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Original Article

NISSL STUDY OF LOWER MOTOR NEURONS OF MYLOHYOID MUSCLE

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ABSTRACT

Objective: To determine the somatotopic organization of lower motor neurons of the mylohyoid muscle in the motor trigeminal nucleus.

Design: Histological study of the rat hind-brain by fluorescence microscopy and Nissl staining.

Setting: Neuro-histological laboratory, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Materials: The mylohyoid muscle in twenty albino rats were injected with retrogradely transported fluorescent material DAPI-Pr. Fluorescence microscopy was performed, followed by subsequent staining of these sections with the Nissl stain, thionine.

Results: The fluorescent cells were found to occupy the rostro-medial part of the ipsilateral motor trigeminal nucleus. Subsequent staining of the sections with the Nissl stain thionine confirmed the rostro-medial location of the labeled cells. The entire lot of cells in this "rostro-medial subgroup" was found labeled, representing the domain area of the lower motor neurons of the mylohyoid muscle.

Conclusion: The rostro-medial subgroup of the trigeminal motor nucleus is the only one to supply the mylohyoid muscle.

KEY WORDS: Mesencephalic, trigeminal motor nucleus, fluorescence microscopy, retrograde tracer.

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INTRODUCTION

The motor nucleus of trigeminal nerve cytoarchitecturally is composed of dorsolateral and ventromedial divisions[1-2]. The former extending craniocaudally almost the whole extent of the nucleus while the latter localized to caudal two thirds of the nucleus[3]. The ventromedial division of the motor trigeminal nucleus along its whole craniocaudal extent provides innervation to the mylohoid muscle[4-6]. Gromysz et al[7] shift this innervation of the mylohyoid muscle to the neurons in the intermediate part of the nucleus in the rabbit. The medial position of the mylohoid motor neurons is intermingling with those described for the anterior belly of the digastric, temporalis and masseter muscles[6,8-10]. Chen et al[6] (1998) found ventromedial location for the lateral pterygoid and ventromedial part of the rostral two thirds of the motor trigeminal nucleus for the medial pterygoid muscle of the rabbit. Apart from the area for the tensor tympani muscle, very little of the motor trigeminal nucleus is exclusive for one muscle[11-12]. Do the stem neurons of the mylohyoid muscle occupy the entire medial extent of the motor trigemi-

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nal nuleus? The adopted technique shows the exact location of the motor neurons supplying the mylohyoid muscle and shows also the unlabelled cells. This is the first study of its kind carried out in the kingdom of Saudi Arabia for identification of the musculotopic organization of lower motor neurons of the mylohyoid muscle.

SUBJECTS AND METHODS

The left mylohoid muscle of twenty adult Wistar albino rats was injected slowly at three different places by means of Hamilton syringe mounted on a micro-drive machine, with 10-50μl of DAPI-Pr (25% of 4'6-Diamidino-2-phenyl indole-2-HCL added to equal amounts of 5% Primuline), after resection of the anterior belly of the digastric muscle under anesthesia. Forty-eight hours after injection, the tissue was fixed in situ by perfusion through the aorta with 10% buffered formalin. The brains were removed after decapitation and kept in the buffered formalin made up with 30 % sucrose solution. The control side of the brainstems were marked with a nick and kept for a few days in the same solution. The hindbrains were cut transversely on the freezing microtome into 8μ thick sections. All the pontine sections were collected, mounted on gelatinized slides and examined without cover-slips under the fluorescence microscope which was fitted with an excitation filter of 365 μ wave length and showed only the fluorescing cells. All these labeled cells were immediately photographed.

In order to show the entire area of these labeled cells among the unlabeled ones, all sections containing them were charted sequentially, stained with 1% thionine, cover-slipped and photographed. The labeled cells were singled-out from the rest by virtue of their charted places and their overall shapes. This technique revealed the exact place as well as the number of the labeled versus the unlabeled cells. Level and place of the labeled cells were identified with reference to photos made of alternating Nissl (1% thionine) stained sections displaying the entire extent of the motor trigeminal nucleus.

RESULTS

Following injection of the mylohyoid muscle with the different amounts (10-50μl) of DAPI-Pr, only two places in the brainstem showed labeled cells, the mesencephalic trigeminal nucleus and the motor trigeminal nucleus. The ipsilateral motor trigeminal nucleus displayed a labeling pattern of its own as revealed by the successive cranio-caudal transverse sections containing these labeled cells (Fig. 1, sections H through A). As seen through the fluorescence microscope, the labeled nerve cells were filled with small granules that sparkled with a white color against a deep blue background. Except for the nucleus of the cell, no other place in the perikaryon was spared from these shiny fluorescing granules. They filled the processes of the motor cells to a variable distance, more evident in the proximal segments of these processes. This microscopy revealed only those retrogradely labeled cells having their axons intact and contacting the area receiving the injected dye. Some hazy and less sparkling cells were also intermingled with the rest. No other element of the neuropil was shown through the fluorescence microscope.

At the level where mesencephalic trigeminal nucleus took a large dimension, the cellular

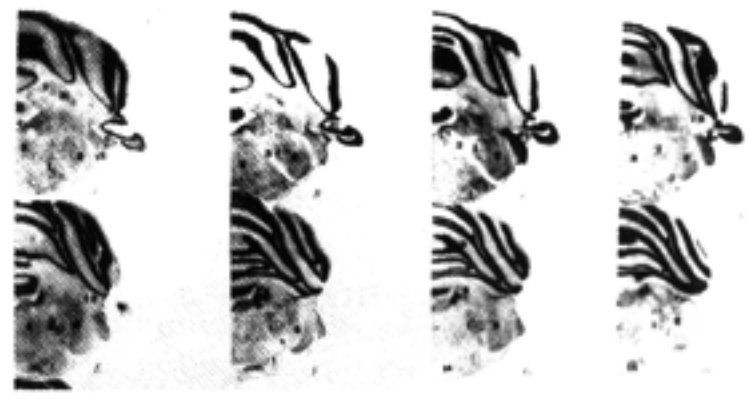

Fig. 1: 1% thionine stained sections of the rat hindbrain (X10), displaying the entire extent of the motor trigeminal nucleus. The photograph shows only one half of the transverse sections, to avoid eventual asymmetry. 1–motor trigeminal nucleus, 2–sensory trigeminal nucleus, 3–internal loop of the facial nerve, 4–fourth ventricle, 5–locus ceruleus, 6–mesencephalic trigeminal nucleus, 7- internal genu of the facial nerve, 8-reticular formation, 9-cerebellum, 10-flocculonodular lobe, 11- cochlear nucleus, 12-middle cerebellar peduncle, 13-inferior cerebellar peduncle, 14-corticospinal tract, 15-deep cerebellar nuclei, 16-abducent nerve.

mass of the medial group of the motor trigeminal nucleus was reduced. All cells of the medial patch were labeled. This indicates that the stem cells of the mylohyoid extend rostrally as far as there is still a medial group. Caudal to this level (at G in Fig. 1) the nucleus is large and the medial group became more distinct. It extended with a smaller part laterally into the ventral portion of the intermediate group. Again at this level, all cells of the medial group were labeled.

The entire cellular lot of the medial division of the nucleus at the middle third of the nucleus (corresponding to E-F of Fig. 1) is labeled following the injection of the mylohyoid muscle. No cell is spared, which would otherwise, indicate a possible overlap of this area by stem cells of other muscles occupying the medial motor division. At the level of the locus Ceruleus, which corresponds to (D and E in Fig. 1) and where the mesencephalic trigeminal nucleus started to appear, the trigeminal motor nucleus is well developed, with an appreciable size and its medial subgroup is quite distinct. This "medial subgroup" seems to be entirely specific for the mylohyoid stem cells as revealed by its sparkling with the fluorescing material. All the cells comprising the medially located "subgroup" at the above-mentioned level i.e., at the middle of the rostro-caudal extent of the nucleus, took up the dye, leaving no cells unlabeled. The medial cellular patch of the motor trigeminal 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.

The most caudal level of the trigeminal motor nucleus, which showed labeled cells following the injection of the ipsilateral mylohyoid muscle, corresponds to sections C and D of Fig. 1. The motor cells lie besides the internal loop of the facial nerve. In addition to the presence of this loop, the caudality is confirmed by the exclusion of any part of the mesencephalic trigeminal nucleus that starts to show up more rostrally. The cells of the motor nucleus that appear at this level are only those of the medial "subgroup". Other cells are not quite distinct from the surrounding reticular formation. Yet under the light microscope, some cells found dorsolateral to the medial "subgroup" resemble, to an extent, those comprising this medial "subgroup", and are assumed to be the caudal part of the intermediate motor part. The medial "subgroup" is less developed than the situation at more rostral levels.

DISCUSSION

The fluorescence technique employed in the present study singles-out the lower motor neurons of the injected mylohyoid muscle from the rest of the motor trigeminal nucleus, and by comparison with the Nissl stain of the same sections, one can determine how much of the cellular lot took up the dye. The boundaries of the "subgroup" allocated for a particular muscle are presumptuous, as it depends on the presence or absence of eventual spaces that separate any motor cellular patch from another similar gathering of neighboring motor cells. Contrary to other studies, which found the ventrolateral cells of the motor trigeminal nucleus labeled by retrograde injection of horseradish peroxidase into the mylohyoid,[2,13-14] the adopted technique in the present study showed it to be the medial group. The medial part of the ipsilateral motor trigeminal nucleus represented itself by leaving a gap free of motor cells from the rest. It looked like a circumscribed patch of cells that was known not to extend as much rostrally as the other "subgroups" of the motor nucleus[3]. The rostral part of this medial subgroup was found to be composed of entirely labeled cells, after injection of the mylohyoid with DAPI-Pr. The labeling of all cells in this group could not be due to simultaneous contamination of neighboring muscles, because the adjacent muscles, liable to contamination occupy other places within the motor trigeminal nucleus, which were found empty of labeled cells. The immediate impression is that all cells of the rostromedial patch are reserved for the mylohyoid muscle. This was confirmed by comparison with the Nissl stained photos, which

showed the presence of only the labeled cells. Therefore, the stated overlap[8,9] was not substantiated by the present study, for at least the rostro-medial part of this "subgroup", and which was found entirely devoted to the mylohyoid muscle. This also confirmed the subgrouping of the trigeminal motor nucleus[1,14-16]. The two mylohyoid muscles are made inseparable by virtue of their midline fusion. Their stem cells are shown to occupy the most medial aspect of their respective motor trigeminal nuclei, a fact that renders their two sides communication easier and more economical[17-18]. The rostral occupation of the stem cells within the medial part of the motor trigeminal nucleus is also justified. Because the two bellies of the digastric muscle, with which the mylohyoid is most associated, are the ones that shall occupy the caudal position within this medial part of the nucleus, as implied by the dual innervation of the digastric muscle by the more caudally lying facial nerve. The presented results show no contralateral labeling, which means that no contamination of the other side took place during the injection of the one side of the mylohyoid muscle. The spreading of the injected dye did not affect the underlying anterior belly of the digastric muscle because that muscle was resected, and no cells were found labeled in the caudal part of the nucleus (the domain area of the digastric muscle).

CONCLUSION

The rostro-medial subgroup of the trigeminal motor nucleus is the only one to supply lower motor neurons to the mylohyoid muscle.

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