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Original Article

Musculotopic organization of the orbicularis oculi within the facial motor nucleus of the albino rat *

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ABSTRACT

Musculotopic organization of the motor neuron pools innervating the orbicularis oculi within the facial motor nucleus of the albino rat was investigated using retrograde fluorescent tracers – Fast Blue and Diamidino Yellow – and histological techniques. The facial motor nucleus consisted of multipolar motor neurons. It had a rostrocaudal extent of 1.07 ± 0.02 cm and consisted of 5 subnuclei – medial, intermediate, dorsolateral, ventrolateral and suprafacial. Retrograde labelling by exposure of the proximal cut end of the nerve to orbicularis oculi to the tracers ipsilaterally and then bilaterally revealed ipsilateral labelling of scattered neurons in all subdivisions of the facial motor nucleus except the suprafacial. Double labelling of a few neurons were observed indicating the origin of some nerves from the contralateral nucleus also. These results confirm previous studies regarding musculotopic organization of the facial motor nucleus and support the fact that sparing of orbicularis oculi in the upper motor neuron lesions could be attributed to the bilateral innervation at the lower motor neuron level. ***Neuroanatomy; 2007; 6: 46–48.***

Key words [Fast Blue] [Diamidino Yellow] [Albino rat] [orbicularis oculi] [facial motor nucleus] [facial nerve]

Introduction

Various neuroanatomical techniques have been used to study the morphology and connections of the facial motor nucleus. These include nerve degeneration studies [1], electrophysiological methods [2,3], mechanical and electrolyte lesions [4–6] and neuroanatomical tract tracing [7–11]. This study was designed to redefine the morphology of the facial motor nucleus and to trace the cells of origin of the branch of facial nerve supplying the orbicularis oculi muscle.

Materials and Methods

Twenty albino rats of both sexes weighing between 150-200 gms were kept under standard laboratory conditions. Two per cent aqueous solution of both dyes Fast Blue and Diamidino Yellow were prepared. The animals were divided into two groups. In the first group, in half the set, Fast Blue was applied to one side while the contralateral side was kept as control and vice versa. In the second group, Fast Blue and Diamidino Yellow were applied on either side in half the set of animals and vice versa in the other half. Under ether anesthesia the branch of the facial nerve to orbicularis oculi was exposed and severed close to the muscle. The proximal cut end was exposed to 3-5 μL of the dye and sealed with wax to avoid spillage. The wound was closed and the animal allowed to survive for 72-98 hours.

Following an optimum survival period of 98 hours for both the dyes the animals were sacrificed. The animals were perfused with 10% formal saline, the brains extracted and immersed in 10% sucrose cacodylate buffer for a few hours. Fifteen μm frozen serial sections and JB-4 embedded 30 μm plastic serial sections were examined under the fluorescent microscope with a combination excitor 360 nm, beam splitter 395 nm and barrier filter 397 nm. Cresyl Fast Violet stained sections were used to determine the morphology of the facial motor nucleus. This study was conducted in conformity with the recommendations from the Declaration of Helsinki and the international guiding principles for biomedical research involving animals. And approval was obtained from the institution's review board.

Results

The facial motor nucleus was situated in the ventrolateral part of pons about 1.2 cm below the junction of midbrain and pons. It extended rostrocaudally for a mean length of 1.07 ± 0.02 mm and 1.1 to 1.5 mm lateral to the median plane. It appeared as a fine cluster of cells arranged in five subgroups – medial, intermediate, dorsolateral, ventrolateral, and suprafacial (Figure 1). Fast Blue labelled neurons of the facial motor nucleus of the first group of animals showed a blue fluorescence of cytoplasm and the initial segments of their processes, and a dark negative

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This paper was presented at the national conference of the Anatomical Society of India held in December 1995 at Wardha, Maharashtra, India.

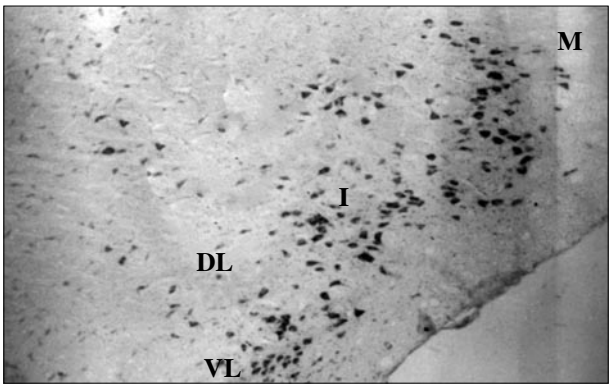

Figure 1. Photomicrograph of the ventrolateral portion of transverse section of right side of pons showing disposition of medial (M), intermediate (I), dorsolateral (DL), ventrolateral (VL) subdivisions of the facial motor nucleus (Cresyl Fast Violet stain x75).

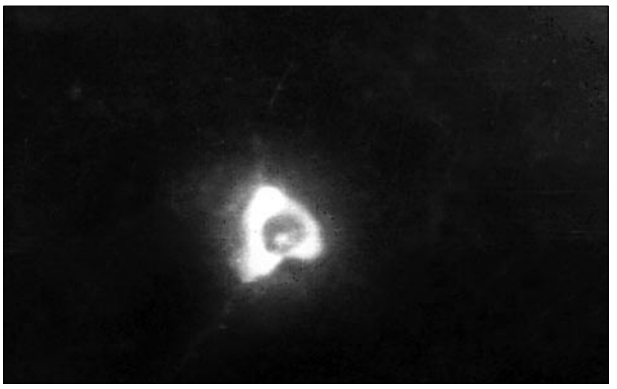

Figure 2. Photomicrograph of a facial motor neuron labelled with Fast Blue of the ventrolateral subdivision showing blue fluorescence of cytoplasm and a dark negative nuclear shadow. (x300)

Figure 3. Photomicrograph of the facial motor neurons labelled with Fast Blue, where the dye was applied to one side only. The labelled cells are seen in the medial, dorsolateral, ventrolateral and intermediate subdivisions of the facial motor nucleus. (x75)

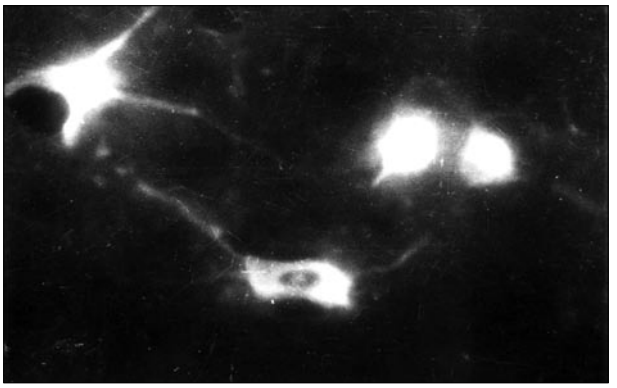

Figure 4. Photomicrograph showing a neuron in the dorsolateral subdivision of the facial motor nucleus labelled with Fast blue (with negative nuclear shadow)and others showing double labelling (no negative nuclear shadow). (x300)

Figure 5. Photomicrograph showing double labelling of neurons in the dorsolateral subdivision and the typical appearance of a Diamidino Yellow labelled neuron (arrow). (x300) Color version of figure is available online.

nuclear shadow (Figure 2). The labelled neurons of this group were not constant in the various subdivisions. The suprafacial subdivision was not labelled in either of the subgroups. The labelling was mainly seen in the ventral tier nuclei, usually the ipsilateral side with an occasional labeled cell on the contralateral side (Figure 3).

In the second group where both dyes were applied on either side of the animal and vice versa, labelling was seen in the ventral tier subdivisions of the nucleus on the ipsilateral side (Figure 4). Occasional double-labelled neurons showed intense blue somata and a bright yellow nucleus in the lateral parts of the rostral half of the nucleus (Figure 5).

Discussion

The nerve to orbicularis oculi seemed to originate from the neurons of the facial motor nucleus as seen by retrograde transport of Fast Blue and Diamidino Yellow. The neurons innervating the orbicularis oculi were located on the ipsilateral side. The labelled motor neurons were not restricted to any specific subdivision but were found scattered throughout the ventral tier nuclei except the suprafacial subdivision. This finding was in contrast to the reports of the other researchers [8,12]. It indicates that the fine cell clusters seen in the facial motor nucleus do not appear to have any morphological significance but can probably be explained on the basis of phenomenon of neurobiotaxis [13].

The presence of tracers in some divisions in a particular study [5,8,9,14] and their absence in other subdivisions

could probably be attributed to the inability to obtain serial frozen sections most commonly done in these tract tracing techniques. It is also likely that some researchers had possibly missed the labelling in particular sections. Some authors have reported contralateral neuronal labelling with HRP (Horseradish peroxidase) in rats but due to limitations of the technique the contributions to the ipsilateral side could not be demonstrated [15].

In the present study, a few neurons were double labelled with both Fast Blue and Diamidino Yellow when nerves of both sides were exposed to either of the dyes. This indicated that in the case of the nerve supplying orbicularis oculi some fibers originated from the contrlateral nucleus and that some neurons gave contributions bilaterally.

Conclusion

An attempt was made to locate the facial motor neurons supplying orbicularis oculi by retrograde fluorescent labelling and to redefine the morphology of the facial motor nucleus, using Cresyl Fast Violet staining. The facial motor nucleus of the albino rat lies in the ventrolateral part of pons as a small cluster of neurons. It extends rostrocaudally for a mean length of 1.07 ± 0.02 mm. Five clusters of neurons were observed – ventrolateral, dorsolateral, intermediate, medial and suprafacial. Fluorescent labelling of motor neurons supplying orbicularis oculi were observed in the dorsolateral, intermediate and medial subdivisions. The dorsolateral subdivision in the rostral parts of the nucleus showed double labelling indicating bilateral collaterals from both left and right facial motor nuclei.

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