THE SOMATOTOPIC ORGANIZATION OF MECHANORECEPTOR PROJECTIONS TO THE CUNEATE-GRACILE NUCLEAR COMPLEX OF THE OPOSSUM

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
THOMAS CHARLES HAMILTON
1971

ABSTRACT

THE SOMATOTOPIC ORGANIZATION OF MECHANORECEPTOR PROJECTIONS TO THE CUNEATE-GRACILE NUCLEAR COMPLEX OF THE OPOSSUM

By

Thomas Charles Hamilton

Microelectrode mapping procedures were used to determine the organization of mechanoreceptor projections to the cuneate-gracile nuclear complex in seven adult opossums under Dial-urethane anesthesia. The projections were determined to show a definite somatotopic organization such that fibers from adjacent regions on the body surface innervated neighboring groups of cells in the ipsilateral nuclear complex. Furthermore, the resulting nuclear representation of body surface areas can, within any coronal section, be visualized as an animal lying on its back with its extremities dorsal, tail dorso-medial and head lateral. There also exists a functional distortion of the degree to which various body parts are represented, such that the volar hand (and to some extent also the foot and tail) receives a larger nuclear representation, with smaller receptive fields, than its body surface area would warrant. No longitudinal differences were observed in the general somatotopic organization nor in the size of receptive fields, although systematic variations occur in the degree to which some body parts are represented at different levels. These observations are consistent with those reported previously for a number of placental mammals and for one reptile.

THE SOMATOTOPIC ORGANIZATION OF MECHANORECEPTOR PROJECTIONS TO THE CUNEATE-GRACILE NUCLEAR COMPLEX OF THE OPOSSUM

By

Thomas Charles Hamilton

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biophysics

1971

ACKNOWLEDGMENTS

I gratefully extend appreciation to my wife, Ruth, for her tolerance, cheerful urging and secretarial skills, particularly typing the final manuscript; to Dr. J. I. Johnson for his guidance and encouragement; and to Andrew Harton, Grace Kim, Emiline Haight, and Kristi Dege for their help in the histological processing. Thanks are also due Dr. Edward M. Eisenstein and Dr. Rudy A. Bernard for their help in the preparation of this manuscript.

I should also like to express my deep gratitude to William Colgan, whose efforts precipitated my introduction to scientific research, and whose advice and friendship have helped immensely in the organization and accomplishment of my educational goals.

This research was supported by NIH Training Grant GM-01422 and NIH Research Grant NS-05982.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

One of the major pathways for large diameter, first order, afferent fibers which relay information from cutaneous mechanoreceptors to higher brain centers is the dorsal column-medial lemniscus system. In this system, which is reviewed in great detail by Norton (1970), the sensory afferent fibers ascend in the extreme dorsal and medial quadrant of the spinal cord. Those fibers from the lower thoracic, lumbar, and sacral segments ascend in the fasciculus gracilis (fasciculus of Goll), which in the upper cord is bordered laterally by the fasciculus cuneatus (fasciculus of Burdach) which contains sensory afferents from the upper thoracic and cervical segments. These fibers synapse in an orderly fashion upon ipsilateral neurons of the cuneate (Cu) and gracile (Gr) nuclei in the medulla. These nuclei lie just ventral to the dorsal columns, but, except rostral to the obex where they migrate more laterally, still occupy the dorsal and medial aspect of the medulla. Laterally and ventrally they are bordered by the spinal trigeminal (Sp Trg) nuclei which recieve projections of cutaneous sensory input from the head. Axons projecting from the Cu-Gr nuclear complex decussate and ascend (via the medial lemniscus) to the contralateral ventrobasal thalamus. This second nuclear region sends projections to the somatosensory cortex.

The projections of the sensory afferents to Cu-Gr are somatotopically organized such that projections from adjacent body regions innervate adjacent regions of the nuclei. Furthermore, the more distal body parts are represented more dorsally (the proximal more ventrally) and the more caudal regions more medially (the rostral more laterally). This somatotopy is continuous between the two nuclei (and at most levels also between Cu and Sp Trg) and thus the somatotopy can be represented diagramatically as an animal lying on its back with its tail located medially and head ventro-laterally. This somatotopic organization has been demonstrated in a number of placental mammals including the cat (Kruger, Siminoff and Witkovsky, 1961; Kuhn, 1949), the raccoon (Johnson, Welker and Pubols, 1968), the rat (McComas, 1962, 1963, 1964; Nord, 1967), the sheep (Woudenberg, 1970) and in a reptile, Alligator mississippiensis (Kruger and Witkovsky, 1961).

The purpose of this study was to determine the organization of projections from the periphery to these cell groups in the opossum; thus extending current knowledge to a marsupial.

In addition to the general somatotopic arrangement within the nuclei, the amount of nuclear mass associated with a given body part has been found to be related to the behavioral importance of that part. In general, distal body parts, which are usually of more functional significance to an animal, have nuclear representations that are, relative to peripheral body surface areas, disproportionately larger than more proximal regions. But the exact relative degree of this functional distortion varies from species to species and reflects the behavioral characteristics of each animal. That is, those body regions which provide sensory information of more relative importance than other regions have an exaggerated nuclear representation (and probably also a greater peripheral receptor density). Thus, the highly developed manipulative abilities of the raccoon are reflected in the extremely detailed forepaw somatotopy which occupies at least 44% of the nuclear volume (Welker, Johnson and Pubols,1964).

It is generally believed that this somatotopic organization exists throughout the nuclei. But it has also been suggested (Gordon and Jukes,1962; Gordon and Seed,1960; McComas,1962,1963) that there is a differentiation of Cu-Gr such that the size of receptive fields from a given body region varies with position along the rostral-caudal axis of these nuclei. The smallest receptive fields were reported to exist in the middle third of the nucleus, slightly larger field sizes in the most caudal third and the largest receptive field sizes in the most rostral portion. However, others have maintained that the only important variable is peripheral receptor densities (Kruger et al.,1961; Nord, 1967; Perl, Whitlock and Gentry,1962; Winter,1965; Woudenberg,1970). Thus, it was also of interest to examine in opossums the extent to which the various body parts are represented at different levels of the nuclei and to determine if any rostal-caudal differences in receptive field size exist.

A comparison of the detail of somatotopic representation in Cu-Gr to that in Sp Trg of the opossum will permit correlation of the representation to the behavioral activities of this animal. Furthermore, a comparison of the representation of body parts in the medulla to that of higher levels of the somatosensory system (Erickson, Jane, Waite, and Diamond,1964; Pubols and Pubols,1966; Lende,1963a; Oswaldo-Cruz and Rocha-Miranda,1971) will also facilitate comparison of the opossum to other vertebrates. Finally, since the opossum brain is at birth a very undeveloped structure (Ulinski, 1971), in which Cu-Gr is not yet formed (Ulinski,1969), the demonstration that the medial lemniscus system of this marsupial is similar to the placental mammals studied thus far would permit the opossum to be used most conveniently as a representative mammal for developmental studies.

Thus, this study consists of a number of objectives: (1) establishing the somatotopic organization in the Cu-Gr of the opossum, a marsupial, (2) examining the possibility of longitudinal variations in receptive field sizes, (3) comparing the somatotopic detail of Cu-Gr to Sp Trg and (4) comparing medullary to previously reported thalamic and cortical representations.

METHODS

Subjects

Seven adult opossums (Didelphis marsupialis), three male and four female, were used for microelectrode studies of the cuneate-gracile nuclear complex. All were wild -- caught in central lower Michigan.

Subject Preparation

The initial subjects were deprived of food for approximately fifteen hours prior to the start of surgery, but low surgery survival percentages resulted. These were countered by allowing the subjects to eat as normal with the addition of a vitamin coated slice of bread the day before experimentation.

All experimental animals were anesthetized by an intraperitoneal injection of sodium hydroxide buffered dial urethane solution (60 mg/kg). As discussed by Lende (1963a,b), dial urethane was a more satisfactory anesthetic than other agents tested; for fewer injections were required and these produced much longer periods of steady anesthesia. Thus, since anesthetic agents have in general been found to interfere only with transmission from Cu-Gr to higher centers (Norton,1970) and since Johnson et al. (1968) found no differences between pentobarbital sodium (another barbituate) anesthetized animals and an unanesthetized decerebrate one, it was assumed that the anesthetic was not a confounding variable in this study.

In addition to the anesthetic, all subjects were injected with atropine (0.15 mg/kg) to reduce secretions, and were tracheotomized and placed on the operating table for a nonsterile exposure of the medulla. Maintenance injections of dial-urethane (one quarter of the initial injection) were administered as required as well as frequent injections of alternately either normal saline (0.9%) or 5% dextrose saline (combined average dosage of approximately 10 ml/hr). The maintenance injections of anesthetic were administered when an eye blink was observed upon tactile stimulation of the cornea.

The exposed medulla was held in position by fixing the skull and axis relative to the reference frame of the recording electrode micromanipulator by means of metal rods fastened to these structures and to a head holder upon which the micromanipulator was mounted. (Special measures to insure immobilization of the medulla were not necessary.)

The dura and epi-pial tissue covering the dorsal surface of the medulla were removed; but the presence of a venous sinus, located between the two layers of dura and which usually extended for the entire length of the nuclei, led to profuse bleeding when the dura and epi-pial tissue were removed. The use of Gelfoam (a clotting agent) and the immediate reflection of the membranous tissue usually helped to control and eventually stop the blood loss.

When necessary (for extremely rostral exposures) the caudal aspect of the cerebellum was removed by aspiration. The exposure was covered with warm mineral oil and photographed. A pictoral record of puncture locations was made on the resulting photograph.

The animal was then straddled over two parallel bars to facilitate access to receptive fields on the entire body surface. Body temperature was monitored rectally and maintained at approximately 34 C by means of a heating tape powered by either batteries or a variable transformer. All animals maintained adequate respiration throughout recording sessions.

Recording Equipment

Glass insulated tungsten microelectrodes, modifications of the Baldwin, Frenk and Lettvin (1965) electrodes, were used. These electrodes had 30 to 40 microns of uninsulated tip and had shaft diameters of approximately 60 to 100 microns.

Recorded responses were passed through a low level A.C. amplifier, low (80 Hz) and high (10 KHz) cut off filters, displayed visually and amplified by an oscilloscope and were also monitored aurally by a speaker system. Evoked activity and voice commentary were recorded on separate channels of a stereophonic tape recorder.

Recording Procedure

The electrode was oriented approximately perpendicular to the dorsal surface of the medulla and punctures were systematically made to map the nuclei in coronal rows spaced either one half or one millimeter apart. Punctures within a given row were between one-half and two-tenths of a millimeter apart. The electrode was advanced in approximately 50 micron steps and the body surface was stimulated mechanically at each position. Areas evoking a neural spike cluster electrical response (see Response Criteria) each time tactile stimulation was applied, were carefully delineated and drawn on photographs of opossum body parts. A written record describing these responsive areas, as well as activity noted at other electrode placements, was also kept. Additionally, a sample of activity from each reliable response area was recorded on tape.

The first row of punctures was made at the rostral end of the area to be mapped and additional rows were made successively more caudal to minimize interruption of ascending dorsal column fibers. The position of each puncture was measured on the coordinate system of the micromanipulator and was recorded on a grid as well as on the photograph of the exposed medullary surface.

Response Criteria

In order for somatic sensory activity to be accepted as unit spike activity the criteria of Johnson *et al.* (1968) were used. Responses from nuclear regions consisted of a cluster of spikes of variable amplitude with a relatively noisy background of smaller spikes that merged to widen the appearance of the baseline. Fiber responses were distinguished from cell body responses by a relatively low noise level from which they could be easily detected, as well as having only a few (if even more than one) spikes of differing amplitudes evident. Furthermore, nuclear responses could be maintained throughout a considerably greater range of electrode movement than fiber responses and could be maximized more easily by this movement.

Histology and Track Localization

At the termination of each experiment the animal was profused with normal saline followed by 10% formalin in saline. The medullas were removed, dehydrated in alcohol, embedded in celloidin, sectioned in 25 micron slices and stained. Even numbered sections were stained with thionin (Nissl method) to show cell bodies and odd numbered sections were stained with hematoxylin (Weil and Heidenhain methods) to show myelinated fibers.

Electrode tracks were identified by projecting slides of the sections and tracing and comparing serial sections. The tracks were then compared to recorded micromanipulator readings for response locations. Occasional microlesions, made during the course of the experiment by passing a small anodal current (usually 15 microamps) through the electrode tip, aided in identifying both punctures and response locations.

Each row of punctures was drawn on a tracing of a section that showed the puncture tracks passing through the Cu-Gr nuclei and was then fitted to a generalized brain (made from one of the seven experimental animals) by comparison of nuclear regions.

RESULTS

Responses and Response Criteria

In addition to requiring that a receptive field evoke neural activity in response to light touch, an evaluation of the neural activity obtained was also made. A number of general types of activity were observed. Single units (SU) were occasionally encountered. These responses could derive from either a single ascending sensory axon (either in a fiber tract or in a nuclear area) or from a single Cu or Gr cell. They were characterized by action potentials of a constant amplitude which were separated in time. A second response catagory was that of cluster responses (CU). These resulted from the simultaneous firing of a large number of neurons such that the recorded action potentials completely merged to widen the oscilloscope displayed baseline and to obscure the detail of all but the largest (greatest amplitude) action potentials. These responses were considered to be nuclear. Intermediate responses (MU) which consisted of action potentials of multiple amplitudes that could still be separated in time were also frequently encountered. In general, the latter two categories were utilized most in determining the somatotopic organization of Cu-Gr, although the SU gave insight into the receptive field sizes of single fibers and thus into the limit of resolution with which the map might be constructed (cf. Johnson et al., 1968). Examples of typical activity are shown in Figure 1.

Slow potentials were also encountered. Their timecourses were much slower than action potentials and resulted from activity in a large number of distant fibers or cells. Therefore, these responses were not used in evaluating the nuclear somatotopy.

The relative frequency of occurence of the various response categories varied between experiments (i.e., between electrodes) since different electrodes had different lengths of uninsulated tip. One animal (70587) also differed because it had received an injury to the dorsal spinal cord (probably during surgery).

Because a somatotopic mapping of only mechanoreceptor projections to Cu-Gr was intended, deep pressure and proprioceptive responses may often have been overlooked. However, if identified, the occurence of these responses was noted.

Of the 190 electrode tracks that were identified histologically 105 passed through Cu-Gr. In these 105 punctures 219 mechanoreceptor responses (150 Cu, 69 Gr) were identified. The actual number of mechanoreceptor responses obtained per puncture varied from zero to seven. Only three punctures penetrated the nuclear area without any responses being obtained, and each contained some weak neural activity associated with mechanical stimulation, but which did not meet the criteria required to be considered a response. Several other

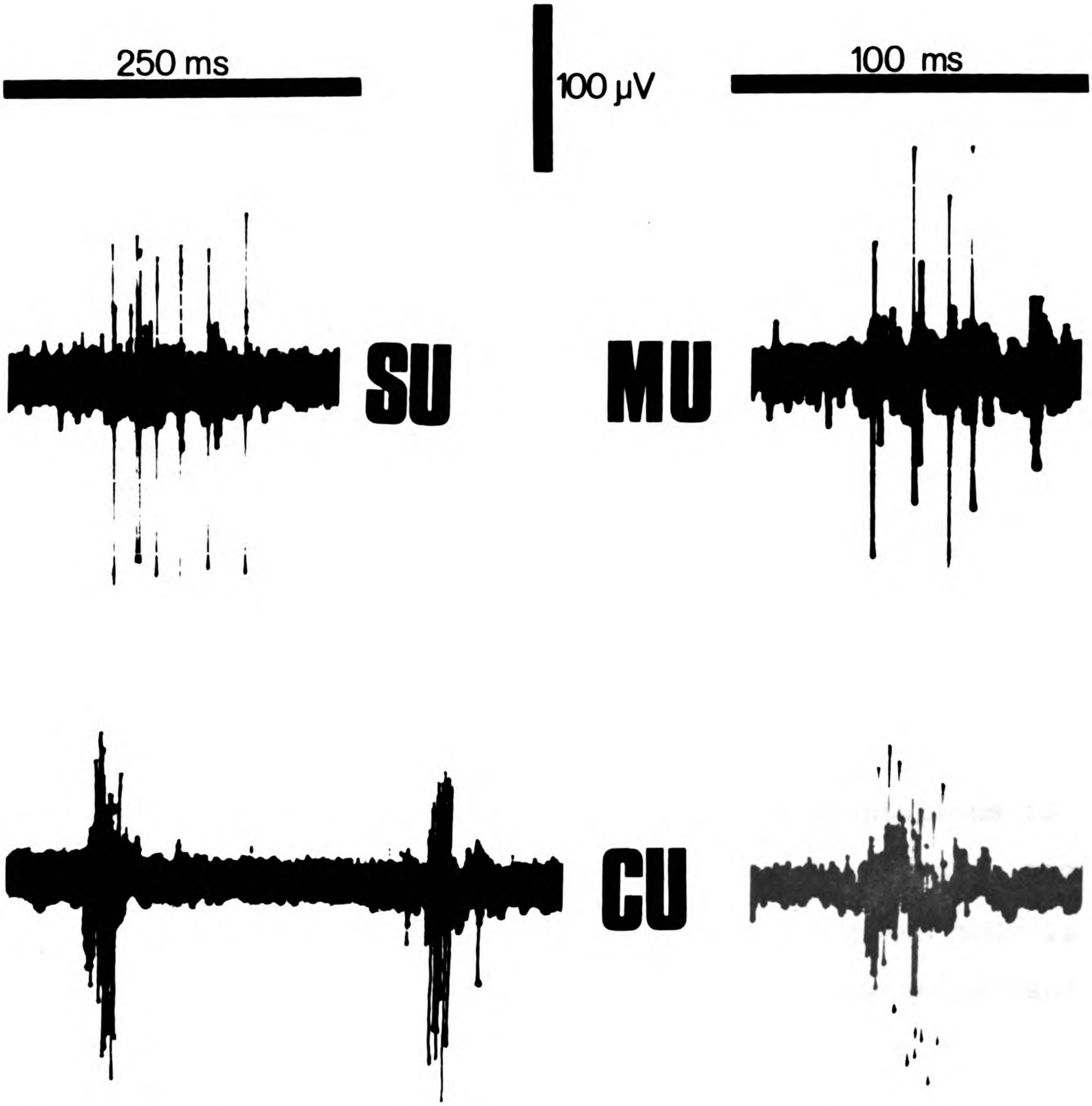

Figure 1. Examples of Cu-Gr neural response classifications.

Top, left: A single unit (SU) response to stimulation of the tail (receptive field 5A in Figure 6).

Top, right: A multiple unit (MU) response to stimulation of the foot and leg (receptive field 5B in Figure 6).

Bottom: A cluster of units (CU) response to stimulation of pad B of the volar hand (part of receptive field 4A in Figure 6). The trace at the right is a more detailed view of the left most CU response in the left trace. The left trace was retouched.

punctures had no mechanoreceptor responses, but did contain proprioceptive and deep pressure responses that are also associated with Cu-Gr.

General Somatotopy

In agreement with previous studies, the organization of mechanoreceptor projections in Cu-Gr of the opossum consists of an orderly arrangement, such that neighboring peripheral body regions are represented in adjacent regions of Cu-Gr. Furthermore, as in the placental mammals, the representation can be visualized as an animal lying on its back with its tail medio-dorsal, extremeties dorsal and neck and ear lateral. The largest volume of the nuclear complex is devoted to representations of the volar forepaw which occupies about half of the Cu. The smaller Gr contains representations of all body parts innervated by the lower thoracic and more caudal dorsal roots. Thus, the resolution of the lower body is not very detailed; and even the resolution of the volar hand does not approach the finer grain receptive fields represented in the larger Sp Trg (Weller,1971).

In general, there is also a migration of receptive fields from pre- to post-axial arm as one progresses from the lateral to the medial portion of Cu. This is in agreement with the same pattern found in other studies (cf. Werner and Whitsel,1968) and seems to follow the order of dermatomal innervation (cf. Pubols and Pubols,1969). Likewise, Gr data obtained was consistent with a representation which follows the order of caudal dorsal root innervation in the opossum (Oswaldo-Cruz,Pagani and Rocha-Miranda,1965).

Finally, Cu, Gr, and Sp Trg were found to be continuously connected at most levels investigated. This indicates that the three may be considered as one functional unit (cf. Woudenberg,1970; Weller,1971).

Detailed Somatotopy

To facilitate the comparison of data from different animals, one of the experimental brains was used as a standard medulla and the data from other animals was, by comparing nuclear topography, matched to the appropriate level of the standard brain. Figure 2, which illustrates a dorsal view of the medulla with the cerebellum removed, has been arbitrarily divided into two millimeter segments which begin at the rostral end of the Cu. The distribution of rows (planes) of data is indicated by the hyphenated numbers. Those rows that will be discussed in more detail are labeled by the figure in which they are represented. Table 1 shows the distribution of responses within the data planes of Figure 2 and identifies the animal in which the plane was analyzed.

The rostral end of Cu-Gr was unintentionally mapped in less detail than the remainder of the nuclei, but the data from this section was consistent with the other levels studied. The fewer number of responses per row (and per puncture) in the rostral portion of the nuclei is felt to be a function of the decrease in nuclear size at this level.

The data in the planes of punctures in segments 2 and 3 illustrate the somatotopic organization quite well. Therefore,

Figure 2. Distribution of data planes in the opossum medulla.

This figure shows a dorsal view of an opossum medulla with the cerebellum removed. The shaded regions indicate the approximate location of the Cu-Gr in the opossum. The dotted region represents the Gr and the stripped region, the Cu. There is a slight overlap of the two nuclear regions, in this projection, along their common border.

The medulla has been divided into 2 mm segments beginning at the rostral end of the Cu. The hyphenated numbers at the left indicate the approximate rostro-caudal location of planes of histologically analysed data. The number preceeding the hyphen corresponds to the particular 2 mm segment in which the data plane is located and the number following the hyphen simply orders the data planes, from the most rostral to most caudal, within each 2 mm segment. Those planes to be described in more detail later are identified by the figure in which they are represented.

The scales on the extreme right and left margins are in mm.

Abbreviations (after Voris and Hoerr,1932):

al. cin.	ala cinerea
a. vest.	area vestibularis
col. fac.	coliculus facialis
corp. rest.	corpus restiformis
r. c. I	root of the first cervical nerve
r. VII	radix facialis
r. VIII c.	radix cochlearis
r. VIII v.	radix vestibularis
r. IX	radix glossopharyngei
r. XI sp.	radix spinalis n. accessorii
tub. ac.	tuberculum acusticum
tub. cun.	tuberculum cuneatum

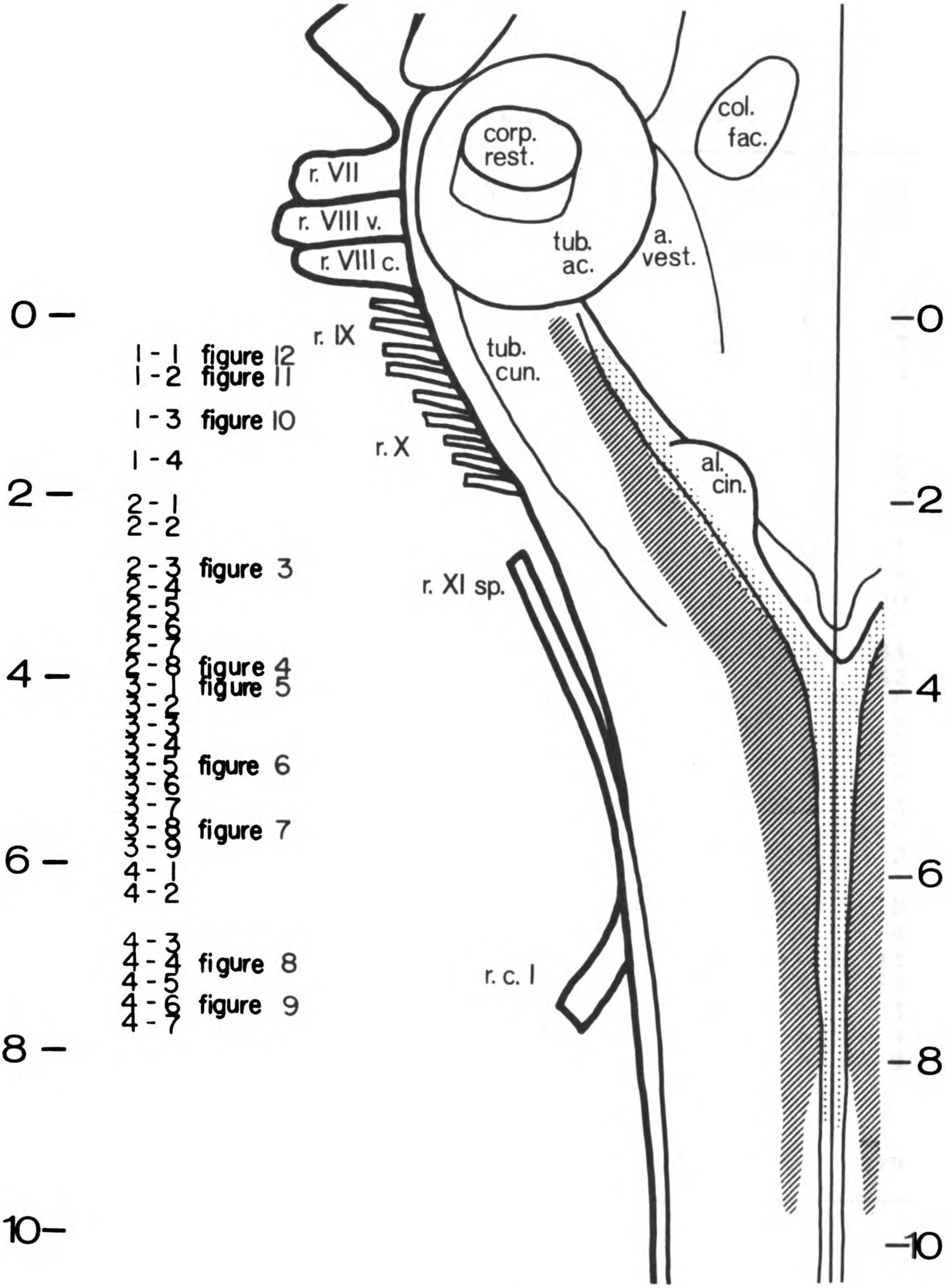
r. VII
r. VIII v.
r. VIII c.
corp. rest.
col. fac.
tub. ac.
a. vest.
r. IX
tub. cun.
r. X
al. cin.
r. XI sp.
r. c. I
0 –
1 - 1 figure 12
1 - 2 figure 11
1 - 3 figure 10
1 - 4
2 –
2 - 1
2 - 2
2 - 3 figure 3
2 - 4
2 - 5
2 - 6
2 - 7
2 - 8 figure 4
3 - 1 figure 5
4 –
3 - 2
3 - 3
3 - 4
3 - 5 figure 6
3 - 6
3 - 7
3 - 8 figure 7
3 - 9
6 –
4 - 1
4 - 2
4 - 3
4 - 4 figure 8
4 - 5
4 - 6 figure 9
4 - 7
8 –
10–
–0
–2
–4
–6
–8
–10

Figure 2

Table 1. Longitudinal distribution of responses.

Plane		Animal number	Number of Cu-Gr punctures	Number of units		
				Cu	Gr	Total
1-1	Figure 12	70503	3	6	2	8
1-2	Figure 11	69583	3	8	0	8
1-3	Figure 10	70503	6	5	2	7
1-4		69583	3	2	1	3
2-1		70501	1	1	0	1
2-2		69583	4	2	1	3
2-3	Figure 3	70501	6	10	6	16
2-4		70587	2	6	1	7
2-5		69581	1	1	0	1
2-6		69583	1	1	0	1
2-7		69581	3	2	2	4
2-8	Figure 4	70501	5	6	4	10
3-1	Figure 5	70501	6	10	1	11
3-2		70587	4	12	0	12
3-3		69581	4	5	1	6
3-4		70501	5	4	4	8
3-5	Figure 6	70502	8	11	4	15
3-6		70587	2	6	0	6
3-7		69581	2	1	2	3
3-8	Figure 7	70502	6	8	7	15
3-9		70587	2	2	0	2
4-1		70501	4	6	2	8
4-2		70502	3	5	4	9
4-3		70502	5	5	6	11
4-4	Figure 8	70588	7	19	4	23
4-5		70501	3	3	0	3
4-6	Figure 9	70502	3	3	6	9
4-7		70588	2	0	9	9
Totals:						
28		7	105	150	69	219

five of these midnuclear planes are presented in detail with photomicrographs and diagrams to identify the medullary level of the data and with figurines* to illustrate the extent of receptive fields at the various response loci. Two planes of data from more caudal levels and three planes from the rostral end of the Cu-Gr are then presented to compare the content and somatotopic organization at these levels to that of midnuclear planes.

Rows of data that were indicated in Figure 2 and Table 1, but which are not presented in detail here, were consistent with the somatotopic organization presented, but in general were not mapped in as much detail as those rows which are presented.

The format used in Figures 3-12 is as follows:

Right page:

Top, left: Above, drawing of an opossum hindbrain showing the approximate level of the plane of data (heavy line); below, tracing of the section shown in the photomicrograph of the facing page. Location of all electrode tracks at this level are indicated. Those having Cu-Gr data are indicated with dashed lines.

Top, right: Enlarged drawing of the region containing data. The approximate position of each response loci is

---

*The figurines used in all figures are oriented, as much as possible, in the same manner (relative to the nuclear area) as is the body representation. This causes some responses to be shown on the contralateral aspect of a figurine. The actual responses, however, were ipsilateral in all cases.

indicated with a black dot and is coded with a letter.

Bottom: Illustration of the receptive field areas (solid black shading) of the response loci indicated above. Fiber responses are indicated by dotted receptive field representations. As mentioned previously, the figurines are oriented to illustrate, as accurately as possible, the somatotopic organization of the nuclear area; and all responses were ipsilateral although they may be located on the contralateral side of the figurine.

Left page:

Top: Photomicrograph of a section about the level at which the electrode tracks passed through the nuclear area.

Bottom: The description under the figure caption discusses the data presented in the figure and should be read in conjunction with the text.

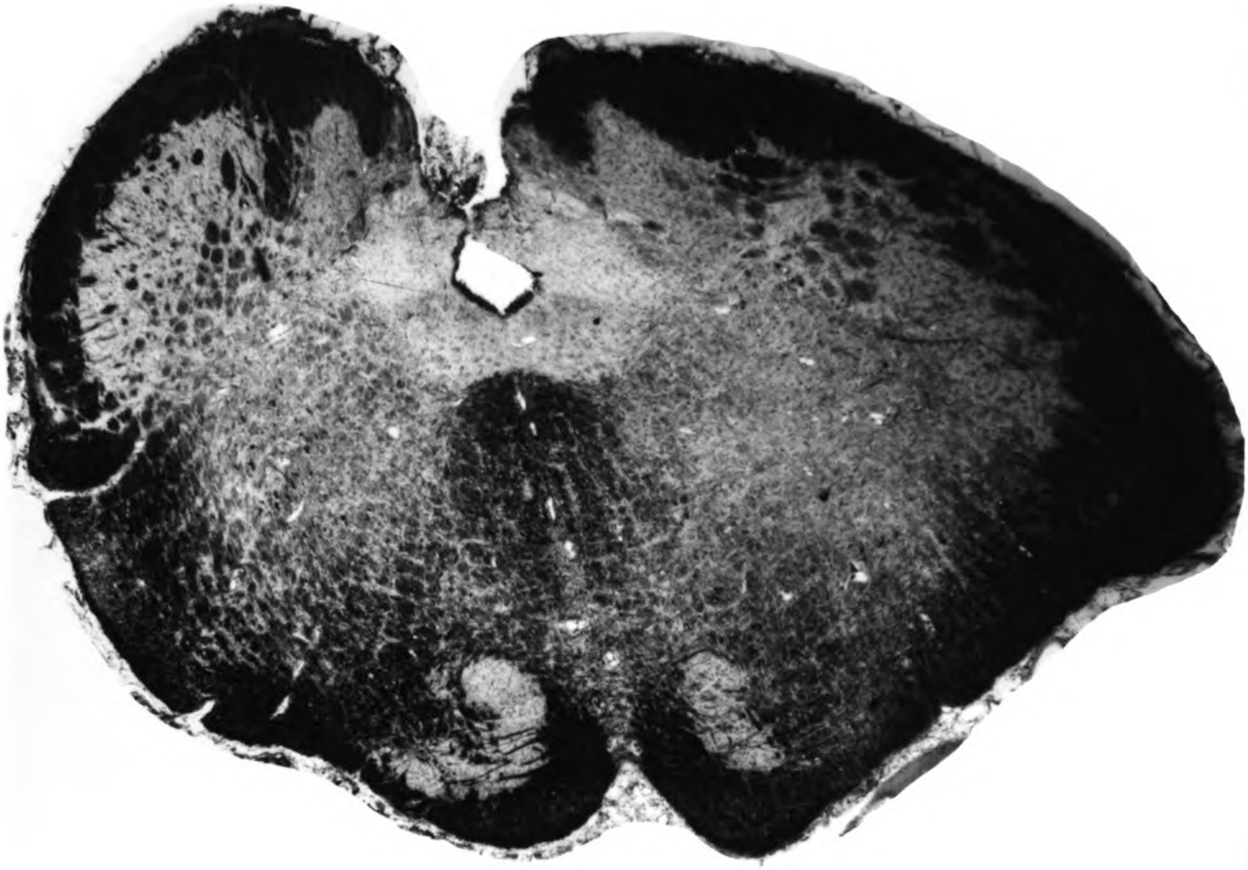

Figure 3b

Figure 3. Response distribution in Plane 2-3.

Figure 3a. The organization of Cu at the level of the obex is consistent with an inverted figure and evidences a large volar hand representation. The organization within Gr is illustrated nicely in this plane. The foot is represented most dorsally. The leg, lumbar and thoracic regions are at successively more ventral locations. Continuity between Cu and Gr is evident. Note also that Cu extends along the ventral border of Gr (response 14E). Thus upper body responses are found below Gr representations and at more caudal segments, also along the midline. This differs from the placental mammals studied previously (Johnson _et al_., 1968; Kruger _et al_., 1961; Kuhn, 1949; McComas, 1962, 1963, 1964; Nord, 1967; and Woudenberg, 1970). The absence of either tail or perianal representation may represent a property of Gr above this level; for no representation of these body parts was found at more rostral levels.

Abbreviation: A Po .... area postrema

Figure 3b. Photomicrograph of section 167 (hematoxylin stained, Heidenhain method). Portions of punctures 11-14 are evident.

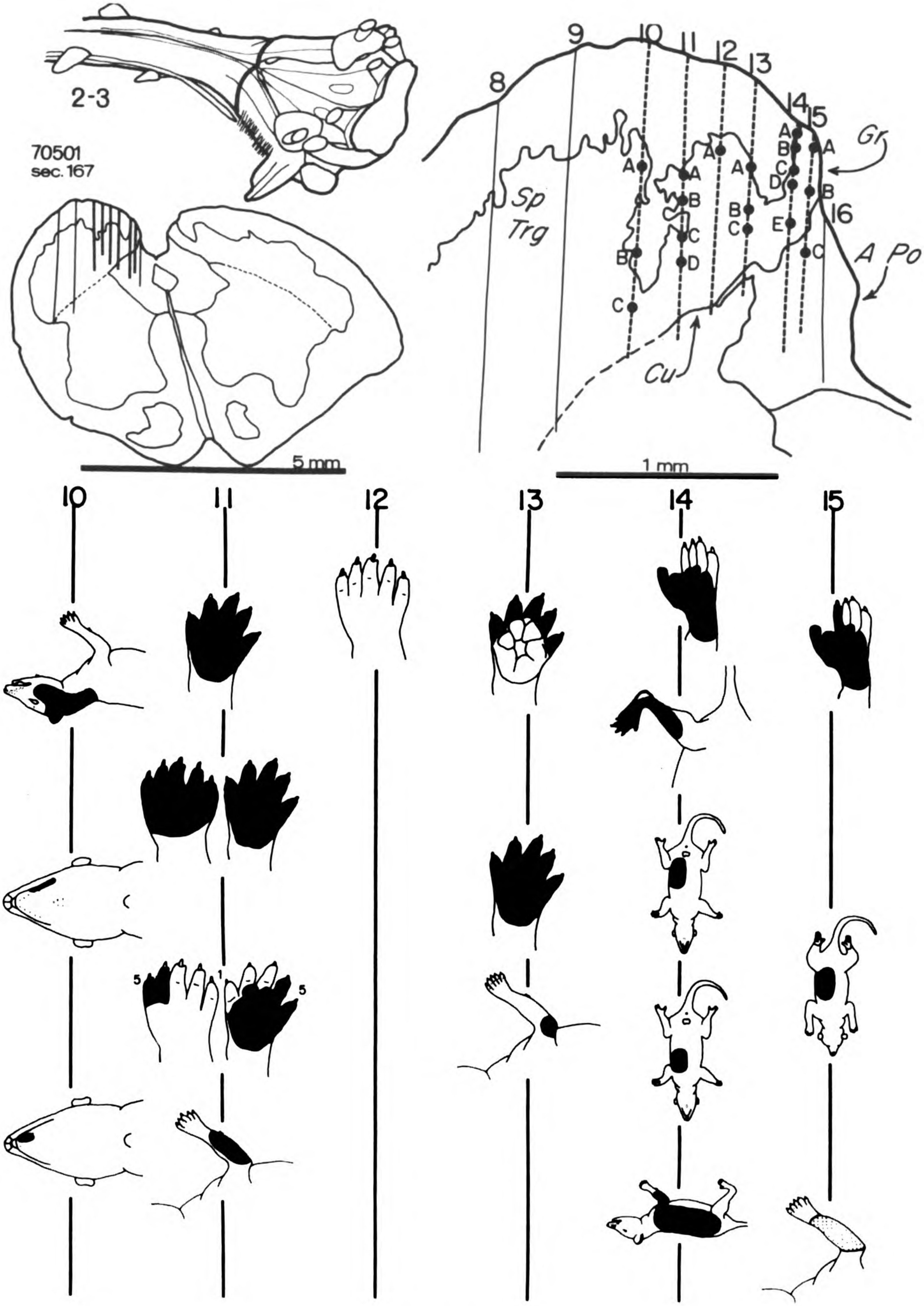
2-3
70501
sec. 167
5 mm
8
9
10
11
12
13
14
15
16
Sp
Trg
Gr
A Po
Cu
A
B
C
D
E
1 mm
10
11
12
13
14
15
5
1
5

Figure 3a

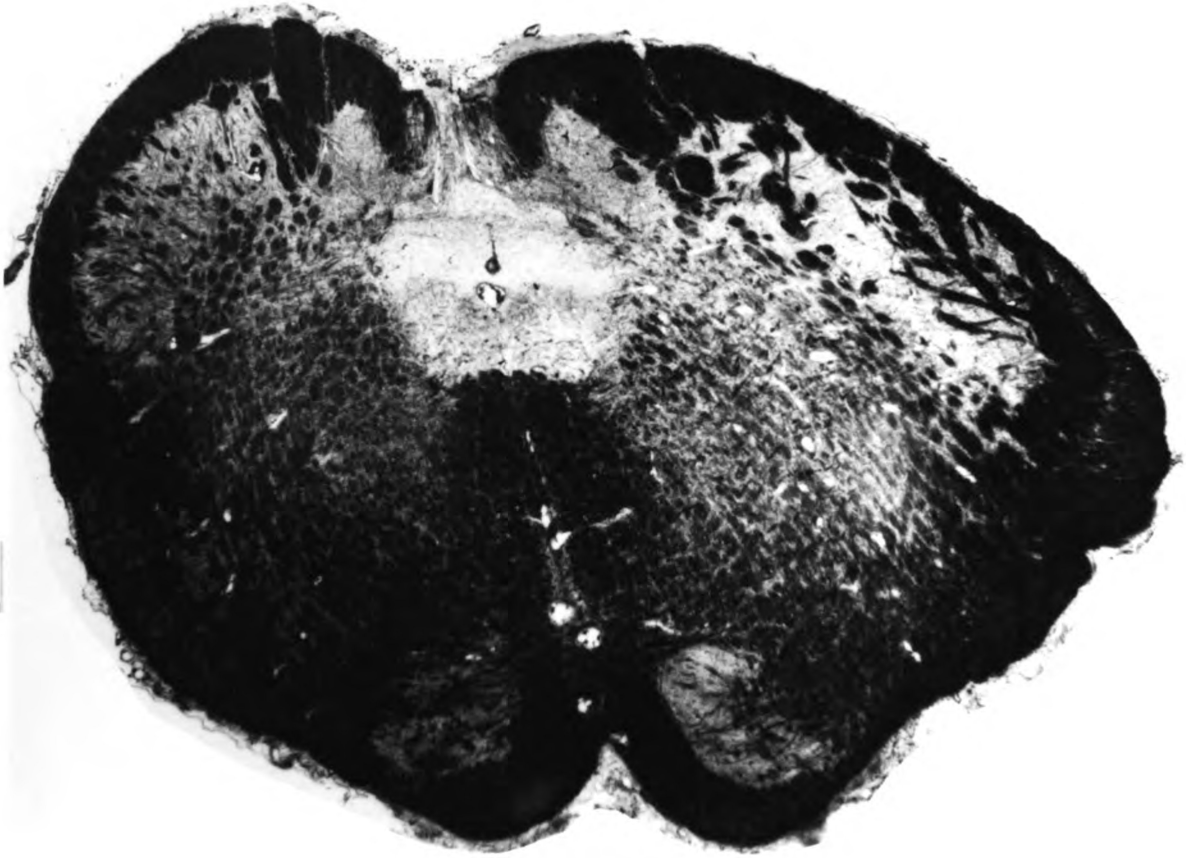

Figure 4b

Figure 4. Response distribution in Plane 2-8.

Figure 4a. At a level one-half mm caudal to the obex the general trend of an inverted figure is again evident. The ear and neck response in puncture 21 (P21) and the shoulder and body responses in P18 also show the continuity between Cu and Sp Trg, and Cu and Gr. Notice also that the tail representation is located in the central hump of the Ψ shaped Gr. The close spacing between punctures (about 200 microns) and the relatively long distance over which the electrodes can record (about 100 microns) make it impossible to categorically state that this central hump is devoted to tail representation, but this interpretation seems likely and it would be consistent with current thought (Chang and Ruch, 1947; Johnson *et al.*, 1968). The lateral to medial shift from pre- to post-axial arm is illustrated very nicely by P20 and P19.

Figure 4b. Photomicrograph of section 183 (hematoxylin stained, Heidenhain method). Portions of all five punctures can be seen.

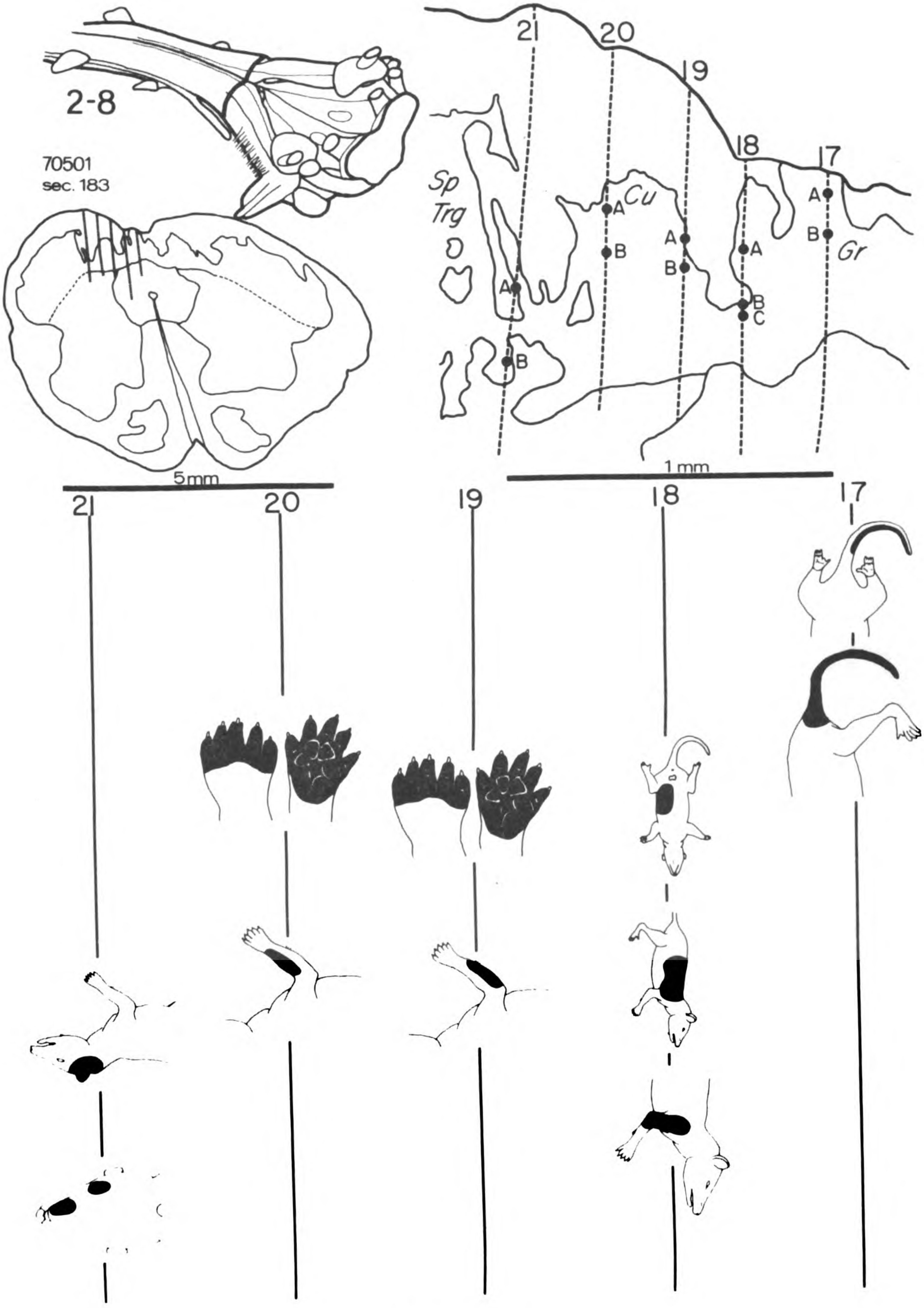

Figure 4a

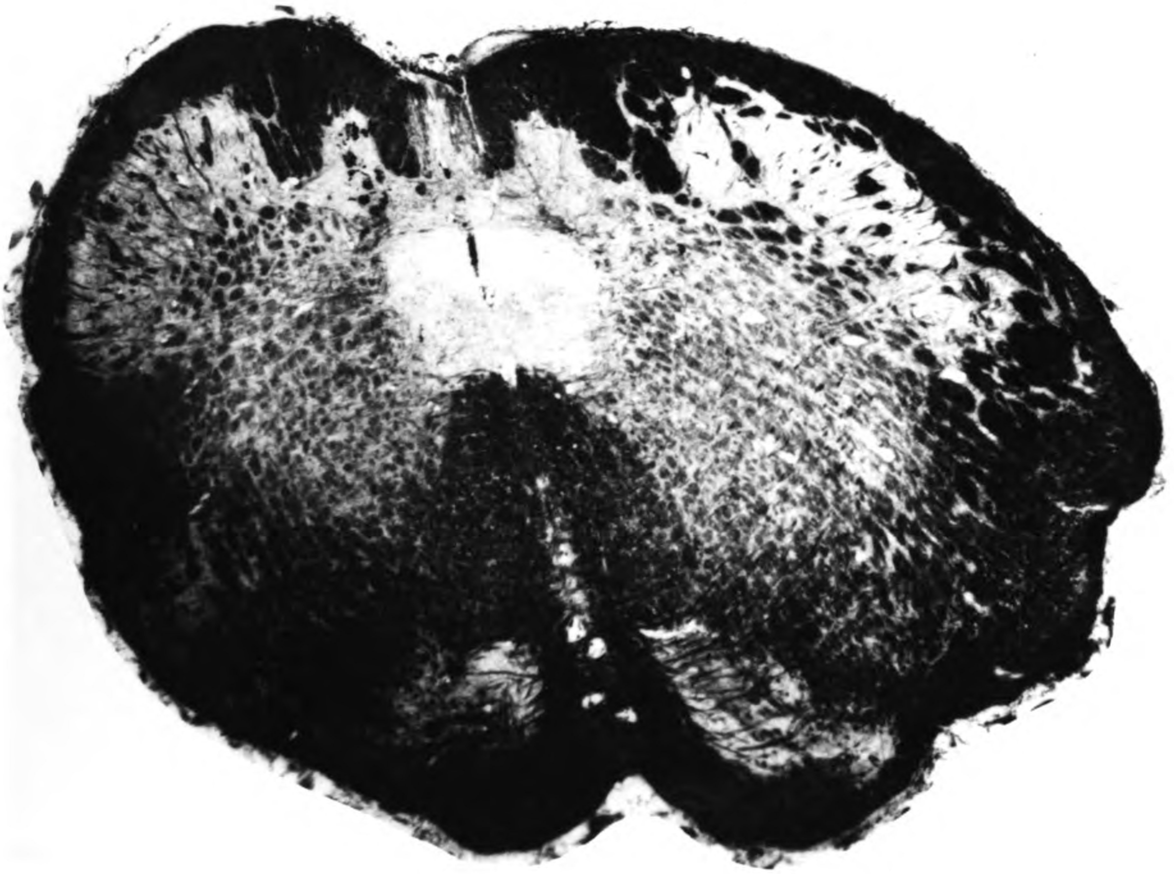

Figure 5b

Figure 5. Response distribution in Plane 3-1.

Figure 5a. This plane illustrates the organization of Cu approximately one millimeter caudal to the obex. Obviously little can be said about Gr from this data. But, the general trend of an inverted figure is evident in the Cu. Also noticable is the large volume of Cu which is devoted to representation of the volar hand and the trend from pre- to postaxial arm as more medial portions of Cu are encountered. Also, although the entire hand seems to have representation throughout most of the dorsal Cu, there is a tendency, somewhat evident here, for the digit 1 side of the hand to predominate laterally and the digit 5 side to predominate medially. An absence of responses is also evident in the ventral part of the central portion of Cu. This seems to be a property of this nuclear area; for all of the other planes of data are consistent with this interpretation.

Figure 5b. Photomicrograph of section 197 (hematoxylin stained, Heidenhain method).

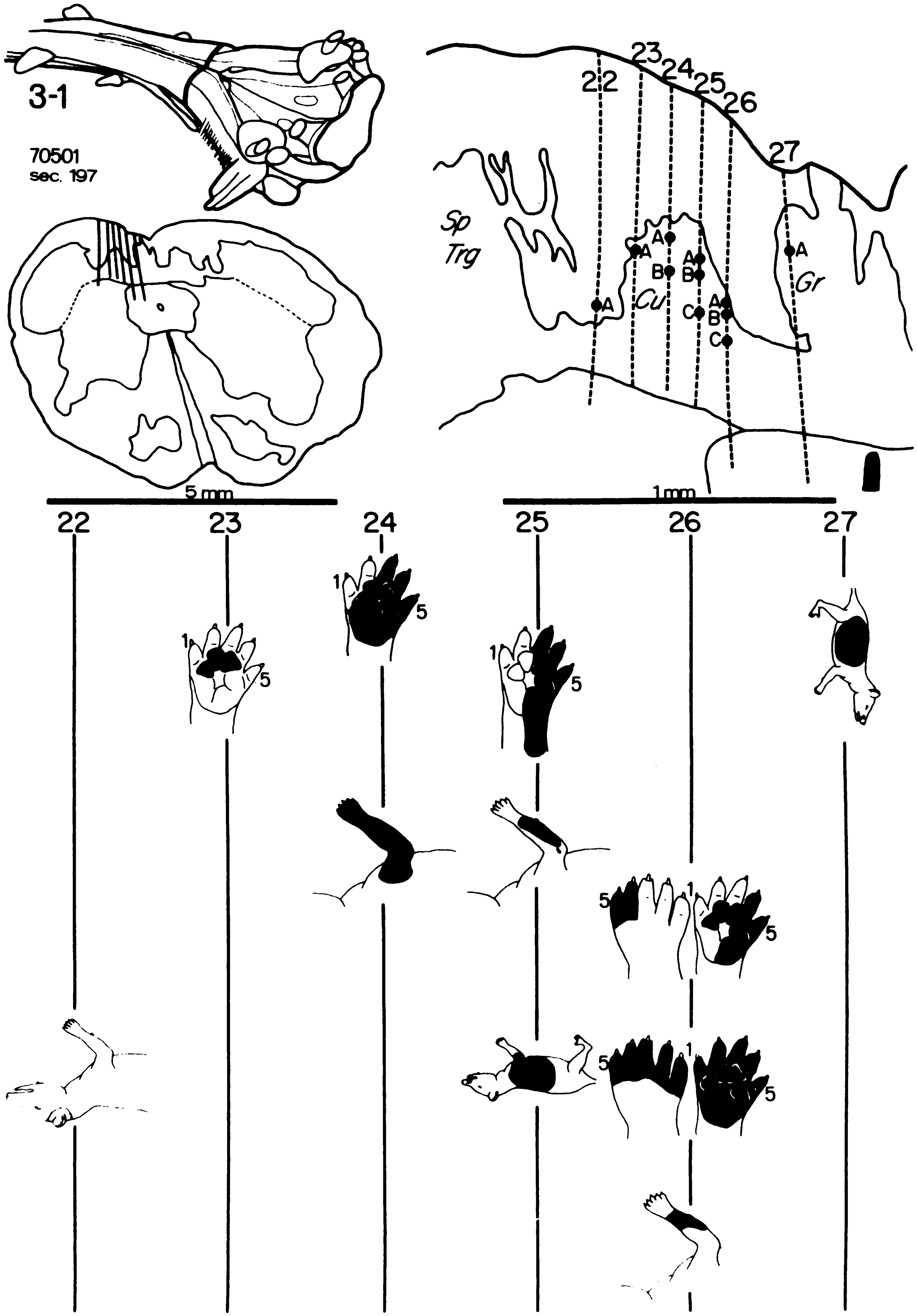

Figure 5a

Figure 6b

Figure 6. Response distribution in Plane 3-5.

Figure 6a. In this plane, located approximately 2 mm caudal to the obex, a number of somatotopic details are evident. P1, P2, and P8 illustrate the relatively large nuclear volume, located in and around the bridge connecting Cu and Sp Trg, devoted to representation of the pinna and upper neck. This is consistent with observations of Weller (1971). Also, P5 and P6 indicate that the lateral portions of the ψ shaped Gr is devoted to representation of the foot and leg.

Figure 6b. Photomicrograph of section 143 (hematoxylin stained, Heidenhain method).


3-5
70502
sec. 143
3 mm
9
8
1
2
3
4
5
6
7
A
B
C
Sp
Trg
Gr
Cu
1 mm
8
1
2
3
4
1
5
1
5
5
5
5
6
7

Figure 6a

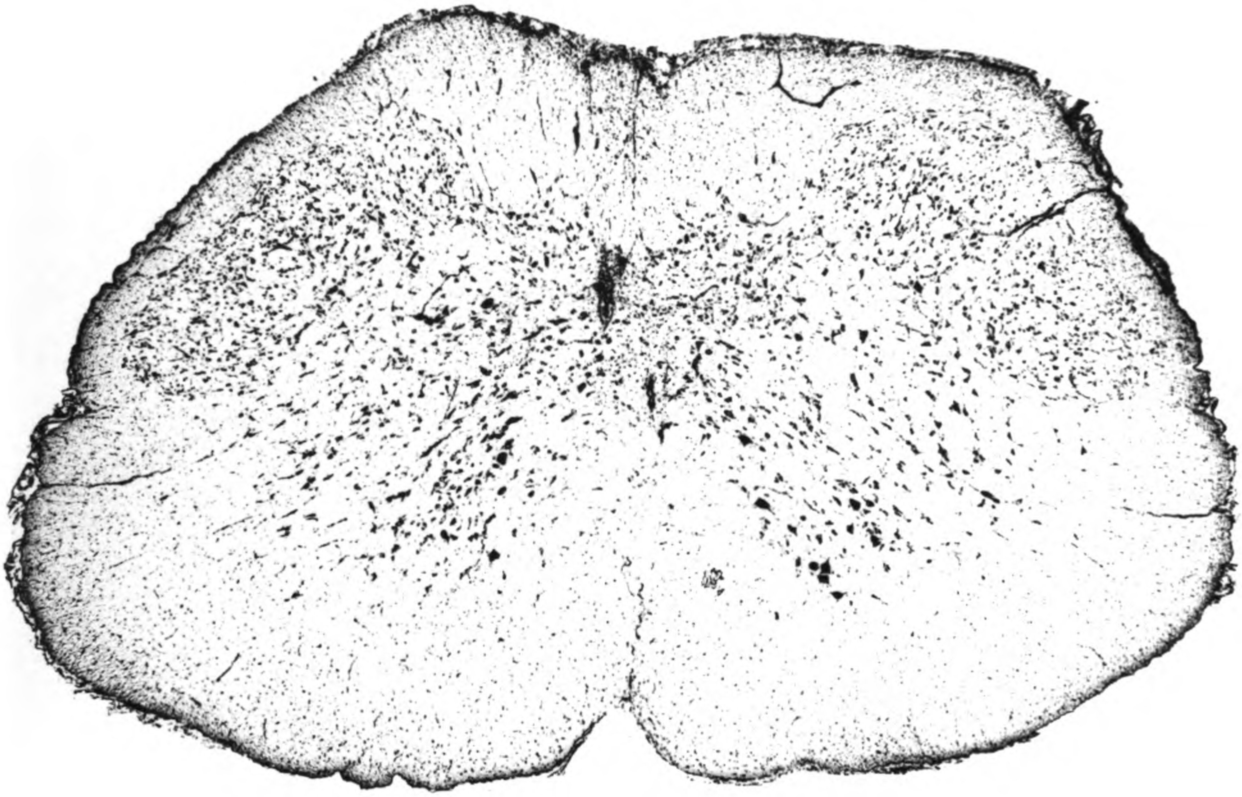

Figure 7b

Figure 7. Response distribution in Plane 3-8.

Figure 7a. This plane illustrates some of the somatotopic organization at a level about 2.5 mm caudal to the obex. P10 shows the representation of the ear and surround in the junction between Cu and Sp Trg. P13 demonstrates the trend toward representation of the digit 5 side of the hand in the medial Cu as well as the more proximal locations of peripheral receptive fields as one records from successively more ventral aspects of Cu. P14 illustrates the location of tail representations (response 14A is bilateral) in the central Gr as well as the location of foot responses in the lateral Gr and also shows the contiguity of Cu-Gr (response 14D). P15 shows the perianal region to be represented at the junction between the lateral and medial arms of the Gr and also illustrates the shift to representation of more rostral body regions in more ventral portions of the Gr.

Figure 7b. Photomicrograph of section 174 (thionin stained, Nissl method). Portions of punctures 10-15 are evident.

Longitudinal Somatotopic Variations

More caudal regions of Cu-Gr are illustrated in Figures 8 and 9. The same general organization as that found at midnuclear levels (Figures 3 through 7) is again apparent. The receptive field sizes are the same and the data again indicates continuity between Cu and Sp Trg. However, the rapidly decreasing size of Gr at these levels causes a spacial separation between Cu and Gr. The somatotopic organization in those portions of Gr that remain is identical to more rostral levels.

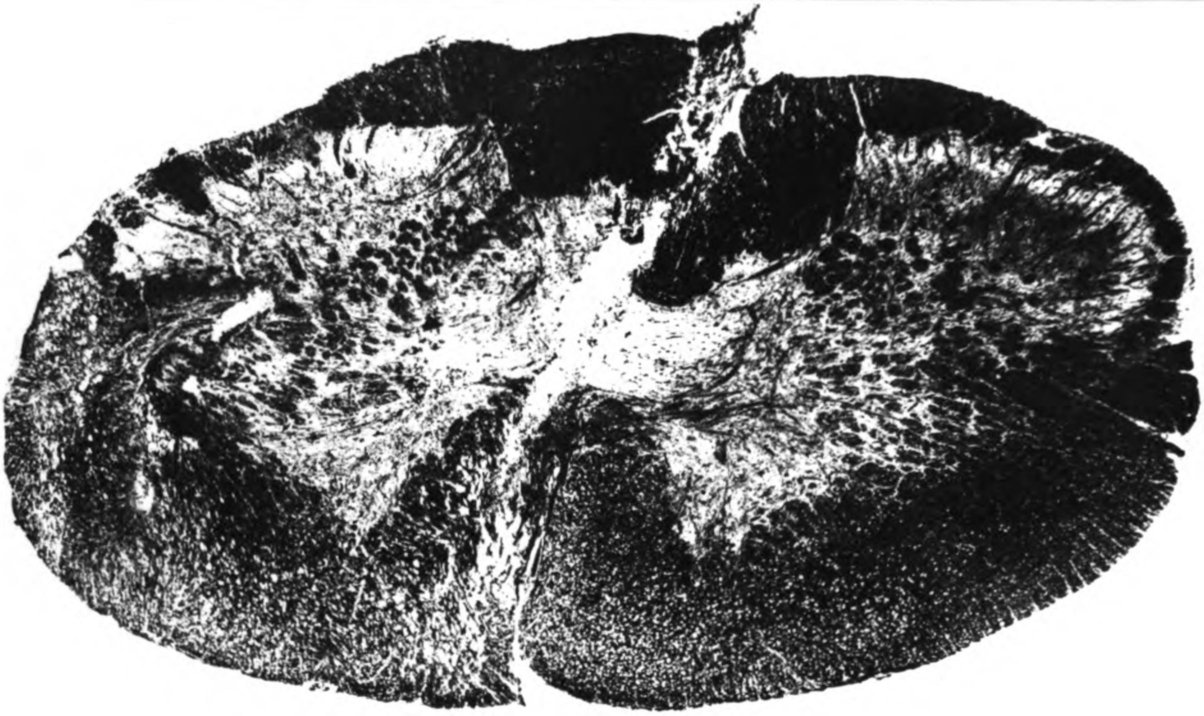

Figure 8b

Figure 8. Response distribution in Plane 4-4.

Figure 8a. This plane illustrates the somatotopic organization approximately 3.5 mm caudal to the obex. The rather large tear in this section obscures Gr organization and makes it difficult to completely determine the organization of Cu. However, the large volume devoted to volar hand and the general trend toward an inverted figure, except in the lateral aspect of the Cu, is still present. The deviation from the inverted figure is seen to occur only in the ear, neck, and arm representation. Since the ear and neck are continuous with the Sp Trg and since the Sp Trg is somewhat dorsal as well as lateral to the Cu at this level, the location of the neck and ear in the dorsal Cu can be understood. The ear seems to lie along the dorsal part of the Cu on its lateral edge and the neck and arm successively more ventral to this. The dorsal hand is found ventral to these body parts and is also generally ventral to the volar hand throughout the remainder of the nuclei. This deviation may also result from the slight tilt of the electrode penetrations which caused the deeper responses in each puncture to also be more medial.

Figure 8b. Photomicrograph of section 47 (hematoxylin stained, Heidenhain method).

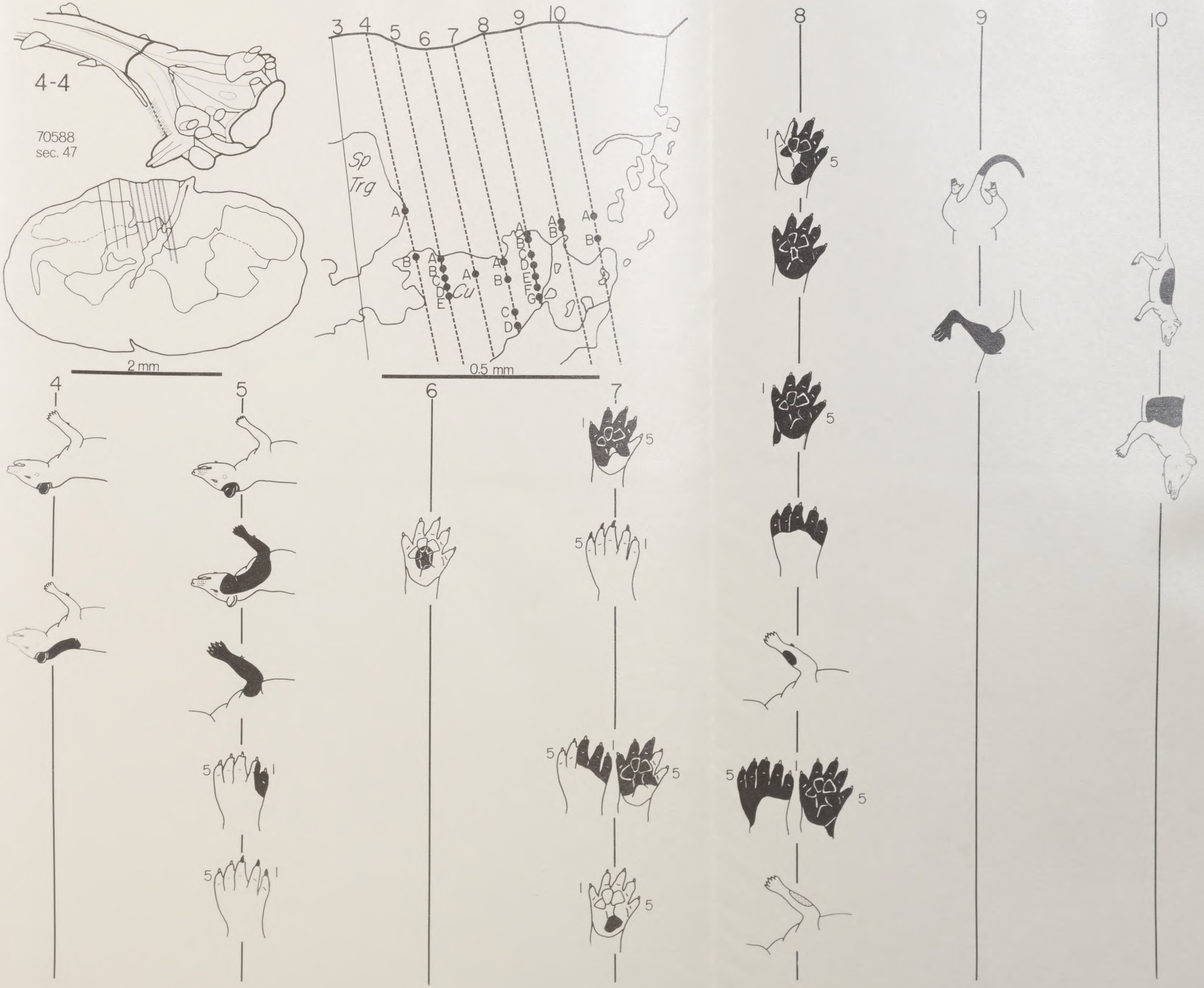

Figure 8a

Figure 9b

Figure 9. Response distribution in Plane 4-6.

Figure 9a. This plane, about 4 mm caudal to the obex, is again consistent with the general somatotopic organization and illustrates the large volar hand representation still present at this level. A decrease in the density (number) of responses per puncture is also beginning to become apparent as it will at the very rostral end of the nuclei. The lower thoracic region is not represented in the caudal Gr as opposed to the absence of caudal body representation in the rostral Gr. The volar hand is the only body area in obvious representation in the caudal Cu with the other upper body area representations decreased significantly. (Three other planes from this level, not illustrated in detail here, are completely consistent with this observation as is the data from this level in one additional animal in which the electrode tracks have not been identified histologically.)

Figure 9b. Photomicrograph of section 259 (hematoxylin stained, Weil method). Portions of all five punctures are evident.

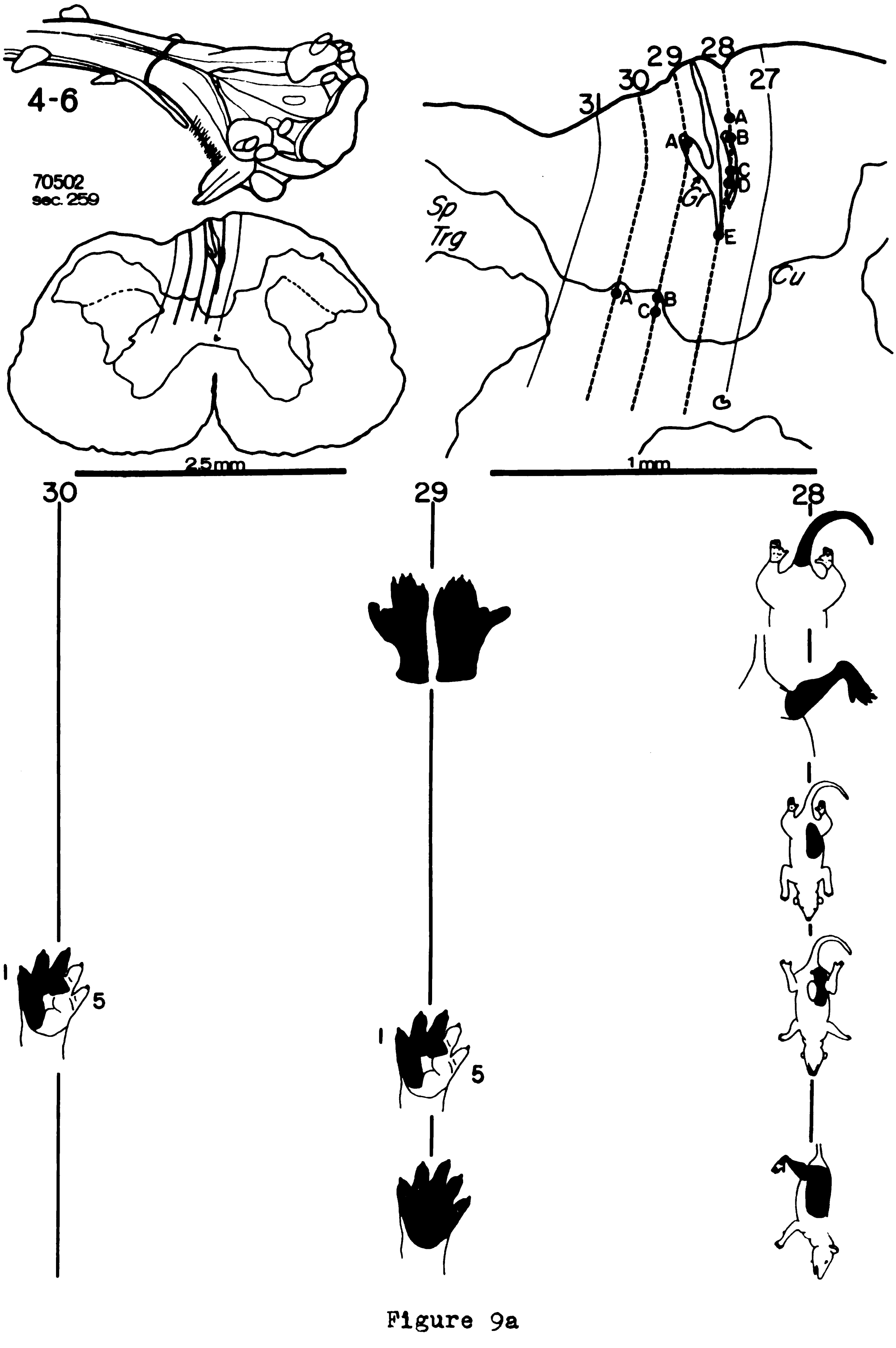
4-6
70502
sec. 259
2.5mm
31
30
29
28
27
A
B
C
D
E
A
A
B
C
Sp
Trg
Gr
Cu
1mm
30
29
28
1
5
1
5

Figure 9a

At the extreme rostral end of Cu-Gr, the same general organization exists, but the degree to which the various body parts are represented has changed. As mentioned previously, the tail and perianal region are not represented above the obex, and indeed, at the very rostral portion of Cu-Gr only the lower thoracic regions are found to be represented in Gr, the representation of lower body regions having stopped in an orderly manner at more caudal levels. Cu has maintained the same apparent organization with the volar hand representation still exaggerated relative to other body parts. No change is apparent in the size of receptive fields, but a decrease in the density (number) of responses per puncture is obvious. These trends, along with the continuing continuity between Cu and Gr and between Cu and Sp Trg are evidenced in all three of the planes of data from this level that are presented in Figures 10 through 12.

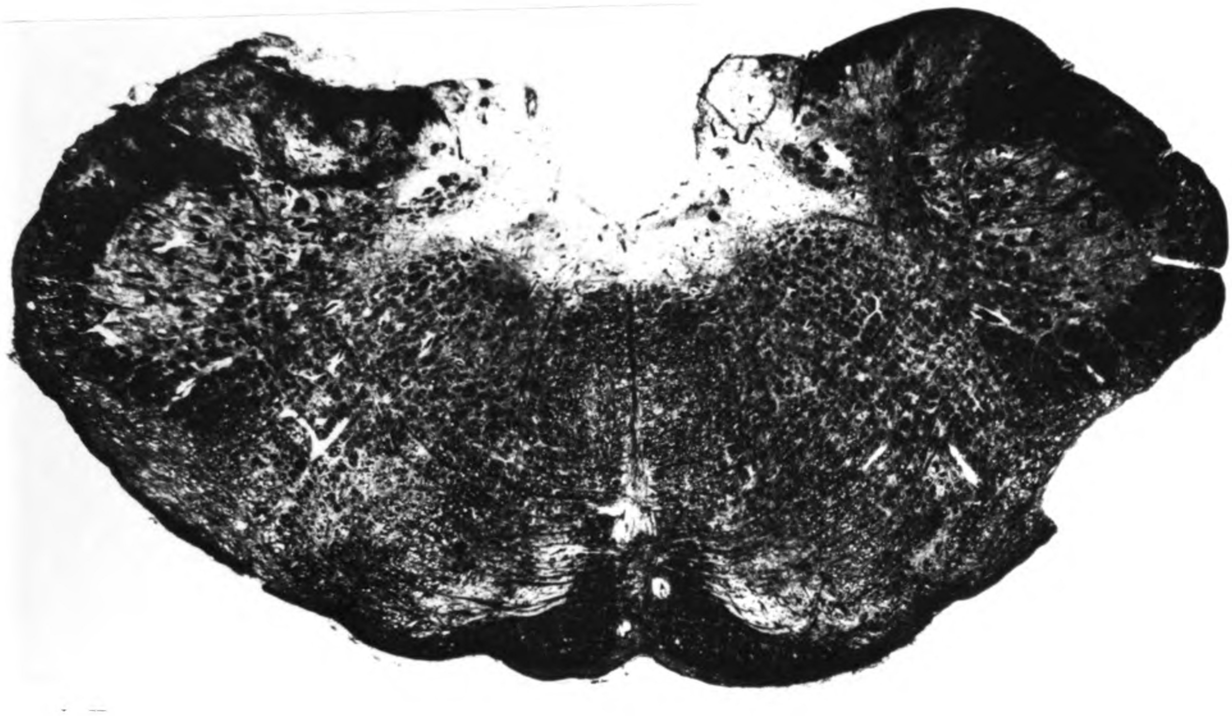

Figure 10b

Figure 10. Response distribution in Plane 1-3.

Figure 10a. As mentioned in the text, the number or responses per puncture has decreased at this level -- about 2 mm rostral to the obex. The fiber response in P28 suggests that the foot may still be represented at this level, but, as mentioned previously, caudal body regions have a reduced representation.

Abbreviation: E.-Cu .... external cuneate nucleus

Figure 10b. Photomicrograph of section 193 (hematoxylin stained, Heidenhain method).

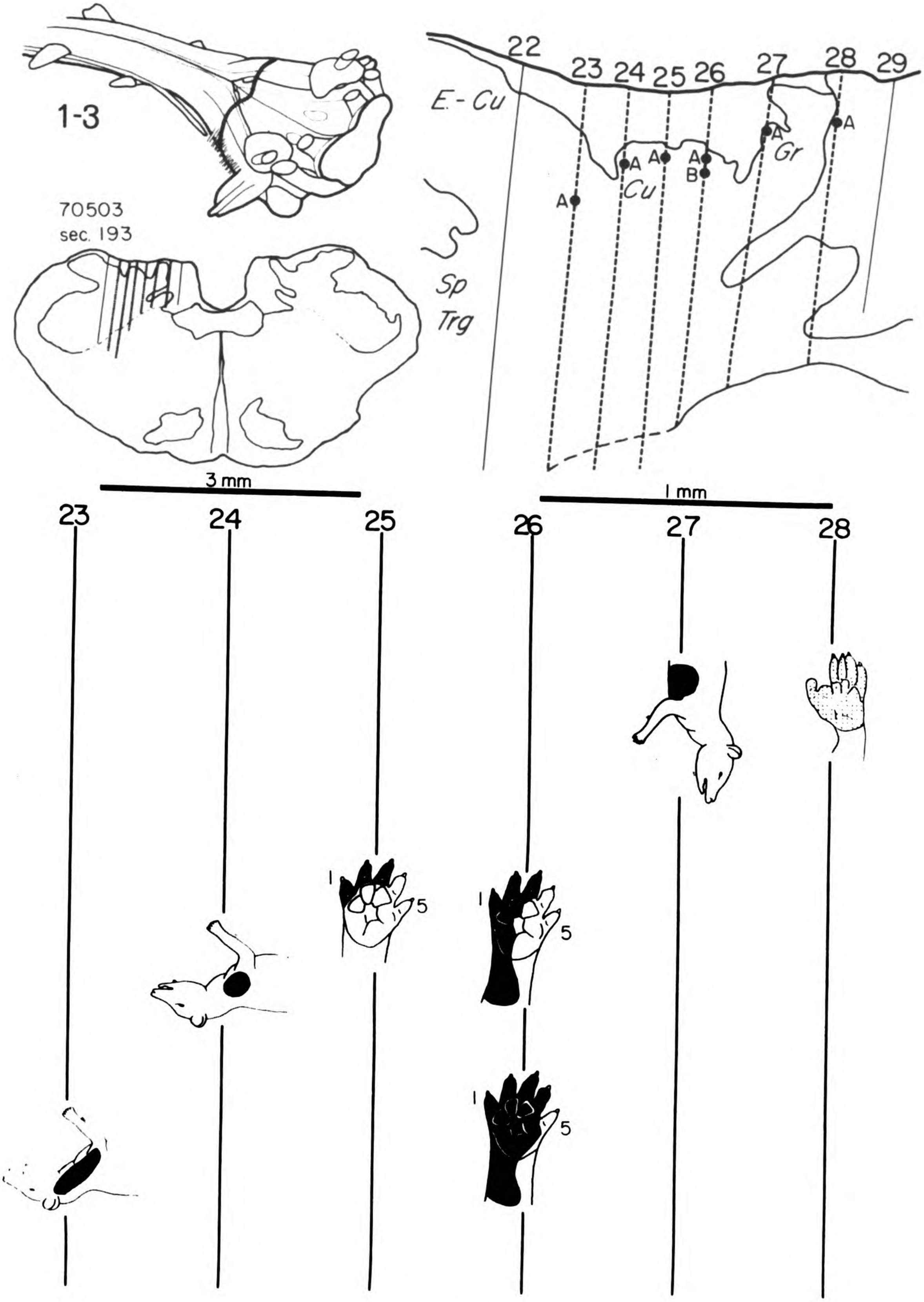
1-3
70503
sec. 193
3 mm
22
23
24
25
26
27
28
29
E.- Cu
Cu
Gr
Sp
Trg
A
B
1 mm
1
5

Figure 10a

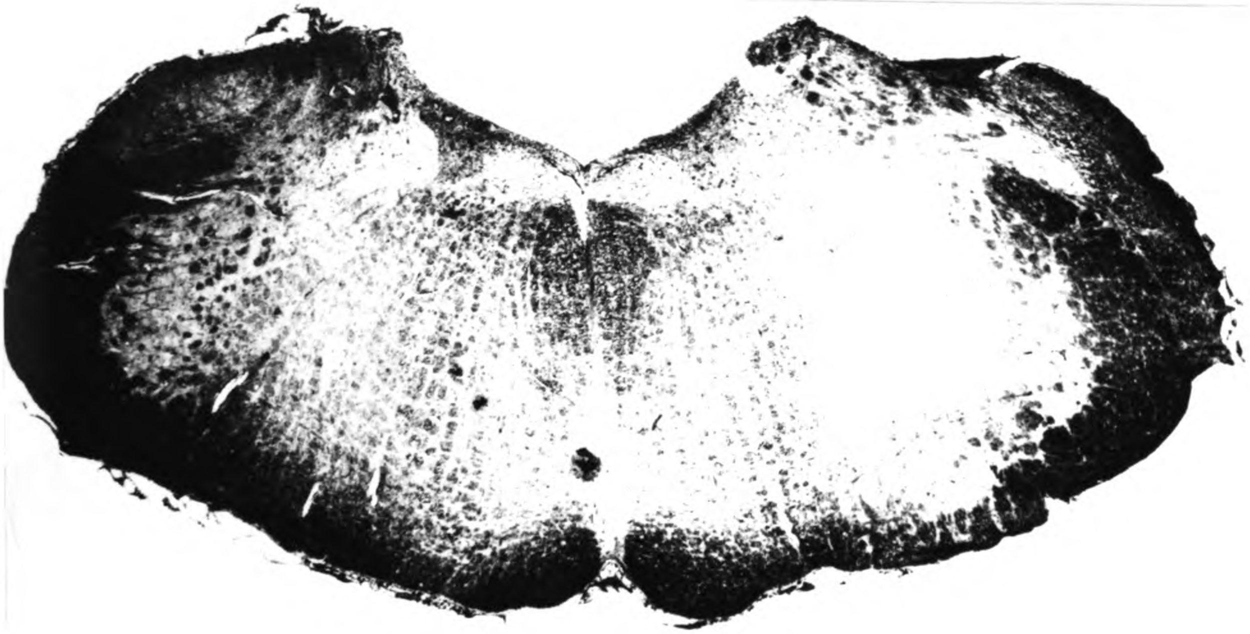

Figure 11b

Figure 11. Response distribution in Plane 1-2.

Figure 11a. At the rostral tip of Gr, about 2.5 mm rostral to the obex, caudal body representations are again noticably absent from Gr, which is greatly reduced in size. The volar hand is still prominent in Cu.
Abbreviation: E.-Cu ..... external cuneate nucleus

Figure 11b. Photomicrograph of section 291 (hematoxylin stained, Weil method).

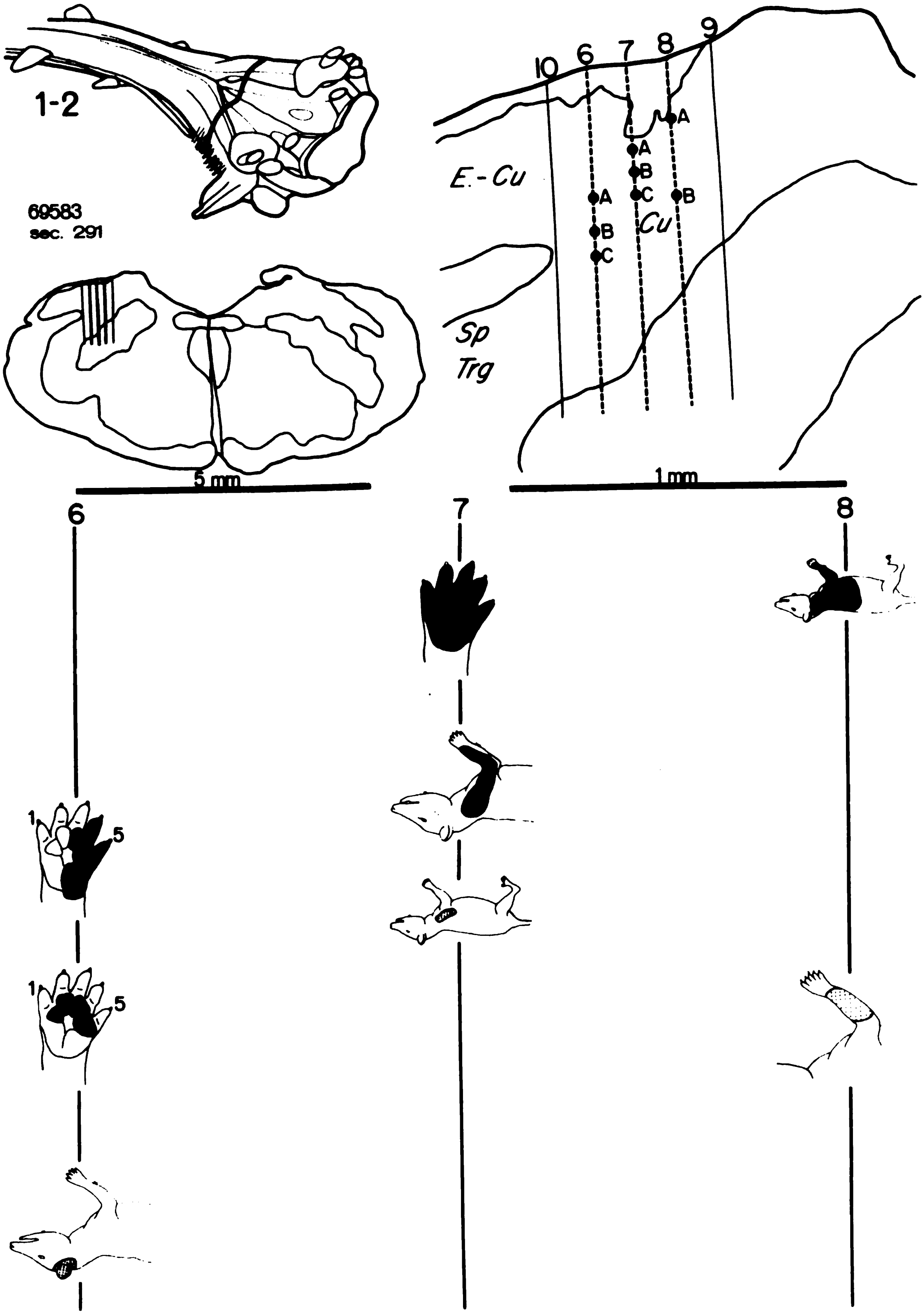

Figure 11a

Figure 12b

Figure 12. Response distribution in Plane 1-1.

Figure 12a. Again about 2.5 mm rostral to the obex, at the rostral tip of Gr, the same trend is evident. Volar hand representations are present in Cu but more proximal body regions are diminished in the degree of representation. In Gr, only representation from the lower thoracic region is observed.

Abbreviation: E.-Cu .... external cuneate nucleus

Figure 12b. Photomicrograph of section 157 (hematoxylin stained, Heidenhain method). Portions of P16 through P18 are visible.

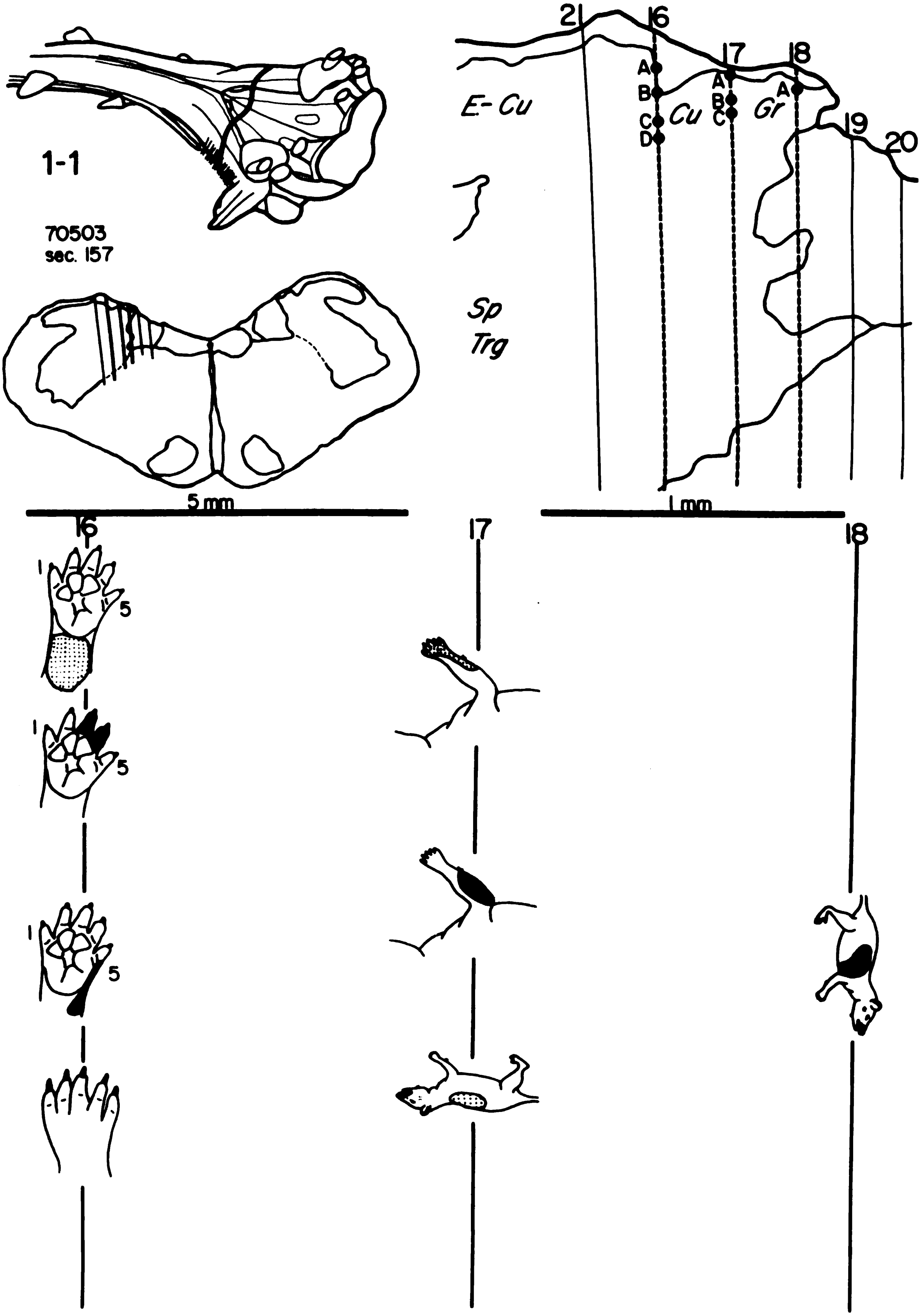
1-1
70503
sec. 157
5 mm
21
16
17
18
19
20
A
B
C
D
E-Cu
Cu
Gr
Sp
Trg
1 mm
16
17
18
1
5
1
5
1
5

Figure 12a

DISCUSSION

Somatotopic Organization

The general somatotopic organization of Cu-Gr found in the placental mammals and the one reptile previously studied has been determined to exist in the opossum. That is, neighboring peripheral body regions are represented in adjacent locations in the nuclear mass. Also, the representation can be visualized as an animal lying on its back with its extremities dorsal and tail dorso-medial. The representations of the various body parts are distorted in such a manner that the peripheral regions having the largest nuclear representation are those which, judging from their usefulness to the animal in mechanoreception, have the greatest behavioral significance. All representations, with the exception of some tail responses, were ipsilateral. Many of the tail responses were asymmetrically bilateral, which is to be expected because the relatively long effective recording radius of the electrodes spans the small tail representation of the central Gr.

In the opossum Cu-Gr the volar hand has the largest representation, but the detail of representation of the vibrissae and rhinerium in Sp Trg is even more elaborate (Weller, 1971). The detail of representation that exists in the medulla is not consistent with the extremely large receptive fields reported for the ventrobasal thalamus by Erickson et al. (1964). But the detail reported here and by Weller (1971) as well as the relative importance of the representation of facial parts over that of the lower body is consistent with the representations found in later microelectrode studies in the ventrobasal thalamus (Pubols and Pubols,1966; Oswaldo-Cruz and Rocha-Miranda,1971) and in an earlier evoked potential study in the somatosensory cortex (Lende, 1963).

Although no attempt was made to determine the somatotopic organization of any but the mechanoreceptor projections, it was noted that the deep pressure and proprioceptive responses were generally found to be more ventral in Cu-Gr than, and except for location in the appropriate nucleus (either Cu or Gr) did not follow the same somatotopic organization as, the mechanoreceptor projections.

One of the major differences between the opossum Cu-Gr and that of most placental mammals studied thus far is the relatively reduced detail in the organization of mechanoreceptor projections in the opossum. Most of the receptive fields covered a relatively large body area. Gr responses often included the entire ipsilateral hindquarter. Even the receptive fields of the relatively more detailed volar hand representation usually included at least several digits and/or pads. The use of predominantly CU and MU responses undoubtedly increased the average receptive field size (cf.

Johnson et al.,1968, p 28); for those SU responses encountered innervated smaller receptive fields; but even these were generally large compared with SU responses in other animals (Welker et al.,1968; McComas,1962; Kruger et al.,1961; Perl et al.,1962) and in the Sp Trg of opossums (Weller, 1971).

The representation of the volar hand also did not show any indications of the fine grain resolution present in the raccoon (Johnson et al.,1968). Indeed, although there was a tendency for the digit 1 side of the hand to be represented laterally in Cu and the digit 5 side medially, and a tendency for the digits to be represented more dorsally than the palm, all parts of the forepaw were represented to some extent in all parts of Cu devoted to hand representation. Thus, a general somatotopic map can be constructed for rather broad peripheral body areas, but a detailed mapping of body topography to the nuclear space is impossible. Such a generalized map is shown in Figure 13 for a Cu-Gr level slightly caudal to the obex. Variations in this map at different nuclear levels are observed to occur only in the extent to which the various body parts are represented (if represented at all at some levels) and not in the relative organization of anatomical representation (somatotopy).

Woudenberg (1970) described the location of the pinna in the sheep to be confined to a bump of cells located between Cu and Sp Trg. The neck was found to be located medial to this and the throat and cheek laterally. The same

Figure 13. Schematic map of the somatotopic organization of Cu-Gr mechanoreceptor projections.

Top: The figurine at the upper left represents the opossum body and has been divided into several areas, each of which is designated by a letter. The drawing represents a coronal section of Cu-Gr about 1 mm caudal to the obex. The nuclear area has been divided into regions that correspond to the various body parts labeled in the opossum figurine. The diagram is partially a summary of the data collected and partially an estimation of the actual somatotopic organization.

Bottom: This illustration is a very schematized figurine of an opossum body that has been drawn into the nuclear area in a manner that approximates the location of corresponding body part representations.

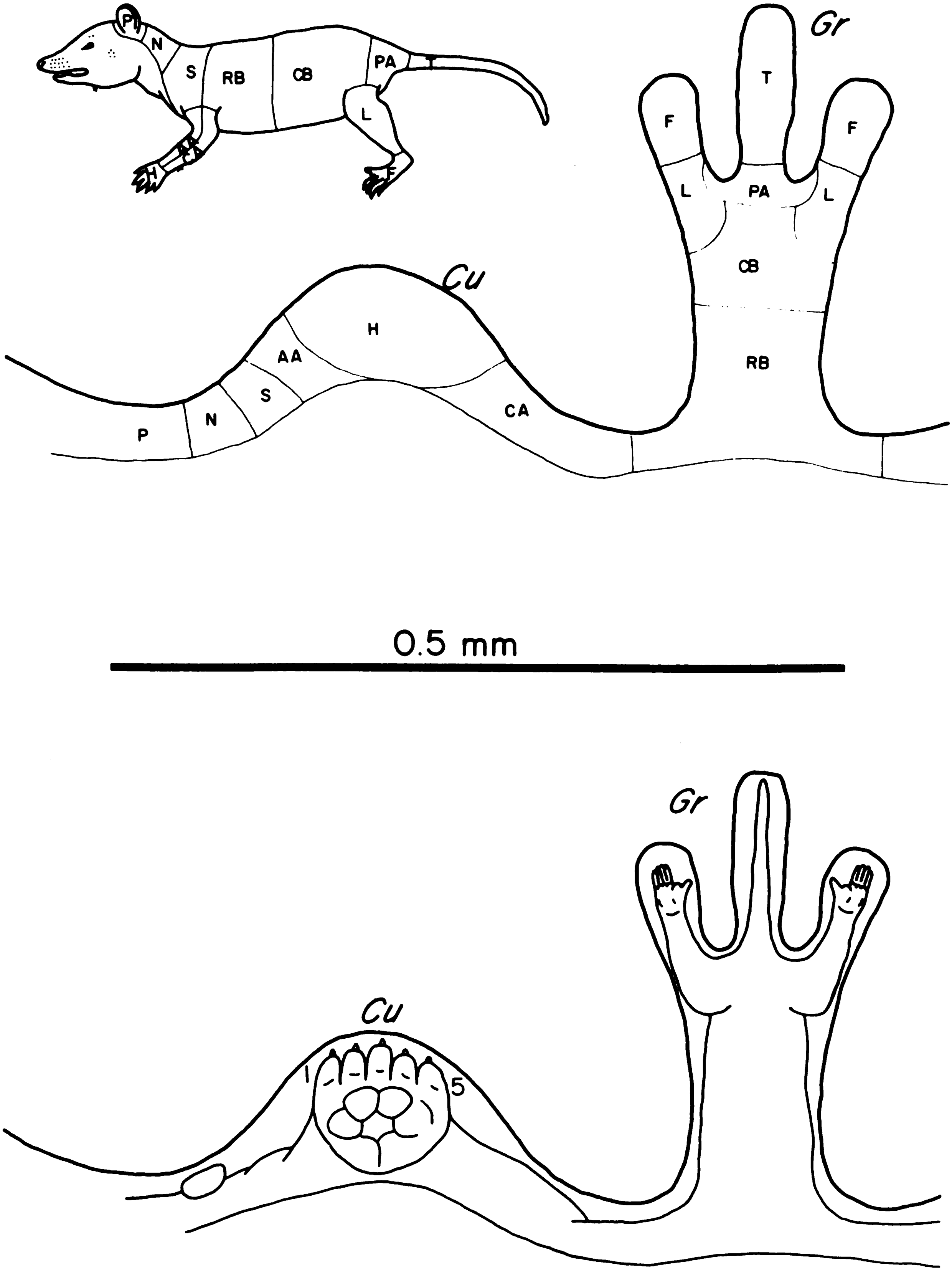
P
N
S
RB
CB
PA
T
L
AA
CA
H
F
Gr
T
F
F
L
PA
L
CB
RB
Cu
H
AA
S
N
P
CA
0.5 mm
Gr
Cu
1
5

Figure 13

situation exists in the opossum except that no bump of cells exists between Cu and Sp Trg and the pinna representation is instead found in the dorsolateral Cu, the dorsomedial Sp Trg, and in the connection between the two nuclei. This has been previously reported by Weller (1971) and is consistent with the pattern found in the rat (Nord, 1967), monkey (Kerr, Kruger, Schwassmann, and Stern, 1968), and raccoon (Johnson *et al.*, 1968). However, the pinna representation in the opossum extends to other regions of the Sp Trg as well, and thus is more widely distributed than has been reported for other animals (Weller, 1971).

In agreement with Weller (1971), the somatotopic representation is continuous between Sp Trg and Cu. The additional demonstration that the representation is also continuous between Cu and Gr shows that the entire body and head have a continuous representation at the level of the medulla. This conflicts with the assertions of Kerr *et al* (1968, p. 133) that such continuity does not exist at brain stem levels.

Longitudinal Variations

The continuity of representations along the longitudinal axis of Cu-Gr has also been subject to controversy. Several investigators (Gordon and Jukes, 1961; Gordon and Seed, 1960; McComas, 1962, 1963) reported a rostral-caudal differentiation of the gracile nucleus on the basis of receptive field size. The smallest receptive field sizes were reported in the middle third of the nucleus and largest fields in the rostral third. Intermediate size receptive fields were reported in the caudal third of the nucleus. But other investigations (Kruger et al.,1961; Nord,1967; Perl et al.,1962; Winter,1965; Woudenberg,1968) find no such differentiation and maintain that the receptive field size is solely a function of the position on the body surface which is being represented. This study on the opossum Cu-Gr agrees completely with the latter assertions; for no differences in receptive field size were evident along the rostral-caudal axis of Cu-Gr. Furthermore, those body areas having the smallest receptive fields occupied the largest nuclear volume.

Similarly, no rostral-caudal differentiation was noted in the general somatotopic organization of Cu-Gr. But the extent to which the various body parts were represented at different nuclear levels did not remain constant. No tail representation was found rostral to the obex and at the extreme rostral end of the Gr, only the lower thoracic regions (the most anterior regions represented in the Gr) are found to be represented. Likewise, the representations of the pinna, neck, and shoulder (the most anterior regions represented in the Cu) are either absent or greatly reduced at the most caudal levels of the Cu. This is consistent with findings in other animals (cf. Norton,1970, p 24).

Suggested Studies

Since Cu-Gr was mapped using mainly CU responses, a more detailed study, employing mainly SU responses would be beneficial for determining the limit of resolution of peripheral representation. It may also help clarify the organization of the volar forepaw representation in the Cu. Particular attention should also be given to deep pressure and proprioceptive responses so that the projections of these modalities of sensory receptors can be compared to the cutaneous mechanoreceptor somatotopy. A SU study should also be capable of clarifying the exact location of tail representations and of investigating more thoroughly the possibility of rostral caudal nuclear differences in receptive field sizes.

Also, as suggested by Weller (1971), additional experiments should be performed in which electrode penetrations deviate from the verticle by as much as 45°. These experiments will give a much better insight into the medial-lateral organization of the nuclei than is possible from the vertical punctures of the present study, and may also help to determine the role of the central portion of the ventral Cu.

BIBLIOGRAPHY

BIBLIOGRAPHY

Baldwin, H.A., S. Frenk, and J.Y. Lettvin. 1965. Glass coated tungsten microelectrodes. Science 148: 1462-1463.

Chang, H.T. and T.C. Ruch. 1947. Organization of the dorsal columns of the spinal cord and their nuclei in the spider monkey. J. Anat. 81: 140-149.

Erickson, R.P., J.A. Jane, R. Waite, and I.T. Diamond. 1964. Single neuron investigation of sensory thalamus of the opossum. J. Neurophysiol. 27: 1026-1047.

Gordon, G. and M.G.M. Jukes. 1962. Correlation of different excitatory and inhibitory influences on cells in the nucleus gracilis of the cat. Nature 196: 1183-1185.

Gordon, G. and W.A. Seed. 1960. Responses of single neurons in nucleus gracilis of the cat to lemniscal and cerebellar stimulation. J. Physiol. 152: 63P-64P.

Johnson, J.I. Jr., W.I. Welker, and B.H. Pubols, Jr. 1968. Somatotopic organization of raccoon dorsal column nuclei. J. Comp. Neurol. 132: 1-44.

Kerr, F.W.L., L. Kruger, O. Schwassmann, and R. Stern. 1968. Somatotopic organization of mechanoreceptor units in the trigeminal nuclear complex of the macaque. J. Comp. Neurol. 134: 127-143.

Kruger, L. and P. Witkovsky. 1961. A functional analysis of neurons in the dorsal column nuclei and spinal nucleus of the trigeminal in the reptile (Alligator mississippiensis). J. Comp. Neurol. 117: 97-106.

Kruger, L., R. Siminoff, and P. Witkovsky. 1961. Single neuron anatomy of dorsal column nuclei and spinal nucleus of trigeminal in cat. J. Neurophysiol. 24: 333-349.

Kuhn, R.A. 1949. Topographical pattern of cutaneous sensibility in the dorsal column nuclei of the cat. Trans. Amer. Neurol. Ass. 74: 227-230.

Lende, R.A. 1963a. Sensory representation in the cerebral cortex of the opossum (Didelphis virginiana). J. Comp. Neurol. 121: 395-403.

__________ 1963b. Motor representation in the cerebral cortex of the opossum (Didelphis virginiana). J. Comp. Neurol. 121: 405-415.

McComas, A.J. 1962. Longitudinal organization of the gracile nucleus. J. Physiol. 161: 21P-22P.

__________ 1963. Responses of the rat dorsal column system to mechanical stimulation of the hind paw. J. Physiol. 166: 435-448.

__________ 1964. Innervation of cells in rat gracile nucleus. J. Physiol. 175: 46P.

Nord, S.G. 1967. Somatotopic organization in the spinal trigeminal nucleus, the dorsal column nuclei and related structures in the rat. J. Comp. Neurol. 130: 343-355.

Norton, Allen C. (ed.). Updated through 12 Dec 1970. The Dorsal Column System of the Spinal Cord, An Updated Review. UCLA Brain Information Service, NINDS Neurological Information Network, National Institutes of Health, U.S. Department of Health, Education, and Welfare.

Oswaldo-Cruz, E., R. Pagani, and C.E. Rocha-Miranda. 1965. Lumbar and sacral dermatomes in the opossum (D. aurita, Wied). An. Acad. Brasileira Cienc. 37: 337-344.

Oswaldo-Cruz, E. and C.E. Rocha-Miranda. 1971. In press.

Perl, E.R., D.G. Whitlock, and J.R. Gentry. 1962. Cutaneous projection to second order neurons of the dorsal column system. J. Neurophysiol. 25: 337-358.

Pubols, B.H. Jr. and L.M. Pubols. 1966. Somatic sensory representation in the thalamic ventrobasal complex of the Virginia opossum. J. Comp. Neurol. 127: 19-34.

__________ 1969. Forelimb, hindlimb and tail dermatomes in the spider monkey (Ateles). Brain, Behavior, and Evolution 2: 132-159.

Ulinski, Phillip Steven. 1969. Normal development of the cuneate-gracile nuclear complex in pouch young opossums. Ph.D. Thesis, Michigan State University, East Lansing, Michigan.

__________ 1971. External morphology of pouch young opossum brains: a profile of opossum neurogenesis. *J. Comp. Neurol. 142*: 33-58.

Welker W.I., J.I. Johnson, Jr., and B.H. Pubols, Jr. 1964. Some morphological and physiological characteristics of the somatic sensory system in raccoons. *American Zool. 4*: 75-96.

Weller, William Lee, Jr. 1971. Patterns of mechanoreceptor projections to the trigeminal nuclei of the opossum. M.S. Thesis, Michigan State University, East Lansing, Michigan.

Werner, G. and B.L. Whitsel. 1968. The topology of the body representation on somatosensory area I of primates. *J. Neurophysiol. 31*: 856-869.

Winter, D.L. 1965. Nucleus gracilis of cat. Functional organization and corticofugal effects. *J. Neurophysiol. 28*: 48-70.

Woudenberg, R.A. 1970. Projections of mechanoreceptive fields to cuneate-gracile and spinal trigeminal nuclear regions in sheep. *Brain Research 17*: 417-437.

General References

Keefe, James F. 1967. *The World of the Opossum*. J.B. Lippincott Company, New York.

Oswaldo-Cruz, E. and C.E. Rocha-Miranda. 1968. *The Brain of the Opossum* (*Didelphis marsupialis*): *A Cytoarchitectonic Atlas in Stereotaxic Coordinates*. Instituto de Biofisica, Universidade Federal do Rio de Janeiro.

Voris, H.C. and N.L. Hoerr. 1932. The hindbrain of the opossum *Didelphis virginiana*. *J. Comp. Neurol. 54*: 277-355.