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THE SYNAPTIC ORGANIZATION OF THE MOTOR
NUCLEUS OF THE TRIGEMINAL NERVE IN THE
OPOSSUM.

THE OHIO STATE UNIVERSITY, PH.D., 1979

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THE SYNAPTIC ORGANIZATION OF THE MOTOR NUCLEUS OF THE
TRIGEMINAL NERVE IN THE OPOSSUM

DISSERTATION

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

By

James E. Hamos, B.A.

* * * * *

The Ohio State University

1979

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LIST OF ABBREVIATIONS

C	-	C-bouton
Coe	-	Locus coeruleus
DC	-	DC terminal
GP	-	Griseum pontis
IP	-	Interpeduncular nucleus
MGN	-	Medial geniculate nucleus
OcM	-	Oculomotor nucleus
P	-	P-bouton
PAG	-	Periaqueductal gray
RaD	-	Nucleus raphé dorsalis
S	-	S-bouton
Tr me t	-	Tract of the mesencephalic nucleus of the trigeminal nerve
Tr Mo	-	Motor nucleus of the trigeminal nerve
TrN	-	Trigeminal nerve
Tro	-	Trochlear nucleus
Tr PS	-	Principal sensory nucleus of the trigeminal nerve
Tr Sp	-	Spinal nucleus of the trigeminal nerve
Tz	-	Trapezoid nucleus
Vst S	-	Superior vestibular nucleus
IV	-	Fourth ventricle

INTRODUCTION

Recent ultrastructural descriptions of several cranial nerve motor nuclei have detailed the morphology of the neurons and the type of synaptic terminals present in these brainstem pools (oculomotor - Tredici et al., '76; trochlear - Bak and Choi, '74; abducens - Spencer and Sterling, '77; Descombes et al., '79; facial - Falls and King, '76a, b; hypoglossal - Takana, '64; Sumner, '75a). The present study examines the fine structure of the motor nucleus of the trigeminal nerve, a motoneuronal region whose ultrastructure has not been described in any species. This area is of particular interest in light of its specific Group Ia afferent input from the mesencephalic nucleus of the trigeminal nerve (for review, see Bratzlavsky, '76). The present findings suggest that the trigeminal motor nucleus has many anatomical features in common with the other cranial nerve motor nuclei. Additionally, the fine structure of all these nuclei resembles that of lamina IX of the ventral horn (Bodian, '64, '66; Conradi, '69; McLaughlin, '72a).

Ramón y Cajal ('52) first described the cytological details of the "principal or masticateur nucleus" of the motor root of the trigeminal nerve. He noted that in Nissl and Golgi preparations the neurons of this nucleus were of the "motor type". Other authors have primarily devoted their efforts to looking at the sizes and groupings of

trigeminal motoneurons (Jacobsohn, '09; Willems, '11; Meesen and Olszewski, '49; Szentagothái, '49; Olszewski and Baxter, '54; Vedral and Matzke, '67; Berman, '68; Oswaldo-Cruz and Rocha-Miranda, '68). More recently, several investigators have examined the specific neuronal representation of the muscles of mastication within the nucleus (Mizuno et al., '75; Batini et al., '76; Limwongse and Desantis, '77; Sessle, '77a; Matsuda et al., '78; Dom, personal communication).

The present investigation correlates the ultrastructure of the opossum trigeminal motor nucleus with details obtained from Nissl sections, Golgi preparations and toluidine blue-stained (1 μ m) plastic sections. Experimental techniques are employed at the electron microscopic level to further elucidate the synaptic organization of this nuclear group. Comparisons are made between the present results and those obtained from studies of the other cranial nerve motor nuclei and the ventral horn. Finally, these morphological findings are analyzed with respect to information from physiological studies on the trigeminal motor nucleus (Nakamura et al., '67; Sauerland and Mizuno, '69; Nakamura et al., '75; Bratzlavsky, '76; Goldberg, '76; Sessle and Greenwood, '76; Sumino, '76; Sessle, '77a, b; Chan, '79).

MATERIALS AND METHODS

The nuclear conformation and the cytological features of trigeminal neurons were initially examined in serial frozen sections of the adult opossum brain that had been cut in either the transverse, sagittal or horizontal plane and stained for Nissl substance with cresyl violet. These sections were also used to measure the range of cell body areas throughout a single trigeminal motor nucleus. For this analysis, 40 μ m thick sections through the nucleus were photographed and pictures were then enlarged to a magnification of X334. At this 40 μ m thickness there is no possibility of measuring a neuron more than once as only neurons whose nucleoli were clearly identifiable were included in the analysis. Subsequently, the enlargements were analyzed on a cybergraphic tablet interfaced with a Hewlett-Packard computer which had been programmed to compute areas of individual, circumscribed regions.

Further details of trigeminal cells were studied in sections of the opossum brainstem which were impregnated by the Golgi-Kopsch technique (Colonnier, '64) and cut in either the transverse, sagittal or horizontal plane. These brains had been fixed by perfusion with either formaldehyde or a mixture of glutaraldehyde and paraformaldehyde and remained in fixative for times in excess of six months. Drawings of

Golgi-impregnated neurons and axons were made using a Leitz projection microscope with either a X54 oil immersion, a X50 water immersion or a X25 objective. Measurements were taken from drawings with a final magnification of X1100 in order to determine average diameters of proximal, intermediate and distal dendrites.

Electron microscopic analysis was performed on sections from adult opossum brains that had been perfused according to the procedure described by Mihailoff and King ('75). In control animals, the right and left trigeminal motor nuclei were removed by employing previously determined coordinates (Oswaldo-Cruz and Rocha-Miranda, '68) to localize the nucleus. These blocks of tissue were then washed in 0.2 N sodium cacodylate, post-fixed in 1% osmium tetroxide, block-stained in saturated uranyl acetate, dehydrated in a graded series of ethyl alcohols, and flat-embedded, in Maraglas, to insure a transverse plane of section. The position of the trigeminal motor nucleus in each block was determined by examination of semi-thin (1 μ m), toluidine blue-stained sections which then served as a guide to trim the blocks down to the nuclear borders. Thin sections were cut on a Sorvall Porter Blum MT-2 ultramicrotome, picked up on copper grids, stained with lead citrate and observed in a Philips EM 300 electron microscope. The majority of electron micrographs which were subsequently examined were taken from dorsal and lateral parts of the trigeminal motor nucleus because of the precise nuclear border which is apparent in these regions (i.e. the axons of the trigeminal nerve). Initially, nineteen trigeminal cell bodies were selected, at their midnuclear level, for fine structural analysis. Features such as the number of synapses, the

shape of synaptic vesicles and the location of cisternal elements were noted in pictures with a final magnification of X50,000. Synaptic elements located between trigeminal cell bodies were quantified by examining fifteen montages of regions with dimensions of 35 μ m X 45 μ m; the final magnification of these pictures was X25,000. Cross-sectioned dendrites within these areas were measured and identified as proximal, intermediate or distal by comparing them with measurements made in Golgi-impregnated material.

In order to identify the synaptic terminals of afferents to the trigeminal motor nucleus, large lesions were placed in either the mid-brain tectum or the contralateral trigeminal complex. All animals with these experimental lesions were perfused within two to seven days postoperatively. The blocks of tissue containing the trigeminal motor nucleus were subsequently removed and processed in the same manner as control material. Thin sections from these blocks were examined for lesion-dependent degeneration. In each experimental case, the areas containing the lesion were removed, sectioned with a freezing microtome, mounted serially on slides and stained for Nissl substance so that the extent of each lesion could be determined.

OBSERVATIONS

Cytoarchitecture

The opossum trigeminal motor nucleus is generally round in shape as is seen in transverse Nissl sections of the dorsolateral pons (fig. 1). The nucleus measures 1.3 mm in its rostral to caudal extent and reaches the pontomesencephalic junction rostrally while its caudal pole is situated near the cranialmost part of the spinal trigeminal nucleus. Throughout its extent, the trigeminal motor nucleus contains a fairly compact arrangement of variably-sized neurons, as well as longitudinally directed fibers. These features are evident in both transverse (fig. 1) and horizontal (figs. 2 and 3) Nissl-stained sections. In the caudal two-thirds of the nucleus there is a discrete group of smaller neurons located in the ventromedial quadrant of the main body of the nucleus (fig. 1, arrow 1). Also situated caudally and approximately 0.2 mm dorsomedial to the main body of the nucleus is a distinct cluster of neurons (fig. 1, arrow 2). These isolated neurons are of comparable size to those of the main body. The dorsomedial (fig. 2, arrow 2) and the ventromedial (fig. 3, arrow 1) cell groups are best seen in the horizontal plane.

Cytology

An analysis of all perikaryal areas in transverse Nissl sections (fig. 4) taken from a single trigeminal motor nucleus shows a range of neuronal cell bodies from 150 to 2650 μm^2 with a peak spectrum of 700 to 1750 μm^2 . Examination of the four quadrants of the nucleus reveals that neurons in its ventromedial part are smaller and more homogenous in size than neurons in the other three quadrants. This correlates with the previously described cytoarchitectural findings (fig. 1, arrow 1 and fig. 3, arrow 1).

When examined in any of the three standard planes of section, trigeminal neurons have a multipolar shape. Additionally, in sections stained with cresyl violet these neurons have intense accumulations of basophilia (fig. 5). Similar staining characteristics representing distinct Nissl bodies are also evident in 1 μm , toluidine blue-stained sections (fig. 6).

Golgi preparations

Trigeminal neurons, which were impregnated by the Golgi-Kopsch technique, have perikarya ranging up to 50 μm in diameter and as much as 2000 μm^2 in area. Centrally located neurons have dendrites which radiate in all planes so that their overall dendritic geometry often forms a sphere having a diameter in excess of 1 mm and extending throughout the conventional borders of the nucleus (figs. 7 and 8). Cells located near the periphery of the nucleus generally have dendrites arching along the nuclear border as well as directed towards the center of the nucleus. Infrequently, a dendrite of a trigeminal neuron leaves

the confines of the motor nucleus to enter the neighboring reticular formation (fig. 7, solid arrows). Similarly, reticular neurons (fig. 7, open block arrow) occasionally send one of their dendrites into the trigeminal nucleus (fig. 7, open arrow). Large proximal dendrites of trigeminal neurons measure 5.0 to 8.0 μm in diameter and each gives rise to two to five intermediate-sized dendrites of 2.5 to 5.0 μm diameter (fig. 8). These dendrites, in turn, narrow into small distal dendrites with diameters less than 2.5 μm (fig. 8).

Spines of various forms are a consistent feature on both the somata and dendrites of trigeminal neurons (figs. 8 and 9). Somatic spines are mainly of the sessile variety (fig. 8, solid block arrow) whereas sessile, long-necked (fig. 9, solid arrow) and complex appendage forms (fig. 9, open arrow) are found on all parts of the dendritic tree. The complex spines often extend as far as 10 to 15 μm from their parent dendrite. Clusters of spines are common, especially at branching points of large diameter dendrites (fig. 8, open block arrows).

The presence or absence of axon collaterals could not be established as axons of trigeminal neurons were never impregnated beyond the initial segment in our material. Axons, which course throughout the nucleus, however, were frequently impregnated. Generally, they form "strings of beads" whose largest varicosities measure 2 to 10 μm in length and are separated by distances of up to 15 to 20 μm (fig. 10). Another type of axon branches into a terminal plexus which ends as numerous bulbous swellings (figs. 11 and 12).

Ultrastructure of neuronal somata

All trigeminal neurons, as seen in micrographs taken at their midnuclear level, display similar fine structural features (fig. 13). Their abundant perinuclear cytoplasm is filled with a normal complement of organelles; dense aggregates of rough endoplasmic reticulum and polysomes are particularly apparent and lipofuscin granules are also common within the cytoplasm. The nuclei of trigeminal neurons range from round to oval in shape and contain prominent nucleoli as well as separate clumps of heterochromatin. The nuclear envelope is most often smooth, although an occasional indentation may be present. Axon hillocks contain few organelles and, after a length of approximately 15 μm , narrow into axonal initial segments with their characteristic dense undercoating (Peters et al., '76).

Boutons usually cover over fifty percent of the somatic surface of trigeminal neurons and in some cases approximate up to two-thirds of this membrane (fig. 13, open and solid block arrows). The remaining surface is most commonly contacted by glial sheaths. In addition, appositions are also found with glial cell bodies, myelinated axons and dendrites. As many as six somatic spines have been found in a single thin section and these most often have a narrow, long (1 μm) neck that expands slightly into a head. When present, these protrusions usually are in contact with a synaptic terminal. The axon hillocks of trigeminal neurons are also postsynaptic to terminals.

Ultrastructure of boutons

Four categories of synaptic terminals may be distinguished in the trigeminal motor nucleus based upon terminal size, shape of their synaptic vesicles, number and size of dense core vesicles, and the presence or absence of an associated subsynaptic cistern. These endings are presynaptic to both the cell body and the dendritic tree of trigeminal neurons.

The first and most frequently encountered type of presynaptic terminal contains spherical synaptic vesicles (S-boutons) and measures 1 to 4 μm in length (fig. 14). In these endings, vesicles are usually aggregated at the one or two synaptic junctions made with the trigeminal neurons where the complexes are of the Gray's type 1 (asymmetric) variety. These S-boutons are commonly found to be expansions along axons containing arrays of microtubules, indicating that at least some of these endings may be boutons en passant.

Boutons containing a population of pleomorphic (i.e. spheroidal, ellipsoidal and/or flattened) vesicles (P-boutons) are the second most common type of terminal in the trigeminal motor nucleus (fig. 15) and are 1 to 2 μm in length. Frequently, synaptic complexes with either an intermediate type of asymmetric junction or a symmetric junction (Gray's type 2) are found between these endings and the neurons. Once again, their appearance as varicosities along axons indicates that some of the P-boutons may be boutons en passant.

A third type of ending (DC terminals) contains a heterogeneous population of vesicles varying in size, shape and granularity (fig. 16). For the most part, these boutons are marked by large dense core vesicles

dispersed among small dense core vesicles and pleomorphic clear vesicles. DC terminals are usually 1 to 3 μm in length and are present as isolated profiles among various elements of the neuropil. Synaptic complexes are rarely found, but when they are seen the junctions are generally of the asymmetric variety.

The most distinctive terminals on trigeminal neurons contain spherical synaptic vesicles and are always associated with a single subsynaptic cistern (C-bouton; fig. 17). These endings are large, measuring from 2 to 5 μm in length. Within the C-bouton, agranular vesicles comprise the majority of the tightly packed vesicles although occasional dense core vesicles are also present (fig. 18, block arrows). For the most part, the subsynaptic cistern is continually and distinctly associated along the length of the overlying bouton. Presynaptic dense projections are occasionally evident along the presynaptic membrane (fig. 19, curved arrows), but an associated postsynaptic density is lacking. Identifiable junctional complexes have only been seen in regions where the cistern is at some distance from the trigeminal membrane (fig. 19, open block arrow). Adjacent C-boutons often interdigitate with each other (fig. 18); however, no membrane specializations have been found at this site of apposition. Similar interdigitations are also commonly present between C-boutons and S- and P-boutons. Although C-boutons are usually less than 5 μm in length, examples up to 10 μm in length have been found on proximal dendrites.

Distribution of synaptic terminals

All four varieties of terminals contact both the somatic surface and the proximal dendrites of trigeminal neurons (table 1) although DC terminals are rarely seen. Boutons cover approximately half of the somatic cell membrane. Of these, fifty-four percent are S-boutons; nearly eight percent of the endings are C-boutons; and the remaining thirty-eight percent are P-boutons. When somatic spines are present, they are usually postsynaptic to S-boutons. The axon hillocks of trigeminal neurons are mainly contacted by S-boutons although P-boutons are also present.

Within the neuropil, large diameter proximal dendrites (greater than 5 μm in cross-sectioned diameters) are covered with boutons containing either spherical or pleomorphic vesicles (fig. 20); C-boutons also frequently contact these dendrites (table 1). Intermediate-sized dendrites (diameters of 2.5 to 5.0 μm) are most commonly covered with S-boutons although P-boutons are not infrequent (fig. 21); these dendrites are never postsynaptic to C-boutons. Finally, distal dendrites (diameters less than 2.5 μm) are most frequently in contact with S-boutons (fig. 22). The varying percentages of S- and P-boutons on trigeminal neurons are reflected in the increasing ratio between these terminals from proximal to distal parts of the dendritic tree (table 1).

Dendritic spines typically contain mitochondria and cisterns of smooth endoplasmic reticulum as well as a flocculent matrix (fig. 23). They are rarely seen in continuity with their parent dendrite due to their length (Golgi preparations; up to 10 μm) and, instead, are

TABLE 1

DISTRIBUTION OF BOUTONS *								
	Cell Bodies **		Dendrites ***				Spines ****	
Synaptic type	%	n =	Proximal	Intermed.	Distal	n =	%	n =
S	54%	427	67%	76%	78%	499	68%	94
P	38%	305	30%	24%	22%	172	32%	43
C	8%	63	3%	0%	0%	5	0%	0
S : P Ratio	1.4 : 1		2.2 : 1	3.1 : 1	3.5 : 1		2.1 : 1	

* DC terminals have not been included due to their infrequency

** Total boutons on cell bodies = 795

*** Total boutons on all sizes of dendrites = 676

**** Total boutons on spines = 137; spines are isolated in the neuropil rather than associated with any specific size of dendrites or with the cell body

usually found as isolated cross sections within the neuropil (fig. 23). Presynaptic elements contain either spherical or pleomorphic vesicles.

In summary, while S-boutons constitute a significant population (54%) on trigeminal perikarya and proximal dendrites, they are the major type of terminals on distal dendrites. P-boutons represent almost forty percent of the terminals on the cell body and proximal dendrites and diminish in number distally. C-boutons are only present on the cell body and proximal dendrites. Isolated spines within the neuropil are contacted by more pleomorphic vesicle-containing terminals than is typical of all dendrites in general (table 1). DC terminals, when seen, are found adjacent to all parts of trigeminal neurons but synapses are rarely present.

Experimental studies

Experimental material was used to determine which synaptic endings degenerate after lesions involving sources of afferents to the opossum trigeminal motor nucleus (as described by Panneton and Martin, '79 and Panneton and Martin, personal communication). Moreover, this analysis also examines the location of the contact between these degenerating terminals and the trigeminal neurons.

Lesions that destroy most of the midbrain tectum and, thus, the majority of trigeminal mesencephalic neurons after survival times of two, three and five days elicit electron-dense degeneration within the trigeminal motor nucleus. Two days after a tectotomy, degeneration is found only in myelinated axons measuring from 1 to 4 μ m in diameter. Tissue from animals with survival times of three (fig. 24) or five days

contains degenerating myelinated axons, as well as degenerative changes in synaptic terminals. For the most part, these degenerating boutons have an electron-dense appearance, contain spherical vesicles and either contact small diameter dendrites or spines (figs. 25 and 26). Some of the degenerating terminals are C-boutons. A series of electron micrographs shows a suggested sequence for the degenerative changes in the C-boutons which contact trigeminal cell bodies and proximal dendrites (figs. 27 through 30). Characteristic electron-dense degeneration is the first sign of the process (fig. 27). Subsequently, the terminals become swollen and are surrounded by glial sheaths (fig. 28) which eventually engulf the bouton (fig. 29). Throughout this process, the characteristic subsynaptic cistern remains closely applied along the length of the previous contact (figs. 27, 28 and 29, small solid block arrows). Very few degenerating axons or terminals are found in the trigeminal motor nucleus seven days after a tectotomy. However, expanded atypical glial sheaths are present in apposition to trigeminal neurons (fig. 30). Frequently, a single degenerating C-bouton (fig. 29, solid block arrow) is found adjacent to an apparently normal bouton (fig. 29, 'C').

Electron-dense degeneration is found two days after stereotaxic lesions which destroy areas around the contralateral trigeminal motor nucleus or which undercut fibers from this region (fig. 31). Dark, degenerating terminals contain either pleomorphic (fig. 32) or spherical (fig. 33) vesicles and approximate intermediate and distal dendrites of trigeminal neurons. In animals with longer survival times, boutons are engulfed by glial elements.

DISCUSSION

Conformation and functional representation within the nucleus

The appearance of the opossum trigeminal motor nucleus approximates that of other species. Three-dimensionally, it is ovoid in shape with its major axis in the horizontal plane. Consequently, when viewed in the transverse plane, most of the nucleus is circular. Cytoarchitecturally, the nucleus has generally been divided into dorsal and ventral divisions (man, Jacobsohn, '09; rabbit, Meesen and Olszewski, '49; cat, Berman, '68). For the most part, the dorsal division contains large, loosely-arranged neurons while the ventral portion is densely packed with smaller neurons. In the opossum, the dorsal and more lateral quadrants of the main body of the trigeminal nucleus are characterized by cells of different sizes, whereas a more compact arrangement of smaller neurons predominates ventromedially. A unique feature of the opossum trigeminal nucleus is the cell cluster located dorsomedially to the main body. This cluster contains neurons of approximately the same size as those located within the main body of the nucleus.

In an attempt to determine the location of motoneurons innervating specific muscles of mastication recent experiments have used the horseradish peroxidase technique. A particular aim of these studies has been to characterize the location of jaw-closers versus that of

jaw-openers. In the cat, the two main jaw-closers, i.e. the temporalis and masseter muscles, have their motoneurons located in dorsal and lateral parts of the nucleus (Mizuno et al., '75; Batini et al., '76). Mylohyoid and anterior digastric motoneurons, i.e. those responsible for jaw-opening, are found in the ventromedial division (Mizuno et al., '75; Batini et al., '76). The same authors have indicated that the two pterygoid muscles have motoneurons located between the other two groups. These muscles are responsible for lateral movements of the jaw as well as for some of its closure. Mizuno et al. ('75) also described the mylohyoid motoneurons as being smaller than those of the anterior digastric muscle, although both are found in the ventromedial division. Unlabelled regions of the nucleus were thought to innervate the tensor veli palatini and the tensor tympani muscles. Analogous findings have been reported for the rat (Limwongse and Desantis, '77) and rabbit (Matsuda et al., '78) trigeminal nuclei.

Dom (personal communication), in the opossum, has recently used the horseradish peroxidase method to determine the location of neurons which supply specific muscles of mastication. As in the cat, motoneurons for all four of the jaw-closers are located in the dorsal and lateral parts of the main body of the trigeminal motor nucleus. However, neurons innervating the jaw-openers are separated: anterior digastric motoneurons are found in the isolated dorsomedial cell cluster, while the smaller neurons which innervate the mylohyoid muscle are in the ventromedial quadrant of the main body of the nucleus. Thus, the unique configuration of the opossum trigeminal motor nucleus reflects

the functional representation of the muscles of mastication in this species.

Light microscopy

Neurons in the opossum trigeminal motor nucleus are identical to those which have been described as being of the "motor type" by Ramón y Cajal ('52). In Nissl sections, they are characterized by their large dimensions, their multipolar shape, and their tightly massed chromatic Nissl bodies. Stereotaxic atlases of the brain (rabbit, Meesen and Olszewski, '49; cat, Berman, '68; opossum, Oswaldo-Cruz and Rocha-Miranda, '68) also note the large, intensely stained neurons of the trigeminal motor nucleus. The present report indicates that, although heterogenous in size, the neurons within the opossum trigeminal nucleus are comparable to those of other species. Trigeminal neurons as seen in Nissl preparations are morphologically similar to the alpha motoneurons in the other cranial nerve motor nuclei (Meessen and Olszewski, '49; Berman, '68; Oswaldo-Cruz and Rocha-Miranda, '68) and in the spinal cord (Conradi, '69; McLaughlin, '72a).

Ramón y Cajal ('52) applied the Golgi method to the rat trigeminal nucleus and observed large, multipolar neurons that give off three or more dendrites which divide and subdivide as they traverse the width of the nucleus. Such neurons are intercalated within a plexus of nerve fibers. Both their cell bodies and dendrites are characterized by thorn-like protoplasmic expansions. The present observations in the opossum conform to this general Golgi characterization.

Ramón y Cajal also described all rat trigeminal axons as being grouped into bundles which leave the brainstem without giving off collaterals. On the contrary, Lorente de Nó ('22), while agreeing that the motor cells of the rat trigeminal nucleus have axons joining the motor root directly, also cited evidence for "associative cells" within this same nucleus. These neurons are regularly distributed throughout the nucleus and their axons course dorsally while giving rise to ascending and descending branches as well as to collaterals. No such Golgi type II neurons have been impregnated by the Golgi-Kopsch method in the trigeminal nucleus of the opossum.

As seen in other motor regions, spinal alpha motoneurons have been found to have recurrent axon collaterals (Cullheim et al., '77) while comparable neurons of the facial motor nucleus do not have such processes (Falls and King, '72a). In our Golgi material, no axons have been impregnated beyond the initial segment of trigeminal neurons, thus no statement can be made relative to the presence of axon collaterals.

As stated earlier, an occasional dendrite leaves the confines of the nucleus and enters the reticular formation (fig. 7, solid arrows). Similarly, reticular neurons frequently send secondary dendrites into the trigeminal motor nucleus (fig. 7, open arrow). These findings alter the traditional notion of a sharp boundary existing between the nucleus and the surrounding reticular formation. Finally, our Golgi preparations do not provide any evidence for dendritic bundles as have been described in the ventral horn of the spinal cord (Scheibel and Scheibel, '70).

Electron microscopy, cytology

Other than this report, there have been no descriptions of the ultrastructure of the trigeminal motor nucleus in any species. Comparisons may be made, however, with other studies which have detailed findings in the spinal cord ventral horn (Bodian, '64; Conradi, '69; McLaughlin, '72a) and in the other cranial nerve motor nuclei (Takana, '64; Bak and Choi, '74; Sumner, '75b; Falls and King, '76a; Tredici et al., '76; Spencer and Sterling, '77; Destombes et al., '79). In general, the cytological features of trigeminal neurons are similar to those of neurons in these other regions in terms of their overall morphology and their somatic contacts.

Although neuronal size varies over a wide range (fig. 4), the fine structural characteristics of all trigeminal neurons appear similar. This contrasts with some findings in the facial (Falls and King, '76a) and abducens (Spencer and Sterling, '77; Destombes et al., '79) nuclei where there are ultrastructural features which distinguish small neurons. Nevertheless, a homogeneity in electron microscopic findings for trigeminal motoneurons is consistent with descriptions of other motor regions. In many studies (Conradi, '69; McLaughlin, '72a; Bak and Choi, '74; Tredici et al., '76) size is the sole anatomical criteria for distinguishing alpha from gamma motoneurons with large and small neurons serving merely as "extensions of a continuous series of morphological variants" (Tredici et al., '76). In their work on the abducens nucleus, Spencer and Sterling ('77) used the technique of retrograde axonal transport of horseradish peroxidase to distinguish alpha motoneurons from projection neurons which send their axons to the oculomotor

complex. The cytology and synaptology of these two functional classes of neurons were shown to be identical or, at least, overlapping.

Lund et al. ('78) have shown the presence of muscle spindles in the two large jaw-closers, the temporalis and masseter muscles. Sessle ('77a) has provided correlative physiological evidence for alpha and gamma motoneurons, as well as for interneurons, in the trigeminal motor nucleus of the cat. As stated previously, there are no morphological clues for such distinctions in the opossum except for possible arbitrary separations on the basis of size. Additional techniques, such as the intracellular injection of an electron opaque material into physiologically identified cells, must be utilized to determine anatomical correlates for the physiological data. Perhaps with this approach more subtle variations in morphology will come to light.

Electron microscopy of boutons and their distribution

We have described four basic varieties of terminals in the opossum trigeminal motor nucleus: (1) S-boutons containing spherical synaptic vesicles, (2) P-boutons containing a population of pleomorphic vesicles, (3) DC terminals containing a heterogenous population of vesicles including many large dense core vesicles, and (4) C-boutons containing spherical vesicles and always associated with a single subsynaptic cistern. All of these bouton types have been identified in one or more of the various motoneuronal pools of the central nervous system. S- and P-boutons represent the great majority of endings in the cranial nerve motor nuclei and the ventral horn. In contrast, terminals similar to C-boutons have been specifically localized in the ventral

horn (Bodian, '66; Conradi, '69; McLaughlin, '72a), the hypoglossal nucleus (Sumner, '75b), the facial nucleus (Falls and King, '76a), and the oculomotor nucleus (Tredici et al., '76). In the two nuclei which innervate single extra-ocular muscles, the trochlear (Bak and Choi, '74) and abducens (Spencer and Sterling, '77; Destombes et al., '79) nuclei, C-boutons have not been reported. Synaptic endings resembling DC terminals have been identified in the ventral horn (Bodian, '66; Conradi, '69), the facial nucleus (Falls and King, '72a), and the trochlear nucleus (Bak and Choi, '74).

While the above four types of terminals are consistently present in the opossum trigeminal motor nucleus, other boutons that have been identified in the ventral horn are not present. There is little evidence for endings related to postsynaptic dense bodies (the "T" and "M" boutons of Conradi, '69 and McLaughlin, '72a); likewise, the normal filamentous endings ("NF_S" or "NF_D") that were reported by McLaughlin ('72a) were not observed.

As is common through the nervous system, it is apparent that both S- and P-boutons are most likely heterogenous groups in the trigeminal motor nucleus (unpublished observations). However, no sub-classifications based on variables such as vesicle size and shape, location of mitochondria and microtubules, and density of axoplasmic matrix have been attempted.

A comparison of motoneuronal regions suggests that the general distribution of synapses on the cell bodies and dendritic trees remains fairly constant. C-boutons, when present, are only found on the soma and proximal dendrites and represent four to ten percent of the total

bouton population (Bodian, '66; Conradi, '69; McLaughlin, '72a; Sumner, '75a; this report). The two other predominant bouton types, S- and P-boutons, constitute almost all of the remaining terminals and are found, to some degree, on all parts of motoneurons. S-boutons are more numerous, especially on distal dendrites, whereas P-boutons tend to comprise a more significant proportion of endings on the cell body and more central parts of the dendritic tree (for summary of data, see table 1).

Uchizono ('65) has correlated the shape of synaptic vesicles and postsynaptic activity in the cat cerebellar cortex and has hypothesized a general functional model for the mammalian central nervous system. This hypothesis is that excitatory terminals contain round or spherical synaptic vesicles while endings that have an inhibitory function contain flattened or pleomorphic vesicles. Generalizing this hypothesis to the present data suggests that the S- and C-boutons, containing spherical vesicles, would be excitatory in nature while P-boutons, containing pleomorphic vesicles, would be inhibitory. Furthermore, according to the previously described "distribution of synapses", excitatory terminals (S- and/or C-boutons) predominate throughout motor regions whereas boutons with an inhibitory nature (P-boutons) are numerically more significant on the cell body and proximal dendrites. At the same time, spines (somatic and dendritic) have a higher proportion of inhibitory terminals presynaptic to them than do dendritic shafts (table 1). The accuracy of this interpretive theorem remains to be determined by physiological methods not only in motor regions, but in other areas of the nervous system as well. This is especially true in light of evidence which has shown that, at least in some cell cultures, terminals

containing round synaptic vesicles may have an inhibitory function (Nelson et al., '78).

Terminals which are similar to the presently described DC terminals are frequently found in areas of the nervous system that receive a monoamine innervation (Chan-Palay, '77). Recently, such an innervation has been shown to come from noradrenergic cell groups to the trigeminal motor nucleus (Silver et al., '79). This correlative information provides a potential source and function for DC terminals in the trigeminal motor nucleus.

Although the "distribution of synapses" is fairly consistent in various motor regions, more subtle variations seem to exist in each region. Thus, if one looks at the ratio of S- to P-boutons on different parts of trigeminal motoneurons, there is a ratio of 1.4:1 on cell bodies as compared to 3.5:1 on distal dendrites (table 1). This compares to ratios of 0.7:1 and 1.4:1, respectively, on similar areas of spinal motoneurons (derived from McLaughlin, '72a). In the abducens nucleus, there are S/P ratios of 1.0:1 on cell bodies and 2.3:1 on dendrites in general (Spencer and Sterling, '77). While the pattern remains constant, therefore, the abducens nucleus and especially the ventral horn seem to have significantly higher percentages of terminals containing flattened or pleomorphic synaptic vesicles than does the trigeminal motor nucleus. This may correlate with known inhibitory pathways that are functionally important in these regions, i.e. Renshaw and inhibitory interneuron feedback to spinal motoneurons and medial vestibular and oculomotor inhibitory input to the abducens nucleus.

Additional reasons for these discrepancies may simply be that they reflect sampling differences or variables in fixation methods.

Experimental fine structure

In the opossum, there are at least three general sources of direct afferent input into the trigeminal motor nucleus (Panneton and Martin, '79; Panneton and Martin, personal communication): (1) the mesencephalic nucleus of the trigeminal nerve; (2) the medullary and pontine reticular formation; and (3) the trigeminal complex itself.

Ramón y Cajal ('52) described the projection from the unipolar neurons of the mesencephalic nucleus to the trigeminal motor nucleus. At least some of these axons are known to complete the reflex arc for jaw-closing muscles, i.e. the jaw jerk (for review, see Bratzlavsky, '76). Many of these mesencephalic afferents are proprioceptive in nature as they carry the Group Ia primary afferent information of jaw-closing (masseter and temporalis) muscle spindles (Karlsson, '76; Lund et al., '78). Other potential areas of distribution for peripheral processes of mesencephalic neurons are periodontal mechanoreceptors (Hannam, '76) and the various types of temporomandibular joint receptors (Storey, '76) including Golgi tendon organs (Lund et al., '78). Electrophysiological data indicates that the mesencephalic input to trigeminal alpha motoneurons is monosynaptic and mostly, if not completely, excitatory in nature (for review, see Bratzlavsky, '76 and Sessle, '77a). Muscle spindles and Golgi tendon organs have not been found in the jaw-opening (anterior digastric and mylohyoid) muscles (Karlsson, '76). The absence of these receptors may be correlated

with the lack of a monosynaptic mesencephalic input to the neurons which innervate these muscles (Sessle, '77a).

After the destruction of the midbrain tectum and, thus, the cell bodies of trigeminal mesencephalic neurons, electron-dense degenerating terminals are present in the trigeminal motor nucleus. The fact that these terminals contain spherical synaptic vesicles which are functionally correlated with an excitatory action provides potential support for Uchizono's hypothesis in the trigeminal nucleus. On intermediate and distal dendrites, mesencephalic terminals form part of the population of S-boutons, while on the cell body and proximal dendrites they give rise to at least some of the C-boutons. All mesencephalic neurons do not have a single common function but, instead, have peripheral processes activated by a variety of receptors (muscle spindles, Golgi tendon organs, free nerve endings; Karlsson, '76; Hannam, '76; Storey, '76). Thus, it is not unreasonable to expect that two morphological classes of boutons, as presented here, are from the mesencephalic trigeminal nucleus.

Earlier attempts to determine the source of C-boutons in the ventral horn of the spinal cord were not successful. Conradi ('69) noted the presence of subsynaptic cisterns which were not associated with an overlying terminal after dorsal root section. However, the inability to find degenerating C-boutons after either dorsal root section or spinal hemisection has led to the conclusion that the origins for these terminals are probably short propriospinal neurons (Conradi, '69; McLaughlin, '72b, c; Bodian, '75; Pullen and Sears, '78). Degenerating C-boutons have only been found in the cat spinal cord in normal

developing material (Ronnevi, '79). The present data suggests that in the trigeminal motor nucleus at least some of the C-boutons originate from the mesencephalic nucleus of the trigeminal nerve and are, potentially, central terminations of Group Ia fibers. Light microscopic data (Panneton and Martin, '79) suggests that there are no other possible sources of afferents to the trigeminal motor nucleus which may have been destroyed by our large tectal lesions. In conclusion, these results either reveal a unique feature of the trigeminal motor nucleus or provide information upon which comparable studies should be performed in other motoneuronal regions.

The experiments involving tectal lesions also provide data about adjacent C-boutons which interdigitate with each other. When two C-boutons interdigitate they are not necessarily terminals of the same axon. Instead, at least some of these pairs seem to be derived from different parent axons as, at times, one degenerates while a neighboring terminal maintains its normal morphology (fig. 29, 'C').

A wide range of sources which exert excitatory or inhibitory polysynaptic influences on trigeminal motoneurons have their input mediated by the reticular formation. Reticular neurons scattered throughout the medullary and pontine tegmental fields project directly to the nucleus (Holstege and Kuypers, '77; Holstege et al., '77; Panneton and Martin, personal communication). Points of origin for these multisynaptic linkages include widespread sites in the periphery which have their cell bodies in the trigeminal mesencephalic nucleus (Group I muscle afferents, Group II mucosal afferents, peridontal mechanoreceptors, temporomandibular joint receptors; Goldberg, '76; Sessle and

Greenwood, '76; Sumino, '76; Sessle, '77b), the cerebral cortex (Nakamura et al., '67; Sessle, '77a), the amygdala (Sessle, '77a), and the cerebellum (Sessle, '77a). These inputs have a wide range of reciprocal and nonreciprocal effects on jaw-opening alpha motoneurons and jaw-closing alpha and gamma motoneurons. While most of these inputs act directly on the trigeminal neurons, an additional, presynaptic mechanism has been suggested for some of the inhibition which is produced after stimulation of a few of these sources (Sauerland and Mizuno, '69; Goldberg, '76). Nakamura et al. ('75) have specifically examined the input into the trigeminal motor nucleus from the medial bulbar reticular formation (MBRF), a region which, in turn, had previously been shown to mediate cortical input from the orbital gyrus (Nakamura et al., '67). In their work, the MBRF was found to have reciprocal, bilateral effects on trigeminal neurons; that is, MBRF stimulation monosynaptically inhibited masseteric motoneurons and excited digastric ones. Recently, it has been reported that the jaw-opening reflex is suppressed by the injection of morphine into a portion of the MBRF, the nucleus reticularis gigantocellularis (Chan, '79). This study suggests that a trigeminal motor reflex may, at least in part, be a pain response, and also illustrates one of the myriad of potential influences that may be mediated through the reticular formation onto trigeminal motoneurons.

To date, no lesion-dependent degeneration has been observed in either the ipsi- or contralateral trigeminal motor nucleus after hemisections of the medulla or pons (survival times of two and three days; unpublished observations). This is most likely due to the scattered locations of the tegmental projection neurons as well as to the

inability to destroy a significant number of these cells or their fibers without involving the motor nucleus itself. Additionally, there is no ultrastructural evidence, such as axo-axonic synaptic junctions between boutons, for any presynaptic mechanism in the trigeminal motor nucleus other than the interdigitations between C-boutons which have been described.

Lorente de Nó (22) first provided evidence for a projection into the trigeminal motor nucleus from the cell groups which are in proximity to the motor nucleus, the supratrigeminal and intertrigeminal nuclei. Since then, an abundance of anatomical and physiological data has suggested that neurons from these nuclei act as interneurons for the trigeminal motor nucleus, both ipsi- and contralaterally (for review, see Mizuno et al., '78). Sumino ('76) has specifically shown that supratrigeminal neurons are involved in a disynaptic pathway that is initiated by intra-oral stimulation and results in inhibition of masseteric motoneurons.

In the opossum, there are no clusters of neurons found in either a supratrigeminal or an intertrigeminal position and, consequently, no similarly named nuclei (Oswaldo-Cruz and Rocha-Miranda, '68; unpublished observations). Injections of horseradish peroxidase into the trigeminal motor nucleus lead to retrogradely labelled neurons within the contralateral trigeminal complex (Panneton and Martin, personal communication). Such neurons are scattered throughout this complex, especially among cells of the principal sensory nucleus of the trigeminal nerve. Based on this data, the labelled neurons are most likely analogous to the supra- and intertrigeminal neurons of other species.

In the present study fibers from the contralateral side have been interrupted by some of our lesions. Following survival of two days, some electron-dense terminals, containing either spherical or pleomorphic vesicles, were found which approximated variably-sized dendrites. These findings are similar to those which have been reported by Mizuno et al. ('78). Due to the proximity of the supra- and intertrigeminal neurons with the trigeminal motor nucleus, ipsilateral connections of these interneurons cannot be detected by methods that are presently available.

There is no information available as to the source of the DC terminals although it has been shown that there is a catecholaminergic innervation, originating from medullary and pontine regions, for the trigeminal motor nucleus (Silver et al., '79). Hemisections of the brainstem caudal to the trigeminal motor nucleus have not resulted in any clearcut degenerating DC terminals. The findings presented in this study have just begun to reveal the synaptic organization of the motor nucleus of the trigeminal nerve. A wide variety of inputs, including a significant proportion of terminals containing pleomorphic vesicles, must reach the cell body of trigeminal neurons and must still be identified. Also, distinctions must be made as to the sources and localization of input onto alpha versus gamma motoneurons as well as onto those neurons innervating jaw-closers versus those for jaw-openers.

ILLUSTRATIONS

Figure 1. A photomicrograph of a transverse section through the trigeminal motor nucleus in the dorsolateral pons. Distinct cellular subgroups are indicated as follows: small ventromedial neurons (arrow 1) which are part of the main body of the nucleus and an isolated dorsomedial cell cluster (arrow 2). Solid lines labelled 2 and 3 indicate approximate levels at which horizontal sections are shown in figures 2 and 3, respectively. Nissl stain, X63.

Figure 2. A photomicrograph of a horizontal section through the trigeminal motor nucleus which passes through level 2 as indicated in figure 1. Arrow 2 indicates the dorsomedial cell cluster as shown in figure 1. Nissl stain, X63.

Figure 3. A photomicrograph of a horizontal section through the trigeminal motor nucleus which passes through level 3 as indicated in figure 1. Arrow 1 indicates a group of small ventromedially-placed neurons which are part of the main body of the nucleus as shown in figure 1. Nissl stain, X63.

- Figure 4. A histogram of perikaryal areas in the trigeminal motor nucleus plotted from the total population of neurons in one nucleus. Area bin widths are $50 \mu\text{m}^2$.
- Figure 5. A high power photomicrograph of a trigeminal motoneuron illustrating its multipolar shape and staining characteristics. Nissl stain, X730.
- Figure 6. A high power photomicrograph of a trigeminal motoneuron illustrating its staining characteristics. One micron plastic section, toluidine blue stain, X730.

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Figure 7. A camera lucida drawing illustrating the position of the neuron in figure 8 within the trigeminal motor nucleus. Solid arrows point to dendrites of the trigeminal neuron which leave the nucleus. The position of a reticular neuron dendrite (open arrow) whose soma (large open block arrow) is outside of the motor nucleus is indicated. IV shows the location of the fourth ventricle and TrN refers to the position of the trigeminal nerve.

Figure 8. A camera lucida drawing of a trigeminal motoneuron. The dendrites are radially oriented and are covered with spines as is the cell body. Open block arrows illustrate clusters of spines located at dendritic branching points. The solid block arrow points to sessile spines on the cell body. The box indicates the area enlarged in figure 9. Golgi-Kopsch preparation, X180.

Figure 9. High power photomicrograph of a dendrite of the trigeminal motoneuron in figure 8 showing a long-necked spine (solid arrow) and a complex appendage (open arrow). Golgi-Kopsch preparation, X800.

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Figure 10. A camera lucida drawing of an axon which courses through the trigeminal motor nucleus and appears as a "string of beads". Open block arrow indicates a varicosity which measures approximately 10 μ m in maximum length. Golgi-Kopsch preparation, X1550.

Figures 11 and 12. High power photomicrographs taken at different depths of field to illustrate the appearance of bulbous terminals on an axon within the trigeminal motor nucleus. Golgi-Kopsch preparation, X770.

10

12

11

10 μm

Figure 13. A profile of a neuronal cell body in the trigeminal motor nucleus. Synaptic terminals cover approximately forty percent of the somatic membrane of this neuron. These endings are indicated as follows: S-boutons - solid arrows, P-boutons - open arrows, C-bouton - solid arrow with 'C'. Electron micrograph, X3900.

Figure 14. A synaptic terminal containing spherical synaptic vesicles and making a Gray's type 1 junction with a trigeminal neuron - S-bouton. Electron micrograph, X59,000.

Figure 15. A synaptic terminal containing a population of pleomorphic synaptic vesicles and making an intermediate type of synaptic junction with a trigeminal neuron - P-bouton. Electron micrograph, X59,000.

Figure 16. A synaptic terminal containing dense core vesicles and a pleomorphic population of agranular vesicles and making a Gray's type 1 junction with a trigeminal neuron - DC terminal. Electron micrograph, X59,000.

- Figure 17. A synaptic terminal containing spherical synaptic vesicles and associated with a subsynaptic cistern located in a trigeminal neuron - C-bouton. Electron micrograph, X54,000.
- Figure 18. Three interdigitating C-boutons are present. The arrows indicate dense core vesicles within a C-bouton. Electron micrograph, X54,000.
- Figure 19. High power electron micrograph showing features of a C-bouton and its associated subsynaptic cistern. Presynaptic dense projections within the ending are indicated by curved arrows. The open block arrow points to a rare synaptic complex marked by a widened synaptic cleft and a postsynaptic density over a region in which the cistern is at some distance from the cell membrane. X155,000.

- Figure 20. A cross-sectioned proximal dendrite which is contacted by a variety of S- and P-boutons. Electron micrograph, X16,500.
- Figure 21. A cross-sectioned intermediate-sized dendrite which is contacted by a variety of S- and P-boutons and is in apposition to a DC terminal. Electron micrograph, X15,500.
- Figure 22. Two cross-sectioned distal dendrites which are contacted by S-boutons. Electron micrograph, X29,000.

Figure 23. A cross-sectioned isolated spine which is contacted by two S-boutons. Electron micrograph, X32,000.

- Figure 24. A line drawing illustrating the extent of a tectal lesion from an animal with a three day survival time. The following structures are labelled for reference: interpeduncular nuclei (IP), medial geniculate nucleus (MGN), nucleus raphé dorsalis (RaD), oculomotor nucleus (OcM), periaqueductal gray (PAG), and trochlear nucleus (Tro).
- Figure 25. A degenerating terminal (solid block arrow) containing spherical synaptic vesicles contacts a spine. Tectotomy, 3 day survival. Electron micrograph, X44,000.
- Figure 26. A degenerating terminal (solid block arrow) containing spherical synaptic vesicles is isolated by glial processes. Tectotomy, 3 day survival. Electron micrograph, X55,000.

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Figures 27
through 30. Sequence of electron micrographs showing suggested stages of degeneration of C-boutons after a tectotomy (3, 5 or 7 day survival). Large solid block arrows indicate the degenerating terminals. Small solid block arrows show the continuing presence of a subsynaptic cistern through much of the degenerative process.

Figure 27. Electron-dense degeneration of a C-bouton. X45,000.

Figure 28. Engulfment of an electron-dense and swollen C-bouton by glial processes. X32,500.

Figure 29. Completed stage of engulfment by glial processes. 'C' indicates a normal C-bouton which has remained intact. X21,500.

Figure 30. A remaining, expanded glial sheath which is not over a cistern. X26,000.

Figure 31. A line drawing illustrating the center of a lesion which has destroyed fibers crossing from the ipsilateral trigeminal complex to the contralateral trigeminal motor nucleus. This animal had a two day survival time. The following structures are labelled for reference: griseum pontis (GP), locus coeruleus (Coe), motor nucleus of the trigeminal nerve (Tr Mo), principal sensory nucleus of the trigeminal nerve (Tr PS), spinal nucleus of the trigeminal nerve (Tr Sp), superior vestibular nucleus (Vst S), tract of the mesencephalic nucleus of the trigeminal nerve (Tr me t), and trapezoid nucleus (Tz).

Figure 32. A degenerating terminal (solid block arrow) containing pleomorphic synaptic vesicles contacts a dendrite. This terminal is within the trigeminal motor nucleus contralateral to the lesion illustrated in figure 31. Electron micrograph, X50,000.

Figure 33. A degenerating terminal (solid block arrow) containing spherical synaptic vesicles is presynaptic to a distal dendrite. This terminal is within the trigeminal motor nucleus contralateral to the lesion illustrated in figure 31. Electron micrograph, X46,000.

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