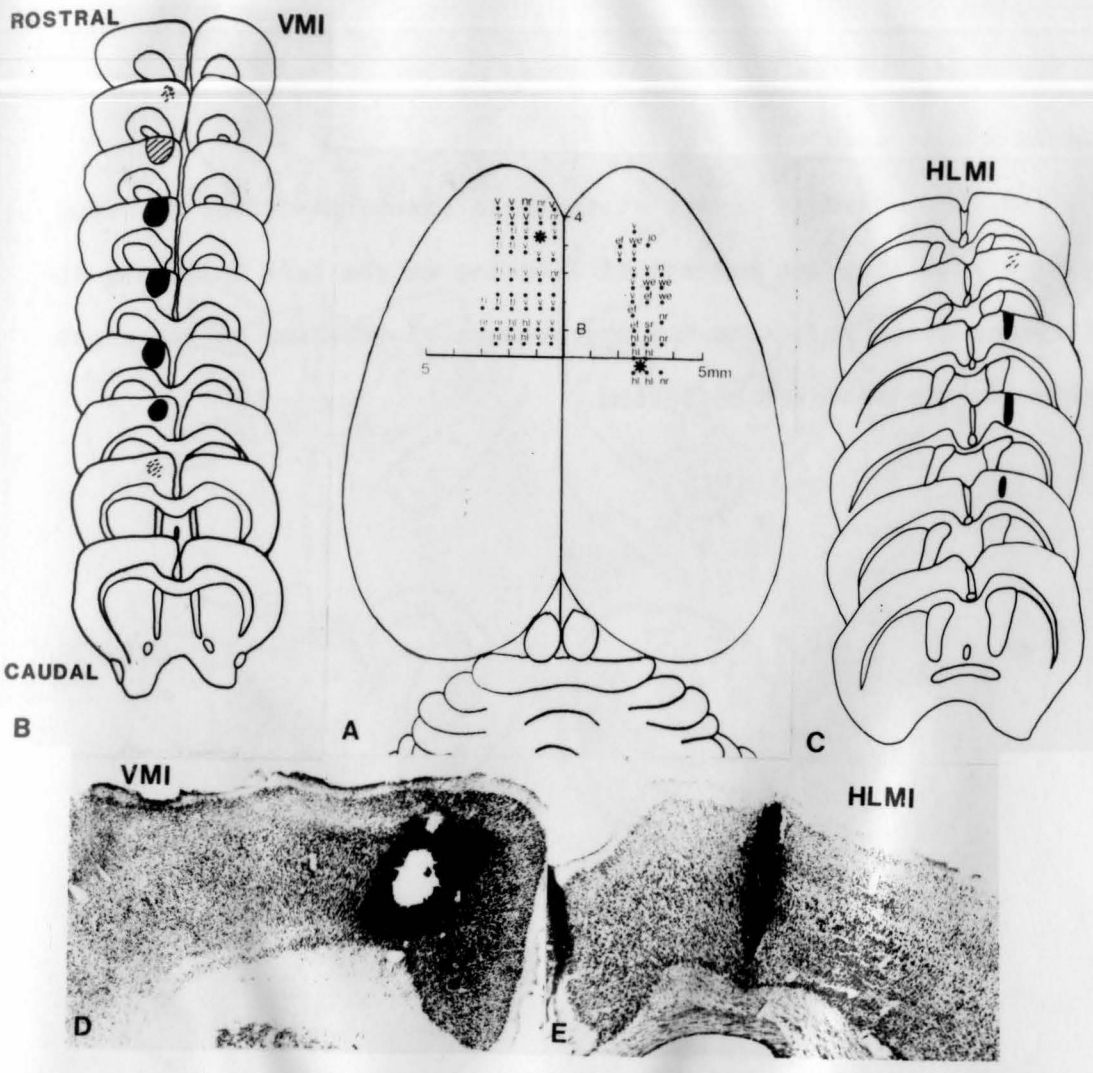

ROSTRAL
VMI
CAUDAL
B
A
HLMI
C
5
4
B
5mm
VMI
HLMI
D
E

Figure 2 Continued

F-M. Rostrocaudal series of thalamic coronal sections at 300um intervals showing the pattern of labeling on the left following the vibrissae motor injection and the pattern of labeling on the right following the hindlimb injection.

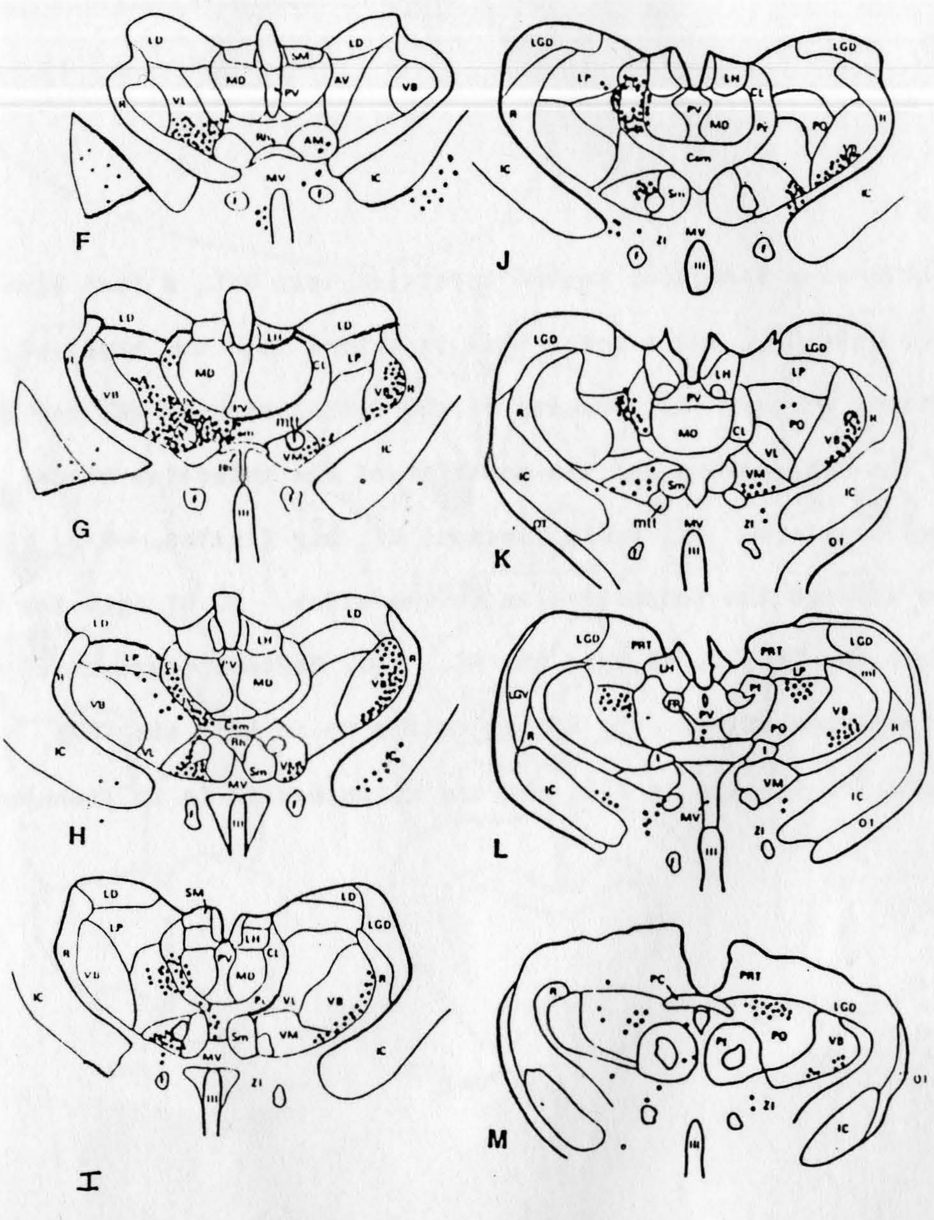


Figure 3

Results of a diamidino yellow injection into RFL, a fast blue injection into CFL, and a Evans blue injection into the hindlimb sensorimotor cortex. A. Drawing of the dorsal view of the rat brain showing the ICMS points and the position of the injection sites, indicated by stars. Af, ankle flexion; Hf, hip flexion. B-D. Stack drawings through the respective injection sites. B. DY into the RFL; C. FB into the CFL; D. EB into the HL. E-G. Photomicrographs of the actual injection sites. The RFL injection is in AG1; the CFL injection site is also in AG1; and the HL injection is in granular cortex.

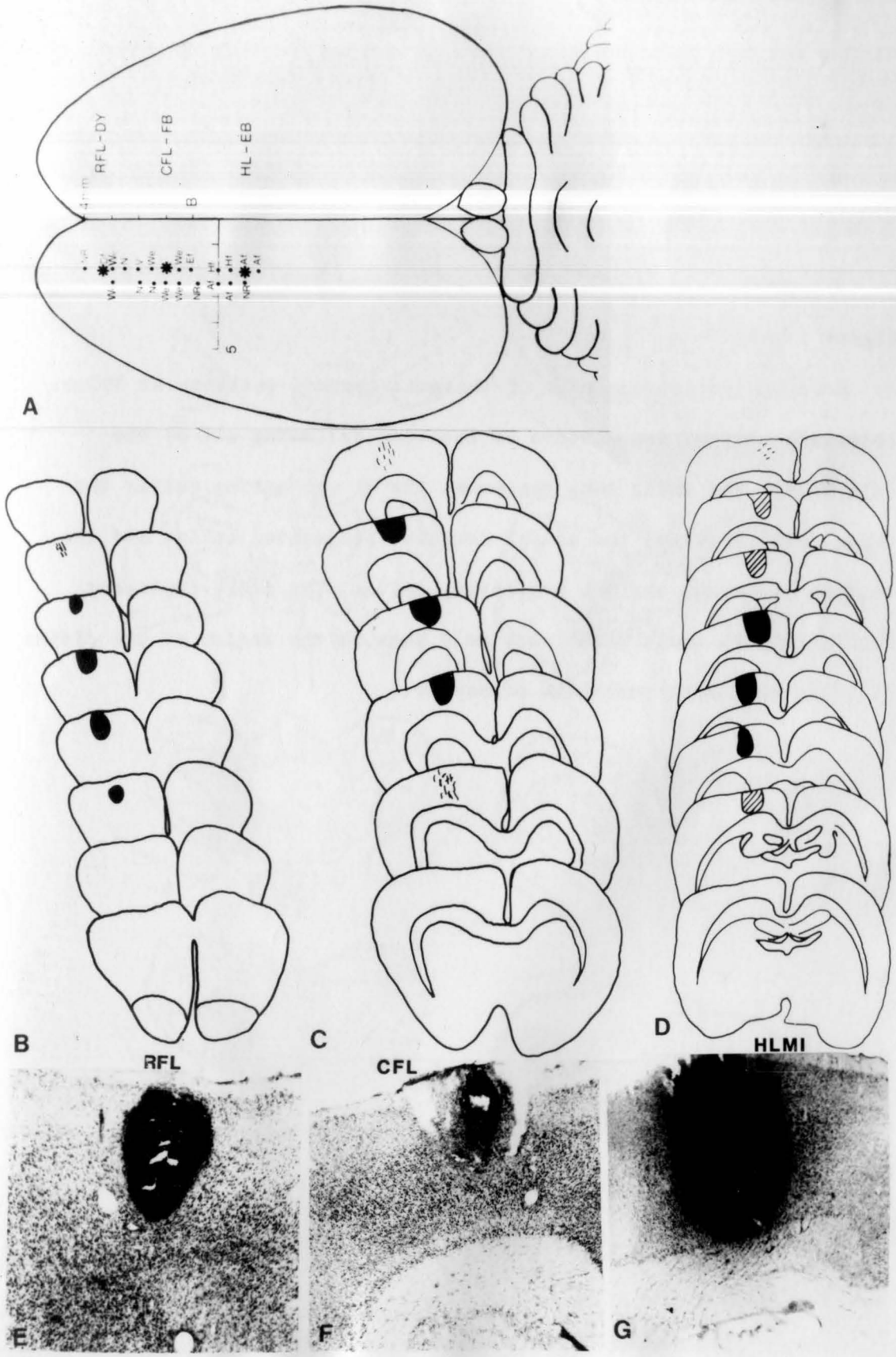

RFL-DY
CFL-FB
HL-EB
B
5
A
B
C
D
RFL
CFL
HLMI
E
F
G

Figure 3 Continued

H-O. Rostrocaudal series of thalamic coronal sections at 300um intervals showing the pattern of labeling following all of the injection. The small dots represent the HL projecting cells; the large dots represent the caudal forelimb projecting cells; and the slashes represent the RFL projecting cells. The stars represent double labeled cells which were only seen in the region of the globus pallidus and basal forebrain of Meynert.

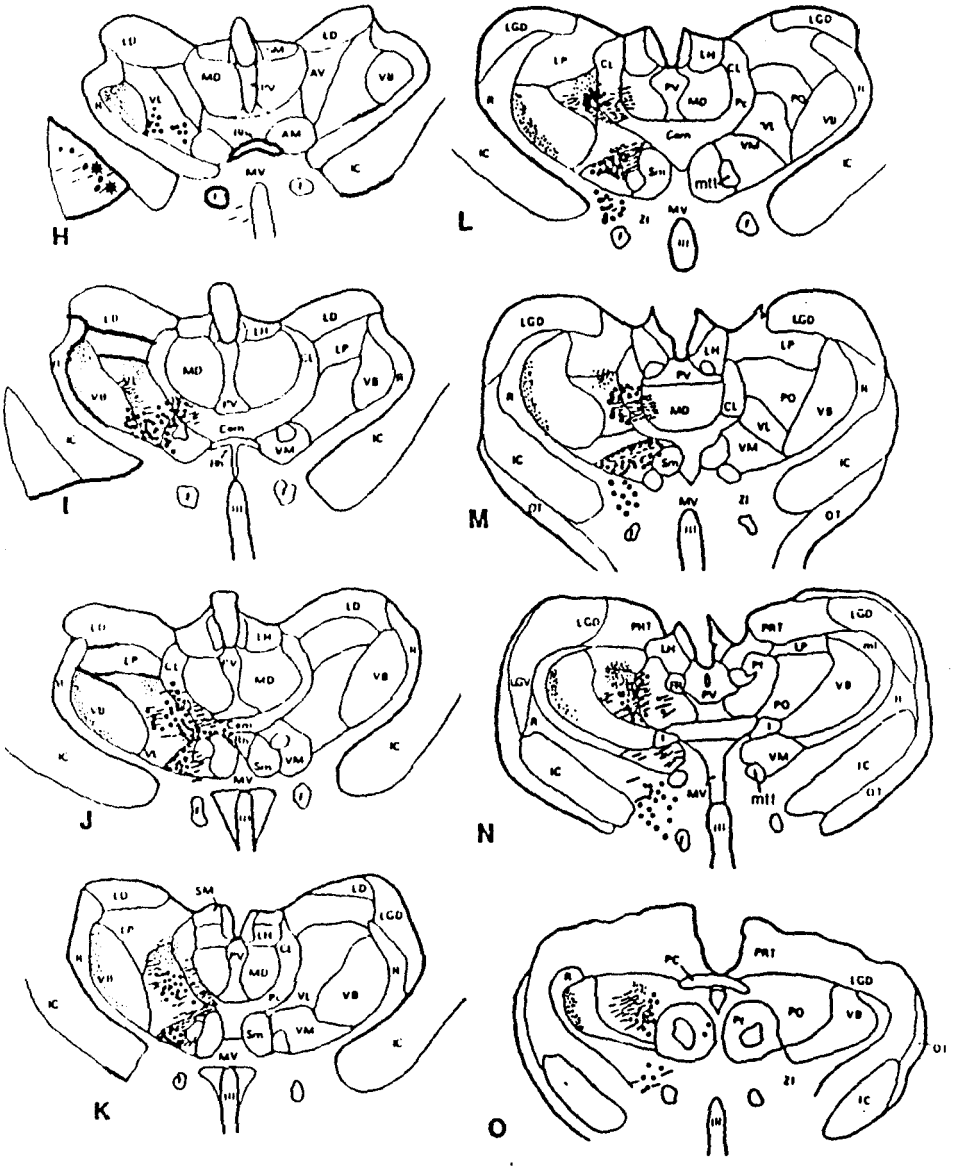

H
L
I
M
J
N
K
O

Figure 4

A. Photomicrograph (6X) of a coronal section through the caudal thalamus at the level of the FR from an animal with a WGA-HRP injection into the CFL. The presence of anterograde labeling as well as retrogradely labeled cells (indicated by arrows) along the FR, especially within the nucleus of Darkschewitsch (NDk) are evident. The area near these labeled cells is darkened by anterograde labeling. Pyronin Y counterstain (15X). B. Higher power photomicrograph (30X) of the same area in A showing the labeling in NDk.

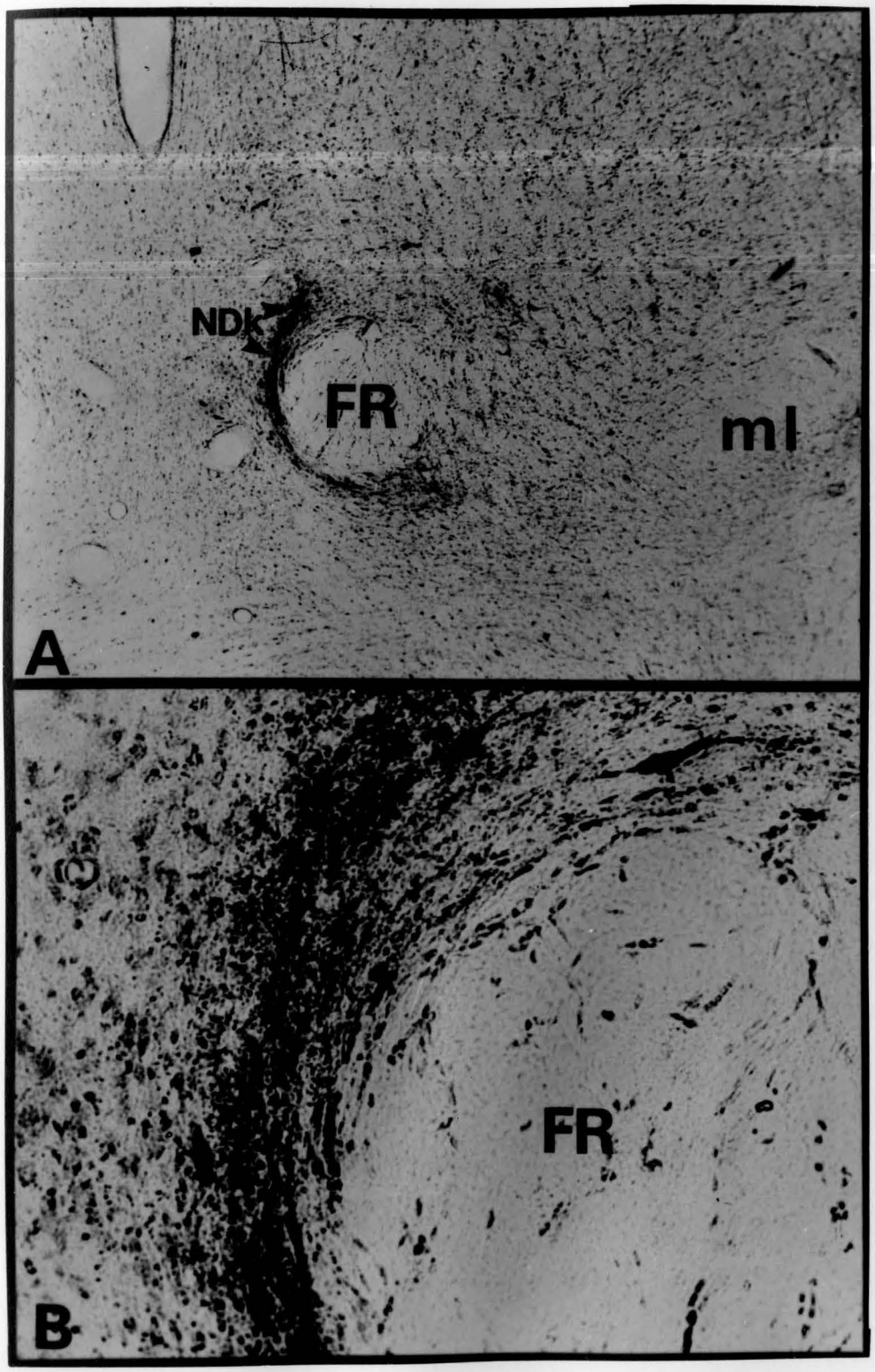
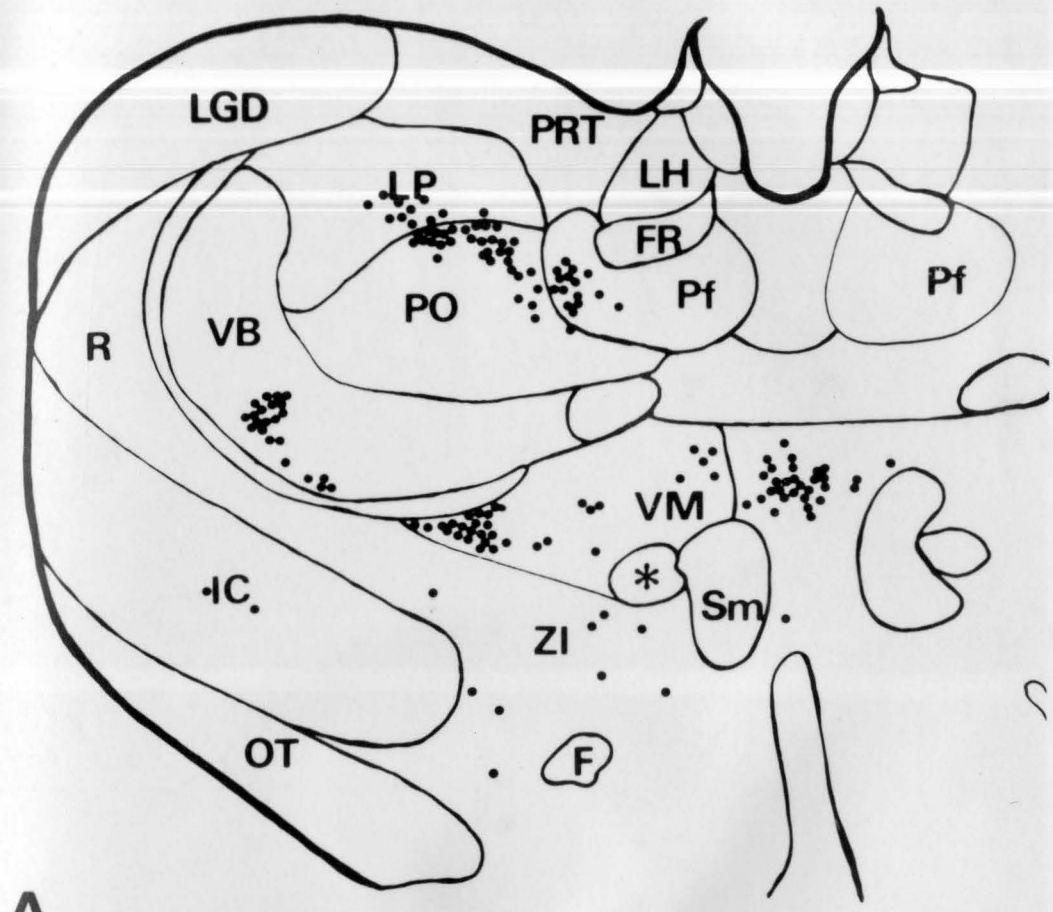
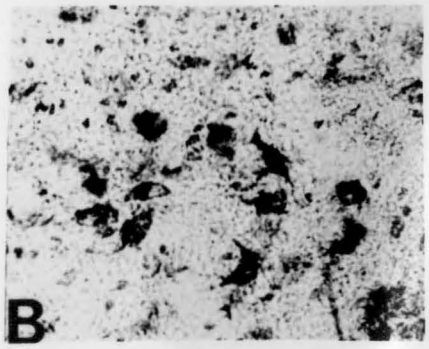

NDK
FR
ml
A
FR
B

Figure 5

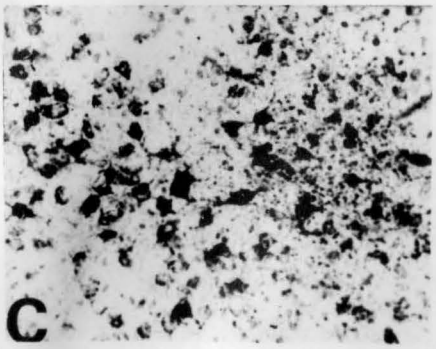
A. Outline drawing of a coronal section through the caudal thalamus of an animal which received a large injection of WGA-HRP into the CFL. Note the presence of labeled cells in the ventrobasal complex which suggests that the injection may have involved part of the laterally adjacent granular senosory cortex. B. Photomicrograph (25X) of labeled cells in VB depicted in A. C. Photomicrograph (25X) of labeled cells in PO and Pf as depicted in A.



A



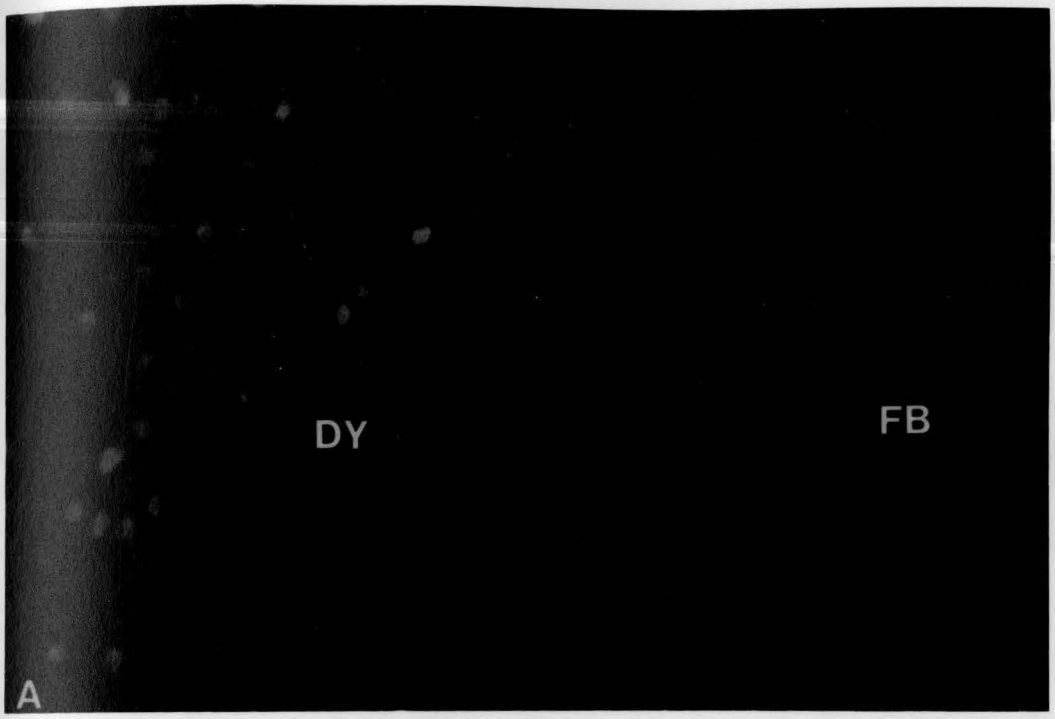
B



C

Figure 6

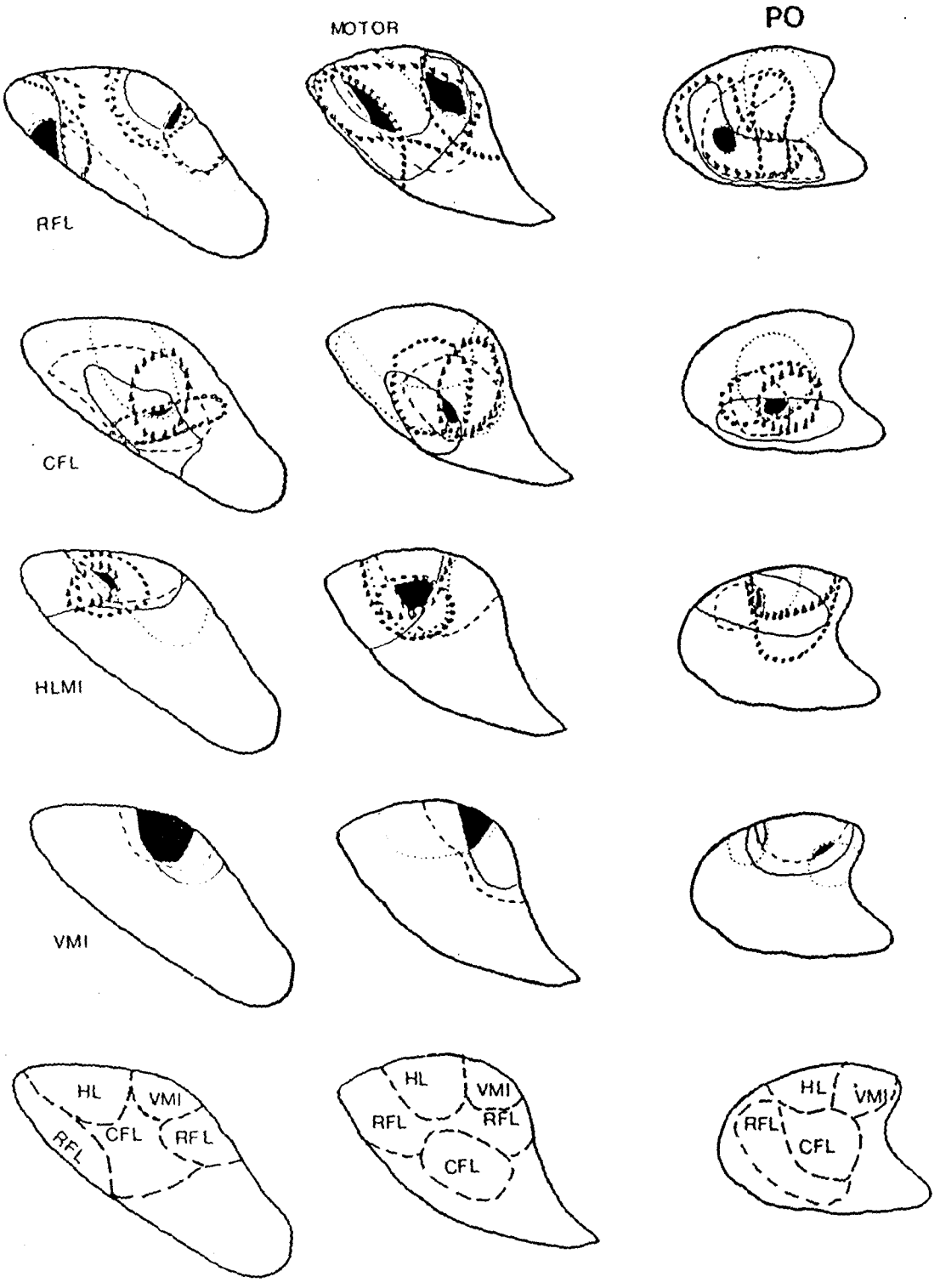
A. Photomicrograph (15X) of fluorescent retrogradely labeled cells in VL following the RFL injection of DY. The more pale staining cells to the right are fast blue cells also in VL, following a CFL injection. B. Photomicrograph (15X) of the few double labeled cells in the basal forebrain following an injection of DY into the RFL and FB into the CFL.


DY
FB
A


B

Figure 7

Summary diagram showing the topographical relation of PO thalamic neurons projecting to RFL,CFL, HL, and VMI areas of motor cortex. A-C. Data from five comparably placed DY injections in the RFL are indicated by differently hatched lines. The solid dark areas represent those areas within PO which were commonly labeled following all five injections illustrated. D-F. Data from five comparably placed FB injections in the CFL, illustrated as described above. G-I. Data from five comparably placed DY injections in the HLMI. J-L. Data from three comparably placed DY injections in VMI. M-O. Illustrates the topographical organization that was found within PO. The hatched lines indicate that the borders for the designated areas are not rigid. These borders were drawn from data obtained from the comparably placed injection sites (A-L above) as well as from data collected from individual experiments. The hindlimb projecting neurons are always dorsal within PO, the CFL projecting cells are ventral to the HL cells, the RFL cells are found on either side of the CFL cells, and the VMI cells are located in the dorsomedial part of PO. This pattern of organization appears to show a somatotopy with hindlimb dorsal, forelimb ventral, and face medial (dorsomedial).


MOTOR
PO
RFL
CFL
HLMI
VMI
HL
VMI
RFL
CFL
RFL
HL
VMI
RFL
RFL
CFL
HL
VMI
RFL
CFL

CHAPTER III

EXPERIMENTS

D. A COMPARISON OF THALAMIC PROJECTIONS TO PRIMARY AND SECONDARY SOMATOSENSORY AREAS IN THE RAT

INTRODUCTION

The primary and secondary somatosensory areas (SI and SII) of the rat neocortex have been shown by electrophysiological recording methods to possess a very precise somatotopic organization (Welker, 1971; Welker and Sinha, 1972; Welker, 1976). This organization reflects the precise topographic distribution of somatosensory pathways into VB (Lund and Webster, 1967a, 1967b; Killackey, 1973; Feldman and Kruger, 1980) and the subsequent orderly relay of sensory information through VB to the neocortex (Jones and Powell, 1969; Hand and Morrison, 1970; Killacky and Leshin, 1975; Saporta and Kruger, 1977). In contrast, several reports demonstrate a complex multisensory convergent input to the thalamic posterior nucleus (PO) with subsequent divergent projections to widespread cortical areas (Lund and Webster, 1967a,1967b; Geisler et al., 1979; Neylon and Haight, 1983). This apparent convergent-divergent circuitry implies a system that is not characterized by a precise topographical organization (Rose and Woolsey, 1949). However, since the topographic SI and SII areas are among the cortical regions receiving PO afferents, this study was undertaken to determine if PO displays a topographic projection to the somatosensory cortex.

Initial studies of thalamocortical connections relied on the method of examining retrograde neuronal degeneration after cortical lesions (Macchi et al., 1959) or used methods that stained for axonal degeneration after thalamic lesions (Jones and Powell, 1969; Hand and

Morrison, 1970). Current methods based on the retrograde transport of tracers injected into cortical areas provide a considerably more precise method to study thalamocortical fibers.

Retrogradely transported fluorescent dyes combine the advantage of small, well defined injection sites with the ability to simultaneously visualize several readily distinguishable transported tracers in the same animal (Kuypers et al., 1979). This makes it possible to inject one dye into a physiologically identified region of SI and a second dye into a physiologically identified region of SII in the same animal, an approach we have utilized in the experiments described below. These experiments also provide a means to study the degree of collateralization of thalamic neurons projecting to somatosensory cortex as well as the topographic pattern of such projections to SI and SII in the rat.

MATERIALS AND METHODS

Fourteen adult male Long-Evans hooded rats weighing between 250-450 grams were used in this study. The animals were anesthetized with sodium pentobarbitol (40 mg/kg, IP) and placed in a Kopf stereotaxic frame. A midline incision was made on the dorsum of the head, and the skin and soft tissues reflected laterally. On the side to be recorded and injected, the temporalis muscle was also reflected and in some animals had to be removed. The cisterna magna was opened to prevent cortical swelling, and a large craniotomy performed revealing the lateral surface of the brain. In order to adequately expose SII, the ear bar had to be removed to extend the craniotomy below the level of the rhinal sulcus. To secure the head of the animal, three small screws were placed into the dorsal convexity of the skull, and dental acrylic was then used to secure a small L-shaped washer between the screws and onto the skull. This L-shaped washer was then grasped with a Kopf vertebral clamp so that the animals head could be suspended securely without the use of the ear bars. Once the craniotomy was completed, the animals head was tilted so that mineral oil could be placed over the exposed cortical area. Additional dental acrylic was used to create a dam for the mineral oil. The vascular pattern on the surface of the brain was then photographed and recording sites were plotted on a photograph of the brain surface.

Peripherally evoked multiunit activity was then monitored using

standard physiological recording equipment and an audio monitor. With the aid of a Zeiss micromanipulator, a glass insulated tungsten microelectrode (30 um or less of tip exposed; cf. Neafsey, 1982) was inserted just below the cortical surface, and the body surface touched and tapped in order to define the receptive field of that point. This multiunit activity was used to define the representations of specific body parts in SI and SII so that partial maps of SI and SII were obtained in each animal (See results below for description of how the boundary between SI and SII was determined). Once the location of SII was identified, injections of retrograde tracers were made into one of the electrode tracks. In twelve animals 0.02 ul of 1% WGA-HRP (n=2), or 0.04 ul of 1% DY (n=7), or 0.04 ul of 1% Rhodamine conjugated microspheres (n=3) were injected into a physiologically defined region of SII (six injections into the forelimb representation, three each into the face and hindlimb representation). In six of these animals, 0.2 ul of 2% fast blue was also injected into the primary sensory cortex, two in the vibrissae representation, two in the forelimb and two in the hindlimb, in order to compare the pattern of projections to SI and SII. In addition, two other animals received 2 injections within SII, DY in its forelimb representation (FLSII) and Rhodamine (ROD) labeled microspheres (Katz et al., 1984) in its hindlimb representation (HLSII) so that the somatotopy of SII thalamic projecting neurons could also be addressed. The animals receiving HRP injections were allowed to survive for 2-3 days and then perfused with

1% paraformaldehyde and 1.5% glutaraldehyde (Rosene and Mesulam, 1978). These brains were removed and subsequently sectioned on a freezing microtome at 50 um intervals in the coronal plane. The tissue was processed for HRP histochemistry according to the TMB procedure of Mesulam (1978) as modified by Gibson et al. (1984). Those animals receiving injections of DY, FB, and ROD were allowed to survive for 2-3 days and then perfused with 4% paraformaldehyde followed by 10% buffered sucrose (Kuypers et al. 1979). The brains were removed and placed in 30% buffered sucrose until they sank, which took about 4-5 days. The brains were sectioned frozen at 50 um intervals in the coronal plane and mounted onto gelatinized slides from 0.005 acetate buffer (Spatz and Grabig, 1983). These sections were then examined on an Olympus microscope equipped with epifluorescent filters providing excitation wavelengths of 330 and 550 nm.

Data was collected from each brain in a systematic fashion. Outline drawings of every fourth section of each brain were made using a Bausch and Lomb projecting microscope. These outline drawings were then used to plot retrogradely labeled neurons using a camera lucida attachment to the Olympus microscope. Once all of the cells were plotted, which was always done within 2-3 days after histological preparation, color slides were taken of the fluorescent labeling and then the sections were counterstained with Pyronin Y; the HRP treated tissue was also counterstained with Pyronin Y. The counterstained

sections were used to parcellate thalamic nuclei in which the labeled cells were found (cf. Bold et al., 1984). The cytoarchitectonic location of the injection sites was also determined using the counterstained sections.

Since different tracers were used in various combinations, it is important to point out that these experiments were primarily directed towards determining regions within various thalamic nuclei that projected to a given cortical area. Although different tracers generally showed quantitative differences after injections into a similar cortical areas, topographic distributions within the various thalamic nuclei were nonetheless very consistent. For example, all injections of WGA-HRP, DY, or ROD into the forelimb SI representation labeled neurons in the intermediate portion of VB where cuneate projections terminate (Feldman and Kruger, 1980). This observation allows comparisons to be made between experiments using different tracers.

RESULTS

Physiological Identification of SI and SII

The vascular pattern on the surface of the lateral parietal cortex (Figure 1) provides an excellent guide to the approximate location of SII. Welker and Sinha (1972) found SII in the region of 2 or 3 large veins which drain into the inferior cerebral vein that courses along the rhinal sulcus. In our experiments, SII was usually found in the triangle formed by the inferior cerebral vein, the most caudal of the two large veins draining into it, and the major branch of the middle cerebral artery, which runs caudomedially from a point just rostral to SII (See Figure 1B).

Once the approximate location of SI and SII was determined, 10 to 20 electrode penetrations were made with a glass insulated tungsten microelectrode to record multiple unit activity in response to gentle tactile stimulation of the face and limbs. Within SI, the vibrissae representation was always found without difficulty, as was the forelimb representation, just medial to the vibrissae area. Within SII, the face was always found most rostrally followed caudally by the forelimb and then the hindlimb. This somatotopy within SII is in aggrement with previous work (Welker and Sinha, 1972). While the SI face representation shares a common or "congruent" border (Nelson et al, 1979) with the SII face representation, these two face

representations could be distinguished because the SII area showed less baseline neuronal activity than recording points in SI, and the receptive fields in SI were more specific for individual vibrissae.

Cytoarchitecture

The cytoarchitecture of the second somatosensory area (SII) of the rat was found to be distinctive in Nissl stained material from the cytoarchitecture of the dorsally contiguous primary sensory area (SI), although both are termed granular cortex. SII (Figure 2) had a much more uniform appearance compared to the prominent lamination seen in SI, and the overall histological contrast of SII was much lighter than in SI. The supragranular layers (II-III) were much thinner in SII than in SI and the density of cells, especially within layer II was considerably less in SII. The prominent acellular light band that underlies the supragranular layers seen in SI was not visible in SII. The layer IV granule cells, although still evident in SII, were also reduced in density and appear more widely spaced and did not form a dense band of cells as seen in SI. Also, there were no layer IV cell aggregates in SII like in SI (Welker, 1976). Layers V and VI within SII were present but are not well differentiated. The large, darkly staining pyramidal cells of layer Vb seen in SI were not evident in SII. Thus, the cytoarchitecture of SII can be best summarized as having an overall decreased cell packing density, a more homogeneous

appearance, less distinct lamination, and no layer IV cell aggregates, all of which are uncharacteristic of the adjacent primary sensory cortex (SI). Also, the large dark staining cells in layer V of the ventrally contiguous claustral cortex were distinctively different from SII. Similar cytoarchitecture of SII has been described by Welker and Sinha (1972) and Zilles et al. (1980) and is probably that area described by Kreig (1946) as area 40 or Area Supramarginalis. The caudally contiguous auditory (temporal) cortex was even more homogeneous than SII with layer IV granule cells wider spaced and globular in appearance (Zilles, et al. 1980).

Forelimb SI and Forelimb SII Injections

In this group (n=3) diamidino yellow (DY) was injected into the physiologically defined forelimb SII (FLSII) area and fast blue (FB) was injected into the forelimb SI area (FLSI) to determine the extent of overlap in the origins of the thalamic projections to these areas. Although the same basic set of thalamic nuclei contained retrogradely labeled cells following both injections, the position of the labeled cells within these nuclei was distinctively different (Figure 3). For example, retrogradely labeled cells were located in the ventrobasal complex (VB, Figure 8A-B) and the posterior nucleus (PO, Figure 8C-E) following both injections, yet there were no double labeled cells in either nucleus and there was very little overlap of labeled cells. In VB, labeled cells from the FLSI injection were most numerous at

rostral thalamic levels and dissipated caudally. In contrast, some labeled cells following the FLSII injection were located rostrally in VB but most were found at caudal levels. At those thalamic levels where both FB and DY labeled cells were found in VB (Figures 3F-G and 8A-B) the FLSI cells were located lateral and ventral to the FLSII labeled cells which were located in the central part of the nucleus. In PO, FLSI labeled cells were located rostromedially (Figure 3E) extending into the intralaminar nuclei. At more caudal levels (Figure 3G-H), FLSI labeled cells were more centrally located within PO. FLSII labeled cells were not found rostrally within PO (Figure 3E) but were found laterally and ventrally within more caudal PO levels (Figure 3F-H). In comparison to the FLSI labeling, FLSII labeled cells were somewhat dorsal and mostly lateral to FLSI cells (Figure 8 D), but at extreme caudal thalamic levels (Figure 3H) FLSII labeled cells appeared to surround the FLSI cells on all sides except dorsally.

In addition to the labeling found in VB and PO, labeled cells were found in the internal capsule (IC), the ventromedial thalamic nucleus (VM), the intralaminar nuclei (ILN) and the zona incerta (ZI). Both forelimb SI and SII labeled cells were located rostrally within the internal capsule (Figure 3A-D), but the forelimb SII labeled cells in IC extended further caudally (Figure 3E). Labeling in VM was dense after the FLSI injection and very sparse following the FLSII injection. In fact, only one cell was found in VM from the FLSII

injection, whereas the entire dorsal part of VM was labeled following the FLSI injection (Figure 3D-F). Retrogradely labeled cells were also located in the ILN after both injections, but the position of these cells was dependent on the injection placement. Forelimb SI labeled cells were located in the central lateral (CL) and paracentral (Pc) nuclei, and FLSII labeled cells were located in the central medial (Cem) and parafascicular (Pf) nuclei (Figure 3D-H). In addition, one FLSI labeled cell was found in the rostral Cem (Figure 3D), and a few FLSI labeled cells were found in the parafascicular nucleus (Figure 3G-H). Within the ZI, FLSI labeled cells were much more abundant than the FLSII labeled cells. FLSI cells in the ZI were located at all levels except the extreme rostral pole and were heaviest at midthalamic levels (Figue 3D-F). In contrast, a few FLSII labeled cells were seen in the ZI rostrally (Figure 3C) and at intermediate levels (Figure 3E) but the labeled cells were heaviest at caudal thalamic levels (Figure 3G).

In summary, labeled cells were found in VB, PO, ZI, and the ILN following both FLSI and FLSII injections, and labeled cells were found in VM only after the FLSI injection. Within VB, FLSI cells were concentrated rostrally while FLSII labeled cells were concentrated caudally. In the middle of VB where the two populations were both present, the FLSI cells extended ventral and lateral to the FLSII labeled cells. Within PO, FLSI labeled cells were medial to FLSII cells at rostral PO levels. Further caudal, FLSI cells occupied a

more central position within the nucleus and FLSII cells surrounded the FLSI cells except dorsally.

Hindlimb SI and SII

Although double retrograde tracer injections into the hindlimb SI (HLSI, n=7) and hindlimb SII (HLSII, n=3) were not obtained in the same animal, the data from two separate animals is plotted on the same set of thalamic drawings (Figure 4) to facilitate comparison. As in the previously described experiment, both VB and PO were differentially labeled following both injections. Labeling in VB following the HLSI injection was quite heavy throughout its rostrocaudal extent. At rostral levels, HLSI labeled cells within VB were located laterally and extended from the dorsal to ventral extent of VB (Figure 4B-C). At further caudal levels (Figure 4D-F) HLSI labeled cells continued to be located along the lateral part of VB but occupied only the ventral half of the nucleus. At still further caudal levels (Figure 4G-H), labeled cells diminished in number and were again laterally and ventrally located. Cells in VB labeled from hindlimb SII were not present at rostral thalamic levels (Figure 4A-B) but were located laterally and ventrally at midthalamic levels (Figure 4C-D) interspersed among the HLSI labeled cells. Further caudally within VB (Figure 4E-F), HLSII cells continued to be intermixed with the HLSI cells with the HLSII cells extending slightly dorsal along

the lateral part of VB.

Within the posterior nucleus (PO), HLSII cells were located more rostrally than HLSI cells (Figure 4E) which is the opposite of the arrangement seen with the FLSI and FLSII injections. Further caudally, both HLSI and HLSII labeled cells were found within PO with the HLSI labeled cells occupying a position somewhat lateral to the HLSII cells, all in the dorsal part of PO. At extreme caudal thalamic levels (Figure 4H) HLSII cells were located across the dorsal part of PO whereas HLSI labeled cells were more laterally positioned in the dorsal part of PO.

In addition to the labeling found in VB and PO, labeled cells were seen in VM, ZI, and the ILN following both injections but only in the internal capsule after the HLSI injection. Within VM, HLSI labeled cells were intermixed with HLSII cells, and all labeled cells in VM following these two injections were either ventrally or laterally located within VM from rostral (Figure 4B) to caudal thalamic levels (Figure 4F). A few labeled cells were also found in the ventral most part of VL following the HLSI injection (Figure 4B-D) and two cells were found in the dorsomedial part of VL following the HLSII injection (Figure 4D). Labeling in the ZI was sparse following both injections except at extreme caudal thalamic levels (Figure 4H) where HLSII labeled cells become numerous. Within the intralaminar nuclei only a few HLSII labeled cells were found at midthalamic levels within CL (Figure 4D) but no HLSI labeled cells were found in any of the

intralaminar nuclei. Labeling in the internal capsule was also very sparse and seen only rostrally following the HLSI injection.

In summary, labeled cells were located in VB, PO,VM, VL, and the ZI after both HLSI and HLSII injections and in the CL nucleus after a HLSII injection. Within VB, HLSI cells were located throughout the rostral to caudal extent of VB and were laterally positioned. HLSII labeled cells tended to be intermixed with HLSI labeled cells. Within PO, HLSI cells were dorsal and medial to the more laterally positioned HLSII labeled cells. Within VM, HLSI cells were ventrally located and HLSII cells were very few in number.

Vibrissae SI and SII

Although the differentiation of the vibrissae areas in SI and SII is difficult because of the close proximity of the two areas, in two animals an attempt was made to identify physiologically both areas in the same rat. Diamidino yellow was then injected into the vibrissae SII area and fast blue was injected into vibrissae SI. The multiunit sensory map for this group was more detailed than other sensory maps so that the boundaries of SI and SII could be determined. The data from one animal are plotted in Figure 6. As with the other double retrograde experiments, labeled cells were located in both VB and PO following both SI and SII injections. Within VB, VSI labeled cells were located dorsally and medially throughout its rostrocaudal extent. In contrast, VSII labeled cells in VB were not seen rostrally but were

located ventromedially in VB from midthalamic levels to the caudal limit of the diencephalon (Figure 6D-H). At all levels where both VSI and VSII labeled cells were present, the VSI labeled cells were dorsal and medial to the VSII labeled cells and no double labeled cells were found.

Within PO, VSI and VSII labeled cells were not present rostrally (Figure 6E) and were intermingled more caudally (Figure 6F-G). However, no double labeled cells were found within PO. At caudal thalamic levels (Figure 6H) VSI labeled cells were surrounded laterally and medially by VSII labeled cells. In addition to the labeled cells located in VB and PO, labeled cells were also located in VM, VL, ZI, ILN, and the internal capsule following both VSI and VSII injections. Within VM, VSII labeled cells were dorsal to VSI labeled cells (Figure 6D-E), but the extent of labeling from the VSI injection was much more extensive in VM than the labeling following the VSII injection. In fact, VSI labeling in VM was present from midthalamic levels to the caudal limit of this nucleus. Face SII labeling, on the other hand, was seen only at midthalamic levels (Figure 6D-F). Within VL, retrogradely labeled cells were found rostrally (Figure 6B-C) following the VSI injection and a few cells were found ventromedially following the VSII injection (Figure 6C). Labeling within the ZI was also heavier following the VSI injection when compared to VSII labeling in the ZI. Face SI labeling in the ZI extended from midthalamic levels (Figure 6C) to the caudal diencephalon (Figure 6G)

where the heaviest VSI labeling within the ZI was found. Labeling in the ZI following the VSII injection was confined to midthalamic levels (Figure 6E) where a few labeled cells were found intermixed with the VSI labeled cells. Labeling in the internal capsule was seen only at rostral thalamic levels (Figure 6A-B) and was heaviest following the VSII injection. In fact, only a few VSI labeled cells were found within the IC and only at one level (Figure 6B). Labeling in the ILN was found in Pc and Cem at rostral levels following a VSII injection and within Pc and CL following a VSI injection. No labeling was found in Pf following either injection.

In summary, labeled cells were located in VB, PO, VM, VL, ILN, ZI, and the internal capsule following both VSI and VSII injections but double labeled cells were nnot seen in the diencephalon. Within VB, VSI labeled cells were medial and dorsal to VSII labeled cells; whereas, VSI labeled cells in VB were found both rostrally and caudally, VSII labeled cells were found only from midthalamic to caudal thalamic levels. Within PO, VSI and VSII labeled were intermingled rostrally but at caudal thalamic levels VSII labeled cells surrounded the VSI labeled cells within PO. Labeling in VM was heaviest following the VSI injection and was found from midthalamic levels to the caudal thalamic limit of VM, with most cells positioned medially within VM. VSII labeled cells in VM were less frequent and occupied a position dorsal to the VSI labeled cells in VM. Within the ILN, VSI labeled cells were present in Pc rostrally and CL at more

caudal levels whereas VSII labeled cells were present in Pc and Cem at midthalamic levels only. Cells in VL were found rostrally, with the VSI cells found in the central part of this nucleus and the VSII cells found ventromedially. Labeling in the ZI was heaviest following the VSI injection and was seen throughout the caudal half of the thalamus. Very few VSII labeled cells were located in the ZI, all at midthalamic levels. Finally, the rostral internal capsule contained a moderate number of VSII labeled cells and only a few scattered VSI cells.

Forelimb SII and Hindlimb SII

In two animals an attempt was made to make two separate injections into SII, one in the forelimb representation (FLSII) and one in the hindlimb representation (HLSII). Once the forelimb and hindlimb areas within SII were identified physiologically, diamidino yellow was injected into the FLSII and rhodamine conjugated microspheres were injected into the HLSII. From this group, the organization of SII thalamic projecting neurons could be determined. Histologically, no spread was evident between injection sites.

Retrogradely labeled cells were located in VB, PO, ZI, and the internal capsule after both FLSII and HLSII injections, in VM, Pc and Cl after HLSII and in Cem following FLSII injections. No labeled cells were found in rostral VB (Figure 7A-C). At midthalamic levels HLSII labeled cells occupied the lateral part of VB and continued in this position caudally. Forelimb SII labeled cells within VB were

found only at caudal thalamic levels, positioned medial and slightly dorsal to the HLSII labeled cells (Figure 7F-H). Within PO, HLSII labeled cells were consistently dorsal to FLSII labeled cells; Within rostral PO, forelimb SII cells were found laterally along the PO-VB border and extended medially from this position into the ventral part of PO to the Pf border (Figure 7G-H).

In addition to the labeled cells found in VB and PO, labeling occurred in VM, ILN, ZI, and IC. Within VM, a few HLSII labeled cells were scattered from midthalamic levels (Figure 7D-E) to more caudal levels (Figure 7F), but no consistent position of these cells was found. Cells in VM labeled from the FLSII injection were fewer in number than HLSII cells; in fact, only one FLSII cell was found caudally (Figure 7F).

Labeled cells in the ILN after the FLSII cortical injection were seen in the CL nucleus at midthalamic levels (Figure 7D-E) and in Pc at more caudal levels (Figure 7F). Hindlimb SII labeled cells in the ILN were confined to one cell in Pc rostrally (Figure 7B), and a distinct cluster in Cem at midthalamic levels (Figure 7E-F).

Within the ZI, the majority of FLSII cells were located caudally (Figure 7G) with scattered cells present at more rostral levels (Figure 7B-E). In contrast, no HLSII cells were seen rostrally within the ZI (Figure 7A-C) and at midthalamic levels a few cells were found widely dispersed within the ZI (Figure 7D-E). At more caudal levels (Figure 7G) a few more ZI labeled cells were seen. Labeling in the

internal capsule was found exclusively at rostral thalamic levels where both FLSII and HLSII labeled cells were seen (Figure 7B-D).

In summary, labeled cells were found in VB, PO, ZI, and the IC following both FLSII and HLSII cortical injections, but none were double labeled. Labeled cells were found in CL and VM only after the HLSII injection and in Cem only after the FLSII injection. Within VB, no cells were found rostrally but from midthalamic levels caudally HLSII labeled cells were consistently lateral and somewhat ventral to FLSII labeled cells. Within PO only HLSII cells were found rostrally while more caudally both FLSII and HLSII labeled cells were seen. The HLSII cells were consistently dorsal to FLSII cells which were positioned laterally and extended medially in the ventral part of caudal PO. Labeling in VM was seen only after the HLSII injection, except for one FLSII cell found caudally. Within the intralaminar nuclei, CL and Pc were labeled following the HLSII injection and Cem following the FLSII injection. Labeling in the ZI was heaviest following the HLSII injection. It was not found rostrally and was heaviest at midthalamic levels. Within the IC, cells were seen only rostrally with a few double labeled cells found at extreme rostral thalamic levels.

Topographic Analysis of VB and PO

Since the primary object of this study was to compare thalamic projections to specific body representations of SI and SII, the topographical organization of such efferents within VB and PO was

examined. This data was compiled in exactly the same way as was done in the study of thalamic projections to motor cortical representations. For each injection type the maximal distribution of labeled cells, representing the sum of individual labeling patterns, was plotted on to three levles of VB and PO. Similarly, the area of labeling common to all animals in each injection type was determined. These maximal and minimal distribution patterns were then compared across injection types in order to determine the degree of overlap and the degree of topographic separation (Figures 9-12).

The obtained analysis of VB projections to SI cooroborates previous studies of somatotopy (Saporta and Kruger, 1978). As shown in Figure 9 (top and bottom set of three levels through VB) the hindlimb SI region is most lateral. The forelimb SI region is medial to the hindlimb and dorsal to the vibrissae SI representation rostrally, but the forelimb SI cells move ventral to the vibrissae cells at middle and caudal levels of VB. The vibrissae SI region is most medial through all levels of VB, but is more ventrally located rostrally and more dorsally located at middle and caudal levels of VB.

In Figure 10, VB's projection to SII are summarized. The hindlimb SII region is also seen laterally but only at middle and caudal levels of VB. The forelimb SII region is seen only at middle and caudal levels of VB. At the middle level of VB, the forelimb SI and SII regions overlap slightly but are largely separate. The vibrissae SII region is located dorsomedially at middle and caudal levels of VB. In

summary, the hindlimb SI and SII regions are lateral, the forelimb SI and SII regions ventromedial at middle and caudal levels, and the vibrissae SI and SII regions are dorsomedial within VB.

The topography of PO's projections to SI established by the same method is illustrated in Figure 11. The hindlimb SI region is located dorsally at all levels. The forelimb SI region is located more medially in rostral PO but moves more centrally at more caudal levels of PO. The vibrissae SI region within PO is located both lateral and medial within PO at rostral thalamic levels but caudally is located only lateral to both the hindlimb and forelimb regions.

The topography of PO's projections to SII is illustrated in Figure 12. The hindlimb SII region is located dorsally at all levels through PO, but is more medially located at rostral and middle levels than at the most caudal level. The forelimb SII region is located in the central part of PO at all levels and extends ventrally at more caudal levels. The vibrissae SII region is located along the lateral border of PO at all levels. In summary, the hindlimb SI and SII regions within PO are dorsal, the forelimb SI and SII regions are centrally located, and the vibrissae SI and SII regions are lateral.

DISCUSSION

The major findings of this study are: 1) the thalamic nuclei VB, PO, and in some cases the intralaminar nuclei project to both SI and SII in the rat; 2) VM projects to all regions of SI but not to all regions of SII; 3) labeled cells in VB and PO were generally arranged in columns or sheets of cells, extending throughout the rostrocaudal extent of each nucleus; 4) the different body parts within SI and SII (i.e., hindlimb, forelimb, face) were represented by topographically organized projecting neurons within VB and PO (those in VB were somatotopic); 5) no double labeled cells, indicative of axonal branching, were found within any of the thalamic nuclei. Since the fluorescent retrograde double labeling technique was used and no double labeled cells were found in the thalamus but were seen elsewhere, it appears that thalamocortical projections to SI and SII arise from separate populations of neurons.

Ventrobasal Complex

Retrogradely labeled cells observed after primary sensory cortical injections were somatotopically organized within VB. HLSI cells were located most lateral and ventral; FLSI cells were found more medially; and VSI labeled cells most medial extending to the dorsalmost part of this nucleus. This topographic arrangement within VB supports previous findings of reported somatotopy (Saporta and

Kruger, 1977). Furthermore, studies using rats (Lund and Webster, 1967a; Smith, 1973) and cats (Berkley, 1980) have found the gracilis, cuneatus, and principal trigeminal nuclear efferents display a lateral to medial topography within VB which corresponds with our observations concerning the topography of projections to the appropriate areas of SI.

The distribution of retrogradely labeled cells seen within VB following injections of the secondary somatosensory cortical area were also somatotopically arranged. While some overlap was observed and if the overlapping regions are subtracted, the topography of SII projecting thalamic neurons is such that VSII projecting cells were dorsomedially located while FLSII projecting cells were more medially and ventrally located and HLSII projecting cells occupied the lateral part of VB. While this topography appeared less precise in comparison to VB projections to SI, like projections to SI no double labeled neurons were found after injections into SII.

Previous reports have not examined the topographic organization of VB neurons projecting to physiologically defined areas of SII in the rat, but this question has been studied in the monkey (Burton and Robinson, 1981), cat (Burton and Kopf, 1984) and the raccoon (Herron, 1983). However, in the rat it has been suggested that there are two distinct areas within VB that corresponded to SI and SII (Emmers, 1965). He reported that the SI region was confined to VB and received contralateral somatosensory inputs while the SII thalamic

region, within the caudal parts of VB, received bilateral cutaneous inputs (Emmers, 1965). Saporta and Kruger (1977), in addition to studying the ventrobasal projections to SI in the rat, also attempted to identify the SII region of Emmers (1965). They reported that no area exists within VB with bilateral receptive fields. Part of the lack of agreement between these two studies may be due to a possible lack of precision in Emmers' (1965) study because the histological reconstructions he made were done on brains other than the ones recorded from. Although the present report does demonstrate a second body representation within VB that projects exclusively to SII, it is likely that the SII thalamic region that Emmers (1965) identified was the representation of the body found in the posterior nuclear complex and not in VB. It is unlikely that the close approximation of the thalamic neurons projecting to SI and SII, which we and others (Jones, 1975) have found could be distinguished with electrode penetrations at 0.25 mm intervals.

Comparing the thalamic projections from VB to SI and SII also reveals a topographical pattern. At rostral thalamic levels the labeling from SI injections is generally separate from the labeling of SII injections, but at midthalamic levels the labeling from the two injection types was often intermixed, yet organized in such a way that the SII labeled cells partially surrounded the SI labeled cells in VB. This was especially true for animals receiving injections in HLSI and HLSII areas.

Previous studies (Rose and Woolsey, 1949; Jones, 1975) have described "essential" and "sustaining" thalamocortical projections. The essential projections were identified as specific thalamic nuclei projecting to specific cortical areas whereas sustaining projections were to more than one area of cortex. Diamond (1983) has recently described the areas receiving essential projections as "core" areas of sensory cortex (SI) and the areas receiving sustaining projections as "belt" areas of sensory cortex (SII). When applied to the thalamus, the core region apparently corresponds to areas containing primary (essential) projecting neurons and the belt region to secondary (sustaining) projection neurons (Diamond et al., 1967). With respect to VB and PO our data do not agree with this concept of essential and sustaining projections because we never found double labeled cells in VB nor PO. In fact, very specific parts of both VB and PO project to precise physiologically defined regions of somatosensory cortex. Furthermore, our data does not agree with the concept of "core" and "belt" because it appears that both SI and SII projecting thalamic neurons arise exclusively from the core region. This is further substantiated by evidence in the cat that the dorsal columns project directly to the core region of VB while the spinal cord and other brainstem nuclei relaying somatosensory information project to areas surrounding the core zone (Berkley et al., 1980). In a previous study (Bold et al., 1983) we have injected WGA-HRP into the dorsal column nuclei and principal trigeminal nucleus, and the regions of PO and VB

that receive the terminations from these nuclei closely correspond to the regions where the somatosensory cortex projecting neurons are located. This observation of several specific thalamic nuclei or parts of them relaying somatosensory information substantiates the theory of parallel processing of somatosensory information (Dykes, 1980).

Posterior Nuclear Complex

A topographical organization of neurons projecting to the primary sensory cortical regions was found in PO. The HLSI region was found most dorsal, the FLSI region more centrally located and the VSI region split into two, one along the lateral border of PO and one ventromedially located. At caudal levels within PO the hindlimb SI region is dorsally located and the vibrissae SI region is lateral in position. Examination of the rostral level of PO shows the hindlimb SI dorsal, forelimb SI more centrally located, and vibrissae SI lateral and ventromedially located. Previous reports have not described this topography, although projections to the primary sensory cortical area have been found in the rat (Jones and Leavitt, 1973; Donoghue et al., 1979), mouse (White and DeAmicis, 1979) and cat (Spreafico et al., 1981; Bentivoglio, 1983; Burton and Kopf, 1984).

Retrogradely labeled cells in PO following injections in physiologically defined areas of SII were also found to be

topographically organized. At the rostral PO level the hindlimb SII region is dorsomedial, the forelimb SII region is centrally located and the vibrissae SII region is lateral. At the middle level of PO, the hindlimb SII region is dorsomedial, the forelimb SII region is again centrally located, and the vibrissae SII region is along the lateral border of PO. At the caudal level of PO a similar pattern is seen with the hindlimb SII region dorsal, the forelimb SII region more ventrally located, and the vibrissae SII region lateral. A comparison of figures 11 and 12 shows the relationship of the regions projecting to SI and SII.

A recent study reported that PO projects more heavily to SII than to SI and that VB projects heavier to SI than to SII in the rat (Weinberg et al., 1984). In our experiments the relative density of labeling in PO is basically the same following both SI and SII injections. We do agree, however, that more VB neurons project to SI than to SII, but this may be because the density of cells projecting to SI is greater.

Other Thalamic Nuclei

In addition to the apparently well organized distribution of VB and PO projections to different regions of SI and SII, retrogradely labeled cells were also found in VM, ZI, and the ILN. Within VM, retrogradely labeled cells were always seen following SI cortical injections but many fewer were seen following SII cortical injections.

Since VM processes information from the basal ganglia (Faull and Carman, 1968; Herkenham, 1978) to the cortex, it appears that SII is not receiving as much of this information as is SI. Although it has been reported that VM projects to layer I of the anterior two-thirds of the neocortex (Herkenham, 1980), our data suggests that the terminations in SII are less dense than in SI. Retrogradely labeled cells in the zona incerta were more numerous following SI cortical injections, but all SII cortical injections labeled cells in the ZI, especially caudally. Labeling in the ILN was typically found rostrally in CL and/or Pc following the SI cortical injections and caudally in Cem and/or Pf following the SII coritcal injections. Although the ILN have been implicated as comprising the "diffuse" thalamic projection system, it appears that their projections to SI and SII are specific, suggesting that these nuclei also have a more "specific" role than previously thought.

Comparisons with Other Species

Studies in the cat concerning thalamic projections to SI and SII initially described only VB neurons projecting to SI (Heath and Jones, 1971; Jones and Leavitt, 1973). However, more recent studies (Spreafico et al., 1981; Bentivoglio, 1983; Burton and Kopf, 1984) show additional afferents from part of the posterior nuclear complex projecting to both SI and SII. Also, these authors have provided evidence that the intralaminar nuclei also project to SII in the cat.

This data is in agreement with the present report where VB, PO, and the ILN were found to send differential projections to SI and SII. The possibility that labeled cells in PO are "displaced VB neurons" (Jones et al, 1979) seems unlikely, because of the significant differences in the size, location and distribution of the retrogradely labeled cells found in PO. In the monkey, displaced VB neurons are large and are located in the dorsolateral and ventromedial parts of PO (Jones et al., 1979). Labeled cells in the rat PO were small cells and were distributed throughout this nucleus with topographical locations associated with the physiologically identified injection site.

One difference between the results reported here and previous studies in the cat (Jones, 1975; Spreafico et al., 1981) was the absence of double labeled cells in the thalamus following combined SI and SII injections. In the cat, Jones (1975) and Spreafico et al. (1981) found 10% of the labeled cells in VB to send collaterals to SI and SII. However, Bentivoglio (1983) found only two or three double labeled cells per animal in the POm nucleus of the cat following separate injections in the somatosensory cortex. In the rat, no double labeled cells were found within VB or PO, and thus, in the rat, neurons from these two thalamic nuclei do not appear to project to both cortical areas.

Our results appear to differ from those described for SI and SII in the raccoon, where Herron (1983) reported that distinctively different

nuclei project to SI and SII in the raccoon without overlap. In fact, he found that only VB projects to SI and not to SII, while VPI, a subnucleus ventral to VB in the raccoon, projects only to SII and not to SI. In the rat, there is no apparent VPI beneath VB, but it is the ventral part of VB, lying just dorsal to the medial lemniscus, where retrogradely labeled cells are seen following all SII cortical injections. Thus, VPI in the raccoon is likely homologous to the ventral part of VB in the rat, as reported in this study. Consistent with Herron's finding of no labeling in the more dorsal aspects of VB, we also failed to label cells in dorsal VB following SII injections. In addition, labeled cells were also seen in PO in the raccoon by Herron (1983, cf. Figure 6-7) following both SI and SII injections. With this interpretation of Herrons's data, it appears that the thalamic projections to SI and SII in the rat and raccoon are actually quite similar.

In the cynomologus, macaque, and most new world monkeys (e.g. squirrel monkey and owl monkey) the primary somatosensory cortex (SI) consists of four separate fields (Areas 3a, 3b, 1, 2), each having distinct cytoarchitecture, specific sensory properties, and specific inputs from different thalamic nuclei (Jones, 1975). Area 3b in the primate appears to corresond to SI in the rodent (Sur et al., 1979; Chapin and Lin, 1984), receiving cutaneous information. Thalamic afferents to 3b in the macaque arise from the ventroposterior lateral (VPL) nucleus (Nelson and Kaas, 1981; Pons and Kaas, 1984) which

appears to be homologous with VB in the rat, and from scattered cells in the intralaminar nuclei (Burton, personal communication). The anterior pulvinar and superior part of the VPL nucleus (VPLs, Pons and Kaas, 1984; Kaas et al, 1984), which appear to correspond to the dorsal part of the posterior nucleus in the primate (Burton, personal communication), do not appear to project to area 3b, but instead project to area 2, an area receiving deep receptor inputs (Pons and Kaas, 1984). Although the deep receptor regions and the cutaneous receptor regions of the rodent SI appear to be distinguishable (Welker et al., 1984; Chapin and Lin, 1984), PO may be the thalamic relay for deep receptor information to SI. However, our cortical injections were not small enough to involve only deep or only the cutaneous receiving regions of SI cortex. In the macaque, where the deep and cutaneous receptors cortical regions within SI are much more separate, the cutaneous region (Area 3b) receives afferents exclusively from VPL while the deep receptor region (Area 2) receives afferents from the anterior pulvinar (pa) and the superior division of VPL (VPLs, Pons and Kaas, 1984), which, based on location and connections appears to be homologous to PO in the rat. The afferents to SII in the macaque arise from the ventroposterior inferior nucleus (VPI) as well as from a few scattered cells in VPLc and a few cells in the dorsal part of the posterior nucleus, POm (Burton, personal communication).

Thalamocortical Organization

The pattern of labeling within the thalamus following all cortical injections consisted of elongated columns or oblate ellipsoids of cells that usually extended throughout the rostrocaudal dimension of the thalamus. This finding has been described previously in rats and other species (Saporta and Kruger, 1977; Kuypers et al., 1977; Bentivoglio, et al, 1979; Jones, 1983; Bold and Neafsey, 1984b), where it sometimes appeared that such columns crossed cytoarchitectonic borders (Kuypers, et al. 1977). In our study, the columns were found to be mostly confined to VB and PO although at caudal thalamic levels they did appear to cross from VB into PO. Jones (1983) has termed this pattern of labeling "rods" and suggested that these rods could be the basis for afferent inputs to the place and modality specific columns within primate SI. The relative size of these retrogradely labeled elongated thalamic arrangements appear to be related to the relative size of the cortical injections (Saporta and Kruger, 1977) and represent the limitations of the present methodology in terms of defining the fine grained patterns of thalamocortical organization.

Summary

This study was undertaken to examine the thalamic sources of projections to two divisions of the rodent somatosensory cortex, SI and SII, and to determine if separate populations of cells project to the same body representations of SI and SII. The organization of thalamocortical projections to SI and SII were studied using the

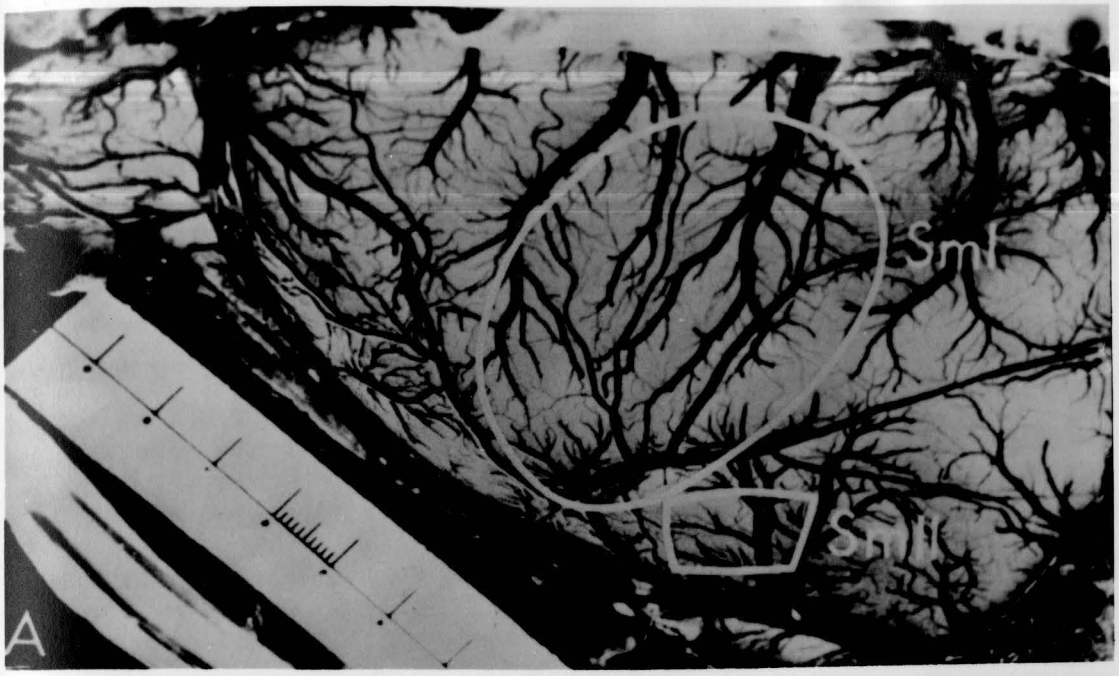
multiple fluorescent retrograde tracing technique, using different combinations of tracers injected into physiologically identified areas of SI and SII. The distribution of thalamic retrogradely labeled cells showed that both VB and PO project to both cortical areas. In addition, it appears that different parts of each nucleus concentrate its efferent projections onto one of the cortical areas and does not send collaterals to the other cortical field. This topographic organization of thalamic neurons projecting to SI and SII in the rat appears to be specific and somatotopic, at least in VB, with the pattern of labeling best described as columns or ellipsoids of cells occupying almost the entire rostrocaudal dimension of each nucleus labeled.

The available information concerning the pattern of thalamic connections to the primary and secondary somatosensory fields in mammals appears to be rather species specific. In SI of some mammals, such as the raccoon and rat, the afferents to SI and SII arise from two separate thalamic nuclei (VB and PO) and these projections appear to be well organized. In the cat, where the thalamus is more differentiated and hence more subdivided, the same two nuclei also project to both SI and SII, but only a specific part of VB (VPL) and an even more speicifc part of PO (POm) project to these different cortical areas. In the macaque, only the VPLc nucleus projects to 3b while VPI, VPLc, and POm cells project to SII. The important fact here is that the homolog of PO does not appear to project to 3b in the

monkeys studied but does project to SII, as well as to area 2.

Figure 1

Location of SI and SII shown on the dorsolateral view of the rat brain. A. Photographic reproduction of Welker and Sinha's (Brain Research 37:132, 1972) vasculature photograph. B. Photograph of experimental hooded rat vasculature shown in this study. Note the similar pattern of blood vessels in the region of SII. The scale is in millimeters in both figures.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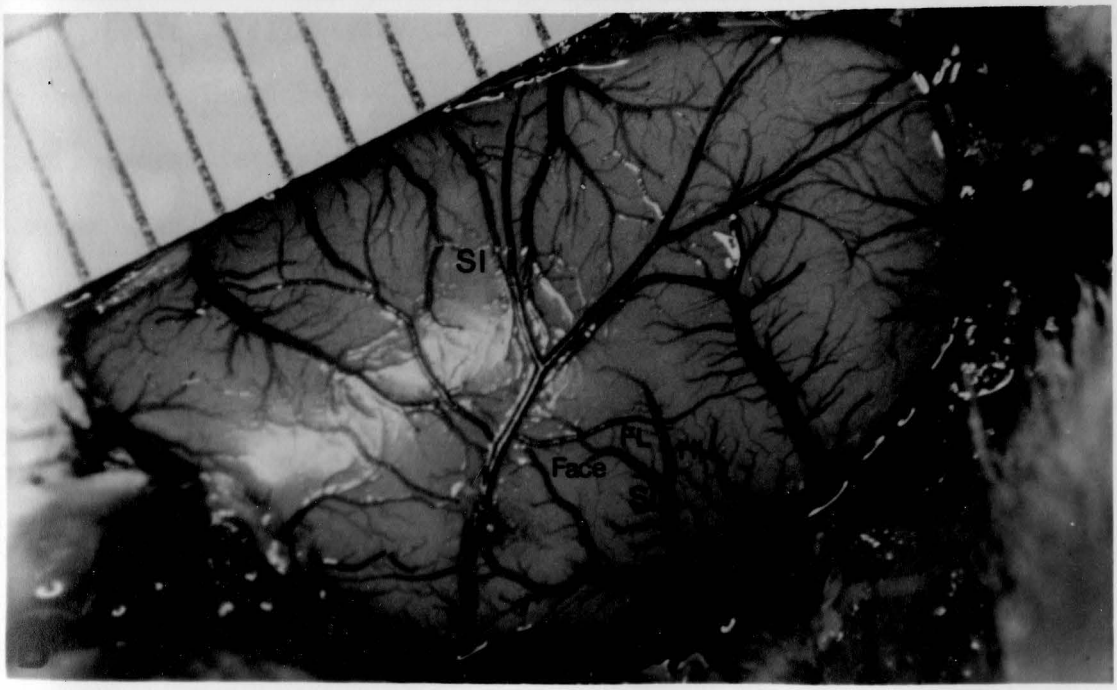
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Figure 1 Continued

C. Dorsolateral view of the rat brain illustrating the somatotopic organization within both the motor and somatosensory cortices, adapted from Hall and Lindholm (1974). The somatotopy within the motor cortex, which is indicated by the solid lines, is based on intracortical microstimulation studies done in Dr. Neafsey's lab. The somatotopy within the somatosensory cortex, which is indicated by the hatched lines, is based on the studies of C. Welker (1971, 1976). Abbreviations are: AUD, auditory cortex; CFL, caudal forelimb motor; FLSI, forelimb sensory; HL, hindlimb motor; HSI, hindlimb sensory; Jo, jaw open; LL, lower lip motor; LLSI, lower lip sensory; N, neck motor; NSI, neck sensory; R, rhinarium or snout; RFL, rostral forelimb motor; RHL, rostral hindlimb motor; SII, second somatosensory area; T, trunk motor; To, tongue out motor; TSI, trunk sensory; ULSI, upper lip sensory; V, vibrissae motor; VIS, visual cortex; VSI, vibrissae sensory

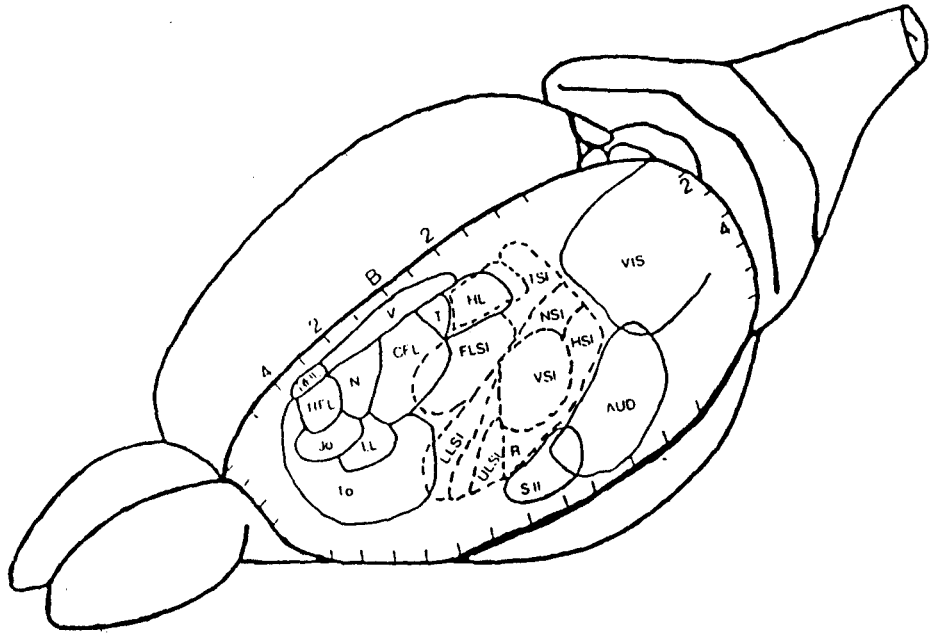
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Figure 2

A-F. A rostrocaudal series of photomicrographs (4X) at 500 um intervals through the second somatosensory area (SII). The sections are stained with thionin (Nissl method). Abbreviations: SI, first somatosensory area; SII, second somatosensory area; Cli, isocortical claustral cortex; Clpri, proisocortical claustral cortex; Clpc, periallocortical claustral cortex; Prpi, prepiriform cortex. These abbreviations are from Zilles et al (1980).

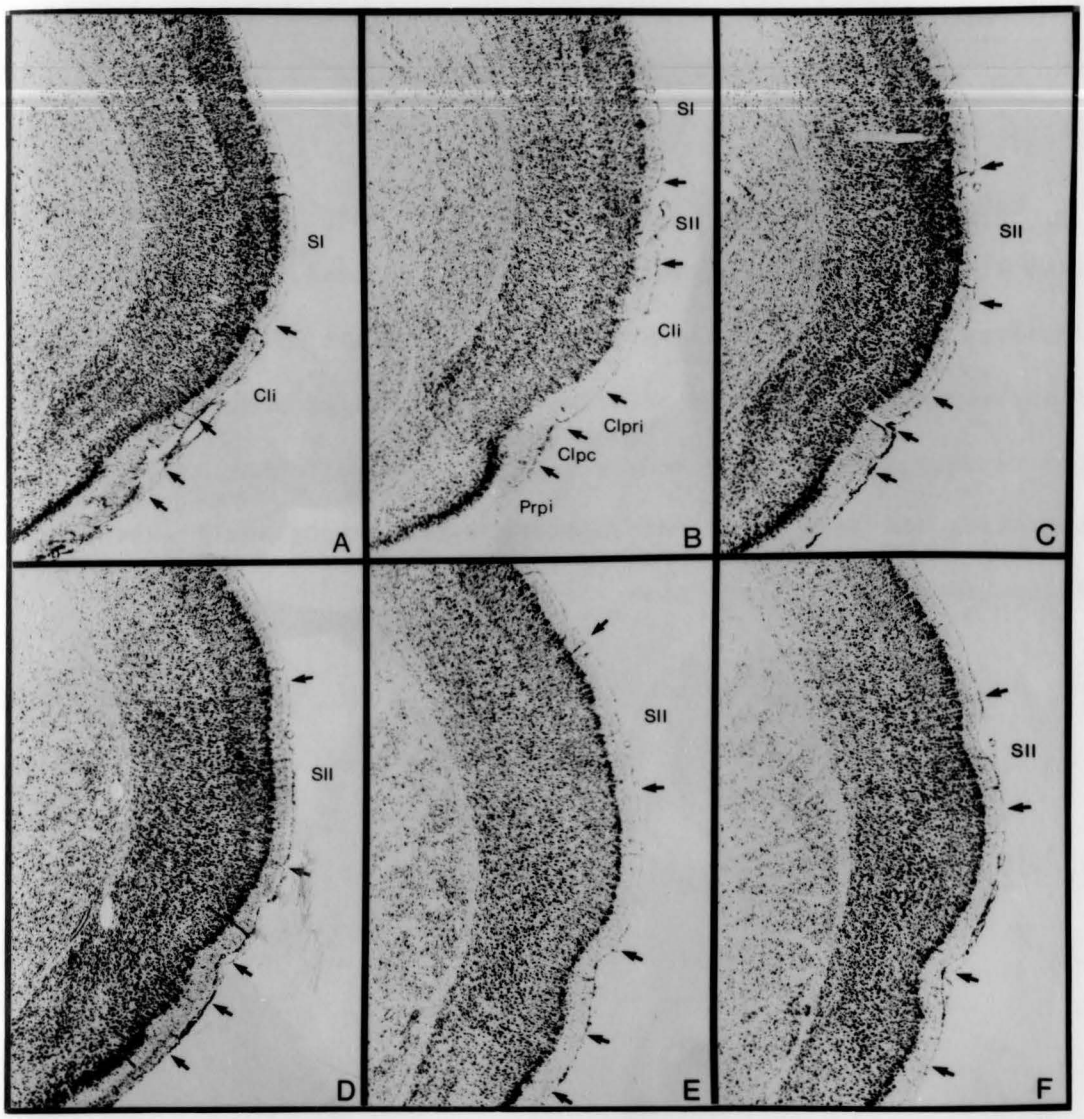
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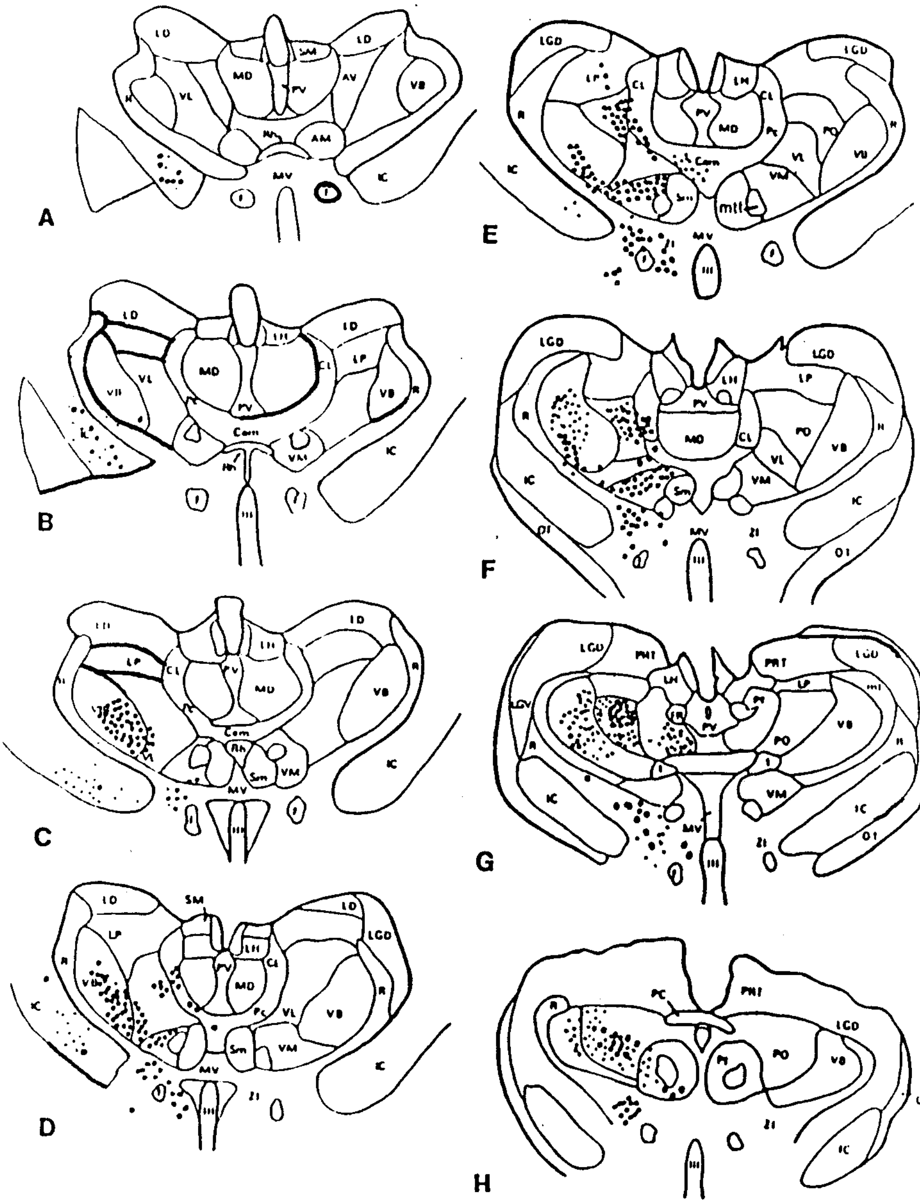
Figure 3

Results of a diamindio yellow injection into the forelimb SII and fast blue into the forelimb SI of the same animal. A-H. Outline drawings of coronal sections at 300 um intervals through the thalamus illustrating the location of retrogradely labeled neurons. Each large dot represents one retrogradely labeled cell following the FLSI injection and each small dot represents one retrogradely labeled cell following the FLSII injection.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H

Figure 3 Continued

I. Dorsolateral view of the brain showing the sensory mapping recording points. The injection placements are circled, one in FLSI and one in FLSII. The open stars represent forelimb SII points, the closed stars represent forelimb SI points, the squares represent vibrissae SII points, the large dots represent vibrissae SI points, and the small dots represent no response points. J. Stack drawing through the injection sites showing the maximal spread of the injected material. Each section represents 200 um.

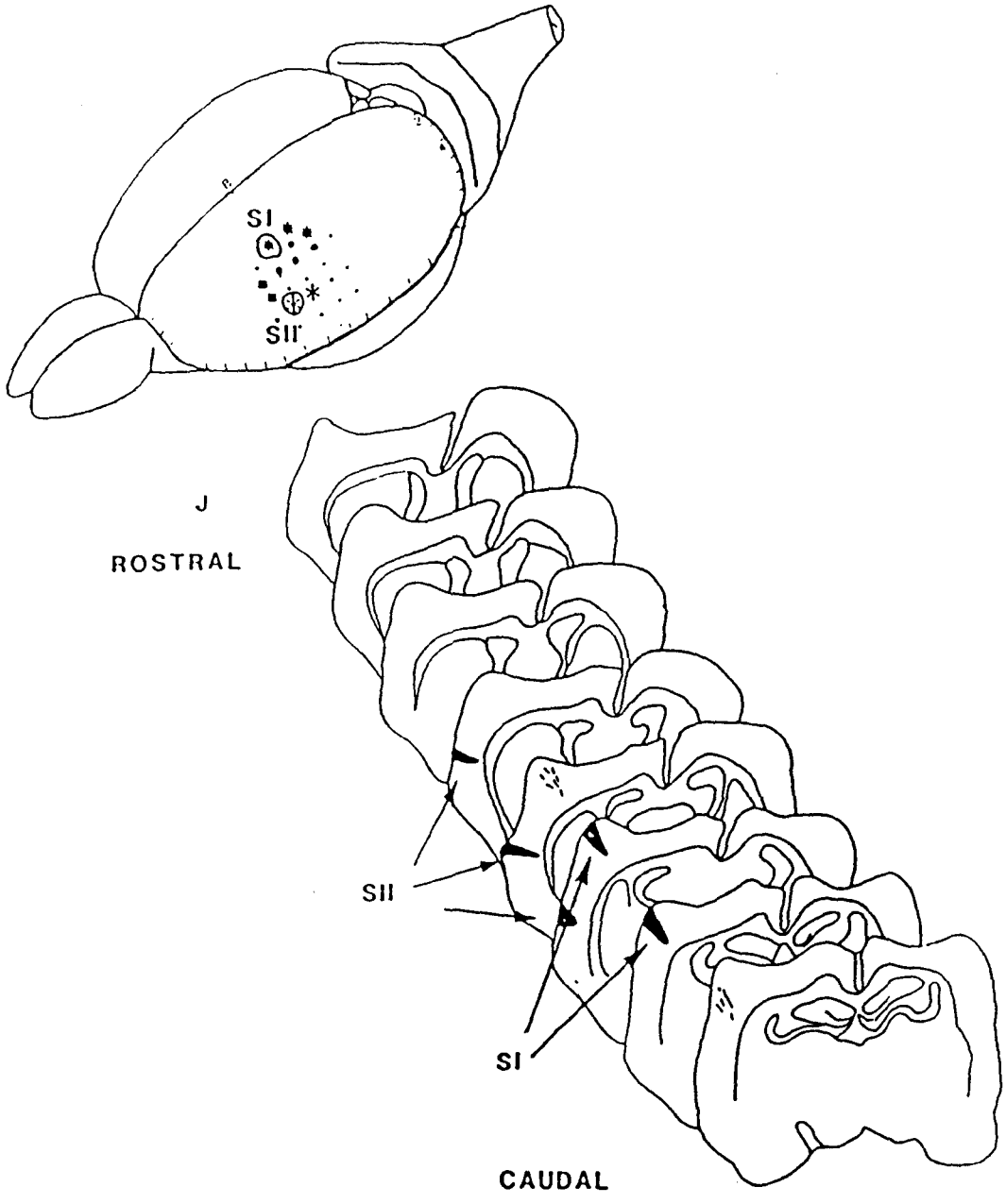

SI
SII
J
ROSTRAL
SII
SI
CAUDAL

Figure 4

Composite experimental data of a diamidino yellow injection placed in hindlimb SII of one animal and fast blue into hindlimb SI of a different animal. A-H. Outline drawings of coronal sections at 300 um intervals through the thalamus illustrating the location of retrogradely labeled cells. Each large dot is a HLSI labeled cell and each small dot is a HLSII labeled cell.

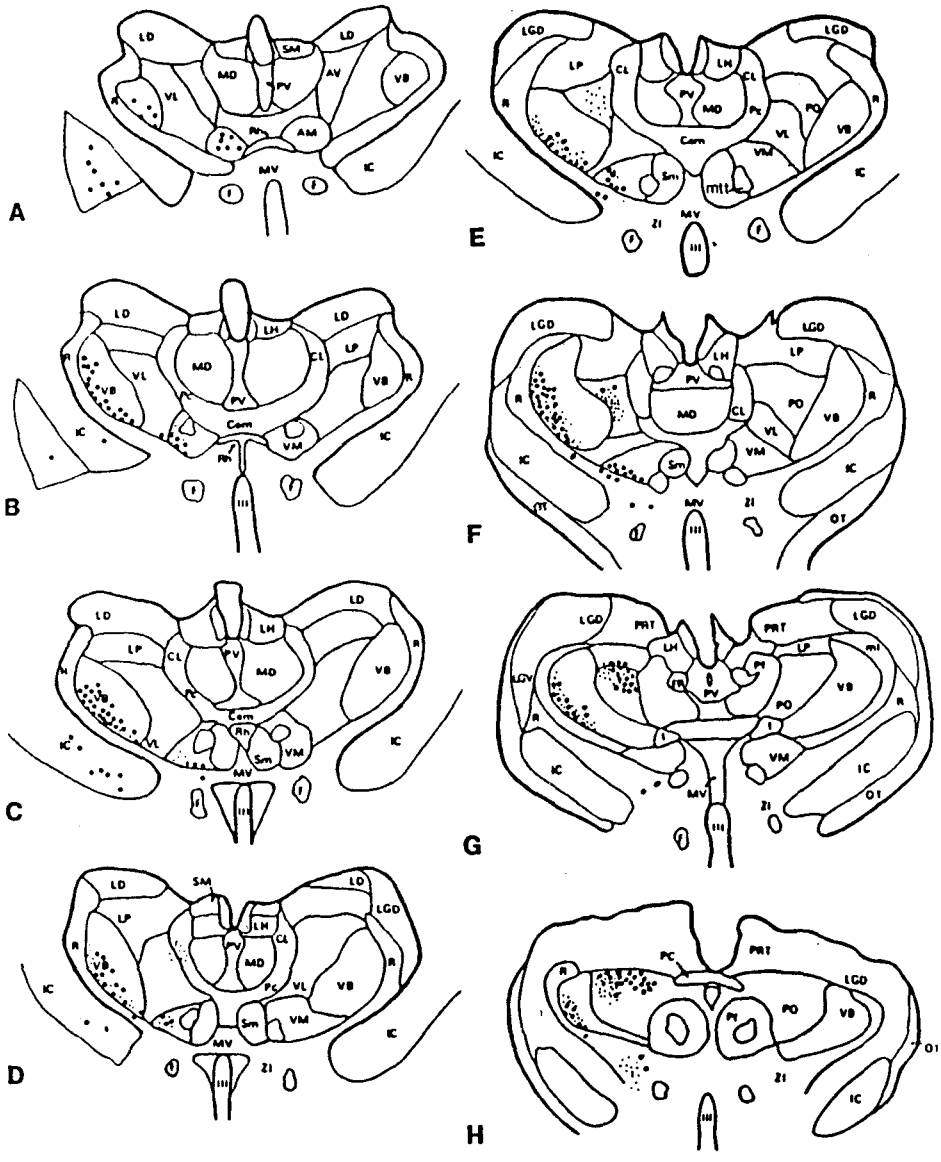
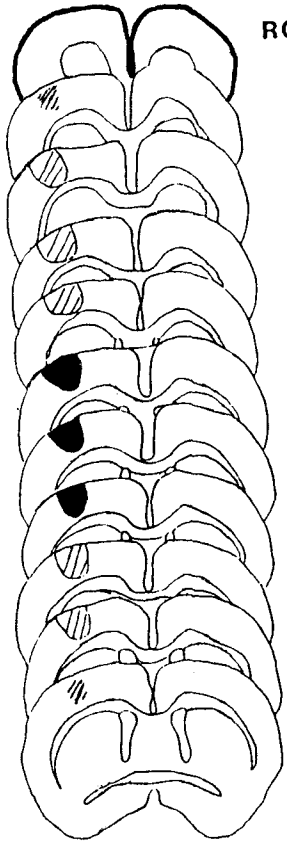

A
LD
SM
LD
MD
AV
VL
PV
VB
Rh
AM
IC
MV
I
II
B
LD
LH
LD
LP
CL
MD
VL
VB
PV
VB
R
IC
Cem
VM
IC
Rh
I
III
I
C
LD
LH
LD
LP
CL
PV
MD
VB
R
Cem
Rh
Sm
VM
IC
MV
I
III
D
LD
SM
LD
LGD
LP
LH
CL
PV
MD
R
VB
Pc
VL
VB
IC
Sm
VM
IC
MV
I
III
ZI
E
LGD
LGD
LP
CL
LH
CL
R
PV
MD
Pc
PO
R
VL
VB
Cem
VM
IC
Sm
mlf
IC
ZI
MV
I
III
I
F
LGD
LGD
LH
LP
PV
R
MD
CL
PO
VB
R
VL
IC
VM
Sm
IC
MV
ZI
OT
III
G
LGD
PRT
PRT
LGD
LH
LP
LV
PV
VB
PO
R
I
I
IC
VM
IC
MV
OT
ZI
III
H
PC
PRT
R
LGD
PV
PO
VB
ZI
IC
III

Figure 4 Continued

I. Stack drawing through the HLSI injection site showing the maximal spread of the injected material. J. Stack drawing through the HLSII injection site, also showing the extent of spread.

I HLSI INJECTION

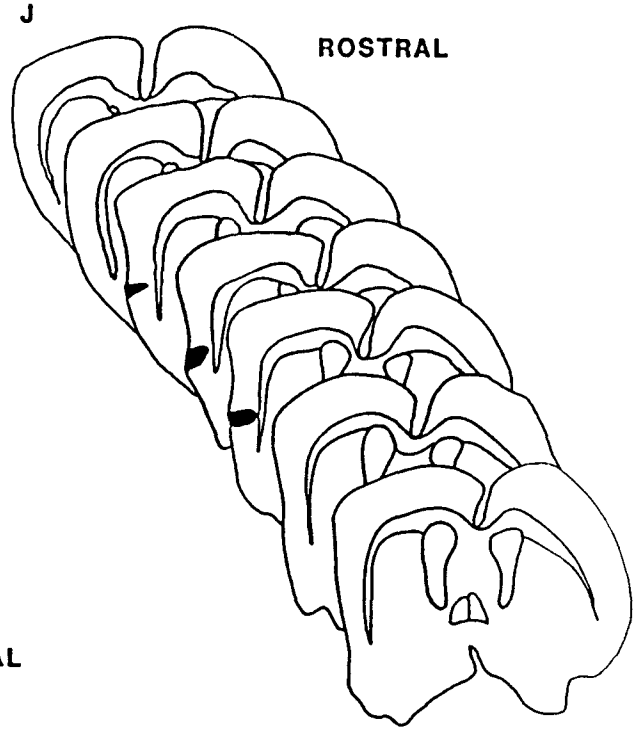


ROSTRAL

CAUDAL

HLSII INJECTION

J



ROSTRAL

CAUDAL

Figure 5

Nissl stained photomicrographs of actual injection sites. Each photograph represents a 50 um section taken from the experimental animals illustrated in figures 3,4,6, and 7. A. Fast blue in VSI (3X). B.Fast blue in FLSI (3X). C.Evans blue in HLSI (3X). D.Diamidino yellow in VSII (1.25X). E. Diamidino yellow in FLSII (1.25X). F. Diamidino yellow in HLSII (1.25X).

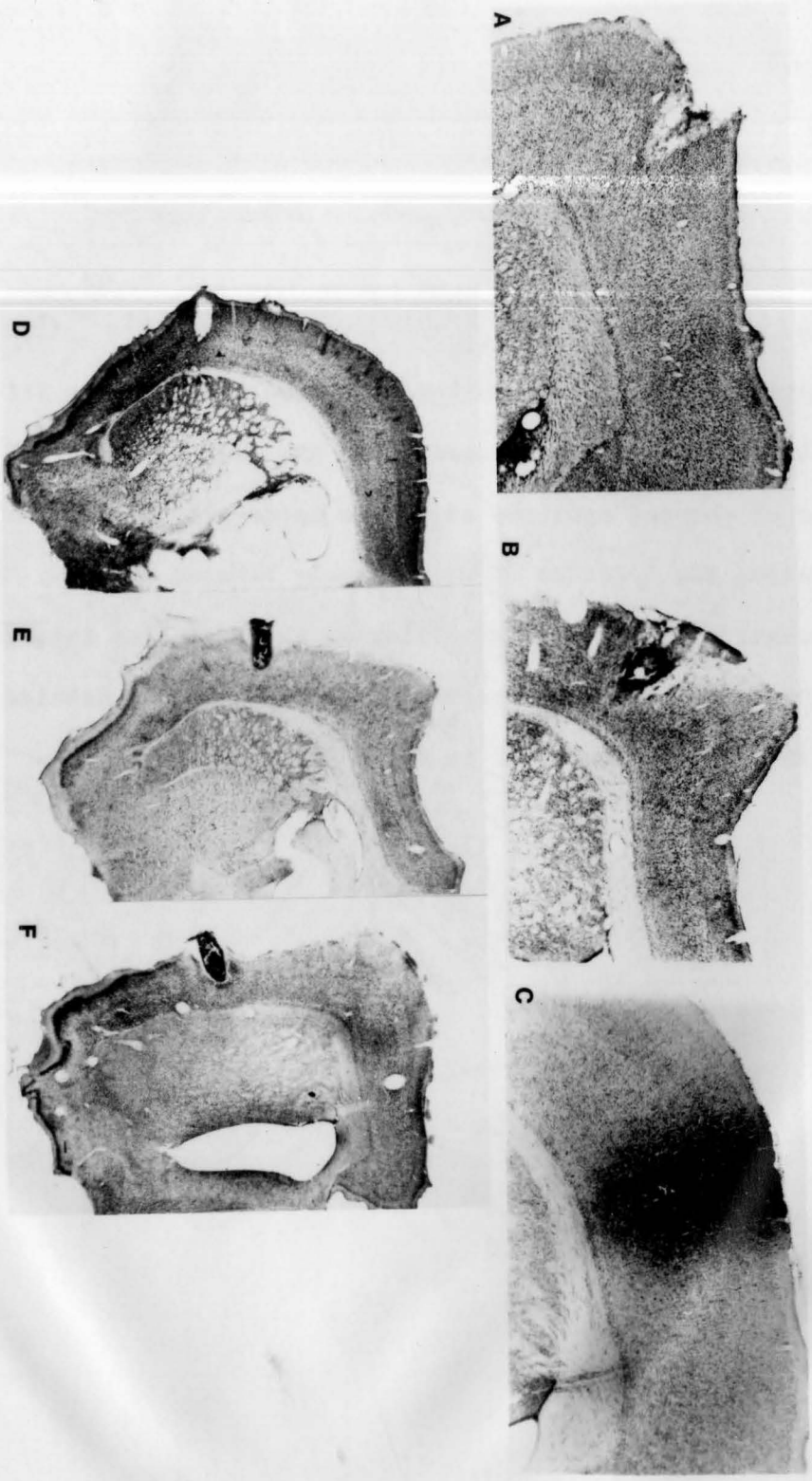

A
B
C
D
E
F

Figure 6

Results of a diamidino yellow injection in vibrissae SII and a fast blue injection in vibrissae SI in the same animal. A-H. Outline drawings of coronal sections at 300 um intervals through the thalamus illustrating the location of retrogradely labeled neurons. Each large dot represents a cell labeled following the fast blue injection in VSI injection. Each small dot represents a retrogradely labeled cell following an injection of DY in VSII.

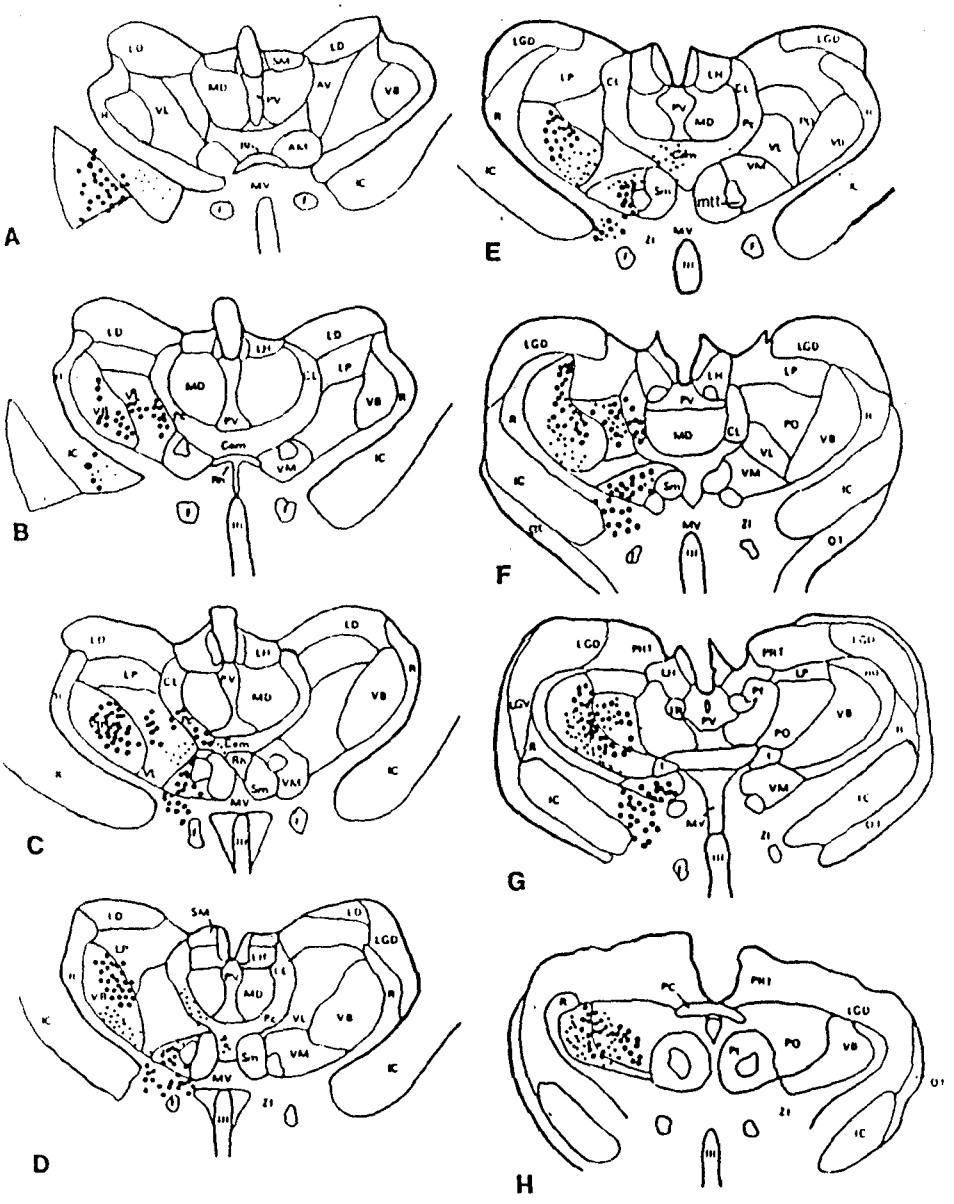

A
B
C
D
E
F
G
H

Figure 6 Continued

I. Stack drawing through the two injection sites showing the maximal spread of the injected material. Each section is section is separated by 200 um.

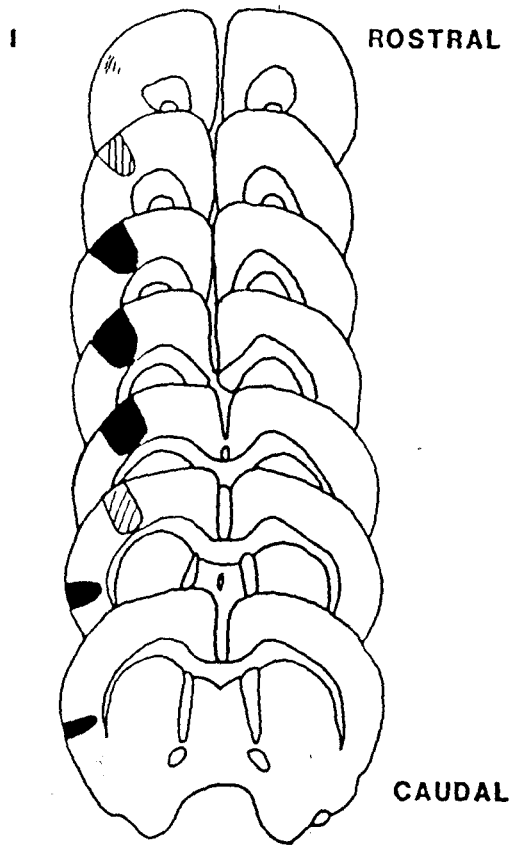

I
ROSTRAL
CAUDAL

Figure 7

Representative material of a DY injection in FLSII and rodamine conjugated microspheres in HLSII in the same animal. A-H. Outline drawings of coronal sections at 300 um intervals through the thalamus illustrating the location of retrogradely labeled cells. Each large dot represents a cell labeled following the HLSII injection and each small dot represents a cell labeled following the FLSII injection.

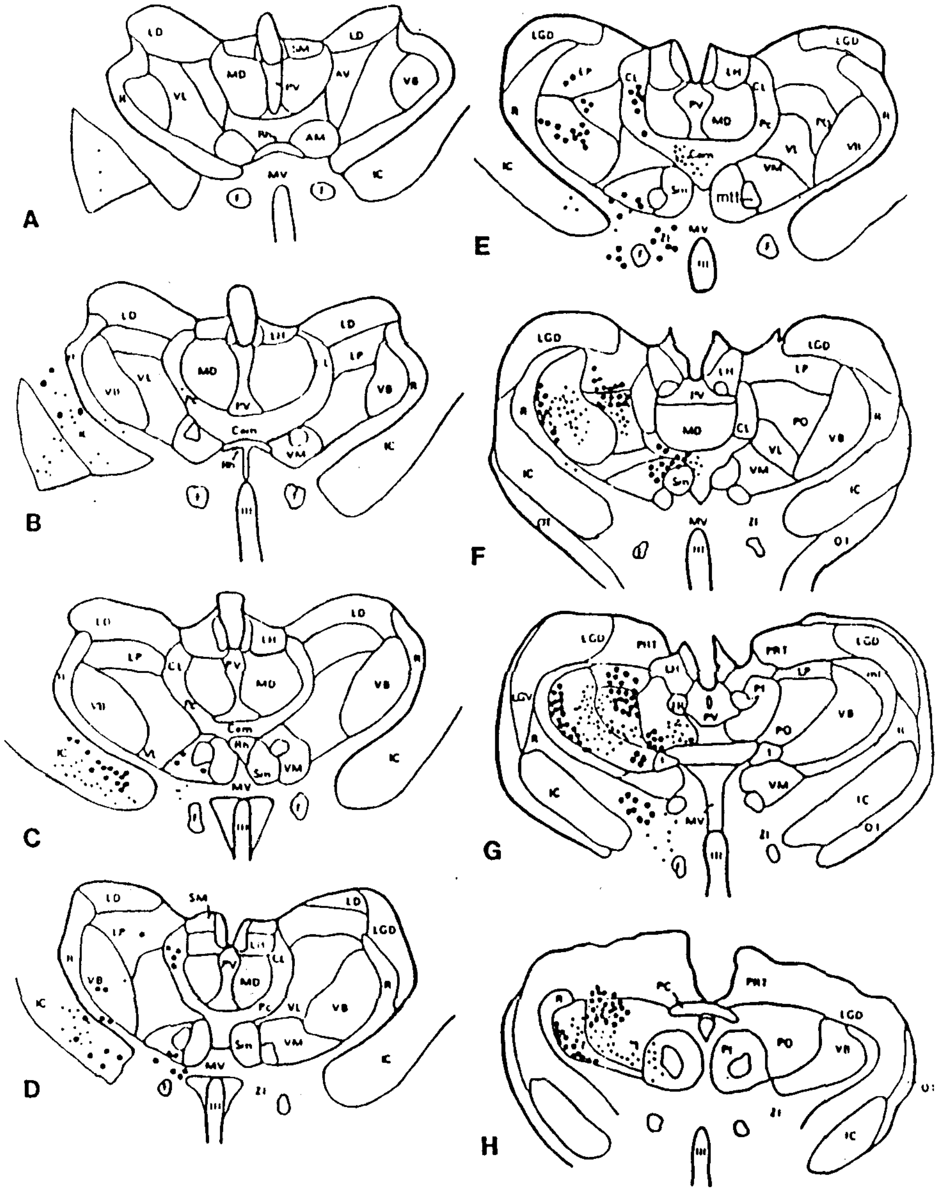

A
B
C
D
E
F
G
H

Figure 7 Continued

I. Stack drawing at 200 um intervals through the two injection sites showing the maximal spread of the injected material.

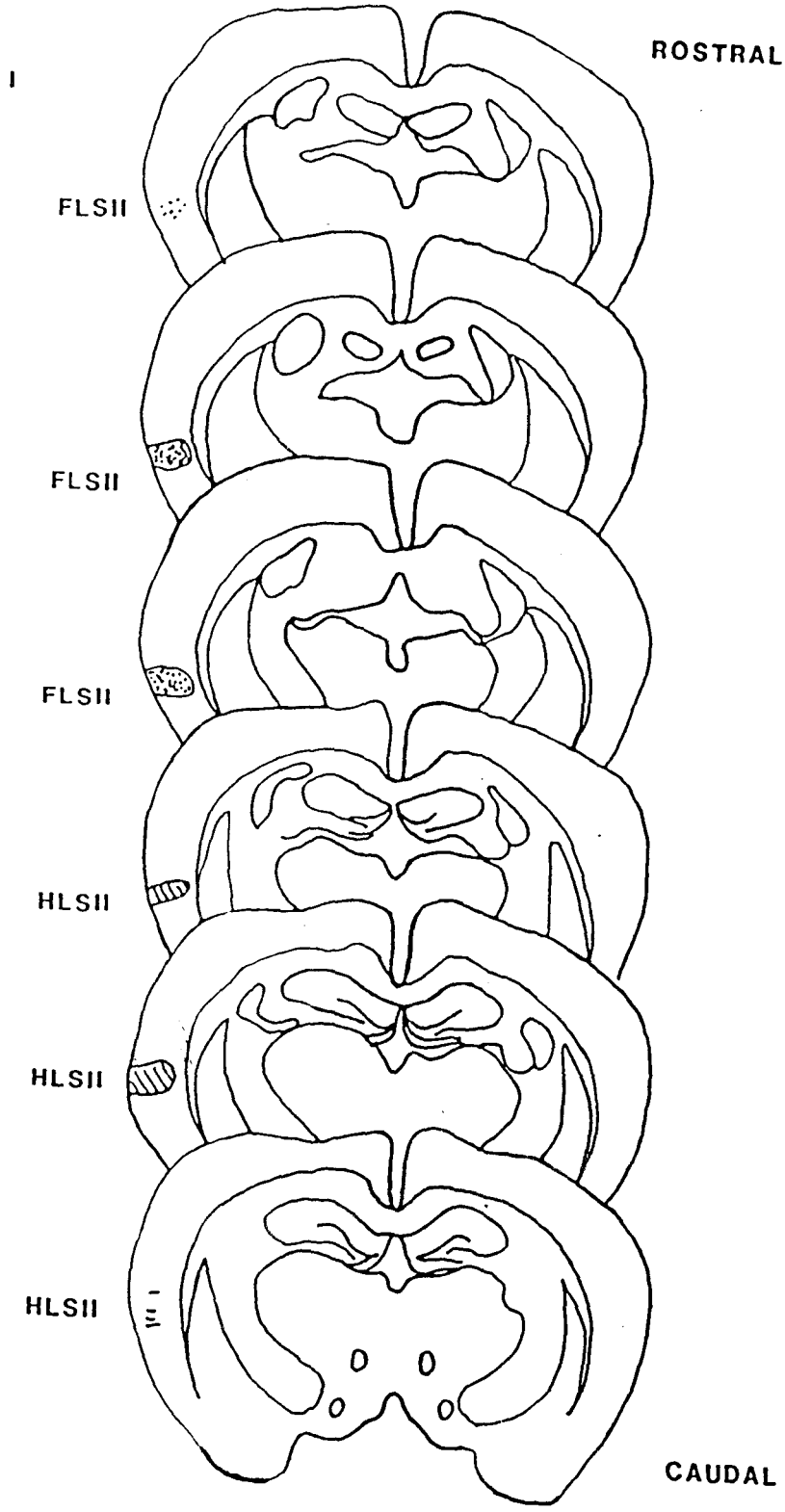
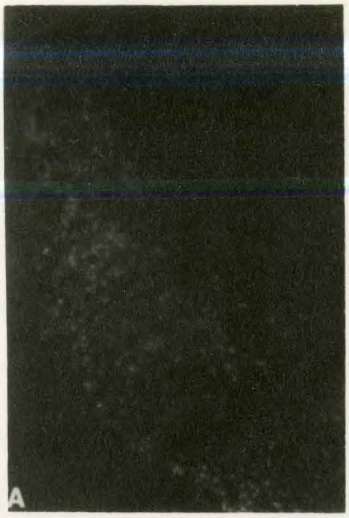
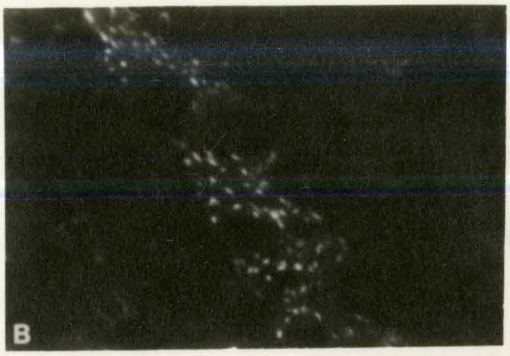
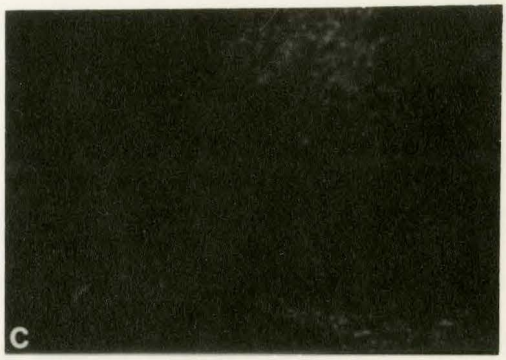

I
ROSTRAL
FLSII
FLSII
FLSII
HLSII
HLSII
HLSII
III
CAUDAL

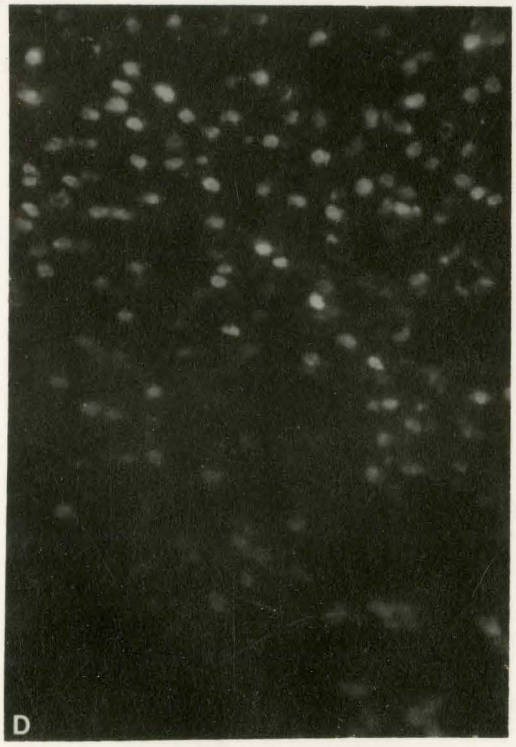
Figure 8

Photomicrographs of flourescent labeled cells. A. DY and FB labeled cells in VB following a DY injection in FLSII and FB in FLSI (6X) B. Higher magnification (15X) of the DY labeled cells in VB seen in A. C. Low power photomicrograph (6X) of labeled cells in VB, VM, and PO. D. Higher magnification (25X) of the labeled cells in PO. E. Labeled cells in PO (25X) showing DY cells (VSII) dorsal to FB cells (VSI).


A


B


C


D


E

Figure 9

Summary diagram showing the topographical organization of VB thalamic projections to primary somatosensory cortex (SI). Data from five comparaly placed DY injections in HLSI are indicated by differently hatched lines in the top row representing three levels through VB. The solid dark areas represent those areas within VB wich were commonly labeled following all five injections illustrated. Data from five comparably placed FB injection in FLSI are indicated by differently hatched lines in the second row of three levels of VB. Data from three injections in VSI are shown in the third of three levels through VB. The bottom row represents the topographical organization that was found in VB when comparisons of the data are made. The hatched lines indicate that the borders for the designated areas are only an approximation. These lines were drawn from data obtained from the comparably placed injections sites as well as from data collected from individual experiments. The topographical organization is somatotopic moving lateral to medial; hindlimb, forelimb, vibrissae.

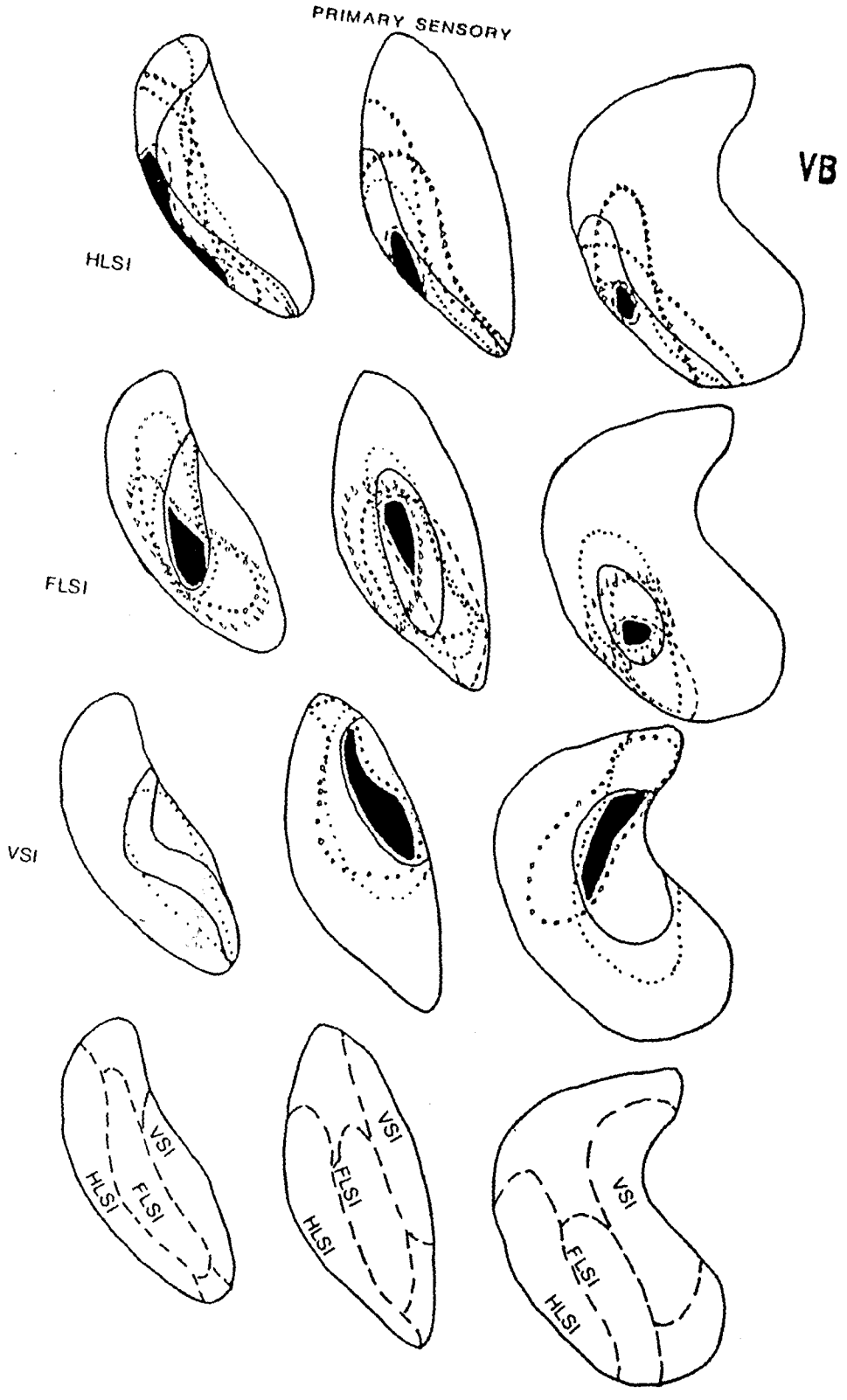

PRIMARY SENSORY
VB
HLSI
FLSI
VSI
VSI
FLSI
HLSI
VSI
FLSI
HLSI
VSI
FLSI
HLSI

Figure 10

Summary diagram showing the topographical organization of VB thalamic projections to secondary somatosensory cortex (SII). The same animals used for the analysis of SI were used for SII. See figure 9 legend for details.

SECONDARY SENSORY

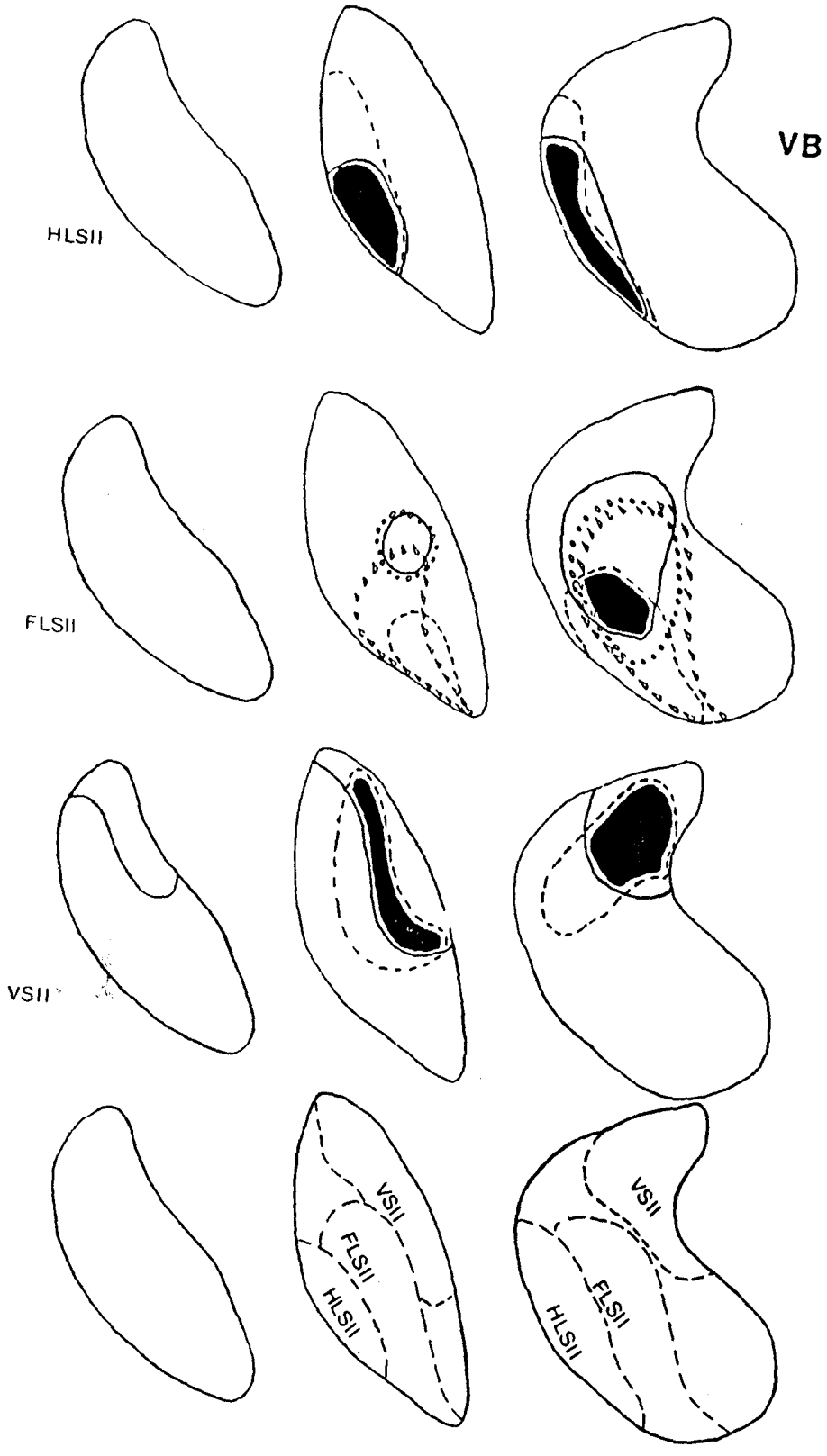


Figure 11

Summary diagram showing the topographical organization of PO thalamic projections to primary somatosensory cortex (SI). The same animals used for the analysis of VB were used for the analysis of PO. See figure 9 legend for details.

PRIMARY SENSORY

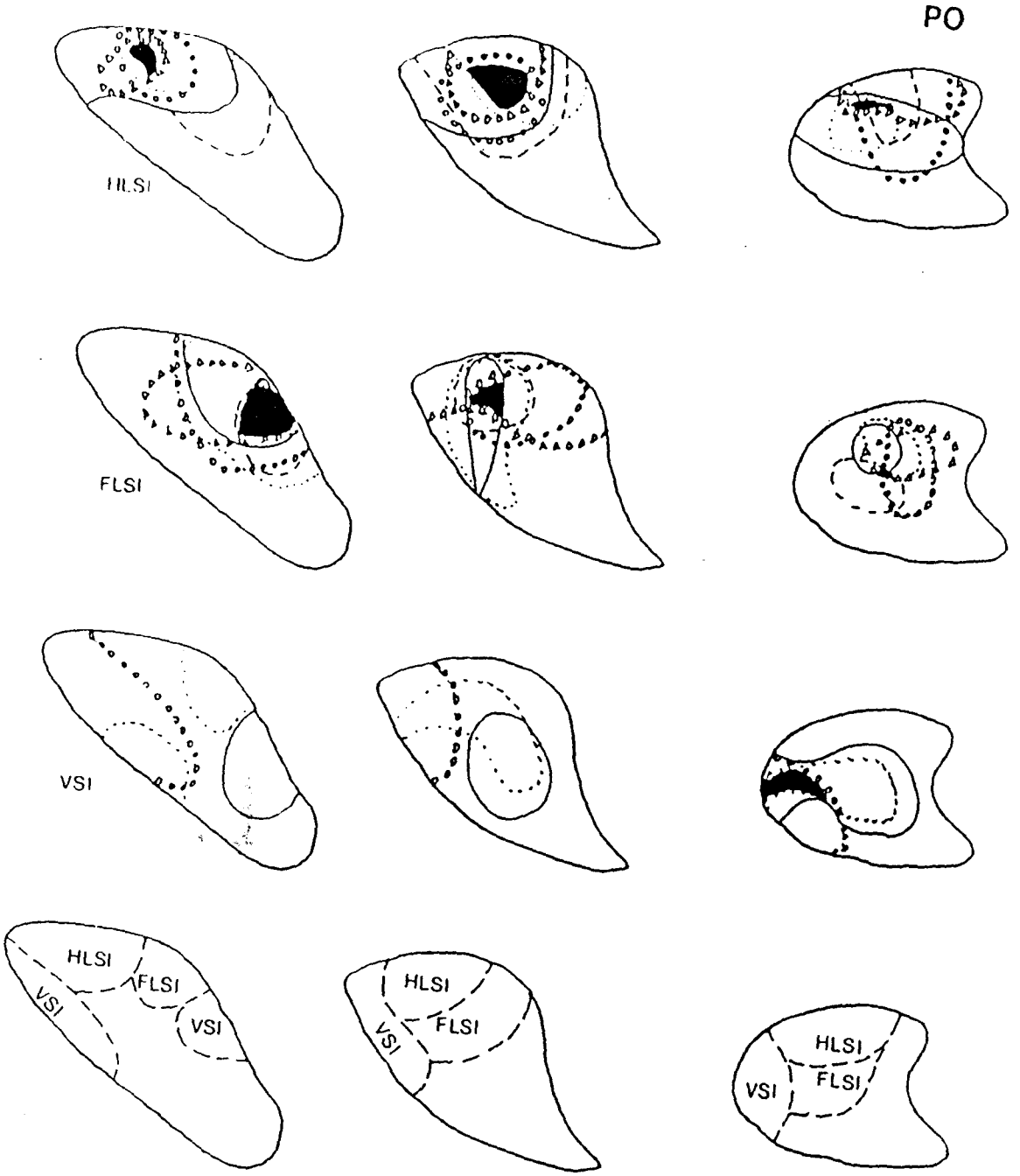
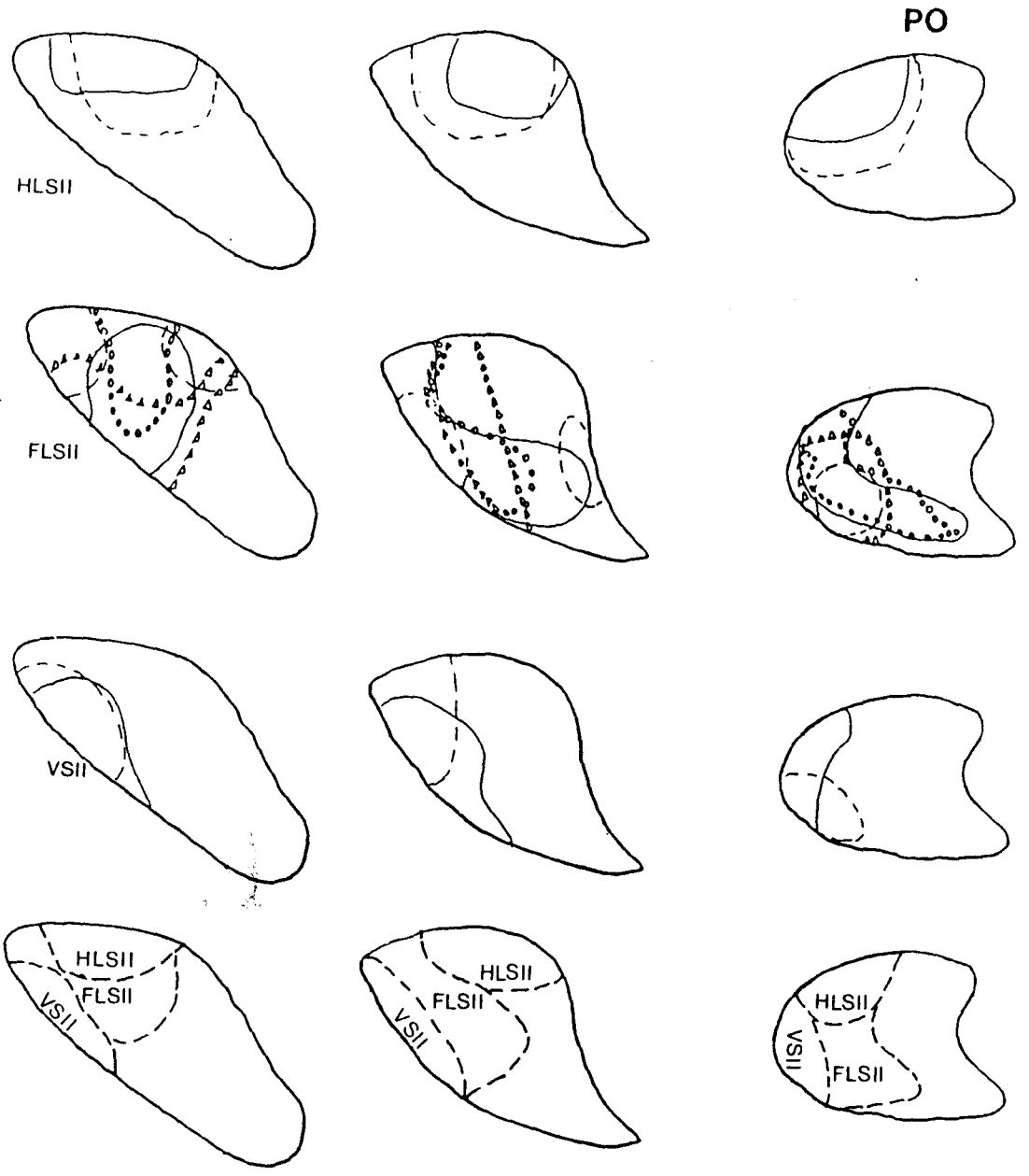


Figure 12

Summary diagram showing the topographical organization of PO thalamic projections to secondary somatosensory cortex (SII). See figure 9 legend for details.

SECONDARY SENSORY



CHAPTER III

EXPERIMENTS

E. THE TOPOGRAPHY OF DORSAL COLUMN NUCLEAR PROJECTIONS TO REGIONS OF THE THALAMIC POSTERIOR NUCLEUS THAT PROJECT TO SENSORIMOTOR CORTEX IN THE RAT

The existence of a short-latency (5-10 ms) peripheral sensory input to the motor cortex was first described by Malis et al. (1953) and has been confirmed by several other investigators (Evarts, 1973; Fetz and Baker, 1969; Rosen and Asanuma, 1972; Lemon and van der Burg, 1979; and others). The function of this afferent input to the precentral cortex is to relay proprioceptive information to that part of the cortex most directly connected with peripheral motor mechanisms (Malis et al., 1953). It has also been reported that cortical potentials recorded in the precentral gyrus evoked by stimulating peripheral nerves are not reduced in amplitude following total ablation of the postcentral gyrus (Malis et al., 1953). This study also found that complete cerebellectomy, including ablation of the deep cerebellar nuclei, did not reduce the cortical potentials evoked on the surface of the motor cortex by peripheral stimulation. This suggests that afferent information to motor cortex is not relayed via the cerebellum but must relay directly through the thalamus.

Poggio and Mountcastle (1963) suggested that those regions of the thalamus which project to sensory cortex in the primate, the caudal part of the ventroposterior lateral nucleus (VPLc), receive afferents from superficial structures such as skin. Those thalamic regions projecting to motor cortex, the oralis division of the same nucleus (VPLo), receive deep afferent inputs from deep structures such as muscles, joint receptors, capsules, and fascia. Anatomical studies have shown that VPLo projects to motor cortex and receives its

afferent input from the deep cerebellar nuclei (Kievet and Kuypers, 1977; Thach and Jones, 1979). The caudal portion of the ventrolateral nucleus (VLc) also receives cerebellar afferents and projects to motor cortex (Strick, 1976). VPLc receives its afferent input form the dorsal column nuclei (Boivie, 1978) and projects to sensory cortical areas (Areas 3,1,2). On the basis of these findings, it was thought that the thalamic relay to motor cortex might be VPLo yet it does not receive afferents from the dorsal column nuclei. Brinkman et al. (1978) transcected the the dorsal column medial lemniscal pathway and this abolished the short latency input to motor cortex. Therefore, the peripheral sensory input to motor cortex apparently travels through the medial lemniscus (Tracey et al., 1980) Tracey and his collaborators attempted to identify other potential relay nuclei that might provide insight into this problem. The work of Malis et al. (1953) tends to eliminate the deep cerebellar nuclei and the corticocortical connections from sensory to motor cortex. The lateral cervical nucleus (LCN), the external cuneate nucleus (ECN), and the vestibular nuclei were also considered until Tracey et al. (1980) determined that all three nuclei send terminations to VPLc and not VPLo. Therefore, the identification of the nucleus relaying this information remains unknown, largely because those areas of the thalamus which project to motor cortex do not appear to receive afferent terminations from any readily identifiable brainstem or spinal cord somatosensory relay nuclei.

Boivie (1978) studied the efferent projections of the DCN and reported that the DCN terminations in the suprageniculate and magnocellular medial geniculate in the macaque were homologous to DCN terminations in POm in the cat (Jones and Burton, 1976). In addition, Boivie (1978) described the spinothalamic tract to project specifically to the medial part of PO (POm) in the macaque. This information leads to the possibility that POm in the monkey may be the elusive thalamic nucleus that provides the relay for peripheral sensory input to motor cortex. This is supported by similar findings in the rat that PO receives terminations of the medial lemniscus (Lund and Webster, 1967a) which is known to carry the short latency input to the motor cortex (Brinkman, 1978).

Our earlier studies in the rat (previous chapters) have clearly demonstrated labeling of a PO region of the rat thalamus following physiologically identified motor cortex injections of WGA-HRP or fluorescent dyes. If this region is homologous to POm in the monkey and cat, then it is possible that PO in the rat could relay sensory input to motor cortex.

To determine whether PO in the rat relays medial lemniscal information to the motor cortex, rats were anesthetized and placed in a stereotaxic frame and the sensorimotor cortex exposed. The motor cortex was mapped using intracortical microstimulation and the sensory cortex was mapped using multiunit recording technique described in the previous papers. In three animals the hindlimb motor area was

injected with fast blue, the forelimb sensory cortex was injected with diamidino yellow and the nucleus gracilis was injected with 0.02 ul of 1% WGA-HRP. In three different animals, the forelimb motor area (caudal forelimb) was injected with fast blue, the forelimb sensory area with diamidino yellow, and the nucleus cuneatus was injected with 0.02 ul 1% WGA-HRP. Following survival times of 1-4 days, the animals were anesthetized with sodium pentobarbitol and perfused transcardially with 0.9% saline followed by a 4% buffered paraformaldehyde solution and then a 10% buffered sucrose solution. Brains were removed and frozen sections were cut 3 days later at 40 um intervals. Every other section was processed for HRP histochemistry according to the protocol of Mesulam (1978) and then examined under epifluorescence and polarized light for retrogradely labeled thalamic neurons and anterograde HRP terminal labeling.

Retrogradely labeled neurons within the posterior nucleus seen following sensorimotor hindlimb cortical injections closely overlapped with nucleus gracilis terminations as demonstrated with anterograde WGA-HRP labeling (Figure 1 left set A-D). Retrogradely labeled PO neurons seen following sensory forelimb cortical injections overlapped with the nucleus cuneatus terminations (Figure 1 right set A-D). Retrogradely labeled PO neurons seen following motor forelimb injections showed virtually no overlap with the terminations of the dorsal column nuclei.

This lack of overlap between DCN projections and PO neurons

projecting to motor cortex suggests PO does not relay sensory information to motor cortex in the rat. However, this conclusion is based on findings that the anterograde terminal labeling was approximately 200 um away from the retrogradely labeled PO neurons. If the dendritic fields of these PO neurons are less than 200 um then our conclusion, using purely anatomical criteria, is correct. If, on the other hand, golgi studies of this thalamic region reveal an extensive dendritic arborization, then PO may relay such information. Physiological studies correlated with anatomical studies are needed to answer this question definitively and will be the focus of future studies.

Figure 1

Experimental data from two separate animals. The data on the left represents that from an animal receiving an injection of 1% WGA-HRP in nucleus gracilis (NG), an injection of diamidino yellow in the forelimb sensory cortex, and an injection of fast blue in the hindlimb sensorimotor cortex. The squares on the left represent fast blue retrogradely labeled cells following the hindlimb injection, the circles represent diamidino yellow labeled cells following the forelimb sensory injection, and the small dots are anterograde terminals from the nucleus gracilis. The data on the right represents that from an animal receiving an injection of 1% WGA-HRP in the nucleus cuneatus (NC), an injection of diamidino yellow in the forelimb sensory cortex, and an injection of fast blue in the forelimb motor cortex. The triangles on the right represent retrogradely labeled fast blue neurons following the forelimb motor injection, the circles are forelimb sensory projecting thalamic neurons, and the small dots are anterograde terminal labeling. The dorsal view of the brain shown at the top left and right show the relative placement of each injection. Abbreviations are listed at the beginning of the dissertation.

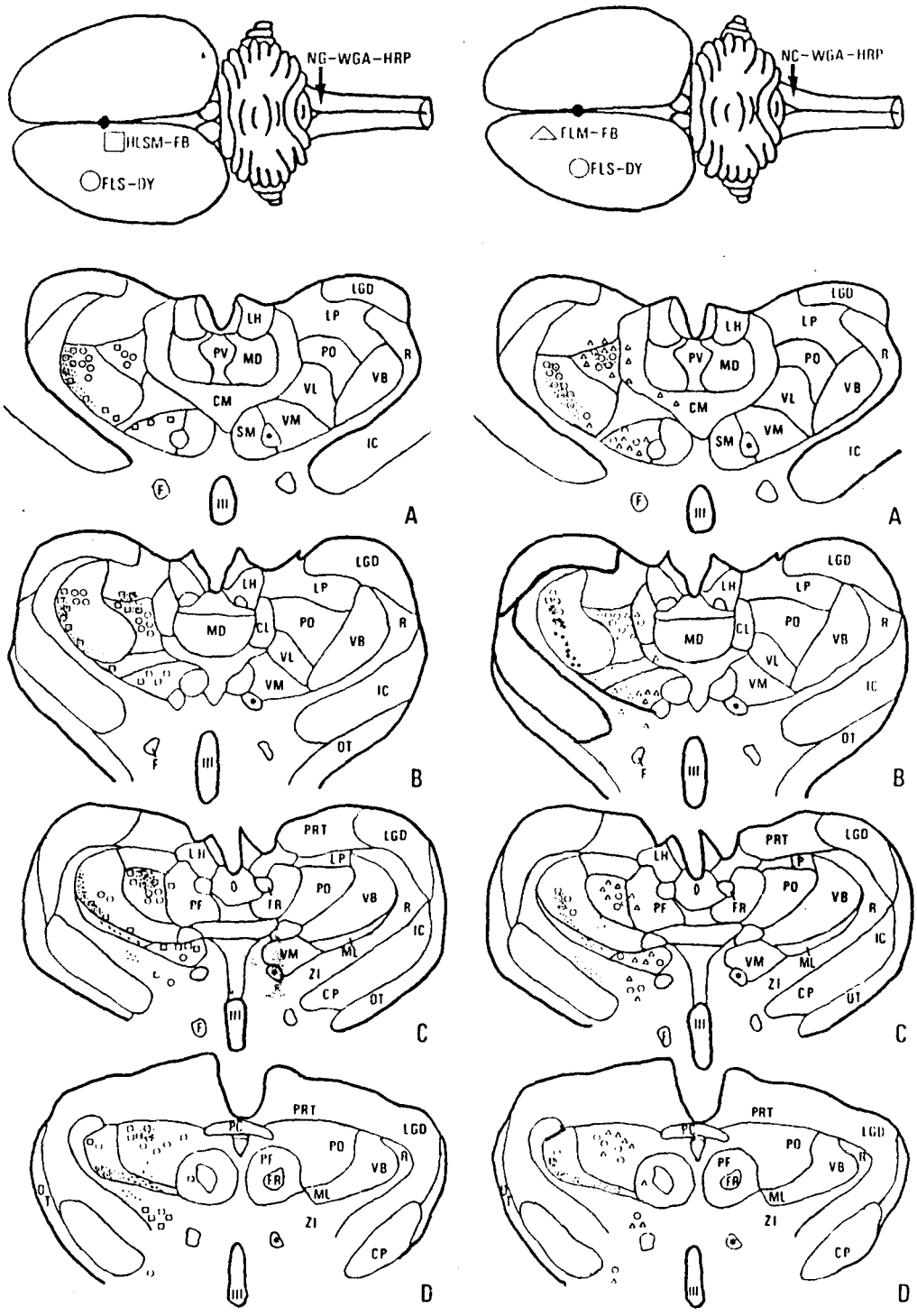
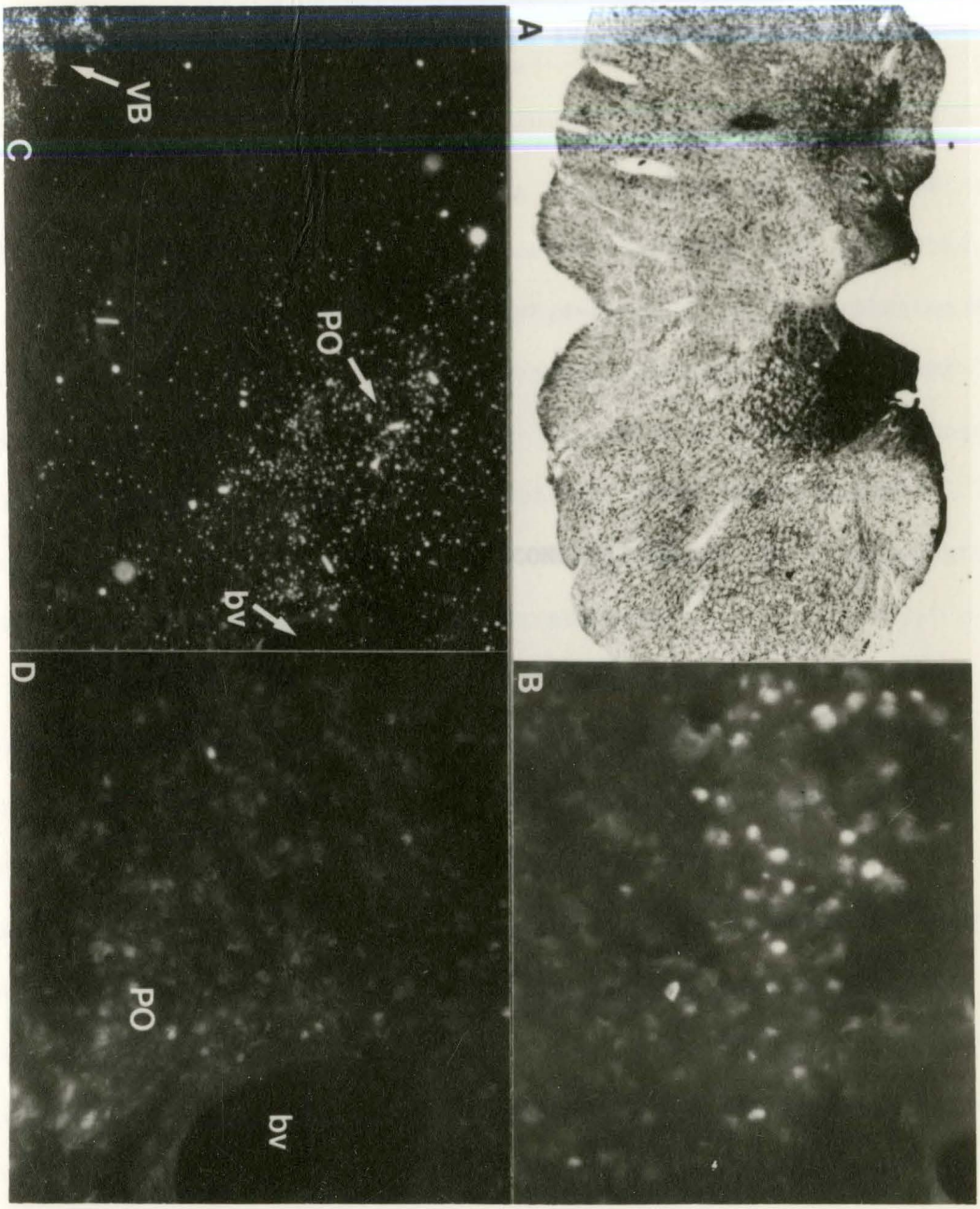

NG-WGA-HRP
HLSM-FB
FLS-DY
NC-WGA-HRP
FLM-FB
FLS-DY
LGD
LH
LP
PV
MD
PO
R
CM
VL
VB
SM
VM
IC
III
A
CL
OT
B
PRT
PF
FR
ZI
CP
C
D

Figure 2

Representative material of experimental data. A. Shows an injection site of 1% WGA-HRP in the dorsal column nuclei, centered in the nucleus cuneatus (1.25X). B. Retrogradely labeled neurons in the posterior nucleus (PO) following an injection of diamidino yellow in forelimb SI and fast blue in forelimb MI (15X). C. Anterograde HRP labeling in PO following a nucleus cuneatus injection (25X). D. Fast blue labeled neurons (bottom right) following a forelimb motor injection (10X). Note the lack of overlap as both pictures are of the same section (note blood vessel).


A
B
C
D
VB
PO
bv
PO
bv

CHAPTER IV

SUMMARY AND CONCLUSIONS

This anatomical study provides a cytoarchitectural description of the rodent thalamus with a particular focus on the afferent and efferent connections of the posterior nuclear complex. Afferents to PO from a variety of areas associated with several sensory modalities have been described and the topographical organization of PO as well as other thalamic neurons projecting to physiologically defined regions of motor and somatosensory cortex has also been described.

The first study described the cytoarchitectonic parcellation of the rat dorsal thalamus using the criteria of cell size, packing density, staining intensity, and the presence or absence of fibers. This study provided a solid basis for the description of our experimental results and provided a reference set of photomicrographs for comparing our results with other studies.

The second study addressed the afferent input to PO, a small cortical projecting nucleus that has been mainly associated with processing somatosensory and possibly nocioceptive information to the cortex (Casey, 1966; Poggio and Mountcastle, 1960; Curry, 1972). The data suggests that PO receives afferent information from seemingly every sensory system represented in the central nervous system. Auditory afferents arise from the magnocellular division of the medial geniculate complex, the inferior colliculus and the auditory cortex, while visual afferents arise from the superior colliculus and the primary visual cortex. Somatosensory afferents originate from the dorsal column nuclei, the trigeminal complex, especially the principal

sensory nucleus, and from the spinal cord as well as from the sensorimotor cortex, including primary sensory and secondary sensory cortical fields. Visceral afferents arise from the parabrachial nuclei and claustral cortex. Vestibular afferents from the superior and medial vestibular nuclei. In addition to sensory inputs, PO receives motor system afferents from the primary and supplementary motor areas as well as the zona incerta and the dentate and interpositus cerebellar nuclei. PO also receives a large number of afferents from the thalamic reticular nucleus and fewer afferents from the dorsal raphe and deep mesencephalic nucleus.

The apparent convergence of multisensory inputs to PO appears consistent with the traditional "non-specific" function associated with this nucleus (Herkenham, 1980) and from this concept one might infer a diffuse or non-specific distribution of thalamocortical projections from PO. However, the detailed analysis of the output of PO to various subdivisions of somatic sensory and motor cortex reveals that this output is indeed not diffuse or non-specific rather, relatively localized clusters of neurons within PO project to specific functional areas within the cortex. This finding of "specificity" within PO is further strengthened by the observation that dorsal column nuclear inputs to PO largely terminate within the clusters of PO neurons that in turn project to somatosensory cortex. Dorsal column afferents to PO avoid neurons that project to motor cortex. The study of other afferent patterns is necessary to determine if this

concept could be extended to determine whether all PO afferents show the same specificity in projection patterns. These data suggest that the organization of PO is much more specific and detailed than previously thought, with various subregions of PO relaying specific types of inputs to particular cortical areas. The specific organization for VB projecting neurons has previously been described (Welker, 1973; Saporta and Kruger, 1978; Feldman and Kruger, 1980) and has been verified in the present studies, and thus serves as an "internal control" for the description of the specific cortical projections of PO. This organization of VB and PO projecting neurons is summarized in Figure 1.

The relay of sensory pathways through PO represents a second or possibly alternate route for the flow of information to the coerbral cortex. The possible functions of this duplicated or redundant parallel pathway through PO is not understood. One hypothesis suggests that PO represents an archetypical thalamus from which modality specific nuclei differentiated (Erickson et al., 1964; Rockel et al., 1972). This is supported by several observations: 1) PO receives afferent inputs from a variety of structures associated with several sensory modalities (present study); 2) PO is among the first neurons to fully differentiate embryologically (Altman and Bayer, 1979); 3) PO is the only somatosensory nucleus found in reptiles and birds (called posterior medialis) (Northcutt, 1984); and 4) there appears to be little overlap in the convergence of the sensory input

within PO as found in the present study.

A second hypothesis suggests that PO relays a different modality of sensory information to the somatosensory cortex than does the ventrobasal nucleus (VB). This concept derives from studies on the somatosensory cortex in primates. Cortical area 2 receives deep receptor information relayed through the superior division of the ventroposterior lateral nucleus (VPS) and the anterior pulvinar nucleus (pa, Pons and Kaas, 1984; Friedman, in preparation). Area 3b, the homolog of rat SI, receives cutaneous input from the caudal division of VPL (VPLc, Thach and Jones, 1979; Nelson and Kaas, 1981). VB and PO project to SI in the rat (present study) and since the primate equivalent of VB (VPL) projects to 3b and what appears to be the primate equivalent of PO (VPS and pa) projects to area 2, it is tempting to speculate that VB in the rat is the relay nucleus for cutaneous information and PO is the deep receptor relay nucleus. Only physiological identification of deep receptor inputs to PO in the rat can verify this notion.

Figure 1

Summary diagram illustrating the hindlimb, forelimb and vibrissae topography through three levels of VB for SI and SII thalamocortical projections. The top row represents the pattern of thalamocortical projections to SI and the bottom row the pattern of projections to SII. The hatched lines indicate that the borders of each representation are an approximation and are not rigid.

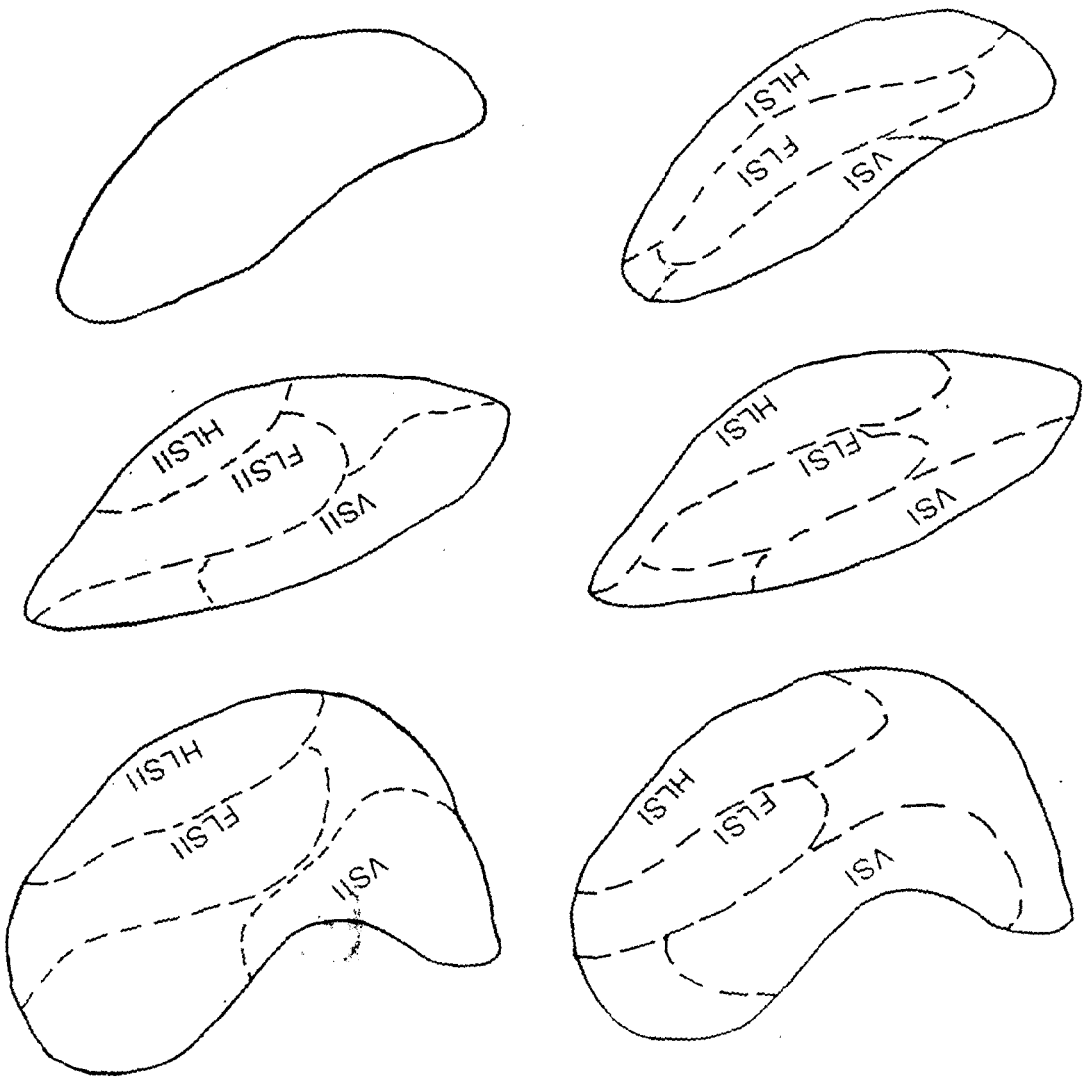
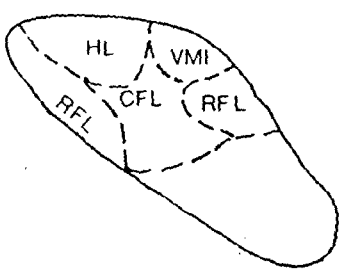

HLSI
FLSI
VSI
HLSII
FLSII
VSII
HLSI
FLSI
VSI
HLSII
FLSII
VSII
HLSI
FLSI
VSI

Figure 2

Summary diagram illustrating the hindlimb, forelimb, and vibrissae topography through three levels of PO for motor, SI and SII thalamocortical projections. The top row represents the pattern of thalamocortical projections to motor cortex. The middle row represents the pattern of projections to SII and the bottom row the pattern of projections to SI.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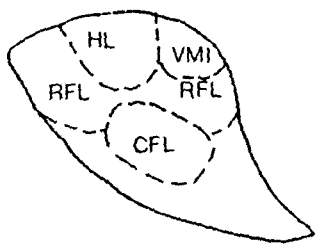
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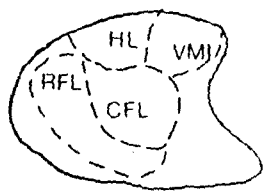
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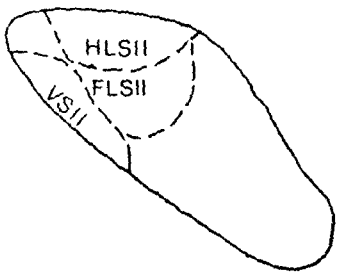
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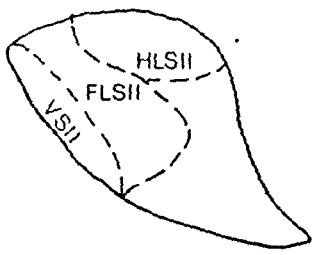
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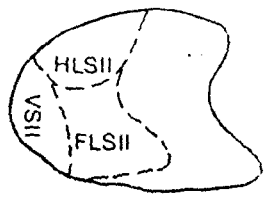
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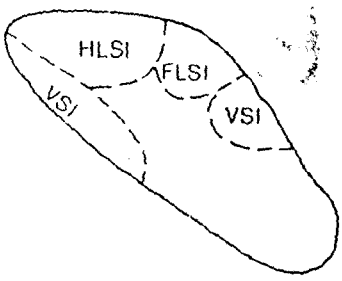
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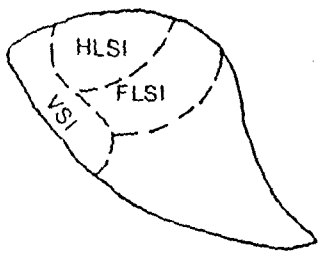
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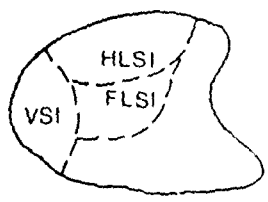
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CHAPTER V

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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 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.

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