# MORPHOLOGICAL AND MORPHOMETRIC ORGANIZATION OF THE TRIGEMINAL MOTOR NUCLEUS IN THE *CEBUS* MONKEY

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■ ABSTRACT: The morphologic and morphometric characteristics of the neurons of the trigeminal motor nucleus were studied in five New World monkeys (*Cebus apella*). Trigeminal motoneurons were identified by injecting a 25% solution of horseradish peroxidase into the masseter, temporal, medial pterygoid, lateral pterygoid, and anterior digastric muscles. Cytoarchitectonically, the trigeminal motor nucleus was composed of lateral and dorsomedial divisions. In Nissl stain sections the somal diameter of trigeminal motoneurons ranged between 16.15 and 52.73 μm, the mean being 32.89 μm (SD ± 4.97 μm). The distribution of parameters such as area, minimum diameter and average somal diameter was unimodal, which precluded morphometric differentiation between alpha and gamma motoneurons.

■ KEYWORDS: Trigeminal nuclei; motoneurons; *Cebus apella*; alpha and gamma motoneurons.

## Introduction

Anatomic studies on trigeminal motor nucleus (Vmot) reveal the existence of two motoneuron populations, identifiable by morphometric criteria[12, 22, 23] and by their electrophysiological properties.[25] These two populations would be formed by alpha (skeletomotor) and gamma (fusimotor) motoneurons, with cell body diameter of 25 and 15 μm respectively. Although physiological studies have confirmed the existence of alpha and gamma motoneurons in the Vmot,[26] recent morphometric studies done on different species[14, 15, 26] have not had success in subdividing these two neuronal populations, which puts in doubt the possibility to differentiate anatomically alpha and gamma motoneurons of brainstem motor nuclei. On the other hand, morphological

heterogeneity observed in the population of motoneurons of the Vmot can be related to other factors, among them to the different histochemical characteristics of the muscle fiber population observed in the muscles supplied by the Vmot. In those muscles, three different types of muscle fibers have been identified[29] and some authors have observed a relationship between the size of the motoneuron and the characteristics of the muscle fiber that it innervates. [4, 5] This fact could explain the difficulty that some authors have encountered in subdividing morphometrically the motoneurons of the Vmot in only two populations.

In the present study we analyze the morphometric distribution of the neuronal population of the Vmot and the citoarchitectonic pattern of this nucleus in five New World monkeys (*Cebus apella*).

## Material and method

### Identification of motoneurons by retrograde transport of horseradish peroxidase (HRP)

Five male, adult Cebus monkeys (*Cebus apella*) weighting 2 to 4 kg, from a colony kept by Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus of Araçatuba, Brazil, were used in this experiment. The animals were anesthetized with an intramuscular injection of Ketamine hydrochloride (10-20 mg/kg) followed by an intra-abdominal injection of pentobarbital sodium (30 mg/kg).

Trigeminal motoneurons were identified by the injection of 25% HRP (Sigma, type VI) dissolved in destilled water, into the masseter, temporal, medial pterygoid, lateral pterygoid, or anterior belly of the digastric muscle, with a 10 μl Hamilton syringe. One muscle on each side in each animal was exposed and injected. To prevent the diffusion of the neuronal tracer into the neighboring structures, the injections were given very slowly, endeavoring to occupy all of the muscular belly. After the injections, the area exposed was abundantly irrigated with saline solution and the surgical wound carefully sutured.

### Histochemical and histological procedures

After a survival period of 48 hours, the animals were deeply anesthetized with pentobarbital sodium (30 mg/kg, intra-abdominal) and initially perfused with 800 ml of saline solution at room temperature, followed by 3000 ml of fixative solution containing 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, also at room temperature. They were subsequently perfused with 500 ml of 5%, 10% and 20% phosphate-buffered sucrose solution at 4°C. The brain stems were

immediately removed and maintained in sucrose solutions of 20 and 30% at 4°C, until complete saturation. The region corresponding to the pons was cut serially at 40 μm in a cryostat. Two alternate series of sections were utilized for the histochemical demonstration of HRP using tertramethylbenzidine as chromogen,[18] then mounted onto gelatinized slides and one of them counterstained with 1% neutral red. A third series, without reaction, for morphometric and morphologic analysis, was mounted and stained with 2% Cresylechtviolet (Nissl method).

For the morphometric analysis, Nissl stained sections were measured by means of a computerized image analyzer system (Mini Mop, Kontron Bildanalyse) associated with a light microscope. The cursor of the analyzer system was equipped with a diode that emitted a light point which was reflected through the drawing apparatus into the microscope. This enabled microscopic images to be evaluated directly from the source.

Certain criteria were utilized to select the neurons to be included in the morphometric analysis: exact demarcation of the cell body, nucleus and nucleolus in focus, and precise localization of the neuron inside the Vmot. Delineating carefully the outline of each motoneuron, some parameters as area, minimum and maximum diameter, were calculated. With the objective of comparing our results to those of other authors, which analyze varied and different parameters, we present the results obtained on somal area, minimum diameter, and average somal diameter (ASD) calculated from: (maximum diameter + minimum diameter)/2. The analysis of frequency distribution of the three parameters mentioned above were represented in histograms (Figure 3).

## Results

HRP injection into the masticatory muscles of the *Cebus apella* following histochemistry treatment resulted in an effective retrograde labelling of the cell body of some Vmot motoneurons (Figure 2). This allowed a reliable identification of this nucleus, thus avoiding any possible confusion to neighboring motor nuclei such as those of the facial and/or oculomotor nerves.

As in other species already studied,[3, 17, 20, 28, 31] the Vmot lies in the mid-lateral tegmentum of the rostral pons, medial to the root fibers of the trigeminal nerve, and near the junction with the midbrain of the *Cebus apella*. The clusters of large and heavily stained motoneurons in Nissl preparations stand out in contrast to the smaller and lesser stained cells of the surrounding neuropil (Figure 1A, B).

It is constituted fundamentally of typical multipolar and spindle-shaped motoneurons of various sizes, each with a large central nucleus, and coarse, deeply stained tigroid bodies (Figure 1B), that when grouped together formed a nucleus of ovoid form, with its rostrocaudal long axis measuring 1.9 mm (SD ± 0.2) in length. When cut transversal, the Vmot presented an oval shape, with a maximum diameter of 1.4 mm (SD ± 0.28). This diameter, when projected in a superior direction, described an angle of approximately 45° in relation to the vertical axis (Figure 1A).

In the rostrocaudal direction a small dorsomedial subdivision was distinguished beginning at the middle third of the main body of the nucleus (Figure 1A). This subdivision was not observed in the rostral third of the Vmot.
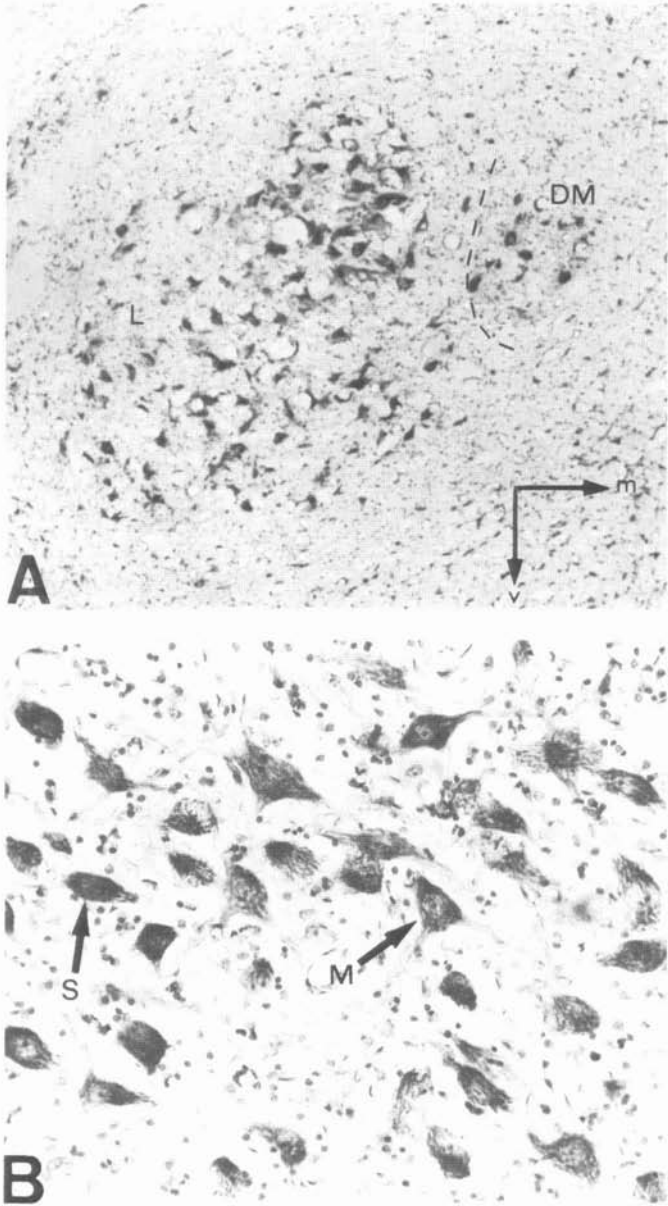


FIGURE 1 – A) Transverse section through the middle level of the trigeminal motor nucleus of the *Cebus apella*. Lateral (L) and dorsomedial (DM) subdivisions are indicated (Nissl stain, x 200, m, medial; v, ventral). B) Different morphological forms (arrowheads) of trigeminal motoneurons: M, multipolar motoneurons; S, spindle-shaped motoneurons (Nissl stain, x 450).
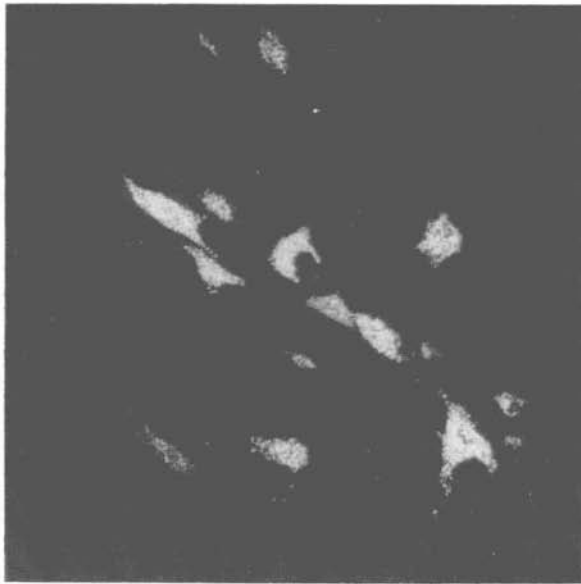
FIGURE 2 – Dark field photomicrograph of retrogradely labeled motoneurons in the Vmot following HRP injection in the masseter muscle (x 450).

In the Nissl stained sections, 1338 trigeminal motoneurons were analyzed. Their mean area measured 700.63 $\mu m^2$ (SD ± 200.29 $\mu m^2$), with a range between 200 $\mu m^2$ – 1510 $\mu m^2$. The minimum diameter varied between 11.23 $\mu m$ and 38.69 $\mu m$, the mean being 23.89 $\mu m$ (SD ± 4.88 $\mu m$). The ASD varied between 16.15 $\mu m$ and 52.73 $\mu m$, the mean being 32.89 $\mu m$ (SD ± 4.97 $\mu m$). The distribution of frequency histograms of each of the analyzed parameters presented a clear unimodal distribution (Figure 3).

## Discussion

In Macaca monkeys, the Vmot has been divided cytoarchitectonically into dorsolateral and ventromedial parts.[20] This same division is observed in the cat,[3,28] rat,[13] guinea pig[31] and corresponds to the γ and β nuclei described in the rabbit.[17] We observed a similar division in the Cebus monkey. In this species, however, the medial, smaller division of the nucleus, was located in the dorsomedial aspect of the nuclear body (Figure 1A).

Yet unpublished anatomical studies made in our laboratory revealed that simultaneously with this architectonic organization, there was a precise musculotopic organization of the trigeminal motoneurons. Jaw closing-muscles had their motoneuronal population clearly segregated in the lateral division of the nucleus, while motoneurons of the jaw opening-muscles were localized in the dorsomedial subdivision.
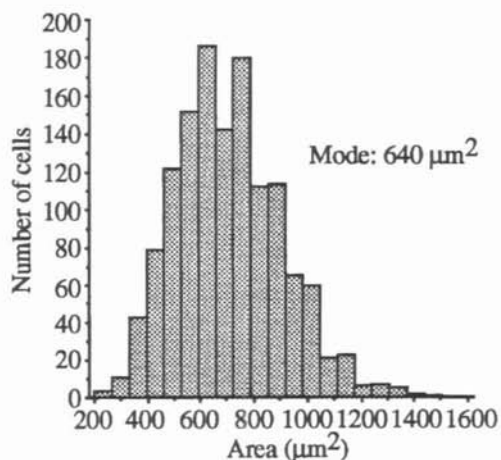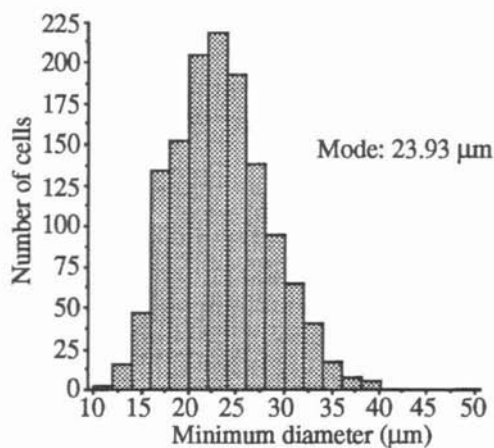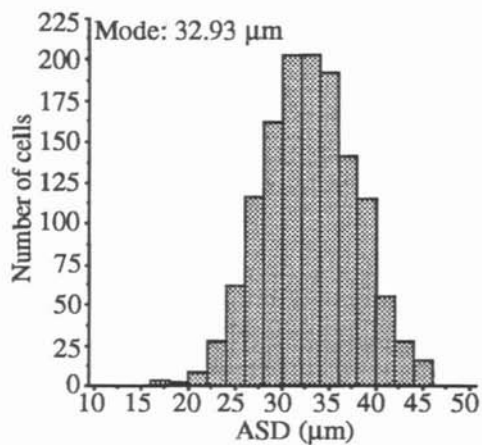
FIGURE 3 – Histograms showing the distribution of averages of ASD, minimum diameter, and area. In the three cases the distribution was unimodal.

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This myotopical arrangement of masticatory motoneurons has been reported in the rat,[13] cat,[19] rabbit,[16] guinea pig[31] and Macaca monkeys,[20] with little pattern variation. Comparative studies reveal that even in lower vertebrates such as Agnathans, Elasmobranchs, Teleosts, Amphibia, Reptilia and birds, the organization of the trigeminal motor column is highly conserved regardless of the huge modifications observed in the structure of the mandibular musculature.[27] This phylogenetically conservative pattern seems to be the result of a similar ontogenetic development of the Vmot among craniate species.[21]

Our results indicate that the measurements of the area, mean and minimum diameter obtained in the neuronal population of the Vmot of the Cebus monkey are similar to those observed by other authors in cranial motoneurons of different species. Previous papers revealed that the mean soma diameter of facial motoneurons was found to be 35.4 μm,[30] tensor tympani motoneurons 26.3 ± 1.8 μm,[8] oculomotor neurons 35.9 ± 6.9 μm,[7] and abducens motoneurons 25 ± 0.5 μm.[9] In the only morphometric paper dealing with trigeminal motoneurons in macaque monkeys, Mizuno et al.[20] obtained a value of 23.1 μm (SD ± 0.33 μm) in the diameter of jaw-opening (mylohyoid) motoneurons. The same authors observed that jaw-opening motoneurons are smaller than the jaw-closing motoneurons. This can also be observed in the Cebus monkey, and probably support the differences in relation to our results, since jaw-closing and-opening motoneurons were both included in our study.

It is interesting to note that, in general, motoneurons of cranial nerve nuclei are smaller than spinal ones. Burke et al.[4, 5] obtained values between 52.5 μm and 55 μm in diameter for spinal alpha motoneurons and approximately 27 μm for spinal gamma motoneurons. So, spinal gamma motoneurons presented a diameter near the average of the majority of the cranial motoneurons. Our trigeminal motoneurons, with a mean soma diameter being 32.89 ± 4.97 μm are smaller than spinal motoneurons, but similar to other groups of cranial motoneurons.

Limwongse & DeSantis[12] distinguished, in accordance to the soma diameter, two populations of motoneurons (bimodal distribution) in the rat Vmot: gamma motoneurons with peaks at 10-16 μm and alpha motoneurons 22-30 μm. Curiously, the population of gamma motoneurons was larger than the population of alpha motoneurons. This result is very disconcerting since physiological studies [25] already indicate that the ratio of masseter and temporalis alpha to gamma motoneurons is approximately 3.5:1. A ratio similar was noted for spinal motoneurons. At variance with the bimodal distribution obtained by Limwongse & DeSantis [12] and by Rokx et al.[22, 23] in rats, our results indicate that in the Cebus monkey, the trigeminal motoneurons present a clear unimodal size distribution (Figure 3), which precluded morphometric differentiation between alpha and gamma motoneurons.

Bimodal distribution of motoneurons has already been observed in spinal motoneurons and also associated to the existence of gamma and alpha motoneurons;[4] however, morphological differences between gamma and alpha motoneurons have not been encountered in some motor nuclei of cranial nerves,[6, 15] even though the

existence of gamma motoneurons in these nuclei have been confirmed through physiological studies.[25, 26] These studies indicate that (1) the conduction velocities of alpha fibers in the Vmot and in other nuclei of cranial nerves is smaller than that observed in spinal alpha fibers and (2) the conduction velocities of gamma and alpha trigeminal fibers overlap each other. These data seem to indicate that cranial motoneurons do not always fall within an alpha or gamma classification. According to these studies, this overlapping in the conduction velocities can be related to morphometric similarities between the two neuronal populations. Our results, which present a unimodal distribution of the motoneurons, are in agreement to these physiological characteristics of the trigeminal motoneurons and to the unimodal distribution of the diameter of trigeminal motor fibers observed in anatomic studies.[10]

The discrepancies as compared to the Limwongse & DeSantis[12] results, can be related with interspecific variations, although more recent papers[11] have shown that even in the rat, bimodal distribution of parameters such as area and average diameter is not very evident, and that other parameters such as major and minor axes of Vmot cell bodies have a clear unimodal representation, which is in agreement with our results.

Other factors besides the existence of alpha and gamma motoneurons, as the existence of histochemical and functionally different types of muscle fibers encountered in the various muscles supplied by the trigeminal nerve, can influence the kind of distribution that we observed in the population of trigeminal motoneurons. In limb muscles, small motoneurons innervate tonic fibers and large and medium size motoneurons innervate fast glycolytic and fast oxidative-glycolytic fibers.[5] Very probably, a similar situation can be observed in the masticatory complex, and in this way, the morphometric heterogeneity of the neuronal population in the Vmot would not only be related to the simple fact of the existence of alpha and gamma motoneurons, but also, with the existence of different types of skeletomotor neurons. Therefore, depending on the characteristic distribution of muscle fibers of each species, related to its masticatory behaviour, a diverse morphometric aspect could be observed in the Vmot. Earlier reports[1, 2] reveal that the distribution of these muscle fibers of masticatory muscles in the Cebus monkey is different from that observed in rodents.[24] This fact could partially justify our divergence with the results obtained by Limwongse & DeSantis.[12]

## Acknowledgments

■ *RESUMO: As características morfológicas e morfométricas dos neurônios do núcleo motor do nervo trigêmeo foram estudadas em cinco macacos* Cebus apella. *Os motoneurônios trigeminais foram identificados pela injeção de* horseradish peroxidase *(HRP) nos músculos masséter, temporal, pterigoideo medial e ventre anterior do músculo digástrico. Do ponto de vista citoarquitetônico, o núcleo motor do nervo trigêmeo foi subdividido numa porção lateral e outra, menor, ventromedial. O diâmetro dos motoneurônios, corados pela técnica de Nissl, variou entre 16,15 e 52,73* μm, *sendo a média de 32,89* μm. A distribuição de parâmetros como área, diâmetro mínimo e diâmetro médio foi unimodal, o que impossibilitou uma diferenciação morfométrica entre motoneurônios alfa e gama.

■ *PALAVRAS-CHAVE: Núcleos do trigêmeo; motoneurônios trigeminais;* Cebus apella; *motoneurônios alfa e gama.*

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