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The Synaptic Organization of the Motor Nucleus of the Trigeminal Nerve in the Opossum

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ABSTRACT The motor nucleus of the opossum trigeminal nerve consists of a main body and a small dorsomedial cell cluster. The cell bodies form a unimodal population with areas that range from 150–2700 μm^2. Golgi impregnations reveal that each neuron has three to six primary dendrites which radiate in all planes from the cell body. Within 300 μm from the soma, the primary dendrites divide into secondary branches and these, in turn, bifurcate into thinner distal dendrites. The overall diameter of the dendritic tree often extends as much as 1 mm, with a rare branch leaving the confines of the nucleus to enter the neighboring reticular formation. Somatic and dendritic spines are often present and are either sessile or complex appendage forms.

The perikarya and initial dendritic trunks of trigeminal neurons are contacted by four types of presynaptic terminals which cover more than 40% of the membrane. Most endings are 1–3 μm long and contain either spherical (S) or pleomorphic (P) synaptic vesicles. Another, less common, type of bouton is marked by large dense-core (DC) vesicles. Approximately 8% of the terminals on trigeminal cell bodies are large (2–5 μm) with spherical synaptic vesicles and are always associated with a subsynaptic cistern (C-boutons). These terminals very often interdigitate with adjacent synaptic endings. S-, P-, and C-boutons synapse on the dendritic tree of trigeminal neurons in the following characteristic pattern: proximal dendrites (greater than 5 μm in diameter) are contacted by all three types of terminals; intermediate-sized dendrites (between 2.5 and 5.0 μm in diameter) are most often contacted by S-boutons although P-boutons are also present; and small, distal dendrites (less than 2.5 μm in diameter) are almost always contacted by S- boutons. Both S- and P-boutons contact spines.

In order to determine the ultrastructural identity of some of the major afferent systems to the trigeminal motor nucleus, adult opossums were subjected to two different types of lesions. Three and 5 days subsequent to lesions which destroyed most of the trigeminal mesencephalic nucleus, degenerating terminals containing spherical vesicles were found. These endings were S-boutons on more distal parts of the dendritic tree while on the cell body and proximal dendrites they were C-boutons. Seven days after a mesencephalic lesion, expanded glial processes approximated the trigeminal cell membrane. Two days subsequent to lesions which transected commissural fibers from the contralateral trigeminal complex, degenerating S- and P-boutons were found in contact with intermediate and distal parts of the trigeminal dendritic tree.

The motor nucleus of the trigeminal nerve is a region favorable for correlative anatomical and physiological studies, as within it there are motoneurons which are segregated in the nucleus for two sets of antagonist muscles (Mizuno et al., '75; Batini et al., '76; Limwongse and DeSantis, '77; Sessle, '77a; Matsuda et al., '78; Dom, personal communication). Thus far, there have been a variety of

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physiological studies on this nuclear group (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) as well as some anatomical descriptions at the light microscopic level (Lorente de Nó, '22; Ramón y Cajal, '52). There have, however, been no studies of the fine structure of trigeminal motoneurons and their surrounding neuropil. This investigation describes the cytoarchitecture and the normal cytology and synaptology of the trigeminal motor nucleus in the opossum. Additionally, we provide a preliminary experimental identification of the sources of specific bouton types and their distribution over the trigeminal cell surface.

MATERIALS AND METHODS

The nuclear conformation and the cytological features of trigeminal neurons of the adult opossum brain were initially examined in serial frozen sections 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. Cell body areas were measured rather than maximum diameter, as areas depended less on plane of section than did diameter. For this analysis, photomicrographs of 40 μm thick sections through the nucleus were enlarged to a magnification of $\times$ 334. Subsequently, these enlargements were analyzed on a cybergraphic tablet interfaced with a Hewlett-Packard computer which had been programmed to compute areas of individual, circumscribed regions. As only neurons with clearly identifiable nucleoli were included in this study, there was no possibility of measuring a neuron more than once at a thickness of 40 μm.

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 a minimum of 6 months. Drawings of both Golgi-impregnated neurons and isolated axons were made using a Leitz projection microscope with either a $\times$ 54 oil immersion, $\times$ 50 water immersion, or a $\times$ 25 objective. In order to determine average diameters of proximal, intermediate, and distal dendrites, measurements were taken from drawings with a final magnification of $\times$ 1100.

Electron microscopic analysis was performed on sections from adult opossum brains that has been perfused according to the procedure described by Mihailoff and King ('75). In 17 control animals, tissue containing the right and left trigeminal motor nuclei was removed by employing previously determined coordinates (Oswaldo-Cruz and Rocha-Miranda, '68) to localize the region of the nucleus. These blocks of tissue were then washed in 0.2 N sodium cacodylate, postfixed in 1% osmium tetroxide, block-stained in saturated uranyl acetate, dehydrated in a graded series of ethyl alcohols, and flat-embedded in Maraglas to ensure a transverse plane of section. The position of the trigeminal motor nucleus in each block was determined by examination of semi-thin (1 μm), 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 analysis of electron micrographs was largely confined to dorsal and lateral parts of the trigeminal motor nucleus because of the well-defined nuclear border which is apparent in these regions (i.e., the axons of the trigeminal nerve). Initially, 19 trigeminal cell bodies from five animals were selected 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 micrographs with a final magnification of $\times$ 50,000. Synaptic elements located between trigeminal cell bodies were quantified by examining 15 montages ($\times$ 25,000) of regions with dimensions of 35 μm $\times$ 45 μm. This sample was taken from nine different animals. 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.

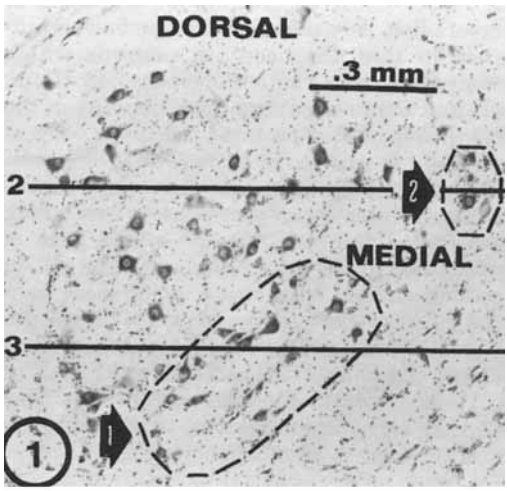
In order to identify the synaptic terminals of major afferents to the trigeminal motor nucleus, large lesions were placed in either the midbrain tectum or the contralateral trigeminal complex. Fourteen animals with these experimental lesions were perfused 2 to 7 days postoperatively. Blocks of tissue containing the trigeminal motor nucleus were

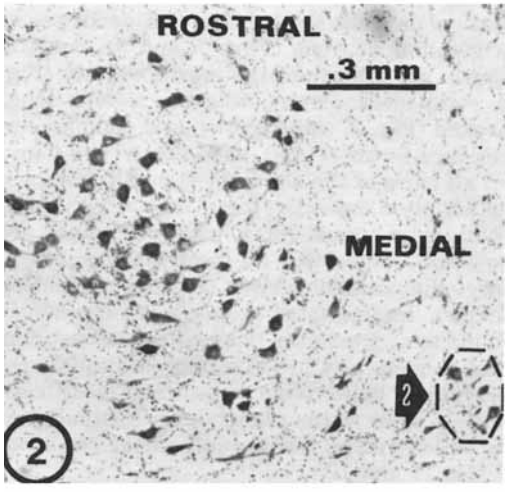
subsequently removed and processed in the same manner as control material. Thin sections from these blocks were then examined for lesion-dependent degeneration. In each experimental case, the area containing the lesion was removed, sectioned with a freezing microtome, mounted serially on slides, and stained for Nissl substance to determine the extent of the lesion.

OBSERVATIONS

Cytoarchitecture and cytology

Located in the dorsolateral pons, the main body of the opossum trigeminal motor nucleus is egg-shaped, measuring 1.3 mm in its rostral to caudal extent and 1.0 mm from its medial to lateral borders. Rostrally the nucleus reaches the pontomesencephalic junction while caudally it reaches the cranialmost part of the spinal trigeminal nucleus. The trigeminal motor nucleus contains a compact arrangement of variably-sized neurons which are distinguished from the surrounding neural tissue by their larger size. This feature is clearly 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 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 to the main body of the nucleus is a cluster of neurons (Fig. 1, arrow 2) comparable in size to those of the main body of the nucleus. The ventromedial (Fig. 3, arrow 1) and dorsomedial (Fig. 2, arrow 2) cell groups are best seen in the horizontal plane and, as shown in ex-





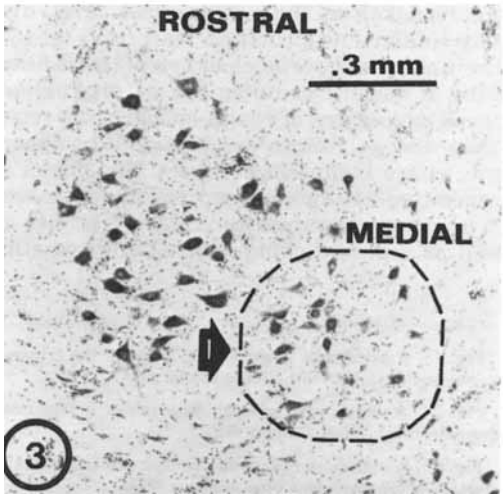


Fig. 1. A photomicrograph of a transverse section through the trigeminal motor nucleus in the dorsolateral pons. Distinct cellular subgroups are indicated as follows: small ventromedial neurons (arrow 1) which are part of the main body of the nucleus and an isolated dorsomedial cell cluster (arrow 2). Solid lines labelled 2 and 3 indicate approximate levels at which horizontal sections are shown in Figures 2 and 3, respectively. Nissl stain, × 56.

Fig. 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, × 56.

Fig. 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, × 56.

periments using the retrograde transport of horseradish peroxidase, have special significance in that they contain neurons which innervate muscles which open the jaw (Dom, personal communication). Motoneurons in the dorsal and lateral parts of the main body of the nucleus primarily innervate jaw-closers.

Trigeminal motoneurons have cell bodies with maximal cross-sectional areas which range from 150 to 2700 μm^2 with a peak of approximately 900 to 1350 μm^2 (Fig. 4). 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). After Golgi impregnation, trigeminal neurons are seen to be multipolar (Figs. 7 and 8) and show no preferential orientation in any plane. 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 arrow). Impregnated axons were frequently found to approximate trigeminal motoneurons and to generally form "strings of beads" representing boutons *en passant* (Fig. 10) although some axons branch into terminal plexes which end as numerous bulbous swellings (Figs. 11 and 12).

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, as are lipofuscin granules. 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 a characteristic dense undercoating (Peters et al., '76).

Dendritic architecture

Trigeminal motoneurons give off three to six primary dendrites which are 5.0 to 8.0 μm in diameter (Fig. 8). These subdivide into two-to-five intermediate-sized branches of 2.5 to 5.0 μm diameter. These dendrites, in turn, narrow into small distal dendrites with diameters less than 2.5 μm. Ultrastructural correlates to these dendrites are found as cross-sectioned profiles in the neuropil of the trigeminal nucleus (Figs. 20, 21, and 22).

Neurons near the center of the trigeminal nucleus give off dendrites radially, thus forming a spherical dendritic field which is approximately 1 mm in diameter (Fig. 8). Cells located near the periphery of the nucleus generally have dendrites that either arch along the nuclear border or are directed toward 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, the reticular neurons (Fig. 7, open block arrow) occasionally send one of their dendrites into the trigeminal nucleus (Fig. 7, open arrow). These findings alter the traditional notion of the existence of a sharp boundary between the motor nucleus and the surrounding reticular formation and should be taken into account in future descriptions of specific components of the trigeminal motor nucleus.

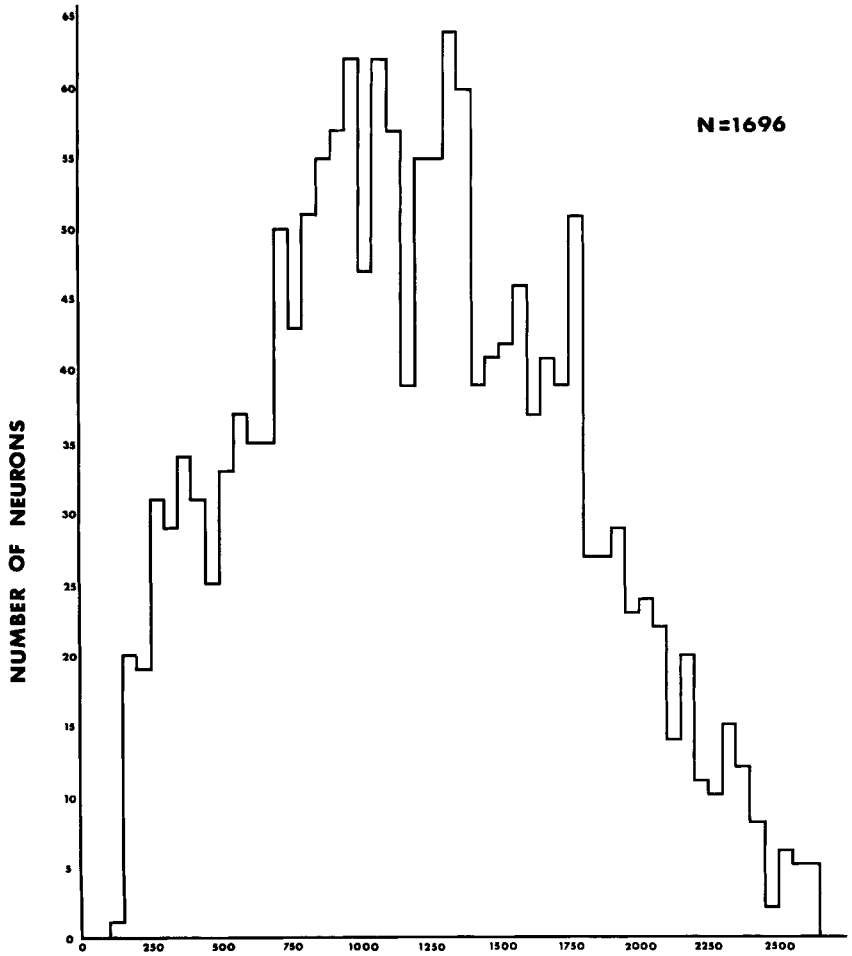
Synaptology

Boutons usually cover 40 to 70% of the somatic surface of trigeminal neurons (Fig. 13, open and solid block arrows) with the remaining surface being most commonly contacted by glial processes. Appositions are also found between the neurons and glial cell bodies, myelinated axons and dendrites. Termi-

Fig. 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 μm^2.

Fig. 5. A high-power photomicrograph of a trigeminal motoneuron illustrating its multipolar shape and staining characteristics. Nissl stain, × 670.

Fig. 6. A high-power photomicrograph of a trigeminal motoneuron illustrating its staining characteristics. One-micron plastic section, toluidine blue stain, × 670.


N=1696
NUMBER OF NEURONS
AREA (μm²)
0
5
10
15
20
25
30
35
40
45
50
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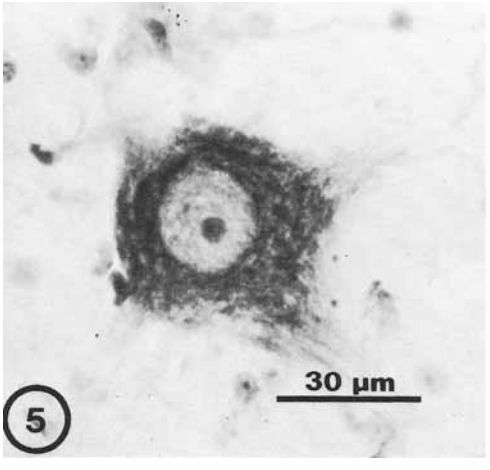
65
250
500
750
1000
1250
1500
1750
2000
2250
2500

4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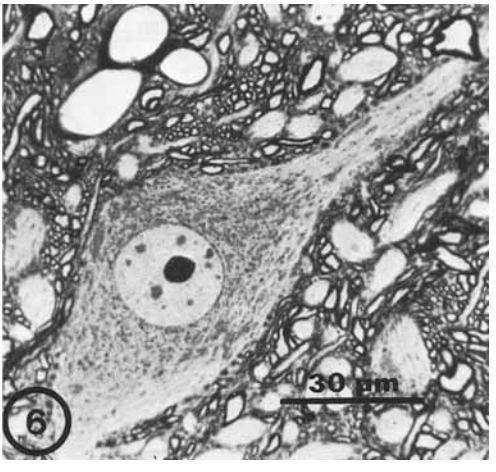
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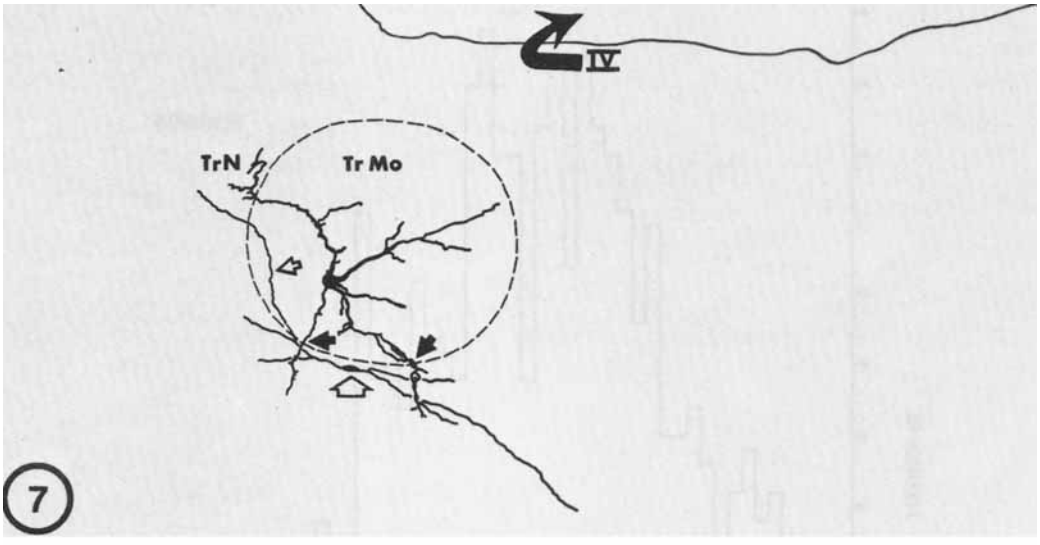
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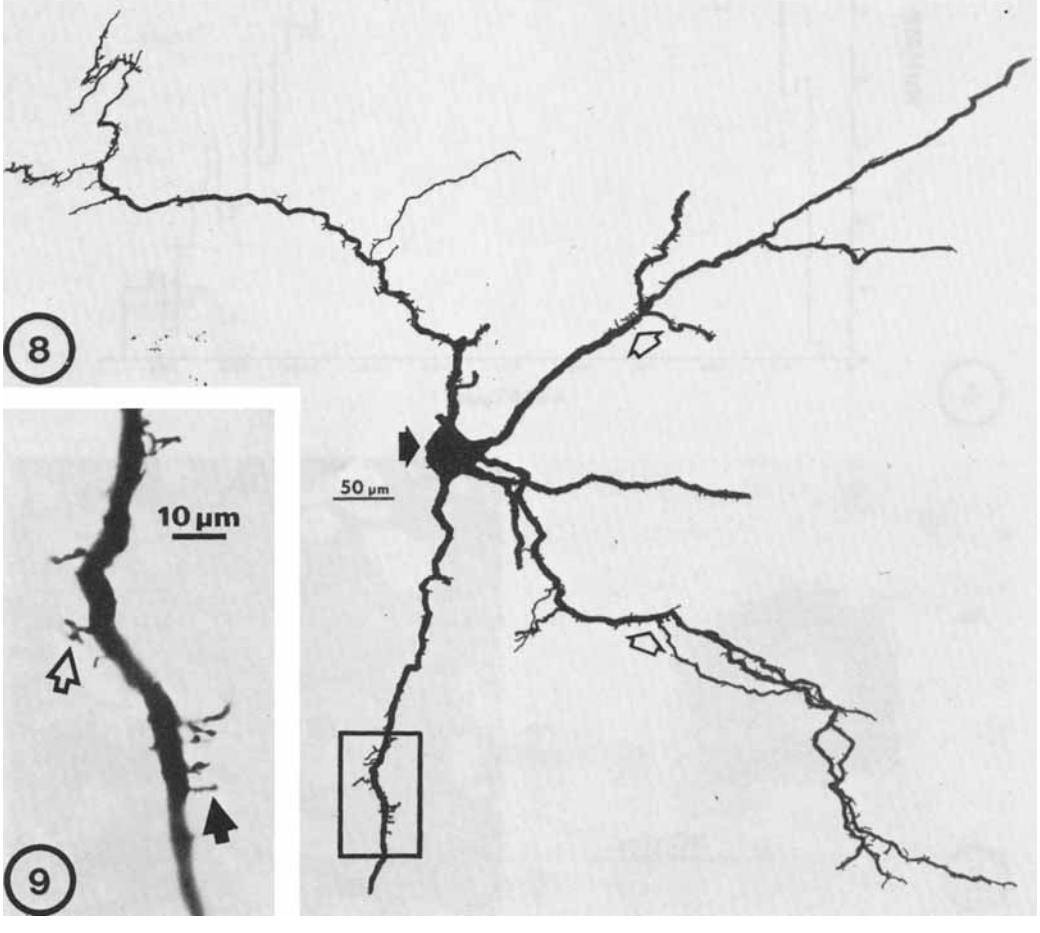

8
50 µm

10 µm
9

nals are also presynaptic to the axon hillocks of trigeminal neurons as well as to much of the available surface of trigeminal dendrites. 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.

The first and most common type of presynaptic terminal contains spherical synaptic vesicles (S-boutons) and measures 1 to 3 μm in length (Fig. 14). In these endings, vesicles are usually aggregated at one or two asymmetric synaptic junctions. 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*. Dense bodies are occasionally present in the trigeminal cytoplasm underlying S-boutons.

The second most frequent type of bouton contains a population of pleomorphic (i.e., spheroidal, ellipsoidal, and/or flattened) vesicles (P-boutons) and are 1 to 2 μm in length (Fig. 15). Frequently, synaptic complexes with either an intermediate type of asymmetric junction or a symmetric junction 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*.

The third type of ending (DC terminals) contains a heterogenous 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 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 fourth and most distinctive type of terminal on trigeminal neurons contains spherical synaptic vesicles and is always associated with a single subsynaptic cistern (C-bouton; Fig. 17). These endings are large, usually measuring from 2 to 5 μm in length; additionally, examples up to 10 μm in length have been found on proximal dendrites. Within the C-bouton, the majority of the tightly packed vesicles are agranular, although occasional dense-core vesicles are also present (Fig. 18, block arrows). For the most part, the subsynaptic cistern is continually and distinctly associated with 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 between the postsynaptic membrane and the subsynaptic cistern. Identifiable junctional complexes have only been seen in rare regions where the cistern is not evident along 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.

The relative distribution of each type of bouton differs for the various parts of trigeminal neurons (Table 1), although DC terminals are rarely seen and are, therefore, not included in the following distributional scheme. On the soma, 54% of the terminals are S-boutons; nearly 38% of the endings are P-boutons; and the remaining 8% are C-boutons. When somatic spines are present, they are usually postsynaptic to S-boutons. The axon hillocks of trigeminal neurons are contacted by both S- and P-boutons.

Within the neuropil, proximal dendrites (greater than 5 μm in cross-sectioned diameter) are mostly covered with S- and P-boutons (Fig. 20); C-boutons also contact these dendrites (Table 1). Intermediate-sized dendrites (diameters of 2.5 to 5.0 μm) are covered with terminals containing either spherical or pleo-

Fig. 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.

Fig. 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, × 160.

Fig. 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, × 700.

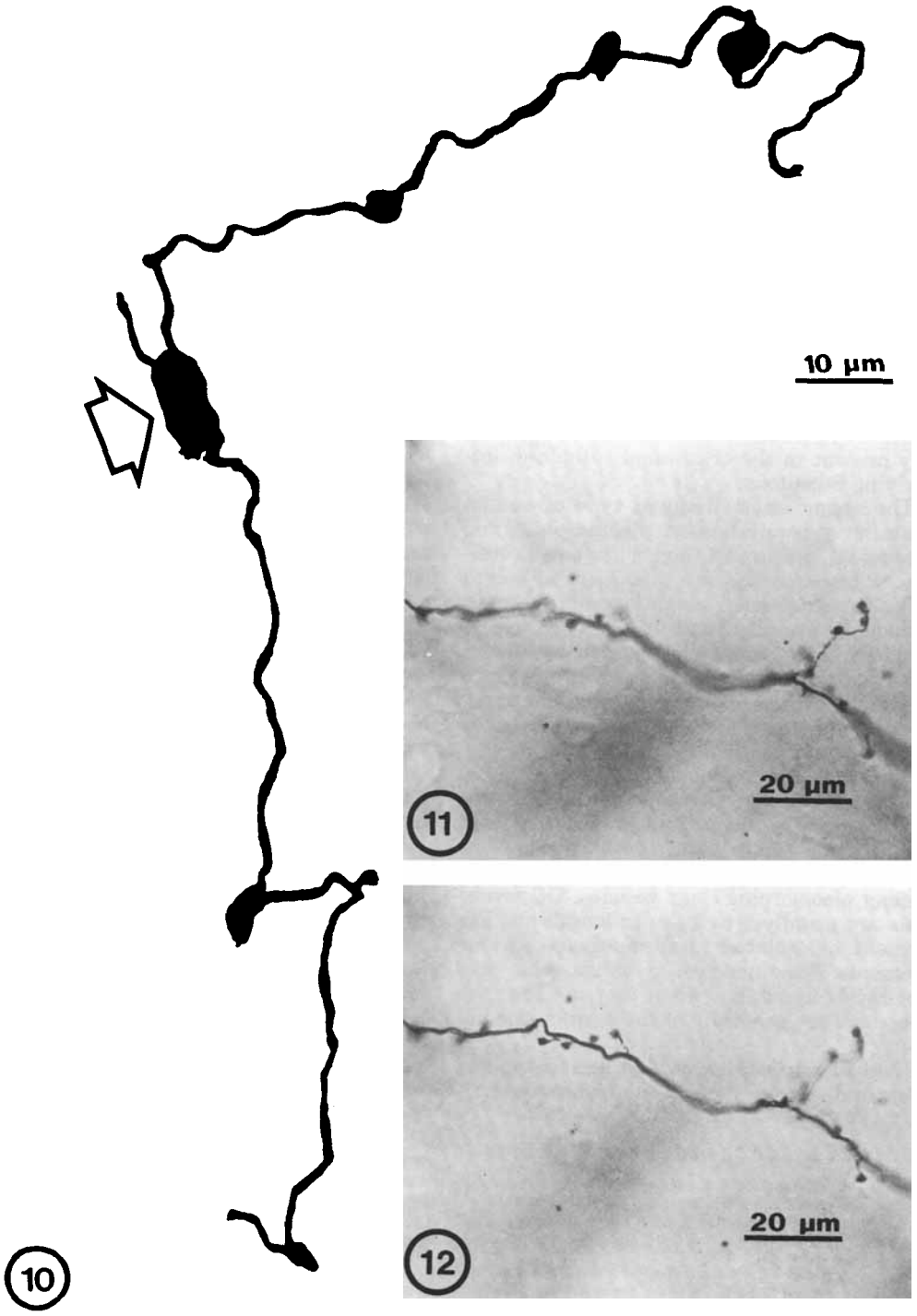


Fig. 10. A camera lucida drawing of an axon which courses through the trigeminal motor nucleus and appears as a "string of beads." Open block arrow indicates a varicosity which measures approximately 10 μm in maximum length. Golgi-Kopsch preparation, × 1400.

Figs. 11, 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, × 700.

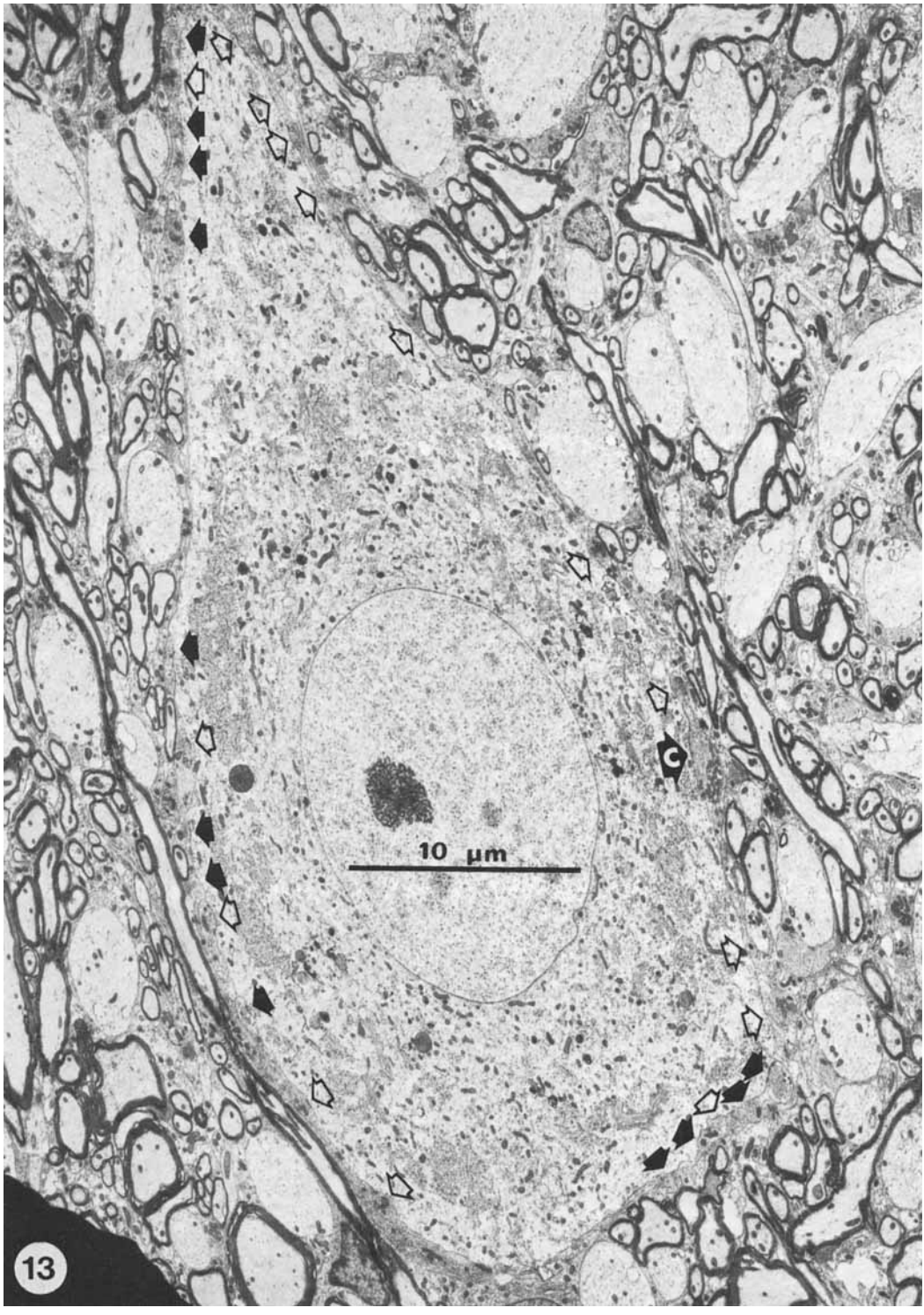
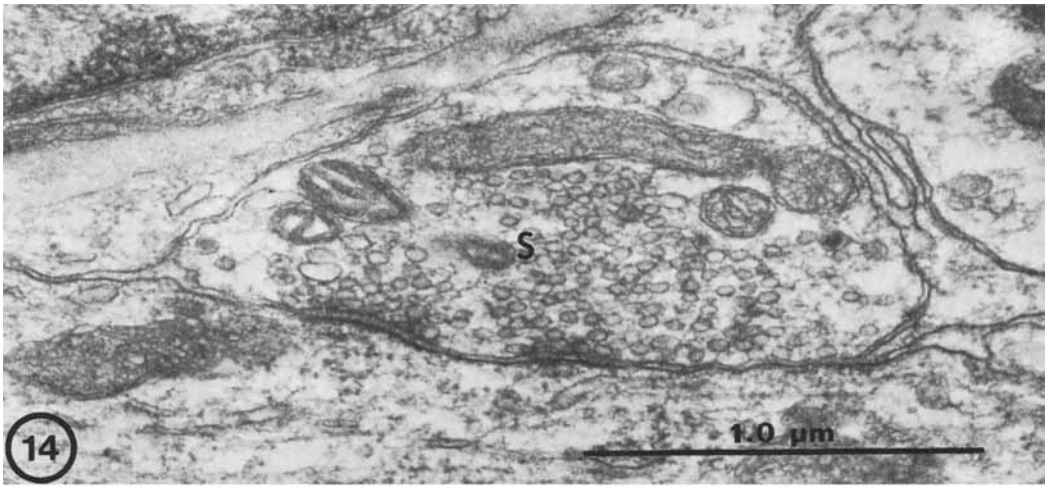


Fig. 13. A profile of a neuronal cell body in the trigeminal motor nucleus. Synaptic terminals cover approximately 40% of the somatic membrane of this neuron. These endings are indicated as follows: S-boutons, solid arrows; P-boutons, open arrows; C-boutons, solid arrow with 'C'. Electron micrograph, × 3500.


S
14
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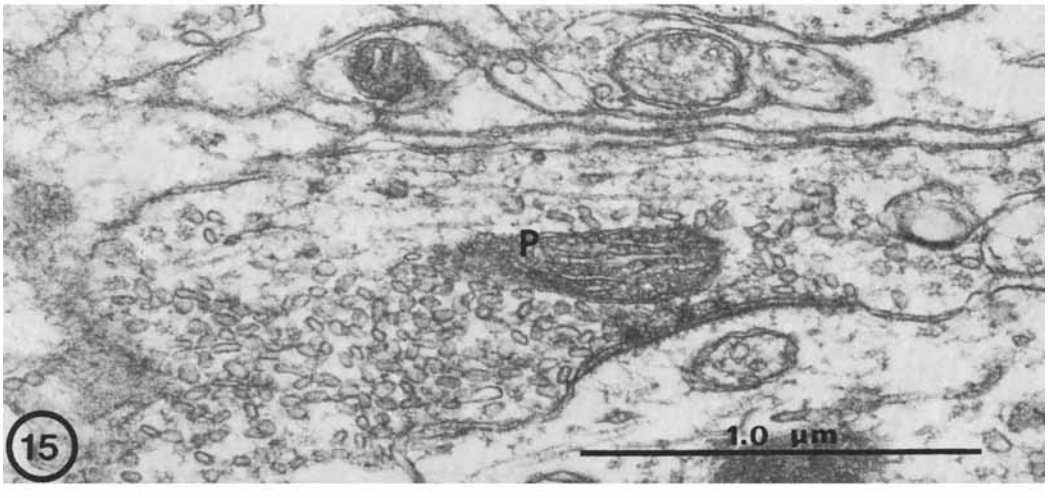
P
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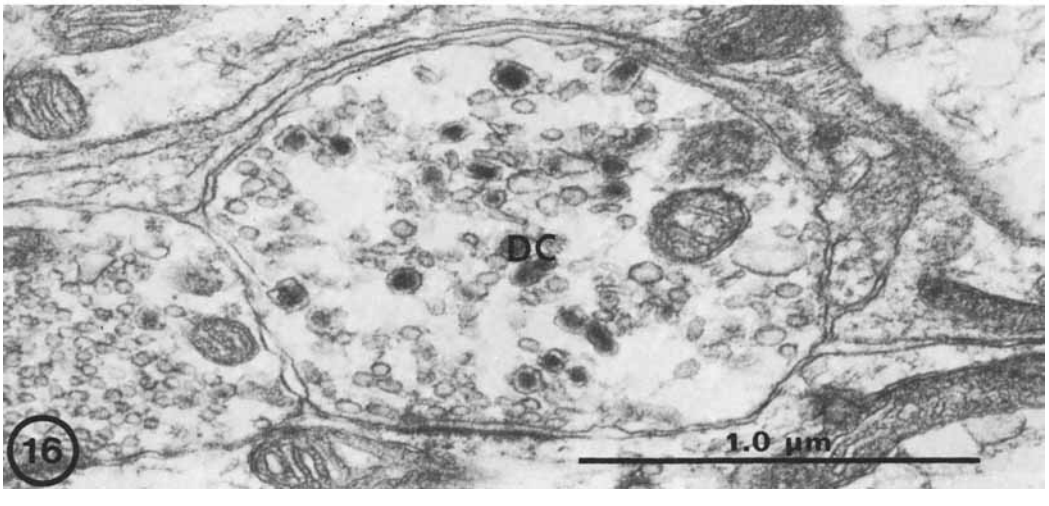

DC
16
1.0 µm

morphic vesicles (Fig. 21); these dendrites are never postsynaptic to C-boutons. Finally, distal dendrites (diameters less than 2.5 μm) are most frequently contacted by 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, seen as isolated profiles within the neuropil (Fig. 23), are postsynaptic to S- and P-boutons (Table 1).

In summary, while S-boutons constitute a significant population of terminals (54%) on trigeminal perikarya and proximal dendrites, they are the predominant type on distal dendrites. Conversely, P-boutons represent almost 40% of the terminals on the cell body and proximal dendrites, but they diminish in number distally. C-boutons are present only on the cell body and proximal dendrites. Isolated spines within the neuropil are contacted by more pleomorphic-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

The majority of trigeminal mesencephalic neurons are destroyed after lesions of the midbrain tectum. At a survival time of 2 days, only large myelinated axons (1 to 4 μm in diameter) show an increase in electron density indicating degeneration. However, at survival times of 3 (Fig. 24) to 5 days there are degenerating myelinated axons as well as changes in synaptic terminals. Most altered boutons are electron-dense in appearance, contain spherical vesicles, and contact either distal 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). The first indication of degeneration is an increase in electron density of the axoplasmic matrix (Fig. 27). Subsequently, the terminals become swollen and are surrounded by glial processes (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). Frequently, a single degenerating C-bouton (Fig. 29, solid block arrow) is found adjacent to an apparently normal bouton (Fig. 29, 'C'). Very few degenerating axons or terminals are found in the trigeminal motor nucleus 7 days after a lesion of the tectum. However, expanded atypical glial processes are present in apposition to trigeminal neurons (Fig. 30).

Electron-dense degeneration is found 2 days after stereotaxic lesions which destroy areas around the contralateral trigeminal motor nucleus or which undercut commissural 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 neurons in the trigeminal nucleus. In animals with longer survival times boutons are engulfed by glial elements.

DISCUSSION

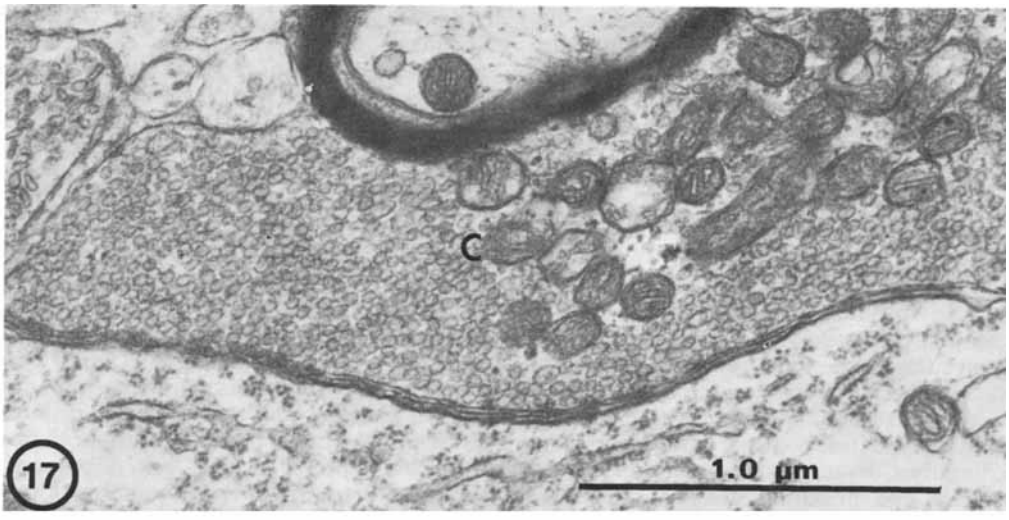
Cytoarchitecture and cytology

It is apparent that neurons of the opossum trigeminal motor nucleus form a homogenous morphological pool of cells and, as might be expected, they have cytological features identical to those of the motoneurons which have been described in the motor regions of many species (Bodian, '64; Takana, '64; Conradi, '69; McLaughlin, '72a; Bak and Choi, '74; Sumner, '75b; Falls and King, '76a; Tredici et al., '76; Spencer and Sterling, '77; Destombes et al., '79). Characteristically, when viewed at the light microscopic level they are large, with numerous Nissl bodies, and concomitantly, their outstanding feature when viewed in the electron microscope is many parallel stacks of rough endoplasmic reticulum. Ultrastructurally, motoneurons also have many axosomatic contacts that are not as prominent in many neuronal cell groups. Despite the homogeneity described above, however, it is im-

Fig. 14. A synaptic terminal containing spherical synaptic vesicles and making an asymmetric junction with a trigeminal neuron–S-bouton. Electron micrograph, × 53,000.

Fig. 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, × 53,000.

Fig. 16. A synaptic terminal containing both dense-core and pleomorphic agranular vesicles and making an asymmetric junction with a trigeminal neuron–DC terminal. Electron micrograph, × 53,000.


C
17
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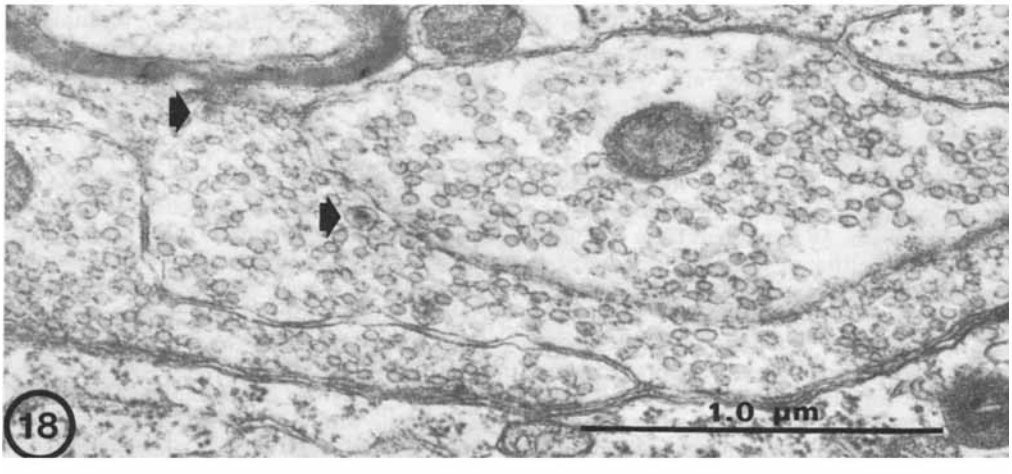
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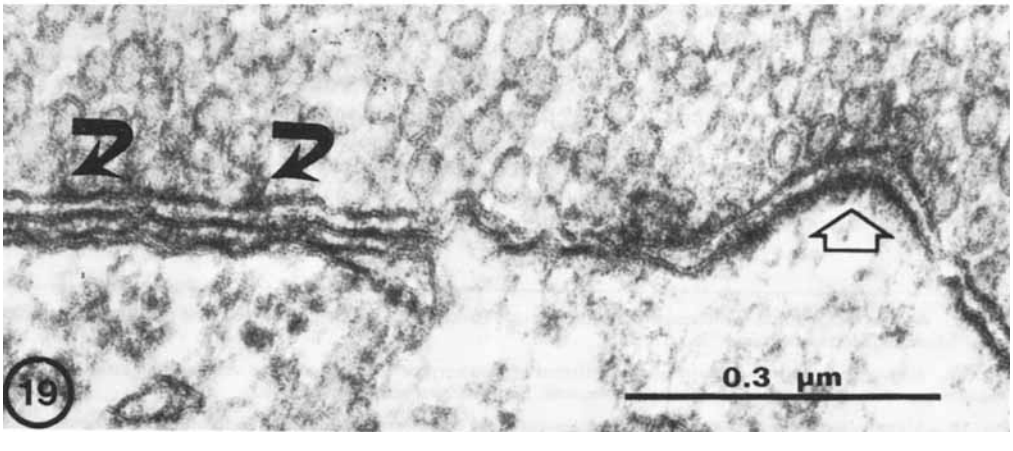
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portant to realize that there are functional subclasses of trigeminal neurons which must have inherently different patterns of synaptic organization dispersed within their domains.

Of particular interest in the trigeminal motor nucleus is the presence of separate neuronal populations which innervate jaw-closers versus those that innervate jaw-openers, these being two major classes of antagonist muscle groups which are innervated by trigeminal motoneurons. Various investigators have attempted to determine the location of motoneurons innervating specific muscles of mastication in the cat, rat, and rabbit, species whose trigeminal motor nuclei do not have a dorsomedial cell cluster (Mizuno et al., '75; Batini et al., '76; Limwongse and DeSantis, '77; Sessle, '77a; Matsuda et al., '78). These studies have localized the jaw-closers (masseter, medial and lateral pterygoids, temporalis) and jaw-openers (anterior digastric, mylohyoid) to the general dorsal and ventral parts of the nucleus, respectively. Dom (personal communication) has examined this question in the opossum and found that the unique cytoarchitectural configuration of the trigeminal nucleus in this species reflects the functional representation of the muscles of mastication. That is, while the jaw-closer motoneurons are, once again, in the dorsal portion of the nucleus, neurons innervating the jaw-openers are separated with cells innervating the anterior digastric muscle present in the isolated dorsomedial cell cluster, while smaller neurons innervating the mylohyoid muscle are in the ventromedial quadrant of the main body of the nucleus. Since the two general muscle groups have antagonist actions the synaptic organization of each subpopulation of neurons must differ even within the overall framework of afferents arriving from identical places in the neuraxis. As the elucidation of such facts would require an experimental paradigm beyond the baseline data which is being presented here, such distinctions are not attempted in this report.

Within the wide class of neurons which innervate muscles there are also two functional subpopulations, i.e., alpha and gamma motoneurons which innervate extrafusal and intrafusal (muscle spindle) fibers, respectively. Lund et al. ('78) have described muscle spindles in the two large jaw-closers, the temporalis and masseter muscles, in the cat, while such specializations have not been found in the jaw-opening muscles of any species (Karlsson, '76). This suggests that there should be gamma motoneurons in jaw-closing regions of the nucleus, and there is correlative physiological evidence showing that there are alpha and gamma motoneurons present in these areas of the trigeminal motor nucleus of the cat (Sessle, '77a). Anatomically, there is very little information which could be used to make any distinctions for cell types in motor regions. In fact, size, which is often an important criteria for distinguishing populations of neurons, has been the sole criteria used to describe the two major physiological classes of motoneurons (Bodian, '64; Takana, '64; Conradi, '69; McLaughlin, '72a; Bak and Choi, '74; Sumner, '75b; Tredici et al., '76). As shown in this study, neuronal size in the opossum trigeminal nucleus varies over a wide range (Fig. 4) without definable separations based on cell body area. Additionally, the fine structural characteristics of all trigeminal neurons appear similar. This ultrastructural homogeneity of trigeminal motoneurons is consistent with description of other motor regions (Bodian, '64; Takana, '64; Conradi, '69; McLaughlin, '72a; Bak and Choi, '74; Tredici et al., '76) although it 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 features which distinguish some small neurons from the characteristic large ones. Overall, then, there are no morphological clues for physiological subclasses of neurons in the opossum trigeminal nucleus except for possible arbitrary separations on the basis of size.

Fig. 17. A synaptic terminal containing spherical synaptic vesicles and associated with a subsynaptic cistern located in a trigeminal neuron–C-bouton. Electron micrograph, × 48,000.

Fig. 18. Three interdigitating C-boutons are present. The arrows indicate dense-core vesicles within a C-bouton. Electron micrograph, × 48,000.

Fig. 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 not evident. × 140,000.

TABLE 1. Distribution of boutons

	Distribution of boutons[1]							
	Cell bodies[2]		Dendrites[3]				Spines[4]	
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	

[1] DC terminals have not been included due to their infrequency.
[2] Total boutons on cell bodies = 795.
[3] Total boutons on all sizes of dendrites = 676.
[4] 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.

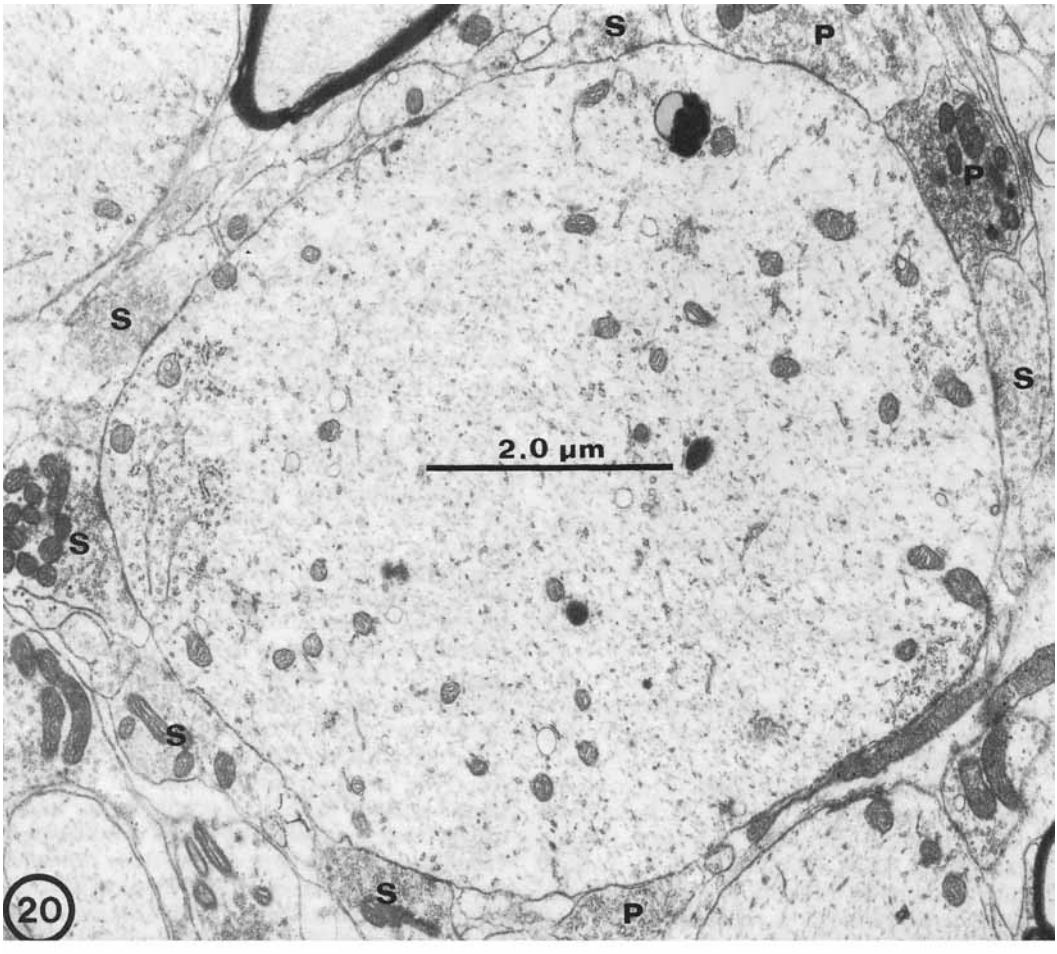
Additional techniques, such as the intracellular injection of an electron opaque material into physiologically identified cells, must be utilized to determine the anatomical correlates for the physiological data. Perhaps with this approach more subtle variations in morphology will come to light.

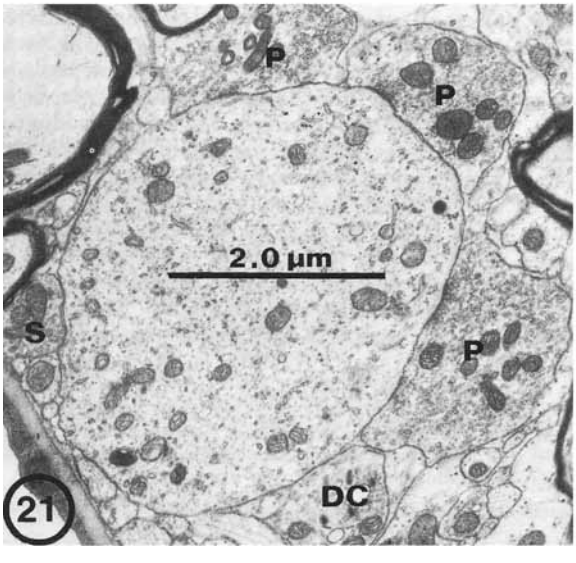
Boutons and their distribution

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. While DC, S-, and P-boutons are commonly found throughout the central nervous system, C-boutons seem to be a type of synaptic endings unique to motor regions. Such terminals which are unmistakenly related to a subsynaptic cistern have been previously specifically localized in the ventral horn (Bodian, '66; Conradi, '69; McLaughlin, '72a), the hypoglossal nucleus (Sumner, '75b), the facial nucleus (Falls and King, '76b), and the oculomotor nucleus (Tredici et al., '76).

A comparison of several motoneuronal regions suggests that the general distribution of synapses on the cell bodies and dendritic trees remains fairly constant. C-boutons, when present, are found only on the soma and proximal dendrites and represent 4 to 10% 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). These findings do not exclude the possibility of differences in the precise pattern of synapses among the various functional subclasses of motoneurons, i.e., alpha versus gamma motoneurons or neurons innervating extensors versus those innervating flexors.

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 vesicles, while endings that have an inhibitory function contain flattened or pleomorphic synaptic vesicles. Generalizing this hypothesis to the present data, 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) are the predominant synaptic type on trigeminal motoneurons, whereas presumed inhibitory boutons (P-boutons) are numerically more significant on the cell body and proximal dendrites. At the same time, spines (somatic and dendritic) receive a higher proportion of inhibitory terminals than do dendritic shafts (Table 1). The accuracy of this interpretive theorem remains to be determined by physiological methods not only in the trigeminal motor nucleus, but also in each area of the nervous system. This is especially true in light of evidence which has shown that, at least in





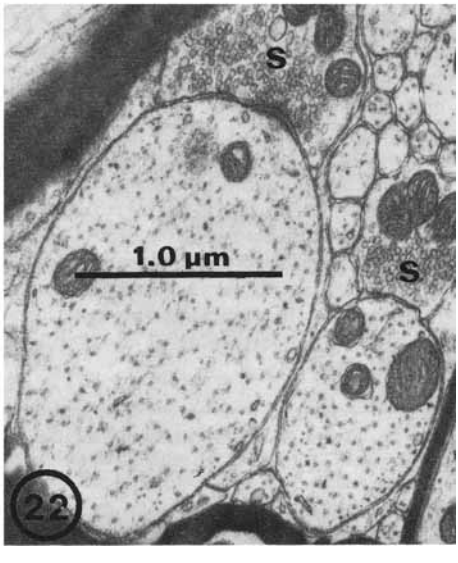


Fig. 20. A cross-sectioned proximal dendrite which is contacted by a variety of S- and P-boutons. Electron micrograph, × 16,000.

Fig. 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, × 14,000.

Fig. 22. Two cross-sectioned distal dendrites which are contacted by S-boutons. Electron micrograph, × 27,000.

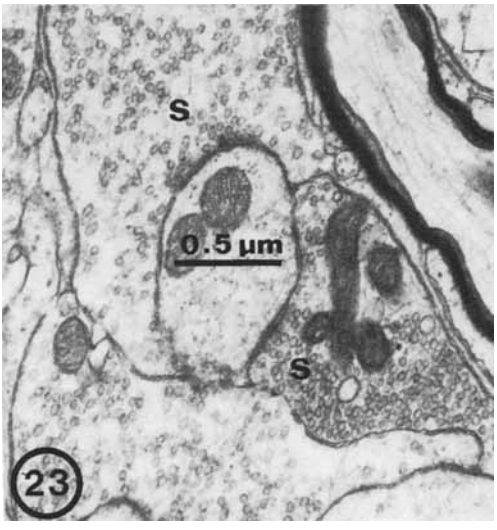


Fig. 23. A cross-sectioned isolated spine which is contacted by two S-boutons. Electron micrograph, × 28,000.

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

Although the "distribution of synapses" shows the same trend in the various motor regions, more subtle variations seem to exist in each area. 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 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., medial vestibular and oculomotor inhibitory input to the abducens nucleus and Renshaw and inhibitory interneuron feedback to spinal motoneurons. Other reasons for these discrepancies may be technical and may simply reflect sampling differences or variables in fixation methods.

Synaptic organization

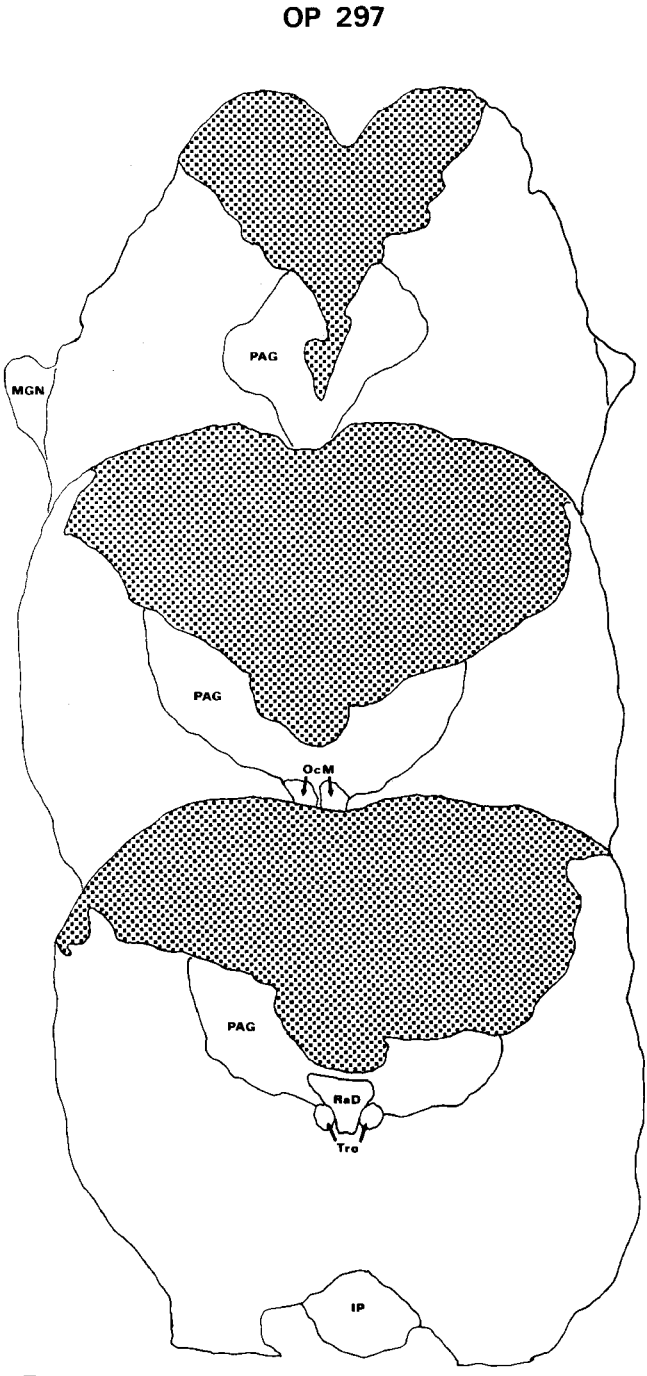
In the opossum there are at least three major sources of input into the trigeminal motor nucleus (Panneton and Martin, '79; Panneton and Martin, personal communication): 1) the mesencephalic nucleus of the trigeminal nerve, 2) the medullary and pontine reticular formation, and 3) the trigeminal complex itself. With our knowledge of the normal morphology of the trigeminal nucleus as a basis, we have used the technique of localizing degeneration at the electron microscopic level in an attempt to determine the wiring pattern of these sources of input on trigeminal motoneurons and, in this way, to provide a useful guide to the synaptic organization of this region. Additionally, in correlation with this anatomical data, some appropriate physiology is considered.

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 a monosynaptic, excitatory reflex arc for jaw-closing muscles, i.e., the jaw jerk (for review, see Bratzlavsky, '76). Many of the 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 the peripheral processes of mesencephalic neurons include periodontal mechanoreceptors (Hannam, '76) and the various types of temporomandibular joint receptors (Storey, '76), including Golgi tendon organs (Lund et al., '78). 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

Fig. 24. A line drawing illustrating the extent of a tectal lesion from an animal with a 3-day survival-time. The following structures are labelled for reference: interpeduncular nuclei (IP), medial geniculate nucleus (MGN), nucleus raphe dorsalis (RaD), oculomotor nucleus (OcM), periaqueductal gray (PAG), and trochlear nucleus (Tro).

Fig. 25. A degenerating terminal (solid block arrow) containing spherical synaptic vesicles contacts a spine. Tectotomy, 3-day survival. Electron micrograph, × 40,000.

Fig. 26. A degenerating terminal (solid block arrow) containing spherical synaptic vesicles is isolated by glial processes. Tectomy, 3-day survial. Electron micrograph, × 51,000.



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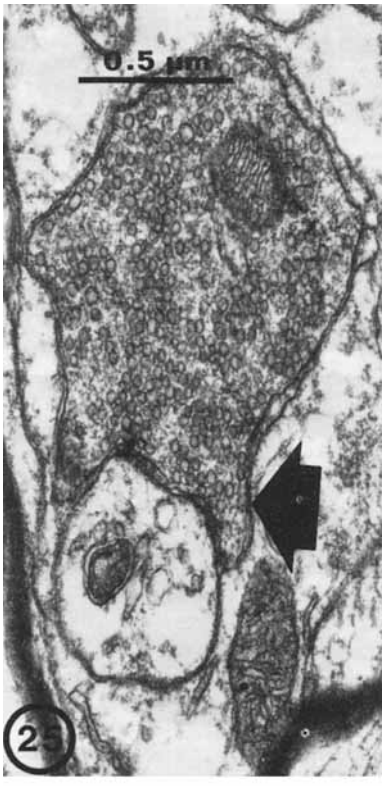

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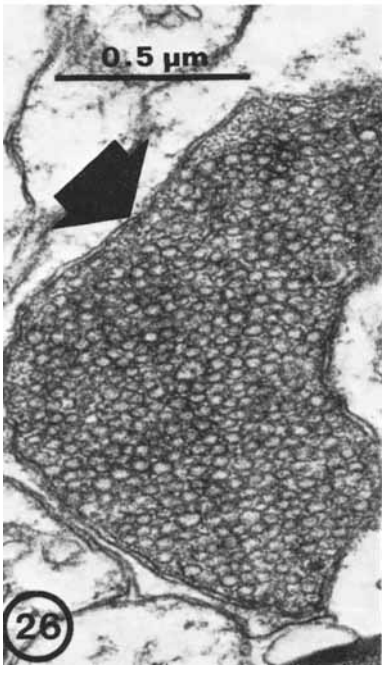

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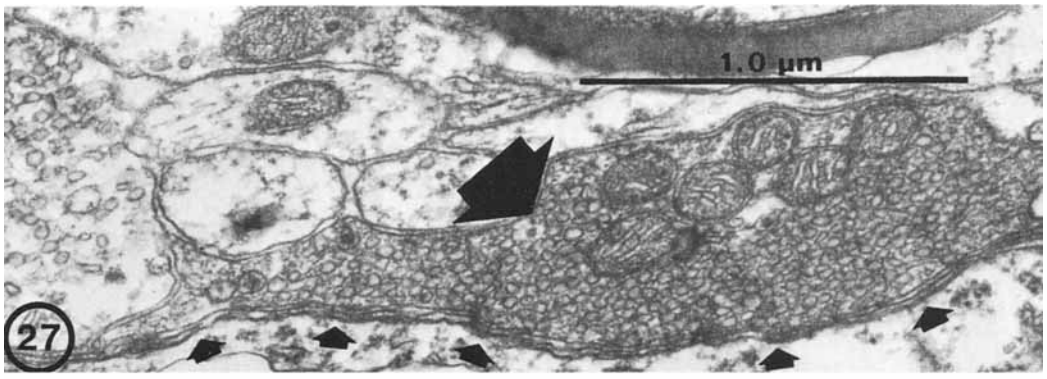
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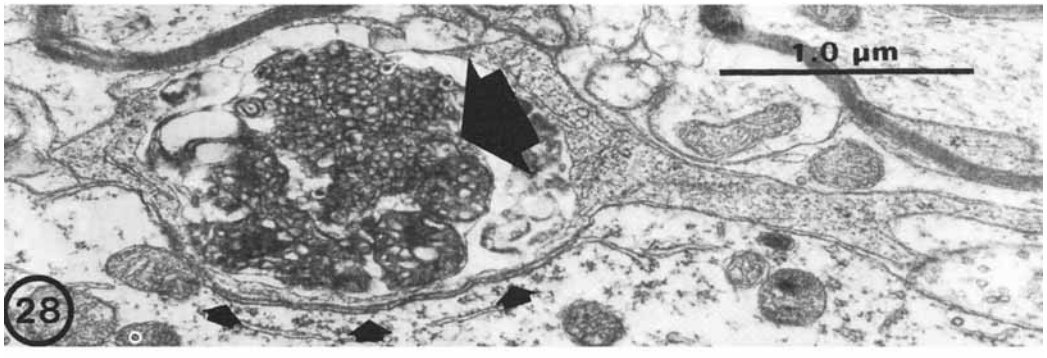
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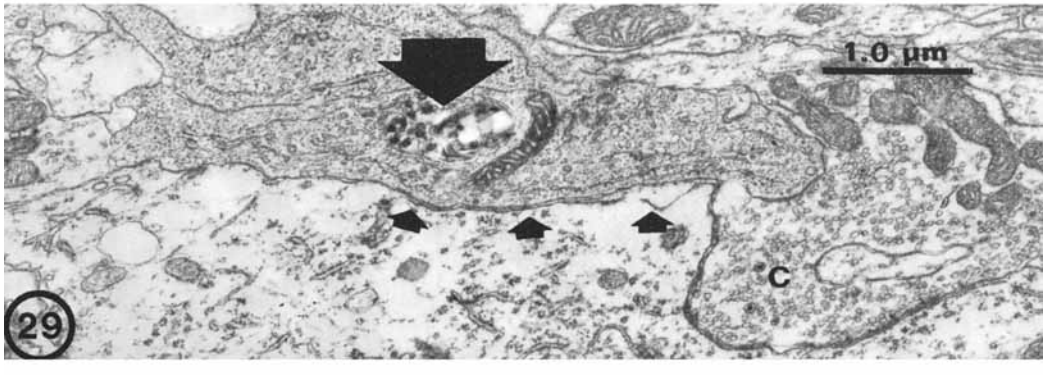
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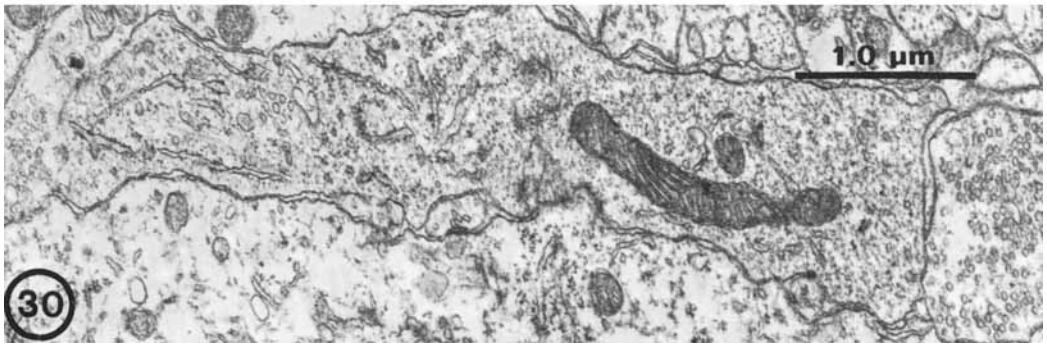
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lack of a monosynaptic mesencephalic input to the neurons which innervate these muscles (Sessle, '77a).

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. 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. Mesencephalic neurons are not a homogenous functional population as they have peripheral receptors activated by a variety of stimuli (muscle spindles, Golgi tendon organs, free nerve endings; Hannam, '76; Karlsson, '76; Storey, '76). Thus, it is reasonable to expect that the central terminations of mesencephalic neurons would differ in some of their cytological characteristics.

Earlier attempts to identify the sources of C-boutons in other motor regions were not successful. Conradi ('69) noted the presence of subsynaptic cisterns which were not associated with an overlying terminal in the ventral horn of the spinal cord after dorsal root section. However, the inability to find electron-dense 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). The present results illustrate that in the trigeminal motor nucleus at least some of the C-boutons originate from the mesencephalic nucleus of the trigeminal nerve and that they are central terminations for some proprioceptive fibers. Connectional data (Panneton and Martin, '79) show that there are no other possible sources of afferents to the trigeminal motor nucleus which may have been destroyed by our large tectal lesions. All in all, these results suggest that there are differences in the synaptic organization of motor regions which have very similar morphology; however, future studies should determine if these differences are due to variables in experimental technique or if they reflect some unique feature of the respective areas.

Divergent areas of the neuraxis 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 (i.e., Group I muscle afferents, Group II mucosal afferents, periodontal 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 certain cortical and intraoral afferents (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 trige-

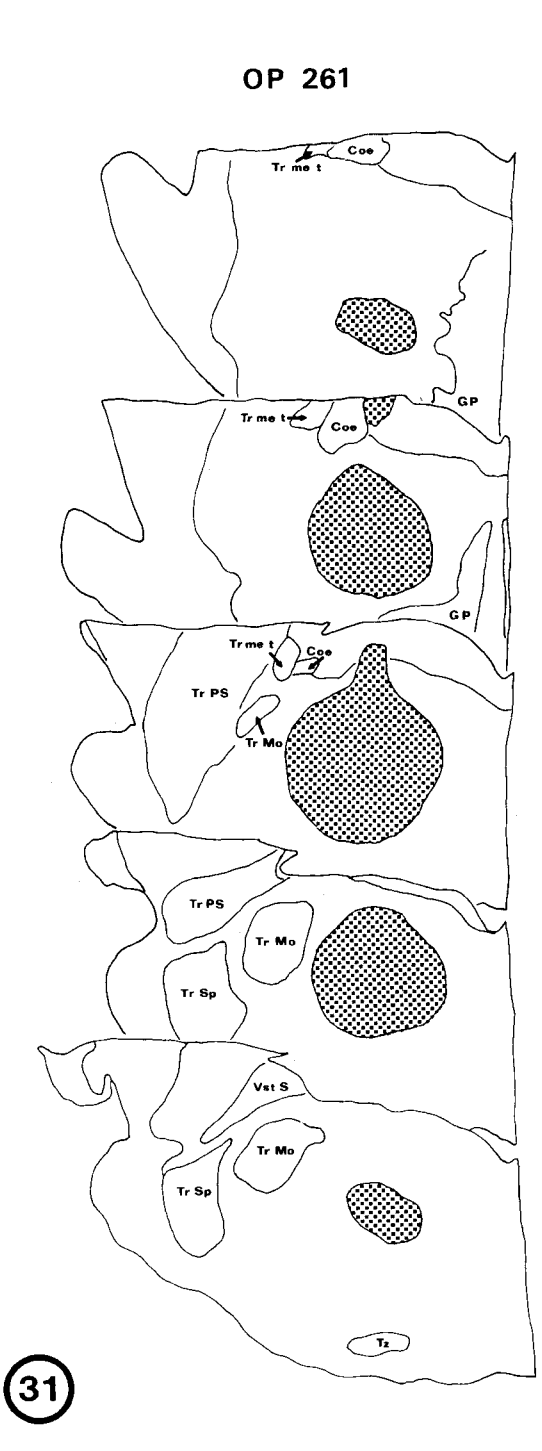
Figs. 27–30. Sequence of electron micrographs showing suggested stages of degeneration of C-boutons after a tectotomy (3-, 5- or 7-day survival). Large solid block arrows indicate the degenerating terminals. Small solid block arrows show the continuing presence of a subsynaptic cistern through much of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

Fig. 27. Electron-dense degeneration of a C-bouton. × 51,000.

Fig. 28. Engulfment of an electron-dense and swollen C-bouton by glial processes. × 33,000.

Fig. 29. Completed stage of engulfment by glial processes. 'C' indicates a normal C-bouton which has remained intact. × 20,000.

Fig. 30. A remaining, expanded glial sheath which is not over a cistern. × 24,000.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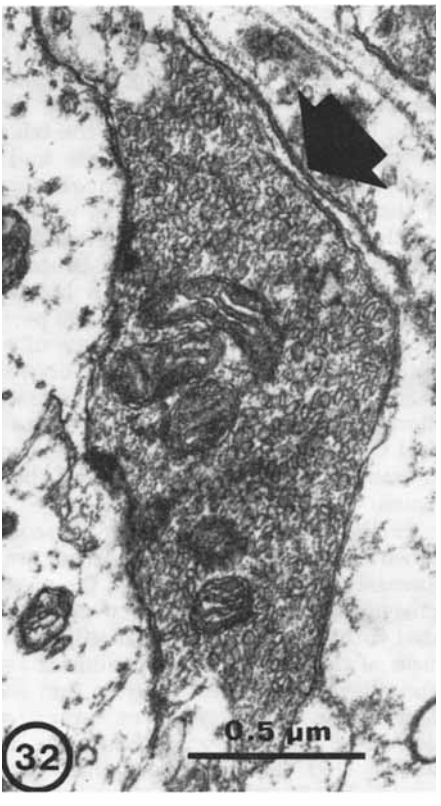
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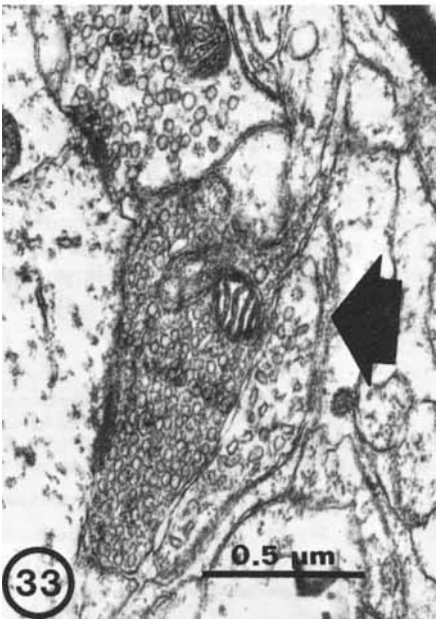
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minal neurons; that is, MBRF stimulation monosynaptically inhibited masseteric motoneurons and excited digastric ones. Recently, it has been reported that the jaw-opening reflex is suppressed by the injection of morphine into a portion of the MBRF, 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 ipsilateral or contralateral trigeminal motor nucleus after hemisections of the medulla or pons (survival times of 2 and 3 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 within the trigeminal motor nucleus as has been suggested by physiological studies (Sauerland and Mizuno, '69; Goldberg, '76).

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 have suggested that neurons from these nuclei act as interneurons for the trigeminal motor nucleus, both ipsilaterally 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 intraoral 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 neuronal labelling 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 these data, the labelled neurons are most likely analogous to the supratrigeminal 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 times of 2 days, some electron-dense terminals containing either spherical or pleomorphic vesicles were found approximating variably sized dendrites. These findings are similar to those reported by Mizuno et al. ('78). Due to the proximity of the supratrigeminal and intertrigeminal neurons with the trigeminal motor nucleus, the ipsilateral connections of these interneurons were not studied.

Terminals which are similar to the presently described DC terminals are frequently found in areas of the nervous system which receive a monoaminergic innervation (Chan-Palay, '77). Recently, a noradrenergic innervation from the A1 and A5 cell groups has been shown for the trigeminal motor nucleus of the cat (Silver et al., '79). These correlative data may provide a potential source and transmitter for DC terminals in the trigeminal nucleus although hemisections of the brainstem caudal to the nucleus, which should cut

Fig. 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 2-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).

Fig. 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, × 46,000.

Fig. 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, × 43,000.

axons of the A1 and A5 neurons, have not resulted in clear-cut degeneration of DC terminals. Some conflicting evidence to this hypothesis has been shown by Card and Moore ('79) in the rat, where after treatment with the neurotoxin 6-hydroxydopamine, statistical studies show that there is a reduction in the populations of some terminals similar to both the S- and P-boutons which have been described in this report.

The findings presented in this study have shown the normal morphology of the opossum motor nucleus of the trigeminal nerve while just beginning to reveal its synaptic organization. A wide variety of inputs, including a significant proportion of terminals containing pleomorphic vesicles, reach the cell body of trigeminal neurons and must still be identified as to their origin. Also, distinctions must be made as to the source and localization of input onto alpha versus gamma motoneurons as well as onto those neurons innervating jaw-closers versus those innervating jaw-openers.

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