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A Comparison of Thalamic Afferents to the Rostral and Caudal Forelimb Regions of Rat Motor Cortex: An Experimental Investigation Using Horseradish Peroxidase

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A COMPARISON OF THALAMIC AFFERENTS TO THE ROSTRAL AND CAUDAL FORELIMB REGIONS OF RAT MOTOR CORTEX

AN EXPERIMENTAL INVESTIGATION USING HORSERADISH PEROXIDASE

by

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VITA

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

In 1973 he graduated from Loyola Academy in Wilmette, Illinois. In September, 1973 he entered Lawrence University in Appleton, Wisconsin and during his tenure at Lawrence spent 10 months at the University of Wisconsin, Madison, in the Neurophysiology Department. In June, 1977 he received the degree of Bachelor of Arts with a major in biology, magna cum laude.

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ABBREVIATIONS

AD - anterior dorsal nucleus of the thalamus

AM - anterior medial nucleus of the thalamus

AV - anterior ventral nucleus of the thalamus

BSC - brachium of the superior colliculus

Cem - central medial nucleus of the thalamus

CL - central lateral nucleus of the thalamus

DMN - deep mesencephalic nucleus

F - fornix

FR - fasciculus retroflexus

GP - globus pallidus

HC - habenular commissure

IC - internal capsule

III - third ventricle

INC - interstitial nucleus of Cajal

IV - interventricular nucleus

LD - lateral dorsal nucleus of the thalamus

LGD _ dorsal nucleus of the lateral geniculate

LGV - ventral nucleus of the lateral geniculate

LH - lateral habenular nucleus

LP - lateral posterior nucleus of the thalamus

MD - mediodorsal nucleus

MDpl- paralamellar division of the mediodorsal nucleus

MGD - dorsal nucleus of the medial geniculate

MGM - magnocellular nucleus of the medial geniculate

MGP - principal nucleus of the medial geniculate

ml - medial lemniscus

MV - medioventral nucleus

NDk - nucleus of Darkschewitsch

NPC - nucleus of the posterior commissure

OT - optic tract

P - pineal gland

PAG - periaqueductal gray

Pc - paracentral nucleus of the thalamus

PC - posterior commissure

Pf - parafascicular nucleus of the thalamus

PO - posterior nucleus of the thalamus

PRT - pretectal region

PT - paratenial nucleus of the thalamus

PV - paraventricular nucleus

R - reticular nucleus of the thalamus

Rh - rhomboid nucleus

RN - red nucleus

SG - suprageniculate nucleus

Sm - submedial nucleus

SM - stria medullaris

SN - substantia nigra

sPf - subparafascicular nucleus

t - gustatory (taste) nucleus of the thalamus

VB - ventrobasal nucleus of the thalamus

VL - ventrolateral nucleus of the thalamus

VM - ventromedial nucleus of the thalamus

ZI - zona incerta

* - mammillothalamic tract

FIGURES

INTRODUCTION

Recent studies on the precise origin of the rat corticospinal tract have reported two regions on the dorsal convexity of the frontal cortex which project to the cervical enlargement (Hicks and D'Amato, 1977). Within the larger and more caudal of these regions (just anterior to bregma) electrical stimulation produces movement of the contralateral forelimb (Woolsey et al, 1952; Hall and Lindholm, 1974). However, stimulation in the smaller, more rostral of these regions near the frontal pole was previously reported to evoke only jaw, tongue, nose, or vibrissae movements (Hall and Lindholm, 1974). More recently, Neafsey and Sievert (1982) have used microstimulation techniques to remap this rostral region and from it they evoked contralateral forelimb movements, especially digit flexion and wrist extension. These authors have termed this region the rostral forelimb area (RFL) of the rat motor cortex to distinguish it from the previously described more caudal forelimb area (CFL). Thus, two morphologically separate yet functionally similar forelimb regions have been described in the rat motor cortex.

Previous neuroanatomical studies of afferents to the rodent frontal cortex (Jones and Leavitt, 1974; Krettek and Price, 1977; Donoghue et al, 1979) have described inputs from several thalamic

nuclei including the ventromedial nucleus (VM, Herkenham, 1979), the ventrolateral nucleus (VL, Jones and Leavitt, 1974), the mediodorsal nucleus (MD, Krettek and Price, 1977), and the intralaminar nuclei (Jones and Leavitt, 1974; Bentivoglio et al, 1981). However, none of these studies physiologically identified the cortical regions to which the thalamic afferents were traced. The present study used microstimulation to determine the location of the rostral and/or caudal forelimb regions of motor cortex and reinvestigated the distribution of projections from different thalamic nuclei to these two regions. The technique of retrograde transport of horseradish peroxidase (HRP, LaVail and LaVail, 1972) or wheat germ agglutinin conjugated with HRP (WGA-HRP, Gonatas et al, 1979) was employed.

MATERIALS AND METHODS

Eight Long Evans hooded rats weighing between 300-500 grams were anesthetized with Ketamine HCl (100 mg/kg, IP) and placed in a stereotaxic frame. The cisterna magna was opened to prevent cortical swelling and a small piece of bone (5mm X 2mm) was removed just rostral to bregma on one or both sides. The rostral and/or caudal forelimb regions were identified by locating the region where intracortical microstimulation (.25 msec. pulses, 100 uamps or less, 300 msec. train @ 350 Hz.) evoked digit or wrist movements rostral or caudal to an area where neck movements were elicted (Neafsey and Sievert, 1982). In this manner, a physiological map of the cortex of each animal studied was obtained before the HRP injections were made, providing data on the "functional" location of the HRP injection site. Once the site for the injection was determined, .01-.02 ul of either a 30% solution of HRP (Sigma VI) or a 5% solution of WGA-HRP (Polysciences and Sigma) in physiological saline was injected into a previously stimulated electrode track with a 1 ul Hamilton syringe fitted with a 50 um diameter pipette tip (typical diameter of injection site = 1.5 mm). The diameter of the rostral forelimb area determined by microstimulation is approximately 1.5 to 2.0 mm (Neafsey and Sievert, 1982). In some animals one injection was made into

either the RFL or CFL area of each hemisphere after it was found that unilateral injections resulted in only ipsilateral labeling. After survival periods of 1 to 2 1/2 days the animals were reanesthetized, transcardially perfused with 1.25% glutaraldehyde and 1% paraformaldehyde and the brains removed and stored overnight in 10% buffered sucrose at 4 degrees C. The next day 50 um frozen sections from the frontal pole to the mesencephalon were cut on a freezing microtome and the tissue processed for HRP neurohistochemistry according to the TMB procedure of Mesulam (1978). After mounting, one series of alternate sections was counterstained with Pyronin Y, a red Nissl stain. Retrogradely labeled neurons were identified under both light and dark field on histological sections utilizing a Leitz photomicroscope fitted with a camera lucida drawing tube. The parcellation of the rat thalamic nuclei was accomplished with the series of Nissl counterstained sections using a projecting microscope.

RESULTS

Normal Rat Thalamus

Before describing the location of retrogradely labeled neurons within the rat thalamus following cortical injections of HRP, the normal cytoarchitecture of the rat thalamus will be briefly described using a rostral to caudal series of photomicrographs (400 um intervals) of celloidin embedded, 33 um coronal sections, stained with cresyl violet (fig. 1A-L). Although several recent studies (Faull and Carmen, 1968 and 1978; Jones and Leavitt, 1974) have described the cytoarchitecture of different regions of the normal rat thalamus, no comprehensive description of thalamic cytoarchitecture using Nissl-stained photomicrographs is available. The early description of the rat thalamus by Gurdjian (1927), although useful and detailed, is illustrated only with line drawings. The purpose in presenting this relatively brief description of thalamic anatomy is to make clear the criteria for delineating the various nuclei and to allow comparison of this work with that of others who may employ different terminology. The terminology is based principally on that used by Faull and Carmen (1978) and Jones and Leavitt (1974) in their studies of the rat thalamus.

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 ventro-medial portion of the dorsal thalamus and rostrally is positioned ventral and lateral to the mammillothalamic tract (MTT; designated by * in fig. 1D-G) as the MTT enters the anteromedial nucleus (AM, fig. 1A-B). At successively more caudal levels, VM remains in about the same position while the MTT moves more ventrally relative to VM (fig. 1D-F). Most caudally the MTT lies within VM (fig.1G, 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. 1D) and more caudally by the central medial nucleus (Cem, fig.1F). At still further caudal levels, VM merges with the prerubral nucleus of Field H of Forel which is located just lateral to the fasciculus retroflexus (fig. 1H, Herkenham, 1979). A constant landmark medial to both VM and MTT is the submedial nucleus (Sm, Jones and Leavitt, 1974). Sm is a nearly spherical nucleus which surrounds the MTT medially and is characterized by a paucity of both cells and fibers. Herkenham (1979) termed this region 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. 1B) and thus forms the most rostral component of the ventral nuclear complex (Faull and Carmen, 1978). More caudally, VL extends dorsally over VB forming a lateral wing (fig. 1C) while at still further caudal levels VL extends over VM forming a medial wing (fig. 1D-E). Caudally, VL is gradually replaced by the posterior nuclear complex (PO, fig 1F).

The ventrobasal complex (VB) is composed of large and medium-sized deeply stained neurons with an abundance of fibers terminating throughout this nucleus. The cells in the dorsomedial aspect of VB appear to be more densely packed than the cells ventrolaterally. VB is relatively large, rounded and lies lateral to VM and medial to the reticular nucleus (R, fig. 1E). At midthalamic levels, VB is bordered dorsomedially by VL, PO, and LP (fig. 1E-F). The medial lemniscus (ml) which terminates within VB can be observed in figure 1G-J. The most medial extension of the ventrobasal complex, presumably the gustatory nucleus (Herkenham, 1979), is observed between VM and the parafascicular nucleus (Pf) at caudal thalalmic levels (t, fig. 1G).

Intralaminar Nuclei-These nuclei are located between the ventral nuclear complex and the mediodorsal nucleus (MD), and include the central medial (Cem), central lateral (CL), paracentral (Pc), and the parafasicular (Pf) nuclei. Both Cem and Pc nuclei contain large,

densely packed cells. The cells in Cem, which are slightly smaller than those in Pc, tend to form a compact rhomboid shape located on the midline between the paracentral nuclei (fig. 1D). The somewhat elongated cells in Pc appear oriented transversely in the direction of the fibers of the internal medullary lamina (fig. 1E, Jones and Leavitt, 1974). The central lateral nucleus (CL) contains neurons, similar in size to Pc but which tend to be more rounded and less tightly packed. Medially, the junction between CL and the paralamellar mediodorsal nucleus (MDpl) is not very distinct (fig. 1D-E), in contrast to the very distinct lateral border of CL adjacent to the smaller celled lateral nuclear complex (LP, LD, fig. 1C-E). 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 is found at caudal thalamic levels, appearing to surround the fasciculus retroflexus (FR, fig. 1F-G). 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 (fig. 1G-H).

Midline Nuclei-The midline nuclei include the medioventral nucleus (MV, also termed the reuniens nucleus, Herkenham, 1978), the paratenial (PT), rhomboid (Rh), paraventricular (PV), and submedial (Sm) nuclei (Jones and Leavitt, 1974). The central medial nucleus

(Cem) is also usually considered a midline nucleus but in the present account was described with the intralaminar nuclei. The medioventral (reuniens) 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 (fig. 1B-F). 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 (FR, fig. 1F). The small, oval-shaped paratenial nucleus (PT) is located at extreme rostral thalamic levels (fig. 1A) 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 rhomboid nucleus (Rh) is positioned between the Cem dorsally and the MV ventrally at rostral thalamic levels (fig. 1C). Rh contains medium-sized densely stained neurons which are very tightly packed. At its rostral extent, Rh is more T-shaped (fig. 1C) but assumes its rhomboid shape at lower levels (fig. 1D). The paraventricular nucleus (PV) is situated subjacent to the third ventricle and extends from rostral thalamic levels all the way to the posterior commissure (PC, fig. 1A-F). This moderately packed nucleus contains many large and medium-sized densely stained neurons. The submedial nucleus (Sm) nucleus 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. 1A). It is bordered medially by SM and the PT nucleus and laterally by AV. The larger AV nucleus demonstrates a similar band-like arrangement of loosely packed medium-sized cells (fig. 1A). AV is bounded medially by AD, laterally by the reticular nucleus (R) and merges with AM ventromedially. AM shows the least densely packed neurons of the anterior nuclear group having fewer, lightly stained cells and is further characterized by the MTT fibers which are entering it ventrolaterally (fig. 1A).

Lateral Nuclear Complex-The lateral nuclear complex includes the lateral dorsal (LD) and the lateral posterior (LP) nuclei, both made up of small cells moderately stained and moderately packed. The LD nucleus is apparent at rostral thalamic levels just dorsal to the rostral cap of VL (fig. 1B) and extends caudally in its dorsolateral thalamic position to the level of the fasciculus retroflexus (fig. 1F-G) where it is replaced by the dorsal nucleus of the lateral geniculate body (LGD). The lateral posterior nucleus (LP) is found just medial to the ventrobasal complex, lateral to the central lateral nucleus (CL), and dorsal to the posterior nuclear complex (PO, fig.

1D-E). It merges with the pretectum dorsomedially and with PO ventrally at the level of FR (fig. 1F-G), so that only PO remains medial to the ventrobasal complex in the caudal thalamus (fig. 1H-I).

Posterior Nuclear Complex-This complex (PO) contains many fibers and is less densely populated with neurons than the more medially located intralaminar nuclei (fig. 1E). The small-celled PO has a distinct lateral border with the more deeply stained VB and is bounded ventrally by the remnants of VL (fig. 1E-F). 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 (fig. 1H-I). The rostral portion of PO has also been termed the dorsomedial division of the ventral nuclear group (Gurdjian, 1927; Konig and Klippel, 1963).

Mediodorsal Nucleus-The mediodorsal nucleus (MD) is an oval-shaped nucleus positioned in the dorsal aspect of the thalamus lateral to the midline paraventricular nucleus. MD is characterized by an abundance of fibers, especially laterally, and contains many small, lightly stained and loosely packed neurons. It appears at rostral thalamic levels (fig. 1B-C) and extends throughout the thalamus becoming wider more caudally (fig. 1D-E). The mediodorsal nucleus 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; Krettek and Price, 1977), but in Nissl preparations only a paralamellar portion (MDpl) in the lateral aspect of MD is easily differentiated within MD.

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. This reticular nucleus also extends ventrally to form the ventralmost border of the thalamus where it blends into the zona incerta (ZI) at midthalamic levels (fig. 1F). The reticular nucleus separates the internal capsule (IC) from the diencephalon for the most part 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 (fig. 1H-I). These nuclei include the nucleus of Darkschewitsch (NDk) which contains large deeply stained neurons located within the ventrolateral PAG and the more laterally positioned interstitial nucleus of Cajal (INC) which also contains large densely stained neurons which are fewer in number and are not found within the PAG proper. The fasciculus retroflexus (FR) can be seen coursing ventrally (fig. 1G-I). Also note that the posterior nuclear complex (PO) is the caudalmost structure of the dorsal

thalamus where it merges with the deep mesencephalic nucleus (DMN, fig. 1I). Dorsal to PO note the location of the nucleus of the posterior commissure (NPC) and the pretectal area (PRT, fig. 1I). 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. 1I).

Figure 1A-F

Photomicrographs (6X) through the rostral two-thirds of the thalamus of the normal rat brain, stained for cells with cresyl violet. Each section is 33 um in thickness and each section represented is 400 um further caudal than the previous section. Each section is oriented with dorsal at the top and lateral to the right.

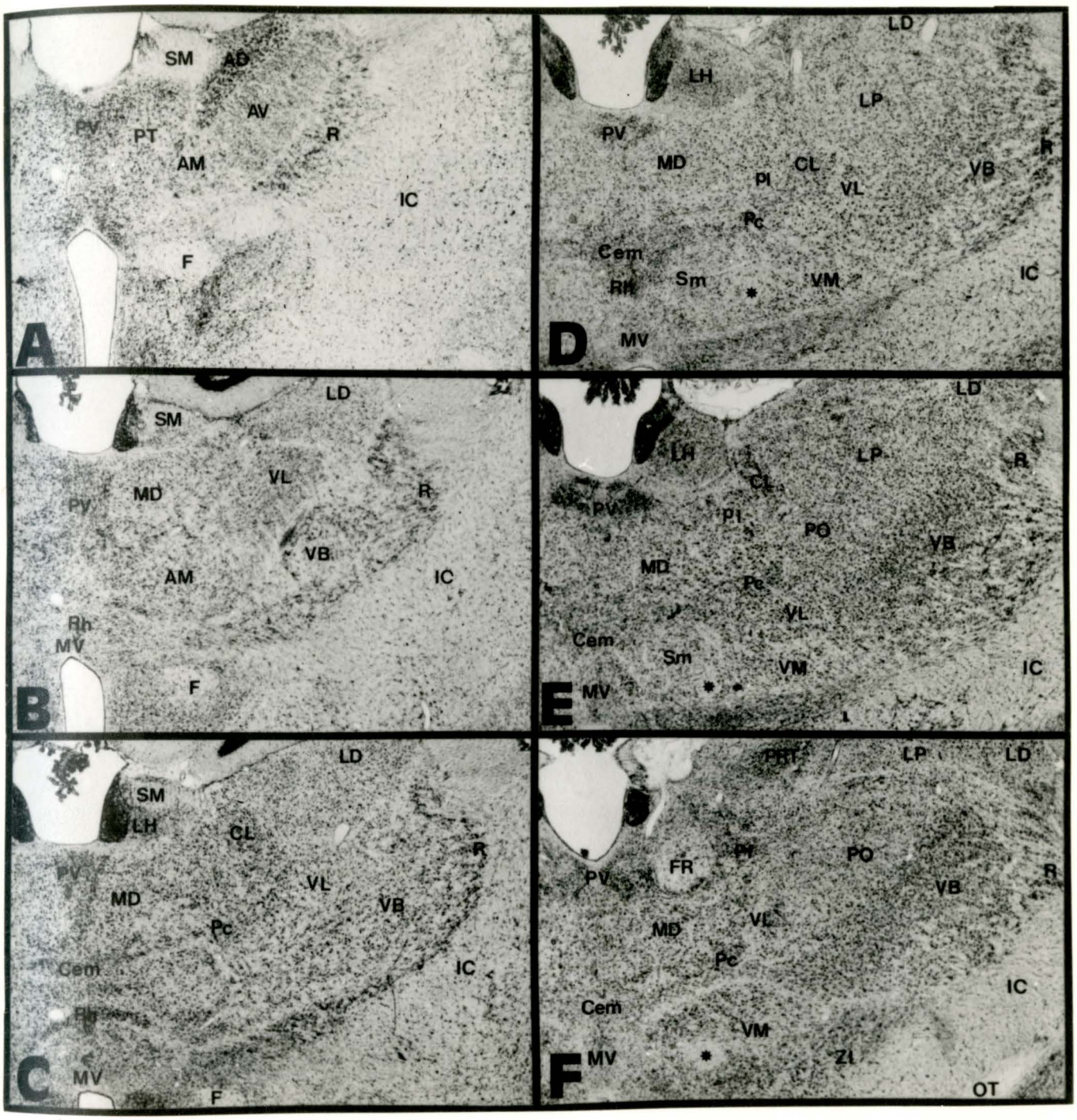
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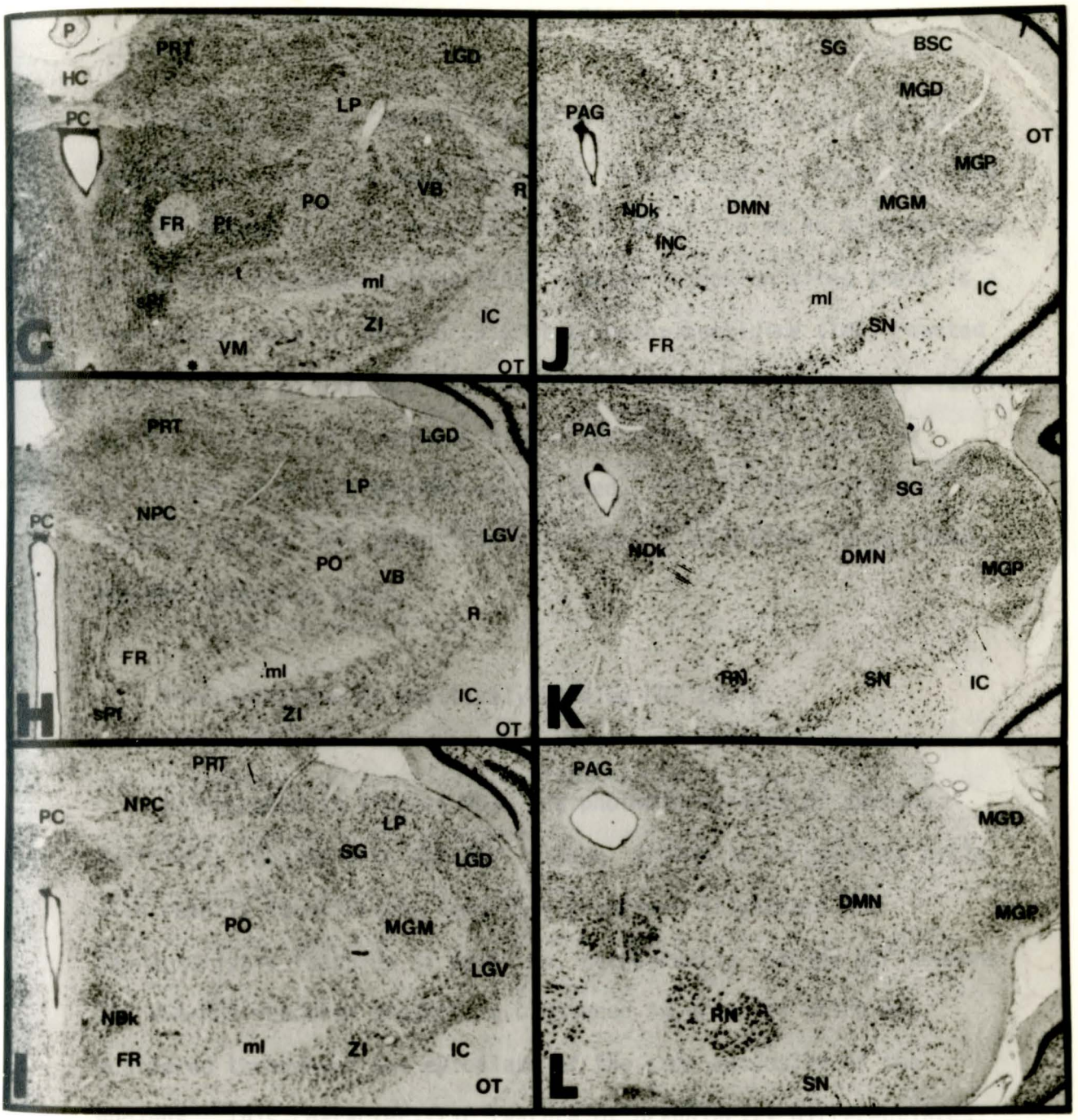
Figure 1G-L

Photomicrographs (6X) through the caudal one-third of the thalamus and the rostral one-half of the midbrain of the normal rat brain, stained for cells with cresyl violet. Each section is 33 um in thickness and each section represented is 400 um further caudal than the previous section. Each section is oriented with dorsal at the top and lateral to the right.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Identification of the Injection Site

A representative WGA-HRP injection site located in the rostral forelimb region of rat motor cortex is illustrated in figure 2B. The spatial extent of this injection site is about 1.5 mm in diameter and unlike 3,3'-diaminobenzidine (DAB) reacted injection sites there is a homogeneous zone of the 3,3',5,5'-tetramethylbenzidine (TMB) reacted material with no distinct central focus. Figure 2A shows the relative position of the two locations within rat motor cortex where the injections of HRP or WGA-HRP were placed. For the same volume of tracer (.02 ul), HRP injections sites were 2 to 3 times larger than WGA-HRP injection sites.

Identification of Positively Labeled HRP Cells

Illustrated in figures 4B-C are photomicrographs of neurons positively labeled with WGA-HRP. These medium to large-sized cells with readily identifiable dendritic processes illustrate the characteristic features of positive, retrogradely labeled HRP neurons. The WGA-HRP reaction product is clustered in granules which are scattered throughout the cytoplasm of the soma exclusive of the nucleus. This characteristic feature of granularity differentiates

positively labeled neurons from pericytes associated with blood vessels which contain endogenous peroxidase. This granularity also is easily differentiated from artifactual material which with the TMB procedure is always crystalline in structure. Also, retrogradely labeled neurons with positive HRP granules are different from anterograde labeling where no distinct reaction product is seen within neuronal cell bodies, but rather provides a background for the retrogradely labeled neurons in the area. The positively labeled WGA-HRP neurons were the result of reaction with TMB which produces a blue reaction product. The reaction product remained visible on Pyronin Y (red staining) counterstained sections.

Experimental Injections

Seven injections (6 WGA-HRP, 1 HRP) were made in the RFL area in one hemisphere. In three of these the injection site was considered too large (the RFL area is relatively small, 1.0 to 1.5 mm in diameter) and, although the locations of labeled cells were plotted in each of these experiments, the present report is based only on the results from the four small injections in the RFL area. Of the three injections (WGA-HRP) made in the CFL region two were small, while one was large (3.0 to 4.0 mm in diameter). Data from this large injection site is described separately. In each of these acceptable injections

(4 RFL, 3 CFL), the location of every labeled neuron on every section was plotted on outline drawings of the thalamus where the thalamic nuclear boundaries had been delineated from the Nissl stained sections (cells labeled on unstained sections were plotted on the outline drawing of the adjacent counterstained section). Labeling in all cases following unilateral injections of HRP or WGA-HRP was strictly ipsilateral. In one animal, therefore, the rostral forelimb area was injected on the left and the caudal forelimb area on the right. The results from this animal will be described in detail since it well illustrates the results obtained in all the other experiments and facilitates comparison of thalamocortical projections to each area (fig. 3A-F).

Rostral Forelimb Injection

A small injection of HRP or WGA-HRP into the physiologically defined rostral forelimb area of motor cortex positively labeled cells in a number of ipsilateral thalamic nuclei, including VL, VM, CL, Pc, Cem, MDpl, Pf, LP, and PO as well as cells in the ZI, MV, GP, and IC. These neurons are plotted on the left side of the cross-sectional drawings in Figure 3. At rostral thalamic levels (fig. 3A-B) labeling was most numerous in the ventrolateral nucleus (VL) where positively labeled neurons were found dispersed throughout the extent of this

nucleus. In addition, a cluster of labeled cells were also found within the central medial nucleus (Cem) of the intralaminar group as well as a few labeled cells dispersed within the lateral aspect of the paracentral nucleus (Pc).

At midthalamic levels (fig. 3C) VL remains heavily populated with positively labeled HRP cells. In addition, some labeled cells appear to spill over into the lateral posterior nucleus (LP) dorsally and VM ventrally. As VL is replaced caudally by PO (fig. 3D), labeled cells in VL appear to diminish in number and more and more cells become labeled within the central lateral (CL) and paracentral (Pc) nuclei and the dorsal aspect of VM. The overall pattern of labeling (seen best in figure 3D) resembles a double wing. The dorsal wing extends from the medial one-half of PO to the central lateral (CL) nucleus. The ventral wing begins in the dorsolateral aspect of VM where VM borders the ventrobasal complex (VB) and extends dorsomedially through the ventral aspect of VL to the intralaminar nuclei where the two wings are joined. It should also be noted that a few small clusters of cells are also seen within central PO at the PO/LP border as well as in the zona incerta (ZI).

At caudal thalamic levels (fig. 3E-F) the double wing pattern is still apparent; however, the union of the dorsal and ventral wings within the intralaminar nuclei is no longer present. The dorsal wing now extends from the medial border of VB across the dorsal aspect of

PO and into CL as well as into the paralemellar mediodorsal nucleus (MDpl, fig. 3E). Two large clusters of positively labeled cells are seen in this dorsal wing (fig. 3E). One large cluster lies within the dorsomedialmost part of PO while the other large cluster occupies the entire medial to lateral extent of central CL. A few scattered positively labeled cells are also seen within Pc and dorsomedial VL. The ventral wing at this level extends across VM and along the border of the submedial nucleus (Sm) into Cem all the way to the midline. In this ventral wing, the cells in VM are arranged in a band across this nucleus with all cells lying dorsal to the mammillothalamic tract (MTT). The cluster of labeled cells within Cem is very prominent and the cells are very densely packed with the WGA-HRP grains (fig. 3E). Again, labeled cells are still dispersed within the zona incerta (ZI).

At the level of the fasciculus retroflexus (FR, fig. 3F) the dorsal wing extends across the dorsal aspect of PO from the LP border into the central region of Pf. Two distinct clusters of labeled cells are seen in PO while a single cluster of cells is seen in Pf. The ventral wing at this caudal level is confined almost exclusively to the VM nucleus although a few cells are seen near the midline in the medioventral nucleus. Cells are still present within the ZI and in the lateral hypothalamus between the MTT and the fornix.

Further caudally in the pretectal region of the rostral midbrain (fig. 3F) the double wing pattern breaks up as the dorsal wing

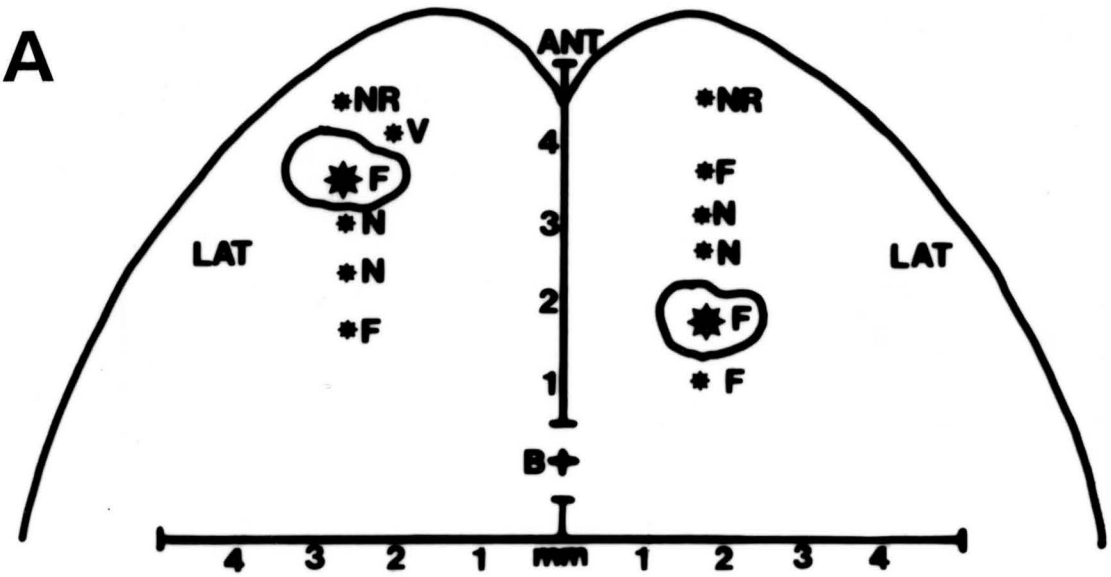
dwindles to a sparse band of cells extending from lateral PO to the fasciculus retroflexus (FR) within Pf. Labeled cells in the ZI are fewer than before and in addition a few labeled cells are seen dorsal and medial to the FR in the nucleus of Darkschewitsch (NDk).

Figure 2

A. Dorsal view of the rat frontal cortex showing the position of the physiologically identified injection sites. Stimulation points where movements were evoked are designated by a small *.Capital letters denote body part moving: F, forelimb; N, neck; V, vibrissae; NR, no response. The large asterick designates the location of injection site into the RFL in the left hemisphere and into the CFL in the right hemisphere. B denotes bregma.

B. Dark Field photomicrograph of a representative WGA-HRP injection site in the RFL area of

A



B

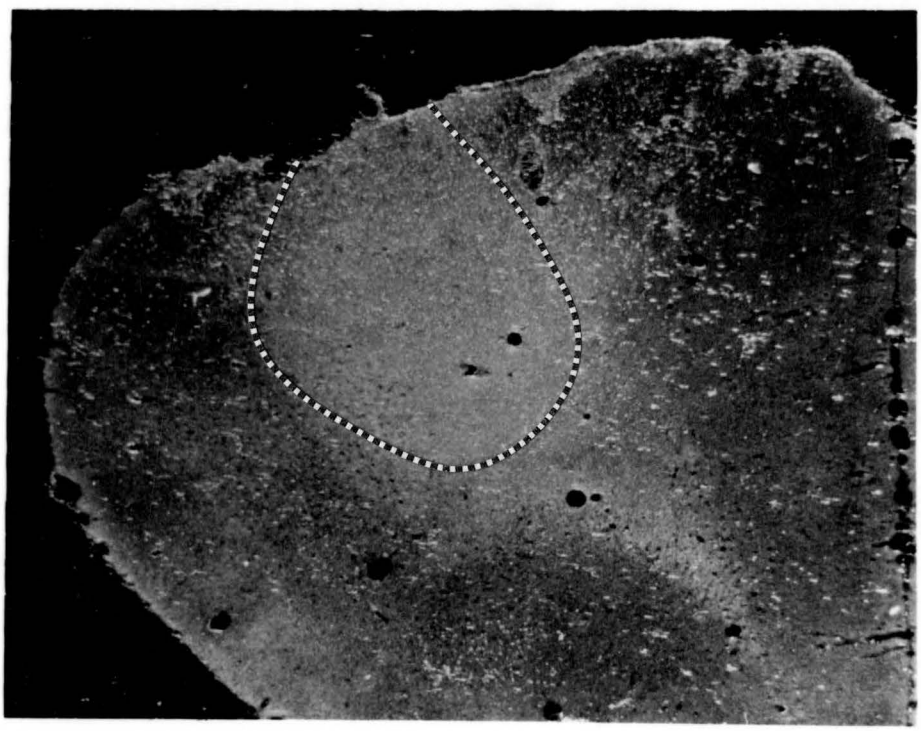
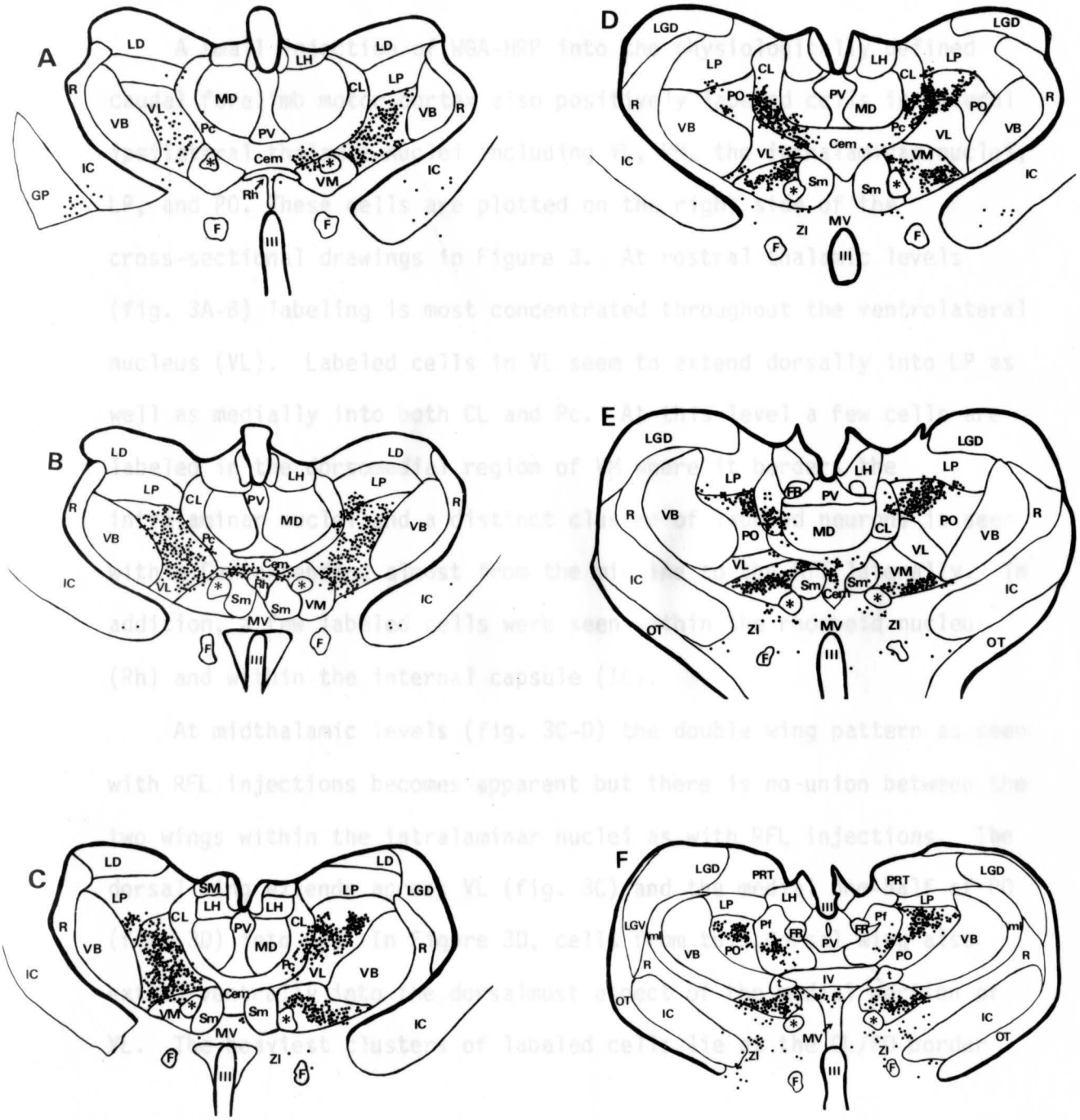


Figure 3

A series of outline drawings of coronal sections through the thalamus of the rat. Labeled cells (represented as a single dot for each cell) on the left side of the figure represent the pattern of labeling following rostral forelimb injections and labeled cells on the right side represent the pattern of labeling following caudal forelimb injections. Sections proceed in the caudal


A
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Caudal Forelimb Injection

A small injection of WGA-HRP into the physiologically defined caudal forelimb motor cortex also positively labeled cells in several ipsilateral thalamic nuclei including VL, VM, the intralaminar nuclei, LP, and PO. These cells are plotted on the right side of the cross-sectional drawings in Figure 3. At rostral thalamic levels (fig. 3A-B) labeling is most concentrated throughout the ventrolateral nucleus (VL). Labeled cells in VL seem to extend dorsally into LP as well as medially into both CL and Pc. At this level a few cells are labeled in the dorsomedial region of VM where it borders the intralaminar nuclei and a distinct cluster of labeled neurons is seen within Cem extending almost from the midline to the MTT laterally. In addition, a few labeled cells were seen within the rhomboid nucleus (Rh) and within the internal capsule (IC).

At midthalamic levels (fig. 3C-D) the double wing pattern as seen with RFL injections becomes apparent but there is no union between the two wings within the intralaminar nuclei as with RFL injections. The dorsal wing extends across VL (fig. 3C) and the medial one-half of PO (fig. 3D) into CL. In Figure 3D, cells from this dorsal wing also extend ventrally into the dorsalmost aspect of the medial portion of VL. The heaviest clusters of labeled cells lie at the CL/PO border

and one cluster within central PO. In addition, a few labeled cells extend dorsally towards and into LP where it borders PO. Scattered labeled cells are also seen in the intralaminar nuclei (Cem and Pc). The ventral wing at this midthalamic level extends from the ventromedial aspect of VB across VM up to and mostly dorsal to the MTT. The largest cluster of cells in this ventral wing is located in the ventrolateral aspect of VM where it borders VB. In addition, a few cells are seen in the ZI between the MTT and the fornix and within the internal capsule.

At more caudal thalamic levels (fig. 3E) the dorsal wing is still present, with the largest cluster of labeled neurons lying within central PO and a few cells extending into CL medially and LP dorsally. Cells within the central lateral nucleus do not continue into MD medially but do occupy almost the entire extent of this nucleus (fig. 3D). The ventral wing is again principally composed of labeled cells in the ventromedial nucleus (VM) and extends from the VB border laterally to the MTT medially. In addition, a few cells appear along the border of and within the submedial nucleus (Sm). A distinct cluster of cells is also found within the Cem nucleus at the midline and scattered cells are seen within the ZI.

At the level of the FR (fig. 3F) the double wing pattern is still evident with the dorsal wing occupying most of the dorsal one-half of PO and extending medially into ventrolateral Pf, dorsally into LP,

and laterally towards VB. The ventral wing at this caudal thalamic level (fig. 3F) fills the central part of VM. Cells within MV are grouped in a distinct cluster at this level while the number of cells in the ZI seems to be increasing. In another animal with a large CFL injection, a cluster of labeled cells was found in the central one-third of VB (VB, fig. 4A). In all other animals no labeled cells were seen in VB.

Further caudally in the pretectal region cells again appear to be positively labeled within NDk (fig. 5A-B). At this level a few cells are also still labeled within the most caudal portion of PO.

Figure 4

A. Outline drawing of a coronal section through the caudal thalamus of rat 80-23A which recieved a bilateral CFL injection. Of interest is the presence of labeled cells within the VB nucleus. This was the only animal which showed labeled cells in this nucleus. Also note the abundance of labeled cells across the dorsal aspect of PO extending dorsally into LP and medially into Pf.

B. Photomicrograph of labeled cells in VB represented in figure 4A (40X).

C. Photomicrograph of labeled cells in PO represented in figure 4A (25X).

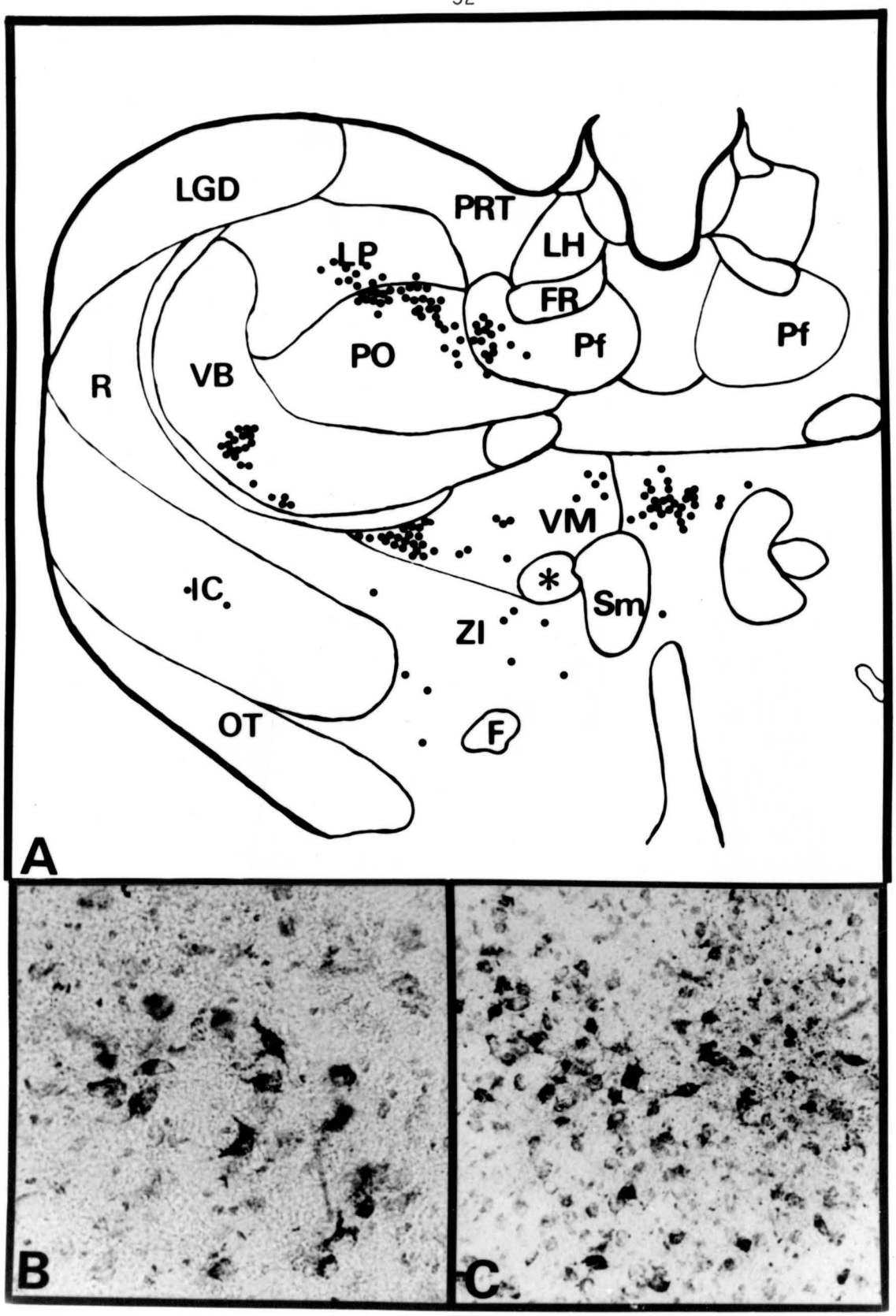
LGD
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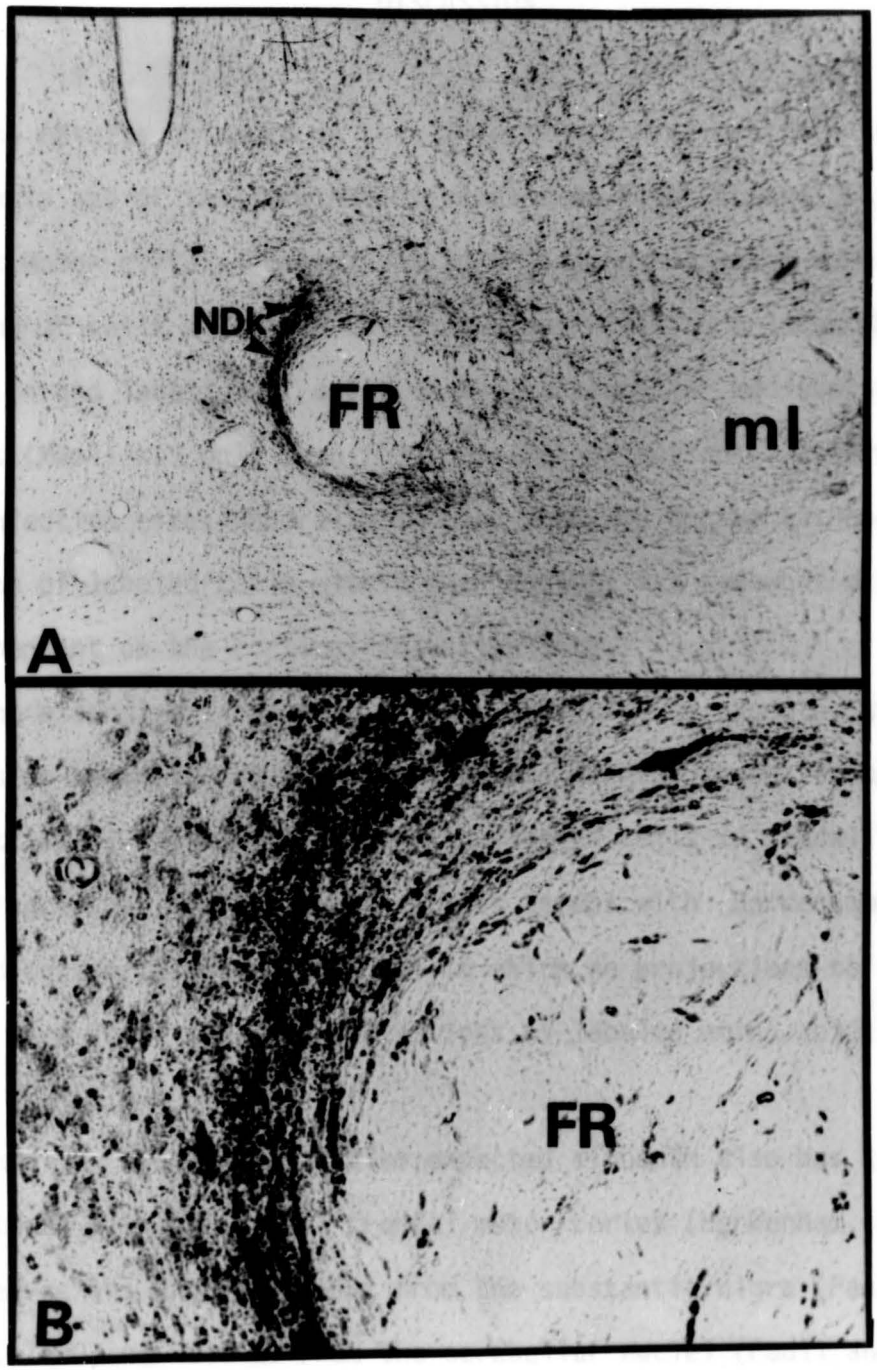
Figure 5

A. Photomicrograph of a coronal section through the caudal thalamus at the level of the fasciculus retroflexus (10X). The presence of labled cells along the FR, especially within the nucleus of Darkschewitsch (NDk) are evident. The area near these labeled cells is darkened by anterograde labeling of WGA-HRP. This animal, 81-23B, received a small CFL injection.


NDK
FR
ml
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FR
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DISCUSSION

The results obtained in this investigation demonstrate that the same basic set of thalamic nuclei project to both RFL and CFL areas of the rat motor cortex. Exceptions to this generalization were that labeling of cells within VB was only seen following a large CFL injection and labeling of cells in the paralamellar mediodorsal nucleus (MDpl) was only seen following RFL injections. Otherwise, both injection sites generally labeled the same nuclei although the location of labeled cells within each nucleus was somewhat different and dependent on the cortical injection site.

The extensive labeling of cells within VL was expected since this nucleus is known to project to the dorsal frontal cortex (Jones and Leavitt, 1974). The failure to find labeled cells in caudal VL following either RFL or CFL injections agrees with Herkenham's (1980) autoradiographic study of the rat in which no projections to frontal cortex were found following injections of labeled amino acid into caudal VL.

Labeling within VM was also expected since VM also has known connections with the dorsal frontal motor cortex (Herkenham, 1979). VM receives its afferent input from the substantia nigra (Faull and Carmen, 1968) as well as from the cerebellar nuclei (Faull and Carmen,

1978). The amount of labeling within VM following the two forelimb cortical injections was comparable; however, the pattern of labeling within VM was markedly different. RFL injections labeled cells in the dorsal aspect of VM whereas CFL injections labeled cells more ventrally within VM. This difference in labeling within VM may indicate some internal organization within VM concerning the input-output relations of this nucleus which has not yet been defined. Although Herkenham (1980) described the projection of VM to be directed almost exclusively to the outer one-half of layer I to a large part of the neocortex, it appears that it also projects to deeper layers (III and V) in the motor area (Herkenham, 1979). Since VM and VL both receive afferent input from the cerebellum in an overlapping fashion, it has been argued that these nuclei together are homologous to the VA-VL complex in the primate (Faull and Carmen, 1978). In addition, VM in the rat appears to be homologous to the nigrothalamic end-stations within the VA-VL complex in the primate (Faull and Carmen, 1978) and hence is a point of convergence for several components of the precortical motor system (Herkenham, 1979).

The ventrobasal complex (VB) showed a few labeled cells only after a large CFL injection. These cells in VB were found only at caudal thalamic levels in the central one-third of this nucleus, the location of the forelimb area of VB in the rat (Saporta and Kruger, 1977). Donoghue et al (1979) have proposed that since in the rat the

sensory (SI) and the motor (MI) cortical forelimb areas partially overlap (Hall and Lindholm, 1974), there should be convergence of VB and VL projections to this cortical area. Since it is possible our larger injection spilled over laterally into the forelimb sensory region, the extent of overlap between cortical sensory and motor forelimb areas remains to be determined. It is clear from our results, however, that there is no overlap between the RFL motor area and the SI forelimb area.

The labeling within the intralaminar nuclei was not surprising (Jones and Leavitt, 1974) and the pattern of labeling following RFL and CFL injections was similar. At rostral thalamic levels, labeling within the intralaminar nuclei was ventrally located within the Cem and Pc nuclei. At successively more caudal thalamic levels the labeling moved more dorsally into CL and continued caudally into the parafasicular nucleus. One exception to this pattern of labeling was the presence of labeled cells ventrally within the central medial nucleus (Cem) along the midline at caudal thalamic levels (fig. 3F). Some differences between the two injections can be seen, however. For example, following RFL injections the dorsal and ventral wings were joined by cells labeled within the paracentral and Cem nuclei, whereas following CFL injections the dorsal and ventral wings were largely separate, although both Cem and Pc contained some labeled neurons. The intralaminar nuclei receive their afferent input from the dorsal

column nuclei, the spinothalamic tract and from the brainstem reticular core (Lund and Webster, 1967a+b; Feldman and Kruger, 1980) and project to layers V and VI of cortex covering the anterior part of each hemisphere including the motor area (Jones and Leavitt, 1974; Herkenham, 1980). Although the intralaminar nuclei have been considered as part of the paleothalamic or cephalic reticular core (Herkenham, 1980), they also have been shown to function in the motor control of head and eye movements (Schlag-Rey and Schlag, 1977; Maldonado and Schlag, 1981). Their possible role in limb movements via their projections to the forelimb areas is not yet known.

Labeled cells in the paralamellar mediodorsal (MDpl) nucleus were seen only after RFL 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 their medial precentral (PrCm) cortical field, located on the dorsomedial shoulder of the hemisphere where the cortex descends into the sagittal fissure. This region appears to correspond with the medial agranular field in rat frontal cortex recently described by Donoghue and Wise (1981). These last authors reported 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, personal communication) and thus the

labeling seen in MD may be due to diffusion away from the injection site into this medial agranular region.

The labeling within the posterior nuclear complex (PO) was quite extensive. Both 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. Also, in all animals, both injection sites showed a few labeled cells extending from PO dorsally into the lateral posterior (LP) nucleus. Projections from PO to the rat sensorimotor cortex have been previously described (Donoghue et al, 1979). Dorsal column nuclei and spinothalamic tract projections to PO in the rat have been described (Lund and Webster, 1967 a+b), suggesting that PO may be involved in transmitting peripheral sensory input to the motor cortex. In the monkey, where this question has been intensely studied, peripheral sensory input appears to reach the motor cortex via the VPLo (VIM) nucleus of the thalamus (Asanuma, 1981). It is tempting to speculate that this part of PO in the rat may be the homologue of the primate VPLo nucleus since both nuclei are located dorsal to VB and caudal to VL and both PO (Herkenham, 1980) and VPLo (Friedman and Jones, 1981) project to layer III of the motor cortex. The existence of dorsal column nuclei projections to VPLo in the monkey has recently been questioned since Tracey et al (1980) only found cerebellar nuclear projections to VPLo. In the rat, the recent autoradiographic study by Feldman and Kruger

(1980) also failed to find dorsal column nuclei projections to this part of PO. At present, then, this question of the thalamic relay for sensory input to motor cortex remains unresolved.

The labeling of cells within the nucleus of Darkschewitsch (NDk) was unexpected since no known projections to the motor cortex from this nucleus has been described previously. This retrograde labeling was seen with both WGA-HRP and HRP injections. Projections from the motor cortex to this nucleus has been previously described (Kunzle, 1978) but our data appears to be the first indicating a reciprocal projection between motor cortex and NDk. The possible function is unknown.

The overall pattern of labeling seen in the thalamus could be described as consisting of dorsal and ventral longitudinal columns of neurons running from rostral to caudal through the thalamus. A similar pattern was described by Kievet and Kuypers (1977) in monkeys, where these columns extended across nuclear borders. This can also be seen in the present study if, for example, one follows labeled cells within VL caudally through CL and CL/PO into Pf. Thus, such a longitudinal columnar organization appears to be present for thalamocortical projections to the forelimb motor cortex of the rat.

Comments on Conjugated HRP

This report is based on the intraaxonal transport of HRP or HRP conjugated with wheat germ agglutinin (WGA-HRP). Wheat germ agglutinin is a lectin derived from plant sources and is one of the newest neuroanatomical tracers first described by Gonatas et al (1979). Wheat germ agglutinin is a simple protein (MW=35,000) which can be covalently conjugated to HRP with glutaraldehyde. WGA-HRP convienently circumvents the most serious problems of the free HRP (FHRP) tracer technique. The problem of diffusion is minimized because the WGA macromolecule binds to a specific glycoprotein site on the neuronal plasma membrane which contains an N-acetyl glucosamine residue. The membrane is then internalized and transported to the cell body as part of smooth ER (Grob et al, 1982). This is obviously more selective than the non-specific bulk pinocytosis of free HRP. The problem of uptake by fibers of passage also appears to be minimized by the WGA-HRP method as evidenced by the work of Grob et al (1982). These investigators found that very little WGA-HRP is taken up by axons of passage following injections of WGA-HRP into the corpus callosum, whereas free HRP was significantly taken up by such fibers following identical injections. This does not imply that axons of passage cannot theoretically take up the WGA-HRP because in fact the axonal membranes and myelin contain glycoproteins which may bind lectins. However, Grob et al (1982) have found that such uptake is considerably less than the uptake of free HRP.

Trojanowski et al (1981) compared the relative sensitivity of free HRP with that of WGA-HRP and HRP conjugated with cholera toxin (CTHRP). They found that the conjugated HRP was 30-50X more sensitive than the free HRP. This was based on the demonstration that 75 ug of free HRP was needed to label cells with the same intensity that 1-5 ug of WGA-HRP would label cells and thus provided evidence for the superior sensitivity of the conjugated HRP. This increased sensitivity of WGA-HRP is attributed to the affinity for specific receptors on the neuronal plasma membrane.

Concerning the question of anterograde labeling, free HRP has been demonstrated to be taken up by cell bodies in a manner similar to retrograde terminal pinocytosis. However, free HRP anterograde labeling is very limited and usually is not readily visible because the quantities of HRP needed to demonstrate such labeling is quite large. WGA-HRP on the other hand, has been shown to be a very effective anterograde tracer as well. The work of Scouten and Malbury (1980) showed that WGA-HRP was as sensitive an anterograde tracer as a retrograde tracer even with very small injections. They found WGA-HRP to produce well localized injections with considerably less diffusion than free HRP and attribute the anterograde sensitivity to the rapid binding of the lectin to its carbohydrate receptor on the membrane and subsequent phagocytosis and transport.

One other advantage provided by conjugated HRP is its reported

usefulness as a transneuronal or trans-synaptic marker within the CNS. Itaya and van Hoesen (1982) have reported WGA-HRP to be an effective marker for transneuronal mapping in the visual system. Such transneuronal transport is further supported by the work of Coulter et al (1980) who also demonstrated transneuronal transport from the vibrissae region to the trigeminal nucleus. Since the trigeminal nucleus is known not to project to this area, the labeling seen in this nucleus must have been the result of transneuronal transport. Nonetheless, transneuronal transport has only been reported following massive (3-50 ul) injections of WGA-HRP and does not appear to be evident with smaller injections (.02 ul) such as those used in this study.

In conclusion, WGA-HRP is a highly sensitive tracer which can be transported both ways down the axon as well as transneuronally. It appears to be both more sensitive and more specific than free HRP since it does not diffuse away from the injection site. In addition, conjugated HRP is not readily taken up by axons of passage, it is not taken up by RBC associated pericytes and it is nontoxic and is easily available.

BIBLIOGRAPHY

Asanuma,H.
The Pyramidal Tract. In: Handbook of Physiology. J.M. Brookhart and V.B. Mountcastle, eds. Waverly Press, Baltimore, Maryland, 1981, pp.703-733.

Beckstead, R.M.
Convergent thalamic and mesencephalic projections to the anterior medial cortex in the rat.
J. Comp. Neurol., 166 (1976) 403-416.

Bucher, V.M. and Nauta,W.J.H.
A note on the pretectal cell groups in the rat's brain.
J. Comp. Neurol., 100 (1954) 287-294.

Coulter, J.D., Sullivan, M.C. and Ruda, M.A.
Lectins as markers for studies of neuronal connectivity.
Neuroscience Abstract, 6 (1980) 339.

Divac, I., Kosmal,A., Bjorklund,A., and Lindvall,O.
Subcortical projections to the prefrontal cortex in the rat as revealed by the horseradish peroxidase technique.
Neuroscience, 3 (1978) 785-796.

Domesick, V.B.
Thalamic relationships of the medial cortex in the rat.
Brain, Behavior, and Evolution, 6 (1972) 457-483.

Donoghue, J.P., Kerman, K.L., and Ebner,F.F.
Evidence for two organizational plans within the somatic sensory-motor cortex of the rat.
J. Comp. Neurol., 183 (1979) 647-664.

Donoghue, J.P. and Wise, S.P.
The motor cortex of the rat: Definition by microstimulation and cytoarchitectonics.
Neuroscience Abstract, 7 (1981) 18.

Emmers, R.
Organization of the first and the second somesthetic regions (SI and SII) in the rat thalamus.
J. Comp. Neurol., 124 (1965) 215-228.

Faull, R.L.M. and Carmen, J.B.
Ascending projections of the substania nigra in the rat.
J. Comp. Neurol., 132 (1968) 73-92.

Faull, R.L.M. and Carmen, J.B.
The cerebellofugal projections in the brachium conjunctivium of the rat: I. The contralateral ascending pathway.
J. Comp. Neurol., 178 (1978) 495-518.

Feldman, S.G. and Kruger, L.
An axonal transport study of the ascending projection of the medial lemniscal neurons in the rat.
J. Comp. Neurol., 192 (1980) 427-454.

Friedman, D.P. and Jones, E.G.
Thalamic input to areas 3a and 2 in monkeys.
J. Neurophysiology, 45 (1981) 59-85.

Gonatas, N.K., Harper, C., Mizutani, T., and Gonatas, J.O.
Superior sensitivity of conjugates of horseradish peroxidase with wheat germ agglutinin for studies of retrograde axonal transport.
J. Histochem. Cytochem., 27 (1979) 728-734.

Grob, P., Buttner-Ennever, J., Lang, W., Akert, K. and Fah, A.
A comparison of the retrograde tracer properties of (125 I) wheat germ agglutinin (WGA) with HRP after injection into the corpus callosum.
Brain Research, 236 (1982) 193-198.

Gurdjian, E.S.
The diencephalon of the albino rat: Studies on the brain of rat #2.
J. Comp. Neurol., 42 (1927) 1-114.

Hall, R.D. and Lindholm, E.P.
Organization of motor and somatosensory neocortex in the albino rat.
Brain Research, 66 (1974) 23-38.

Herkenham, M.
The connections of the nucleus reuniens thalami: Evidence for a direct thalamo-hippocampal pathway in the rat.
J. Comp. Neurol., 177 (1978) 589-610.

Herkenham, M.
The afferent and efferent connections of the ventromedial thalamic nucleus in the rat.
J. Comp. Neurol., 183 (1979) 487-518.

Herkenham, M.
Laminar organization of thalamic projections to the rat neocortex.
Science, 207 (1980) 532-535.

Hicks, S.P. and D'Amato, C.J.
Locating corticospinal neurons by retrograde axonal transport of horseradish peroxidase.
Exp. Neurol., 56 (1977) 410-420.

Itaya, S.K. and van Hoesen, G.W.
WGA-HRP as a transneuronal marker in the visual pathways of monkey and rat.
Brain Research, 236 (1982) 199-204.

Jones, E.G. and Leavitt, R.Y.
Retrograde axonal transport and the demonstration of nonspecific projections to the cerebral cortex and striatum from thalamic intralaminar nuclei in the rat, cat and monkey.
J. Comp. Neurol., 154 (1974) 349-378.

Kievit, J. and Kuypers, H.G.J.M.
Organization of the thalamo-cortical connections to the frontal lobe in the rhesus monkey.
Exp. Brain Research, 29 (1977) 299-322.

Konig, J.F.R. and Klippel, R.A.
The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.
R.E. Krieger Publishing Co. Huntington, NY, 1963.

Krettick, J.E. and Price, J.L.
The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat.
J. Comp. Neurol., 171 (1977) 157-192.

Lashley, K.S.
Thalamocortical connections of the rat's brain.
J. Comp. Neurol., 75 (1941) 67-121.

LaVail, J.H. and LaVail, M.M.
Retrograde axonal transport in the central nervous system.
Science, 176 (1972) 1416-1417.

Leonard, C.M.
The prefrontal cortex of the rat: I. Cortical projection of the mediodorsal nucleus; II. Efferent connections.
Brain Research, 12 (1969) 321-343.

Leonard, C.M.
The connections of the dorsomedial nuclei.
Brain, Behav. Evol., 6 (1972) 524-541.

Lund, R.D. and Webster, K.E.
Thalamic afferents from the dorsal column nuclei: An experimental anatomical study in the rat.
J. Comp. Neurol., 130 (1967a) 301-312.

Lund, R.D. and Webster, K.E.
Thalamic afferents from the spinal cord and trigeminal nuclei: An experimental anatomical study in the rat.
J. Comp. Neurol., 130 (1967b) 313-328.

Maldonado, H. and Schlag, J.
Head and eye movements evoked by electrical stimulation of thalamic gaze center.
Neuroscience Abstract, 7 (1981) 775.

McAllister, J.P. and Wells, J.
The structural organization of the ventral posterolateral nucleus in the rat.
J. Comp. Neurol., 197 (1981) 271-301.

Neafsey, E.J. and Sievert, C.
A second forelimb motor area exists in rat frontal cortex.
Brain Research, 232 (1982) 151-156.

Powell, T.P.S. and Cowen, W.M.
The connections of the midline and intrlaminar

nuclei of the thalamus of the rat.
J. Anatomy (London), 88 (1954) 307-319.

Saporta, S. and Kruger, L.
The organization of thalamocortical relay neurons in the rat ventrobasal complex studied by the retrograde transport of horseradish peroxidase.
J. Comp. Neurol., 174 (1977) 187-208.

Scheibel, M.E. and Scheibel, A.B.
The organization of the ventral anterior nucleus of the thalamus. A Golgi study.
Brain Research, 1 (1966) 250-268.

Schlag-Rey, M. and Schlag, J.
Visual and presaccadic neuronal activity in thalamic internal medullary lamina in cat: A study of targeting.
J. Neurophysiology, 40 (1977) 156-173.

Scouten, C.W. and Malsbury, C.W.
The use of a wheat germ agglutinin-horseradish peroxidase conjugate for anterograde and retrograde tracing in the CNS.
Neuroscience Abstract, 6 (1980) 733.

Shintani, Y.K.
The nuclei of the pretectal region of the mouse brain.
J. Comp. Neurol., 113 (1959) 43-60.

Tracey, D.J., Asanuma,C., Jones, E.G. and Porter, R.
Thalamic relay to motor cortex: Afferent pathways from brainstem, cerebellum and spinal cord in monkeys.
J. Neurophysiology, 44 (1980) 532-554.

Trojanowski, J.Q., Gonatas, J.O. and Gonatas, N.K.
Conjugates of horseradish peroxidase (HRP) with cholera toxin and wheat germ agglutinin are

superior to free HRP as orthogradely transported markers.
Brain Research, 223 (1981) 381-385.

Ullan, J. and Artieda, J.
Somatotopy of the corticospinal neurons in the rat.
Neuroscience Letters, 21 (1981) 13-18.

Veazey, R.B. and Severin, C.M.
Efferent projections of the deep mesencephalic nucleus (pars lateralis) in the rat.
J. comp. Neurol., 190 (1980) 231-244.

Wise, S.P., Murray, E.A., and Coulter, J.D.
Somatotopic organization of corticospinal and corticotrigeminal neurons in the rat.
Neuroscience, 4 (1979) 65-78.

Woolsey, C.N.
Organization of somatic sensory and motor areas of the cerebral cortex.
In: Biological and Biochemical Basis of Behavior.
H.F. Harlow and C.N. Woolsey, eds.
University of Wisconsin Press, Madison, WI, 1958, pp. 63-81.

APPROVAL SHEET

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

The thesis is therefore accepted in partial fulfullment of the requirements for the degree of Master of Science.

Date 4/21/82

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