Location of the motoneurons of the mylohyoid muscle in the rat

A fluorescence and Nissl study

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ABSTRACT

Objectives: To locate the neuronal motor cells of the mylohyoid muscle and discuss their topographical organization.

Methods: The present study was conducted at the Department of Anatomy and Histology, Faculty of Medicine, University of Jordan, Amman, Jordan between 2002 and 2003. The mylohyoid muscle in 15 albino rats was injected with 15 µliter of a retrogradely transported fluorescent material DAPI-Pr. After a survival period of 48 hours, animals were sacrificed, fixed in situ and brains harvested. The caudorostral transverse sections of the hindbrains were examined under the fluorescence microscope to detect the fluorescing cells, which were immediately photographed. Sections containing the labeled cells were charted, stained with 1% thionine and photographs obtained through light and fluorescence microscopes at different magnifications. The place and shape of all labeled cells were singled out by asset of their charted referring photographs of hindbrain sections, which display the entire motor trigeminal nucleus.

Results: The results showed that the fluorescent cell increase was found to occupy the rostromedial part of the ipsilateral motor trigeminal nucleus. The nucleus was large at its caudal third; the labeled cells are mainly those of the medial "subgroup". These cells are rationally distinct and lie alongside the internal loop of the facial nerve. At the middle third, most of the medial "subgroup" was found labeled. At its middle, the nucleus found was well developed, attained an appreciable size and its medial "subgroup" was somewhat distinct. Whereas, at the rostral third, the nucleus was larger, the medial group was more distinct and all cells were labeled. The medial cellular mass of the nucleus showed reduced labeled cells at the rostral end.

Conclusion: This study demonstrates that the rostromedial part of the motor trigeminal nucleus represents the absolute territorial domain of the mylohyoid muscle motoneurons.

Neurosciences 2005; Vol. 10 (1): 85-89

The ventromedial part of the motor trigeminal nucleus of the mouse was found to provide the nerve supply to the mylohyoid muscle.[1] Similar results were documented in the rat but restricted to the caudal one-third of the motor trigeminal nucleus.[2] Gromysz et al[3] admitted its caudality, but shift this innervation of the mylohyoid muscle to neurons in the intermediate part of the nucleus in the rabbit.[3] Others reported that, these neurons are medially located and intermingling with those described for the anterior belly of the digastric muscle.[4,5] The medially positioned neurons allocated in the rat were found to be related to other muscles such as the temporalis and the masseter.[2,6] The ventromedial part of the nucleus provide neurons to the lateral pterygoid muscle in the rat,[2] whereas, the

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Received 15th March 2004. Accepted for publication in final form 5th June 2004.

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ventral and the ventromedial parts of its rostral two-thirds provide neurons to the medial pterygoid in the rabbit.[7] Apart from the tensor tympani muscle, all tiny areas of the motor trigeminal nucleus are not exclusive for one muscle.[8]

Do the stem neurons of the mylohyoid muscle occupy the entire medial extent of the motor trigeminal nucleus? The adopted technique showed the exact location of the motor neurons supplying the mylohyoid muscle and, eventually, unlabeled cells in the same area. The controversy in the literature concerning this localization is introduced, and the topography of the stem cells of the mylohyoid muscle is discussed.

Methods. The present study was conducted at the Department of Anatomy and Histology, Faculty of Medicine, University of Jordan, Amman, Jordan, from January 2002 to June 2003. Fifteen Wistar albino rats of either sex weighing 300g were used. Each was anesthetized by an intra-abdominal injection of pentobarbitone sodium (30mg/kg). The mylohyoid muscle was exposed at one side via skin incision followed by resection of the anterior belly of the digastric muscle. The mylohyoid muscle was injected with a predetermined efficient dose of 15 µliter of 2.5% of 4'6-Diamidino-2-phenyl indole 2 hydrochloric acid added to equal amounts of 5% Primuline (DAPI-Pr) for 5 minutes by Hamilton syringe mounted on a micro-drive machine. The needles were left in place for another 3 minutes then slowly withdrawn, the covering tissues readjusted and the wound sutured carefully. Injection dose of less than 10 µliter was found inappropriate for muscles of that thickness and size. After a survival period of 48 hours, the animals were sacrificed by an overdose of pentobarbitone sodium. The animals were fixed in situ by 10% buffered formalin infused through the aorta and left for 6 hours. Their brains were harvested and the brainstems were marked with a nick opposite the injected muscle and stored in 10% buffered formalin with 30% sucrose solution for 4 days. Caudorostral transverse sections of the hindbrains were cut by the freezing microtome at 60-micrometer thickness. All pontine sections were collected then mounted on gelatinized slides. Slides were examined without cover slips under fluorescence microscope, fitted with 365-micrometer wavelength excitation filter to show the fluorescing cells. All labeled cells were immediately photographed, to illustrate them among the unlabeled cells. Sections containing labeled cells were charted, stained with 1% thionine, cover-slipped and photographs obtained through light microscope at different magnifications. The labeled cells were singled out by virtue of their charted places and overall by their shapes. The place and level of labeled cells were identified by referring photographs of the hindbrain sections stained by alternating Nissl (1% thionine), displaying the entire motor trigeminal nucleus. **Figure 1** demonstrates only half the photographs of these transverse sections. The other halves were mirror imaged by their photocopies and were labeled as earlier published.[9-12]

The adopted method revealed the factual place of these cells and disclosed the labeled versus the unlabeled cells from the cellular patch under study. The present technique also shed light on the type of cells that supply the muscle.

Results. Only the motor trigeminal nucleus and the mesencephalic trigeminal nucleus showed labeled cells in the brainstem after injecting the mylohyoid muscle with DAPI-Pr. The ipsilateral motor trigeminal nucleus displayed a labeling pattern of its own, revealed by consecutive caudorostral transverse sections (**Figure 1**). At the caudal third, the trigeminal motor nucleus is quite large and showed distinct labeled cells lying beside the internal loop of the facial nerve (**Figure 1B - 1D**). The cells that appear at this level are almost only the motoneurons of the medial "subgroup". Caudality is confirmed by the exclusion of any part of the mesencephalic trigeminal nucleus that starts to appear more rostrally. At the middle third, the greater part of the medial "subgroup" was found labeled (**Figure 1E - 1F**). This group of cells is evidently the most medial, and beyond which the cytoarchitecture of the surrounding medial neuropil is very different and could not be counted as trigeminal motoneurons. At the middle of its caudorostral extent, where the locus ceruleus appears prominent and the mesencephalic trigeminal nucleus becomes visible, the trigeminal motor nucleus appeared well developed, attained an appreciable size and its medial "subgroup" was quite distinct (**Figure 1F**). At the rostral third, the trigeminal motor nucleus is comparatively large, the medial group is more distinct and all cells are labeled (**Figure 1G & Figure 2A - 2C**). Clusters of a few labeled cells showed advancement laterally to the intermediate territory of the nucleus (**Figure 2B**). This lateral extension of the labeled cells bears the possible meaning of an overlap from the side of the mylohyoid with stem cells of the intermediate column supplying other muscles of mastication. Under fluorescence microscope, these labeled cells appeared filled with small granules that sparkled with white color. The sparkling granules fill the entire perikaryon of the motor cells, except the nucleus (**Figure 2C**). The medial cellular patch of the nucleus at this level represents the absolute territorial domain of the mylohyoid muscle; no other muscle is sharing or overlapping this area of the nucleus. At the rostral end of the trigeminal motor

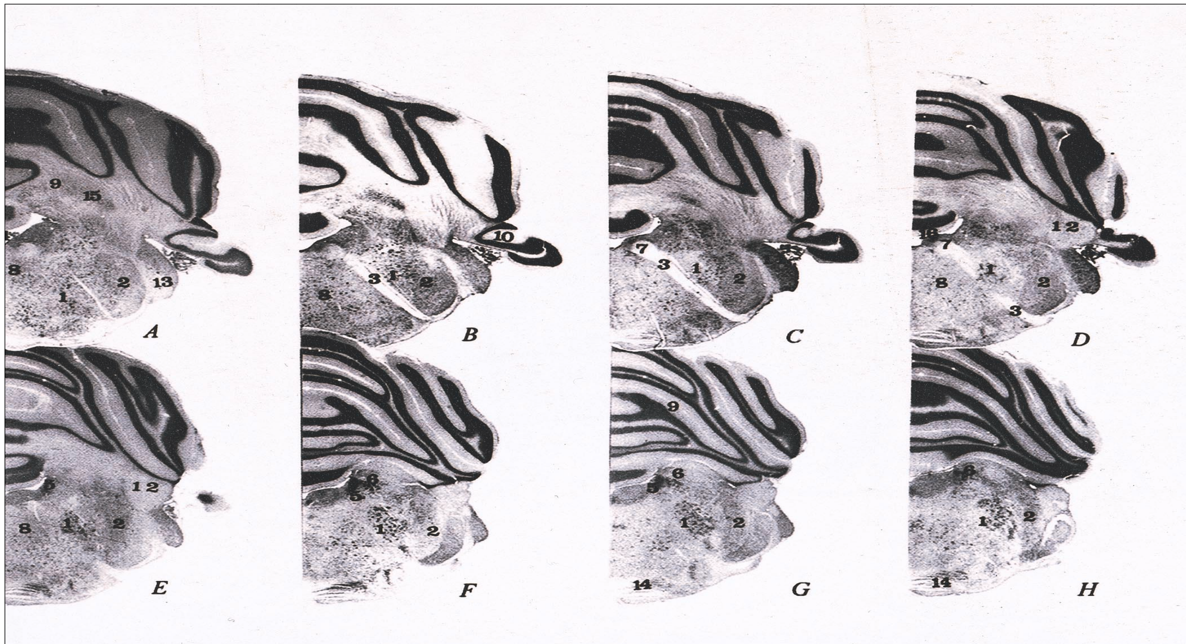

Figure 1 - Light microscopic photographs of alternating Nissl (1% thionine) stained sections of the hindbrain of the rat. It displays the entire extent of the motor trigeminal nucleus in one-half of these sections. The other half was omitted to avoid an eventual asymmetry. 1. Motor trigeminal nucleus, 2. Sensory trigeminal nucleus, 3. Internal loop of facial nerve, 4. Fourth ventricle, 5. Locus ceruleus, 6. Mesencephalic trigeminal nucleus, 7. Internal genu of facial nerve, 8. Reticular formation, 9. Cerebellum, 10. Flocculonodular, 11. Cochlear nucleus, 12. Middle cerebellar peduncle, 13. Inferior cerebellar peduncle, 14. Corticospinal tract, 15. Deep cerebellar nuclei, 16. Abducent nucleus. (Nissl X 13).

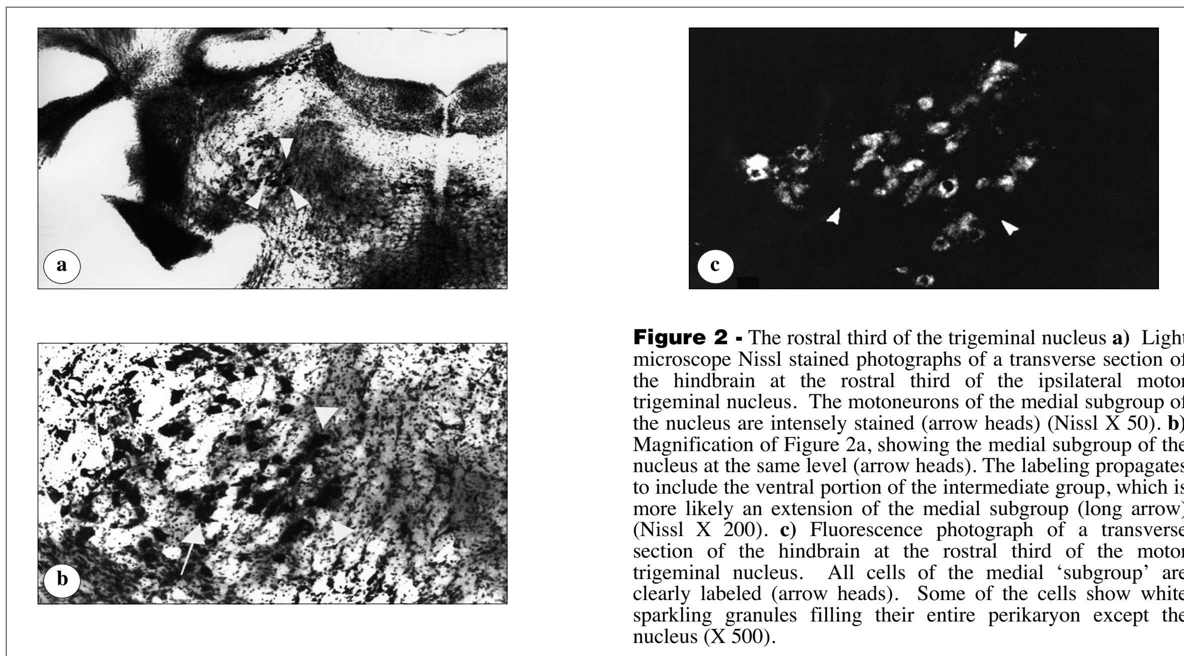

Figure 2 - The rostral third of the trigeminal nucleus **a)** Light microscope Nissl stained photographs of a transverse section of the hindbrain at the rostral third of the ipsilateral motor trigeminal nucleus. The motoneurons of the medial subgroup of the nucleus are intensely stained (arrow heads) (Nissl X 50). **b)** Magnification of Figure 2a, showing the medial subgroup of the nucleus at the same level (arrow heads). The labeling propagates to include the ventral portion of the intermediate group, which is more likely an extension of the medial subgroup (long arrow) (Nissl X 200). **c)** Fluorescence photograph of a transverse section of the hindbrain at the rostral third of the motor trigeminal nucleus. All cells of the medial 'subgroup' are clearly labeled (arrow heads). Some of the cells show white sparkling granules filling their entire perikaryon except the nucleus (X 500).

nucleus where the mesencephalic trigeminal nucleus takes a quite large dimension, the medial cellular mass showed reduced labeled cells (**Figure 1H**). This indicates that the stem cells of mylohyoid extend rostrally as far as there is still a medial group.

Discussion. The fluorescence technique employed in the present study, singles out the neuronal stem cells of the injected mylohyoid muscle from the rest of the motor trigeminal cells. By comparison with the same normal Nissl stain sections, which reveal all cells of the nucleus, one can determine how much of the cellular batch took up the dye. The boundaries of the "subgroup" allocated for a particular muscle is presumptuous, as it depends on the presence or absence of eventual spaces that separate any motor cellular patch from a similar gathering of neighboring motor cells.

When horseradish peroxidase was utilized in the mylohyoid muscle, the labeled cells were detected in the ventrolateral part of the motor trigeminal nucleus,[13,14] whereas, the adopted technique showed that the labeled cells are those of the medial group. The medial part of the ipsilateral motor trigeminal nucleus groups more closely by leaving a gap free from motor cells from the rest. It appears like a circumscribed patch of cells that are known not to extend as much rostrally as the other "subgroups" of the motor nucleus.[13] Labeling of all cells in this group could not be attributed to simultaneous contamination of neighboring muscles, as adjacent muscles, likely to contamination, that occupy other places within the nucleus were found empty of labeled cells. The intermediate place of this motor nucleus was ascribed to the temporalis, and the masseter muscles.[1,15,16] These 2 muscles are neither alleged to occupy this intermediate place nor are topographically close enough to be contaminated during the mylohyoid injection. The instant proposal is that all cells of the rostromedial patch are reserved for the mylohyoid muscle. Comparison with Nissl photographs confirmed the presence of only the correspondent images of labeled cells. Therefore, the stated overlap[4,5] is not substantiated in the present study, at least for the major rostromedial part of this "subgroup" and found entirely devoted to the mylohyoid muscle. This also confirms the sub grouping of the trigeminal motor nucleus.[13,14,17]

The 2 mylohyoid muscles are made inseparable by virtue of their midline fusion. Their stem cells are revealed to occupy the most medial aspect of their motor trigeminal nuclei, a fact that renders their 2-side communication easier and more economical.[18] The rostral occupation of the stem cells within the medial part of the motor trigeminal nucleus is also justified, as the 2 bellies of the digastric muscle, which are associated most with the mylohyoid, are the ones that shall occupy a caudal position within this medial part of the nucleus. This is implied by the dual innervation of the digastric muscle and further caudality of the facial nerve.[19,20]

Decontamination of the contralateral mylohyoid muscle in the present study was indicated by the absence of labeling cells in the other side. Dispersion of the injected dye did not affect the underlying anterior belly of the digastric muscle, as this muscle was resected and no cells were found in the caudal part of the nucleus (the domain area of the digastric muscle). Also, the labeled cells in the mesencephalic trigeminal nucleus were found to occupy a place other than that reported for the anterior belly of the digastric muscle.[21,22]

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