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# KINESTHETIC AND CUTANEOUS MECHANOSENSORY REGIONS OF THE VENTROBASAL THALAMUS OF RACCOONS AS DETERMINED BY ELECTROPHYSIOLOGICAL MAPPING OF PROJECTIONS IN RELATION TO DISTRIBUTIONS OF CYTOCHROME OXIDASE ACTIVITY, ACETYLCHOLINESTERASE ACTIVITY AND NISSL CYTOARCHITECTURE

presented by

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By

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# ABSTRACT

# KINESTHETIC AND CUTANEOUS MECHANOSENSORY REGIONS OF THE VENTROBASAL THALAMUS OF RACCOONS AS DETERMINED BY ELECTROPHYSIOLOGICAL MAPPING OF PROJECTIONS IN RELATION TO DISTRIBUTIONS OF CYTOCHROME OXIDASE ACTIVITY, ACETYLCHOLINESTERASE ACTIVITY AND NISSL CYTOARCHITECTURE

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In order to determine the presence, organization and architecture of kinesthetic, as compared with other, mechanosensory projection zones in the thalamus of raccoons, finely detailed maps of mechanosensory projections were compared with three types of staining in the same tissues. Unit-cluster responses to mechanical stimulation of the postcranial body were mapped electrophysiologically in the thalami of 14 raccoons. A distinct zone of kinesthetic projections was found in the rostral and dorsal aspects of the mechanosensory projection zone. These projections are somatotopically organized, with projections from axial structures dorsal-most and projections from successively more distal limb regions located more ventrally in the thalamus. The kinesthetic forelimb representation is enlarged and lies rostro-dorsal to a large central core of forepaw glabrous digit projections. Evoked responses from light tactile stimulation of the forepaw hairy skin, claws and glabrous palm were found superjacent to the central core region. A zone containing projections

from cutaneous receptive fields of the axial body and hairy skin of the limbs is located dorsally at the caudal aspect of the central core. This cutaneous body representation has a somatotopic organization distinct from, but similar to, that of the kinesthetic projection zone. Cutaneous tail and glabrous hindlimb projections are on the lateral aspect of the mechanosensory projection zone. Responses from combinations of glabrous skin, hairy skin, claw or kinesthetic receptive fields usually occurred at borders between zones containing only one of these response types.

Electrophysiologically mapped tissues were frozen, sectioned and alternate section series were reacted for Nissl substance, cytochrome oxidase activity or acetylcholinesterase activity. Electrolytic lesions placed as tissue markers reveal that: 1) the mechanoreceptor projection zone has higher cytochrome oxidase and lower acetylcholinesterase staining than neighboring regions, 2) The kinesthetic projection zone is often separated from other mechanoreceptor projections by bundles and laminae of myelinated fibers, as are zones of cutaneous projections from distinct body parts. These subdivisions are particularly well marked by the cytochrome oxidase stain which is moderate to strong in neuropil and cell bodies but absent in myelinated fibers.

This is dedicated to
Pamela Carmen Iraneta.

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The raccoons and mice that I killed to realize this.

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# FOREWORD

This thesis is organized into three complementary communications. Chapter I is a section entitled _Literature review and statement of the problem_. Chapter II is an article entitled _Organization of kinesthetic and mechanosensory projections to the ventrobasal thalamus in raccoons: Mapping of evoked unit cluster electrical activity_. Chapter III is an article entitled _Architecture of the kinesthetic and cutaneous mechanosensory projection zones in the ventrobasal thalamus of raccoons: Distributions of cytochrome oxidase activity, acetylcholinesterase activity and Nissl substance in electrophysiologically mapped tissues_.

Each chapter has its own references and references within Chapters II and III to the "accompanying paper" refer to Chapters III and II respectively.

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Weakly staining, dorsoventrally oriented fibrous laminae separate the heavily stained projections from the respective forepaw glabrous digits as well as from the hindlimb and head. These laminae are a distinguishing characteristic of these cutaneous projection zones. The region dorsal to this does not contain the fibrous laminae and displays evoked electrical activity following stimulation of (deep) muscles (K) and joints (J), light touch to hairy skin (H) or stronger and visible displacement of the skin (P). In this plane of section, fiber bundles appear to course mediolaterally within the plane of section in the region containing kinesthetic projections and those from hairy skin. In the glabrous forepaw projection zone, the bundles course more perpendicular to the plane of section. Open white circle and open black circle in Figure 3.4B indicate the points used as reference limits for quantitation of stain density in Figure 3.8A. As is shown in Figure 3.8A, the mechanosensory projection zone and VL have dense staining while the neighboring LP, VMP and VPI have less dense staining. Scale bars = 1 mm.

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Figure 3.8A.0-40% no shading; 40-63% stippled; 63-85% cross-hatched; 85-100% blackened.

Figure 3.8B.0-38% no shading; 38-56% stippled; 56-72% cross-hatched; 72-100% blackened.

Intense cytochrome oxidase activity is found in the kinesthetic and cutaneous mechanoreceptor projection zones (cf. Figures 3.4B and 3.6B). In Figure 3.8A high levels of cytochrome oxidase are also found in the ventrolateral nucleus dorsally. Weaker stain levels are found in the LP, VMP and VPI which border the mechanosensory projection zone.

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## ABBREVIATIONS

Abbreviations for anatomical structures follow Berman and Jones (1981). The following occur in the text of Chapter I:

| | |
|---|---|
| D.im. | Dorsal intermediate nucleus |
| H | Head representation |
| L | Leg representation |
| LA | Lateral anterior nucleus |
| LCN | Lateral cervical nucleus |
| LD | Lateral dorsal nucleus |
| LG | Lateral geniculate nucleus |
| LP | Lateral posterior nucleus |
| mm | Millimeter |
| n. | Nucleus |
| Po | Nucleus polaris |
| POm | Medial part of the posterior nuclear complex |
| RE | Reticular nucleus |
| SI | Primary somatosensory cortex |
| ST | Spinothalamic tract |
| sTN | Spinal trigeminal nucleus |
| sTNi | Nucleus interpolaris of sTN |
| VA | Ventral anterior nucleus |
| VB | Ventrobasal complex |
| VBX | External nucleus of VB |
| VI | Ventral intermediate nucleus |
| V.im. | Ventral intermediate nucleus |
| VL | Ventrolateral complex |

| | |
|---|---|
| VLC | Caudal part of VL |
| VLo | Oral part of VL |
| VM | Ventromedial nucleus |
| VMb | Basal ventromedial nucleus |
| VMp | Principal ventromedial nucleus |
| VP | Ventroposterior nucleus |
| VPI | Ventroposterior inferior nucleus |
| VPL | Ventroposterior lateral nucleus |
| VPLc | Caudal part of VPL |
| VPLo | Oral part of VPL |
| VPM | Ventroposterior medial nucleus |
| VPMpc | Parvocellular part of VPM |
| $\mu$m | Micrometer ($10^{-6}$ meter) |

The following occur in the text of Chapter II:

| | |
|---|---|
| Cm | Centimeter |
| DAB | Diaminobenzidine |
| DCN | Dorsal column nuclei |
| HRP | Horseradish peroxidase |
| Hz | Hertz (cycles per second) |
| IP | Intraperitoneal |
| kg | Kilogram |
| kHz | Kilohertz |
| LP | Lateral posterior nucleus |
| M | Molar |
| mg | Milligram |
| ml | Milliliter |
| mm | Millimeter |
| POm | Medial part of posterior nuclear complex |
| VA | Ventral anterior nucleus |
| VBX | External nucleus of ventrobasal complex |
| VL | Ventrolateral complex |
| VLc | Caudal part of VL |
| VPI | Ventroposterior inferior nucleus |
| VPL | Ventroposterior lateral nucleus |
| VPLo | Oral part of VPL |
| µm | Micrometer ($10^{-6}$ meter) |

The following occur in the Figures of Chapter II:

| | |
|---|---|
| C | Caudal |
| CA | Caudate nucleus |
| D | Dorsal |
| GLAB | Glabrous skin |
| L | Lateral |
| M | Medial |
| OB | Olfactory bulb |
| R | Rostral |
| T | Thalamus |
| µm | Micrometer ($10^{-6}$ m) |
| V | Ventral |
| 1,2,3,4,5 | Digit with respective number (Thumb = 1, etc.) |

The following occur in the text of Chapter III:

| | |
|---|---|
| AChE | Acetylcholinesterase |
| DAB | Diaminobenzidine |
| ECuMt | Medial tongue of the external cuneate nucleus |
| HRP | Horseradish peroxidase |
| iso-OMPA | Isopropyl pyrophosphoramide |
| CM | Centre médian nucleus |
| LP | Lateral posterior nucleus |
| VB | Ventrobasal complex |
| VBX | External nucleus of VB |
| VL | Ventrolateral complex |
| VMP | Principal ventromedial nucleus |
| VPI | Ventroposterior inferior nucleus |
| VPL | Ventroposterior lateral nucleus |
| VPLc | Caudal part of VPL |
| VPLo | Oral part of VPL |
| μm | Micrometer ($10^{-6}$ meter) |

The following occur in the Figures of Chapter III:

| | |
|---|---|
| AChE | Acetylcholinesterase |
| AD | Anterior dorsal nucleus |
| AV | Anterior ventral nucleus |
| C | Claw |
| CA | Caudate nucleus |
| CLN | Central lateral nucleus |
| CM | Centre median nucleus |
| D | Dorsal |
| G | Glabrous skin |
| GLAB | Glabrous skin |
| H | Hairy skin |
| IC | Internal capsule |
| J | Joint |
| K | Kinesthetic muscle |
| L | Lateral |
| LD | Lateral dorsal nucleus |
| LGD | Lateral geniculate nucleus |
| LP | Lateral posterior nucleus |
| M | Medial |
| m | Meters |
| OB | Olfactory bulb |
| P | Cutaneous pressure |
| PO | Posterior nucleus |
| PP | Pes Pedunculi (Cerebral peduncle) |
| PUL | Pulvinar nucleus |

| | |
|---|---|
| R | Rostral |
| RE | Reticular nucleus of the thalamus |
| SUB | Subthalamic body |
| T | Thalamus |
| μm | Micrometer ($10^{-6}$ meter) |
| V | Ventral |
| VL | Ventrolateral complex |
| VMP | Principal ventromedial nucleus |
| VPI | Ventropostero-inferior nucleus |
| 1, 2, 3, 4, 5 | Digits with respective numbers (Thumb = 1) |

Small case letters identify microelectrode penetrations in respective figures.

# CHAPTER I

# LITERATURE REVIEW AND STATEMENT OF THE PROBLEM

# INTRODUCTION

Our laboratory is currently investigating the organization of the neural systems which transfer and transform electric signals originating at kinesthetic receptors and related mechanosensory units in the raccoon forepaw. This system is especially useful since in raccoons the cortical, thalamic and medullary representations of this somatic sensory system are outstanding in size and internal differentiation (Welker and Seidenstein, 1959; Welker and Johnson, 1965; Johnson, Welker and Pubols, 1968; Johnson, 1980; summary in Welker, Johnson and Pubols, 1964). Many portions of these representations have easily identified subdivisions related to distinct parts of the forepaw.

We are engaged in a series of experiments designed to elucidate the pathways carrying electrical signals which are elicited by stimulation of somatic receptors for submodalities of mechanosensory inputs from regions including skin, muscle, tendon, joint, claw and hair. Three spatially discrete zones have been reported to exist in the raccoon primary somatosensory cortex (Johnson, Ostapoff and Warach, 1982). which are respectively characterized by responses to low threshold stimuli of:

1) muscles, joints and tendons (referred to as "deep" or "kinesthetic"),
2) small, specific regions of glabrous skin with preservation of information about spatial location, and
3) a "heterogeneous" population of mechanoreceptive fields including dorsal hairy hand, claws and the fields described in 1) and 2).

## Anatomy of the thalamus

General structure of the mammalian thalamus. This description follows that of Brodal (1981). The dorsal thalami of mammals are a pair of ovoid masses separated by the third ventricle. The internal medullary lamina is a series of sheets of myelinated fibers (or white matter) which subdivides each thalamus into three cell masses or nuclear groups (as shown in Figure 1.1 for the human). These nuclear groups are the anterior, the medial and the lateral thalamic masses. In some literature the lateral mass is referred to as the ventral mass instead. This is not suggested here since it may cause confusion with the ventral thalamus which is a separate entity from the dorsal thalamus (which consists of these three thalamic masses). In addition, there are small nuclei which are sometimes grouped with one of the three masses. Within the internal medullary lamina are the intralaminar nuclei. The midline nuclei, sometimes considered a fourth nuclear group of the dorsal thalamus, lie just beneath the ependymal lining of the third ventricle and within the interthalamic adhesion which connects the two thalami. The thinly layered reticular nucleus of the thalamus, considered part of the ventral thalamus, is separated from the outer borders of the dorsal thalamus by the external medullary lamina.

Within the nuclear groups, nuclei and subnuclei have traditionally been distinguished based upon structural criteria including cell body distribution (cytoarchitecture), myelinated fibers (myelo-architecture), fiber patterns and more recently, glial architecture. The lateral nuclear group is bordered by the internal medullary lamina medially and the external medullary lamina laterally. The lateral nuclear group is subdivided into dorsal and ventral tiers of nuclei. From rostral to

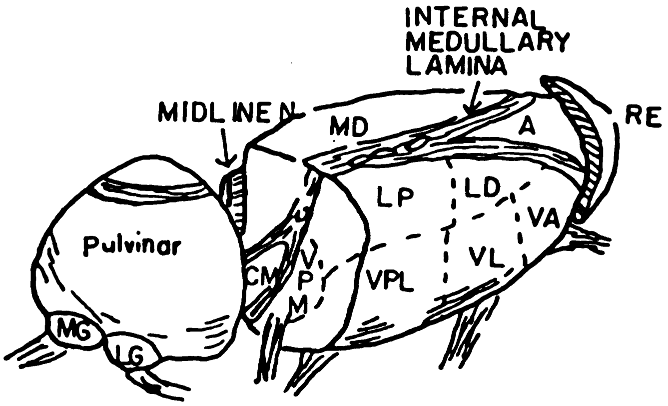


Figure 1-1

Diagram of human right thalamus as seen from the dorsolateral aspect (after figure 2-14 of Brodal, 1981). Abbreviations of nuclei: A- anterior group; CM- centre median; LD-lateral dorsal; LG-lateral geniculate; LP-lateral posterior; MD- medial dorsal; MG-medial geniculate; VA-ventral anterior; VL-ventral lateral; VPL-ventroposterior lateral.

caudal, the dorsal tier contains the lateral dorsal nucleus (LD), the lateral posterior nucleus (LP) and pulvinar (see Figure 1.1). From rostral to caudal, the ventral tier contains the ventral anterior nucleus (VA), the ventral lateral complex (VL), the ventrobasal (VB) or ventral posterior (VP) nuclear complex. Most caudal in the ventral tier of the dorsal thalamus are the lateral and medial geniculate bodies. The latter are sometimes referred to as a distinct division, the metathalamus.

### Anatomy of the lateral mass of the thalamus in the class Carnivora, with special reference to the ventrobasal complex.

In order to gain perspective of the significance of studies in the raccoon, the organization of the ventrobasal thalamus (which responds to somatosensory stimulation) in other mammals will be discussed. Contrasting the specializations of the somatosensory thalamus of other mammals, including primates, with those of the raccoon will help to distinguish characteristic features of the raccoon thalamus. Although the divergence of the carnivore line from that of the primates is ancient, members of these two orders will be shown to share many anatomical, connectional and functional principles in their somatosensory systems. In later sections, the somatosensory system of the raccoon will also be considered as a possible model for human sensory processing systems on the basis of similar behavioral and ecological specializations.

**Dog.** On the basis of "differences in the types and arrangements of the cells," Rioch (1929) delineated the ventrobasal thalamus of the dog, but referred to it in terms of its components, the nucleus ventralis, pars externa in its lateral aspect and the nucleus ventralis, pars arcuata in its medial aspect. These both lie in his ventral group of the thalamus which also includes VA and VL in its pars anterior. The pars externa and pars arcuata compose the "principal portion" of the ventral group. Rioch's ventral group also includes a pars medialis (apparently the ventromedial nucleus of more current nomenclatures), as well as pars commissuralis or nucleus inter-ventralis medial-most. The cells of the pars anterior were described as large and polygonal, while cells of the pars externa were both large and medium-sized, roundly polygonal and densely staining. An "indefinite" fiber layer was observed between the pars arcuata and pars externa. Fibers also course "longitudinally and laterally" concentric to the outer surface of the nucleus while still other fibers course in horizontal planes.

Cat. Rinvik (1968) includes the ventrobasal complex in his ventral group (i.e. the lateral mass) of thalamic nuclei in the cat. [See Figure 1 of Rinvik (1968)]. The nucleus ventralis anterior (VA) is the rostral-most member of the group and contains large polygonal cells which are moderately scattered and which contain several cytoplasmic processes. Caudal to VA is the nucleus ventralis lateralis (VL). Rinvik (1968) avers that the VL of the cat corresponds to the nucleus arcuatus in the cat as described by Ingram et al. (1932) and Jasper and Marsala (1954). Dorsal to VL is the nucleus lateralis anterior (LA). At the caudal part of VL, the rostral end of the nucleus lateralis posterior (LP) lies between VL and LA. The nucleus ventralis medialis (VM) is located medial to VL in the rostral thalamus. As VL decreases in size caudally, the ventrobasal complex (VB) appears and widens from its lateral position toward the internal medullary lamina. The ventrobasal complex is made up of the nucleus ventralis posteromedialis (VPM) and the nucleus ventralis posterolateralis (VPL). VB is characterized by large polygonal cells in a network of myelinated fibers. Medial to VPM proper is the parvocellular nucleus of VPM (VPMpc). Ventral to VPM and VPMpc is the nucleus ventralis posterior inferior (VPI), which contains small, widely scattered cells.

In the cat, Jones and Burton (1974) found a region of transition between the VL and VB complexes that is not well defined cytoarchitectonically. The region was characterized by its position, the dominant population of large cells, the presence of spinothalamic projections and its appearance of receiving group I muscle afferent projections from limbs. This region was termed the spinal part of the ventrolateral complex (VLsp). More recently, this region has been referred to as the transitional region between the ventrolateral and ventrobasal complexes (Berman and Jones, 1982).

Raccoon. Sakai (1982) presents an excellent description of the anatomical organization of the lateral mass of the raccoon thalamus. See Figure 1.2. VA and VL form a complex in the anterior thalamus. The more rostrally located VA has a lower cell density than VL. Sakai (1982, pp. 240 and 243) describes further:

> "Ventral to VA, VL emerges from the dorsolateral edge of the external medullary lamina. As VA diminishes caudally by occupying the dorsalmost cap of the ventral thalamic complex, VL expands so as to occupy a wide mediolateral extent of the thalamus. Cytoarchitecturally, VL is characterized as a heterogeneous nuclear mass containing darkly stained cells organized into small, irregular clusters which are segregated by fiber bundles."
>
> "In the raccoon, the largest and most distinctive component of the ventral thalamic tier is the ventrobasal complex (VBC). VBC emerges ventral to VL hugging the external medullary lamina. The VL/VBC border is easily recognized by an increase, in VBC, in the cellular packing density and the presence of large clusters of cells with darkly stained cell bodies. Mediolaterally, the delineation of VBC subdivisions is marked by the presence of large fiber fascicles. More caudally, VL occupies the region dorsomedial to VBC for some distance."
>
> "Medial to VA, VL and VBC is the ventromedial nucleus. This nucleus consists of the principal ventromedial nucleus (VMp) and the basal ventromedial nucleus (VMb; Hendry et al., 1979). In the raccoon, VMp lies medial to VA and VL and is composed of widely spaced small cells which stain lightly. VMb appears as a nuclear mass medial to the arcuate division of VBC. Its cells are small and more densely packed than those of VMp." (End of excerpt).

Anatomy of the lateral mass of the thalamus in primates.

Monkey. In Walker's (1938) description, the lateral nuclear mass of the monkey (this term will describe the rhesus monkey, Macaca mulatta) thalamus lies between the internal and external medullary laminae and anterior to the pulvinar. Rostralmost this region is the nucleus ventralis anterior (VA). The remainder of the rostral half of the lateral nuclear mass is the nucleus ventralis lateralis (VL). VL is bordered

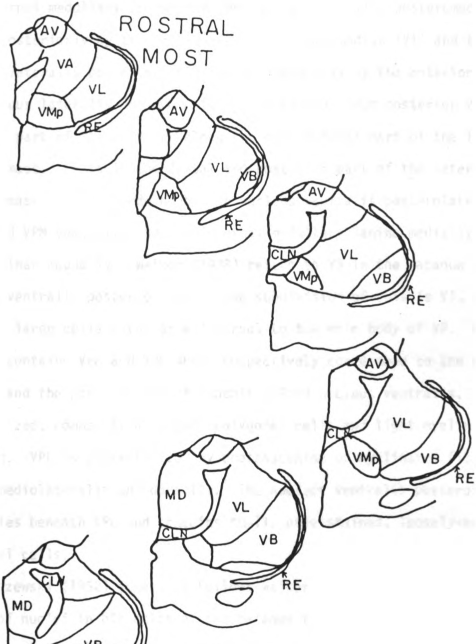


Figure I-2. Diagrams of frontal sections of raccoon right thalamus from rostral (at top of page)to caudal (at bottom of page) after Sakai (1982). Abbreviations for nuclei: AV-anterior ventral; CLN-central lateral; MD-medial dorsal; RE-reticular; VA-ventral anterior; VB-ventrobasal complex; VL-ventrolateral complex; VMp-principal ventromedial.

anteriorly by VA, laterally by the thalamic reticular nucleus, medially by the internal medullary lamina and the nucleus ventralis posteromedialis (VPM), posteriorly by the nucleus ventralis intermedius (VI) and the nucleus lateralis posterior (LP) and dorsomedially by the anterior half of the nucleus lateralis dorsalis (LD). LD extends from posterior VA to the anterior part of the pulvinar along the dorsolateral part of the lateral nuclear mass. LP is in the dorsal and posterior part of the lateral nuclear mass. It is bordered by the nucleus ventralis posterolateralis (VPL) and VPM ventrally, by the internal medullary lamina medially and by the pulvinar caudally. Walker (1938) refers to VB in the macaque as the nucleus ventralis posterior (VP). One subdivision of this is VI, a thin sheet of large cells anterior and dorsal to the main body of VP. Walker's VP also contains VPM and VPL which respectively correspond to the pars arcuata and the pars externa of Rioch's (1929) nucleus ventralis. VPM has medium-sized, compactly arranged, polygonal cells and light myelin staining. VPL is characterized by crosshatching of myelinated fibers which course mediolaterally and dorsally. The nucleus ventralis posteroinferior (VPI) lies beneath VPL and contains small, pale-stained, loosely-arranged polygonal cells.

Olszewski (1952) generally follows Walker's (1938) descriptions and naming of nuclei in his atlas of the macaque thalamus. [See Plates 26 and 27 of Olszewski (1952)]. He finds VA to be gradually displaced in its caudal extent by the pars oralis of VL (VLpo or VLo) which expands from a ventrolateral position dorsally and medially. A border region labelled area X is described as cytoarchitectonically homogeneous and distinctive, lying between VA, the pars caudalis of VL (VLc), and the internal medullary lamina. In its caudal extent, VL is replaced ventrally by VPL.

Within VPL, Olszewski finds the controversial pars oralis (VPLo), as well as the pars caudalis (VPLc), and the pars medialis (VPLm). Although he admits that it is difficult, Olszewski (1952, p. 19) finds the border between VPL and VL transitional subnucleus observable in horizontal sections. This confusing border region which is of particular interest to us lies between VLc, which contains large cells, and VPL, which contains large and small cells. Olszewski (1952) renames Crouch's (1934) VI as VPLo. VPLo contains very large cells which are multipolar and contain dark, finely granulated Nissl substance. The VPLo division of VPL in the macaque is problematic since, (as will be discussed later,) unlike the VPL of carnivores, it receives cerebellar afferents and no lemniscal afferents. While these subdivisions of VPL are claimed to be cytoarchitectonically distinct, a fourth, the pars postrema (VPLps) is topographically distinctive.

Kalil (1981) reviews the organization of VB in the rhesus monkey. VLo, with its clusters of deeply staining cells, is distinguished from VPLo, with its sparse but evenly distributed cells, and from VPLc with its smaller, densely packed cells. Kalil has difficulty distinguishing VLc from VPLc along the dorsal border of VPLc in her transverse sections. However, LP is differentiated from VPLc by virtue of its smaller cells and the orientation of cell bodies along horizontally coursing fiber bundles.

Human. This description of the nuclei of the lateral mass of the human thalamus is based upon that of Andrew and Watkins (1969) which is in general agreement with the work of Hassler (1959). See Schaltenbrand and Bailey (1959). Unfortunately, the nomenclature in the human is quite different than that of cat, raccoon and monkey. The nucleus polaris (Po) caps the rostral-most portion of the lateral thalamic mass. Caudal to Po is the nucleus oralis which extends further laterally than Po. Nucleus oralis is subdivided into a pars internus (O.i.) and a pars externus (O.e.), which in turn are divided into dorsal and ventral regions. Further caudal are the intermediate nucleus and the nucleus caudalis. The intermediate nucleus is divided into dorsal (D.im.) and ventral (V.im.) regions. In sagittal sections, V.im. can be distinguished from its rostral and caudal neighbors, the ventral oral and ventral caudal nuclei respectively, by its large, darkly staining and particularly laterally, scattered cells. D.im. is distinct from V.im. but is not so easily differentiated from the dorsal caudal nucleus. The caudal nucleus is subdivided into four divisions: the ventral caudal n., pars externus, the ventral caudal nucleus, pars internus, the ventral caudal nucleus, pars parvocellularis and the ventral caudal nucleus, pars portae. In addition there is a poorly defined caudal dorsal nucleus. Pulvinar is caudal-most in the lateral mass.

The relationships between the nuclei of the animals discussed above are outilned in Table I. The reviews by Crosby, Humphrey and Lauer (1962) and Jones (1981) were consulted in the construction of the table. In some cases, homologies are not exact and borders overlap. Regions with totally uncertain homologies are not listed. In general, more subnuclei are delineated in monkey and man than in the cat. Fewer entries appear in the column of human nuclei only because homologies are not as well substantiated.

Parallels with somatosensory cortex of mammals. In order to gain a broader perspective in understanding the ventrobasal thalamus, the somatosensory cortex will be discussed. As is the case for other specific thalamic nuclei in mammals, the somatosensory nuclei of the ventrobasal thalamus receive fibers (which carry signals) from other parts

Table I. Homologies of nuclei of the lateral (or ventral) mass of the dorsal thalamus of some mammals.

| Carnivore | Monkey | Human |
|---|---|---|
| (Jones, 1981) | (Olszewski, 1952) | (Hassler, 1959) |
| VA | VAp<br>VAmc<br>X | nucleus ventralis pars oralis (nucleus polaris?) |
| VL | VLo<br>VPps<br>VLc<br>VPLo | nucleus ventralis oralis, pars caudalis |
| VM | VMp (VLm) | nucleus zentrolateralis? |
| VPMpc | VMb (VPMpc) | |
| VP (VB) | | n. ventrocaudalis |
| (Following are portions of VB and n. ventrocaudalis) | | |
| VPL | VPLc | n. ventralis caudalis, pars extrema |
| VPM | VPM | n. ventralis caudalis, pars interna |
| VPI | VPI | |

Crosby, Humphrey and Lauer (1962) and Jones (1981) were consulted in the construction of this table. Citations below animal group names refer to source of terminology.

of the nervous system (discussed later) and have reciprocal relations with regions of the cerebral cortex. All short-latency signals conveying sensory inputs (except olfaction) reach the cortex exclusively through thalamic connections. The cortex, in turn, makes connections with many other important sites including alpha motorneurons which execute physical behavior.

Throughout the class of mammals, somatosensory cortical specialization parallels that of the thalamus. In those mammals wherein they appear, cytoarchitectonic zones 3b, 1 and 2 are recipients of cutaneous signals. Area 3a, located in the central sulcus which separates the sensory and motor cortices, is electrically activated with deep stimuli in cats and primates (reviewed by Jones and Porter, 1981). In the cat, this region is distinguished cytoarchitectonically by an attenuating internal granular layer, or layer IV (which extends anteriorly from area 3b) overlying a pyramidal cell layer, or layer V (as found in the further anterior area 4) (Hassler and Muhs-Clement, 1964). Jones and Porter (1981) redefine area 3a of primates as the cortical focus which is activated by group I muscle afferent fibers with a short latency of response. While granule cells are consistently found in this region, pyramidal cells do not serve as a reliable guide to its location.

## Somatosensory pathways and tracts.

Overview of the mammalian plan. Mammals have three pathways that carry signals via the medial lemniscus to the thalamus from the periphery (reviewed by Angel, 1977). The lemniscal pathway consists of primary afferent fibers which form synapses in the trigeminal-cuneate-gracile complex. The spinocervicothalamic tract forms the projection from second-order sensory cells in the spinal grey and courses through the dorsal funiculus of the spinal cord. The spinocervicothalamic system is more developed than in primates (Ha and Morin, 1964; Truex et al., 1970). The spinothalamic tract consists of fibers of second-order sensory neurons in the spinal cord. These decussate in the cord and then project to the thalamus.

Primary afferent nerve fibers of the dorsal column-lemniscal system enter the spinal cord via its dorsal roots (see Figure 1.3). These fibers penetrate the dorsomedial border of the dorsal horn to reach a variety of depths. The primary afferents then bifurcate to form an ascending branch and a descending branch. The ascending projection fibers traverse the fasciculi cuneatus (forelimb projections) and gracilis (hindlimb projections) of the spinal cord to terminate in the respective nuclei of the same name in the dorsal medulla. Within these columns, these projections have a topographic organization with caudal-originating fibers lying medial to more rostral originating fibers. The dorsal column nuclei (the cuneate and gracile nuclei) send projections to VPL, the posterior nuclear group, zona incerta and the ventral part of the lateral geniculate nucleus in the hedgehog (Schroeder et al., 1968). In more developed animals (Malaysian tree shrew, Slow loris and marmoset, respectively) these terminals are progressively more restricted to VPL. The VPL afferents retain somatotopic (i.e. ordered in relation to topographic relations of peripheral source) organization, with gracile projections rostrolateral, cuneate caudomedially and trigeminal projections located more medially in VPM.

The second-order nuerons of the spinocervicothalamic system are located in Rexed's laminae III, IV and V (Bryan et al., 1974). Their fibers ascend in the dorsolateral funiculus to terminate in the lateral cervical nucleus (LCN). Projections from the LCN decussate at the spinal

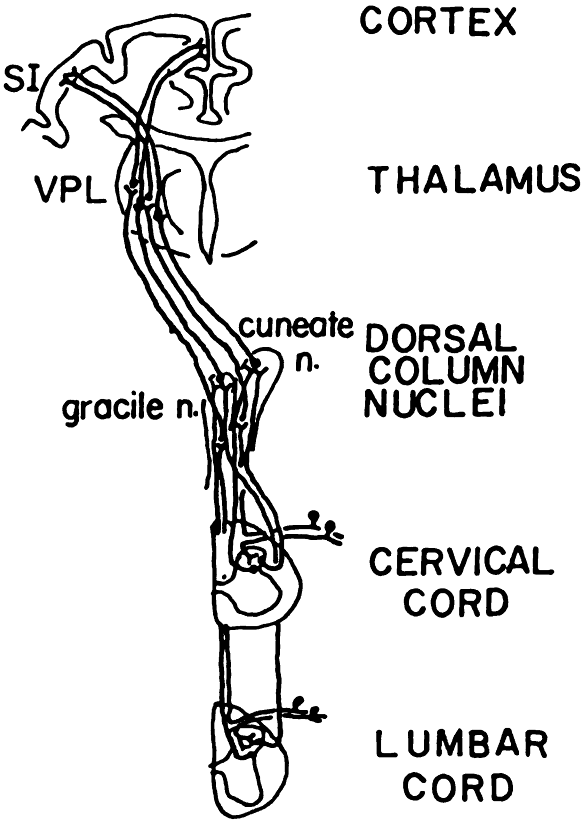


Figure 1-3. Diagram of dorsal column-lemniscal pathway for low-threshold cutaneous receptor input signals as discussed in text (after figure 2-10 of Brodal, 1981). Abbreviations: n.-nucleus; SI- primary somatosensory cortex; VPL- ventroposterior lateral nucleus.

cord-medulla junction to join the medial lemniscus in its lateral aspect and terminate in the VB. The LCN has been observed in the cat (Rexed and Strom, 1952), the dog (Kitai et al., 1965), raccoon (Ha et al., 1965) and monkeys (Ha and Morin, 1964; Mizuno et al., 1967).

The spinothalamic tract is composed of at least three components:

1) the spinobulbar, which terminates in the medullary reticular formation,
2) the paleospinothalamic which projects to the reticular formation and the thalamus, and
3) the neospinothalamic which converges with the medial lemniscus to terminate in the thalamus.

Spinothalamic projections can arise from secondary sensory neurons throughout the spinal grey matter, but they are especially found in laminae IV and V (but also in laminae I, VI, VII and VIII). The fibers decussate via the anterior commissure and ascend in the anterolateral (or ventrolateral) quadrant of the spinal cord. Fibers which extend to the diencephalon pass through the medulla dorsolateral to the inferior olive to ascend dorsal to the medial lemniscus. Thalamic terminals include VB, the posterior thalamic group and the intralaminar nuclei.

In addition to inputs from the medullary trigeminal-cuneate-gracile complex, spinal cord neurons and the LCN, VB receives the terminals of nerve fibers from the midbrain reticular formation, vestibular nuclei and sensory cortex. These connections and some possible circuit properties that they may express are proposed by Welker (1973).

On the basis of the organization of ascending afferent fibers in the cat and monkey, Berkley (1980) proposes that the ventrobasal complex is composed of a dense core of terminals which is surrounded by an uneven shell of less dense somatosensory terminals. On a functional level, the core has mainly cutaneous responses while the shell has joint and muscle responses rostrally at the VB-VL border and multimodal responses dorsally and posteriorly in POm. VPI is also included in the shell ventrally although its response fields are not well established. On a connective level, the afferents which converge in the core region retain territoriality in their non-overlapping clusters of terminals (see below) while termination patterns do not appear so complex in the shell regions.

Cat. In experiments with simultaneous use of anterograde nerve degeneration and tritiated amino acid transport techniques for tracing fiber tracts, Berkley (1980) studied the terminals of ascending fibers from the dorsal column nuclei (DCN), lateral cervical nucleus (LCN), spinal cord (ST) and spinal trigeminal nucleus (sTN) in the diencephalon of the cat. The DCN projections to VB are generally confined to VPL with gracile terminals lateral (in a zone designated the pars lateralis of VPL, abbreviated VPLl) to cuneate terminals (in pars medialis of VPL or VPLm) with some scattered overlap. Dense clusters (of size 45-210 μm) of terminals were found in VB, with larger clusters more ventral. The highest density of DCN projections was found in central VB. In cases of injections or ablations of the DCN which included the rostral-most or ventral-most portions (including part or all of nucleus z), labelling or degeneration was also found in the most lateral, rostral and dorsal borders of VB. This included the VPL/VL border rostrally, the VPL-VPM/lateral posterior nucleus border dorsally and the VPL/lateral geniculate nucleus border laterally. Other terminals of DCN projections were found in zona incerta, the posterior pretectal nucleus and the medial division of the posterior group complex (POm).

Berkley (1980) observed that sTN projections extend to VPM, rostral brainstem, and the lateral part of the anterior pretectal nucleus.

Terminals from sTN projections overlap with those of the DCN in caudal and medial parts of POm and zona incerta. The terminals from the nucleus caudalis (sTNc) and the nucleus interpolaris (sTNi) of sTN form clusters 85-250 μm in diameter in VPM, with denser labelling from sTNi.

In this same study, LCN projections were found to extend primarily within the ventromedial border of POm where it meets the medial portion of the pars medialis of the medial geniculate nucleus. There are also LCN projections to the lateral and dorsal portions of VB. Terminal clustering is found in the same region of VB as DCN terminal clusters. However, it is rare that the same neurons are contacted by both LCN and DCN terminals. In the borders of VB, LCN projections are found further dorsal, lateral and rostral than DCN projections. In the cases of extensive DCN injections or ablations, however, the labelled or degenerating terminals are found to be coextensive but sparser than the LCN terminals.

The spinothalamic (ST, also called spinodiencephalic) tract was studied in cats with ventral funiculus lesions placed at a level between C3 and C5 of the spinal cord. Terminals were found in the central lateral nucleus of the thalamus (CL) and adjacent portions of the mediodorsal and paracentral nuclei. Although few ST terminals were found in VB, more were located in regions along its ventral, rostral, dorsal and lateral borders. Beyond the borders of VB, VL and LP each have a high density of ST terminals. ST terminals were also found in VPI.

In the cat, signals from group I muscle afferents of the hindlimb are carried via nucleus z (Landgren and Silfvenius, 1971; Grant, Boivie and Silfvenius, 1973) through the medial lemniscus to a transitional region around the anterodorsal aspect of VB. This region is called transitional since it is on the border between VB and VL. VL receives afferents from the deep cerebellar nuclei and cortical area 4. The muscle afferent region of the thalamus sends projections to area 3a of the cortex. Since the VB/VL border region is not easily distinguished on the basis of Nissl cytoarchitecture, Rinvik (1968) suggests the use of afferent connections as a supplementary way to mark this anatomical subdivision.

Raccoon. According to Welker and Johnson (1965), fibers leaving the thalamus are oriented mediolaterally, while the entering fibers form sagittal planes as they sort out to their respective terminals. This crossing of fibers oriented in two different planes produces the lobulated appearance of VB. (See Figure 1.4). Projections have been found from VB to the primary sensory cortex (SI); from VPI to secondary sensory cortes (SII) (Herron, 1983); from VL to primary motor cortex and from the dorsomedial nucleus to the secondary motor cortex (MII) (Sakai, 1982). Other pathways involving the somatosensory thalamus are currently under investigation and will be reviewed in the Discussion of Chapter II.

Monkey. In the monkey, VPL is divided into two (Nissl) cytoarchitectonically distinct regions: the pars oralis (VPLo) which receives cerebellar and cortical afferents and the pars caudalis (VPLc) which receives lemniscal afferents and cortical afferents distinct from those of VPLo. Tracey, Asanuma, Jones and Porter (1980) observed that the two zones were not easily distinguished when histological preparations of the monkey thalamus were sectioned in the frontal plane. The sharp borders that they found in other planes of section were attributed to non-overlapping divergence of the terminals of afferents from distinct sources.

Figure 1.4. A. Diagram of frontal section through left somatosensory thalamus of raccoon from portion of brain shown in Figure 1.4B. Divisions between representations of different body parts appear in tissue as myelinated fiber laminae. These give this region a lobulated appearance. Figures 1.4C and D are similar diagrams of horizontal planes in this region. (After Figures 4 and 5 of Welker and Johnson, 1965). Abbreviations: CA-caudate nucleus; H-head representation; L-hindleg representation; OB-olfactory bulb; RE-reticular nucleus; T-thalamus; VPI-ventroposterior inferior nucleus.

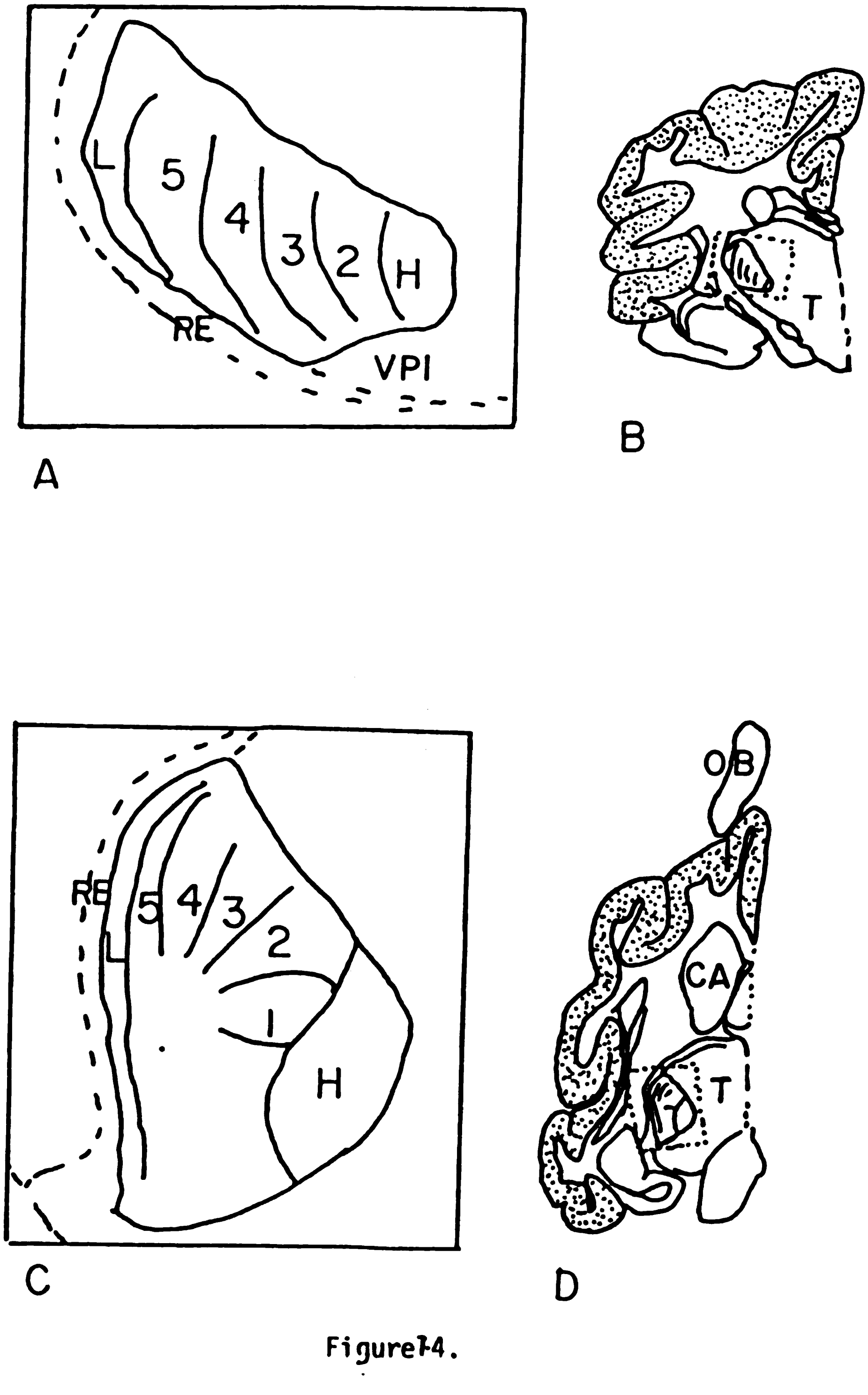


Figure 1-4.

Tracey et al., (1980) also found that VPLo receives fibers from the deep cerebellar nuclei, spinal cord, and area 4 of the cerebral cortex. No VPLo connections with cortical area 3a were found. Kalil (1981) observed cerebellar nuclear projections to VPLo, VL and caudal-most VA in the rhesus monkey. The terminals of these projections are oriented in longitudinal strips.

Kalil (1981) observed projections from the trigeminal-cuneate-gracile complex to terminate in a diffusely scattered manner in VPLc but not in VPLo at all. This projection is spatially ordered such that cuneate nucleus projections are medial to those of the gracile nucleus. Boivie (1978) made lesions in the monkey dorsal column nuclei and found subsequent degeneration of terminals in VPL, POm and zona incerta. In Kalil's (1981) lesions of the dorsal column nuclei (DCN), the degenerating fibers were observed to enter the internal arcuate system after exiting the DCN, then cross the midline at the decussation of the medial lemniscus and then pass through the medial lemniscus to arrive at the caudal thalamus. The fibers then course medial to the medial geniculate body to enter the caudal VPLc by skirting the oral division of the pulvinar. The fibers terminate throughout VPLc. No terminal clustering was found. Injections of tritiated amino acids into the DCN also produced sparse labelling in the suprageniculate and the oral portions of the pulvinar nuclei.

Berkley (1980) observed fiber tracts ascending to the diencephalon of squirrel and rhesus monkeys with the combined techniques of fiber degeneration and tritiated amino acid transport. DCN projections were found to terminate densely in ventral and lateral portions of the pretectal area and zona incerta, less densely in POm and caudal and lateral VPI and hardly at all in rostral POm. Within VB, the terminals of DCN projections form clusters, but not as distinctly as in the cat. Berkley (1980) suggests that these clusters are not apparent in massive injections or ablations because the density of the label or degeneration obliterates the cluster pattern. Although the DCN projections appeared to be confined to VPL, more rostral levels also show terminals in experiments involving massive ablations or injections.

Berkley (1980) also found that the lateral cervical nucleus (LCN) projects to dorsal and lateral portions of POm, caudal and ventral portions of VPL, caudal portions of VPI and the adjoining ventral part of VPM, and the middle part of the central lateral nucleus. Spinothalamic (ST) fibers project densely to clusters in VB which do not overlap with the DCN cluster projections. Other ST projections are to caudal POm and adjacent areas, as well as lateral and rostral parts of VPI.

Topographic arrangements of peripheral representations were first observed in early degeneration studies. Walker (1934) made small cortical ablations limited to individual representation zones (such as face, arm and leg) in both the pre- and post-central gyri of the monkey cortex. The subsequent degeneration revealed a mediolateral arrangement of the face, arm and leg in the nucleus ventralis posterior of the thalamus.

In an axonal transport study of afferents to the VPL of the monkey, Tracey et al., (1980) observed projections to VPLc from the trigeminal-cuneate-gracile complex, areas 3, 1 and 2 of the cerebral cortex as well as the vestibular nuclei.

Lin et al., (1979) observed that deep receptor afferents project via cells lying on the border of VP to cortical cytoarchitectonic zone 2. In a horseradish peroxidase tracer experiment, Whitsel et al., (1978) found that the central part of the macaque VP projects to cortical areas 3b and 1, while the rostral and caudal parts of VP, as well as associated border zones, project to cortical areas 3a and 2 respectively.

Structure and function in somatosensory thalamus.

A set of characteristics of VB thalamus are reviewed by Welker (1973). Mechanical stimulation of the body is the most effective way to evoke cellular activity in this region from the periphery. In many cases, unit activity from adjacent sites in the ventrobasal thalamus (VB) is evoked by stimulation of adjacent regions in the periphery (*e.g.*, Mountcastle and Henneman, 1949). Some mammals have been found to have highly specialized and enlarged topographic representations of those peripheral regions used in active somatic exploration of the environment (Welker, 1973).

*Cat*. In pentobarbital-anesthetized cats, Mountcastle and Henneman (1949) made systematic recordings with ordered rows of microelectrode penetrations. Their grid had 0.5 mm by 1.0 mm spacings. Response fields were observed at 0.25 mm intervals within the individual penetrations. The electrodes measured 400 μm (0.4 mm) in diameter along the insulated shaft and tapered to 40 μm diameter at their tips which had exposed lengths of 50-100 μm. Usually two to three hundred recording sites were observed within a single thalamus in one of their experiments. In pars arcuata (*i.e.* VPM) and pars externa (*i.e.* VPL) of their ventral nuclear group, abrupt increases in background electrical activity relative to that of surrounding regions were found.

Many topographic patterns of peripheral representations were found within the thalamus. Face and mouth responses were located medially and neck, trunk and tail were found in a contiguous progression laterally along the dorsal portion of the somatic sensory responsive zone.

Responses from the extremities were found in the ventral part of this zone with more distal portions more ventral to proximal ones, again with a contiguous progression of responses. This contiguous progression of response zones responding to contiguous peripheral zones defines an organizational principle of the mammalian VB called somatotopy (Welker, 1973). In Mountcastle and Henneman's (1949) study, the representation of the postaxial side of each extremity were found lateral in the thalamus to the representation of the preaxial side. All representations were of contralateral body parts with the exception of some ipsilateral face responses which were found medial to contralateral face and tongue responses. The authors observed that "the degree of localization, the intensity of the response and the volume (of) representation vary directly, and the extent of overlap (between zones responsive to different body regions) indirectly with the peripheral innervation density." The responses were proposed to be carried to the thalamus via ascending projections and not from the cortex since the responses were the same in chronically decorticated cats.

In this same study, thin sections of the thalamus were Nissl stained and recording sites were determined in the tissues by direct measurements based upon stereotactic coordinates of the recording sites (with appropriate accounting for tissue shrinkage). Figurines representing the peripheral zone for effective stimulation are shown in their appropriate locations on a tracing of cytoarchitectonic borders derived from the histological preparations (see Figure 3 of Mountcastle and Henneman, 1949). It is interesting that the pars externa of the ventral nuclear group in their rostral-most plane of section illustrated in the inlay to Figure 3 of Mountcastle and Henneman (1949) contains a dorsal and lateral band which has no cutaneous responses. This may correspond to a muscle or joint responsive region. In summary, Mountcastle and Henneman (1949) describe the general organization of the cutaneous responsive zone of the thalamus as semiconcentric curved lamellae, each representing a body region, nested around one another.

Mallart (1968) and Millar (1973) have also studied the somatosensory thalamus of the cat with microelectrode mapping of response fields. Their results will not be reviewed extensively here since these are not well documented with histological data. In both of these works, muscle afferent projections to the VPL were studied physiologically, with Millar (1973) noting that these responses are located "on the antero-dorsal edge of VPL."

Raccoon. Like monkey and man, the raccoon has large central representations of its forelimbs. It is capable of executing delicate and complex behaviors involving sensorimotor discrimination with its forelimbs (discussed below).

Welker and Johnson (1965) mapped peripherally evoked responses in the raccoon VB. Penetrations were spaced 0.5 to 1.0 mm apart and records were taken at 0.5 mm spacings within the dorsal to ventral passage of the microelectrode. The main body of VB was found to register responses to stimulation of the volar forepaw. (See Figure 1-4). In myelin stained tissues, the zones in which physiological responses were evoked by stimulation of the respective individual digits appeared separate from one another and from the rest of VB. Within the individual digit representations, the radial side of the digit was found medial to the ulnar side representation in the thalamus. Forepaw claw responses were found ventral to this cutaneous digit region while deep tissues and joints of the palm and proximal digits were represented in a "more dorsal . . . " region. Within the thalamic somatosensory region, higher density and higher amplitude microelectrode recording signals were found in comparison to white matter as well as other thalamic regions. Those responses found in VL as well as the posterior nuclear region were characterized by low amplitude signals and no background signal and required large but poorly localized stimulus fields and vigorous stimulation. In the posterior nuclear region, vibratory, auditory and ipsilateral stimuli were also effective. Sharp cytoarchitectonic distinctions were observed between VB and both VL and VA.

Monkey. In the monkey, the ventral posteromedial nucleus (VPM) receives somatic sensory information from the face and the ventral posterolateral nucleus (VPL) receives sensory input from the limbs and trunk (Walker, 1938). In microelectrode mapping experiments, Mountcastle and Henneman (1952) found responses to light tactile stimulation of the periphery in the VB thalamus of the monkey. Somatotopic organization of the response zones was found. Responses to cervical, thoracic, lumbar, sacral and caudal segments were found in more lateral lamellae of thalamic tissue. Axial regions (e.g. shoulder, hip) had more dorsal representations in VB while apical regions (e.g. hand, foot) had more ventral representations. Although somatotopic organization was present, some discontinuities were found in the thalamic representation of the body. The authors suggested that the vast expansion of the hand representation causes these discontinuities. The somatic responsive zones were found to be cytoarchitectonically well-defined in relation to the nonresponsive neighboring areas of the thalamus.

Loe, Whitsel, Dreyer and Metz (1977) studied the anatomical distribution of single unit responses to peripheral stimulation in VPL of the macaque. Deep stimuli evoked responses in both the anterior and posterior borders of the nucleus while the intermediate, main body of the nucleus responded to cutaneous stimuli. A dorso-ventral organization of response sites was found. In the deep responsive regions, effective stimulus sites progressed from hindlimb successively through trunk, shoulder and upper and lower arm as the electrode descended from a dorsal to more ventral positions. As the electrode descended further, cutaneous responses were found to progress in a reverse order than that of the deep response region to find hindlimb ventral-most. Responses from individual peripheral regions were interpreted as being located in individual curved lamellae of neurons extending continuously from the dorsal boundary of VPL to the ventral boundary, with each lamella devoted to the representation of a single body region. This group had difficulty correlating cytoarchitectonic boundaries with peripheral response zones in their frontal sections.

In awake monkeys, Horne and Porter (1980) found many cells in the caudal part of the VL, pars oralis (VLo) and rostral VPLo cells firing with arm movement. Half of the responses were to contralateral stimuli and half to ipsilateral stimuli. Most ventrocaudal VPL cells responded to contralateral deep stimuli. Limb manipulation and cutaneous stimulation evoked responses in VPLo and VPLc.

Friedman and Jones (1981) observed that there is a 300-500 μm thick "shell region" in the anterior aspect and the dorsal part of VPLc in the macaque. Deep stimuli such as joint movement and manipulation of tendons and muscles elicited responses in their shell region.

Maendly, Ruegg, Wiesendanger *et al*., (1981) studied short latency field potentials in a region of the VPL of the macaque. The stimulus that they used was electrical stimulation of group I muscle afferent fibers. The responsive region included what these authors termed the "rostral cap" of VPL. This region consists of a zone 3 mm long (anterior to posterior direction), 2 mm wide (mediolateral) and a maximal dorsoventral extent of 5 mm. The stimuli which were effective in evoking responses in this region also evoked responses in area 3a of the cortex.

Dykes *et al*., (1981) studied the locations of fields responsive to different stimuli specific for either quickly adapting cutaneous, slowly adapting cutaneous, deep or Pacinian receptors in the VPL and VPI of the squirrel monkey, *Saimiri sciureus*. Separate body representations were found for each modality of receptor. The authors feel that these are individual representations since the "receptive field locus move(s) significantly with each change in response class." An example of the progression of response modalities with dorsoventral passage of the electrode is: high threshold tap->deep->quickly adapting cutaneous-> slowly adapting cutaneous->quickly adapting cutaneous. In many cases, modality changes were seen at cytoarchitectonic boundaries of thalamic subdivisions. Responses were found within 100 μm of entering a given region. Deep responses were found in a "region extending 500 μm immediately above VPL." Convergent quickly adapting responses from many peripheral locations were sometimes found in the layer between the deep and slowly adapting response zone. The order of modality response zones encountered by an electrode passing in the dorsoventral direction remained quite constant between penetrations and deviations in this order were due to the absence of a response type rather than a change in order.

Human. In the human, as in the monkey, there are histologically and physiologically distinctive zones responsive to forelimb cutaneous and deep stimuli. The volar cutaneous responsive zone is centrally located in the ventral caudal thalamic nucleus while the deep responsive zone is more rostral and dorsal (Tasker et al., 1972) in the ventral intermediate nucleus. Ohye (1978) observes that the ventral intermediate nucleus is a 3-4 mm thick layer of cells with a dorsoventral extent of 10 mm. Although extensive, systematic mapping experiments are not done in individual humans, there are indications that this proprioceptive region of the thalamus is somatotopically organized (Ohye, Fukamichi and Narabayashi, 1972).

## Parkinsonism and the kinesthetic thalamus of humans.

Parkinsonism, or paralysis agitans, is a group of neurological disorders characterized by bradykinesia (or hypokinesia), tremor, muscle rigidity and sometimes excessive perspiring and feelings of heat. This discussion concerns the slowly progressive form of Parkinsonism which occurs late in life and is of unknown etiology. A common symptom in Parkinsonism is a resistance to passive movements involving both flexors and extensors. This resistance is fairly constant throughout the range of the movement and is independent of the speed of the movement (Brodal, 1981). Tendon reflexes are not altered. This indicates that the phasic stretch reflex is not exaggerated in Parkinsonism rigidity. Tonic properties of the stretch reflex are exaggerated however (Shimazu et al., 1962; Jansen, 1962). Increased muscle spindle sensitivity has been suggested to be the cause of this spasticity since procaine infiltration of muscles in Parkinsonism patients abolishes rigidity (Walshe, 1924; Rushworth, 1960).

Post-mortem examinations of the brains of Parkinsonism patients show loss of nerve cells and depigmentation in the substantia nigra. Changes have been observed in both the pars compacta and the pars reticulata of the substantia nigra as well as in the locus coeruleus. Neurons in the pars reticulata are not dopaminergic and have projections to the thalamus. Neostriatal dopamine levels are below normal. The dopamine depletion and depigmentation led researchers to try treating patients with L-DOPA, a dopamine precursor which crosses the blood-brain barrier. This treatment is most successful for relief of bradykinesia and secondarily for rigidity symptoms. Apomorphine, a dopamine receptor agonist, is primarily effective for tremor and less effective for rigidity and bradykinesia (Barbeau, 1976). These drug therapies are very popular in the U.S. despite their serious long-term side effects and the fact that they only serve to control symptoms for a few years.

Many authors have observed rhythmic neural activity in synchrony with tremor in the deep receptor afferent region as well as the cerebellar afferent region (VL) (e.g. Cordeau, 1966; Jasper and Bertrand, 1966; Albe-Fessard et al., 1967; Hardy, Bertrand and Thompson, 1979; Ohye and Narabayashi, 1979). The early reports may have prompted the use of thalomotomy procedures for relief of tremor. Stereotactic surgery for the relief of tremor and rigidity in Parkinsonism patients is rarely performed in the U.S. any longer. The operations have ceased here because of problems with continued bradykinesia and impaired postural reflexes as well as one to three year post-surgical recurrences of tremor and rigidity symptoms (Markham, Brown and Rand, 1966).

A review of the effects of the many types of clinical lesions of the thalamus for the relief of Parkinsonism symptoms cannot be done here.

Worthy of note is the fact that the most effective lesion therapies to date were reported by Narabayashi and Ohye (1980). Small coagulative lesions of diameter 3 mm in V.im. were found to abolish tremor in Parkinsonism patients as well as in patients with postural or intention tremor due to midbrain or cerebellar pathology. The Japanese workers suggest that those patients for whom thalamic lesions were not effective may have been lesioned in the VL or other nuclei than the V.im. In order to assist in correct placement of the electrode in V.im. during the surgical procedure, the lightly anesthetized patient is requested to move body parts in order to evoke neural activity. (The lesioning electrode is recorded from at this time.) Remarkable successes have been shown with this procedure allowing totally incapacitated individuals to resume normal levels of physical activity.(Narabayashi and Ohye, 1980; personal communication, H. Imai).

The reason for the effectiveness of microthalamotomy procedures for the relief of tremor is not yet known. The V.im. nucleus of the thalamus is not usually implicated or discussed in theories of the etiology of Parkinsonism. Rather neurotransmitter metabolism and nigrostriatal pathways are emphasized. However, as Brodal (1981) reasons, since the main nuclei showing pathological changes in Parkinsonism (substantia nigra and globus pallidus) have few descending efferents, their influence upon motorneurons must involve other nuclei and pathways. Ablation of precentral cortex, cerebral peduncle and dorsolateral funiculus are effective in treatment of tremor and other dyskinesias. However, hemiparesis always occurs as well. These results indicate that corticospinal pathways are involved in these movement disorders. However, the significance of the alleviation of symptoms following the placement of lesions is difficult to determine without precise information about the region destroyed (including fibers of passage).

## Phylogeny and comparative anatomy of the somatosensory system of raccoons.

Phylogeny. The raccoon is a member of the order Carnivora, superfamily Canoidea and family Procyonidae. Within the Carnivore order are also bears (family Ursidae) and dogs (family Canidae) as well as the less closely related cats (family Felidae). Through the discovery of a fossil skull of the now extinct Phlaocyon of the lower Miocene, some authors suggest that the Procyonidae descended from broad molar-possessing canids (Scott, 1937; Ewer, 1973). The primate and carnivore orders descended from an arboreal stock of small shrew-like insectivores in the early part of the Cenozoic era. In addition to the fossil data from which the above conclusions were derived, cytogenetic data support the canid ancestry of the raccoon (Wurster and Benirschke, 1968).

Evolutionary theory provides several justifications for raccoons and humans to have similar neural substrates and processes for gaining information about the environment via forelimb mechanoreception. As mammals, both have a common evolutionary heritage which provides the genetic potential for similar karyotypic features to form during nervous system ontogeny. Furthermore, the raccoon and primates (and presumably early hominids) both have (or had) arboreal habitats which have been "postulated by evolutionists as an influencing factor in the development of primate intelligence" (Johnson and Michels, 1958).

The primate-like characteristics which the raccoon has developed include prehensile extremities, a proclivity for manipulation and long- and short-term memory (discussed below). These similarities may be related to the (human-like) omnivorous eating habits of the raccoon. This must be considered in light of the importance of dentition patterns in our interpretation of fossil data. The eating habits of an animal define the type, and degree, of sophistication with which the animal must deal with its environment. For example, a predatory lifestyle requires forms of sensorimotor coordination which is not needed in herbivorous ruminants. An omnivorous lifestyle requires a more diverse repertoire of food capture and feeding behaviors than either the carnivorous or herbivorous modes. Another common trait of human and raccoon are the notable lack of running skills or extraordinarily deadly or protective physical features. In order to survive in their environments, raccoon and man must have both experienced selective pressures to evolve advanced mental abilities.

Comparative Neuroanatomy. Neural tissues serve as a substrate for physiological processes which determine and shape sensory, cognitive and behavioral actions of animals. By comparing the structure of these tissues and the related activity patterns between animals from different taxonomic groups, we may gain insight into the evolution of neural structures and their functions. The correlations which are provided by such studies do not take the form of predictive, cause-and-effect laws of the form "if animal A has structure B it must then execute function C" or the converse. Rather than observing laws relating structure and function, we will find correlations of varying degrees of reliability. This is because neural structures are involved in a great variety of functions which may well not be best described by the discrete behaviorally based categories that are conventionally used. The data presented may also appear incomplete because of gaps in the fossil record (raccoons may have been too smart to be trapped in fossil-preserving tar pits; see Stock, 1929, p. 288) and because of the great deal of research yet to be done in this field.

The raccoon has a larger number and proportion of neurons devoted to carrying signals from receptors in the volar surface of the forepaw than many other mammals (Welker et al., 1964). The receptive fields of afferent units in the volar forepaw are smaller than those of both cat and monkey (Pubols, Pubols and Munger, 1971). Pubols, Welker and Johnson (1965) found a higher degree of ventral forepaw representation in the dorsal root fibers than that of coatimundi (also a member of the Procyonid family) and cat. Although these authors claim that the raccoon has a greater ratio of cutaneous to deep afferent units in the forelimb, only the raccoon vs. cat comparison appears to show significant differences. Johnson, Welker and Pubols (1968, p. 28) observe that the raccoon has a more extensive forepaw representation in the medullary dorsal column nuclei than does the cat. Johnson (1980) illustrates the proportionately greater representation of the forepaw in the raccoon medulla compared to sheep and opossum. The hand representation also predominates in the raccoon somatosensory thalamus compared to cat and macaque.

The raccoon's expanded cortical representation of the forepaw is of particular interest since one of the human's most notable neurological features is its hypertrophied cerebral cortex. From a comparative viewpoint, it is tempting to link the human's and the raccoon's propensity for sensory discrimination with this structural feature. The "mushroom-like" (Welker and Seidenstein, 1959) growth of sulci and gyri during ontogeny serves to enlarge the surface of the cortex. Since the geometry of the neural circuits of the cortex is such that the functional unit is a columnar aggregate of cells oriented perpendicular to the surface, increased surface area means increased capacity for functional units.

Woolsey (1959) compared fissuration of the cortices of cat, dog and raccoon. In the brains of cat and dog, sensory and motor areas are distinct, but no central sulcus separates them. However, the coronal sulcus separates arm and face sensory representations. The raccoon has an incomplete central sulcus separating the sensory and motor cortices. The post-central sensory region has a large forepaw representation (Welker and Seidenstein, 1959). While the brain of the raccoon is intermediate in size to that of dog and cat, the hand cortical representation has a surface area exceeding that of both these animals. In addition, the raccoon's cortical representation of the hand has four times the surface area of the homologous region in the monkey (see Table II). The raccoon has a larger proportion of its sensory cortex devoted to the hand representation than any other primate observed by Welker and Seidenstein (1959). The peripheral projections to the forepaw sensory representation in the raccoon cortex are also more sharply defined and specifically localized by gyral and sulcal patterns than those of any other animal.

Table II. Area of cortical sensory zones in cat, dog, raccoon and monkey (references in text).

| Total sensory area (SI) in cortex | Animal | Area (mm$^2$) | % of SI |
|---|---|---|---|
| | Cat | 150-200 | |
| | Dog | 500-600 | |
| | Raccoon | 1000-1100 | |
| Hand sensory area in SI cortex | Cat | 45-60 | 30 |
| | Dog | 100-120 | 20 |
| | Raccoon | 500 | 50 |
| | Rhesus monkey | 125 | |

Raccoon behavior. The raccoon forepaw anatomy determines its range of activity in tactile sensation and exploratory behavior. The thumb is not opposable. The ventral surfaces of both the fore- and hind-paws are hairless and have glabrous skin like those of primates including humans. The digits are long and well separated. The claws are long, sharp, curved and nonretractible. When an object is manipulated, the forepaw moves as a unit with no individual digit motions. Small objectives are usually grasped with two hands with the object contacting volar surfaces. For grasping a small object with one hand, the digits are all flexed in unison and the object is held between the digits and the palm. Small objects like coins can also be grasped between adjacent digits. Rue (1964) has observed raccoons to extract several coins from a pocket simultaneously in this manner. He also notes that they can pull corks, unfasten bottle tops, open door latches and turn door knobs. Cole (1912) has observed raccoons touching objects to their noses in addition to manipulating the objects.

Racoons often live and forage near moving bodies of water. Their omnivorous palates enjoy meat, grain, insects, berries, fruit and eggs. Food is often found on the ground. Raccoons will hunt in shallow pools, turn over rocks and dig into rotten logs. They will eat crayfish, clams, fish, insects, frogs, snakes and mice as well as small squirrels, rabbits and muskrats. The eating behaviors related below were described by Davis (1907), Rue (1964), Schwarts and Schwarts (1959), Scott (1937) and Welker and Seidenstein (1959).

The forepaws figure importantly in the raccoon's food acquisition and handling behaviors. Raccoons have been observed to catch insects in flight with their forepaws. Food is sometimes picked up in minute morsels and nimbly conveyed to the mouth. The digits are dextrous enough to locate food by touch alone, without the use of vision. The raccoon rarely looks when fishing for tadpoles, minnows and crayfish in shallow water. Buxton and Goodman (1967) also found that the raccoon manipulates objects without visually observing them. While manually searching for prey in the water, the raccoon may scan riverbanks and treetops or just stare straight ahead. In a shallow pool, the raccoon may just wade in and sit down. The bottom is then scratched at stirring up the sediment. Fish which lodge in the racoon's fur are grabbed and eaten. Larger fish may be hooked from shallow pools with the claws. Crabs may be lured to clasp the tail of the raccoon, at which point the crustacean is flung onto dry land and consumed.

There is a vast anecdotal literature documenting the raccoon's problem-solving abilities and capacity for associative memory. Anthropomorphic descriptions of raccoon behavioral attributes include: "fear," "greed," "play," "washing," "attention" and "curiosity." Davis (1907) tested the raccoon's ability to solve puzzles and its memory with a variety of locking devices singly and arranged in tandem. He found typical learning curves. As the animals repeatedly worked on a puzzle lock, they eliminated useless movements that they employed in earlier attempts. Older animals appeared more efficient in their problem-solving strategies. Most animals appeared to respond to small differences in complex relations within the different lock problems. Davis (1907) felt that associative learning was taking place since new lock problems were solved faster when a previously solved one was similar. Hence there was some generalization of what was learned. Kitzmiller (1934), in a brief note, describes trial-and-error learning of escape from a "puzzle box." The raccoons remembered the solution of the puzzle (as evidenced by briefer solution time) for short and long periods (one to two years).

Rue (1964) observes that vocalization patterns in raccoons appear to convey messages. Furthermore, he credits raccoons with a greater variety of calls with a higher incidence than any other animal he has observed.

The raccoon has earned its name in German and Latin for its "hand-washing" behavior. The forepaws are immersed in water and splashed about, sometimes while clasping an object or piece of food. Rue (1964) supports the contention that the raccoon is not a washer, but a "dunker," "feeler" and "investigator" (see also Lyall-Watson, 1963). Contrary to early food-washing theories, raccoons have been observed to eat food without immersion in water, even with a good supply of water readily available. Welker and Seidenstein (1959) observed in their electroanatomical study of the raccoon cortex that moistening the tips of the digits reduced the minimal receptive field size. Hence the sense of touch is heightened when the digits are wet.

The raccoon's forelimbs are also suited to a diverse repertoire of locomotor activities whether foraging on the ground and in the water or residing in its arboreal habitat. The raccoon has a plantigrade stance like man and the print of the hindpaw resembles that of a small child. A raccoon foraging for food has a slow shuffling walk which is frequently interrupted as it picks up and manipulates objects while seated on its haunches (Rue, 1964). The raccoon can also stand balanced on its hindlimbs. The tail (not prehensile) is used for balance in climbing and in turning sharp corners and may be used as a brace when seated on its haunches. In addition to the slow shuffling walk, Rue (1964) has observed raccoons in a faster dogtrot with left limbs and right limbs alternating and at their fastest, abound with hindlimbs and forelimbs moving in respective synchrony. The raccoon can't keep this fastest pace going for longer than 15 minutes and will climb a tree to escape persistent enemies.

Raccoons are expert climbers and often make their homes in (usually hollowed out trunks of) trees (Schwartz and Schwartz, 1959). Raccoons, like their carnivore relatives the martens, cats and coatimundis, have claws which allow a secure hold while climbing. But once in the branches, they walk with their normal flat-footed stance with no toe-grasping or claw-clinging. They may descend head or tail first and often jump (Schwartz and Schwartz, 1959). When descending a tree, the feet alternate and the claws clench the bark. The hindfeet may be turned out or positioned completely backwards in tree climbing. Rue (1964) observes that if the raccoon loses its footing in a tree, it will try to catch a branch with a claw or a foot and hang upside down. If a fork is nearby it will pull itself upright; otherwise it may crawl along the branch upside down in a sloth-like manner. Rue (1964) also observes that raccoons can swim well.

The raccoon makes extensive use of its forepaws for manipulation and tactile exploration of the environment compared with other carnivores such as the dog and cat (Davis, 1907; Cole, 1912; Goldman, 1950; Whitney and Underwood, 1952; Welker and Seidenstein, 1959; Welker, Johnson and Pubols, 1964). An electrophysiological map of the motor cortex (MI) of the raccoon revealed a greater variety of motions associated with exploratory behaviors than could be elicited in dog or cat (Hardin, Arumugasamy and Jameson, 1968). However, the monkey and human have much greater mobility in the use of the hand. Rensch and Ducker (1963) found that tactile discrimination in the raccoon is of the same order as that of humans in tests where the subject distinguishes between various textured and sized spheres.

Welker and Campos (1963) compared the closely related raccoon and coatimundi which respectively employ the forepaw and rhinarium in exploratory behavior. The predominant evoked response in the primary somatosensory cortices of these animals are from these respective regions. Welker, Johnson and Pubols (1964) compared enlargements in the cortical sensory representation between the coatimundi and the raccoon. They found that the degree that a body region figured in tactile exploratory behavior is proportional to the receptor density of that body region.

Statement of the problem.

The raccoon shows outstanding development of 1) its neural substrates and processing, 2) its sensory cognitive and behavioral activities and 3) its level of environmental adaptation associated with the uses of its forepaws. This conclusion is supported by comparing characteristics and features related to forepaw use in the raccoon to those in cat, dog, monkey and man. Expansion and specialization in the forepaw-related somatic sensory system of the raccoon parallel the general proliferation of forebrain systems in man. The raccoon forepaw sensory system appears to be an accessible model system for the study of types of higher brain function (and possibly pathology) which are found in man due to the similar hypertrophy of cortical pathways and sophistication of behavior. Unfortunately, many of the studies reviewed above provide findings of limited value since they are difficult to integrate into a common body of knowledge. Nomenclature has not been standardized. Borders from respective anatomical studies involving connectional or functional mapping have yielded results which are difficult to integrate with one another. A comprehensive series of experiments which simultaneously test the interaction of these various aspects of this system is needed to provide integrated understanding. It is not known how the raccoon somatosensory thalamus and cortex processes information received from various sources including deep, hair, claw and cutaneous receptors. Are there separate zones that processes signals from these submodalities? What are their locations and relations to other regions of the thalamus? Are they all relatively large like the volar cutaneous representation? How are projections from different body regions organized within them? How may they be distinguished with histological and histochemical techniques from other functionally responsive regions? How do these anatomical, connectional, biochemical and behavioral aspects interact?

Approach.

Responses evoked from deep stimulation are recorded with microelectrodes from penetrations ordered in a fine grid in the thalamus. This shows the location and organization of a deep responsive region(s). Subsequent histochemical treatment of the regions recorded from shows the architectonics of the recording sites and their surroundings. The results from this study are correlated with those from parallel studies currently being carried out in this laboratory concerning the fiber connections of the respective somatosensory submodality projection zones of the thalamus with those of the cortex, medulla and spinal cord. This provides for the first time, an integrated view of location and arrangement of specific sensory projections, their sources and subsequent destinations in the somatosensory pathway and how these relate to the architectonics of cell bodies, myelinated fibers, mitochondria and acetylcholinesterase-containing structures.

Methods.

Nissl staining of neural tissue outlines the soma and occasional proximal portions of dendrites of neurons. Glial cells are also stained. Nuclei, subnuclei and other anatomical subdivisions can be characterized by soma sizes, orientations and distributions, soma packing densities and degree of clustering. The hypothesis that regions with distinctive somatosensory responses have distinctive organizations of Nissl staining is tested.

Myelin staining is used since myelinated fibers form particularly distinctive borders between the individual digit as well as hindlimb cutaneous representations in the ventrobasal complex of the raccoon thalamus (Welker and Johnson, 1965). The hypothesis that other types of response fields will also be bordered and subdivided by myelinated fibers is tested.

Enzyme histochemistry offers the advantage of quantitatively staining substances with specific biochemical activities and, in some cases, known metabolic functions. Although Nissl substance stains and myelin stains are more frequently used in light microscopic tissue studies, enzyme histochemical procedures are used here to complement the conventional stains in order to determine if they demarcate functional borders in the tissues. The hypothesis that functionally different regions of the thalamus, as determined by electrophysiological mapping with biochemical and metabolic differentiation, appear distinctive in the enzyme activity staining is tested.

Cytochrome oxidase (cytochrome c oxidase; ferrocytochrome c; oxygen oxidoreductase EC 1.9.3.1) is a mitochondrial enzyme involved in the electron transport pathway of cellular oxidative metabolism. Cytochrome oxidase activity levels within specific sensory pathways (as evidenced by histochemical staining) have been shown to reflect long-term levels of sensory-evoked neural activity. This has been shown through experiments where surgical disruption of the auditory, visual or somatosensory pathways caused reduction in cytochrome oxidase staining activity one to several synapses away from the surgery site (Wong-Riley, Merzenich and Leake, 1978; Wong-Riley et al., 1978a; Wong-Riley, 1979 and Wong-Riley and Welt, 1980). In the auditory system this reduction in cytochrome oxidase activity can be reversed by electrical stimulation (Wong-Riley et al., 1978b).

Cytochrome oxidase activity staining was selected for this study of the raccoon somatosensory thalamus since cytochrome oxidase staining in mouse cortical barrel fields, which receive vibrissal somatosensory projections, reflects neuropil organization, as well as peripheral input levels at a critical stage in development and indicates the location of the respective vibrissal projections (Wong-Riley and Welt, 1980). Disruption of the particular whisker vibrissae at a certain time period during development disrupts the respective cortical barrel fields as evidenced by staining for cytochrome oxidase activity. Thus cytochrome oxidase staining indicates sensory input levels of specific subunits of the zone of projections from mouse vibrissae, which are involved in exploratory behaviors. Hence, we questioned whether the dominance of the use of the forepaw in raccoon exploratory behaviors is reflected by cytochrome oxidase staining at the thalamic level of the somatosensory pathway to the raccoon cortex. Although Wong-Riley and Welt's (1980) findings were in the cerebral cortex, we were encouraged by the work of others with succinate dehydrogenase, another mitochondrial energy-transducing enzyme, staining in the thalamus. With this stain, the projections from individual mystacial vibrissae are apparent in the ventrobasal thalamus as well as in three separate locations in the medulla, and in the cortex of the neonatal rat (Belford and Killackey, 1980; Killackey and Belford, 1979).

Acetylcholinesterase histochemistry has been used for many years and has been recently modified to yield high sensitivity and to be compatible with horseradish peroxidase histochemistry in the same tissues (Hardy, Heimer, Switzer and Watkins, 1976). In general, neurotransmitter metabolic enzyme stains indicate relative levels of innervation by afferents which employ the respective transmitter.

However, the significance of acetylcholinesterase staining is not entirely clear since its distribution does not always coincide with that of choline acetyltransferase. In order for acetylcholine release at synaptic terminals to be useful for neural signal processing, the appropriate synthetic and inactivating metabolic enzymes must be nearby. The presence of active agents that catalyze the requisite chemical reactions (perhaps in addition to other activities) is not sufficient evidence that the related transmitter is functional at local synapses. But the absence of such enzyme activities in a given region is strongly suggestive that said transmitter is not employed.

In this study, acetylcholinesterase staining is employed in electrophysiologically mapped tissues to determine if this stain demarcates the borders of the mechanosensory projection zone with other thalamic regions. There are several reasons to expect this to occur. From the illustrations of Jacobowitz and Palkovits (1974), it appears that acetylcholinesterase activity occurs along the dorsal and caudal borders of the ventrobasal thalamus of the rat. Acetylcholinesterase staining is sparse in the ventrobasal thalamus of the dog relative to that of unidentified regions which appear to border it dorsally and medially (Sakai and Tanaka, personal communication). In the monkey, Parent _et al._, (1977) observed "a few lightly stained acetylcholinesterase containing neuronal somata are scattered within the caudal part of the lateral territory mainly occupied by the (ventro)posterolateral and (ventro)posteromedial nuclei . . ." These data indicate that acetylcholinesterase may demarcate the borders of the mechanosensory projection zone.

_Fine grain electrophysiological mapping_ is a specialty of this laboratory and has been used for over a decade here (_e.g._ see Welker and Johnson, 1965 and Haight, 1971). The importance of this technique in the present work is that it allows sampling of the neural activity of a small region (approximately 50-100 μm radius, depending upon electrode characteristics and signal source amplitude) while preserving the histological integrity of the surrounding tissue. The electrodes are constructed of etched tungsten ensheathed in glass (in the manner of Cabral and Johnson, 1971 and Baldwin, Frenk and Lettvin, 1965). This sturdy design permits the shaft diameter of the electrodes to measure between 60 to 100 μm, in contrast with those of earlier mapping studies (Mountcastle and Henneman, 1952 and Welker and Johnson, 1965). Since the recordings are all extracellular and from a large region (from a single neuron vantagepoint), a wide variety and large number of recording sites can be recorded from in a relatively short amount of time. This provides a large data base which will facilitate the resolution of organizational principle.

The stimulation procedures used to evoke thalamic somatosensory responses were selected on the basis of accuracy, simplicity, rapidity and similarity to actual environmental stimuli. In these procedures, peripheral somatic stimuli are produced by direct manipulation and with small wooden applicator sticks with sharpened tips. A systematic series of procedures are used to identify those peripheral zones which elicit activity at the microelectrode recording site. The experimenter first quickly strokes the contralateral side of the animal's body. This includes moving the extremities about as well as stroking the head, tail and belly. Ipsilateral stimulation is periodically attempted especially in the head representation region. If no responses are elicited, the experimenter tries to elicit auditory and visual responses with handclaps and occluding light from the subject's eyes. If no responses are found the microelectrode is advanced to the next recording site. At sites where a response appeared to be elicited from stimulation of a particular region, the effective stimulus was further identified. In glabrous skin and claw regions, light touch was applied by brushing the pencil point-sized tip of a wooden applicator stick across a small patch of the skin or claw surface. In hairy regions, only a few hairs were displaced with the applicator with care not to displace the skin surface. If these stimuli were effective, the extent of the responsive region were recorded along with the identity of its modality (skin, claw or hair). If light stimuli failed to produce a response at a site where the initial stroke was effective, more vigorous stimuli were used. Deep rubbing, deep rapid poking and joint movements were the main effective stimuli for what we term "deep responses."

In intact, undissected subjects, peripheral response fields are very difficult to accurately determine, especially for deep responses. In many response fields, regions other than those under study may be inadvertently stimulated by: 1) passive forces on the subject's body due to its resting position or gravity, 2) motion transmitted mechanically through the body to regions other than the direct stimulus site during active stimulation, 3) the experimenter displacing part of the body in order to better expose a region for stimulation. These problems are best avoided by conscientious application of controlled stimuli to the region of interest using several different approaches and positions. For deep stimuli it is critical to determine whether the response is truly from a muscle, tendon or joint or if it is a high threshold or pain response from skin receptors. In order to resolve this problem, all responses to poking, rubbing or kneading were elicited with at least two different regions of skin overlying the stimulated region. The looseness of the raccoon's skin on its trunk as well as arms and legs down to the ankle and wrist allow the skin to be displaced in this manner. In the undissected raccoon, some response fields remain ambiguous with these techniques. These include deep and high threshold cutaneous stimuli to forepaw, hindpaw and head, receptive fields with specific vibration sensitivities and cutaneous responses to hairy skin surfaces as opposed to hairs only.

In order to process the types of information conveyed from the mechanoreceptors of muscles, tendons and joints, the neural architectonics will be studied to find if they take on a specialized configuration which differs from that of the regions receiving cutaneous receptor signals. Such a specialized configuration would serve as a substrate for the particular signal processing required to carry out the unique sensory processes that this region must perform. An alternative, but unlikely possibility is that the same types of thalamic regions which process sensory information about the location, direction and intensity of stimulation at cutaneous receptors are involved in processing the inputs from deep receptors which convey information of many other types including for example, feedback useful in motor coordination. The significance of the lobulated expanded representation of the volar forepaws in the raccoon may be better understood by comparison with other regions of the thalamus which represent other modalities and body parts which are involved in other types of behaviors.

# CHAPTER II

# ORGANIZATION OF KINESTHETIC AND MECHANOSENSORY PROJECTIONS TO THE VENTROBASAL THALAMUS IN RACCOONS: MAPPING OF EVOKED UNIT CLUSTER ELECTRICAL ACTIVITY

# INTRODUCTION

The organization of those neural systems in mammals which transfer and transform electric impulses signalling peripheral kinesthetic (deep muscle, joint and tendon) stimulation has recently been under intense investigation. Studies usually focus on relevant anatomical structures such as cell aggregates of the somatosensory nuclei of the medulla, thalamus and the somatosensory cortex, and the fiber pathways connecting them as well as the distribution of functional electrophysiological response fields within these structures.

The forepaw representation in the raccoon somatosensory system is a model system for advanced mammalian mechanoreception (this term includes cutaneous and kinesthetic somaticensation) because: 1) in the raccoon, relatively large portions of the entire nervous system, as well as of the somatosensory system, are devoted to conveying and receiving projections from cutaneous mechanoreceptive fields of the glabrous forepaw (see, e.g., Welker, et al., 1964), 2) Raccoons express complex active exploratory and problem-solving behaviors with their forepaws (e.g., Davis, 1907; Kitzmiller, 1934; Lyall-Watson, 1963; Rue, 1964). In addition to providing a model for higher brain function, the raccoon is also easy to procure and maintain.

The kinesthetic region of the somatosensory thalamus is also of interest since Narabayashi and Ohye (1980) observed that lesions of this region in humans relieves symptoms of Parkinson's disease as well as postural and intention tremors. Study of the kinesthetic region of the thalamus may further understanding of the etiology of, and therapies for these disorders.

Here we describe the organization of mechanoreceptor projection fields of the raccoon thalamus with special attention to those arising from kinesthetic receptors and those from combinations of glabrous skin, hairy skin and claws. In related papers, the medullary sources of input, the neocortical targets of output, and the anatomical and histochemical properties, of these thalamic fields are described (Ostapoff and Johnson, 1983; Ostapoff, Johnson and Albright, 1983; Johnson, 1984; the accompanying paper).

The raccoon has expanded representations of the hand throughout the somatosensory system. Welker and Johnson (1965) found large, distinct zones within the somatosensory thalamus of the raccoon which yield unit-cluster electrical responses to light tactile stimulation of the respective digits of the volar forepaw. Responses to the radial side of the digit were found medial to the ulnar side representation. Forepaw claw responses were found ventral to this cutaneous digit region while deep tissues and joints were represented in a "more dorsal" region. Somatotopic organization was found to be a dominating principle throughout the cutaneous mechanosensory projection zone of the thalamus with rostral body regions represented medial to more caudal body regions. Our extensions from that study include:

1) Microelectrode penetrations, as well as recording sites within individual penetrations, were spaced more closely together to uncover finer organizational details, 2) Kinesthetic receptive fields were carefully stimulated in addition to the cutaneous mechanoreceptors.

Recently, Johnson, Ostapoff and Warach (1982) observed three types of mechanoreceptive projection zones in the anterior portion of the primary somatosensory (SI) cortex of the raccoon. These are from 1) glabrous skin, 2) muscles and joints and 3) "heterogeneous receptive fields ... (including) single and multiple claws and dorsal hairy surfaces of digits and proximal hand along with additional projections from volar surfaces." One of the goals of this study was to find possible thalamic analogs to these three mechanoreceptor projection zones of the cortex.

# EXPERIMENTAL PROCEDURES

The subjects were 14 adult raccoons (Procyon lotor) of both sexes weighing between 3.9 and 10.9 kg when trapped in the wild in the vicinities of Michigan State University and Purdue University. The subjects were immobilized with an intramuscular injection of ketamine hydrochloride (15-25 mg/kg) and xylazine (Rompun, 2.0-2.5 mg/kg) to facilitate subsequent intraperitoneal (IP) injection of diallylbarbituric acid buffered with sodium hydroxide (37-45 mg/kg) and urethane (150-175 mg/kg). Supplemental doses were 1/5 to 1/2 of the original dose of each agent as needed to eliminate nociceptive reflexes. In long term experiments, the subjects were administered 5% dextrose in saline IP at six to eight hour intervals. Experiments extended from 8 to 60 hours.

Surgical procedures. In several subjects, the trachea was cannulated with a Y-tube to facilitate respiration. Usually 2-3 $cm^2$ of brain surface was exposed. In several animals, the suprahippocampal cortex dorsal to the thalamus was aspirated. The exposed brain surface was protected under a layer of warm mineral oil. The skull was oriented with a custom-built stereotaxic device which aligned the bottom of the orbit with the ear canal in the horizontal plane and the other stereotaxic planes in their respective (orthogonal) relations. Screws placed in the skull were cemented with dental acrylic to metal bars attached to a stable surface.

Recording procedures. Microelectrodes were constructed of etched tungsten ensheathed in glass (in the manner of Cabral and Johnson, 1971 after Baldwin, Frenk and Lettvin, 1965). The tapered shafts had diameters ranging from 20-60 μm with uninsulated tip exposures of 10-20 μm.

An electrical ground was inserted in neck muscles. The microelectrode signals were amplified, filtered at 80 Hz (low) and 10 kHz (high), and displayed through an oscilloscope and an audio monitor.

Rows of microelectrode penetrations were spaced 0.5 mm apart and were all oriented in either parasagittal or coronal planes in each subject. Within the rows, penetrations were separated by approximately 0.1-0.8 mm and coursed in a stereotaxic dorso-ventral direction. Within somatosensory responsive zones, a micromanipulator was used to advance the microelectrode 50 to 300 μm between recording sites. Prior to many penetrations, the microelectrode tip was dipped in a 30-50% solution of horseradish peroxidase (HRP; in 0.05 M Tris buffer, pH 8.3). In order to mark the locations of recording sites in the thalamus, small lesions were made in the tissue by passing DC current through the recording electrode.

<u>Stimulation procedures</u>. Cutaneous (including glabrous skin, hairy skin and claw) mechanoreceptors were stimulated mechanically to evoke unit cluster (and occasional single unit) electrical activity in the contralateral thalamus with the "microelectrode method of electrophysiological mapping" described by Welker and Johnson (1965). However, if light tactile stimuli failed to evoke a response, deep probing and rapid poking were employed. If this evoked a response, the skin overlying the receptive field was displaced and the effective stimulus was again applied. If the effective receptive field did not move with the displaced skin, the response was identified as <u>muscle</u>, <u>deep</u> and <u>kinesthetic</u>. (Except for the face and extremities, raccoons have a loosely hanging, easily displaced skin, making this identification easy and unequivocal, particularly

for the limbs.) In those recording sites in thalamus where neither light tactile nor deep muscle stimulation evoked unit cluster activity, but joint movement was effective, then the response was identified as _joint_, _deep_ and _kinesthetic_. A category of _cutaneous pressure_ response is used to describe those responses evoked by weak but visible displacement of the skin.

_Histological procedures_. Subjects were administered a lethal dose of anesthetic. The dorsal aorta was clamped and the left ventricle injected with heparin (500 units) and 5 ml of 1% sodium nitrite, usually while the heart was still beating. Ventricular persusion proceeded with saline, followed with 4% formaldehyde or 2% paraformaldehyde-2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and then with 10% sucrose in phosphate buffer. The brain was blocked in the manner of Johnson, Rubel and Hatton (1974) to yield sections in the planes of the rows of electrode penetrations. Further tissue preparation and histochemical procedures are given in the accompanying paper.

_Data analysis_. Electrode tracks were identified by 1) the product of the reaction of diaminobenzidine (DAB) with HRP, which had been deposited by the microelectrode. This was visualized in the Nissl substance and acetylcholinesterase stained tissues only; 2) the product of the reaction of DAB with blood in the latter two series of tissues as well as in the cytochrome oxidase stained tissue, and 3) Movement of glia into wounded tissue. Electrode tracks were identified for correspondence to receptive field recording data by the relative location of the point of entry of the tracks in the dorsal-most aspect of the brain and by the appropriate location of lesions, when present. Lesions were identified by: 1) the presence of holes in the tissue which were not lined with endothelial cells (as blood vessels are), 2) glial infiltration of the surrounding edges of the hole, and 3) loci corresponding to the stereotaxic coordinates recorded when the lesions were made.

Figurine maps were constructed in the manner of Rose and Mountcastle (1952). These reconstruct a plane of electrode tracks as they would appear in a tissue section with a drawing of the effective receptive field at each point along the track that unit cluster activity was evoked. The relative locations of the electrode tracks were determined stereotaxically and confirmed in the histological preparations. Since these figurine maps effectively summarize the organization of functional response fields in the thalamus, they were used as an intermediate data base. Figures 2.1-2.4 were constructed by tracing the loci of the penetrations and recording sites from these figurine maps and then encircling regions with similar receptive fields.

Materials. Ketamine hydrochloride was purchased from Parke-Davis as Vetalar. Xylazine was from Haver-Lockhart with the proprietary name Rompun. HRP was a mixture of Sigma type VI and Boehringer Mannheim, grade I. Heparin was from Abbott Laboratories and is marketed under the proprietary name Panheprin.

# RESULTS

Data was recorded from those 223 of the electrode penetrations which contained responsive recording sites numbering 2570. Additional electrode penetrations did not encounter recording sites. All recordings were in the left thalamus and were evoked by contralateral body stimulation.

General organization of the somatosensory thalamus.

The region of the raccoon thalamus which receives mechanosensory projections is an ovoidal mass which flattens dorsally and is located on the lateral aspect of the thalamus. The largest division of this is a central core region receiving projections from cutaneous receptors of low threshold from the glabrous skin of the forepaw (e.g., Figure 2.1F; cf. Welker and Johnson, 1965). Projections from mechanoreceptors in other body regions extend to one of several shell-shaped zones which wrap partially around this central core. Kinesthetic projections form a rostral, dorsal and lateral shell (shaded region in Figures 2.1, 2.2 and 2.4). Projections from the glabrous hindpaw as well as hairy trunk and tail form a lateral shell which, in its more dorsal aspects, is subjacent to portions of the kinesthetic projection zone (Figures 2.1 and 2.2). Projections from cutaneous receptors in all hair-covered regions of the body project to a region dorsal and caudal to the central core region of glabrous forepaw projections (Figure 2.3). Projections from forepaw claws and dorsal digits (hairs and skin) are contained in a series of regions concentrically superjacent, anterior or subjacent to some borders of the glabrous forepaw digit representations. These claw and dorsal digit projection zone is subjacent or posterior to those portions of the kinesthetic

Figure 2.1. In this and the following Figures, planes are coronal (with the exception of Figures like 1F which are marked accordingly). Vertical lines represent microelectrode penetrations as determined stereotaxically and by examination of tissues. Horizontal bars along penetration lines represent recording sites as determined stereotaxically, whose peripheral receptive fields are categorized by general location in a particular body part. However most recording sites do receive projections from unique receptive fields. All receptive fields are in the contralateral (right) half of the body. Heavy lines circumscribe these projection fields and are dashed to indicate the possibility of further extension of projection zones medially and laterally. Regions shaded with dots and labelled 'DEEP' receive kinesthetic muscle and joint projections and yielded evoked unit cluster electrical activity with kneading, joint motion and tapping to the respective receptive field but did not respond to cutaneous stimulation. Diagonally cross-hatched regions receive forepaw projections from glabrous skin as well as from claw, hair or deep receptive fields. 'HAND' refers to forepaw and 'ARM' refers to forelimb, excluding forepaw.

Figures 2.1A, B, C, D and E are diagrams of somatosensory projections to the rostral most portions of the left thalamus of raccoon 507. Data was recorded from sagittally oriented rows of penetrations and have been re-oriented to approximate coronal planes. Figure 2.1F illustrates the locations of these coronal planes from a hypothetical horizontal section of the left half of the brain (adapted from Welker and Johnson, 1965). The view from the horizontal plane illustrates that the projections from the head are medial and those from the leg are lateral, while the individual forepaw digits have distinct projection zones (digit 1 = thumb, etc.). Caudal to these lie the forepaw palm and cutaneous body projections (Welker and Johnson, 1965). Planes are separated by 0.25-0.3 mm rostrocaudally with that of Figure 2.1A rostralmost. Kinesthetic projections from respective body parts form a discrete region which overhangs the rostral aspect of the somatosensory thalamus occupying much of the rostrolateral pole of the mechanosensory projection region. Kinesthetic projections from more caudal receptive fields are represented lateral to those from successively more rostral receptive fields. Tissue sections from the approximate level of Figure 2.1D are illustrated in Figure 1 of the accompanying paper. Scale bracket = 0.5 mm.

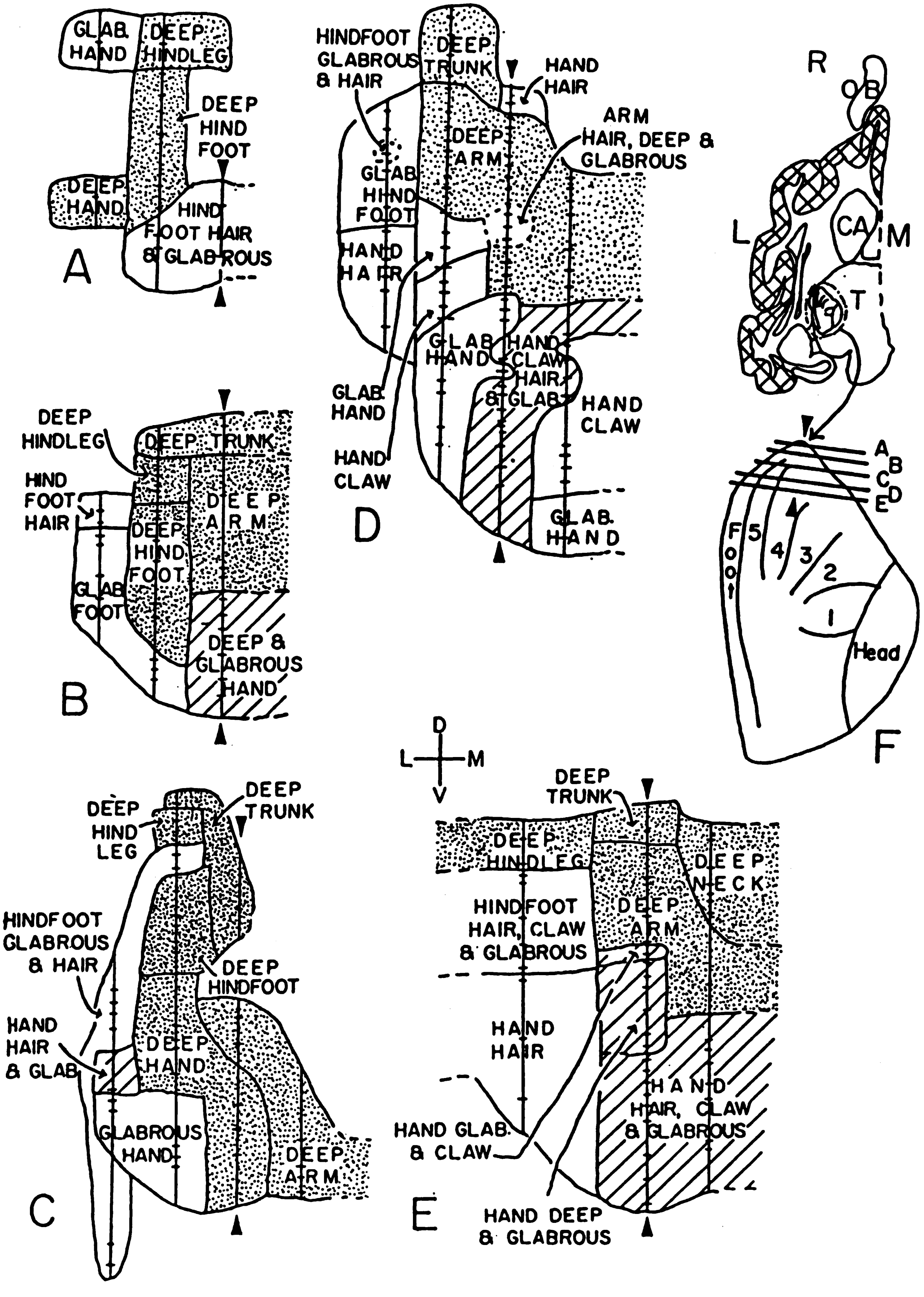
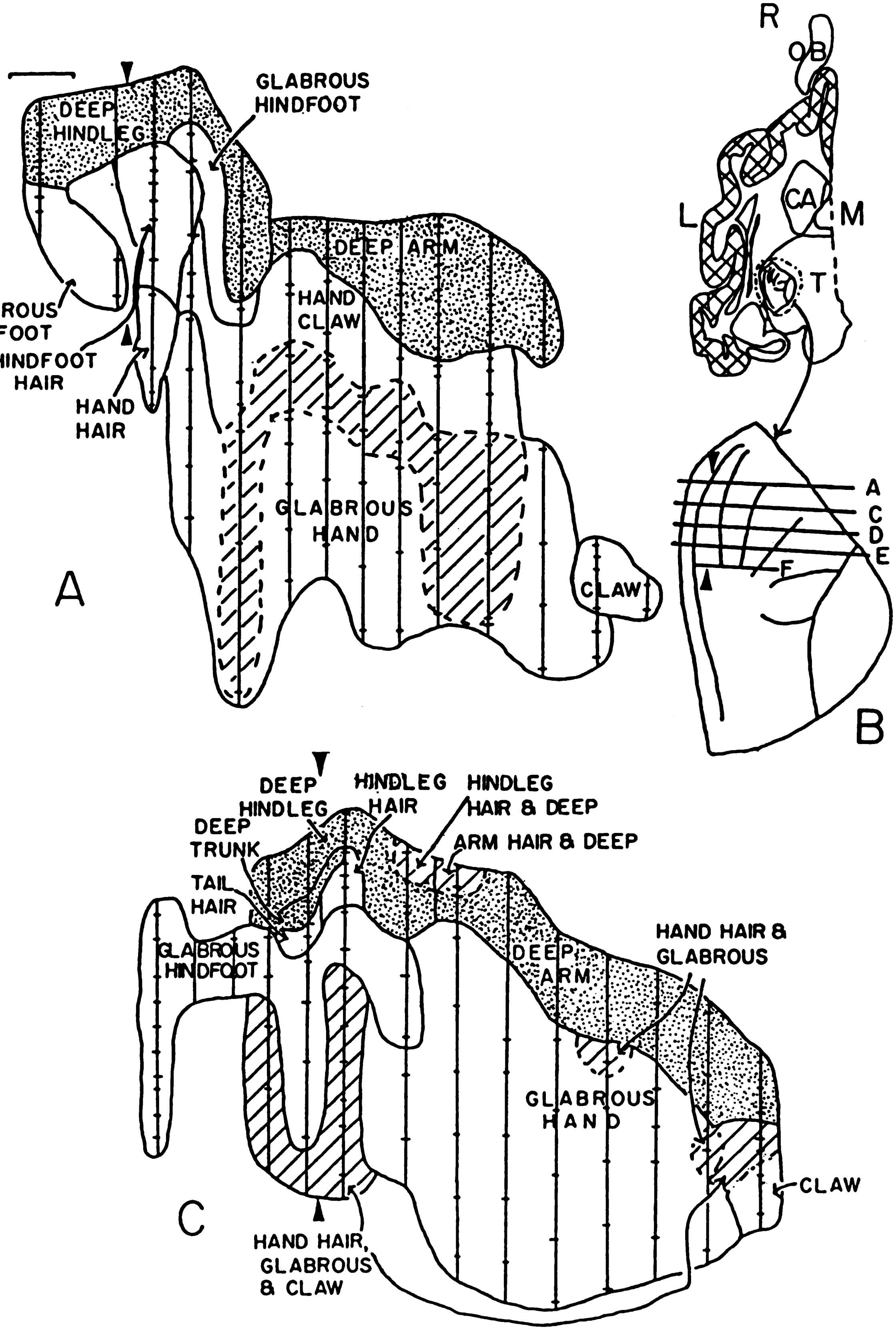
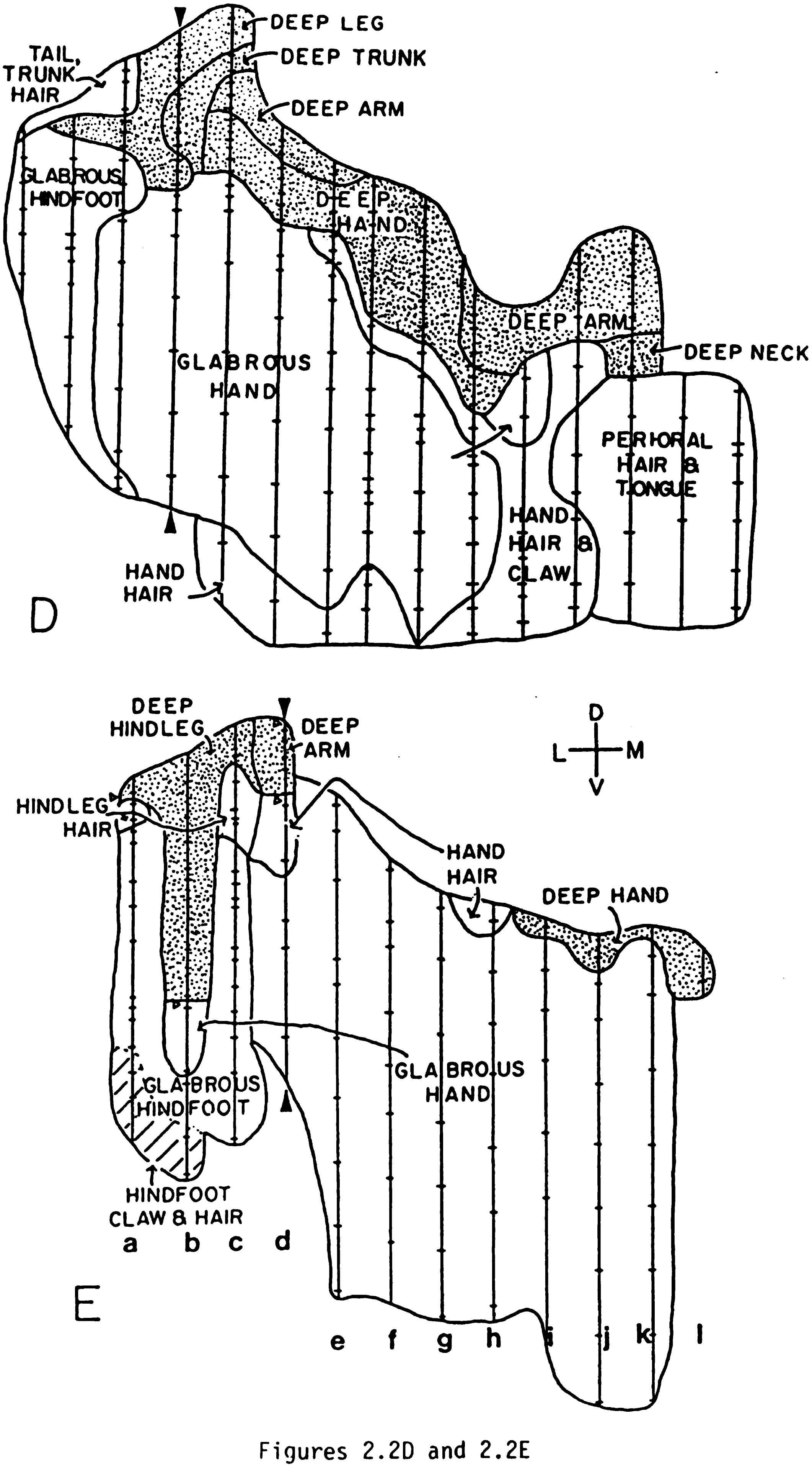

GLAB. HAND
DEEP HINDLEG
DEEP HIND FOOT
DEEP HAND
HIND FOOT HAIR & GLABROUS
A
HINDFOOT GLABROUS & HAIR
DEEP TRUNK
HAND HAIR
ARM HAIR, DEEP & GLABROUS
DEEP ARM
GLAB HIND FOOT
HAND HAIR
GLAB HAND
HAND CLAW HAIR & GLAB
HAND CLAW
GLAB. HAND
HAND CLAW
GLAB. HAND
D
R
OB
L
CA
M
T
A
B
C
D
E
Foot
5
4
3
2
1
Head
F
DEEP HINDLEG
DEEP TRUNK
HIND FOOT HAIR
DEEP ARM
DEEP HIND FOOT
GLAB FOOT
DEEP & GLABROUS HAND
B
D
L
M
V
DEEP HIND LEG
DEEP TRUNK
HINDFOOT GLABROUS & HAIR
DEEP HINDFOOT
HAND HAIR & GLAB
DEEP HAND
GLABROUS HAND
DEEP ARM
C
DEEP TRUNK
DEEP HINDLEG
DEEP NECK
HINDFOOT HAIR, CLAW & GLABROUS
DEEP ARM
HAND HAIR
HAND HAIR, CLAW & GLABROUS
HAND GLAB. & CLAW
HAND DEEP & GLABROUS
E

Figure 2.1

Figure 2.2. Somatosensory projections to the left thalamus of raccoon 511 as determined from evoked unit cluster activity in coronal rows of microelectrode penetrations in the rostral part of the mechanosensory region but caudal to those shown in Figure 2.1. Same conventions, symbols and abbreviations as in Figure 2.1. Figure 2.2B shows the locations of the respective coronal planes in a hypothetical horizontal section of the left half of the brain. Coronal planes A, C, D, E and F are respectively separated by 0.5 mm rostrocaudally with plane A rostral most. Kinesthetic projections form a dorsal shell overlying the forepaw and hindpaw cutaneous and claw projections. Projections form rostrocaudally continuous fields representing respective parts of the body. Kinesthetic arm and hand projection zones are extraordinarily large. Projections from hair and claws of the hand lie adjacent to projections from contiguous receptive fields on the glabrous forepaw. Bracket = 0.5 mm. Figures 2.2G and H are Nissl stained and cytochrome oxidase activity stained tissues containing electrode penetrations recorded in Figure 2.2E. Small case letters identify penetrations. (No evoked responses were recorded in penetration 'n'.) In Figure 2E hollow triangles indicate locations of marking lesions and lie to their left (lateral). Tissue sections from the approximate levels of Figure 2.2E and F in other animals are shown in Figures 2.3 and 2.4 of the accompanying paper.



Figures 2.2A, 2.2B and 2.2C



Figures 2.2D and 2.2E

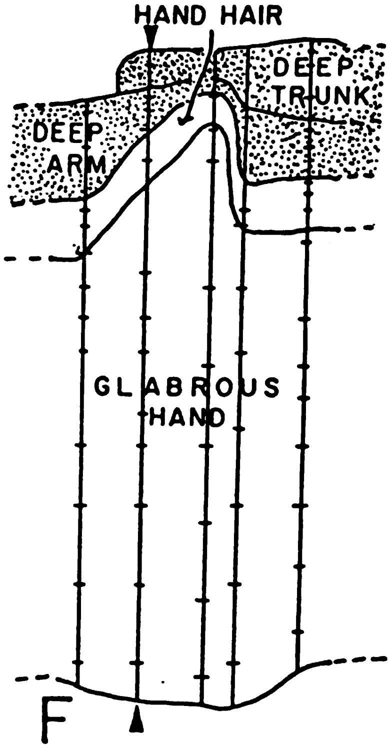


Figure 2.2F*

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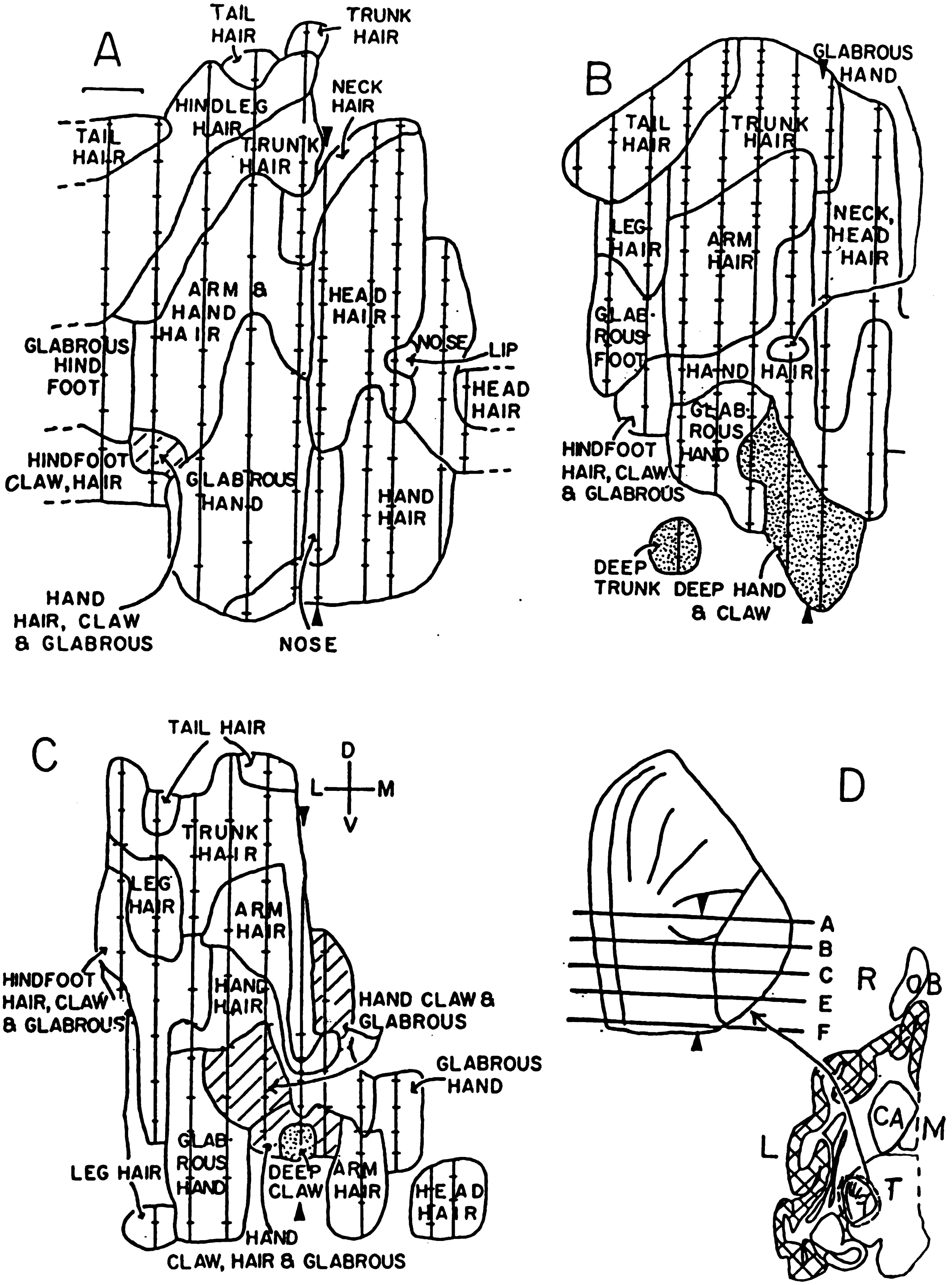
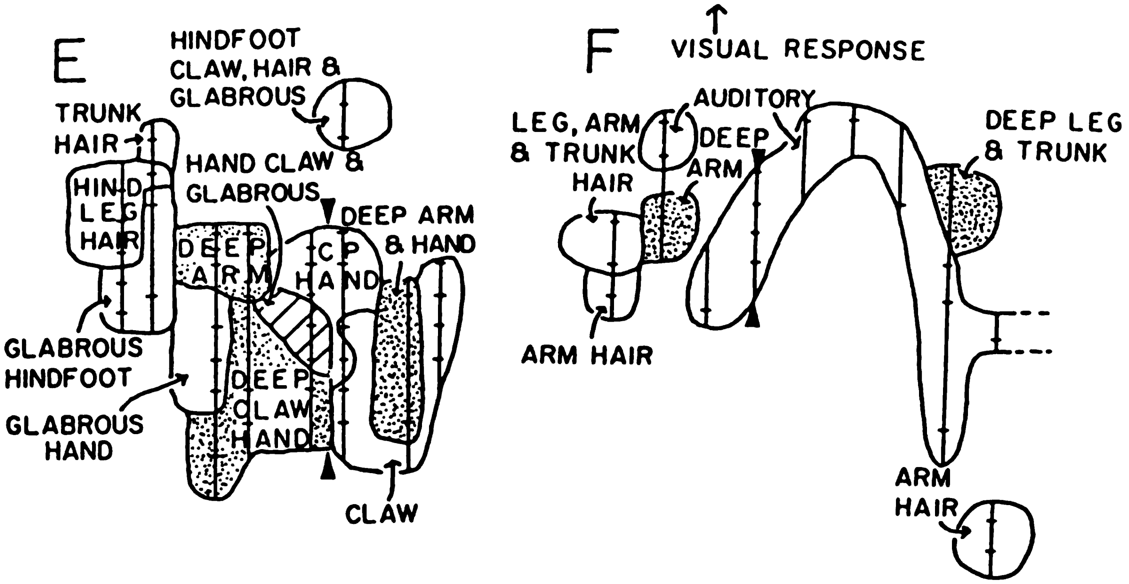
*Figures 2.2G and 2.2H appear in Chapter III as Figures 3.2A and 3.2B respectively.

Figure 2.3. Somatosensory projections to left thalamus in raccoon 537 as determined from evoked unit cluster activity in coronal rows of microelectrode penetrations. Figure 2.3 D indicates that the respective coronal planes are located caudally as shown in a hypothetical horizontal section of the left somatosensory thalamus. Coronal planes A, B, C, E and F are respectively separated by 0.5 mm rostrocaudally with plane A rostralmost. Projections from the cutaneous receptive fields of the body are somatotopically organized. Projections from the caudalmost receptive fields are located dorsal and lateral. Successively more rostral receptive fields are respectively more medial and ventral. Only scattered kinesthetic projections are found. 'CP' in Figure 2.3E indicates that unit cluster responses are evoked only by visible displacement of the skin. Tissue sections from another animal at the approximate levels of Figures 2.3A and B are illustrated in Figure 3.5 of the accompanying paper. Conventions, symbols and abbreviations are the same as for Figure 2.1. Bracket = 0.5 mm.



Figures 2.3A, 2.3B, 2.3C and 2.3D



Figures 2.3E and 2.3F

Figure 2.4. Somatosensory projections to the rostral portion of the somatosensory region in the left thalamus of raccoon 507 as determined from evoked unit cluster activity in sagittal rows of microelectrode penetrations. Abbreviations and symbols are the same as in Figure 2.1. Arrowheads indicate penetrations at the same rostrocaudal level. The penetration demarcated by arrowheads in Figure 2.4A is the same penetration that appears immediately lateral to the arrowheads in Figure 2.1D. Figure 2.4C shows the relative locations of the respective sagittal planes in a hypothetical horizontal section of the left half of the brain (adapted from Welker and Johnson, 1965). Sagittal planes A and B are separated by 0.5 mm with B medialmost. Figure 2.4A is somewhat medial to the plane of Figure 3.6 of the accompanying paper. Figure 2.4B is somewhat lateral to the plane of Figure 3.7 of the accompanying paper.

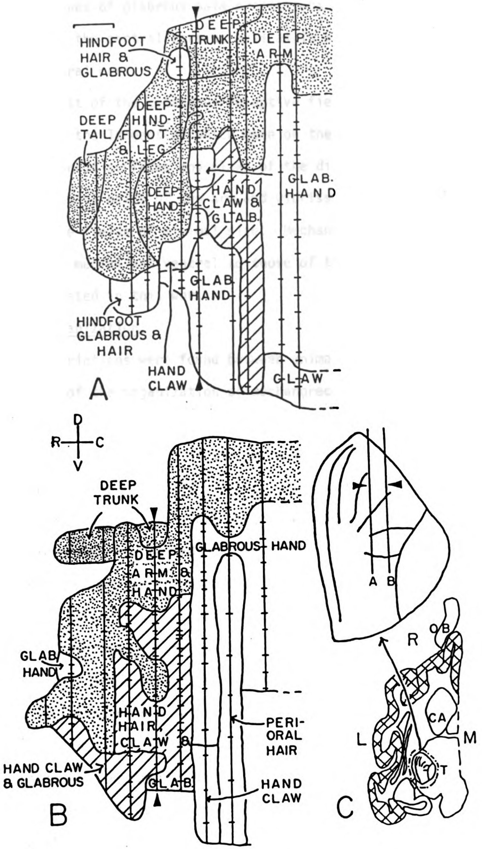

HINDFOOT HAIR & GLABROUS
DEEP TRUNK
DEEP ARM
DEEP HIND-FOOT & LEG
DEEP TAIL
GLAB. HAND
DEEP HAND
HAND CLAW & GLAB.
GLAB HAND
HINDFOOT GLABROUS & HAIR
HAND CLAW
GLAW
A
D
R
C
V
DEEP TRUNK
DEEP ARM & HAND
GLABROUS HAND
GLAB HAND
HAND HAIR, CLAW &
PERI-ORAL HAIR
HAND CLAW & GLABROUS
GLAB.
HAND CLAW
B
A
B
R
OB
CA
L
M
T
C

Figure 2.4

Projection zone which it contacts (Figures 2.2B, D and E, 2.3 and 2.4. Small zones of glabrous palm projections also can be found on peripheral edges of the central core as well as in a larger region caudal to the central core.

Most of the cutaneous receptive fields were not discontinuous regions, the largest of which were on the trunk and the smallest on the glabrous surfaces and claws of the digits, especially of the hand. Guard hairs of the arm and vibrissae of the face also form receptive fields of minimal size. Mechanosensory projections from the head are medial and ventral to those of the body and are not investigated in this work.

### Fine Organization.

Variations were found between animals regarding the exact details of the organization of mechanoreceptor projections. Hence, the following is a composite description of the shapes, orientations and relative positions of the response fields from several animals. The terms response fields, representation, responses, projection(s) and projection zone all are used interchangeably to describe a group of microelectrode recording sites where similar evoked responses were found. Those results which were also elaborated by Welker and Johnson (1965) will only be briefly noted.

Core cutaneous forepaw region. Each forepaw digit has a large, individual projection zone. More laterally (ulnar) located digits are represented more laterally in the thalamus than more medial (radial) digits. Afferents from ulnar digits five, four and three project more rostrally in the thalamus than those of radial digits two and one and the glabrous palm pads (Figure 2.1F). Projections from the glabrous palm are also located in the perimeter of the central core region adjacent to the representation(s) of the proximal portions of the respective peripherally adjacent digit(s).

Kinesthetic region. Deep forelimb responses dominate the kinesthetic afferent zone (Figures 2.1, 2.2 and 2.4). These forelimb projections lie in a rostrocaudally oriented, dorsoventrally flattened band which thickens dorsoventrally in the rostral aspect of the somatosensory thalamus (Figures 2.1, 2.2 and 2.4). In some regions, representations of more proximal portions of the forelimb lie in shells superjacent to those of successively more distal forelimb segments (Figure 2.2D). The kinesthetic hindlimb projections are lateral to those of the forelimb, lying dorsal and occasionally lateral to the forepaw glabrous digit five and the glabrous hindpaw representations (Figure 2.1E). The deep hindlimb projections are organized in a manner similar to the deep forelimb representation, with proximal hindlimb responses dorsal to distal ones (Figure 2.1C). The deep hindlimb projection zone is considerably smaller than that of the deep forelimb (Figures 2.1 and 2.2). Kinesthetic projections from the trunk are contiguously located medial to the deep hindlimb zone and lateral to the forelimb projection zones (Figures 2.1B, C, D, E, 2.2C and D) Caudal trunk representations are successively lateral to those of the rostral trunk. Deep scapula and upper trunk projections, while always dorsal to deep forelimb projections, may lie in one of two zones (not shown). These are respectively located medial and lateral to the deep forelimb representation. Kinesthetic responses from the caudal trunk are found only in one continous zone in the thalamus.

Most joint responses were recorded in a rostrocaudally oriented strip flanked by muscle projections laterally, medially and occasionally dorsally (not shown). Forelimb joints predominated in this region also. Laterally, hindlimb joint responses were also present.

A second, very small, caudal thalamic region with kinesthetic projections, mostly from forepaw muscles and joints, was found. This is located ventral to the cutaneous hairy body projections (described below) in the caudal portions of the somatosensory thalamus, lateral to the cutaneous head projections (stippled regions of Figures 2.3B, C, E and F).

Glabrous hindfoot region. The glabrous hindlimb projections form a distinct rostrocaudally oriented, mediolaterally flattened band lateral to the cutaneous forepaw digit five representation (Figures 2.1, 2.2 and 2.3). Receptive fields in the glabrous hindpaw representation are larger than those of the forepaw representation. Fields often extended over several digits and the sole. Sites receiving projections from combinations of hindpaw glabrous skin, claws and hair were found, especially in the rostral and caudal poles of this zone (Figures 2.1C, 2.1D, 2.1E, 2.2E and 2.3).

Cutaneous hairy body region. Projections from cutaneous receptors in hairy regions of the body (this expression shall refer to all cutaneous body surfaces excluding glabrous skin and the head) are located caudal to the kinesthetic projection zone and dorsal to the glabrous forepaw digit representation. As the latter extends toward the caudal pole of the somatosensory thalamus, it becomes occluded by the ventral expansion of the cutaneous hairy body projections (Figures 2.3A, B, C and D). Cutaneous tail, trunk and hindlimb projections occupy the more dorsal and lateral aspects of this region. In addition, cutaneous tail, caudal trunk and hindlimb (discussed above) projections extend to the rostral pole of the somatosensory thalamus in a region dorsal and medial to the glabrous hindpaw projection zone (Figure 2.2A, C, D and E). In some instances, these tail, caudal trunk and hindlimb cutaneous projections lie adjacent to the respective kinesthetic projections from these regions.

Within the main portion of the zone of projections from the cutaneous hairy body in the caudal somatosensory thalamus, the organization parallels that of the kinesthetic zone (Figures 2.3A, B and C). Trunk projections are dorsalmost. Projections from caudal trunk and tail are lateral to those of more rostral body regions. Often, midline projections are located dorsally and those of more lateral regions are successively further ventral. Proceeding further ventrally, responses to hindlimb stimulation are found lateral to forelimb responses. Successively more distal limb representations are ventral and medial to more proximal limb representations. Forepaw digit hairy skin responses are found ventrally in this region adjacent to glabrous digit responses as well as in the more rostral zones described below.

<u>Forepaw digit hair and claws region</u>. Projections from the claws and dorsal hairy portions of the individual forepaw digits form thin shells which lie in variable locations, but always adjacent to the respective glabrous digit representations (Figures 2.1D, E, 2.2A, B, C, 2.3A, B, C and E). The digit hair and claw projections may extend from the dorsalmost to ventralmost borders at the rostral pole of the glabrous representations of digits five, four and three, on the medial and ventral borders of the glabrous representations of digits three, two and one lateral to the head projection zone as well as on the lateral, ventral and dorsal borders of the glabrous

forepaw digit projection zone.

At the caudal pole of the thalamic mechanoreceptor projection field where it borders with regions responsive to visual and auditory stimuli, responses were found to stimulation of combinations of multiple forepaw glabrous digits and palm, claws and hairy regions as well as kinesthetic stimulation (some are shown in Figures 2.3E and F). These projections did not appear to be somatotopically organized.

### Organizational trends.

Within each of the five zones described there were somatotopic organizational trends. That is, projections from contiguous body regions were adjacent. In many cases somatotopy was found across the borders of these five mechanosensory projection zones. For example, projections from the cutaneous forelimb were found adjacent to kinesthetic projections from the forelimb. However some exceptions to the trend of somatotopy were found when large jumps in peripheral receptive fields, for example from hindlimb to forelimb, occurred with only a slight displacement of the recording microelectrode.

Within individual zones, more distal parts of limbs and more lateral parts of the trunk were represented ventral to more proximal and medial regions. Projections from caudal body regions were lateral to those of more rostral body regions. The general structure is that of a central core of glabrous forepaw projections surrounded by slab-shaped zones of projections from other regions.

# DISCUSSION

A distinct zone of kinesthetic projections was found in the rostral and dorsal aspects of the mechanosensory projection zone. These projections are somatotopically organized, with projections from axial structures dorsal most and projections from successively more distal limb regions located more ventrally in the thalamus. The kinesthetic forelimb representation is enlarged and lies rostro-dorsal to a large central core of forepaw glabrous digit projections. Evoked responses from light tactile stimulation of the forepaw hairy skin, claws and glabrous palm were found superjacent to the central core region. A zone containing projections from cutaneous receptive fields of the axial body and hairy skin of the limbs is located dorsally at the caudal aspect of the central core. This cutaneous body representation has a somatotopic organization distinct from, but similar to, that of the kinesthetic projection zone. Cutaneous tail and glabrous hindlimb projections are on the lateral aspect of the mechanosensory projection zone. Responses from combinations of glabrous skin, hairy skin, claw or kinesthetic receptive fields usually occurred at borders between zones containing only one of these response types.

## Parallels between the organization of raccoon thalamic and medullary mechanosensory projections.

The principal kinesthetic projection zone of the medulla, the external cuneate nucleus, merges with the medullary cutaneous projection zone, the cuneate-gracile nuclear complex (CuGr) at the junction where deep forepaw digit projections meet cutaneous forepaw projections, including hairy dorsal hand and digits (Johnson, *et* al., 1968). This is similar to the case in the thalamus. Within the external cuneate nucleus, projections from successively more distal forelimb fields are successively more medial while in the kinesthetic thalamus, these were successively more ventral. The external cuneate nucleus has a medially extending tongue region which passes through the CuGr and spreads out along its base rostromedially. The medial tongue region receives projections from the deep forelimb. Kinesthetic projections are also found in the basal cuneate, ventral to CuGr. The relatively large representation of the deep forelimb in the raccoon medulla parallels that of the thalamus.

The organization of the cutaneous mechanosensory projection zone of the raccoon medulla also closely parallels that of the thalamus. Within the CuGr, the volar glabrous hand representation is large. Instead of the thick slab-like projections found in the thalamus, the projections from the respective cutaneous digits form rostrocaudally extended slender columns. Representations of the dorsal hand and claws, lie in a band dorsal to the volar hand representation similar to the projections from these receptive fields which lie in a shell surrounding the cutaneous digit projections in the thalamus. Claw projections are found between the dorsal and volar cutaneous digit projections. The cutaneous body projections of the medulla form a band lateral and medial to the volar digit projections and is thickest rostrally. Projections from adjacent receptive fields on the arm are separated by the large glabrous forepaw representation. Medially in the CuGr, the glabrous and hairy hindfoot form a central core of projections which are surrounded by cutaneous ankle and leg projections. Caudally glabrous foot projections extend as an isolated subnucleus with a tail representation forming another isolated subnucleus located dorsally along the midline.

The organizational trend of somatotopy is found both in the thalamus and medulla. Furthermore, the topographic arrangements of projections in the two are remarkably similar as exemplified by: 1) The distinct kinesthetic projection zones in dorsal locations which contact the cutaneous projection zones in the hand representations, 2) Parallel degrees of enlargement of forelimb representations of both cutaneous and kinesthetic receptive fields, 3) Separation of projections from adjacent receptive fields of the cutaneous forelimb. Previously established differences between the medullary and thalamic mechanosensory projection zones include: 1) The location of projections from caudal receptive fields laterally in the thalamus and medially in the medulla, related to the decussation of the medial lemniscus as it passes from medulla to thalamus, 2) variations of the shapes of the respective projection zones and the apparent larger volume of the mechanosensory projection zone in the thalamus (Welker, *et al.*, 1964).

### Parallels between the organization of raccoon thalamic and cortical mechanosensory projections.

Welker and Seidenstein (1959) mapped the primary somatosensory cortex of the raccoon and found individual gyri receiving projections from cutaneous receptive fields in the five digits of the hand, the palm pads of the hand, the arm, hindlimb and head. This represents a greater degree of differentiation of cutaneous projections than that found in the medulla and thalamus, which do not have such readily distinguishable subdivisions for the palm pads and arm. Claws of the hand send projections to the periphery of the volar hand gyrus.

Projections from the cutaneous receptive fields of the dorsal digits were found between the lateral edges of two adjacent digit projections. The dorsal hand projections were separated into two regions located respectively at the junctions of the medial and the lateral cutaneous arm projections with the volar palm representation. No kinesthetic projections were reported in this study.

Johnson, Ostapoff and Warach (1982) examined the cortical gyrus representing digit four of the hand of the raccoon with microelectrode mapping methods using unit clusters. They found three spatially discrete zones which are respectively characterized by low threshold stimuli of: 1) muscles, joints and tendons, 2) small, specific regions of glabrous skin with preservation of information about spatial location and 3) a "heterogeneous" population of projections from mechanoreceptive fields including hairy dorsal hand, claws along with fields such as those described in 1) and 2). The zones described by 1) and 2) above as well as those described in the earlier work of Welker and Seidenstein (1959) all have homologues in the thalamus and in the medulla. Several possible candidates exist as thalamic homologues of the heterogeneous zone of the primary somatosensory cortex. These include: 1) the mixed response zones (cross-hatched in Figures 2.1, 2.2 and 2.3) which occur at the borders between single modality response zones, 2) the occasional nonsomatotopic responses observed in the periphery of the mechanosensory projection zone of the thalamus. Alternatively, regions of the mechanosensory thalamus with separate modality and location responses may send projections that converge in this cortical region. The possibility of these convergent responses in the cortex resulting from intracortical connections can not be ruled out either (Johnson, Ostapoff and Warach, 1982). We have observed (in studies in progress) that horseradish peroxidase injections, whose sites include the kinesthetic region at the anterior border of the somatic sensory cerebral cortex, cause retrograde labelling of cells in the kinestic region in the rostrodorsal shell of the ventrobasal thalamus.

In summary, the organizations of somatosensory projections to the raccoon medulla, thalamus and cortex in succession, are generally consistent with an isomorphic mapping of projections with preservation of modality and place information.

### Discontinuities in the somatotopy of mechanosensory projections to thalamus.

In most cases, projections were adjacent to others with contiguous receptive fields. The organization found is consistent with a single body map, if one considers the muscles and joints to be body loci distinct from the skin. Discontinuities such as adjacent hand and leg projections appear to violate somatotopy. This topological arrangement may originate in the following manner: the greater density of forelimb innervation produces a greater density of forelimb projections; these projections are compacted with the other mechanosensory projections into a discrete volume in the thalamus. (This is similar to the situation in the cortex; Johnson, Ostapoff and Warach, 1982). A simple interpretation of the double representation of cutaneous dorsal hand and digits and the glabrous palm is that these were once a unified projection zone subsequently split by expansion of the zone of projections from the glabrous digits during development. In most cases where responses from more than one modality or body part were found, they were at the borders between two respective

In the monkey, hand and hindfoot projection zones are more alike. As in the monkey, the cutaneous leg and tail projections of the raccoon form lamellae which extend along the lateral edge of the somatosensory projection zone from its rostral to its caudal pole.

While projections from cutaneous receptive fields of the trunk and limbs are found dorsal to the glabrous digit projections and ventral to the kinesthetic projections in the monkey, cutaneous body projections in the raccoon are found in a shell extending caudally from the termination of the kinesthetic projection zone and overlying the remainder of the glabrous digit projections. Many of the lamellae of cutaneous body part representations are flattened dorsoventrally in the raccoon as compared to mediolaterally in the monkey. In some cases, the lamellae of these projections in the raccoon narrow mediolaterally to assume the form of thalamic rods as observed in the monkey (Jones, Friedman and Hendry, 1982).

In both the monkey and the raccoon, kinesthetic projections are found in a dorsal and rostral shell (Friedman and Jones, 1981, 1982; Friedman, Jones and Hendry, 1982; Loe, Whitsel, Dreyer and Metz, 1979). However, the shell of kinesthetic projections in the raccoon does not thicken caudally as in the monkey. The shell of kinesthetic projections in the raccoon also extends along the lateral edge of the thalamus abutting the external medullary lamina. The somatotopic organization and patterns of kinesthetic receptive field projections are quite similar in the two animals. However, the kinesthetic arm projections appear to occupy a relatively larger volume in the raccoon. This parallels the raccoon's extensive behavioral specialization in the use of the forepaw in active exploration of the environment.

the peripheral innervation density." The general organization of these somatosensory projections to the cat thalamus as well as the organizational trends including somatotopy are quite similar to those of the raccoon.

No evoked responses to joint or muscle stimulation were reported in the Mountcastle and Henneman (1949) paper. Kinesthetic projections to the cat thalamus have been reported in more recent microelectrode mapping studies (Mallart, 1968; Millar, 1973). Mallart (1968) found that electrical stimulation of group I muscle afferents of the forelimb evokes short latency potentials in the "dorso-medio-rostral" part of VPL (VPL: the ventroposterolateral nucleus of the thalamus, which receives cutaneous mechanoreceptor projections from the post-cranial body in its central core). Although Millar (1973) found no deep forelimb evoked activity in the cat thalamus, some deep hindlimb responses were found in an anterodorsal-lateral zone of VPL. These kinesthetic projections are similar to those of the raccoon in their location on the rostrodorsal border of the cutaneous projection zone. The raccoon appears to vary from the cat in the more vast expansion and greater differentiation of forelimb projections of both cutaneous and kinesthetic receptive fields.

Thalamic mechanosensory projections in monkey. In both the (rhesus) monkey and the raccoon somatosensory thalami, there exist central cores of cutaneous projections from the volar glabrous digits. In the raccoon, the forepaw digits have a more extensive projection zone. The glabrous hindfoot projection zone is less differentiated and has much larger receptive fields than does that of the hand.

other receptive fields, both cutaneous and kinesthetic, respectively form thin lamellae which are convex laterally as has been well documented in the monkey (e.g., Poggio and Mountcastle, 1963; Mountcastle and Henneman, 1952) and human (Guiot, *et al.*, 1973). In the raccoon, we have shown that such lamellae wrap concentrically around, with their concave face towards, the glabrous digit projections.

*Thalamic mechanosensory projections in cats.* Mountcastle and Henneman (1949) made systematic recordings of electrical activity evoked by cutaneous stimulation. In the thalami of pentobarbital-anesthetized cats. Similar to the present study, they made ordered rows of electrode penetrations but the spacings on their grid were 0.5 mm by 1.0 mm. Response fields were observed at 0.25 mm intervals within the individual penetrations. Face and mouth responses were found medially and neck, trunk and tail responses were located successively more lateral along the dorsal portion of the somatic sensory projection zone. Continuous somatotopic organization of projections was observed. Cutaneous projections from the limbs were located ventral to the axial body projections, with more distal parts of the limb projecting more ventral than proximal ones in a somatotopic manner. The cat thalamic body representation was summarized as "a somewhat distorted figure of a cat crouching upright in the thalamus" (Mountcastle and Henneman, 1949). The representations of the head and extremities were found to be expanded with that of the foreleg exceeding the hindleg in volume. The authros observed that "the degree of localization, the intensity of the response and the volume (of) representation vary directly, and the extent of overlap (between zones responsive to different receptive fields) indirectly with zones as has been found in the monkey (Loe, et al., 1979). Exceptions to the trend for somatotopy and modality segregation were found in scattered projections along the periphery of the somatosensory thalamus and comprised an estimated less than 1% of all recording sites. This may represent recording from intermingled (unsorted) fibers of the entering medial lemniscus.

While the majority of our evoked responses were rapidly adapting, some recording sites with slowly adapting responses were found in each receptive field modality and location. There did not appear to be an aggregation or a distinct zone containing the slowly adapting projections (cf. Dykes, et al., 1981). These response sites were located adjacent to rapidly adapting projections from the same or contiguous receptive fields. It must be emphasized that our stimulation methods were not well suited for accurate discrimination of slowly and rapidly adapting receptive field properties and our stimulation protocol was not designed to systematically map such responses.

### Comparison of organization of mechanoreceptor projections to the thalamus of raccoon with that of other mammals.

Several reviews deal with the organization of mechanoreceptor projections in mammals (e.g., Boivie and Perl, 1975; Bombardieri, et al., 1975; Brodal, 1981; Jones, 1981; Welker, 1973). This organization will be discussed as it appears in cat, monkey and human in the context of the present findings. In these animals, a core region is composed of glabrous digit projections which, in the raccoon, form thick slabs with that of the thumb medial-most and that of other digits successively more rostral and lateral (Welker and Johnson, 1965; see Figure 2.1D). Projections from

Thalamic mechanosensory projections in humans. In the human thalamus, projections from contralateral mechanoreceptors of caudal and rostral body parts have the same respective lateral to medial organization (Guiot, *et al*., 1973) as in the other mammals described. Tasker *et al*. (1982) summarize this human thalamic organization as composed of "several successive anterior-posterior bands of neurons responding to different modalities of somatosensory stimulation each fitted into a rather complex homuncular pattern...". Most ventral and caudal are units responding to light touch and hair bending (Bertrand *et al*. 1967; Fukamichi *et al*., 1973; Guiot, *et al*., 1973; Jasper and Bertrand, 1966). Further rostral is a band of units classified as "deep sensation neurons" (Bertrand *et al*., 1967; Jasper and Bertrand, 1966) which extends rostrally into the ventralis intermediate nucleus. The ventral intermediate nucleus is rostral and dorsal to the ventral caudal nucleus (probably homologous to the VPL of cat, the cutaneous projection zone of raccoon and pars caudalis of VPL (or VPLc) of monkey) and is caudal to the ventral oral nucleus (probably homologous to the ventral lateral nucleus of cat, raccoon and monkey, as well as pars oralis of VPL (or VPLo) in the latter. In the caudal three mm of this second band (in the ventrocaudal nucleus portion only) deep pressure responses are found. More rostral and dorsal to the ventrocaudal nucleus in the ventral intermediate nucleus, units respond to active and passive movement (Albe-Fessard, 1973; Bertrand *et al*., 1973; McComas *et al*., 1970; Ohye *et al*., 1972). Further rostral in the ventral oral nucleus, pars caudalis, a third band of responses are evoked by muscle stretching and squeezing in the caudal part of this region and in its rostral part by voluntary movement only (Jasper and Bertrand, 1966). The deep pressure zone of the human thalamus appears similar in description to the cutaneous pressure responses occasionally encountered on the border of the kinesthetic and cutaneous projection zones of the raccoon thalamus. While the anesthetic used in our experiments prevented the measurement of responses during active or voluntary movements, a few responses were apparently evoked by involuntary tremors while recordings were made in the raccoon kinesthetic projection zone. The passive movement response of the human appears to parallel the deep, joint responses reported here while "muscle stretching and squeezing" corresponds to the deep, muscle response we recorded. The position of this kinesthetic projection zone in the human is similar to that found in the cat, raccoon and monkey.

The ventral intermediate and ventral caudal nuclei are distinguished by a high level of background electrical activity (Fukamichi _et_ _al_., 1977; Nakajima _et_ _al_., 1978). We observe a level of background electrical activity in the kinesthetic and cutaneous projection zones of the raccoon thalamus that is consistently higher than that of adjacent regions. This was also reported previously in the cutaneous projection zone of the raccoon thalamus (Welker and Johnson, 1965).

Mechanosensory projections to the thalamus in other mammals. The organization of mechanoreceptor projections to the thalamus has also been studied with techniques similar to those of the present study in the agouti (Campos _et_ _al_., 1972), hedgehog (Erickson _et_ _al_., 1967), two opossum species (Pubols and Pubols, 1966;

Sousa et al., 1971), owl monkey (Lin et al., 1979), rabbit (Rose and Mountcastle, 1952), rat (Emmers, 1965), sheep (Cabral and Johnson, 1971), slow loris (Krishnamurti et al., 1972), spider monkey (Pubols, 1968) and squirrel monkey (Dykes et al., 1981). General principles of ventrobasal complex organization (cf. Welker, 1973) include somatotopy, lateral to medial location of projections from successively more rostral body parts and an expanded volume of neural tissue receiving projections from parts of the body used in food-handling and exploratory activity. In some of these investigations, the question of kinesthetic projections to the thalamus was apparently not investigated.

In order to place the present discussion of the mechanosensory projections of the raccoon in context, this organization will be briefly noted in other animals. The cuneate-gracile complex of the hedgehog sends projections to the ventroposterolateral nucleus (VPL), the posterior nuclear group and the ventral part of the lateral geniculate nucleus (Schroeder et al., 1968). However, in the Malaysian tree shrew, the slow loris and the marmoset, these projections are progressively more restricted to VPL. In the North American opossum, Pubols and Pubols (1966) found no distinct subcutaneous responses in the ventrobasal complex. However, light cutaneous and deep stimulation of the nose, mouth and forelimb evoked responses in the zona incerta, posterior nuclear region and parafascicular-subparafascicular complex. In the present study, some kinesthetic responses were also found in the caudal and ventral aspects of the cutaneous projection zone. In the South American opossum, Sousa et al. (1971) found tapping or pressure to ipsilateral and contralateral body surfaces evoked responses in the dorsal border of the ventrobasal complex. The kinesthetic receptive fields were not correlated with the more ventral cutaneous receptive fields. Kinesthetic responses were reported throughout the extent of a "shell-like region capping the dorsal and dorsolateral borders of the (ventrobasal complex) including parts of the ventrolateral complex and nucleus C..." (Sousa *et al*., 1971; cf., Oswaldo-Cruz and Rocha-Miranda, 1968). While no ipsilateral kinesthetic response have been reported in the cat, raccoon, monkey or man, the location of kinesthetic responses is similar. In the ventrobasal complex of the spider monkey, kinesthetic projections predominate in the hindlimb representation (Pubols, 1968). Kinesthetic projections from the forelimb and tail were also prevalent. Although a distinct kinesthetic projection zone was not found, deep responses appeared somewhat more rostrally in the thalamus than cutaneous responses. In contrast to the data discussed in the above sections, "no dorsoventral gradient in modality was found" in the spider monkey (Pubols, 1968). In the squirrel monkey, Dykes *et al*. (1981) reported a distinct zone receiving kinesthetic projections as well as respective zones for quickly adapting, slowly adapting, Pacinian and "tap" mechanoreceptors.

### Fiber tract connections of the mechanoreceptor projection zone of the thalamus in several mammals.

This topic has been reviewed extensively (*e.g*., Angel, 1977; Boivie and Perl, 1975; Brodal, 1981). The discussion here will center on those patterns which appear relevant to studies of the raccoon and which possibly represent organizational principles of mammalian somatosensory tracts.

Fiber connections of the ventrobasal complex of the cat. The external nucleus of the ventrobasal complex (VBX) of the cat receives terminals from medial lemniscal afferents (Mehler, 1966; Boivie, 1971; Jones and Burton, 1974; Berkley, 1975; Groenwegen, Boesten and Voogd, 1975) carrying cutaneous mechanosensory information from the contralateral postcranial body. Terminals from the spinothalamic pathway reside rostral to those of the medial lemniscus (Boivie 1971a; Jones and Burton, 1974). Spinocervicothalamic tract terminals were found in a restricted shell capping the lateral part of VBX rostrodorsally (Landgren, Nordwall and Wengstrom, 1965; Boivie, 1970). Individual cells in this rostrodorsal shell of VBX also receive terminals of lemniscal as well as spinocervicothalamic projections (Andersen, Andersson and Landgren, 1966). The transitional region between the ventrolateral complex (VL) and VBX receives projections from second order Group 1 muscle afferents from the contralateral fore- and hindlimbs (Andersson, Landgren, and Wolsk, 1966; Landgren and Silfvenius, 1970; Grant, Boivie, and Silfvenius, 1973). Projections from group I muscle afferents of the forelimb terminate in the deep part of the cuneate nucleus (Rosen, 1969, 1969a), which is presumably homologous to the basal cuneate nucleus in the raccoon (Ostapoff, Johnson and Albright, 1983). Projections from these medullary neurons terminate in the dorsomedial part of the VBX/VL transitional region in the cat (Andersson et al., 1966). Projections from group I muscle afferents of the hindlimb project to nucleus z of the medulla (Pompeiano and Brodal, 1957; Landgren and Silfvenius, 1979. These medullary neurons then send projections to the anterolateral portion of the VBX/VL transitional region (Grant et al., 1973). Group I muscle afferents from the forelimb were reported in the external cuneate nucleus and the "cluster zone" of the cat cuneate nucleus (Millar, 1979). Berkley (1980) found terminals from dorsal column nuclear projections in a dense central core in the VBX of the cat and a lower density of terminals in more peripheral portions of VBX. These clusters form dense clusters (of size 45-210 μm), with larger clusters more ventrally located. Injections of anterograde transport tracers or ablations of the dorsal column nuclei including its rostral-most or ventral-most portions (including part or all of nucleus z) provided labelling or degeneration in the most lateral, rostral or dorsal borders of VB. These terminal zones included the VBX/VL border rostrally, the border of VBX with the lateral posterior nucleus dorsally and the border of VBX with the lateral geniculate nucleus laterally. Dorsal column nuclear projections also were found to terminate in other thalamic regions.

Terminals of projections from the lateral cervical nucleus were found in lateral and dorsal portions of the cat VBX (Berkley, 1980). Clustering of terminals from lateral cervical nuclear projections were observed in the same core region as those from the dorsal column nuclei. In the borders of VBX, terminals from lateral cervical nuclear projections were found more rostral, dorsal and lateral than those of the dorsal column nuclei. Only sparse spinothalamic projections were observed in the main body of VBX with more terminals on its ventral, rostral, dorsal and lateral borders and in the ventropostero-inferior nucleus (VPI). A high density of spinothalamic terminals was found in VL and LP.

VL receives projections from the deep cerebellar nuclei (Rinvik and Grofova, 1974; Hendry, Jones and Graham, 1979). The transitional region between VL and VBX of the cat receives projections from the primary somatosensory cortex (Rinvik, 1968; Jones and Burton, 1974) and the transitional region reciprocates with projections to area 3a (Strick, 1973).

*Fiber connections of the kinesthetic projection zone of the raccoon thalamus.* Ostapoff *et al*. (1983) traced the sources of projections conveying kinesthetically evoked activity from the forelimb to the thalamus in the raccoon. Medullary sources of thalamic projections were identified following horseradish peroxidase (HRP) injections at sites with kinesthetically evoked electrophysiological activity. Labelling was found in cell group z, the external cuneate nucleus and its medial tongue, the basal portion of the cuneate nucleus, and the reticular portion of cell group x Less dense labelling was found in the infratrigeminal nucleus, the lateral cervical nucleus and the rostral portion of layer VI of the spinal cord.

The ventrolateral complex of the thalamus of the raccoon, which lies rostral and dorsal to the kinesthetic projection zone, sends fiber connections to the primary motor cortex (Sakai, 1982). Ablations of the primary somatosensory cortex yield degeneration in the central core of the thalamic mechanosensory projection zone (Welker and Johnson, 1965). HRP tracer studies have shown that the primary (SI) and secondary (SII) somatosensory cortices of raccoons receive ordered projections from the ventrobasal complex and the ventroposterior inferior nuclei respectively (Herron, 1983). Projections originating in cells of the kinesthetic projection zone of the thalamus of the raccoon appear to terminate in the anterior border zones of the raccoon primary somatosensory cortex (Johnson, 1984). Evoked responses to kinesthetic stimulation have also been found in these cortical regions which appear to correspond to area 3a of the cat and monkey cortices (Johnson, Ostapoff and Warach, 1982).

Fiber connections of the ventrobasal complex of the monkey. Kalil (1981) observed projections from the trigeminal-cuneate-gracile complex to terminate in a diffusely scattered manner in the pars caudalis of the ventroposterolateral nucleus (VPLc) of the VBX but not in the pars oralis of the ventroposterolateral nucleus (VPLo) at all. Boivie (1978) made lesions in the monkey dorsal column nuclei and found subsequent degeneration of terminals in VPL, POm and zona incerta. Berkley (1980) observed fiber tracts ascending to the diencephalon of squirrel and rhesus monkeys with the combined techniques of fiber degeneration and tritiated amino acid transport. DCN projections were found to terminate densely in ventral and lateral portions of the pretectal area and zona incerta, less densely in POm and caudal and lateral VPI and hardly at all in rostral POm. Within VB, the terminals of DCN projections form clusters, but not as distinctly as in the cat. Berkley (1980) suggests that these clusters are not apparent in massive injections or ablations because the density of the label or degeneration obliterates the cluster pattern. Although the DCN projections appeared to be confined to VPL, more rostral levels also show terminals in experiments involving massive ablations or injections.

Berkley (1980) also found that the lateral cervical nucleus (LCN) projects to dorsal and lateral protions of POm, caudal and ventral portions of VPL, caudal portions of VPI and the adjoining ventral part of the ventroposteromedial nucleus (VPM), and the middle part of the central lateral nucleus. Spinothalamic (ST) fibers project densely to clusters in VB which do not overlap with the DCN cluster projections. Other ST projections are to caudal POm and adjacent areas, as well as lateral and rostral parts of VPI. Boivie (1979) observed spinothalamic projections unevenly distributed throughout VPL, dense in its outskirts and a profusion of terminals in POm.

In the monkey, projections from the external cuneate nucleus extend to a rostrodorsal zone of the VPL (Boivie, Grant, Albe-Fessard and Levante, 1975; Boivie and Boman, 1981). The external cuneate nucleus has been shown to receive projections from group I muscle afferents of the forelimb (Cooke, Larson, Oscarsson and Sjolund, 1971).

Tracey _et al_. (1980) also found that VPLo receives fibers from the deep cerebellar nuclei, spinal cord, and area 4 of the cerebral cortex. No VPLo connections with cortical area 3a were found. Kalil (1981) observed cerebellar nuclear projections to VPLo, VL and caudalmost VA in the rhesus monkey. The terminals of these projections are oriented in longitudinal strips.

In an axonal transport study of afferents to the VPL of the monkey, Tracey _et al_. (1980) observed projections to VPLc from the trigeminal-cuneate-gracile complex, areas 3, 1 and 2 of the cerebral cortex as well as the vestibular nuclei.

Lin _et al_. (1979) observed that deep receptor afferents project via cells lying on the border of VP to cortical cytoarchitectonic zone 2. In a horseradish peroxidase tracer experiment, Whitsel _et al_. (1978) found that the central part of the macaque VP projects to cortical areas 3b and 1, while the rostral and caudal parts of VP, as well as associated border zones, project to cortical areas 3a and 2 respectively.

Jones and Friedman (1982) observed that the inner core of the monkey VBX projects to cortical area 3b. More dorsal, ventral and posterior parts of this core project to areas 3b and 1. Cells of the rostrodorsal shell of kinesthetic projections (or "deep shell"), which overlies the core region of VBX, sends projections to cortical areas 3a and 2. Anteroposteriorly elongated columns of cells in VBX project to small regions in these cortical areas.

Fiber connections of the ventrobasal complex of humans. In studies of degeneration in the brains of patients with brain lesions Mehler (1971) reports that spinal and lemniscal fibers terminate in the ventral intermediate nucleus of the human, while the terminals of cerebellar projections were found in the ventral oral nucleus and the ventral intermediate nucleus. Further studies with higher resolution will be required to firmly establish these conclusions.

Correlation of fiber connections of the mechanoreceptor projection zones of the thalamus in the cat, raccoon and monkey.

In each of these animals, the central core of the VBX receives projections primarily from the cells in the main body of the cuneate-gracile complex which convey cutaneously-evoked electrical activity. Connections between this zone and the primary somatosensory cortex have been shown in each case. To varying degrees, the kinesthetic projection zone of the thalamus in each animal receives projections from border zones of the cuneate-gracile complex as well as from the external cuneate nucleus, the lateral cervical nucleus, and nucleus z of the cuneate gracile complex and second order spinal neurons. In all cases, the kinesthetic projection zone of the thalamus sends projections to a cortical region on the anterior aspect of the primary somatosensory cortex abutting the primary motor cortex. The kinesthetic projection zone of the thalamus is distinguished from its rostrally and dorsally neighboring nucleus which receives cerebellar input and has connections with the primary motor cortex.

The organizational pattern that emerges here is that the pathway carrying kinesthetic information diverges at the level of the medulla to extend to: 1) the cerebellum, and 2) the rostrodorsal aspect of the cutaneous mechanosensory projection zone of the thalamus. Cerebellar projections extend to a region rostrally adjacent to the kinesthetic mechanosensory projection zone of the thalamus. These two thalamic regions then send projections to adjacent regions of the cortex.

Further experiments are encouraged to determine whether those non-mechanosensory thalamic regions adjacent to the kinesthetic projection zone (cat and raccoon: ventrolateral complex (VL); monkey: VPLo and VLc; human: ventral oral nucleus) receive projections from those cerebellar neurons which are functionally related (by cerebellar circuitry) to the cerebellar recipients of kinesthetic projections from medullary nuclei. Let us assume that: 1) the cerebellar pathway functions as a discriminator of certain qualities (perhaps temporal; cf. Pellionisz and Llinas, 1979) of kinesthetic afferent signals, or as an integrator of kinesthetic with vestibular- or motor- related neural activity. (We will refer to such function as *cerebellar-processed*.) and 2) the lemniscal pathway (medullary nuclei   thalamic nuclei   cortex) and its component nuclei serve, in one of its capacities, to discriminate some spatial qualities, such as location and size, of the stimulated kinesthetic receptive field. Then the appearance of projections of these parallel pathways in adjacent thalamic and cortical fields, as reviewed above, may facilitate (by virtue of their proximity) the interaction of independently processed spatially-discriminated and cerebellar-processed components of kinesthetic sensory stimuli, perhaps through collaterals and association fibers. Hence we propose that in mammals there is a divergence (in the medulla) and a re-apposition of respective pathways which process different informational aspects (such as spatial vs. cerebellar-processed) of kinesthetic stimuli.

# CHAPTER III

# ARCHITECTURE OF THE KINESTHETIC AND CUTANEOUS MECHANOSENSORY PROJECTION ZONES IN THE VENTROBASAL THALAMUS OF RACCOONS: DISTRIBUTIONS OF CYTOCHROME OXIDASE ACTIVITY, ACETYLCHOLINESTERASE ACTIVITY AND NISSL SUBSTANCE IN ELECTROPHYSIOLOGICALLY MAPPED TISSUES

# INTRODUCTION

We are currently investigating the organization of the neural systems which transfer and transform mechanically-evoked electrical signals which originate at peripheral receptors and related structures in the raccoon forepaw and project to cerebral cortical regions. This system is especially informative and tractable since, in raccoons, the cortical, thalamic and medullary representations of this somatosensory system are outstanding in size and internal differentiation (Welker and Seidenstein, 1959; Welker, _et al_., 1964; Welker and Johnson, 1965; Johnson, _et al_., 1968; Johnson, 1980; Johnson, _et al_., 1982 the accompanying paper). Each of these mechanosensory projection zones have histologically and electrophysiologically identifiable subdivisions related to distinct parts of the forepaw as well as other body regions.

Johnson, _et al_. (1982) found that mechanosensory projections to the forepaw digit 4 representation of the raccoon neocortex are spatially segregated into three populations of submodalities and that the locations of these populations are aligned in accord with sulcal folds. Architecturally distinct regions of the raccoon medulla also have been shown to receive cutaneous and kinesthetic mechanosensory projections respectively (Johnson, _et al_. 1968; Ostapoff, Johnson and Albright, 1983). A goal of this work is to determine the architecture of those regions of the raccoon thalamus receiving projections from mechanosensory submodalities including postcranial receptive fields located in muscles and joints, claws and hairy skin, and glabrous skin as mapped electrophysiologically in the accompanying paper.

Welker and Johnson (1965) showed that regions of the thalamus wherein electrophysiological activity is evoked by light tactile stimulation of the glabrous skin of individual forepaw digits are separated from one another as well as from projections from the hindlimb, tail and head by laminae of myelinated fibers. Our extensions from that study include: 1) Application of kinesthetic as well as light tactile stimuli; 2) Placement of electrolytic lesions at the borders of functional response zones; 3) Dipping of electrodes in horseradish peroxidase (HRP) to mark the locations of electrode tracks in the thalamic tissues; and 4) Reaction of tissues for cytochrome oxidase (E.C. 1.9.3.1) and acetylcholinesterase (E.C. 3.1.1.7) activities as well as for HRP, Nissl substance and myelin.

In this study, like Welker and Johnson (1965), recording sites are examined in tissues reacted for Nissl substance and myelin in order to reconcile electrophysiological response fields with cell body and myelinated fiber formations. Enzyme histochemistry is employed as a complementary anatomical technique to further correlate specific biochemical activity distributions with these functional response fields.

Subunits of a somatosensory system have been shown to stain differentially for cytochrome oxidase, a mitochondrial enzyme involved in the electron transport pathway of cellular oxidative metabolism. Individual mystacial vibrissae of the mouse send sensory projections to terminate in anatomically distinct 'barrel fields' of the cerebral cortex (Woolsey and Van der Loos, 1970). The cell-free centers of these barrels can be visualized with histochemical procedures for cytochrome oxidase as well as succinate dehydrogenase, another mitochondrial enzyme (in the neonatal rat: Killackey and Belford, 1979). Blockage of sensory input via disruption of the vibrissa at critical stages of development markedly affects cortical barrel fields, as visualized by cytochrome oxidase histochemistry in the mouse (Wong-Riley and Welt, 1980). This treatment also affects the structure of cortical, thalamic and three sets of medullary projection fields from the vibrissae, as visualized by succinate dehydrogenase histochemistry in the neonatal rat (Belford and Killackey, 1980). Cytochrome oxidase histochemical staining has also been shown to reflect decreases in sensory input over long periods of time in visual and auditory systems (Wong-Riley, 1979; Wong-Riley, _et_ _al_., 1978). In the latter system, decreases in cytochrome oxidase activity were reversible with electrical stimulation of the nerve (Wong-Riley, _et_ _al_., 1978a). "Convergent pathway-specific and/or excitatory synaptic input levels" in respective laminae and regions of the hippocampus are reflected in their reactivity in cytochrome oxidase histochemistry (Kageyama and Wong-Riley, 1982).

Cytochrome oxidase histochemistry is employed in these experiments to answer the following questions: 1) How are mechanosensory projections from respective body parts of the thalamus of the raccoon stained - is the situation analogous to mouse and neonatal rat vibrissal projections? 2) How is the relative density of cytochrome oxidase staining distributed - are mechanosensory projection zones higher in staining density, reflecting a convergence of specific sensory input?

Thalamic tissues were reacted for acetylcholinesterase as a marker for another specific biochemical activity. This stain was employed since preliminary experiments with a variety of histochemical procedures indicated that acetylcholinesterase activity may mark some of the borders of the ventrobasal thalamus. Confirmation of this observation was sought with electrophysiological and tissue marking methods. The significance of acetylcholinesterase as a positive indicator of cholinergic neurotransmission is unclear since choline acetyltransferase activity does not always coincide with acetylcholinesterase activity. However any region devoid of acetylcholinesterase activity could not support cholinergic neurotransmission.

# EXPERIMENTAL PROCEDURES

Animal preparation, electro-anatomical recording, tissue preparation and electrode track and lesion identification procedures are described in the accompanying article. The fixed tissue block was transferred successively through 20% and 30% sucrose solutions in phosphate buffer. The block was frozen and sectioned at the 40 μm setting in a plane parallel to that of the rows of electrode tracks. Four alternate section series were reacted: 1) Nissl substance and HRP, 2) myelin, 3) cytochrome oxidase activity, and 4) acetylcholinesterase activity and HRP. The Nissl stain was thionine. This was accompanied by a diaminobenzidine (DAB) reaction for HRP with cobalt intensification (Adams, 1977). The myelin stain (hematoxylin, Weil method) followed the procedures described by Emmers and Akert (1963). The cytochrome oxidase procedure was that of Wong-Riley (1979). The acetylcholinesterase and HRP reactions were those described by Hardy, *et al*. (1976) with the modification of using 1 x $10^{-6}$ M isopropyl pyrophosphoramide (iso-OMPA) as an inhibitor (Parent, *et al*. 1977).

*Quantitative cytoarchitectonics*. The broadest profile of each nucleolated cell body stained for Nissl substance within square thalamic regions with sides of 260 μm (and hence areas of 67,600 $\mu m^2$) were traced using a camera lucida (1320x magnification). Regions were selected to include cutaneous projections, kinesthetic projections or a region dorsal to where kinesthetically-evoked unit cluster activity was recorded, which we identify as the ventrolateral nucleus of the thalamus (VL). Those regions selected for tracing were clearly identified by marking lesions. Also regions with a relatively low fiber density were preferentially selected. Cross-sectional areas of cells were measured with a digital planometer implemented on a DEC PDP-11 computer with programs written by Dr. M. R. Park of the Anatomy Department, Michigan State University.

Cytochrome oxidase activity was quantitated at the Pattern Recognition and Image Processing Laboratory at Michigan State University using Spatial Data Systems software. The television image of a cytochrome oxidase stained tissue section was digitized and converted to a pseudocolor image. The pseudocolor image indicates relative staining levels of regions of the thalamus.

Materials. Iso-OMPA was from ICN Pharmaceuticals.

# RESULTS

The mechanosensory (including kinesthetic and cutaneous) projection zone of the raccoon thalamus forms a histochemically distinct region, as viewed by the three methods presented below. The identities of anatomical structures were determined with the Berman and Jones (1982) atlas of the cat thalamus and forebrain unless otherwise noted.

## Cytochrome oxidase

The mechanosensory projection zone is dramatically marked by the cytochrome oxidase stain. Cytochrome oxidase activity is more dense in the mechanosensory projection zone than in the more moderately staining neighboring regions including: ventrally, the ventroposterior inferior nucleus (VPI: Rinvik, 1986); medially, the principal ventromedial nucleus (VMP); dorsally and medially (except in the rostral aspects of the mechanosensory projection zone) the posterior nuclei (PO) and the lateral posteri or nucleus (LP) (Figures 3.1-3.8). Staining is absent in the external medullary lamina. Other thalamic regions with moderate to strong cytochrome oxidase activity include the ventrolateral nucleus, the medial and lateral geniculates and the subthalamic mass within the mechanosensory projection zones, the most dense staining was found in what appear to be leg and tail projections (lateral most dark bands in the thalamus in Figures 3.1B, 3.3B, 3.4A, 3.4B and 3.8A). The cytochrome oxidase activity stain reveals two structural features: 1) Mitochondria are stained with the diaminobenzidine (DAB) reaction product to highlight neuropil as well as cell bodies (Wong-Riley, 1979; Kageyama and Wong-Riley, 1982); 2) Myelinated fiber tracts stain only very weakly and their architecture is highlighted

Figure 3.1. Adjacent coronal sections of left diencephalon of raccoon 536 reacted for: (A) Nissl substance (thionin reaction) and (B) Cytochrome oxidase activity. Figure 3.1C is a tracing of these sections which illustrates the receptive fields projecting to the sites traversed by the electrode tracks. In this and following Figures, all receptive fields are illustrated in a schematic drawing of the right side of the body with the palm facing out. Vertical lines represent electrode tracks, horizontal bars are sites where unit cluster electrical activity was evoked by mechanical stimulation, and arrows mark the locations of marking lesions. Letters adjacent to recording sites indicate receptive field characteristics (see Abbreviations). Figure 3.1D indicates that the approximate locations of penetrations (indicated by arrow heads) and sections (horizontal line) shown in Figures 3.1A, B and C are rostral in the mechanosensory region in a hypothetical horizontal section of the left half of the brain. The view from the horizontal plane (adapted from Welker and Johnson, 1965) illustrates that the projections from the head are medial and those from the leg are lateral, while the individual forepaw digits have distinct projection zones (digit 1 = thumb, etc.). Caudal to these lie the forepaw palm and cutaneous body projections. This figure is at the same approximate level as Figure 2.1D of the accompanying paper (Wiener _et al_., 1983).

Four lesions (indicated by arrows in the photographs and in the diagrams) circumscribe the kinesthetic projection zone. Large myelinated fiber bundles (which stain very weakly for cytochrome oxidase activity; indicated by double arrows in Figure 3.1B) separate the cutaneous (G and C in Figure 3.1C) from the kinesthetic (K and J) projection zones. Fiber architecture and more intense cytochrome oxidase activity distinguish the somatosensory projection zone from neighboring thalamic regions. (A third electrode track lies between the two indicated by arrowheads and is not discussed here.) Scale bars = 1 mm. The three small boxes in Figure 3.1A indicate the approximate sites that cell area measurements were made in this section or a section 120 μm rostral to it (see Figure 3.9). The most medial box is in VL, where we encountered no reliable responses to mechanical stimulation; the box lateral to that is in the kinesthetic projection zone (two sites were sampled) and the most ventral box is in the cutaneous projection zone.

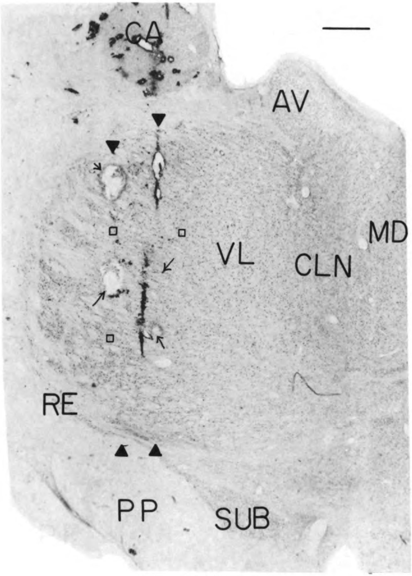


Figure 3.1A

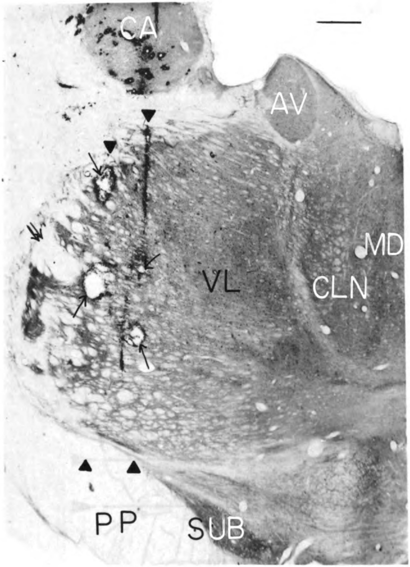
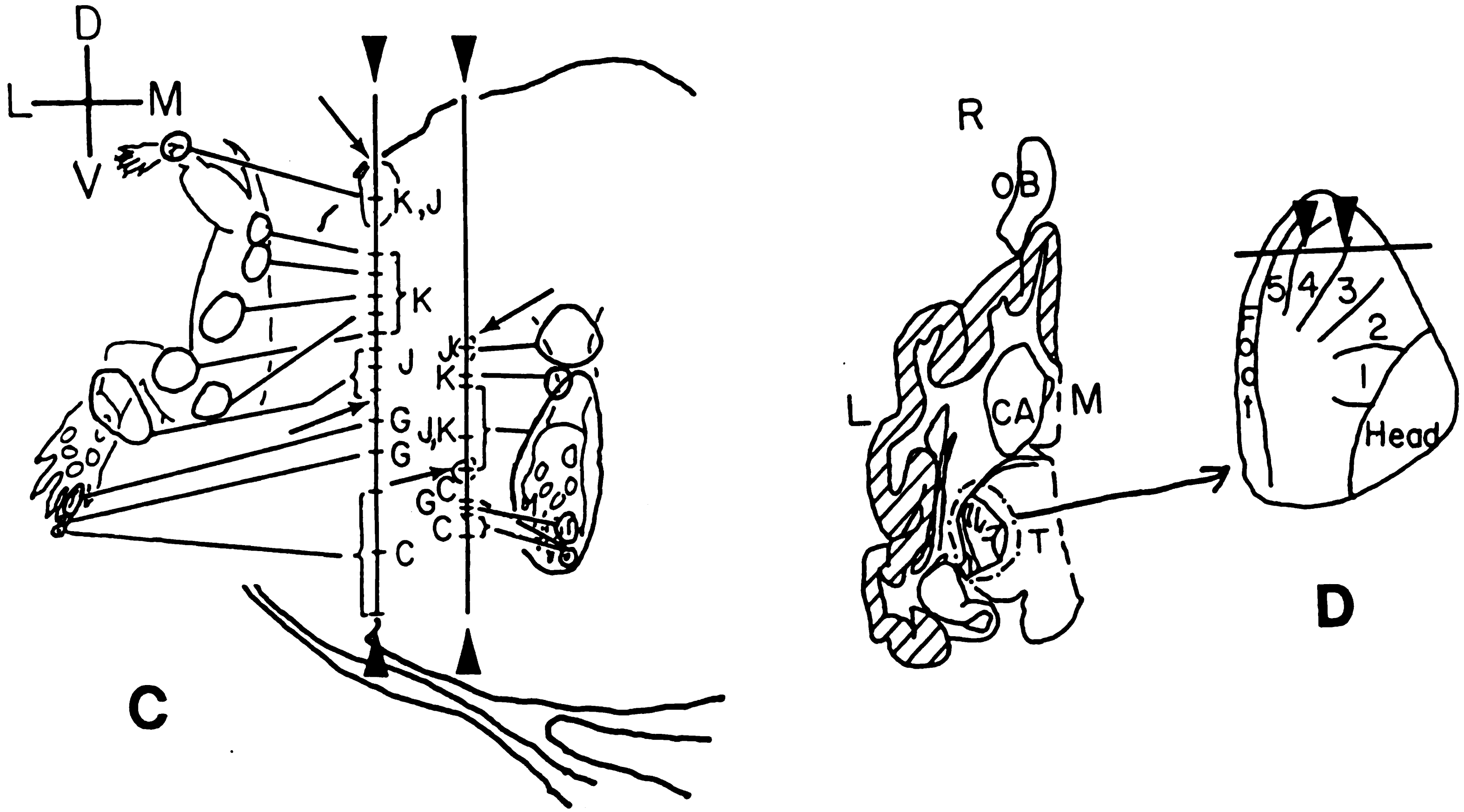


Figure 3.1B



Figures 3.1C and 3.1D

Figure 3.2. Coronal sections through left diencephalon of raccoon 511 containing microelectrode penetrations (indicated by arrowheads) and marking lesions (arrows). Conventions, symbols and abbreviations are the same as for Figure 3.1. Figure 3.2A is stained for Nissl substance (thionin) and 3.2B is stained for cytochrome oxidase activity. Figure 3.2C illustrates the peripheral receptive fields projecting to the sites traversed by the electrode tracks in the tissue as well as marking lesions in penetrations a, b and d. Figure 3.2D shows the relative locations of these sections and penetrations in a hypothetical horizontal plane. In all cases lesions marking the borders of the kinesthetic projection zone lie in close proximity to fiber bundles (marked by double arrows). Dorsal portions of the somatosensory projection zone which receive kinesthetic and non-glabrous cutaneous projections are distinguished by large clusters of fiber bundles while glabrous forepaw as well as cutaneous head projection zones have more evenly spaced fiber bundles as well as the thin dorso-ventrally oriented fibrous laminae separating the individual digit as well as head representations. These sections are from the same plane represented in Figure 2.2E of the accompanying paper and are identical to those of Figures 2.2G and H of the accompanying paper and penetrations are labelled with the same small case letters. Scale bars = 1 mm.

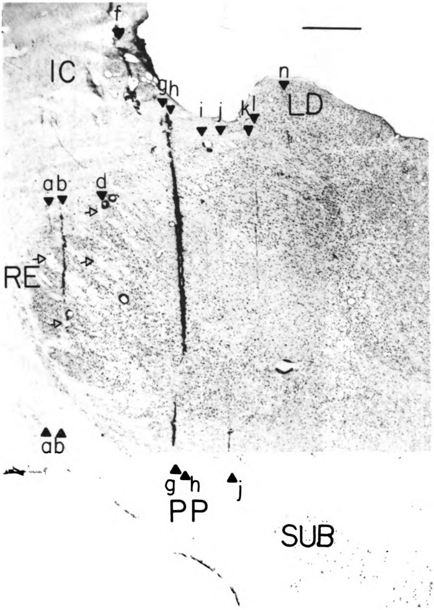


Figure 3.2A
(also Figure 2.2G)

Figure 3.2. Coronal sections through left diencephalon of raccoon 511 containing microelectrode penetrations (indicated by arrowheads) and marking lesions (arrows). Conventions, symbols and abbreviations are the same as for Figure 3.1. Figure 3.2A is stained for Nissl substance (thionin) and 3.2B is stained for cytochrome oxidase activity. Figure 3.2C illustrates the peripheral receptive fields projecting to the sites traversed by the electrode tracks in the tissue as well as marking lesions in penetrations a, b and d. Figure 3.2D shows the relative locations of these sections and penetrations in a hypothetical horizontal plane. In all cases lesions marking the borders of the kinesthetic projection zone lie in close proximity to fiber bundles (marked by double arrows). Dorsal portions of the somatosensory projection zone which receive kinesthetic and non-glabrous cutaneous projections are distinguished by large clusters of fiber bundles while glabrous forepaw as well as cutaneous head projection zones have more evenly spaced fiber bundles as well as the thin dorso-ventrally oriented fibrous laminae separating the individual digit as well as head representations. These sections are from the same plane represented in Figure 2.2E of the accompanying paper and are identical to those of Figures 2.2G and H of the accompanying paper and penetrations are labelled with the same small case letters. Scale bars = 1 mm.

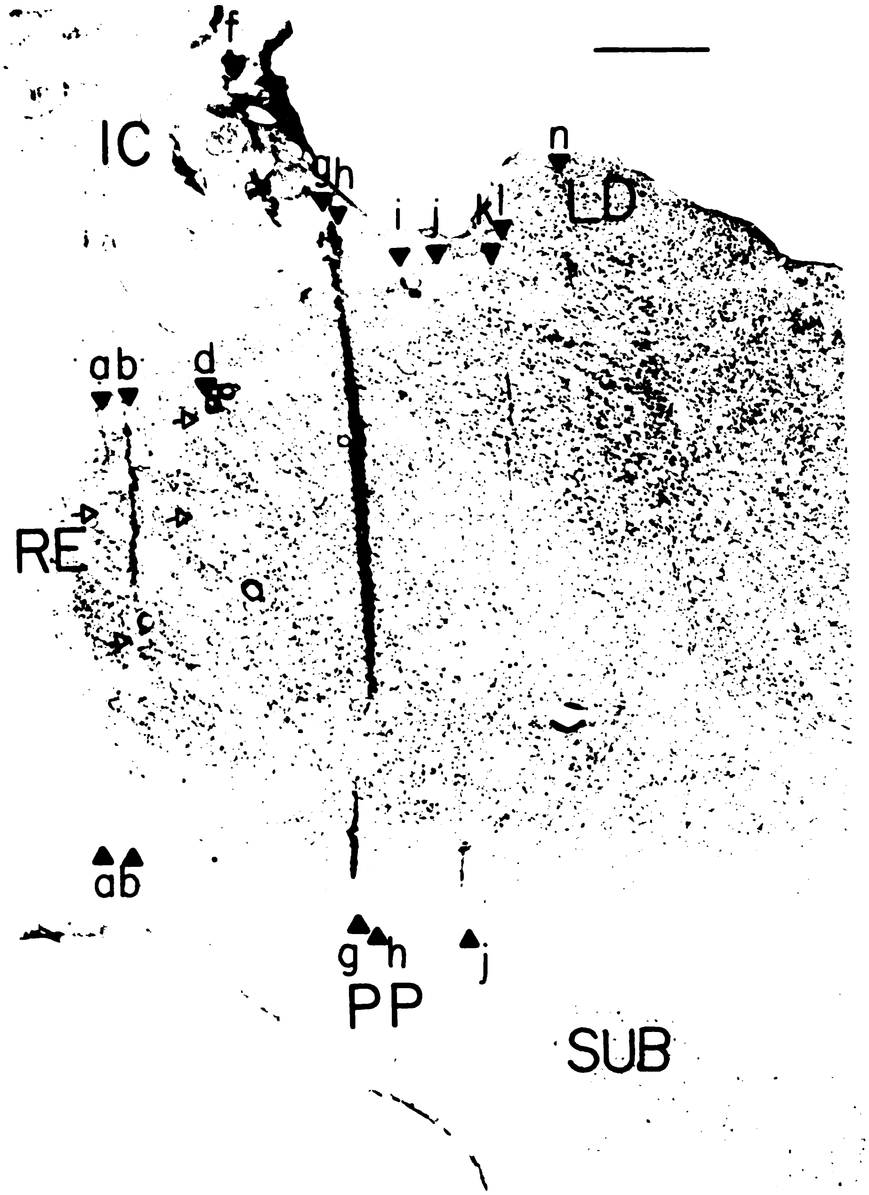


Figure 3.2A
(also Figure 2.2G)

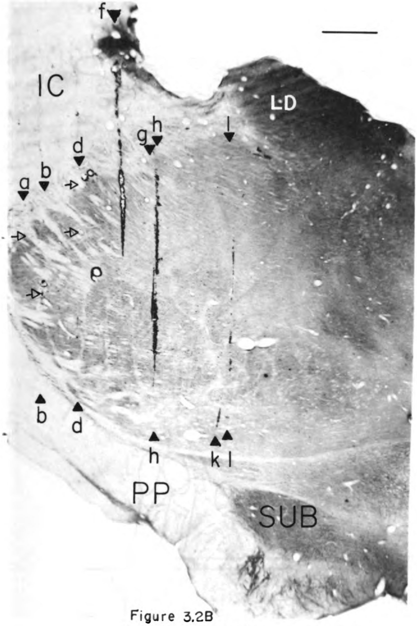
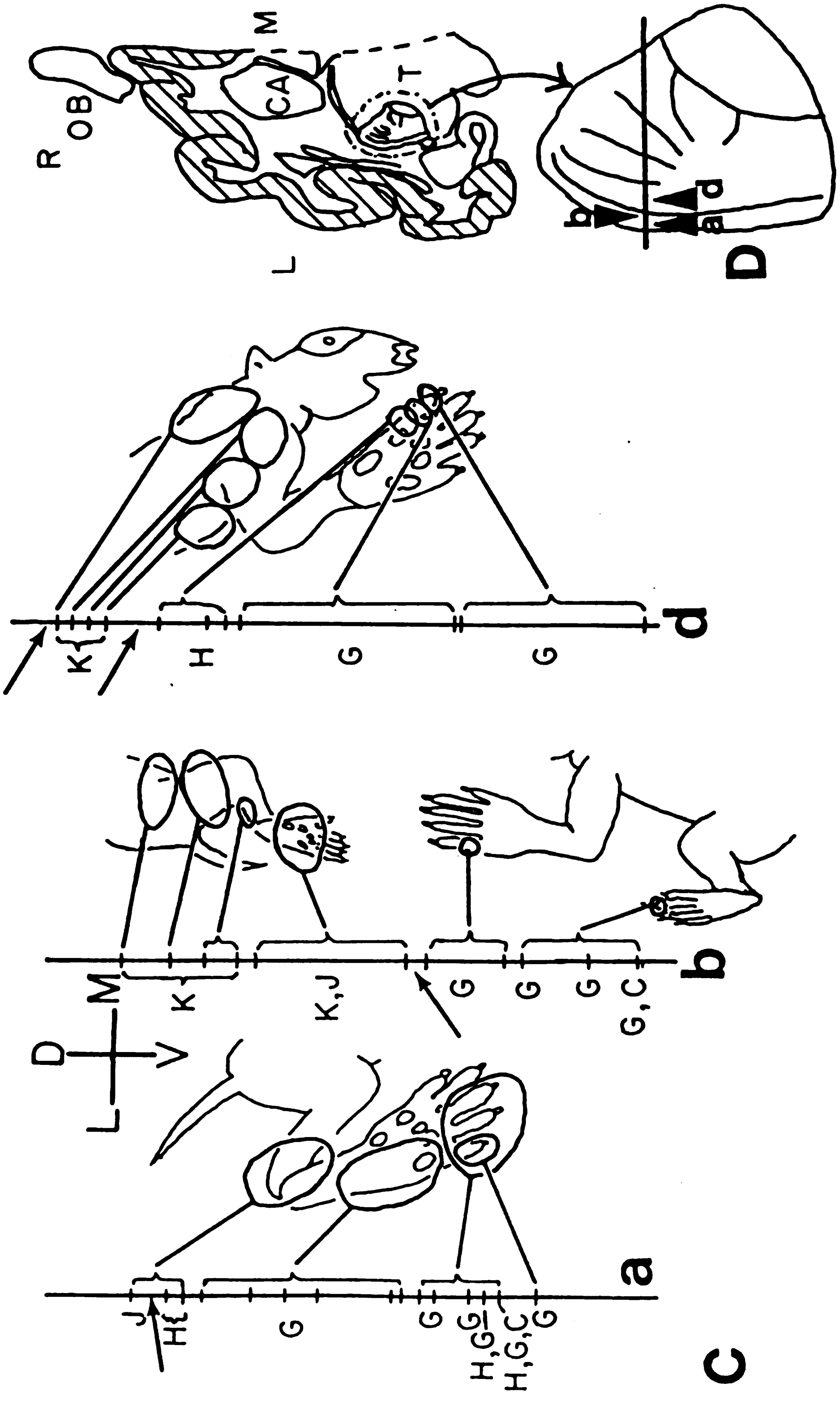


Figure 3.2B
(also Figure 2.2H)



Figures 3.2C and 3.2D

Figure 3.3. Adjacent coronal sections through the somatosensory thalamus of raccoon 537 stained for: (A) Nissl substance (thionin stain), (B) cytochrome oxidase activity and (D) acetylcholinesterase activity. Conventions, symbols and abbreviations are the same as for Figure 3.1. In 3.3B, double arrows indicate the location of fiber fundles on the border of the kinesthetic and cutaneous mechanosensory projection zones. The course of a single microelectrode penetration is indicated by the vertical bar. Figure 3.3C illustrates receptive fields on the forelimb sending projections to the sites traversed by the electrode track in the tissue. Figure 3.3E indicates the relative locations of sections and penetrations, caudal to those of Figure 3.2, in a hypothetical horizontal plane. These sections are at approximately the same level as Figure 2.2F of the accompanying paper. The cytochrome oxidase stained tissue (Figure 3.3B) reveals fibrous laminae in the glabrous digit projection zone as found by Welker and Johnson (1965). These laminae do not extend dorsally into the kinesthetic projection zone. Cytochrome oxidase staining is weaker in VPI and LP than in the bordering mechanosensory projection zone. Acetylcholinesterase staining appears along the dorsal edge of the kinesthetic portion of the mechanosensory projection zone where it abuts LP as well as in VPI while it only appears in scattered cell bodies throughout the mechanosensory projection zone. Scale bars = 1 mm. Flattening of LD in 3.3A and B is artifactual.

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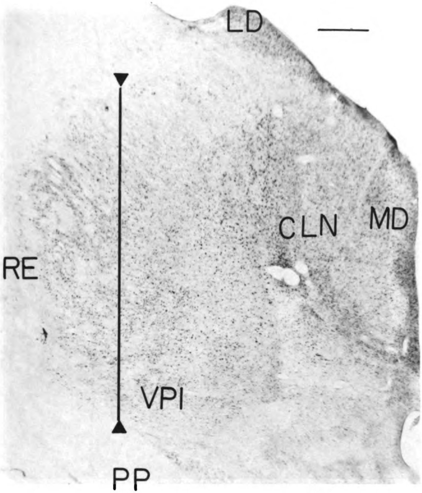


Figure 3.3A

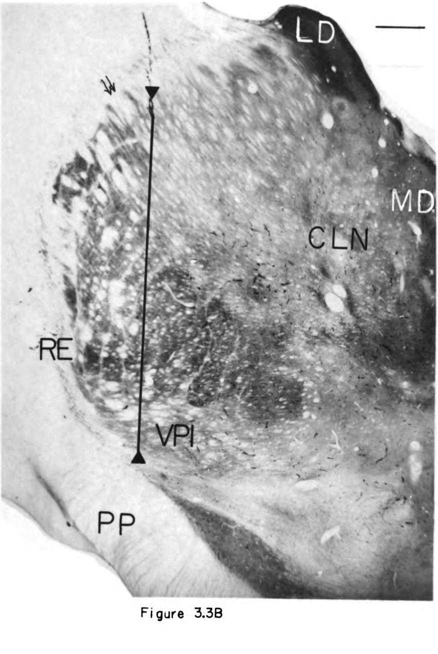


Figure 3.3B

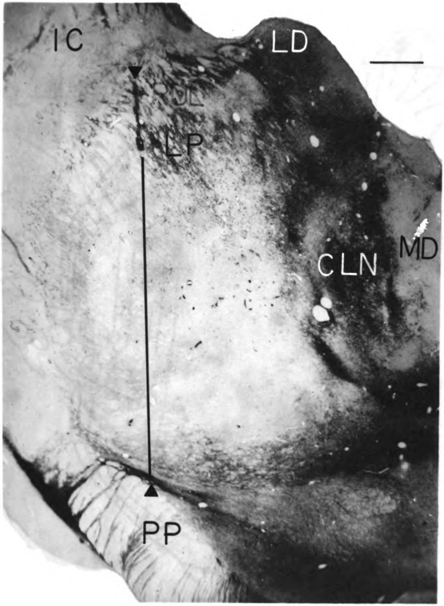
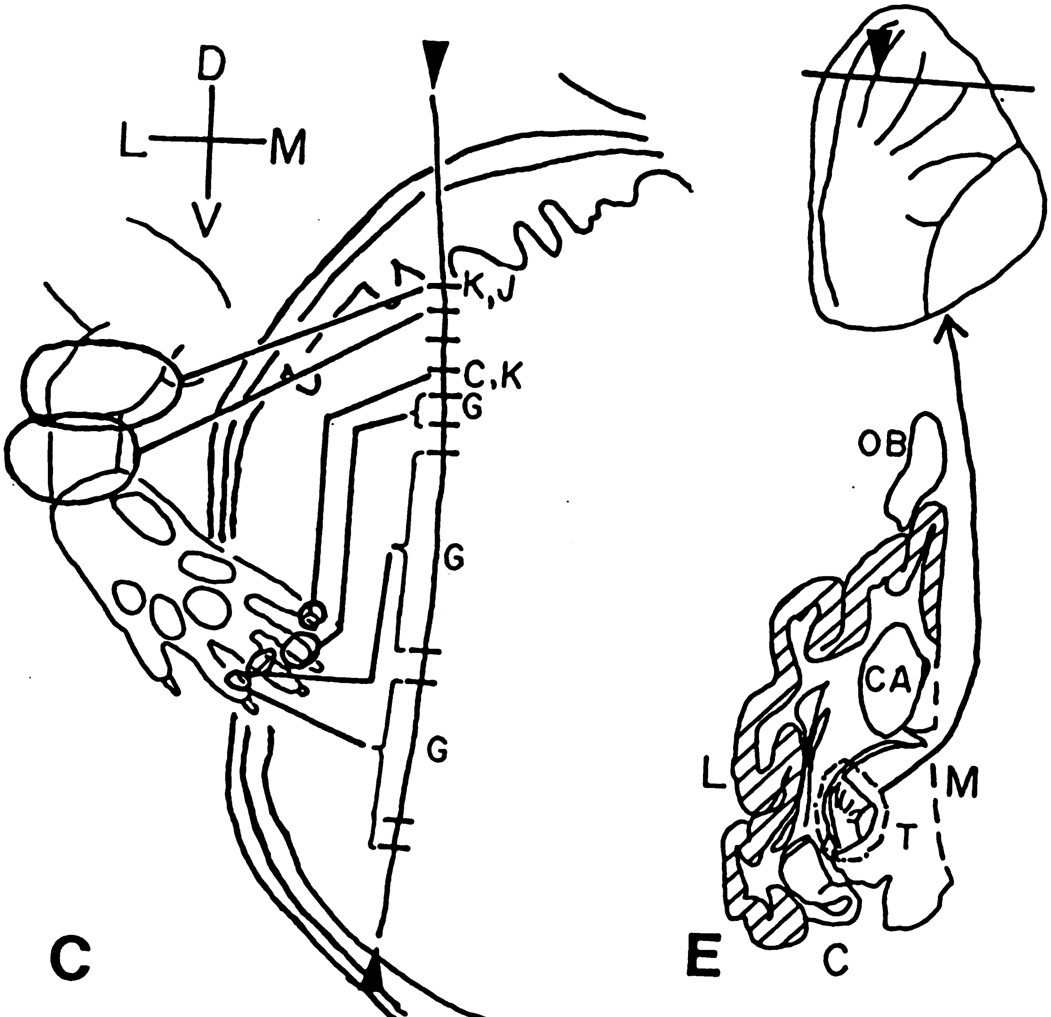


Figure 3.3D



Figures 3.3C and 3.3E

Figure 3.4. A and B are cytochrome oxidase stained coronal sections cut 120 μm apart in the left thalamus of raccoon 536. These are approximately 1.1 mm caudal to those of Figure 3.1. Figure 3.4C shows the relative locations of penetrations and sections in a hypothetical horizontal section of the left half of the brain. This Figure is at the same approximate level as Figures 2E and F of the accompanying paper and is slightly oblique to the stereotaxic coronal plane. Figure 3.4D is a schema of the loci of the electrode tracks and marking lesions in the sections of Figures 3.4A and B. Figure 3.4E illustrates the receptive fields projecting to the sites traversed by the electrode tracks in the tissues of Figures 3.4A and B. Small case letters identify the individual electrode tracks. Conventions, symbols and abbreviations are the same as in Figure 3.1.

Weakly staining, dorsoventrally oriented fibrous laminae separate the heavily stained projections from the respective forepaw glabrous digits as well as from the hindlimb and head. These laminae are a distinguishing characteristic of these cutaneous projection zones. The region dorsal to this does not contain the fibrous laminae and displays evoked electrical activity following stimulation of (deep) muscles (K) and joints (J), light touch to hairy skin (H) or stronger and visible displacement of the skin (P). In this plane of section, fiber bundles appear to course mediolaterally within the plane of section in the region containing kinesthetic projections and those from hairy skin. In the glabrous forepaw projection zone, the bundles course more perpendicular to the plane of section. Open white circle and open black circle in Figure 3.4B indicate the points used as reference limits for quantitation of stain density in Figure 3.8A. As is shown in Figure 3.8A, the mechanosensory projection zone and VL have dense staining while the neighboring LP, VMP and VPI have less dense staining. Scale bars = 1 mm.

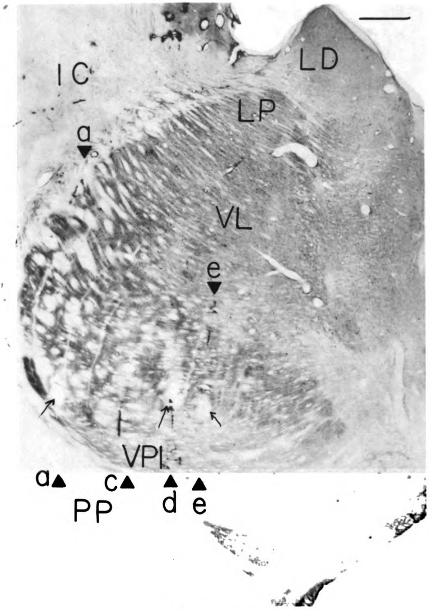


Figure 3.4A

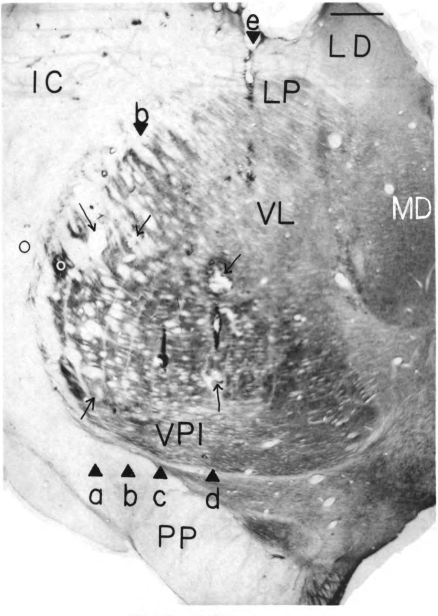
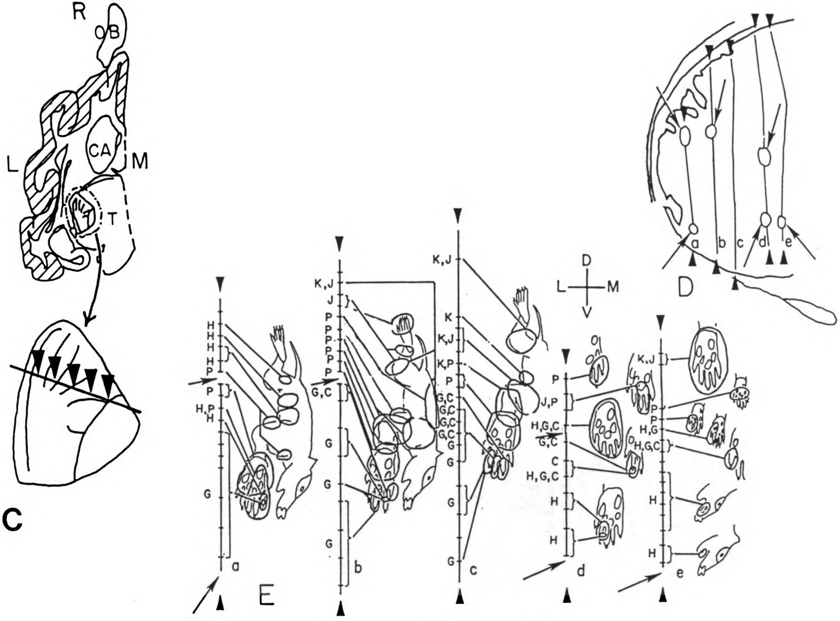


Figure 3.4B



Figures 3.4C, 3.4D and 3.4E

Figure 3.5. Adjacent coronal sections through the caudal part of the left mechanosensory thalamus of raccoon 528 stained for: (A) Nissl substance (thionin stain), (B) cytochrome oxidase activity and (C) acetylcholinesterase activity. Figure 3.5D illustrates the receptive fields projecting to the sites traversed by the electrode track in the tissue. Figure 3.5E indicates relative locations of the sections and the penetration in a hypothetical horizontal plane. This Figure is at approximately the same level as Figures 2.3A and B of the accompanying paper. In the cytochrome oxidase stained section, the fiber architecture of the somatosensory projection zone takes on a characteristic cross-hatched appearance from the mediolateral coursing of myelinated fibers and the dorsoventrally oriented fiber lamina (marked by double arrows). (The stainfree zone in the middle of the line indicating the electrode track is artifactual.) In the acetylcholinesterase stained section, the somatosensory projection zone is free of reaction product. Dense acetylcholinesterase staining is found in the pulvinar, LP and CM bordering the mechanosensory projection zone which is virtually free of staining. Symbols, abbreviations and conventions are the same as in Figure 1. Scale bars = 1 mm.

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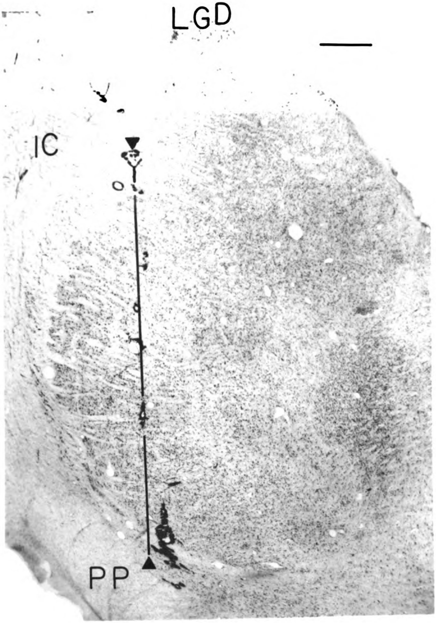


Figure 3.5A

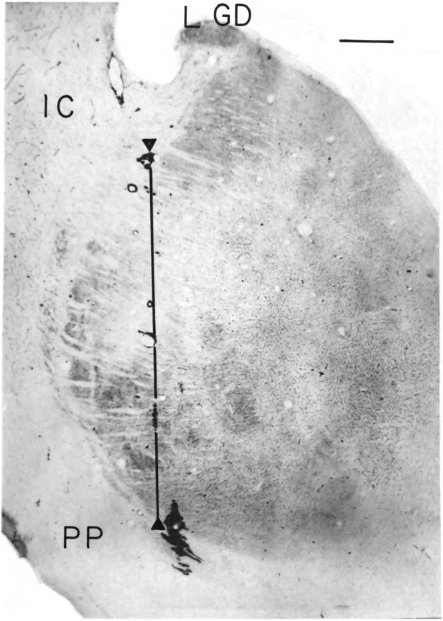


Figure 3.5B

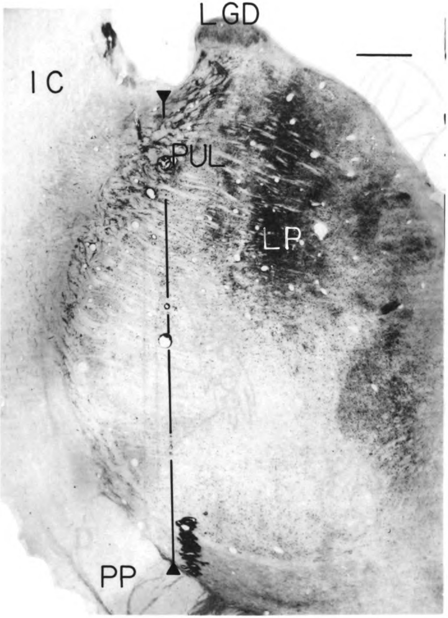
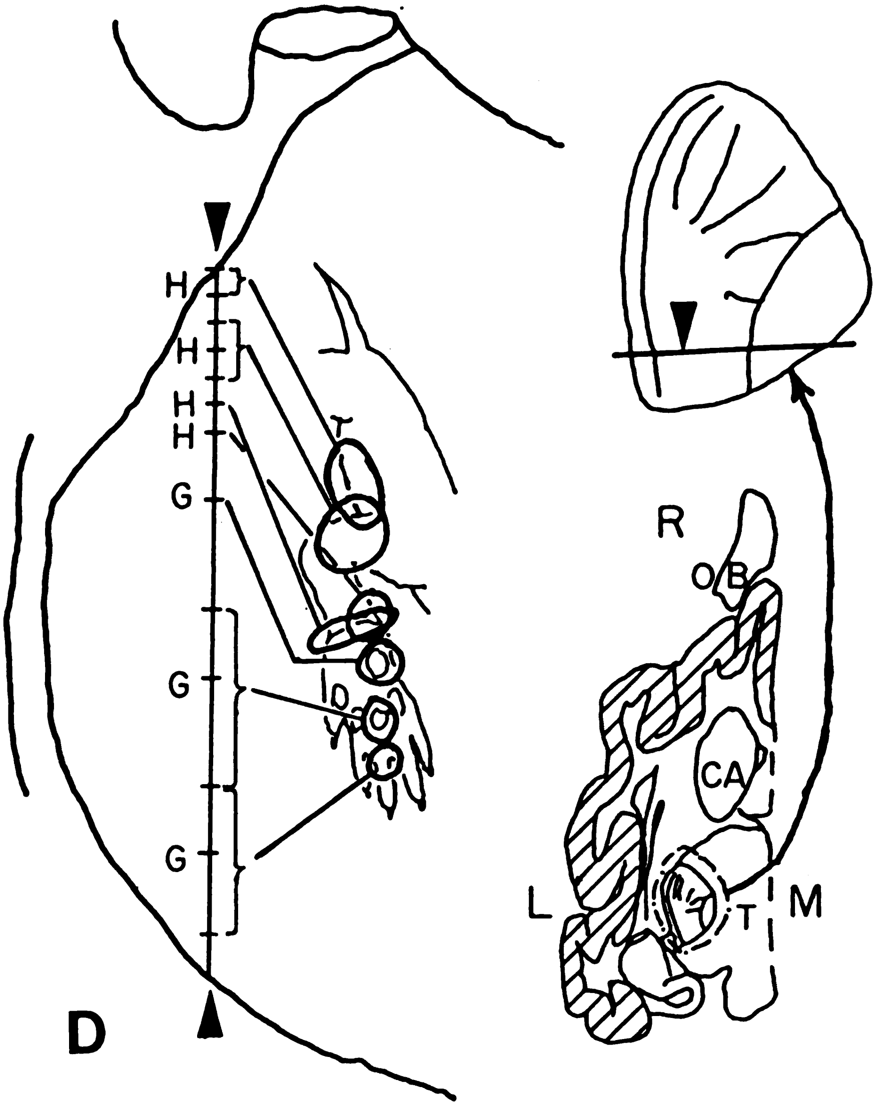


Figure 3.5C



Figures 3.5D and 3.5E

Figure 3.6. Parasagittal section of the left diencephalon of raccoon 519 containing microelectrode penetrations (arrowheads) and marking lesions (arrows). (Other lesions and penetrations are present but not indicated with symbols.) Tissues are stained for (A) Nissl substance (thionin stain) and (B) Cytochrome oxidase activity. Figure 3.6C illustrates the receptive fields projecting to the sites traversed by the electrode track in the tissues shown in Figures 3.6A and B. Figure 3.6E shows the relative locations of the sections and penetrations in a hypothetical horizontal plane. Figure 3.6 is in a plane somewhat lateral to that of Figure 2.4A of the accompanying paper. Large myelinated fiber bundles, (double arrows) which have been marked by electrolytic lesions separate the kinesthetic zone into a distinct region of cell bodies and neuropil which lies in a flattened band dorsal to the cutaneous projection zone in its rostral aspect (Figure 3.6D). Symbols and abbreviations are the same as in Figure 3.1. Boxes in Figure 3.6A indicate approximate locations in this, or a section 120 μm medial to this, from which cell areas were measured for cytoarchitectonic analysis (Figure 3.9). The more rostrodorsal box is in the kinesthetic projection zone; the more caudal and ventral box is in the cutaneous projection zone. Open white circle and open black circle indicate points used for reference limits in digital quantitation of staining intensity in Figure 3.8B. As shown in Figure 3.8B, dense cytochrome oxidase staining occurs in the mechanosensory projection zone, the medial geniculate nucleus, and the lateral geniculate nucleus. Weaker staining occurs in zones neighboring the mechanosensory projection zone: the posterior complex dorsally and caudally and an unidentified zone ventrocaudally. Scale bars = 1 mm.

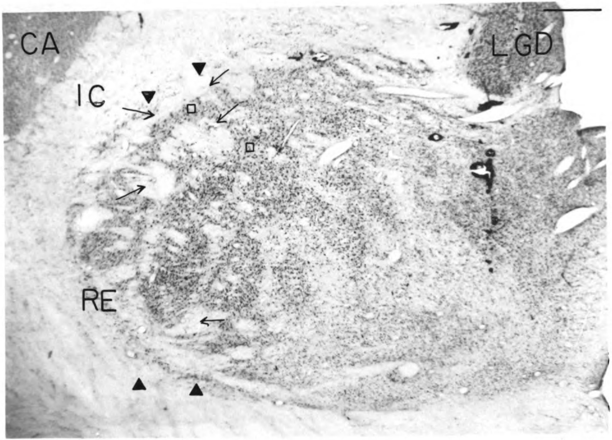


Figure 36A

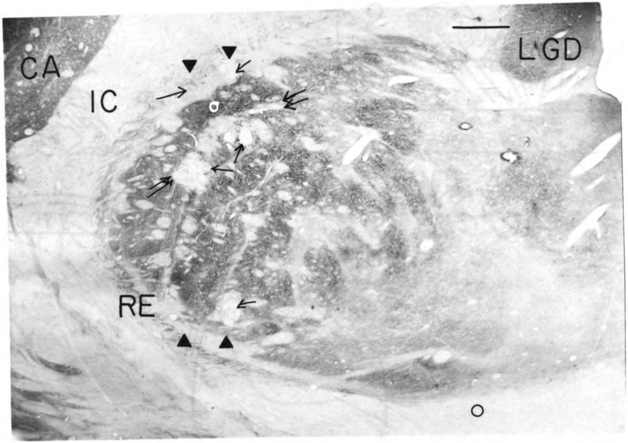
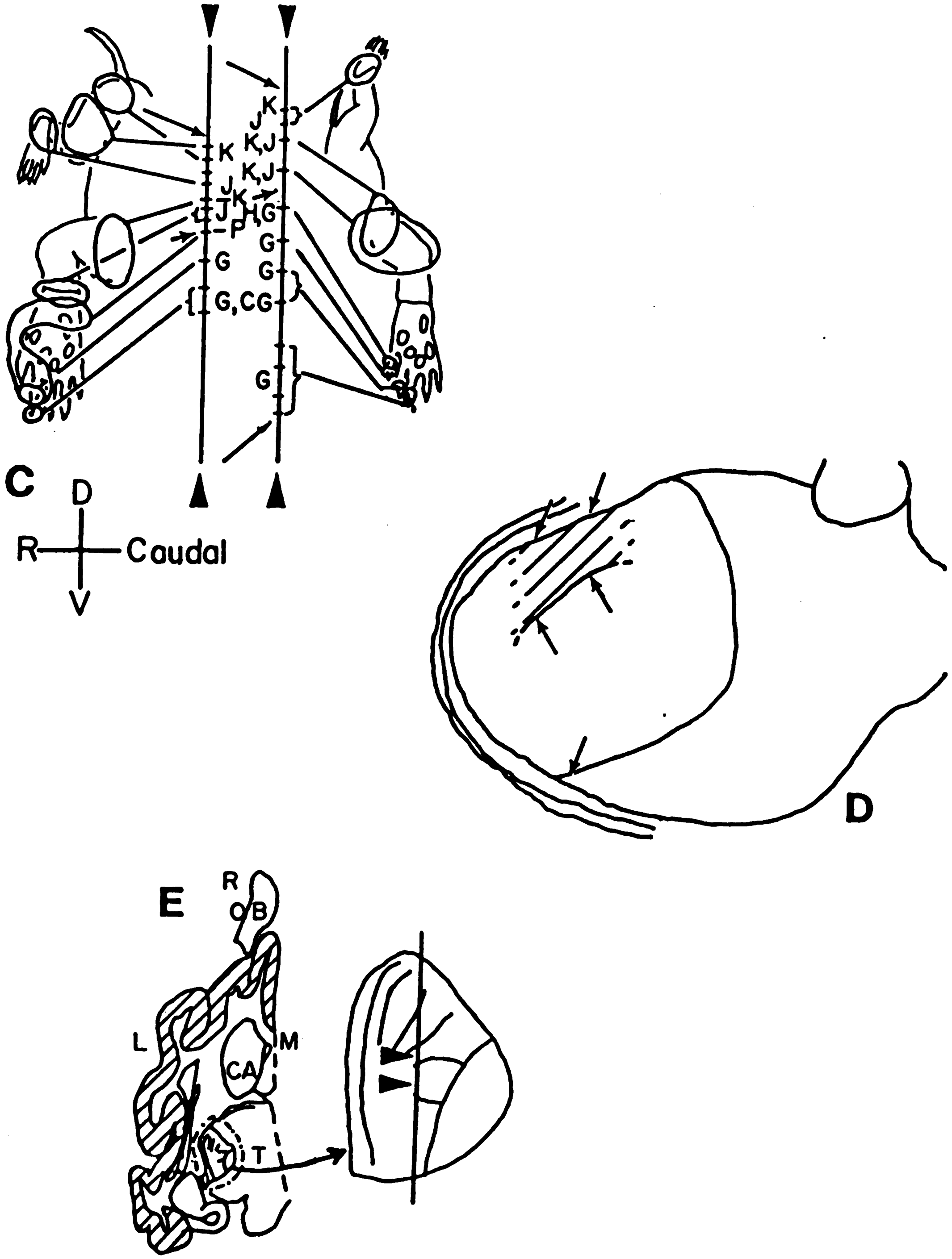


Figure 36B



Figures 3.6C, 3.6D and 3.6E

Figure 3.7. Sagittal sections of the left diencephalon of raccoon 519 located approximately 1.4 mm medial to those of Figure 3.6. Tracks of two electrode penetrations (arrowheads) as well as four marking lesions (arrows) are indicated. Sections are stained for: (A) Nissl substance (thionin reaction), (B) acetylcholinesterase activity and (C) cytochrome oxidase activity. Figure 3.7D illustrates the receptive fields projecting to the sites traversed by the electrode tracks in the tissues of Figures 3.7A, B and C. Figure 3.7E indicates relative locations of sections and penetrations in a hypothetical horizontal plane. These sections are slightly medial to those of Figure 2.4B of the accompanying paper. In Figure 3.7C, the kinesthetic projection zone is bounded as outlined by marking lesions in the rostral penetration. These lesions lie in a fibrous lamina which forms an ovoid mass (outlined by hollow triangles) slight dorsally elevated in its caudal aspect, we believe this mass corresponds to the kinesthetic projection zone. Rostrally, what we believe is the ventrolateral nucleus is separated from the mechanosensory projections by a fibrous lamina (marked by double arrows). In the acetylcholinesterase stained section (Figure 3.7B), the lateral posterior nucleus bordering the unstained somatosensory projection zone dorsally and the medial geniculate bordering it caudally are densely stained. Weaker staining in the posterior complex marks the caudal borders of the cutaneous digit and head projection zones in the cytochrome oxidase stained section (Figure 3.7C). Symbols and abbreviations are the same as in Figure 3.1. Scale bars = 1 mm.

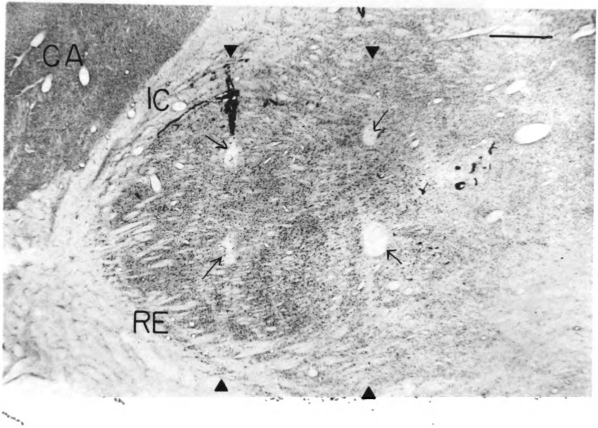


Figure 37A

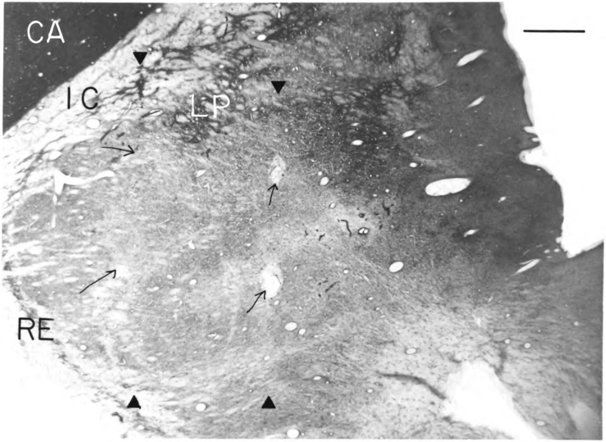
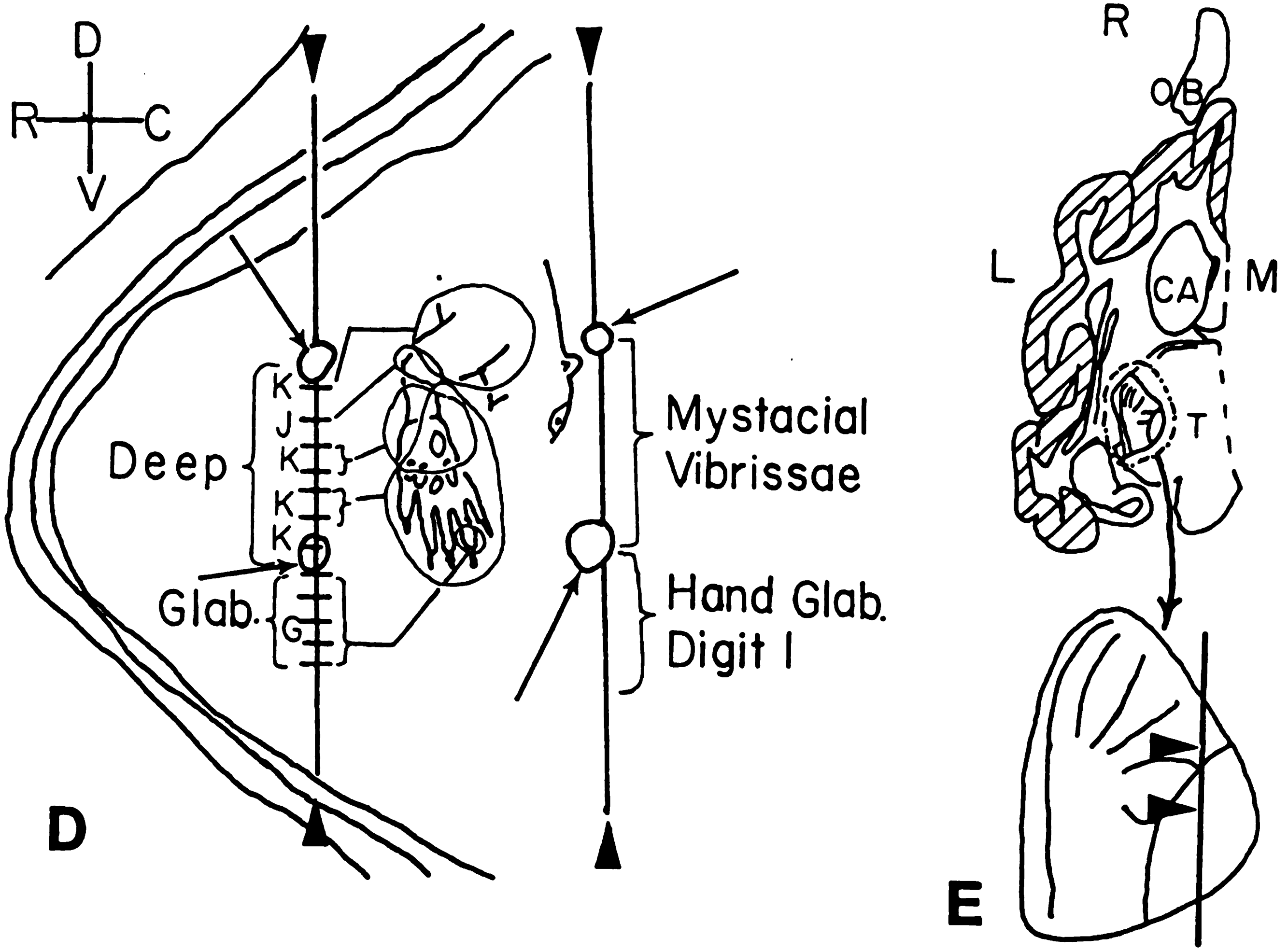


Figure37B

Figure37C



Figures 3.7D and 3.7E

Figure 3.8. Quantitation of cytochrome oxidase activity staining in the raccoon thalamus: A) From the transverse section shown in Figure 3.4B; B) From a sagittal section 120 μm medial to that shown in Figure 3.6B. The images of actual histological sections were digitized with a vidicon camera and a computer and were represented as pseudocolor images. (Pseudocolor shows different intensity levels as different colors and has recently been popularized in brain scan data representations.) Picture elements were visually selected for reference levels of lowest (shown with open black circles in Figures 3.4B and 3.6B) and highest (shown with open white circles in Figures 3.4B and 3.6B) values on the digital intensity scale in order to optimize contrast in the regions of interest. Picture elements with intensities outside these limits were assigned the respective limit values. The pseudocolor image was then traced (with human visual integration) to summarize the staining intensity levels within the section according to the following scales. (0% is the lightest and 100% is the darkest staining intensity.)

Figure 3.8A. 0-40% no shading; 40-63% stippled; 63-85% cross-hatched; 85-100% blackened.

Figure 3.8B. 0-38% no shading; 38-56% stippled; 56-72% cross-hatched; 72-100% blackened.

Intense cytochrome oxidase activity is found in the kinesthetic and cutaneous mechanoreceptor projection zones (cf. Figures 3.4B and 3.6B). In Figure 3.8A high levels of cytochrome oxidase are also found in the ventrolateral nucleus dorsally. Weaker stain levels are found in the LP, VMP and VPI which border the mechanosensory projection zone.

In Figure 3.8B, intense levels of cytochrome oxidase activity are also found in the lateral geniculate and medial geniculate of the thalamus. Moderate staining is found in the caudate nucleus. Weak staining occurs in the posterior complex at the caudal border of the mechanosensory projection zone.

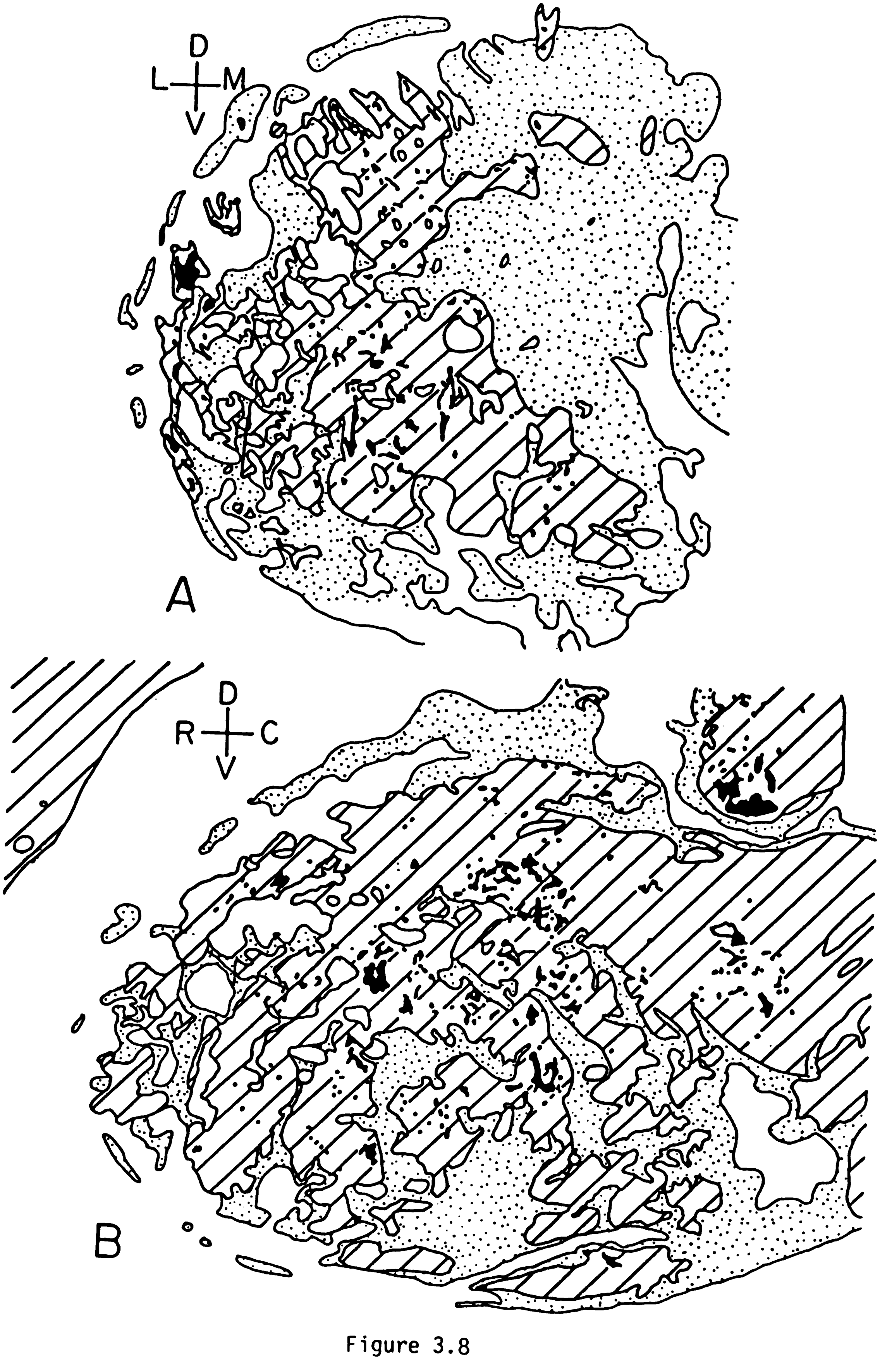


Figure 3.8

Figure 3.9. Histograms showing distributions of areas of nucleolated cell bodies in square sample regions with sides of 260 μm in the thalamus. Sample regions were selected on the basis of clear identity from electrolytic lesions made at borders of zones displaying mechanically-evoked electrical activity. Regions with lower density of myelinated fibers were also selected preferentially. Sample regions were in the cutaneous projection zone (left column), kinesthetic projection zone (middle column) and the ventrolateral (VL) nucleus (right column) of the thalami of three raccoons (in respective rows). As shown in the upper right corner, abscissa is cell body area ($\times 10^{-11}$ $m^2$) and ordinate is number of cells within respective range of areas. Since VL was not present in the plane of this sagittal section it is not shown in the top row. No clear distinctions appear in the cell area distributions of the three respective regions. Although not statistically tested, a trend appears for lower cell density in the kinesthetic projection zone. The histogram from the kinesthetic projection zone of animal 82536- row 2, column 2- is made up of measurements from two square sample regions with sides measuring 260 μm each, while all others are from one such area. Hence values of N reflect cell density. Area measurement means (filled triangles) and medians (open triangles) are computed from histogram values. Means (and medians), of area measurements expressed as $10^{-11}$ $m^2$, are summed from the histogram values of the three animal samples: Cutaneous projection zone- 15.1 (13.5); Kinesthetic projection zone- 13.7 (13.5); Ventrolateral nucleus- 12.4 (13.5).

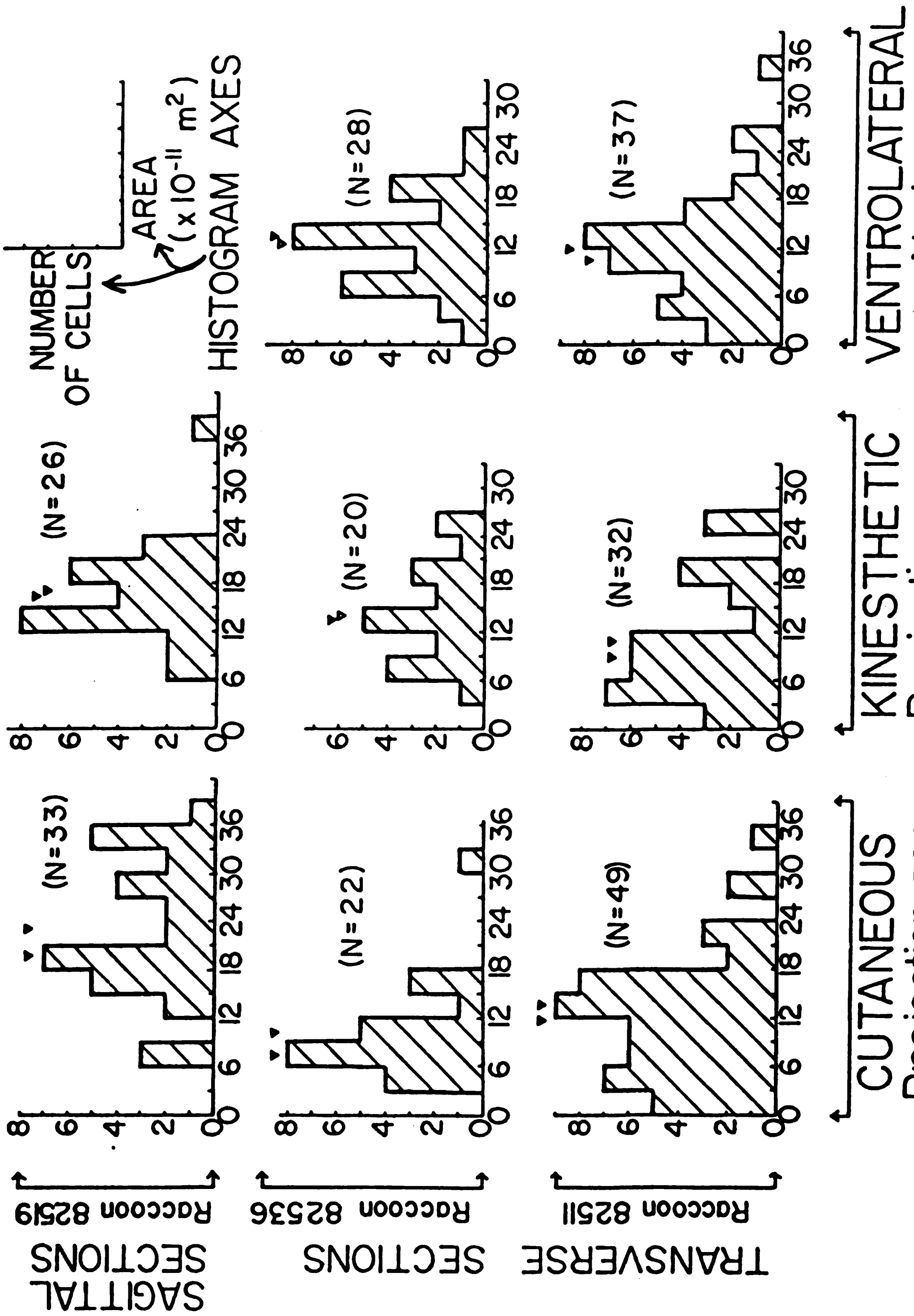

NUMBER OF CELLS
AREA (x10$^{-11}$ m$^2$)
HISTOGRAM AXES
SAGITTAL SECTIONS
Raccoon 8519
SECTIONS
Raccoon 8536
TRANSVERSE SECTIONS
Raccoon 8511
(N=33)
(N=26)
(N=22)
(N=20)
(N=28)
(N=49)
(N=32)
(N=37)
0
2
4
6
8
0
6
12
18
24
30
36
CUTANEOUS Projection zone
KINESTHETIC Projection zone
VENTROLATERAL Nucleus

Figure 3.9

in a manner more graphically and reliably than the hematoxylin reaction in our hands. The absence of cytochrome oxidase activity correlated well with hematoxylin staining in those cases where the latter was successful. (Hence we do not present hematoxylin stained specimens here.)

## Myelinated fiber architectonics

At all levels of the thalamic mechanosensory projection zone, mediolaterally and rostrocaudally oriented fiber bundles appear (Figures 3.1-3.7). Such bundles are often found along the border between the kinesthetic and cutaneous projection zones (double arrows in Figures 3.1-3.3, and 3.6). The caliber and orientation of fiber bundles are characteristic for respective rostro-caudal levels within the mechanosensory thalamic region. The widest caliber (among the widest in all the thalamus) are located in the more rostral, dorsal and lateral aspects of the mechanosensory projection region. Caudally, the caliber decreases, but is still wider than those seen in most other thalamic regions. The fiber bundles are oriented such that they appear to radiate rostrally and laterally from a (hypothetical) central focus on the medial aspect of the mechanosensory thalamus.

A second type of structure composed of myelinated fibers occurs at those levels containing cutaneous projections from the hindlimb, glabrous digits of the forepaw, head or tail. These are dorsoventrally oriented, thin laminae of myelinated fibers which extend rostrocaudally and mediolaterally (Figures 3.2-3.7). These laminae separate the projections from respective glabrous digits of the forepaw, distal hindlimb, head and tail (Welker and Johnson, 1965). These laminae do not extend dorsally and rostrally into the kinesthetic projection zone which caps these projections at their rostral levels nor into the zones of cutaneous projections from the body which cap them at the caudal levels (Figures 3.2, 3.3, 3.4, 3.6 and 3.7, see also accompanying paper). Thus, the fiber laminae appear in all but the most rostral, caudal and dorsal aspects of the mechanosensory projection zone. In Figure 7C it appears that a fibrous lamina separates the glabrous digit representation from the ventrolateral complex rostral to it.

Acetylcholinesterase

Acetylcholinesterase (AChE) activity in the control levels of the raccoon thalamus is very weak in the ventrolateral (VL) and ventral anterior (VA) nuclei, and moderate to strong in the reticular nucleus of the thalamus. Very weak AChE staining is also found in the mechanosensory projection zone. Progressing caudally, AChE activity is found in the lateral dorsal and the central lateral nuclei (Figure 3.2D). Weak to mederate AChE activity is found in VPI. A region containing AChE activity also appears dorsal to the mechanosensory projection zone in the middle of its rostrocaudal extent in the lateral posterior nucleus abutting the caudalmost aspects of VL (Figures 3.2D and 3.7B). Further caudally, AChE activity extends progressively more ventrally and laterally along the borders of the mechanosensory projection zones in the lateral posterior nucleus, the pulvinar and possibly the posterior nuclei (Figures 3.5C and 3.7B). Further caudally, AChE activity extends progressively more ventrally and laterally along the borders of the mechanosensory projection zones in the lateral posterior nucleus, the pulvinar and possibly the posteriot nuclei (Figures 3.5C and 3.7B). It is not clear if AChE is present in the caudalmost levels of the mechanosensory projection zone.

### Nissl substance

Cell bodies appear to contain more dense thionin reaction product in the mechanosensory projection zone than in the neighboring VPI, LP, VMP and CM (Figures 3.1, 3.2, 3.3 and 3.6). Visual inspection reveals that clusters containing large as well as small cells appear in the mechanosensory projection zones. The results of cell cross-section area measurements from the cutaneous as well as kinesthetic projection zones and VL are documented in Figure 3.9. No strongly consistent trends of segregation of cell area distributions are apparent in these data. However, the kinesthetic projection zone appears to have a lower cell density than the neighboring cutaneous projection zone and the ventrolateral complex. We observed no mechanosensory projections in the ventral postero-inferior (VPI) nucleus.

# DISCUSSION

The principal findings of this study are:

1) Zones of mechanosensory projections to the raccoon thalamus from respective body regions form discrete masses of neuropil and cell bodies which are distinguished by high levels of cytochrome oxidase activity. These discrete masses receive projections from the following body parts respectively: head, individual glabrous digits of the hand, glabrous foot, kinesthetic receptive fields and the hairy body.

2) Thin laminae of myelinated fibers and, in some cases, thick myelinated fiber bundles separate these distinct zones receiving projections from respective body loci.

3) Acetylcholinesterase activity is found along and outside the dorsal and medial borders of the thalamic mechanosensory projection zone in its caudal aspect where it abuts the lateral posterior nucleus, the pulvinar and possibly the posterior nuclei.

4) Distinctive populations of cell sizes were found neither in the ventrolateral nucleus (VL), nor in the cutaneous nor the kinesthetic projection zones of the raccoon thalamus.

## Cytochrome oxidase

The high density of cytochrome oxidase activity in the mechanosensory projection zones of the raccoon thalamus is in accord with other reports of cytochrome oxidase as a long-term neural activity indicator in somatosensory and other specific sensory pathways (Wong-Riley, *et al.*, 1978, 1978a; Wong-Riley, 1979; Wong-Riley and Welt, 1980). Chronic deprivation (through surgical intervention) of the auditory (Wong-Riley *et al.*, 1978a) systems result in histochemically demonstrable and reversible decreases in cytochrome oxidase activity in the respective specific sensory projection nuclei from one to several synapses away.

Kageyama and Wong-Riley (1982) found that levels of somatic reactivity to cytochrome oxidase in hippocampal neurons correlate positively with reproted cell firing rates (Vinogradova, 1975). CA3 pyramidal cells and various interneurons were observed to stain more intensely than CA1 pyramidal cells and dentate granule cells. Regional and laminar distributions of intense cytochrome oxidase activity correlated positively with reported intensity of convergence of major extrinsic afferent inputs to the hippocampus (see Kageyama and Wong-Riley for details and references). Dense cytochrome oxidase staining also correlates positively with presumed excitatory, and negatively with presumed inhibitory, neurotransmitter localization in the hippocampus (Kageyama and Wong-Riley, 1982 and references cited therein). In summary, the presence of dense cytochrome oxidase activity is interpreted as being positively correlated with regions with: 1) higher cell firing rates, 2) convergence of afferent inputs and 3) excitatory synaptic input. These correlations appear to hold in the raccoon thalamus also. The spontaneous rate of firing always increased as our electrodes entered the mechanosensory projection zones of the thalamus and decreased as they exited this zone. The unit cluster evoked responses appeared to be excitatory. Many of the thalamic regions in which we observed high levels of cytochrome oxidase activity are expected to receive convergent and presumably excitatory inputs: lateral geniculate nucleus (visual), medial geniculate nucleus (auditory), ventrolateral nucleus (cerebellar), pulvinar (visual). Of the three factors correlated with more dense cytochrome oxidase staining in the mammalian hippocampus (Kageyama and Wong-Riley, 1982), a higher convergence of inputs would most simply explain the greater intensity of cytochrome oxidase staining in the thalamic cutaneous hindfoot and tail projection zones than in other thalamic mechanosensory projection zones of the raccoon.

The staining reaction for cytochrome oxidase activity reveals something of neuropil architectonics. Myelinated fiber structures are also shown negatively by lack of staining. Kageyama and Wong-Riley (1982) confirm that myelinated fibers stain very weakly with this reaction in ten mammals. We believe this is true in the raccoon also. By process of elimination, we infer that neuropil as well as cell bodies are stained in the tissues under study here since 1) neuropil, cell bodies, myelinated fibers and vascular tissue are the main components of neural tissue, 2) myelinated fibers are not stained and 3) tissues reacted for Nissl substance (which is contained mostly in cell bodies) and for cytochrome oxidase do not show identical staining patterns. High power (1250x) light microscopic analysis of cytochrome oxidase stained tissues reveal cell bodies containing reaction product. In addition, strong activity appears between cell bodies indicating neuropil staining. Artifactual staining due to red blood cells was only found in isolated instances, usually along electrode tracks. (Red blood cells react strongly to the diaminobenzidine chromogen and are not disrupted by our histological procedures.)

With the cytochrome oxidase stain we have visualized distinct groupings of neuropil (and cell bodies) in the raccoon thalamus which receive projections from body regions which have distinct peripheral locations (kinesthetic versus cutaneous, digit 1 versus digit 2, etc.).

Other examples of neuropil architecture forming distinct zones related to peripheral sources of projections occur in the somatosensory barrel fields (which receive individual vibrissal projections) of 1) the mouse cerebral cortex (Woolsey and Van der Loos, 1970) and 2) the cortex, ventrobasal thalamus and of the neonatal rat, (e.g., Belford and Killackey, 1980). The latter was shown with a stain for succinate dehydrogenase which, like cytochrome oxidase, is a mitochondrial energy-transducing enzyme.

## Myelinated fiber architectonics

The separation of functionally distinct somatosensory regions of the nervous system by myelinated nerve fibers is frequently evident (Johnson, 1980). In the raccoon thalamus, this architectural trend is manifested in two types of structures composed of myelinated fibers. Thin fibrous laminae determine the borders between zones receiving projections from head, the respective glabrous digits of the forepaw, the hindlimb and the tail. Thick fiber bundles often lie between kinesthetic and cutaneous projection zones. These specializations of myelinated fiber architecture parallel those of the raccoon somatosensory cortex. In the expansion of cortical area to form the gyri and sulci there are related extensions and shortenings of innervating myelinated fibers. This may be interpreted as a cortical specialization of myelinated fibers which also distinguish the projections from the respective parts of the body and delineate the somatosensory submodality projection zones (Welker and Seidenstein, 1959; Johnson, 1980; Johnson, Ostapoff and Warach, 1982). The various subdivisions of medullary sources of the thalamic somatosensory projections are also distinguishable by virtue of their myelinated fiber architecture (Johnson, Welker and Pubols, 1968). We have observed this in raccoon medullas stained for cytochrome oxidase activity.

The separation of thalamic projection zones from the glabrous digits of the hand, as well as head, hindfoot and tail by fibrous laminae in the raccoon is in agreement with Welker and Johnson (1965). Similar laminar subdivisions between projections from respective body parts has been found in the prosimian, slow loris (Krishnamurti, *et al.*, 1972). In the owl monkey, four portions of the VPL are separated by fiber laminae (Lin, *et al.*, 1979).

### Acetylcholinesterase

The absence of acetylcholinesterase staining in the raccoon mechanosensory projection zones is in agreement with reports that acetylcholine is not a major neurotransmitter of the ventrobasal thalamus. The few cholinergic inputs to the ventrobasal thalamus arise from the midbrain reticular formation (Kelly, 1979). The appearance of acetylcholinesterase activity at the medial and dorsal borders of the mechanosensory projection zone in its caudal aspect reveals biochemical differentiation between the somatosensory thalamus and its neighbors, the lateral posterior nucleus, the pulvinar and possibly the posterior nuclei.

The patterns of acetylcholinesterase activity in the raccoon thalamus are similar to those found in other mammals. From the illustrations of Jacobowitz and Palkovits (1974), it appears that acetylcholinesterase activity occurs along the dorsal and caudal borders of the ventrobasal complex of the rat. In a study of acetylcholinesterase-containing somata (following pharmacological depletion of acetylcholinesterase activity in the neuropil), Parent and Butcher (1976) found the VPL of the rat to stain "minimally or moderately" with more intense staining caudalmost. Dense acetylcholinesterase activity is found in the ventrobasal complex of neonatal rat (from birth to six days of age), but not in surrounding thalamic nuclei (Solomon and Kristt, 1982). This activity later disappears. Hence this appears to reflect a temporary developmental process. Kimura, _et al_. (1981) report the absence of cells with choline acetyltransferase-immunoreactive perikarya in the cat diencephalon. However "cholinoceptive" cells (with choline acetyltransferase-immunoreactive terminals and boutons overlying perikarya with no such activity) were found to form a prominent mass in the pulvinar and were found in moderate numbers in the VPL and lateral posterior (LP) nucleus. In the monkey, Parent _et al_. (1977) observed "a few lightly stained acetylcholinesterase containing neuronal somata are scattered within the caudal part of the lateral territory mainly occupied by the (ventro)posterolateral and (ventro)posteromedial nuclei...".

## Thalamic Nissl cytoarchitecture

The absence in our data sample of cytoarchitectonically distinct zones which distinguish the ventrolateral nucleus of the thalamus (VL), the kinesthetic and the cutaneous mechanosensory projection zones may have one of several causes. Our measurements may accurately reflect the absence of distinct populations of cell body areas in these respective regions. In samples of this size we see no differences in distributions of cell sizes and densities nor promising trends that quantitatively distinguish the ventrolateral nucleus, the kinesthetic and the cutaneous mechanosensory projection zones of the ventrobasal complex of the raccoon thalamus. Since the null hypothesis cannot be proven, (if there are no differences, infinite sample sizes will never show them), we abandoned this approach. Those who believe there are differences are welcome to attempt larger and more systematic samples. Nevertheless we present our data to show why we did not pursue this further.

The lack of an orthogonal orientation in the cells (as is found for example in the cortex, where layers are found parallel to the surface) may have confounded our efforts. While our histological sections are in orthogonal stereotaxic planes, the functional organization of the mechanosensory projection zone more closely approximates a hybrid of radial geometries with raccoon body topology. If cytoarchitectonic boundaries are oriented along functional boundaries, our histological observations were not in the optimal planes.

Our subjective observations concur with those of Sakai (1982) that VL appears heterogeneous with dark staining cells organized in small irregular clusters segregated by fiber bundles. In our estimation, this description also fits the mechanosensory projection zone with the exception of clusters appearing somewhat larger than in VL. However the fiber bundles are thinner and more evenly spaced in VL than in the mechanosensory projection zone. Sakai (1982) contends that the cutaneous mechanosensory projection zone is distinguished from VL by an increase in cell packing density and the presence of large clusters of darkly staining cells. Our measurements (Figure 8) indicate that the cell densities in these two regions are approximately equal. The appearance of an increase in cell packing density may be explained by the greater caliber of the neuron-free fiber bundles and hence larger (but fewer) cell clusters which appear densely packed only due to their composition of greater numbers of cells.

We found no clear Nissl cytoarchitectonic boundaries for the kinesthetic projection zone. Candidates have been proposed in other mammals as possible homologues of this region.

Somatosensory thalamus cytoarchitecture of other animals

Cat. An early possible suggestion of the cytoarchitectonically-based location of a non-cutaneous projection zone in the anterodorsal aspect of the cat somatosensory thalamus may be found in Mountcastle and Henneman's (1949) electrophysiological mapping study of mechanically evoked responses. Thin sections of thalamic tissue were Nissl stained and recording sites were determined by direct measurements in the tissues based on stereotaxic coordinates of the recording sites (with appropriate accounting for tissue shrinkage). In their Figure 3, which represents their rostralmost plane of section, tracings of cytoarchitectonic borders are superimposed on figurine representations of effective peripheral receptive fields for evoked responses. In the region that their label as pars externa of the ventral nuclear group (VBX of Berman and Jones, 1982; see below), there is a dorsal band with no cutaneous responses. This may correspond to a zone of kinesthetic projections.

A transitional region between the ventrolateral complex (VL) and the ventrobasal complex (VB) was first recognized as such by Rinvik (1968). Millar (1973) found the zone in the anterodorsal-lateral part of VPL which receives kinesthetic hindlimb projections to contain densely stained cells similar to those of VPL. However, the cells in the zone were described as more scattered and diffuse than in the main body of VPL due to the presence of myelinated fibers. In the cat, Jones and Burton (1974) observed a region of transition between VL and VB that is not well defined architectonically. This region was defined by its position, the dominant presence of large cells, the presence of spinothalamic projections and the appearance of receiving Group I muscle afferent projections from the limbs. This region was termed "the spinal part of the ventrolateral complex" (VLsp). Berman and Jones (1982) refer to this as the "transitional region between the ventrolateral and ventrobasal complexes." The ventrobasal complex of the cat is described as containing both large and medium-sized cells which stain densely with thionin. Berman and Jones (1982) concur with Rioch (1929) and LeGros Clark (1932) in observing bundles of myelinated fibers penetrating this nuclear complex. The cells of VL are described as approximating those of VB in size and staining intensity for the most part. However the cells of VL in the cat appeared more angular and less tightly packed to these authors. In the transitional region between the VL and VB complexes, large angular cells typical of VL were found among a substantial population of small cells, similar to those found in VB. Berman and Jones note that the posterior borders, as well as the anterior borders, of the external nucleus of the ventrobasal thalamus (VB) are difficult to define with the Nissl stain.

In the prosimian, Galago, Pearson and Haines (1981) observed a distinct nucleus lying between VL and the ventrobasal complex. This nucleus has cells slightly larger than those of the main body of ventroposterolateral nucleus (VPL) as well as those of VL and its cells are more scattered than in VPL. Somatotopic organization was not found with HRP tracer studies (Pearson and Haines, 1980). These authors suggest that this is a "VPL-VL transitional nucleus" indicating this is an indistinct region between two distinct zones.

Monkey. In the rhesus monkey, Olszewski (1952) describes the pars oralis of the ventroposterolateral nucleus (VPLo), the thalamic projection zone from cerebellum, as containing large cells which are sparsely and evenly distributed. The pars caudalis of the ventroposterolateral nucleus (VPLc), which receives lemniscal, mechanosensory afferents, is characterised as having a higher cell density than VPLo and contains small cells as well as large. Olszewski (1952, p. 19) finds the VPL/VL borders and related subnuclei easiest to observe in horizontal sections. This border zone may correspond to Walker's (1938) ventral intermediate nucleus which is described as "a quite narrow, almost vertical, sheet of cells which are distinguished by their large size and deep staining." Other authors note difficulty in distinguishing VPL/VL borders in coronal sections (Tracey, et al. 1980; Kalil, 1981). Friedman and Jones (1981) find a zone of cytoarchitectural change between VPLc and VPLo that is distinguishable in horizontal and sagittal sections but not in frontal sections. Maendly, et al. (1981) found forelimb group I muscle afferents project to the border region between VPLo and VPLc which they term "the rostral cap of VPL." In this group I projection zone in the monkey thalamus, they observe a wider variety of cell sizes and a higher cell density in the caudal aspect of this zone than in two further rostral zones which respectively did and did not receive such projections.

Human. Cutaneous mechanosensory projections to the human thalamus are located in the ventral caudal nucleus, while kinesthetic projections are rostral and dorsal to this in the ventral intermediate nucleus. According to the description of Andrew and Watkins (1969), which follows that of Hassler (1959), the ventral intermediate nucleus can be distinguished from its rostral and caudal neighbors, the ventral oral and ventral caudal nuclei, respectively, by its large, darkly staining and, particularly on its lateral aspect, scattered cells. It appears that the ventral oral nucleus is homologous to VL of cats and the conbination of VL and VPLo of monkeys.

Hirai, et al. (1982) made extensive measurements of cell dimensions and density in 7 nuclear divisions in proximal to and including the ventral intermediate nucleus of the human. The boundary of the ventral intermediate nucleus with the ventral oral nucleus is reportedly clear in horizontal sections and in sagittal sections but is problematic in coronal sections. While the ventral oral nuclei are composed mainly of densely packed groups of medium-sized (200-400 $\mu m^2$) neurons, the ventral intermediate nucleus contains neurons ranging, for the most part, from 300-700 $\mu m^2$ medially and 200-1100 $\mu m^2$ laterally. Throughout the ventral intermediate nucleus, large dark staining neurons were found with a lower cell density in its lateral portion.

In the ventral caudal nucleus, there is some heterogeneity of cell areas with a larger population of small neurons and less large neurons than the ventral intermediate nucleus. Hirai, et al. (1982) conclude that the ventral intermediate nucleus of the human, which receives kinesthetic projections is similar in location and cytoarchitectonics to that of the VPLo of the rhesus monkey as determined by Olszewski (1952). This is in agreement with the findings of Maendly, et al. (1981) that a region of the group I muscle afferent focus in the monkey thalamus is cytoarchitectonically similar to VPLo.

Relations of somatosensory thalamus architectonics in raccoon with those of other animals. The zone of mechanosensory projections from postcranial cutaneous receptive fields to the thalamus of the raccoon appears to be cytoarchitectonically similar to the external nucleus of the ventrobasal complex (VBX) of the cat, the pars caudalis of the ventroposterolateral nucleus of the monkey (VPLc) and the ventral caudal nucleus of the human. In all of these regions, there are populations of darkly staining cells with heterogeneous size distributions. These regions are also reported to receive projections from cutaneous mechanosensory receptive fields. The data presented here indicate that the thalamic zone of projections from postcranial kinesthetic receptive fields in the raccoon is cytoarchitectonically indistinct from the cutaneous projection zone and from the ventrolateral nucleus (defined here as the zone immediately dorsal and rostral to the kinesthetic projection zone in which kinesthetically-evoked electrical activity is not reliably found). Zones which may be homologous to the kinesthetic projection zone of the raccoon are reported 1) in the cat as the transitional region between the ventrolateral and ventrobasal complexes (Berman and Jones, 1982), 2) the rostral cap of VPLc in the monkey (Friedman and Jones, 1981; Maendly, et al. 1981) and 3) the ventral intermediate nucleus of the human (Ohye, 1978; Hirai, et al. 1982). The degree of reported cytoarchitectonic distinction of the respective zones progresses from 1) questionable to 2) marginally distinct, with characteristics of VPLo in its rostral portions, but not caudally to 3) quite distinct by virtue of cell size populations and cell density. In the monkey and human, these zones are best distinguished in sagittal and horizontal sections. In the raccoon this zone is distinct in sagittal sections, but on the basis of fiber architectonics, not cytoarchitectonics.

In each of our ssamples a lower cell density was found in the kinesthetic projection zone than in the cutaneous projection zone. A lower cell density was reported in VL than in VBX of the cat (Berman and Jones, 1982) but we are aware of no data on the cell density of the VBX/VL transition zone. In the rhesus monkey, Maendly, et al. (1981) measure a lower cell density in rostral aspects of the group I muscle afferent focus than in its caudal aspect. An equally low cell density is reported in a zone anterior to this focus. In the human, scattered cells are reported on the lateral aspect of the ventral intermediate nucleus. Hence in the monkey and human, a lower cell density is reported in protions of the presumed homologue of the raccoon kinesthetic projection zone.

Patterns of staining with cytochrome oxidase and acetylcholinesterase in thalamic tissues from the cat, monkey and human have not yet been reported. Comparison of staining distributions for these (as well as other, see below) tissue elements should assist in the further evaluation of the homologies proposed above. Furthermore, the cytochrome oxidase and acetylcholinesterase stains may complement the use of Nissl cytoarchitectonics (cf. Berman and Jones, 1982) in determining the dorsal and medial borders of VBX in its caudal aspects, as well as homologous zones in other animals, as they do in the raccoon.

Medullary relations. Kinesthetic projections to the raccoon medulla have been shown in the external cuneate nucleus (ECu) and its medial tongue (ECuMt) (Johnson, Welker and Pubols, 1968) which is distinguished by its location and its large cell population. Thalamic kinesthetic projections have been traced to cell bodies in the ECuMt, and also to the reticular portion of cell group x, cell group z, and the basal portion of the cuneate nucleus (Ostapoff, Johnson and Albright, 1983); kinesthetic stimuli evoke unit cluster electrical activity in all these regions. In the medulla borders are distinct between each of the kinesthetic projection zones and the cutaneous projection zone with regard to stimulus parameters of evoked electrical activity. But these borders are not clear cytoarchitectonically in the medulla as is the case in the thalamus.

### Piecing together the rest of the puzzle.

Earlier investigations (Welker and Johnson, 1965) focussed on the functional organization and architecture of the raccoon somatosensory thalamus with methods that discriminated on the order of 500 μm. This and the accompanying paper extend that work one order of magnitude to measure details in functional organization and architecture as small as 50 μm. The factors limiting spatial resolution in this study include electrode size, micromanipulator sensitivity and the size of our marking lesions. Further studies are suggested to reveal structure-function relationships on other orders of magnitude to ultimately provide us with a working understanding of dynamics of thalamic neural activity. Logical further steps in this progression of experimental approaches would involve measurements of evoked activity in individual cells, followed by filling the cells and examining their ultrastructure. Then, in further studies the synaptic organization as elucidated by electron microscopy should be correlated with physiologically determined electrical dynamics of the neural circuits and with ion flux dynamics of the neurotransmitters and their receptors. In order to determine the local interactions of these nested processes, all of this information on each of these orders of magnitudes should then be integrated into a comprehensive model. A systems simulation of this model would then illustrate how neural structures process somatosensory information. Such a simulation also would provide a prototype with which experimental manipulations may be made which are not feasible in biological subjects.

## Chemoarchitectonics meets electroanatomy: What's next?

The mechanosensory projection zone of the thalamus is distinguished by its lack of acetylcholinesterase staining as well as its intense cytochrome oxidase activity. Hence, a region of the nervous system with specific sensory processing functions shows distinctive architectural properties when reacted with histochemical procedures indicating energy metabolism and neurotransmitter-related biochemical processes. Studies that will reveal further evidence of biochemical and metabolic differentiation in this and in other distinct regions of the nervous system are encouraged. An example of such differentiation is the observation of glutamic acid decarboxylase (GAD)-like and motilin-like immunoreactivities in distinct populations of cerebellar neurons (Chan-Palay *et al.*, 1981). Specific sensory projection zones of the thalamus may be further distinguished with histochemical and immunohistochemical procedures for the neurotransmitters (and related metabolic enzymes) for projection neurons from cerebral cortex, 'relay' nuclei and other afferent groups. Other histochemical methods such as acid phosphatase activity staining may also be helpful as empirical approaches to distinguish brain regions since in Figure 57 of Manocha and Shantha (1970), acid phosphatase distinguishes the border between what are labelled as VL and VPL in the thalamus of the rhesus monkey.

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