

# Somatotopic Organization and Response Properties of Neurons of the Ventrobasal Complex in the Opossum<sup>1</sup>

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**ABSTRACT** Thalamic responses to somatic stimulation were studied in 47 specimens of the South American opossum, *D. marsupialis aurita*. Topographic representation of the body surface in the ventrobasal complex was determined by recording unit cluster responses to natural stimulation in sodium pentobarbital anesthetized animals. The pattern of representation observed was similar to that reported in the North American subspecies and comparable to the findings in eutherian mammals.

The analysis of single units recorded with extra-cellular microelectrodes showed that the great majority of neurons are place and modality specific. A predominance of skin (vs. deep) sensibility was observed. Units activated by displacement of hairs and vibrissae displayed receptive fields similar to those reported in higher mammals. Of the units isolated within the anatomical boundaries of the ventrobasal complex only 1% showed wide receptive fields, or bilateral representation. None of the units could be activated by auditory stimulation. Neurons displaying wide, discontinuous or bilateral receptive fields showing auditory-somatic convergence were isolated in nucleus parafascicularis, subparafascicularis and in the Posterior group.

Using anatomical techniques it has been shown, in a wide variety of mammals, that components of the ventrobasal complex (xVB), as defined by Rose and Mountcastle ('52) are the main site of termination of the medial lemniscus. Recent anatomical literature related to this problem was reviewed by Bowsher ('65).

After the introduction of electrophysiological recording methods, a precise organization of the somatic sensory projections within the anatomical boundaries of this nucleus was demonstrated in several species (rat: Angel, '64; Davidson, '65; Emmers, '65; rabbit: Rose and Mountcastle, '52; Mountcastle et al., '52; raccoon: Welker and Johnson, '65; sheep: Cabral and Johnson, '71; rhesus monkey: Mountcastle and Henneman, '52; cynomolgus monkey: Albe-Fessard and Bowsher, '65; spider monkey: Pubols, '68). It was shown that the body surface is represented in a circumscribed region within the ventrobasal complex. In this representation peripheral regions displaying a greater in-

ervation density are related to a larger volume of thalamic tissue.

Single unit recording from this region in cats showed that the neurons receiving lemniscal projections are place and modality specific (Rose and Mountcastle, '54). Poggio and Mountcastle ('63) demonstrated, by recording more than 1,000 units from the xVB in chronically denervated monkeys, that this restricted input is not due to the use of anesthetic agents.

In eutherian mammals results differing from this pattern have been occasionally reported. Besides cortico-dependent neurons the ventrobasal complex has been shown to include elements that survive decortication (Clark and Powell, '53; Bava et al., '66). They possibly represent the interneurons described in the posterolateral xVB by Andersen et al. ('64).

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By determining the site of termination of the spinothalamic tract, it was shown that the ventrobasal complex is not the only region receiving somatic projections. The small calibre fibers of this tract were shown to project to a wider area, which includes the caudal pole of the ventrobasal complex, the posterior group (Po, as defined by Rose and Woolsey, '58), the parafascicular-center median region, as well as some of the intralaminar nuclei (Mehler et al., '60).

Single unit analysis of the Po region in the cat (Poggio and Mountcastle, '60; Whitlock and Perl, '59; '61) shows that the functional properties of its component cells are different from those reported in the ventrobasal complex. The projections to this region are attributed to the anterolateral spinal tract. A large percentage of its component cells display not only wide receptive fields, often bilateral, but also convergence of different sensory modalities.

Similar properties and functional organization were found in the cortical sensory areas; SI displaying properties akin to those of the ventrobasal complex (Mountcastle et al., '57; Mountcastle and Powell, '59; Powell and Mountcastle, '59), while SII can be related to the properties displayed by Po (Andersson, '62; Carreras and Andersson, '63; Berman, '61a,b). All of the above results were obtained in various species of eutherian mammals.

The functional properties displayed by these divisions of the somatic sensory mechanisms conducted by (1) the dorsal column-medial lemniscus and by (2) the anterolateral pathway have been correlated by Rose and Mountcastle ('59) with respectively, (1) the epicritic and (2) protopathic concepts expressed by Head et al. ('20).

In view of the proposed theories regarding the evolution of the sensory systems and that of the thalamus (Bishop, '59; Diamond and Chow, '62), it would be of interest to extend these studies to primitive mammals, representatives of the metatheria group.

The common opossum (*Didelphis marsupialis*, Linnaeus, 1758), being a species of wide ecological distribution in the continent, has been the subject of numerous

neuroanatomical investigations (for a complete review see Oswaldo-Cruz and Rocha-Miranda, '68). The morphological features of the opossum thalamus, which have been described by various authors (Bodian, '39; Diamond and Utley, '63; Oswaldo-Cruz and Rocha-Miranda, '67a,b, '68), are similar to those of higher mammals.

Recording from single units in the xVB of the North American subspecies, Erickson et al. ('64) reported a large percentage of units displaying wide receptive fields and convergence of sensory modalities. This result would support the idea that the metatheria display a primitive form of thalamic organization, intermediate between the reptilian and the higher mammalian pattern. On the other hand, using the same species but recording unit cluster responses to tactile stimulation, Pubols and Pubols ('66) reported a precise somatotopical organization within the boundaries of the xVB, similar in all respects to the results obtained in eutherian mammals.

In view of the conflicting results obtained in the study of the opossum thalamus, the following work was carried out to clarify the points in variance.

The functional organization of the xVB was studied in a South American subspecies (*D. marsupialis aurita* Wied, 1826). In this species the cytoarchitectonic organization of the central nervous system has been shown to be identical to that of the North American variety by detailed anatomical and statistical studies (Oswaldo-Cruz and Rocha-Miranda, '67a,b, '68).

Our description of the ventrobasal complex closely corresponds with Bodian's ('39) *nucleus ventralis pars principalis*, except for the possible inclusion of part of our *nucleus ventralis thalami lateralis* and *nucleus C* (see Oswaldo-Cruz and Rocha-Miranda, '67a,b, '68). This complex, ovoid in shape, extends from A 5.50 to A 3.17. The rostro-caudal axis of this nucleus is ventrally tilted in respect to the Horsley-Clarke basal plane. In the medio-lateral direction it attains a maximum width of 2.2 mm at its mid-portion; the dorso-ventral extent reaching its maximum of 1.5 mm at the same level.

The medial border of this complex is with *nuclei ventralis thalami medialis* except at its caudal tip, where *nuclei subpara-*

*fascicularis* and *posterioris thalami* replace the latter. The ventro and lateral borders are given by the well developed *lamina medullaris externa*, except at posterior planes where *nucleus posterioris thalami* is found capping the caudal pole of the xVB. Dorsally, in its rostral pole, the complex is related with *nuclei intralaminaris*, *nucleus lateralis thalami intermedius* and *nucleus ventralis thalami lateralis*. At a caudal level *nucleus C* makes its appearance as the dorsal border reaching the caudal pole of the complex.

The rostral limit of this complex is difficult to determine in anatomical grounds, for it gradually merges with *nuclei ventralis thalami lateralis*.

#### MATERIAL AND METHODS

Two experimental series are included in the present work. In the first group of 19 animals, unit cluster activity was recorded in order to establish the topography of projections of the body surface in the xVB. A second group, comprising 28 animals, was used to study the functional characteristics of single units in the xVB and in neighboring structures. Included in this group are four animals in which lesions in the dorsal column and dorsolateral quadrant of the spinal cord were made.

A total of 47 adult specimens of *Didelphis marsupialis aurita*, trapped in the wilds in and around Rio de Janeiro, were used.

*Preparation of the subject.* All animals used for unit cluster recording were anesthetised with an initial intraperitoneal dose of sodium pentobarbital (30 mg/kg), maintenance doses being supplied through an intravenous cannula at a rate of approximately 10 mg every 30 minutes. For single unit recording 21 animals received identical treatment, while five animals were anesthetised with Dial-urethane (100–500 mg/kg) and two animals with  $\alpha$ -chloralose (80 mg/kg).

The anesthetised animal had its fur closely clipped, and following the insertion of tracheal and venous cannulae, the animal was fixed to a Horsley-Clarke instrument specially modified to accommodate this species (Rocha-Miranda et al., '68). A bilateral craniotomy was made, and a dam of quick setting dental impression compound erected around the opening was

filled with warm Ringer or mineral oil. The dura was incised and, in all animals used for microelectrode recording, the cortical gray overlaying the thalamic region undergoing exploration was removed by sub-pial suction.

Penetrations were oriented according to the stereotaxic atlas developed in this laboratory for both the North and South American subspecies (Oswaldo-Cruz and Rocha-Miranda, '68).

In four animals the dorsal column and/or dorsolateral quadrant were sectioned at high cervical level. The pia-arachnoid membrane was dissected and the lesion accomplished by means of a razor blade fragment, the extent of the lesion being later determined by microscopic inspections of paraffin embedded, Nissl stained frontal sections.

*Recording methods.* Unit cluster responses were recorded using steel needles prepared from dental broaches, approximately 300  $\mu$  in diameter, showing a gradual taper towards the tip. The electrodes were varnish-insulated except at the tip, where a region of approximately 150–250  $\mu$  was left exposed.

The electrical activity recorded by these electrodes was led, through a Grass high impedance probe (HP-5), to a high gain differential amplifier (Grass P5). The indifferent electrode was connected to the stereotaxic frame. The electrical activity was displayed in a dual beam Tektronix 502 oscilloscope and simultaneously fed to an audio-monitor system. The characteristic change in the xVB background noise, resulting from peripheral tactile stimulation, was the main criterion to determine the active region.

Single unit activity was recorded by means of indium-filled, gold and platinum plated electrodes, of the type described by Dowben and Rose ('53). For the last experiments of the microelectrode series the electrodes were prepared by etching fine tungsten wire (Hubel, '57). After gold plating of the tip, the electrodes were varnish insulated and oven baked. In order to reduce tip impedance platinum black was deposited just before use. The ability of these electrodes to isolate unitary activity was comparable to that of the indium type.

The activity recorded by the microelectrode was fed to a solid state field-effect probe, and, after further amplification, to the display system. Slow activity was filtered at the amplifier stage. A test signal could be introduced through the preparation at any time during the experiment to evaluate the electrode impedance. In order to facilitate the isolation of single units, a differential amplitude discriminator was incorporated to the system. In addition, interval timers and EPUT meters were used to supplement information concerning latency values and discharge rate of single units isolated by the discriminator.

It is a well established fact that initially negative units are less liable to display signs of injury (Mountcastle et al., '57). The majority of units were studied while displaying negative spikes, and only a small percentage of initially positive units were taken into consideration.

*Stimulation.* Natural stimulation was applied by gentle displacement of hairs and vibrissae, by stroking restricted portions of the body surface with a camel hair brush or by touching these areas with blunt rods. In order to stimulate deep structures, pressure was applied through the cutaneous surface, and passive displacement of the joints was carried out manually. "Propathic" projections were tested by pricking with a pin or by pinching the skin with jeweler's forceps. In order to establish latency values and responsiveness to repetitive stimulation, uninsulated needles were thrust into the receptive field, and electrical stimulation was applied through a stimulus isolation unit (Grass SIU). In nine animals electrical stimulation was applied to different points of the body surface, duplicating the procedure used by Erickson et al. ('64). Uninsulated needles were inserted bilaterally in the rhinarium, region of the snout vibrissae, palm of the hand and pedimanus. Electrical stimulation of the tail was not systematically employed. Electrical stimuli consisted of 0.2–0.3 msec pulses, of amplitude of 20V or more, delivered at a repetition rate of 0.5–1.0 per second. The uninsulated needles inserted in the above mentioned body regions were connected to a selector switch interposed in the stimulation circuit. This

switch permitted simultaneous stimulation of all points or restricted the electrical pulse to a selected single point.

Auditory stimulation was accomplished by a short pulse fed to a loud speaker, resulting in a *click* sound, or by hand clapping in the immediate vicinity of the animal. Visual stimulation was achieved by means of short flashes of a glow modulator tube or by mechanical interruption of the light beam of a tungsten filament lamp, directed into the eye. Monitoring of visually evoked responses at the superior colliculus, the optic mesencephalic tract, the dorsal geniculate body, and the pretectal nucleus was used to establish functional reference points to determine the depth of the penetrations.

*Mapping procedures.* In order to study the topographic arrangements in the xVB, macroelectrodes were lowered in steps of 50–100  $\mu$ , and the entire body surface explored by natural stimulation, at each step. The location and relative intensity of the evoked response were recorded in a series of figurines, including detailed views of the head, hand and pedimanus. The criterion for establishing the active region was the magnitude of the neural background activity evoked by natural stimulation, as judged by CRO display and audio-monitoring.

Penetrations were made at 0.5 mm intervals in the mediolateral plane and at 0.75–1.0 mm in the AP plane. Penetrations at smaller intervals disrupted the cellular pattern, rendering the nuclear borders uncertain. All penetrations, in a given AP plane, were lowered to the same horizontal level whenever possible. This precaution, together with reference markings, reduced the margin of error in estimating the true recording site.

A mechanical micromanipulator having adjustments in all three planes was used to position the microelectrodes, vertical displacements were carried out at a 1  $\mu$  resolution. The electrode was slowly advanced until the activity of a single unit was isolated. Isolation of initially negative units was frequently possible only after resorting to the differential amplitude discriminator. After isolation, the unit was assigned a code number and its receptive field, as well as other functional characteristics, deter-

mined. Units lost before the gathering of complete data on its functional characteristics, or those showing signs of injury were dropped.

*Histological procedures.* After completion of the experiments, the animals were deeply anesthetized and perfused with warm saline followed by liberal amounts of Baker's buffered formalin. After an adequate hardening period, the brains were blocked, in situ, along the plane of penetration, using a special stereotaxically oriented macrotome (Rocha-Miranda et al., '65). Serial frontal sections were stained with Chroma's Cresylecht Violet.

The 19 brains of the first series were embedded in paraffin, the remaining 28 were processed by the frozen technique, receiving otherwise identical treatment.

*Treatment of data.* In a preliminary survey of the slides the penetrations were identified and the bottom of the tracks determined. A reconstruction of each penetration was made based on the enlarged images ( $10\times-15\times$ ) of selected sections. Locations of recording sites were established taking into consideration corrective factors described in greater detail in a previous publication (Oswaldo-Cruz and Rocha-Miranda, '68). The tracks of fine microelectrodes were, at times, exceedingly difficult to determine with precision. This task was greatly facilitated by diagonal illumination as suggested by Poggio and Mountcastle ('60). A better result is obtained by means of low aperture dark field technique.

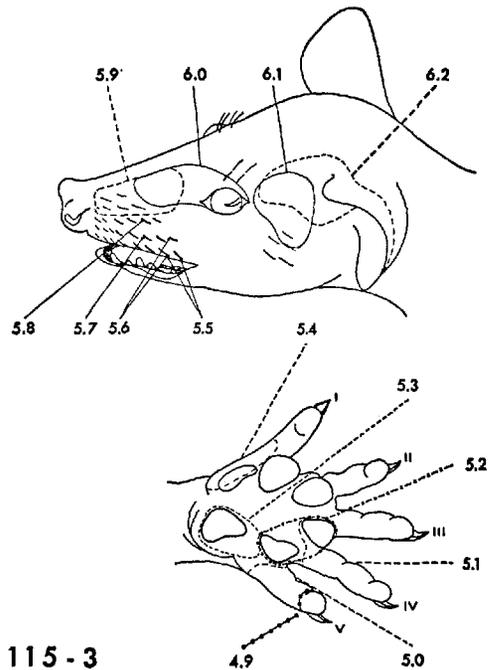
Using the above mentioned procedures it was possible to correlate recording sites with functional properties. This correlation was achieved by plotting all available data in standard planes based on the atlas for this species (Oswaldo-Cruz and Rocha-Miranda, '68).

### RESULTS

When an electrode was slowly advanced towards the ventrobasal complex, there was a characteristic change in the background noise as the recording tip approached its dorsal border. The background discharge was then modified by tactile stimulation of a restricted region of the contralateral body surface. With further advancement of the electrode, in small increments of  $100\ \mu$  or less, the peripheral region acti-

vating the neural population shifted to another site.

The results of a typical penetration are shown in figure 1. The reconstruction of the track of this penetration indicated that the electrode traversed the xVB at 2.2 mm from the midline at the plane A 4.2 mm. When the electrode tip entered the dorsal border of the xVB complex, the neural activity was seen to be driven by stimulation of a region extending from the rostral edge of the pinna to its base. In the next  $300\ \mu$ , the activating region moved towards the zygomatic and periorbital regions, reaching the upper row of vibrissae (H5.9). In the following  $400\ \mu$  the activating site consisted of the region of the mystacial vibrissae, gradually shifting from the dor-



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Fig. 1 Results of a macroelectrode descent into the ventrobasal complex at Horsley-Clarke plane A 4.2, L 2.2. As the electrode is lowered the receptive field gradually shifts along the head in the caudo-rostral direction, from the pinna to the periorbital region and finally to the vibrissae. With further advancement of the electrode (5.4) the receptive field moves abruptly to the palmar surface, at the base of the first digit. At the ventral border of the xVB the projections are restricted to the tip of digit V. The numbers indicating vertical displacement of the electrode do not correspond to the Horsley-Clarke vertical axis scale.

sal to the ventral rows, near the angle of the mouth (H5.5). Further advancement of the electrode resulted in an abrupt change of the receptive field, now found to occupy a restricted region of the palmar surface (H5.4). At still lower levels, the activating site was found near digits IV at H5.1 and V at H5.0, and finally at H4.9 it was restricted to the tip of digit V. Further displacement of the recording electrode resulted in an abrupt change of the background, as its tip left the ventrobasal complex.

Comparable results were obtained in 87 penetrations traversing the xVB. The results of 34 of these penetrations, distributed at different planes of the xVB, are depicted in figure 2.

The results obtained showed that the body surface is represented with its rostro-

caudal axis along the M-L axis of the nucleus, rostral portions of the body represented medially in the thalamus; the axial parts of the body are represented at the dorsal border of the nucleus, its ventral border being occupied mostly by projections from the extremities of the limbs. The trigeminal division is represented throughout the whole extent of the nuclear complex, the remaining body surface is represented laterally at the central portion of the nucleus.

Near the midline, somatically evoked activity was restricted to the xVB, and no response could be obtained in its medial neighbor, xVM.

A summary of the results obtained in all penetrations is depicted in figure 3, which includes a schematic thalamic "didelphunculus" represented at AP plane

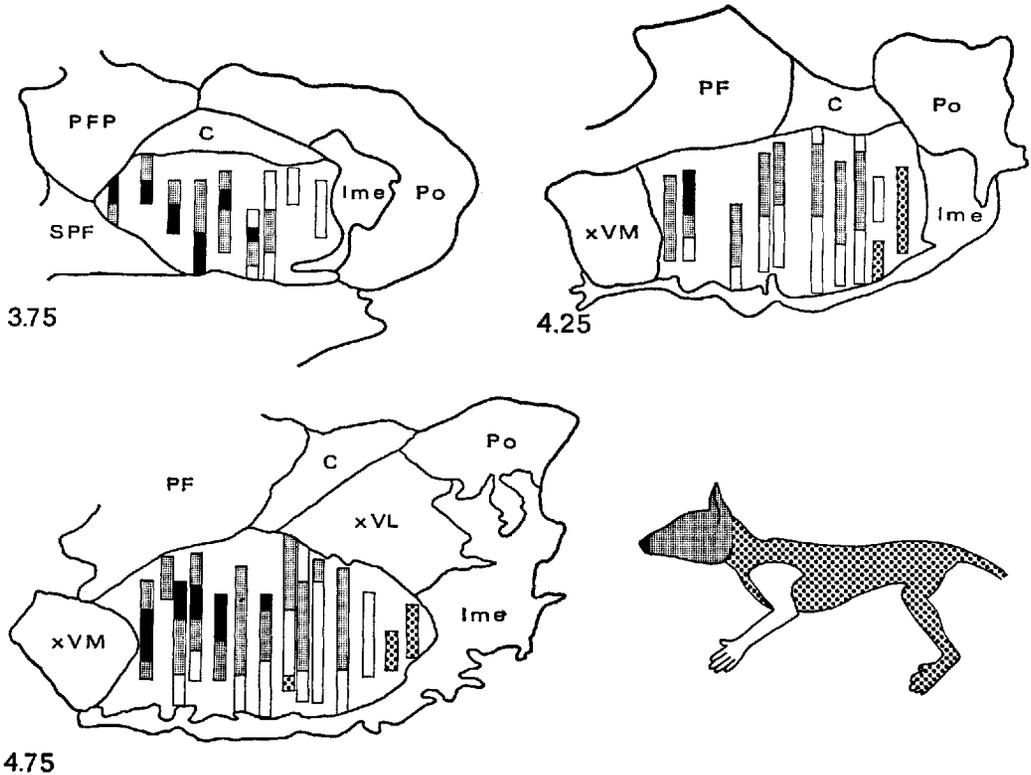


Fig. 2 The observations made in 34 of the 87 penetrations traversing the xVB are illustrated at three different AP planes. Responses evoked by natural stimulation of the rhinarium (dark areas), head (light stippling), forelimb (clear areas) and trunk, hindlimb and tail (heavy stippling), as shown in the figurine, are indicated in columns representing the various penetrations in the xVB. The surrounding structures are labeled: C, nucleus C; Ime, lamina medullaris externa; PF, nucleus parafascicularis; Po, nucleus posterioris thalami; xVL, nuclei ventralis thalami lateralis; xVM, nuclei ventralis thalami medialis.

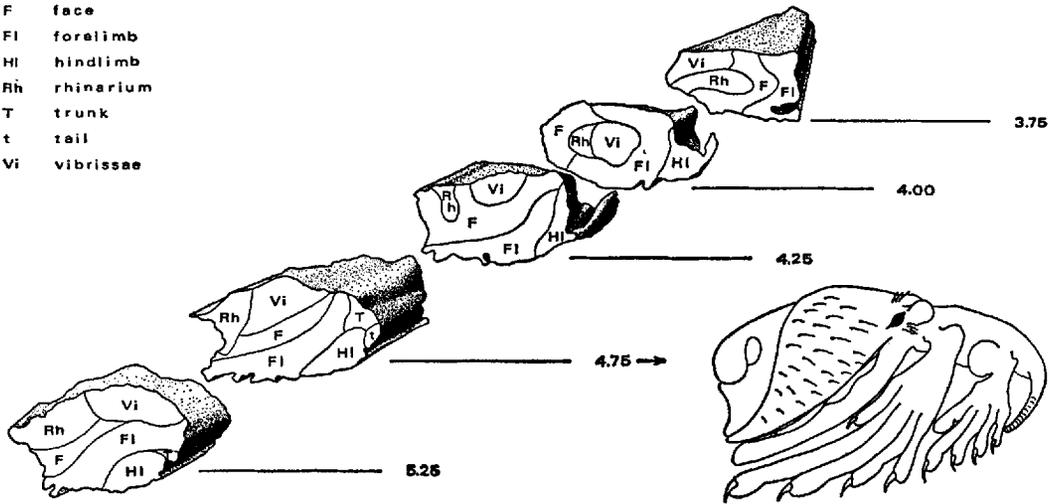


Fig. 3 Semidiagrammatic reconstruction of the ventrobasal complex between the planes A5.25 and A3.75 indicating the areas of representation of the various body regions. Result obtained from the data of 87 penetrations. A thalamic "didelphunculus" is included representing the total body surface as observed in plane A4.75.

4.75. From this reconstruction it is clear that the body surface is represented at this level as a distorted image reflecting the functional significance of its various parts, the trigeminal component occupying more than 50% of the volume of the nucleus.

Using natural stimulation the activity evoked within the xVB was restricted to the contralateral side. Occasionally some response could be obtained from ipsilateral stimulation in the rhinarium, interior of the mouth, or tip of the lips. Ipsilateral projections from the rhinarium in the opossum were reported by Pubols and Pubols ('66) and by Bombardieri and Johnson (personal communication). The latter investigators also found ipsilateral projections to xVB from the upper incisors; however, all other oral projections were contralateral.

A complete coverage of the whole volume of the ventrobasal complex was achieved through the 87 penetrations, no silent area being observed upon natural stimulation. Displacement of vibrissae, hairs or light tactile stimulation in glabrous regions was effective in evoking unit cluster responses, suggesting a high predominance of this type of somatic sensibility. In the caudal half of the body a predominance of deep sensibility was observed, except at the pedimanus. In this

region thalamic responses were readily evoked by tactile stimulation.

Similar predominance of deep receptors in the lumbar and sacral regions was observed recording first order afferent fibers at the dorsal root in this same species (Oswaldo-Cruz et al., '65).

Neural activity with different characteristics could occasionally be evoked by light tapping, by pinching or by applying pressure to large areas of the body surface, contra- and ipsilateral to the recording site. Correlation with the anatomical data indicates that this activity was recorded at *nuclei C*, *parafascicularis* (PF), *ventralis thalami lateralis* (xVL), *posterioris thalami* (Po) and occasionally at *zona incerta* (ZI).

The results obtained from recording unit cluster responses to tactile stimulation in this species are similar to those described by Pubols and Pubols ('66) in the North American variety, and comparable to those reported in various eutherian mammals by other authors.

Based on a single unit analysis and in neuroanatomical evidences Erickson et al. ('64), reported that the ventrobasal complex of the North American opossum presented a primitive form of thalamic organization in which this complex displayed properties akin to those found in the pos-

terior group (Po) of cats as described by Poggio and Mountcastle ('60).

Recording unit cluster activity in a population biased by a high predominance of tactile units would tend to obscure a minority of units displaying wide receptive fields and polysensory convergence.

In order to clarify this matter a second group of experiments was carried out to evaluate the functional properties of single units isolated within the boundaries of the ventrobasal complex.

The results of a typical microelectrode penetration are shown in figure 4. In this particular penetration 14 units were isolated and studied. Additional information regarding multiunit (MU) recording was also included in the original protocol. This penetration traversed the xVB at an AP plane similar to that of figure 1, but approximately 0.5 mm medial to the latter.

From the MU records and from the receptive fields of the isolated units a gradual shift of the active site becomes clear. At this particular penetration all units isolated displayed rapid adaptation and were driven either by hair displacement or by lightly touching the skin with a von Frey type hair stimulator.

The predominance of trigeminal innervation in this species is reflected in the high percentage of units related to the head, representing approximately 65% of the units isolated, the remaining body surface being represented to a lesser degree (see table 1). In the head region the vibrissae and other tactile hairs correspond to a high proportion of the population; the remaining receptive fields on hairy regions, rhinarium and lips displayed round or oval shape, similar in area and in distribution to those reported in other species (Darian-Smith, '64). Typical receptive fields of the head region are illustrated in figure 5. Of the 286 units studied 41% were activated by mechanical stimulation restricted to a single tactile hair; of these some responded to a specific direction of displacement. Of the tactile hairs present in the head region the snout vibrissae displayed the highest innervation density. Units related to the supraorbital, zygomatic and mandibular tactile hairs were also isolated, comprising 6% of the population studied.

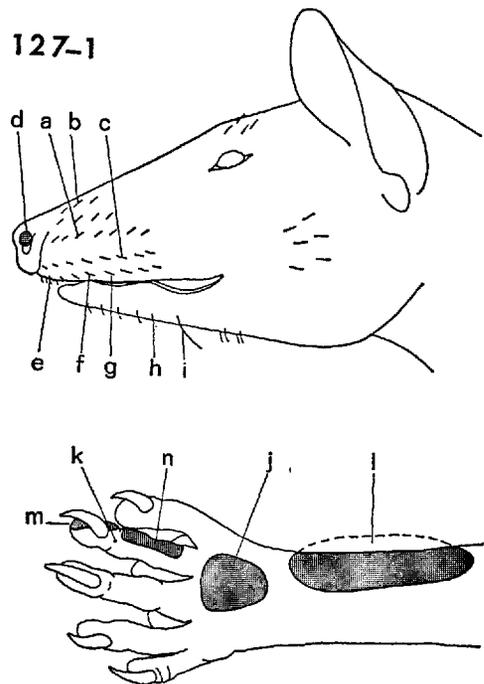


Fig. 4 Localization and extent of the receptive fields of 14 units isolated along one microelectrode penetration. On the head region eight units were activated by displacement of vibrissae or tactile hairs, all of them showing quick adaptation. Another unit displayed a restricted field located at the rhinarium. Of the five units isolated in the forelimb, three were activated by hair displacement in restricted fields on the pre-axial surface (units j, l, n), one m, by light touch on the glabrous skin of the tip of digit II. Unit k responded to deep stimulation.

Receptive fields on the pinna and on the hairy surface of the head were elongated, being oriented along the anatomical axis.

Tactile receptive fields in the glabrous skin of the rhinarium and lips measured only a few square millimeters, being at times punctiform.

The receptive fields in other body regions cover wider areas being larger at the trunk and axial portions of the limbs. Examples of such receptive fields are given in figure 6. The receptive fields of the forelimb are larger in its axial portion, decreasing in size towards the extremities, as reported in other eutherian mammals (Mountcastle et al., '57; Pubols et al., '65). The receptive fields on the hindlimb occupy a larger area except at the pedimanus

TABLE 1

|          |     | %    |          |                 |    | %    |
|----------|-----|------|----------|-----------------|----|------|
| Head     | 186 | 65   | Face     | rhinarium       | 22 | 7.7  |
|          |     |      |          | vibrissae       | 87 | 30.4 |
|          |     |      |          | face            | 77 | 26.9 |
| Forelimb | 87  | 30.4 | Forelimb | hand            | 69 | 24.1 |
|          |     |      |          | arm and forearm | 18 | 6.3  |
| Trunk    | 7   | 2.4  | Trunk    |                 | 7  | 2.4  |
| Hindlimb | 6   | 2.1  | Hindlimb |                 | 6  | 2.1  |

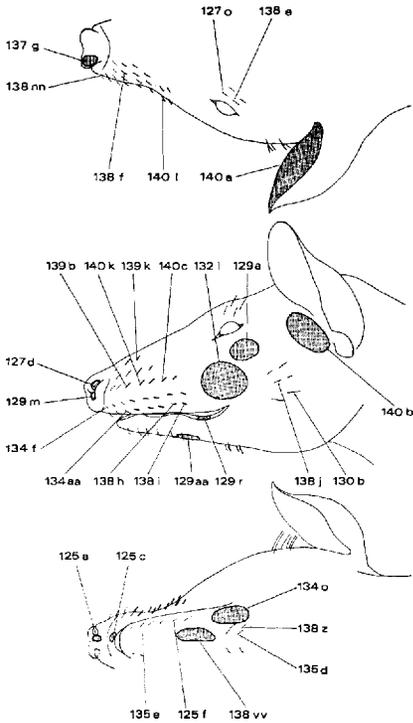


Fig. 5 Receptive fields located in the head region of 11 animals, illustrating 32 units activated by displacement of tactile hairs, by stroking the fur in restricted areas, or by light touch in glabrous skin of the pinna, rhinarium and lips.

where they are similar in size to those found in the hands. Of the 286 isolated units, approximately 80% were driven by surface stimulation, either by hair displacement or light touch (see table 2). Stimulation of deep structures activated 16% of the units but, surprisingly, no units were found to be driven by joint displacement.

Of the 286 units isolated within the anatomical boundaries of the ventrobasal complex, only three, representing approxi-

mately 1% of the population, would not fit this account. These units were driven by ipsilateral stimulation or displayed discontinuous fields.

In a group of nine animals the xVB was explored while the animals were electrically stimulated, simultaneously or singly at eight different points (see Material and Methods). Units isolated under these conditions were found to have the same characteristics as those studied under natural stimulation. Under electrical stimulation, units within the ventrobasal complex were activated only when their restricted receptive field extended over one of the stimulation sites. In this series of experiments and in the previous one, no units within the xVB were activated by auditory stimulation.

In order to rule out the depressing action of sodium pentobarbital in multisynaptic pathways, five animals were anesthetised with dial-urethane and two animals with  $\alpha$ -chloralose, which is known to enhance multisensory responses (Kruger and Albe-Fessard, '60).

"Protopathic" projections are conducted by the antero-lateral system, the fibers of this system projecting also to the xVB, as shown by anatomical and electrophysiological techniques in other species (for a review see Bowsher, '65). These responses are possibly controlled or overridden by the lemniscal projections. In order to ascertain the nature of these projections in this marsupialian ventrobasal complex a series of experiments were carried out with the dorsal columns sectioned at high cervical levels, as suggested by Pubols and Pubols ('66).

In two animals the dorsal column was sectioned bilaterally at a level rostral to the first rootlet of C<sub>1</sub>. In the animals multiunit records and ten isolated units

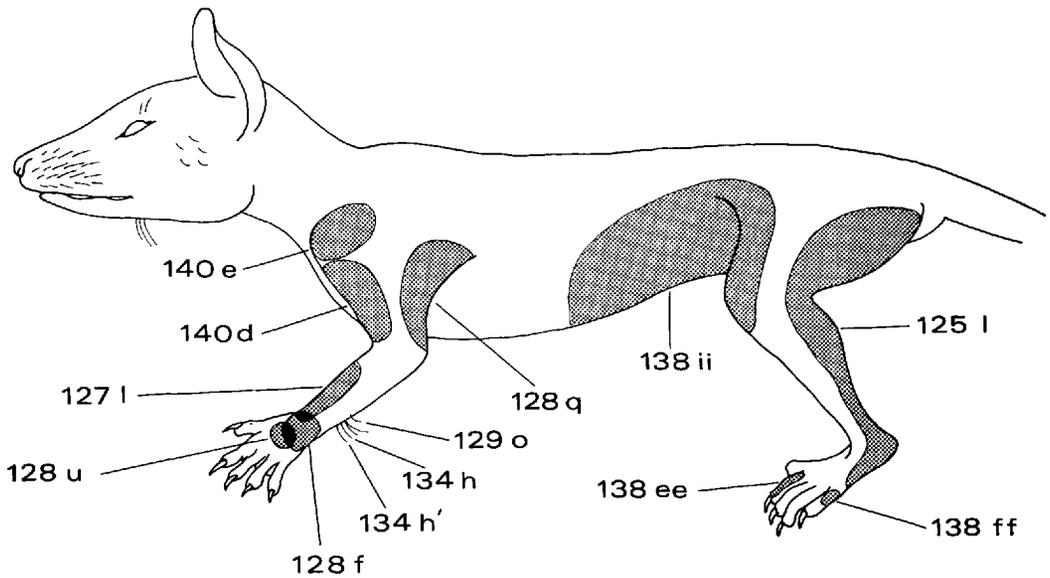


Fig. 6 Localization of the receptive fields of 12 units in the trunk and limbs in seven experiments. There is a gradual decrease in size in the receptive fields towards the extremities of the limbs. At the digits they reach a size as small as those observed in the rhinarium. This reduction in size is more evident in the hindlimb, as comparing the fields of units 125, or 138ii to those of units 138ee and 138ff.

TABLE 2

|     | Skin  |       | Deep  |       | Other |
|-----|-------|-------|-------|-------|-------|
|     | hair  | touch | joint | deep  |       |
| 163 | 72    | 0     | 46    | 3     |       |
|     | 57.4% | 25.4% | 0%    | 16.2% | 1.1%  |

displaying receptive fields in the trunk and limbs, showed properties similar to those observed in animals with intact dorsal columns, with a predominance of tactile units. Although the background activity evoked by natural and electrical stimulation of the trunk and limbs was less intense than in animals with intact dorsal columns, "lemniscal" projections still reached the ventrobasal complex. It is well known that the spino-cervico-thalamic tract displays a predominance of the lemniscal type of projection (Ha et al., '65; Morin et al., '63; Gordon and Jukes, '63; Oswaldo-Cruz and Kidd, '64; Kitai et al., '65; Horrobin, '66) and that the ventrobasal complex is the thalamic site of termination of this pathway (Morin and Catalano, '55; Morin and Thomas, '55; Busch, '61; Landgren et al., '65; Oswaldo-Cruz, '65; Andersen et al., '66).

An anatomical investigation in newborn and in adult specimen of the opossum demonstrated the presence of a lateral cervical nucleus located at the first cervical segment (unpublished results). The functional properties of the lateral cervical nucleus in this species is undergoing study in this laboratory.

In order to rule out the effects of the spino-cervico-thalamic projections two additional animals were prepared with the dorsal column sectioned at C<sub>1</sub> and both dorsolateral quadrants sectioned rostral to C<sub>2</sub>. Under these circumstances projections to the xVB were then restricted to the spino-thalamic tract which is rudimentary in the opossum (Mehler, '57).

In these animals 19 units located within the ventrobasal complex were isolated and studied. This sample showed a predominance of units responding to stimulation of deep structures of the limbs and trunk achieved by applying pressure, by tapping or occasionally only by electrical stimulation. Receptive fields observed in this sample were all contralateral and comparable in area to those isolated in intact animals.

None of the above mentioned units responded to auditory stimulation.

In this series units responding to electrical stimulation of widely dispersed areas were also observed lying in surrounding structures.

Responses to somesthetic stimulation were also recorded in nuclei surrounding the ventrobasal complex. As the electrode approached the dorsal border of the xVB neural activity could occasionally be evoked by tapping or applying pressure to the ipsi and contralateral body surface. The region promoting neural discharges was not correlated with the body region represented in the underlying ventrobasal complex. In various penetrations weak activity evoked by intense stimulation of the forelimb was abruptly substituted by typical ventrobasal activity related to the head area. This type of activity observed at the dorsal border of xVB can be seen in the schematic representation of some penetrations depicted in figure 2. These penetrations, represented by columns, interrupted at the dorsal boundary of the ventrobasal complex, show regions activated by intense forearm stimulation dorsal to the representation of the head region. This extension of atypical activity into the limits of the ventrobasal complex possibly results from errors in estimating the bottom of the tract and in applying the corrective factors involved in the establishment of the vertical scale. Another cause of error lies in the transposition of the various penetrations, performed in different experiments, to a single "standard plane."

Activity of this type was found in a shell-like region capping the dorsal and dorsolateral borders of the xVB including parts of the ventrolateral complex and nucleus C, regions described in detail in previous publications (Oswaldo-Cruz and Rocha-Miranda, '67a, '68). Similar results were also obtained by various authors under different experimental conditions (Kruger and Albe-Fessard, '60; Oswaldo-Cruz, '61; Albe-Fessard and Bowsler, '63; Albe-Fessard and Bowsler, '65).

Activity evoked by natural or electrical stimulation of the body surface was also recorded in the region of the posterior group. In the opossum a region with characteristics homologous to the posterior

group in cats has been described by various authors (Diamond and Utley, '63; Morest, '65; Oswaldo-Cruz and Rocha-Miranda, '67a, '68). Although no systematic analysis has been made in this region, the observations of a large number of isolated units yielded results identical to those described in the cat and monkey (Poggio and Mountcastle, '60; Perl and Whitlock, '61; Whitlock and Perl, '59, '61). A small percentage of the units displayed restricted peripheral fields, similar in all respects to those observed within the ventrobasal complex. Other units displayed wide receptive fields, often discontinuous and frequently bilateral. A large number of units showed sensory convergence being also driven by auditory stimuli. At the level of the caudal pole of the ventrobasal complex, units identified as belonging to *nuclei parafascicularis* and *subparafascicularis* presented extensive receptive fields under electrical stimulation. An example of one of these units isolated in the parafascicular nucleus is shown in figure 7. This cell was activated by ipsi- and contralateral electrical stimulation of the forelimbs, rhinarium and face.

#### DISCUSSION

According to the evidence presented by Herrick ('48) and the interpretations of Bishop ('59) and Diamond and Chow ('62), the thalamus evolves from an undifferentiated cell group receiving fibers from different sources, as seen in lower forms, to an aggregate of differentiated nuclei, each receiving restricted projections, as found in higher mammals. According to these arguments marsupials, being "primitive" mammals, should display an intermediate form of thalamic organization.

Anatomical investigations of the thalamus of the common opossum have shown an organization akin to that displayed in other mammals, and homologies have been drawn between the nuclear masses found in the opossum and those described in eutherian mammals (Bodian, '39; Walker, '38). These "primitive" mammals which show a low degree of neo-cortex development (Gray, '24) present a thalamo-cortical organization similar in various aspects to that of more developed mammals (Loo, '30, '31; Bodian, '42). The eutherian pat-

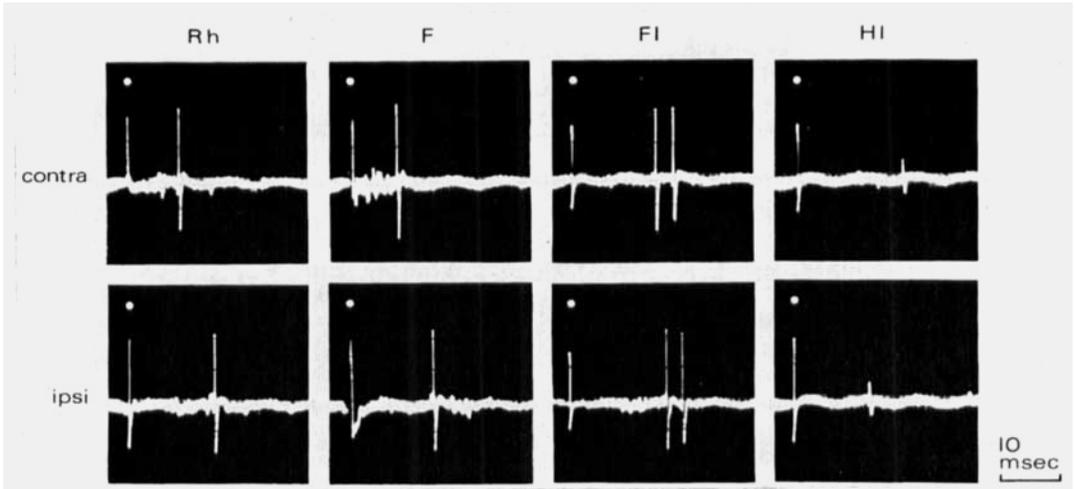


Fig. 7 Responses of unit 148p isolated in *nucleus parafascicularis*, to contralateral (contra) and ipsilateral (ipsi) electrical stimulation of the four limbs, face and rhinarium. (Rh, rhinarium; F, face; Fl, forelimb; Hl, hindlimb). This unit responds to bilateral stimulation of the rhinarium, face and forelimbs, being silent to stimulation of the hindlimb. Another unit, of smaller amplitude, responds to bilateral stimulation of the hindlimbs.

tern of thalamo-cortical organization was not found by Diamond and Utley ('63) in the North American opossum. According to these authors all thalamo-cortical connections in this subspecies are of the sustaining type. Different results may stem from a spread of the lesion to the white matter, as consequence of the peculiar vascular arrangement in this species (Wislocki and Campbell, '37).

In spite of the fact that the opossum shows a poorly developed neo-cortex, well-circumscribed sensory receiving areas have been shown both in the North and South American subspecies (Lende, '63a; Rocha-Miranda and Oswaldo-Cruz, unpublished results), as well as in the closely related marsupial, *Didelphis azarae azarae* Temmink, 1825 (Saraiva and Magalhães Castro, '69; Magalhães Castro and Saraiva, '69). On the other hand the opossum shows a marked departure in the neural organization when compared to the higher mammalian pattern; somato-sensory and motor representation overlap at cortical level. This overlap of function has been reported in other marsupials (Adey and Kerr, '54; Lende, '63a,b).

From the papers discussed above it becomes clear that marsupials conform in most respects to the neural organization

displayed by higher mammals. Nevertheless certain characteristics, such as cortical architectonics and the overlap of motor and somatosensory cortical representation, suggest a primitive pattern of organization. Regarding the functional organization of the xVB in the opossum, conflicting results are found in the literature (Erickson et al., '64; Pubols and Pubols, '66). The results obtained in the present experimental series lend support to those reported by Pubols and Pubols ('66).

*Somatotopic organization.* Our recording of unit cluster responses in the dorsal thalamus of the *D. marsupialis aurita* showed a precise somatotopic organization. The body surface displays a distorted representation reflecting, in a striking way, the functional significance of each body area, regions of greater innervation density occupying a larger thalamic volume. In this representation the head projects to the medial portion of the nucleus and the tail occupies an anterolateral position. This pattern is similar to that described in the cat (Mountcastle and Henneman, '49) and in the rat (Emmers, '65). A caudolateral representation of the tail region was reported by Welker and Johnson ('65) in the raccoon. Our results are substantially in agreement with those obtained by

Pubols and Pubols ('66) except for the fact that these authors did not find a tail representation, possibly due to the limited number of penetrations in the lateral region of the ventrobasal complex. Bombardieri and Johnson (personal communication) have also found a tail representation in the xVB of the North American opossum. Small discrepancies between our results and Pubols' on the topographical organization at various AP planes may be attributed to differences in the adopted plane of sectioning.

*Relation to cytoarchitecture.* No correlation could be established between subdivisions based in the cytoarchitecture of this complex and the projections of the various body regions as observed in other species, which present a clear lamination pattern within this nuclear mass (see Welker and Johnson, '65).

*Question of diffuse projections.* Diffuse projections resulting in convergent responses at a given site of the xVB have been reported by several authors recording with macroelectrodes (Cohen and Grundfest, '54; Gaze and Gordon, '54; and others). These results can be attributed to the spread of electrical fields in a volume conductor (see Woodbury, '62). The synchronous firing of a large neuronal population of the skin or nerve trunks will generate an extensive electrical field. With the macroelectrodes employed in the present study all responses recorded in the ventrobasal complex showed a discrete point to point representation, activity being evoked by somatic stimulation, while visual and auditory stimuli were ineffective.

*Responses from neighboring structures.* Small amplitude responses to intense ipsilateral or bilateral somatic stimulation showing gross somatotopic organization were found in nearby structures above the dorsal border of the ventrobasal complex. These responses are possibly homologous to those reported in rodents (Libouban-Letouze, '64), carnivores (Kruger and Albe-Fessard, '60) and primates (Oswaldo-Cruz, '61; Albe-Fessard and Bowsher, '65). This type of evoked activity is greatly enhanced by the use of  $\alpha$ -chloralose as anesthetic agent, being absent or of small magnitude in animals anesthetised with barbiturates.

Similar observations were made ventral to the xVB in the region occupied by *zona incerta* and the lateral portion of *Campi Forelli*. Activity evoked by somatic stimulation has also been reported in this region in different species under various anesthetic conditions (Oswaldo-Cruz et al., '56; Albe-Fessard et al., '59; Kruger and Albe-Fessard, '60; Oswaldo-Cruz, '61; Darian-Smith, '64; Denavit et al., '64; Albe-Fessard and Bowsher, '65). Units isolated within *zona incerta* display wide receptive fields, often bilateral. These regions have been found to receive spinal afferents from the lateral columns (Nauta and Kuypers, '57; Hand and Liu, '66; see also Herrick, '26).

At the posterior border of the ventrobasal complex convergent type of activity was recorded in the posterior group and in the parafascicular nuclei. A great percentage of units isolated in Po and the majority of those in *nuclei parafascicularis* and *subparafascicularis* were activated by stimuli noxious in character. Units presenting somato-auditory convergence were observed in the posterior group.

#### CONCLUSIONS

The topographic representation of the body surface in the ventrobasal complex of the opossum, revealed by gross electrode exploration, was confirmed and supplemented by single unit recording. The functional properties of units isolated in this complex are identical to those reported in eutherian mammals, i.e., they display place- and modality-specificity. A great predominance of skin representation was observed throughout the nucleus, receptive fields being comparable in size and orientation to those reported in carnivores and primates, both in the trigeminal and in the spinal territories (for a review see Mountcastle and Darian-Smith, '68). When compared to other species, the opossum shows a smaller percentage of deep projections, and although special care was taken in this respect, no units were found to be activated by joint displacement. Of all units studied, only a small percentage comprising approximately 1% of the population, displayed properties differing from the lemniscal type of activity. A large number of units with identical properties were recorded in nearby structures, and so a possibility arises that these units allocated

to the ventrobasal complex belong in reality to the xVL complex, nucleus C or other nearby structures.

Our mapping experiments using unit cluster responses and the observations of multiunit records and the receptive fields of isolated single units are in close agreement to the results obtained by Pubols and Pubols ('66) in *D. virginiana* being in total variance to the results reported by Erickson et al. ('64). The discrepancies observed could stem from four sources: (1) sampling technique; microelectrodes of different type, displaying different recording properties, could introduce a bias by rejecting part of the neuronal population, i.e., "smaller neurons"; (2) stimulation technique; sampling of a population driven by natural stimulation could place a special emphasis on those units readily activated by natural stimuli, leaving undetected those displaying wide receptive fields or those activated by more intense stimulation; (3) preferential activation of the dorsal column-medial lemniscus system caused by natural stimulation would override and obscure the effect of sensory information conducted by small diameter fiber spectrum; (4) sodium pentobarbital and other barbiturates tend to block preferentially some types of response and have also been shown to reduce and modify receptive fields of cells in the posterior group (Poggio and Mountcastle, '60).

In a series of experiments we tried to duplicate the conditions in which Erickson et al. ('64) reported a high percentage of wide fields units in the ventrobasal complex in the opossum. Single unit activity was recorded in opossums anesthetised with Dial urethane in response to electrical stimulation applied simultaneously or singly to different points of the body surface. The results obtained were identical to those observed under natural stimulation in animals anesthetised with sodium pentobarbital. Resorting to  $\alpha$ -chloralose, an anesthetic known to enhance polysensory responses, yielded the same negative results. Summarizing, using different recording and stimulating techniques and different anesthetic conditions (Nembutal, Dial-urethane and  $\alpha$ -chloralose) identical results were obtained. In order to ascertain the possible role exerted by the dorsal

column-medial lemniscus system, single unit recording of the ventrobasal complex was made in animals after sectioning of the dorsal columns. Under these conditions projections to the ventrobasal complex were restricted to the neo- and paleospinothalamic tracts. Recording in animals with lesions restricted to the dorsal columns demonstrated that although there was a marked reduction in the number of elements activated by natural stimulation of the body surface below the level of sectioning, the great majority of units recorded displayed lemniscal properties. Additional lesions in the dorsolateral quadrant, contralateral to the recording site, suggested a projection similar to the spino-cervico-thalamic tract as described in carnivores (for a review on the subject see Bowsler, '65). The results obtained in animals with the thalamic input restricted to the ipsilateral anterolateral quadrant were similar to those described by Whitlock and Perl ('59) in the cat. Under these conditions there was a considerable reduction in the number of active elements that can be justified by the poor development of the spino-thalamic tract in the opossum, as shown by Mehler ('57).

The results obtained in the various types of experiments indicated that the ventrobasal complex of the South American opossum, *D. marsupialis aurita*, displays a functional organization similar to that described in eutherian mammals. Our results confirm and supplement the topographical organization reported by Pubols and Pubols ('66) in the North American subspecies. The results of the single unit analysis are in complete variance to those presented by Erickson et al. ('64) in the opossum, as well as with those reported by the same group in the posterior dorsal thalamus of the hedgehog (Erickson et al., '67). The observed discrepancies cannot be attributed to differences in technical procedures involved in stimulation and recording methods. Single units displaying properties similar to those reported by Erickson et al. ('64) in the ventrobasal complex were observed in structures surrounding this nuclear mass.

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