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OF THE OPOSSUM INFERIOR OLIVARY NUCLEUS.

The Ohio State University, Ph.D., 1972
Anatomy

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THE CONFORMATION, CYTOLOGY AND
SYNAPTOLOGY OF THE OPOSSUM
INFERIOR OLIVARY NUCLEUS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Michael Higgins Bowman, B.S., M.S.

* * * * *

The Ohio State University
1972

Approved by

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.	ii
CURRICULUM VITAE	iii
LIST OF TABLES	vi
LIST OF ILLUSTRATIONS.	vii
INTRODUCTION	1
MATERIALS AND METHODS.	4
OBSERVATIONS	7
DISCUSSION	25
ILLUSTRATIONS.	44
LITERATURE CITED	74

LIST OF TABLES

Table		Page
1	Distribution of the two most commonly found synaptic profiles on the inferior olivary neuron	19

LIST OF ILLUSTRATIONS

Figure		Page
1	An artistic representation of the dorsal aspect of the adult opossum inferior olivary nucleus	48
1A-1L	A series of line drawings of the inferior olivary nucleus in the transverse plane	48
2	An artistic representation of the lateral aspect of the adult opossum inferior olivary nucleus	50
2A-2L	A series of line drawings of the inferior olivary nucleus in the transverse plane	50
3	Photomicrograph of a transverse section of the inferior olivary nucleus near the rostral pole (Nissl preparation).	53
4	Photomicrograph of a transverse section of the inferior olivary nucleus near the caudal pole (Nissl preparation)	53
5	A projection drawing of two olivary neurons impregnated by the Golgi technique.	53
5A	Photomicrograph of an olivary neuron (Nissl preparation)	53
5B	Photomicrograph of the olivary neuron drawn on the left in Figure 5 (Golgi preparation) . . .	53
5C	Photomicrograph of an olivary neuron (Nissl preparation)	53
5D	Photomicrograph of the olivary neuron drawn on the right in Figure 5 (Golgi preparation)	53

Figure		Page
6	Electron micrograph of an olivary neuron cell body	56
6A	Photomicrograph of an olivary neuron (semi-thin section, toluidine blue stain) . . .	56
7	Electron micrograph of subsurface cisternae . .	56
8	Electron micrograph of glial lamellae near the plasma membrane	56
9	Electron micrograph of two boutons adjacent to the plasma membrane of the cell in Figure 6.	56
10	Electron micrograph of a bouton in synaptic contact with the soma of an olivary neuron.	59
11	Electron micrograph of somatic spines and associated axospinous boutons	59
12	Electron micrograph of a somatic spine and associated axospinous bouton.	59
13	Electron micrograph of an intermediate dendrite and associated boutons	59
14	Electron micrograph of two boutons in synaptic contact with intermediate dendrites	63
15	Electron micrograph of boutons in synaptic contact with distal dendrites	63
16	Electron micrograph of a large bouton in synaptic contact with numerous distal dendrites	63
17	Electron micrograph of a complexly organized area of the neuropil.	63

Figure		Page
17A	Photomicrograph of a portion of an intermediate dendrite with an excrescence (Golgi preparation)	63
18	Electron micrograph of a complexly organized area of the neuropil.	67
19	Electron micrograph of desmosomoid contacts between contiguous dendritic spines	67
20	Electron micrograph of two fibrous profiles . . .	67
21	Electron micrograph of a fibrous profile.	67
22	Electron micrograph of a fibrous profile.	67
23	A line drawing of the inferior olivary nucleus in the transverse plane	70
24	A projection drawing of a marginal reticular neuron.	70
24A	Photomicrograph of a marginal reticular neuron (Nissl preparation).	70
24B	A photographic montage of the marginal reticular neuron drawn in Figure 24	70
25	Electron micrograph of a marginal reticular neuron cell body.	73
25A	Photomicrograph of a marginal reticular neuron (semi-thin section, toluidine blue stain)	73
26	Electron micrograph of one of the boutons adjacent to the plasma membrane of the cell in Figure 25.	73
27	Electron micrograph of one of the boutons adjacent to the plasma membrane of the cell in Figure 25.	73

INTRODUCTION

In order to establish the necessary structural substrate for the physiological events occurring within a particular region of the central nervous system, anatomical analysis of such an area should attempt to: (1) disclose the morphological features of each type of indigenous nerve cell, (2) classify the associated synaptic terminals, and (3) determine the differential focus of termination upon selected portions of each neuron by the various extrinsic and intrinsic synaptic endings. This type of investigation necessitates the concomitant use of light and electron microscopy as has been employed in the perscrutation of a few neural regions such as the cerebellum (Hámori and Szentágothai, '66; Fox, et al., '67; Chan-Palay and Palay, '70, '71a, '71b) and the dorsal lateral geniculate nucleus (Szentágothai, et al., '66; Guillery, '69; Famiglietti, '70; Wong-Riley, '72a, '72b; Famiglietti and Peters, '72).

A previous structural and ultrastructural study of the opossum red nucleus (King, et al., '71) served as a basis for analysis of the differential mode of its cortical (King, et al., '72a) and cerebellar (King, et al., '72b) inputs. The intended

sequel of these prior studies is to determine the precise pattern of termination of rubrofugal axons which distribute to the spinal cord and/or certain areas of the brainstem. However, a necessary prerequisite is the precise analysis of the intrinsic morphology of the sites of rubrofugal axon termination. Consequently, the present investigation attempts to define the structural characteristics of one suspected rubrofugal brainstem target, the inferior olivary nucleus. It is anticipated that the data presented in this communication might serve as the foundation for subsequent examinations of the precise mode of termination of olivopetal fibers arising not only from the red nucleus but from other supraolivary and infraolivary loci.

Although the *corpora olivares* (olivary bodies) were first identified and so named by Gabriel Fallopius near the middle of the sixteenth century (Willis, 1664), more than 300 years passed before the neurons that produce these protuberances on the ventrum of the caudal brainstem were described. Vencenzi (1886) was the first to characterize the inferior olivary nerve cells and to portray their unique, highly ramified dendritic arbors. Although Klliker (1893) and van Gehuchten ('05) elaborated upon the cytological features of the olivary nucleus, it was Ramon y Cajal ('09) who most thoroughly described this brainstem area. Considered in Ramon y Cajal's description are the olivary neurons with their

peculiar dendritic arbors, the trajectory of the olivofugal axons and a classification of the termination of various afferent fiber bundles. More recently, Scheibel and Scheibel ('55) have extensively investigated the inferior olivary nucleus of the cat by Golgi impregnation methods and have described additional cytological features. However, the aforementioned accounts have not been augmented by a thorough electron microscopic analysis of the neurons and neuropil of this nuclear area. The ultrastructural studies by Walberg ('63, '64) are limited in their range of sampling and are restricted to a description of certain intracellular features and a classification of the axon terminals in accordance with Gray's nomenclature ('59).

The inferior olivary complex is usually described as consisting of dorsal and medial accessory nuclei as well as a principal nucleus. Since such nuclei are structurally indistinct in the hindbrain of the opossum, great difficulty was encountered in attempting to orient oneself within the confines of this irregularly shaped nucleus. It is recognized that an exceedingly important parameter in a study such as this is the ability to thoroughly sample all areas of the nuclear domain. Therefore, the opossum inferior olivary complex has been reconstructed to scale and this model has served as a valuable guide for the localization of samples obtained for analysis with light and electron microscopic techniques.

MATERIALS AND METHODS

The three-dimensional configuration of the inferior olivary nucleus of the opossum (*Didelphis marsupialis virginiana*) was studied by the use of serially sectioned, Nissl stained (Windle, '43; Fernstrom, '58) hindbrains cut in either the frontal, horizontal or parasagittal planes. Projection drawings of frontal sections were transposed onto low density polyethylene plastic sheets (thickness 0.08 in $\pm$ 5%). The nuclear profiles were cut out and serially realigned using three regional point-coordinates to insure proper orientation. Dorsal and lateral views of the constructed model were illustrated by an artist.

The Nissl preparations also were used to study the cytology of the olivary neurons. Additional observations were made utilizing Golgi impregnations of hindbrains sectioned in each of the aforementioned planes. The Golgi impregnations included adult opossum brains processed by the method of Fox, et al. ('51) and young opossum brains (1 to 2 weeks out of the pouch) prepared by the method of Porter and Davenport ('49).

Adult opossums (2.5kg average body weight) were used to obtain tissue for electron microscopy. After anesthesia was

achieved (sodium pentobarbital, 36 mg/kg body weight) a tracheotomy was performed, a polyethylene cannula inserted into the trachea and the opossum artificially ventilated by a small animal respirator (1200 cm³/min.). Intravascular injections of sodium heparin (0.65 mg/kg body weight) and sodium nitrite (0.09 mg/kg body weight) were immediately administered via the femoral vein. Five minutes later a thorocotomy was performed, the ventral thoracic wall reflected, the parietal pericardium dissected from the heart and a 13 gauge needle inserted into the left ventricle. The cardiovascular system was thoroughly flushed by perfusion (100 mmHg., 75 cm³/min.) with 200-300 cm³ of normal saline (35°C.) followed by 1000-1200 cm³ of 4% glutaraldehyde, 2% paraformaldehyde solution (25°C.) prepared with 0.2N sodium cacodylate buffer. The final fixative solution had been previously adjusted to a pH of 7.2. A mean time of 16 minutes was required to perfuse the animal with the fixative. The brain was immediately removed and immersed in the fixative perfusate for 2 hours, after which the inferior olivary nucleus was excised with the aid of a dissecting microscope. By employing the coordinates of Oswaldo-Cruz and Rocha-Miranda ('68), the entire nucleus and subjacent ventral brainstem was removed as a rectangular polyhedron of medullary tissue. Small, transversely sectioned blocks of this tissue were washed in 0.2N sodium cacodylate buffer, post-fixed in aqueous 1% osmium tetroxide, block stained in saturated aqueous uranyl

acetate, dehydrated in ethyl alcohols and embedded in such a manner that each subsequent thin and semi-thin section would be cut in the transverse plane. The location of the inferior olivary nucleus was verified in toluidine blue stained semi-thin sections (1-1.5 μ) from each small block. In addition to the nucleus, each semi-thin section contained the subjacent pyramid and ventral border of the brainstem which aided in orientation for subsequent trimming of these blocks. Precise areas of the nucleus were thus isolated for analysis by electron microscopy. Thin sections of the blocks were cut on a Reichert ultramicrotome, picked up on copper grids, stained with lead citrate (Venable and Coggeshall, '65) and observed with an RCA EMU-3F electron microscope.

OBSERVATIONS

The caudal pole of the inferior olivary nucleus is located approximately 1mm caudal to the obex. The rostral pole is found approximately 2mm rostral to the obex and at the level of the rostral terminus of the lateral reticular nucleus. The largest dorsoventral dimension of the inferior olivary nucleus is about 0.53mm, while its greatest mediolateral breadth is about 0.75mm.

Conformation of the inferior olivary nucleus

At the rostral pole, the inferior olivary nucleus consists of a dorsomedially oriented cell cluster (Figs. 1A and 2A). Just caudal to the rostral pole, the ventral portion of the nucleus begins to enlarge in a lateral direction (Figs. 1B and 2B) and forms a horizontal lamella of cells, the ventral lamina (Figs. 1C and 2C, vl). At approximately this latter location, the dorsal portion of the nucleus likewise begins to extend laterally to form a horizontal lamella of cells, the dorsal lamina (Figs. 1D and 2D, dl). The resulting reversed C-shaped configuration is characteristic of most of the rostral one-half of the opossum inferior olivary nucleus. An additional feature of the rostral one-half of the nuclear mass is the inconsistent, incomplete division of the ventral

lamina into a lateral and medial portion (Figs. 1E and 2E, l and m).

Near the midpoint of the rostrocaudal extent of the nucleus, the lateral tip of the dorsal lamina becomes an independent dorsal cell column (Figs. 1G and 2G, dcc). Once the dorsal cell column is formed, the dorsal lamina abruptly diminishes in its mediolateral breadth until only a small spur of cells remains cohesive with the dorsomedial extension of the ventral lamina. The dorsal cell column continues caudally and becomes continuous with the ventral lamina as a dorsally directed hillock (Figs. 1H and 2H). This union exists for a short distance, whereupon the dorsal cell column once again becomes an independent entity (Figs. 1I and 2I, dcc) which extends caudally to finally rejoin the ventral lamina (Figs. 1J and 2J).

The most conspicuous characteristic of the caudal one-half of the opossum inferior olivary nucleus is the singular ventral lamina (Figs. 1K and 2K, vl). Unlike the rostral segment, the caudal ventral lamina exhibits no division into medial and lateral regions. However, two prominent protuberances typify the caudal ventral lamina. The first is a well defined ventromedial extension (Figs. 1I and 2I, vme), and the second is a conspicuous dorsomedial extension (Figs. 1I and 2I, dme). More rostrally, this latter enlargement unites the ventral lamina with the dorsal lamina. The irregularly oval ventral lamina ends near the caudal pole by dividing into two small clusters of cells (Figs. 1L and 2L).

Light microscopic structure
of the inferior olivary neuron

Scrutiny of all areas of the inferior olivary nucleus (Figs. 3 and 4) disclosed what appeared to be a single class of neurons. Typical olivary neurons are polymorphic and range in size from 10 to 30 μ in their greatest dimension. After Golgi impregnation the cyton may appear pyramidal, oval, round or flask-shaped and often exhibits somatic spines (Fig. 5, S.S.). Cresyl violet stained preparations disclose a diffuse and lightly basophilic Nissl substance (Figs. 5A and 5C) in the modest perinuclear cytoplasm, and a round, eccentrically located nucleus with a lightly staining nucleolus (Figs. 5A and 5C).

Golgi impregnations of all areas of the nucleus (Figs. 3 and 4) revealed neurons with dendritic arbors which arise circumferentially from the cell body. In each of these areas the extent of the dendritic branching can be included within a range, the extremes of which are represented by the two examples in Figure 5. Although most dendrites remained within a closely circumscribed area (up to 200 μ in diameter) near the perikaryon (Figs. 5, 5B and 5D), examples were found in which one of the terminal dendrites extended beyond the estimated perimeter of the nucleus and into the surrounding reticular formation.

Olivary neurons characteristically give rise to several dendrites, although examples of monodendritic cells were

observed (i.e. flask-shaped cell). These latter cells were found near the margin of the nucleus and their dendritic tree ramified centripetally into the nucleus. More commonly, olivary neurons have dendrites that arise from 2, 3 or more loci (Fig. 5). The proximal dendritic trunks measure from 5 to 8 μ in their greatest transverse dimension and occasionally possess dendritic thorns. The large dendrites taper rapidly into intermediate dendrites that range in size from 2 to 4 μ and thence into undulative distal dendrites that measure from 0.5 to 1.5 μ . The spines found on intermediate and distal dendrites were often independent (Fig. 5, SP.), although sometimes the spines were arranged in clusters (Fig. 5, solid block arrows). Another type of intermediate and distal dendritic excrescence is indicated by the open block arrows in Fig. 5. This outgrowth is characterized by a short stalk (approximately 2 μ) capped with a crown of thorns and will be subsequently referred to as a spine crowned appendage.

Ultrastructure of the
inferior olivary neuron and
associated axosomatic boutons

Electron microscopic examination of samples taken from all areas of the inferior olivary nucleus confirmed the prior observation of a ubiquitous single class of neurons. Profiles of typical olivary neurons are readily identified in electron micrographs (Fig. 6) and semi-thin plastic sections (Fig. 6A). A representative

neuronal profile is seen in Figure 6 and can be compared with those neurons previously described in Nissl and Golgi preparations on the basis of its size (10-30 μ), the diffuse nature of the rough endoplasmic reticulum and polysomes (Nissl substance) and the presence of somatic spines (Figs. 6, 11 and 12, S.S.). Furthermore, the large, smoothly contoured and oval nucleus is characteristically eccentric and contains a single nucleolus.

Somatic spines of these cells may appear as simple excrescences (Fig. 11, S.S.), pedunculated bulbs (Fig. 11, S.S.₁) or ramified appendages (Fig. 12, S.S.). The neuronal cytoplasm contains typical organelles as well as a remarkable number of subsurface cisternae (Fig. 6, solid block arrows). Two such cisternae are enlarged in Figure 7 and demonstrate that whereas some may appear as a single saccule (Fig. 7, solid block arrow), others may be multilaminar (Fig. 7, open block arrow) and continuous with a strand of rough endoplasmic reticulum (Fig. 7, rer).

One of the most striking features of olivary neuron profiles is their nearly complete envelopment by glial processes. Although some of these cytoplasmic projections emanate from perineuronal oligodendrocytes (Fig. 6, ol), they are most commonly derived from astrocytes. A portion of the plasmalemma demarcated by the rectangle in Figure 6 is enlarged in Figure 8 and clearly shows the laminar nature of the astroglial investment.

Another characteristic attribute of the olivary neuron is the relative paucity of synaptic terminals in contact with the cell surface and somatic spines. In Figure 6 only 3 synaptic profiles abut against the plasma membrane of the cell body and visible dendrites (Fig. 6, white block arrows). Two of these profiles (Fig. 6, white block arrows, b_1 , b_2) are enlarged in Figure 9 and demonstrate that one (Fig. 9, white block arrow, b_1) contains a homogeneous population of round and oval vesicles and exhibits a synaptic junction. In contrast, the second bouton (Fig. 9, white block arrow, b_2) is populated by pleomorphic vesicles that range in shape from round to flat. A synaptic junction is not seen in this plane of section. However, in Figure 10 a bouton (Fig. 10, white block arrow, b_2) containing pleomorphic vesicles is shown to have an extended site of synaptic contact with the surface of an olivary neuron. Likewise, synaptic terminals in contact with somatic spines can be categorized as boutons containing a homogeneous population of round and oval vesicles (Fig. 11, white block arrow, b_1), and boutons containing pleomorphic vesicles (Figs. 11 and 12, white block arrows, b_2).

Thus, two distinct types of synaptic endings terminate upon the soma and somatic spines. The first type of terminal (Fig. 9, white block arrow, b_1) ranges in size from 0.5 to 2.0 μ and contains a homogeneous population of round and oval vesicles measuring 350

to 450 Å. In almost every terminal profile of this type, one encounters an occasional round or oval vesicle that measures as small as 250 Å or as large as 600 Å. In addition, dense core vesicles are seen in such endings. The second type of ending (Fig. 9, white block arrow, b_2) also measures from 0.5 to 2 μ but contains a population of pleomorphic vesicles. The round and oval vesicles of these terminals correspond to the sizes given for similarly shaped vesicles in the previous type of ending. Furthermore, numerous flattened vesicles which measure approximately 250 by 500 Å are found in this second type of terminal. Dense core vesicles also have been encountered in this type of synaptic ending.

Ultrastructural
characterization of the proximal,
intermediate and distal dendrites
and associated synaptic profiles

Electron microscopic analysis of samples taken from all nuclear areas reveals a remarkable similarity. Therefore, the description presented in this section is consistent for all portions of the nucleus unless otherwise indicated.

The two distinct types of synaptic terminals that contact the soma and somatic spines also end upon proximal, intermediate and distal dendrites and their thorns. Consequently, these terminals will be subsequently referred to as types 1 and 2 which correspond, respectively, to those boutons containing exclusively round and oval vesicles and those boutons populated with pleomorphic vesicles.

The proximal dendrites of olivary neurons measure from 5 to 8μ in their greatest cross-sectional dimension and only occasionally are seen to emit a simple spiny process. The proximal dendrites, like the soma, are enveloped by glial processes and only rarely are contacted by a synaptic ending. These synaptic terminals can be classified as either type 1 or type 2.

Dendritic profiles which range in size from 2 to 4μ in their greatest transverse dimension are considered to be intermediate dendrites (Fig. 13). In contrast with proximal dendrites, the intermediate dendrites display abundant simple spines (Fig. 13, SP.) and a notable decrease in glial investment (Fig. 13). Concomitantly, a much greater surface area of the shaft and thorns is contacted by synaptic profiles (Fig. 13, b) of types 1 and 2.

A third type of terminal contacts the shaft of intermediate sized dendrites located in the caudal portion of the ventral lamina. These infrequently encountered endings (Fig. 14, white block arrows, b_3) range in size from 0.5 to 1.5μ and contain flattened vesicles that measure approximately 250 by 500 Å. In addition, occasional vesicles are found that measure up to 600 Å in length. Although each terminal did exhibit a few round vesicular profiles (Fig. 14, small, white-on-black triangles), they correspond to the approximate diameter of the flattened vesicles. An occasional

dense core vesicle is present in this type of synaptic ending.

Profiles of distal dendrites measuring from 0.5 to 1.5 μ are plentiful in the neuropil (Fig. 15, d and d'). Fortunate longitudinal sections of distal dendrites reveal single thorny excrescences as well as multiple clusters of spines. Distal dendritic shafts and their spines are copiously contacted by synaptic profiles of both type 1 (Fig. 15, white block arrows, b_1) and type 2 (Fig. 15, white block arrow, b_2). In addition, a distal dendrite may serve as the receptor for converging dissimilar terminals (Fig. 15, white block arrows, b_1 and b_2); and two independent distal dendrites may be simultaneously contacted by a single terminal (Fig. 15, white block arrow, b_2). A further distinction can be made between these two types of endings when they are found to synaptically contact distal dendrites. Under these circumstances, type 1 endings display a marked asymmetry of their synaptic junctions (Fig. 15, white block arrows, b_1); whereas type 2 endings exhibit a prominent symmetry (Fig. 15, white block arrow, b_2). Furthermore, in a random sample of an equal number (575) of type 1 and 2 endings, asymmetrical synaptic junctions appear to be more extensive (up to 1 μ) than the symmetrical junctions (up to 0.5 μ). Moreover, type 1 endings frequently contact distal dendrites at 2 adjacent areas (Fig. 15, b_1 in contact with d'); while similarly located synapses by type 2 endings were always singular. Finally, dense projections were

routinely encountered at the synaptic junctions of both types of profiles, but subjunctional bodies were only seen in association with type 1 endings (Fig. 15, b_1 in contact with d').

A fourth type of synaptic ending (Fig. 16, white block arrow, b_4) contacts numerous distal dendrites in the same plane of section (Fig. 16, arrows). Terminals of this type have been infrequently observed in all areas of the olivary nucleus. These large (up to 5μ) irregularly shaped profiles contain round and oval vesicles which measure from 350 to $450 \text{ \AA}$ and an occasional dense core or flattened vesicle (Fig. 16, open triangles). The synaptic junctional specializations of one large terminal may show both a symmetrical and an asymmetrical conformation (Fig. 16, arrows).

In samples taken from the dorsal lamina and dorsal cell column, apparent synaptic contacts (Figs. 20 and 21, curved arrows) have been noted between small dendritic profiles (Figs. 20 and 21, d) and vesicle-containing, fibrous processes (Figs. 20 and 21, fp). The profiles of these fibrous processes range in size from 0.5 to 2.5μ and contain pleomorphic vesicles (Figs. 20 and 21, open block arrows). The round and oval vesicles of these endings measure from 400 to $500 \text{ \AA}$, and the flattened vesicles measure approximately 250 by $500 \text{ \AA}$. Dense core vesicles also are present in the fibrous processes. Although the density of the

fibrous matrix varies between profiles (compare the fibrous processes (fp) in Figure 20 with the example (fp) in Figure 22), such processes consistently contain a perimeter of microtubules subjacent to the plasma membrane (Figs. 20, 21 and 22, small arrows) as well as a variable number of mitochondria. The orderly arranged fibrillary matrix of the fibrous process (Fig. 20, fp and fp', and Figs. 21 and 22, fp) can be compared and contrasted with the contents of a typical dendrite (Figs. 21 and 22, d), an unmyelinated axon (Fig. 22, a) and an astroglial process (Fig. 22, solid block arrow, f).

Besides the previously described morphological substrate for chemical interactions, a few examples of desmosomal-like contacts have been observed between dendrites in all areas of the neuropil. Moreover, spines from neighboring dendrites have been frequently observed to approach one another and form distinct desmosomal-like contacts. Two examples of these spinospinous contacts are shown in Figure 19 (circles).

Distribution of synaptic profiles on the cell body and dendrites of the inferior olivary neuron

An attempt was made to quantitate the relative numbers of type 1 and 2 synaptic profiles found within the inferior olivary nucleus. Because of their sparsity the large efflorescent terminals (Fig. 16, white block arrow, b₄), the flattened vesicle

terminals (Fig. 14, white block arrow, b_3) and the fibrous process profiles (Figs. 20, 21 and 22) were excluded from the following calculations.

Electron micrographs were randomly chosen from groups representing each morphological area of the nucleus. The number of type 1 and 2 endings in synaptic contact with either the soma and somatic spines or the shaft and spines of various sized dendrites was compiled for all areas of the nucleus and the percentages calculated. The results were similar for all areas of the nucleus. Therefore, the following values are felt to represent the approximate proportional pattern of termination of each of the two types of endings (types 1 and 2) upon selected portions of the olivary neuron.

A total of 1886 terminals of both types were observed on the soma and somatic spines (9.7%), the proximal dendrites and their thorns (6%) and the intermediate and distal dendrites and their simple excrescences (84.3%). A comparison of the particular types of terminals in synaptic contact with each of these areas appears in Table 1. One conspicuous feature is the preponderance of type 2 synaptic terminals on the soma (62%), somatic spines (86%), proximal dendrites (58%) and proximal dendritic spines (83%). In striking contrast, the number of type 1 and 2 profiles on intermediate and distal dendrites and their spines is approximately equal.

TABLE 1
DISTRIBUTION OF THE TWO MOST COMMONLY FOUND SYNAPTIC
PROFILES ON THE INFERIOR OLIVARY NEURON

Location	Non-spinous Postsynaptic Surface				Spinous Postsynaptic Surface			
	Type 1		Type 2		Type 1		Type 2	
	#	%	#	%	#	%	#	%
Soma	50	38	83	62	7	14	43	86
Proximal dendrite	30	42	42	58	7	17	34	83
Intermediate and distal dendrite	587	51	562	49	208	47	233	53

Complex synaptic island in the neuropil of the inferior olivary nucleus

The relatively simplistic morphology of the presynaptic and postsynaptic interrelations thus far demonstrated can be sharply contrasted with areas of the olivary neuropil which display a much greater complexity in their structural organization. Such regions characteristically contain an intermediate to distal

sized dendrite (Fig. 17, d) which emits an irregularly shaped excrescence (Fig. 17, S.C.A.) measuring approximately 2μ in length and displaying spiny processes at its apex (Fig. 17, SP.). It is believed that these profiles correspond to sections of the unusual dendritic outgrowths observed in Golgi preparations and termed spine crowned appendages (Fig. 17A, S.C.A.).

In addition to the irregular shape of the spine crowned appendage (Fig. 17, S.C.A.), it consistently contains an amorphous fluffy matrix, an occasional mitochondrion and an inconsistent number of irregularly arranged tubuli (Fig. 17, small arrows within S.C.A.). These tubules measure $550 \text{ \AA}$ in diameter and often contain a moderately dense material (Fig. 17, small arrows). An additional feature of the tubules, seen in profiles identified as spine crowned appendages, is the frequent dilation of one of their ends (Fig. 18, small arrows). These morphological characteristics permit the identification of some of the neighboring profiles as spine crowned appendages (Fig. 17, S.C.A.₁ and Fig. 18, S.C.A.), although they cannot be seen to arise from their parental dendrites.

In the immediate vicinity of the spine crowned appendages (Fig. 17, S.C.A. and S.C.A.₁) one can identify a number of small profiles (Fig. 17, asterisks) that cannot be categorized with certainty but may correspond to sections taken through spines, small terminal dendrites or through portions of spine crowned

appendages. In Figure 17 a spine (Fig. 17, SP') and a spine crowned appendage (Fig. 17, S.C.A.) are emitted from an intermediate dendrite (Fig. 17, d). This spine (Fig. 17, SP') becomes tangentially applied to the surface of another spine (Fig. 17, SP'') which has arisen from an unknown source. Desmosomal-like contacts are often observed between profiles of adjacent spine crowned appendages (Fig. 17, circles), spine crowned appendages and neighboring spines or small dendrites (Fig. 18, circle), and between profiles that may each represent spines and/or small dendrites (Fig. 18, double circle).

Such clusters of profiles are consistently surrounded by, and in synaptic contact with, numerous boutons (Fig. 17). The synaptic profiles can be classified as either type 1 (Fig. 17, b_1) or type 2 (Fig. 17, b_2). Additional boutons (Fig. 17, b) do not exhibit enough synaptic vesicles to permit classification. Therefore, they could represent profiles of either of the two most commonly found endings or alternatively could represent another type(s) of bouton. This complexly organized area is partially isolated from the surrounding neuropil by a mantle of astroglial lamellae (Fig. 17, solid block arrows).

It is believed that fortuitous sections of intricately arranged areas of the neuropil, similar to that seen in Figure 17, result in profiles of synaptic islands as seen in Figure 18. The

core of this complex synaptic area contains spine crowned appendages (Fig. 18, S.C.A.) and other intimately associated profiles of spines and/or small dendrites (Fig. 18, asterisks). This cluster of profiles is partially encapsulated and synaptically contacted by numerous boutons (Fig. 18, b_1 and b_2). Once again, these endings can be classified as type 1 (Fig. 18, b_1) or type 2 (Fig. 18, b_2). A consistent feature of these synaptic islands is the presence of a peripherally placed dendrite (Fig. 18, d) which measures from 1 to 2.5μ . A dendritic profile such as this (Fig. 18, d) may correspond to a section taken through a dendrite similar to the one observed in Figure 17 (d) that gave rise to a spine crowned appendage. Inconsistently, the profile of a spine crowned appendage (Fig. 18, S.C.A.) or another unidentifiable process (Fig. 18, asterisk) may appear near the periphery of the synaptic island. Finally, to a greater extent than seen in Figure 17, this organized synaptic complex is isolated from the adjacent neuropil by an investment of astroglial lamellae (Fig. 18, solid block arrows).

Light microscopic structure
of a marginal reticular neuron

Large nerve cells are seen in juxtaposition to the inferior olivary nucleus but never within its adjudged limits. Although the marginal neurons are few in number, they are present around the outer perimeter of the nucleus (Fig. 23) at all levels. Moreover,

such neurons are never found along the inner border of the dorsal and ventral laminae where these lamellae form the reversed C-shaped configuration (Fig. 23).

These peripherally located neurons are typically oval or elongate in shape and measure approximately 20 by 50 μ in their greatest dimension (Figs. 24, 24A and 24B). In Nissl preparations, the marginal cells have abundant cytoplasm studded with numerous well defined aggregates of Nissl substance (Fig. 24A, N.B.). In Golgi impregnations, the large dendrites can be seen to emanate in a somewhat polar fashion from the soma (Figs. 24 and 24B). The long (up to 650 $\text{\AA}$) tapered dendrites are relatively unbranched and emit comparatively few thorns (Figs. 24 and 24B). A vast majority of the marginal cells studied in Golgi preparations send a dendrite into the confines of the inferior olivary nucleus (Figs. 24 and 24A, d.).

Ultrastructure of a marginal reticular neuron

Profiles of marginal cells seen in electron micrographs (Fig. 25) and in semi-thin plastic sections (Fig. 25A) are readily identified on the basis of their size (approximately 20 by 50 μ) and numerous aggregates of rough endoplasmic reticulum and polysomes (Nissl substance) present throughout the abundant cytoplasm (Figs. 25 and 25A, N.B.). The usual complement of cytoplasmic organelles

are present. The round to oval nucleus is enclosed by a slightly irregular nuclear membrane (Fig. 25) and contains a single nucleolus (Fig. 25A). Numerous axosomatic synaptic endings contact the surface of the cell body (Fig. 25, arrows). The axosomatic boutons contacting the neuronal profile in Figure 25 are partially outlined in ink to facilitate their identification. Due to the apparent absence of somatic spines, axospinous endings have not been observed in electron micrographs of these cells.

Two classes of synaptic endings have been observed to terminate on the soma of the marginal neuron. An example of each type of terminal is identified in Figure 25 by large, solid block arrows numbered 1 and 2. These endings are enlarged, respectively, in Figures 26 and 27. The first type of terminal (Fig. 26, solid block arrow, 1) contains only round and oval vesicles; whereas the second kind of ending (Fig. 27, solid block arrow, 2) is populated by pleomorphic vesicles.

The proximal dendrite passing beyond the upper border of the electron micrograph (Fig. 25) has been followed for a distance of 22μ . The surface of this dendritic profile is almost entirely covered with synaptic endings (Fig. 25, arrows in proximal dendrite) which, like the terminals on the soma, can be classified by their vesicle populations into the two aforementioned categories.

DISCUSSION

Conformation of the inferior olivary nucleus

A review of the conformation of the inferior olivary nucleus from a phylogenetic perspective was presented by Ariëns Kappers, et al. ('36). However, the single most comprehensive study of the architecture of this nuclear complex was accomplished by Kooy ('17), who modeled and described this nucleus in specimens from nearly every order of each vertebrate class. More recently, attempts to analyze the shape of the nucleus from a comparative standpoint have been made utilizing birds (Vogt-Nilsen, '54) and various mammals (Muskens, '34; Tateishi, '59; Korneliussen and Jensen, '64; Sako, '64; Verhaart, '70). Still other studies deal with the morphology of the olivary nucleus in a single mammalian form such as the opossum (Kankeleit, '13; Voris and Hoerr, '32; Oswaldo-Cruz and Rocha-Miranda, '68), bat (Schober, '59), anteater (Verhaart, '63), rat (Zeman and Innes, '63), dog (Yoda, '40), cat (Brodal, '40; Taber, '61), artic fox (Fiedoruk, '68), horse (Milart, '65), goat (Szteyn and Welento, '66), sheep (Pattison and Holman, '43), domestic pig (Lewandowski, '51; Breazile, '67), wild boar (Welento and Szteyn, '67), cow (Goller, '65), gorilla (Noback, '59) and human (Braak, '70).

The conformation of the inferior olivary nucleus of the opossum as described in this study generally coincides with the accounts given by Kankeleit ('13), Kooy ('17), and Voris and Hoerr ('32). However, these prior reports have employed a terminology for the inferior olive which is based upon analogy between certain areas of the undivided nuclear mass of the opossum and the structurally distinct principal and accessory subnuclei of higher Eutherian forms. A more recent report has described the inferior olivary nucleus of the opossum (Oswaldo-Cruz and Rocha-Miranda, '68) as consisting of 3 columns of cells: a dorsal inferior olivary nucleus, a principal inferior olivary nucleus, and a ventral inferior olivary nucleus. Furthermore, the latter authors consider the ventral nucleus to be divided by the principal nucleus into 2 parts, a dorsomedial portion and a ventrolateral portion.

Some of the confusion in the descriptive nomenclature appears to be the result of attempting to describe the nucleus without the benefit of a three-dimensional reconstruction. Further difficulty is encountered when one attempts to make analogies between various regions in the opossum inferior olive and the distinct olivary subnuclei described for other mammals, including Man. The inferior olivary nucleus of the opossum is represented by an irregularly shaped, singular mass of cells that is difficult to

equate with the morphologically multipartite nuclei that comprise the inferior olivary complex of Eutherian animals. However, it is reported that spino-olivary fibers in the cat (Brodal, et al., '50) and monkey (Mehler, et al., '60) distribute mainly to the dorsal accessory olive and the caudal portion of the medial accessory olive (see Mizuno, '66 for an extensive review of this subject). Sinze Hazlett, et al. ('72) reported that spino-olivary fibers in the opossum terminate in portions of those areas presently described as the dorsal lamina, dorsal cell column and ventral lamina (Figs. 1 and 2, dl, dcc, and vl), at least a portion of this continuous cellular mass may be comparable to parts of the dorsal and medial accessory nuclei of Eutherian mammals. Because comprehensive hodological studies of other afferent and efferent connections of the opossum olivary nucleus are lacking, further comparisons cannot be made between the opossum and other forms. Consequently, a descriptive nomenclature was adopted in this investigation in order that erroneous analogical implications could be avoided and the nuclear features accurately described.

The inferior olivary neuron:
light and electron microscopy

Cell body.--The present investigation has included light microscopic observations of both Nissl and Golgi preparations as well as electron microscopic examination of samples taken from all areas of the nuclear mass. The combined results reveal a

ubiquitous single type of neuron within the inferior olivary nucleus of the opossum. In all of the aforementioned analyses, the olivary neurons appear as polymorphic cells that measure from 10 to 30 μ . Correlative light microscopy of Nissl preparations and electron microscopy disclose that the perikaryon contains a sparse accumulation of diffusely scattered Nissl substance and a large, polarized nucleus.

Prior studies employing either Golgi impregnations (Ramon y Cajal, '09; Scheibel and Scheibel, '55) or electron microscopy (Walberg, '63, '64) have not described somatic spines on olivary neurons. In contrast, perikaryal excrescences were a consistent feature of Golgi impregnated olivary neurons and of their ultrastructural profiles. Moreover, such outgrowths assume a number of morphological contours which may appear as simple thorns, pedunculated bulbs or ramified appendages.

Two additional ultrastructural features of olivary nerve cell bodies, as well as their proximal processes, are the paucity of synaptic terminals in contact with these surfaces and the nearly total investment of these portions of the neuron by glial processes. Some of these enveloping glial processes emanate from perineuronal oligodendrocytes, a cell not found in the fine structural studies of the feline inferior olive (Walberg, '63, '64). More commonly, however, the encompassing glial prolongations were of astrocytic

origin and isolate the neuronal perikaryon from neighboring profiles in the neuropil.

A further fine structural peculiarity of olivary neurons is the remarkable abundance of subsurface cisternae. These structures, first described in detail by Rosenbluth ('62), are most often present as a single cisterna but occasionally appear multilaminar and continuous with strands of rough endoplasmic reticulum. Although the function of these organelles remains undetermined, it has been suggested that they might represent sites of metabolic exchange (Rosenbluth, '62; Herndon, '63). Siegesmund ('68) has found that a majority of these structures lie adjacent to glial processes abutting against the neuronal plasmalemma, a fact well substantiated in the present investigation.

Dendrites.--The inferior olivary neurons of the opossum characteristically display a radial projection of dendrites from numerous somatic loci. However, occasional flask-shaped cellular profiles are observed near the perimeter of the nucleus and were noted to emit a single dendritic shaft which ramifies conically into the nuclear mass.

The dendritic arbors of opossum olivary neurons are typically confined to a closely circumscribed area. Although isolated dendrites have been seen to pass for long distances (400 μ), the average diameter of the dendritic arbor is about 280 μ . This figure can be compared with similar data presented

by the Scheibels ('55) for the cat (211 μ), the macaque (152 μ) and the human (115 μ), and clearly supports the generalization that "...as the mammalian scale is ascended there is apparently progressive decrease in the size of the dendrite arbors [of olivary neurons]..." (Scheibel and Scheibel, '55).

A single type of polymorphous olivary neuron with a variable pattern of dendritic branching has likewise been reported by Vencenzi (1886), Kölliker (1893), van Gehuchten ('05) and Ramon y Cajal ('09). However, Scheibel and Scheibel ('55) state that 2, and possibly more, types of neurons exist in the cat's inferior olivary nucleus. The latter authors differentiation of the neuron types is primarily based on the pattern of dendritic arborization. Thus, the Scheibels ('55) describe one neuron with a highly ramified and spatially restricted dendritic arbor which is primarily found in the principal olive, and another neuron with straight and unramified dendrites which is only found in the accessory olivary nuclei. Neither of the two cells illustrated by these authors are identical to the omnipresent olivary neuron described in the present study. The branched, compact arbor of the first type of neuron (Scheibel and Scheibel, '55) is quite unlike that of the opossum olivary neuron because of the former's extreme ramification and remarkable tendency to fold back towards, and enmesh, the cell from which it arose. Likewise, the unbranched, diverse arbor of

the second cell type (Scheibel and Scheibel, '55) is different than the dendritic arbor of the neurons described in the current investigation because of the latter's tendency to display moderate branching and an occasional recurrent dendrite. Hence, the neurons observed in Golgi preparations of the opossum inferior olivary nucleus appear to be somewhat intermediate between the two divergent neurons illustrated by the Scheibels ('55). The nerve cell in the cat, which may be similar to the one described in the opossum, may be represented by the "transitional cell" mentioned by Scheibel, et al. ('56), but not illustrated. Moreover, this latter cell type (Scheibel, et al., '56) has been found throughout the cat's olivary nuclear complex and in this respect is quite comparable to the neuron typical of the opossum's inferior olivary nucleus.

Although a variation in dendritic branching is evident, several morphological features are common to the dendrites of each of the neurons examined in the current investigation: (1) the dendrites exhibit a moderate waviness; (2) dendrites of all sizes have been observed to emit single thorns, which is in agreement with the description of olivary neuron dendrites given by Ramon y Cajal ('09); (3) dendrites that are classified as intermediate or distal display clusters of spines and unique excrescences termed spine crowned appendages. Thus, based on these

similarities as well as the consistency of somatic features, it is concluded that the inferior olivary nucleus of the opossum contains a single type of neuron which displays a diverse and moderately ramified and snarled dendritic arbor. This corroborates the observation by Ramon y Cajal ('09) that in several Eutherian mammals the degree of branching and entanglement of olivary neuron dendrites diminishes as one descends the phylogenetic scale.

Electron microscopic analysis of the neuropil has confirmed the presence of single spines on proximal, intermediate and distal dendrites. Furthermore, longitudinal sections of intermediate and distal dendrites have revealed clusters of simple thorns arising from one side of the dendritic profile. Llinás and Hillman ('69) have proposed that the very high longitudinal resistance in the neck of a spine would allow for a minimal amount of current to be introduced into the parent dendrite. Thus, a single afferent fiber discharging upon a spine would result in a weak postsynaptic potential. This phenomenon has been considered by Chan-Palay and Palay ('70) who propose two possible compensatory mechanisms. First, a large number of similar synaptic terminals could converge upon numerous dendritic thorns. Thus, the summation of many, if not most, of the postsynaptic potentials resulting from the discharge of these terminals could produce a strong depolarization in the postsynaptic neuron. Because the number of isolated spines on a

single olivary dendrite is low in comparison to the number found on the dendrites of other neurons, such as the Purkinje cell (Fox and Bernard, '57; Fox, et al., '67), this mechanism is probably not utilized in the inferior olivary nucleus.

The second mechanism suggested by Chan-Palay and Palay ('70) involves the clustering of dendritic spines which increases the probability that a greater number of thorns will be contacted by each axon ending. Therefore, the discharge of a much smaller number of afferent fibers in this restricted locus would result in a local summation of each of the small synaptic potentials causing an increase in the effectiveness of the incoming volley. This mechanism might play a role in the activity of olivary neurons because frequent examples of spine clusters have been observed on the intermediate and distal dendrites of these cells.

Additional structures found on the intermediate and distal dendrites of each neuron consist of short protrusions which are capped with a crown of thorns. Consequently, these excrescences have been termed spine crowned appendages. Profiles have been identified in electron micrographs that correspond to these appendages in size, shape and origin. The irregularly shaped ultrastructural profiles of spine crowned appendages contain an amorphous, fluffy matrix and numerous tubuli. The tubuli of spine crowned appendages may collectively represent a type of spine apparatus (Gray, '59) similar to that found in the thorns of

numerous other neurons. However, the spine apparatus is usually described as saccular rather than tubular and somewhat symmetrically arranged rather than irregularly disposed. Furthermore, based on cross-sectional diameter, these tubuli ($550 \text{ \AA}$) do not correspond to the typical microtubules ($200\text{-}260 \text{ \AA}$) found in the cytoplasm of the cell body and dendrites. Thus, these tubular elements appear to be uniquely characteristic of the dendritic excrescences herein termed spine crowned appendages.

Complex synaptic islands

The fine structural analysis of complex synaptic islands in the neuropil has shown that 2 or more spine crowned appendages closely approximate one another and, moreover, are often intimately related by desmosomal-like contacts. Consistently adjoining these unique structures are a number of profiles which may correspond to small terminal dendrites or spines. Desmosomoid contacts also have been observed between these latter elements, as well as between these profiles and adjacent spine crowned appendages. The sum total of these components forms a large, spatially organized core of potential postsynaptic substratum around which a number of synaptic profiles and a single dendrite are located. The encompassing endings, which synaptically contact the elements of the dendritic core, can be categorized as boutons containing only round and oval vesicles (type 1) and boutons containing pleomorphic

vesicles (type 2). The peripheral dendritic profile is believed to emit one or more spine crowned appendage which contributes to the dendritic core. These synaptic islands are encapsulated by a mantle of astroglial processes and, thus, may be termed glomeruli (Szentágothai, '70).

Olivary glomeruli similar to those described here have been previously mentioned in a brief communication by St. Němeček and Wolff ('69). Although the arrangement of the glomerulus is the same in the cat (St. Němeček and Wolff, '69), including the presence of desmosomoid attachments between the core elements, no differentiation between presynaptic terminals or postsynaptic profiles was reported.

Glomeruli characterized by a central bouton(s) and a peripheral mantle of dendrites and other axon terminals have been identified in various locations of the central nervous system including the cerebellum (Gray, '61; Kirsche, et al., '65), the dorsal lateral geniculate nucleus (Szentágothai, '63; Peters and Palay, '66; Famiglietti and Peters, '72), the medial geniculate nucleus (Majorossy and Réthelyi, '68), the lateral vestibular nucleus (Sotelo and Palay, '70), the main sensory trigeminal nucleus (Gobel and Dubner, '69) and various thalamic nuclei (Pappas, et al., '66; Jones and Powell, '69). An inversion of this organization occurs in the glomeruli of the inferior olive, the thalamic pulvinar and lateral posterior nuclear group

(Majorossy, et al., '65), and the ventrobasal complex of the thalamus (Ralston and Herman, '69). Although the intrinsic organization is similar, the centrum of these latter glomeruli contains only the profile of a single, large dendritic protuberance or one to two small dendrites; whereas the core of the olivary glomerulus is made up of numerous small profiles. Thus, the multiple elements of the central core, as well as the apparent lack of intrinsic axoaxonic synapses (a feature shared only by the cerebellar glomerulus), make the olivary glomerulus a unique example within the range of central nervous system glomeruli thus far encountered.

A discussion of the potential physiological mechanisms that might be active in the olivary glomerulus cannot be undertaken with the information presently available. Further Golgi impregnation studies are necessary to unravel the origin(s) of the elements in the central core. Concomitantly, selective ultrastructural degeneration studies must be employed to identify the individual axons impinging upon this core of dendritic substrate. Finally, much more electrophysiological data must be obtained concerning the unitary responses of olivary neurons during activation from the various sites of origin of olivopetal fibers.

In addition to the desmosomal-like attachments found between elements of the central core of the olivary glomerulus,

other isolated examples of nonsynaptic interneuronal specializations have been observed. Thus, a few dendodendritic contacts, similar to those described in the feline olive (Walberg, '63), have been seen in the present investigation. Moreover, distinct desmosomoid contacts have been frequently observed between contiguous spines. At these contact points synaptic vesicles are lacking in both of the spines, the intercellular cleft is maintained, and a generally equal accumulation of dense, amorphous material is found on both opposing membranes. Consequently, such spinospinous contacts can be classified as *puncta adhaerentia* (Farquhar and Palade, '63). Although such contacts have been reported between numerous other neuronal elements (Peters, et al., '70), it is believed that this is the first report of such contacts between spines.

Distribution of synaptic terminals

A quantitative determination of the proportional distribution of synaptic terminals upon the olivary neuron indicates that relatively few synaptic profiles end on either the soma and somatic spines or upon proximal dendrites and their thorns. Moreover, of those terminals so disposed, the vast majority are populated with pleomorphic vesicles (type 2) while only a few endings contain exclusively round and oval vesicles (type 1). In striking contrast, the intermediate and distal dendrites are extensively contacted by an approximately equal percentage of type 1 and type 2 terminals.

Likewise, both isolated and clustered spines, as well as olivary glomeruli, are impinged upon by a relatively even proportion of both types of endings. Walberg ('66) has described boutons with 2 different vesicle populations in a restricted region of the feline inferior olivary nucleus. One of these contains round vesicles and corresponds to the profiles identified as type 1 in the present investigation. The other type of bouton (Walberg, '66) contains either a mixture of round and flattened vesicles or exclusively flattened vesicles. The mixed vesicle boutons correspond to the endings referred to as type 2 in the current study. The boutons containing only flattened vesicles are equatable with similarly described endings in the present study (see below). However, Walberg ('66) did not determine the specific sites of termination on the olivary neuron, nor did he refer to the symmetry of the synaptic junctional specialization. In the current study, type 1 terminals that end upon distal dendritic surfaces have been observed to display an apparent asymmetry at the site of synaptic contact. In contradistinction, type 2 terminals exhibit a marked symmetry at similar sites of synaptic interaction. The correlation of vesicle population and degree of symmetry at the synaptic junction was first made by Colonnier ('68) in the cerebral cortex and subsequently by numerous other investigators in other neuronal regions (Ralston, '68; Ralston and Herman, '69; Guillery, '69;

Clark, '69; Rinvik and Grofová, '70; Westrum and Black, '71; Wong-Riley, '72a).

In addition to the 2 aforementioned types of synaptic terminals, the distal dendrites of olivary neurons are contacted by large, efflorescent terminals containing predominately round and oval vesicles. Although infrequently encountered, these terminals have been seen in all areas of the nucleus. Moreover, in a single section such terminals are observed to contact numerous dendritic profiles. Profiles of this type of ending may correspond to sections of rosette terminals similar to those described in Golgi preparations of the inferior olivary nucleus of the cat (Scheibel and Scheibel, '55).

An additional type of terminal has been identified in samples taken from the caudal portion of the ventral lamina. These rare, small endings contain flattened vesicles and have only been observed to synaptically contact the shaft of intermediately sized dendrites. Thus, the terminals conform in vesicle morphology (flattened vesicles) and site of synaptic termination (shaft) with previously described inhibitory axon endings (Uchizono, '65, '67; Mugnaini, '69; Sotelo, '69). If the endings seen here are likewise inhibitory, then their discharge could "functionally amputate" (Llinás and Hillman, '69) the distal regions of the dendrite upon

which they terminate, and thus mitigate the effects of this distal subsystem upon the functional state of the neuron.

An unusual profile has been observed in the dorsal lamina and dorsal cell column. These fibrous processes frequently contain pleomorphic vesicles and appear to establish synaptic contact with the shaft of a distal dendrite. The consistent identification of these profiles in a very restricted area of the nucleus of numerous animals, along with the rather orderly arrangement of the intrinsic fibrils and peripheral microtubules, combine strongly to suggest that such profiles do not represent filamentous degenerating axons. However, these profiles do not conform with the typically identifiable ultrastructural profiles of any other element of the central nervous system. Instead, the appearance of vesicles polarized toward an apparent synaptic junction on an identifiable dendrite tends to suggest that these profiles represent an heretofore unidentified type of presynaptic axon or dendrite.

Therefore, at least 2, and possibly more, types of synaptic profiles end upon individual neurons located throughout the olivary nucleus. The 2 morphologically divergent terminals (types 1 and 2) may represent 2 general categories of incoming axons. In support of this, Scheibel, et al. ('56) have reported that the bushy arborescent afferent fibers of Ramon y Cajal ('09) are present throughout the nucleus and, moreover, intricately overlap in their terminal

arborizations. Obviously, two morphologically different axon terminals could either arise from different nuclei or could emanate from the same source. Strong support for the former possibility comes from the recent electrophysiological studies of Sedgwick and Williams ('67), Crill and Kennedy ('67), Pumain and Buisseret ('69) and Crill ('70). These investigators have demonstrated that axons from different sources converge upon single olivary units. However, some of the synaptic endings observed in the present study could represent the terminals of recurrent axon collaterals. Although collateralization has not been seen in the Golgi preparations employed in this study, Vencenzi (1886) and Ramon y Cajal ('09) illustrated recurrent collaterals which arose from olivary axons as they course within their nucleus of origin. The final interpretation of the mode of areal overlapping and possible functional integration must await a careful ultrastructural mapping of olivopetal fibers following the selective ablation of each of the various sources of such axons.

The marginal reticular neuron:
light and electron microscopy

A distinctly different type of neuron has been observed in very close approximation to the inferior olivary nucleus. In sharp contrast with the typical olivary neuron, these large (approximately 20 x 50 μ), elongate nerve cells contain numerous Nissl bodies and

are synaptically contacted by a large number of boutons which end upon their soma and proximal dendrites. Their large, rectilinear dendrites have few branches, emit few spines, and pass for great distances into the nearby reticular formation. In addition, many of these neurons seen in Golgi preparations were noted to send a dendrite into the confines of the inferior olivary nucleus.

Similar neurons were observed by Ramon y Cajal ('09) who believed they represented reticular formation nerve cells displaced or trapped by the developing inferior olivary nucleus. Ramon y Cajal ('09) likewise states that these marginal neurons sometimes send a dendrite into the olivary nucleus. Similarly, Valverde ('61) illustrates reticular formation neurons which transmit a dendrite into the olivary nucleus but makes no mention of such a dendritic termination.

The neuronal features depicted in Golgi impregnations classify these nerve cells as a part of the isodendritic core of the brainstem (Ramon-Moliner and Nauta, '66). In addition, their ultrastructural features correspond to the polydendritic neurons observed in the gigantocellular region of the medullary reticular formation (Bowsher and Westman, '70), the caudal bulbar nucleus medulla oblongatae centralis and the mesencephalic nucleus commissurae posterioris (Bowsher and Westman, '71). Thus, the marginal neurons and the polydendritic reticular neurons correspond

in size, Nissl pattern and in the abundant presence of axosomatic and proximal axodendritic synaptic endings. Moreover, the synaptic endings on both neurons consist of boutons with round and oval vesicles and boutons containing elongate (pleomorphic) vesicles.

Some of the profiles encountered in electron micrographs taken in this investigation may represent sections of a dendrite that has passed from the marginal reticular neuron into the olivary nucleus. The possibility that this factor would alter the proportional pattern of synaptic distribution is mitigated by the low frequency of encounter expected in a random sampling of all areas of the nucleus.

The function of the infrequently encountered marginal reticular neuron is unknown. However, their location near the perimeter of the nucleus at all levels and the intraolivary projection of some of their dendrites, suggest that these nerve cells may receive synaptic terminals similar to those ending upon olivary neurons in the same restricted locality.

ILLUSTRATIONS

Abbreviations

a	- unmyelinated axon
b	- bouton (unclassified as to vesicle type)
b ₁	- bouton with round and oval vesicles
b ₂	- bouton with pleomorphic vesicles
b ₃	- bouton with flattened vesicles
b ₄	- large, efflorescent bouton
CAUD.	- caudal
d, d', d''	- dendrites
dcc	- dorsal cell column
dl	- dorsal lamina
dme	- dorsomedial extension
DOR.	- dorsal
f	- astroglial fibrils
fp, fp'	- fibrous processes
i.o.n.	- inferior olivary nucleus
l	- lateral part of ventral lamina
LAT.	- lateral
m	- medial part of ventral lamina
MED.	- medial
N.B.	- Nissl body
ol	- oligodendrocyte

pt	- pyramidal tract
rer	- rough endoplasmic reticulum
ROST.	- rostral
S.C.A.,) S.C.A. ₁)	- spine crowned appendages
SP., SP', SP''	- spines
S.S., S.S. ₁	- somatic spines
VENT.	- ventral
vl	- ventral lamina
vm	- ventromedial extension

PLATE I

- Fig. 1 An artistic representation of the dorsal aspect of the adult opossum inferior olivary nucleus. The drawing was made from a scaled reconstruction of this nuclear mass. The arrows A-L refer to the line drawings of selected transverse sections from the respective levels indicated. Particular portions of the nucleus are labelled as follows: dl, dorsal lamina; dcc, dorsal cell column; dme, dorsomedial extension; vl, ventral lamina.
- Figs. 1A-1L A series of line drawings of the inferior olivary nucleus in the transverse plane. Each drawing represents the approximate shape of the nucleus at the respective point labelled in Figure 1. Particular portions of the nucleus are labelled as follows: l, lateral part of the ventral lamina; m, medial part of the ventral lamina; dl, dorsal lamina; dcc, dorsal cell column; dme, dorsomedial extension; vme, ventromedial extension; vl, ventral lamina. (X 24)

PLATE II

- Fig. 2 An artistic representation of the lateral aspect of the adult opossum inferior olivary nucleus. The drawing was made from a scaled reconstruction of this nuclear mass. The arrows A-L refer to the line drawings of selected transverse sections from the respective levels indicated. Particular portions of the nucleus are labelled as follows: dl, dorsal lamina; dcc, dorsal cell column; dme, dorsomedial extension; vl, ventral lamina.
- Figs. 2A-2L A series of line drawings of the inferior olivary nucleus in the transverse plane. Each drawing represents the approximate shape of the nucleus at the respective point labelled in Figure 2. Particular portions of the nucleus are labelled as follows: l, lateral part of ventral lamina; m, medial part of ventral lamina; dl, dorsal lamina; dcc, dorsal cell column; dme, dorsomedial extension; vme, ventromedial extension; vl, ventral lamina. (X 24)

PLATE III

Fig. 3

A low power view of a transverse section through the inferior olivary nucleus at a point 1mm caudal to the rostral pole. The configuration corresponds most closely to section E in Figures 1 and 2. Identifiable nuclear features include the dorsal lamina (dl) and the lateral (l) and medial (m) parts of the ventral lamina. The pyramidal tract (pt) is labelled for orientation. Nissl preparation. (X 4 objective)

Fig. 4

A low power view of a transverse section through the inferior olivary nucleus at a point 0.6mm rostral to the caudal pole. The configuration corresponds most closely to section I in Figures 1 and 2. Identifiable nuclear features include the dorsal cell column (dcc), the dorsomedial extension (dme), the ventral lamina (vl) and the ventromedial extension (vme). The pyramidal tract (pt) is labelled for orientation. Nissl preparation. (X 4 objective)

- Fig. 5 A projection drawing of two olivary neurons impregnated by the Golgi technique. The sections were cut in the transverse plane. The dendrites display individual spines (SP.), clusters of spines (solid block arrows) and spine crowned appendages (open block arrows). The cell on the right has numerous somatic spines (S.S.).
- Fig. 5A An olivary neuron comparable in size and shape with the nerve cell drawn on the viewer's left in Figure 5. Nissl preparation. (X 54 oil immersion objective)
- Fig. 5B A photograph of the actual neuron drawn on the viewer's left in Figure 5. Golgi preparation. (X 12.5 objective)
- Fig. 5C An olivary neuron comparable in size and shape with the nerve cell drawn on the viewer's right in Figure 5. Nissl preparation. (X 54 oil immersion objective)
- Fig. 5D A photograph of the actual neuron drawn on the viewer's right in Figure 5. Golgi preparation. (X 12.5 objective)

20μ

0.25mm

3

5

0.25mm

4

PLATE IV

Fig. 6

A profile of an olivary neuron cell body.

Intracellular features include non-aggregated Nissl substance, subsurface cisternae (solid block arrows) and somatic spines (S.S.). Two oligodendrocytes (ol) abut against a portion of the neuronal plasma membrane. Most of the cell surface is covered with astroglial lamellae, a portion of which is demarcated by a black and white rectangle and enlarged in Figure 8.

Possible axosomatic boutons are indicated by the large block arrows (white outlined in black) and 2 of these (b_1 and b_2) are enlarged in Figure 9. (electron micrograph)

Fig. 6A

An olivary neuron. One micron plastic section stained with toluidine blue. (X 54 oil immersion objective)

- Fig. 7 A high magnification of typical subsurface cisternae found within an olivary neuron. One consists of a single cisterna (solid block arrow) and the other (open block arrow) is bilaminar and continuous with a strand of rough endoplasmic reticulum (rer). Small triangles delineate the plasma membrane of the cell. (electron micrograph)
- Fig. 8 A higher magnification of the plasma membrane and adjacent glial lamellae circumscribed by the black and white rectangle in Figure 6. (electron micrograph)
- Fig. 9 A higher magnification of two boutons adjacent to the cell membrane in Figure 6. The one (b_1) contains only round and oval vesicles and is in synaptic contact with the somata. The other (b_2) contains pleomorphic vesicles but is not in synaptic contact. Some of the flattened vesicles in the latter bouton (b_2) are identified by open triangles. A single subsurface cisterna is indicated by the solid block arrow. (electron micrograph)

PLATE V

Fig. 10 Illustrated is a bouton (b_2) with pleomorphic vesicles that is in synaptic contact with an olivary neuron. Some of the flattened vesicles in this profile are indicated by open triangles. The extent of a single subsurface cisterna is demonstrated by solid block arrows. (electron micrograph)

Fig. 11 Two adjacent somatic spines are labelled on an olivary neuron. Whereas the simple spine (S.S.) is free of axospinous contacts in this section, the other dilated spine (S.S.₁) is contacted by one bouton that contains only round and oval vesicles (b_1) and by a second bouton that contains pleomorphic vesicles (b_2). Flattened vesicles are indicated in the latter bouton (b_2) by open triangles. (electron micrograph)

Fig. 12 A branched somatic spine (S.S.) is shown emanating from an olivary neuron and invaginating a bouton that contains pleomorphic vesicles (b_2). Some of the flattened vesicles of this synaptic profile are disclosed by open triangles. Both axospinous and axosomatic contacts are accomplished by this terminal. The limits of a subsurface cisterna are displayed by solid block arrows. (electron micrograph)

Fig. 13 The profile of an intermediate dendrite sectioned in a transverse plane. Three spines (SP.) extend from the shaft, two of which are in synaptic contact with boutons (b). Numerous other boutons (b) are closely applied to approximately two-thirds of the dendritic surface. Although not distinguishable at this magnification, these terminals either contain only round and oval vesicles or pleomorphic vesicles. The remaining plasma membrane is covered by astroglial lamellae. (electron micrograph)

PLATE VI

Fig. 14 Illustrated are two boutons (b_3) that contain mostly flattened vesicles. Each terminal is in synaptic contact with the shaft of an intermediately sized dendrite. The electron dense synaptic junction appears to be symmetrical. A few round profiles can be identified in each terminal (white-on-black triangles) but probably correspond to cross-cut flattened vesicles. (electron micrograph)

Fig. 15 Profiles of the 2 typical types of terminals in the neuropil. Two boutons (b_1) contain only round vesicles and are in synaptic contact with the shaft of two distal dendrites (d, d'). Notice the asymmetry of the electron dense synaptic junctions. Another bouton (b_2) contains pleomorphic vesicles and is in synaptic contact with the shaft of two distal dendrites (d', d''). The synaptic junction on both dendrites (d', d'') is visibly symmetrical. Some of the flattened vesicles in the latter bouton (b_2) are indicated by open triangles. (electron micrograph)

Fig. 16 A profile of a large efflorescent bouton (b_4) in synaptic contact with numerous distal dendrites (solid arrows). The upper two arrows point to synaptic junctions that appear to be asymmetrical; whereas an adjacent arrow (just above micron bar) points to a contact that appears to be symmetrical. The vesicles in this ending are predominantly round, but an occasional flattened vesicle can be identified (open triangles). (electron micrograph)

Fig. 17 A profile of an intermediately sized dendrite (d) from which emanates a spine crowned appendage (S.C.A.). In this section, the S.C.A. exhibits two apical spines (SP.) and several tubular structures (arrows) cut longitudinally or transversely. A second spine crowned appendage (S.C.A.₁) also contains tubular structures (arrows) and closely adheres to the first S.C.A. by desmosomoid contacts (circles). Several small unidentified profiles (asterisks) as well as numerous boutons (b , b_1 , b_2) are present. Two of these boutons (b_1) contain only round and oval vesicles while another (b_2) has a pleomorphic

population of vesicles. In this latter bouton (b₂) some of the flattened vesicles are indicated by open triangles. Astroglial processes (solid block arrows) incompletely surround this complex synaptic region. (electron micrograph)

Fig. 17A

A spine crowned appendage (S.C.A.) arising from an intermediately sized dendrite. Golgi preparation. (X 54 oil immersion objective)

PLATE VII

Fig. 18 A complex synaptic area is circumscribed by astrocyte processes (solid block arrows). The core of this area consists of spine crowned appendages (S.C.A.) containing tubular structures (arrows), and small, irregularly shaped dendritic or spinous profiles (asterisks). Such profiles often exhibit desmosomal-like contacts (circles). These structures are surrounded by a number of boutons, a distal dendrite (d), a spine crowned appendage (S.C.A.) and a small, irregular spinous or dendritic profile (asterisk). Some of the boutons (b_1) contain only round and oval vesicles while others (b_2) contain pleomorphic vesicles. Some of the flattened vesicles are identified by open triangles in these latter boutons (b_2).
(electron micrograph)

Fig. 19 Illustrated are profiles of 2 distal dendrites (d' , d'') cut in the transverse plane as well as 1 dendritic profile (d) sectioned somewhat obliquely. Spines (SP.) from two of the dendrites (d , d') form a spinospinous contact (circle).

Another spine (SP') from the obliquely sectioned dendrite (d) forms a second spinospinous contact (circle) with a spine (SP.) of unknown origin. Other adjacent structures include boutons (b₁) containing only round vesicles and others (b₂) with pleomorphic vesicles. A few of the flattened vesicles found within this latter bouton (b₂) are identified by open triangles. (electron micrograph)

Fig. 20

The profile of a fibrous process (fp) containing vesicles (open block arrow). An apparent synaptic contact (curved arrow) is made with a distal dendrite (d). Another, somewhat smaller process (fp') lacks vesicles and shows no synaptic relationship with any other profile. A number of peripherally placed microtubules (small arrows) can be seen in both processes. (electron micrograph)

Fig. 21 The profile of a somewhat obliquely sectioned fibrous process (fp). A number of vesicles are located within the process (open block arrow) and help create an apparent synaptic contact (curved arrow) between the process and a distal dendrite (d). Two microtubules (small arrows) are seen in the fibrous process (fp). (electron micrograph)

Fig. 22 The profile of a transversely sectioned fibrous process (fp). Although no vesicles are present in this section, a number of peripheral microtubules can be identified (small arrows). The structural features of this process can be compared with an adjacent dendrite (d), several unmyelinated axons (a) and the fibrils of an astroglial process (solid block arrow - labelled f). (electron micrograph)

PLATE VIII

- Fig. 23 A line drawing of the inferior olivary nucleus (i.o.n.) viewed in the transverse plane. The configuration most closely corresponds to point E in Figures 1 and 2. Four marginal reticular cells are schematically shown around the perimeter of the nucleus, and each sends a dendrite (d) into the nuclear confines. (nucleus X 7, cells X 30)
- Fig. 24 A projection drawing of a transversely sectioned Golgi preparation illustrating a marginal reticular neuron. This cell is located along the dorsal surface of the inferior olivary nucleus (i.o.n.) and crosses its boundary (broken line) with a ventrally directed dendrite (d). Relatively few spines can be seen on the dendrites of this neuron. One of the dendrites is marked by an open block arrow for orientation with the cell in Figure 24B.
- Fig. 24A A marginal reticular neuron comparable with the cell drawn in Figure 24. Numerous, well defined Nissl bodies (N.B.) are visible. Nissl preparation. (X 54 oil immersion objective)

Fig. 24B

A photographic montage of the actual neuron drawn in Figure 24. The dendrite (d) is directed ventrally into the inferior olivary nucleus. For orientation, the open block arrow indicates the similarly marked dendrite in Figure 24. The solid block arrow points to a dendrite that appears to branch from the dendrite (d) passing into the olivary nucleus. However, this dendrite (solid block arrow) actually continues, out of the plane of focus, in a dorsolateral direction beyond the intraolivary dendrite (d). Golgi preparation. (X 12.5 objective)

PLATE IX

- Fig. 25 The profile of a marginal reticular neuron cell body containing well defined Nissl bodies (N.B.). Numerous synaptic terminals, partially outlined in ink and labelled with arrows, are located on both the soma and proximal dendrite. The solid block arrows labelled 1 and 2 indicate synaptic endings that are shown at a higher magnification in Figures 26 and 27, respectively. (electron micrograph)
- Fig. 25A A marginal reticular neuron with well defined Nissl bodies (N.B.). One micron plastic section stained with toluidine blue. (X 54 oil immersion objective)
- Fig. 26 A higher magnification of the synaptic ending distinguished in Figure 25 by a solid block arrow labelled 1. This terminal is typical of those boutons on the soma and proximal dendrites that contain only round and oval vesicles. (electron micrograph)

Fig. 27 A higher magnification of the synaptic ending distinguished in Figure 25 by a solid block arrow labelled 2. This terminal is typical of those boutons on the soma and proximal dendrites that contain pleomorphic vesicles. Some of the flattened vesicles are identified by open triangles. (electron micrograph)

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