

The organization of the somatic cell nuclei within the oculomotor nuclear complex in rats

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Abstract

Although there is detailed knowledge on the organization of the motor neurons supplying the extraocular muscles, there are still some discrepancies concerning the results of different studies. This study is planned to reexamine the distribution of the motor neurons in the oculomotor nucleus of the rat. In the present study we used 20 young adult Sprague-Dawley rats in four groups which represent four extraocular muscle groups innervated by oculomotor nerve, namely medial rectus, inferior rectus, superior rectus and inferior oblique muscle groups. For each rat 1-2 μ l of 30% Horseradish Peroxidase (HRP) is used as a tracer. Multiple injections were made to fully infiltrate the individual muscles. After determining the position of the medial rectus subgroup, injections were applied into more than one muscle in a single experiment in order to decide the proper localization of the motor neuron subgroups in relation to each other. For each of the muscle groups five rats were injected with HRP. The rostral end of the nucleus begins with a compact neuron group belonging to medial rectus subgroup. However, at the caudal end the neurons were scattered in a wider area gradually decreasing in number. Neurons innervating the medial rectus muscle were located ipsilaterally within the ventral and ventrolateral portions of the nucleus extending throughout its rostrocaudal length. Motoneurons belonging to the inferior rectus subgroup were observed ipsilaterally within the gap between the median raphe and medial end of the medial rectus subgroup. Motoneurons of the inferior oblique subgroup formed an ovoid cell mass ipsilaterally and slightly dorsal to the medial and inferior rectus subgroups. Neurons of the superior rectus subgroup were localized contralaterally within the caudal two thirds of the nucleus. The neurons of this subgroup were located at the ventral and ventromedial parts of the inferior oblique subgroup and dorsal to the inferior and the medial rectus subgroups.

Key words: oculomotor nuclear complex, rat, HRP, organization

Introduction

There is a number of studies concerning the distribution of the neuronal subgroups of the oculomotor nuclear complex. By using the retrograde degeneration technique on monkeys, Warwick [1] and Tarlov et al. [2] reported the most reliable results dealing with this subject. In more recent studies, Gacek [3], Akagi [4], Glicksmann [5] and Labandeira Garcia [6] studied on various species using retrograde axonal transport of the tracer horseradish peroxidase and some fluorescent dyes.

Although there is detailed knowledge on the distribution of motor neurons supplying the extraocular muscles there are still some discrepancies concerning the results of different studies. This study is planned to reexamine the distribution of the motor neurons in the oculomotor nuclear complex in rat.

Materials and Methods

Twenty young adult Sprague-Dawley rats (body weights 300-350 g) of both sexes were used in this study. The rats were anaesthetized with intraperitoneal injections of ketamine/xylazine at a rate of 3mg/kg xylazine and 90mg/kg ketamine providing approximately 30 minutes of surgical anaesthesia after 10-15 minutes induction. Under an operating microscope, the muscle was exposed by retracting

the eyelids, partially collapsing the eyeball, and making a conjunctival incision. 1-2 μ l of 30% Horseradish Peroxidase (HRP) (Sigma type VI) was injected into the muscle by use of a microsyringe. After 36-48 hours of survival time, the rats were deeply anaesthetized and perfused through the ascending aorta with isotonic saline solution followed by 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer containing 5% sucrose (pH 7.4). After the perfusion is completed, the calvaria is revealed and the parietal and the temporal bones were removed in order to reach the brain and brain stem. Mesencephalon, including the oculomotor nerve nuclei, is carefully removed and then dura mater is separated from the surface of the tissue. Eventually, 40-60 μ m sections were obtained by a vibrotome. In order to ascertain whether or not HRP had escaped from the injected muscle, non-injected extraocular muscles that are innervated by the oculomotor nerve were also removed and stored for 24 hours in phosphate buffer containing 30% sucrose. 40-60 μ m frozen sections of the tissue were reacted with tetramethylbenzidine and examined under a light microscope. In the present study we used 20 young adult Sprague-Dawley rats in four groups which represent four extraocular muscle groups innervated by oculomotor nerve, namely medial rectus, inferior rectus, superior rectus and inferior oblique muscle groups. For each of the muscle groups five rats were injected with HRP. In this study, at

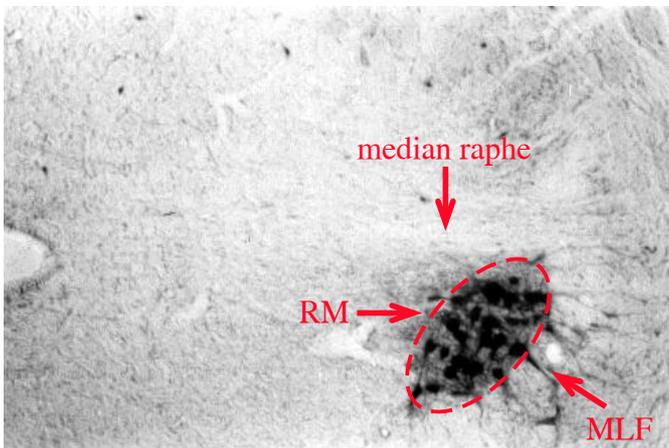


Figure 1 Photograph showing the localization of the medial rectus (RM) muscle subgroup within the most upper part of the oculomotor nerve nucleus. Medial longitudinal fasciculus (MLF) is anterolaterally located.

first we applied HRP into medial rectus muscle and detected the position of this subgroup. Then, differently from previous studies, we applied HRP both into medial rectus muscle and into another extraocular muscle at the same time. So that we had the chance to detect the positions of these two subgroups according to each other. This procedure was done for all muscle subgroups. At the next step, again differently from previous studies, we applied HRP into three extraocular muscles at the same time and found the positions of these three muscle subgroups according to each other. Hence the possible overlapping between the subgroups on the same plane was detected better.

Results

The rostral end of the nucleus begins with a compact neuron group belonging to the medial rectus subgroup. However at the caudal end the neurons were scattered in a wider area gradually decreasing in number. The degree of overlap among the subgroups was found to be very little. The localizations of individual motor neuron subgroups are as follows:

MEDIAL RECTUS MUSCLE SUBGROUP (RM)

At the most rostral levels of the nucleus, the neurons were belonging only to the medial rectus muscle subgroup. More distally the neurons of this subgroup were located at the ventral and ventrolateral portions of the nucleus. The most lateral neurons tended to direct dorsally thus forming a dorsolateral extension. Especially at the middle third of the nucleus, although a boundary could be drawn, the

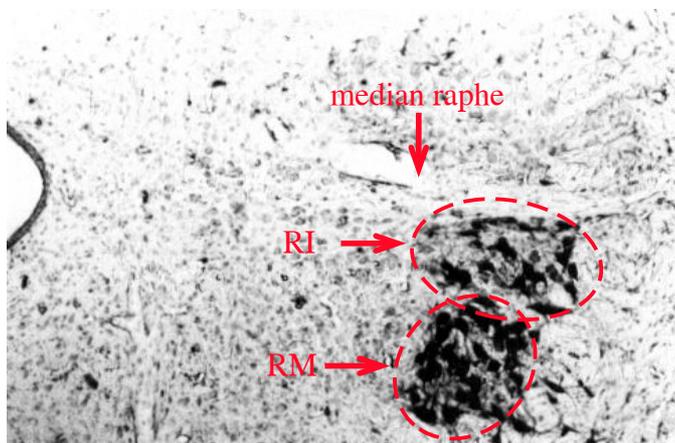


Figure 3 Photograph showing the localization of the medial (RM) and inferior rectus (RI) muscle subgroups at the mid-level of the oculomotor nerve nucleus.

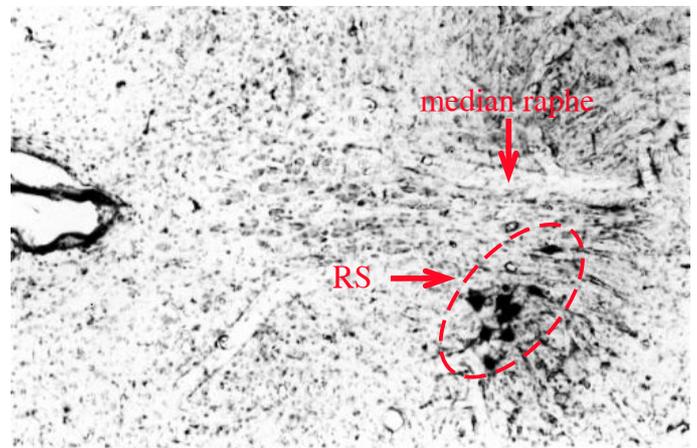


Figure 2 Photograph showing the localization of the superior rectus (RS) muscle subgroup at the mid-level of the the oculomotor nerve nucleus.

medial end of the medial rectus subgroup showed a slight degree of overlap with the inferior rectus subgroup neurons. Some neurons were observed among the fibers of medial longitudinal fasciculus (MLF) beginning from the upper second quarter (Figure 1).

SUPERIOR RECTUS MUSCLE SUBGROUP (RS)

Neurons of this subgroup were localized contralaterally within the caudal two thirds of the nucleus. The neurons were located at the ventral and ventromedial parts of the inferior oblique subgroup and dorsal to the inferior and the medial rectus subgroups (Figure 2).

INFERIOR RECTUS MUSCLE SUBGROUP (RI)

Motoneurons belonging to this subgroup were observed ipsilaterally beginning from the rostral 3/4 of the nucleus extending to the caudal end. The neurons were located within the gap between the median raphe and the medial end of the medial rectus subgroup. (Figure 3, 4, 5)

INFERIOR OBLIQUE MUSCLE SUBGROUP (OI)

The rostral tip of the inferior oblique motoneurons were located nearly at the level of the rostral tip of the inferior rectus subgroup. The caudal pole was extending to the caudal end of the nucleus. Motoneurons of this subgroup formed an ovoid cell mass on the ipsilateral side and located dorsal to the medial and inferior rectus subgroups leaving a gap between them. A "U" shaped area having a dorsal concavity, depleted of neurons was observed between this subgroup and the medial and inferior rectus subgroups (Figure 4, 5).

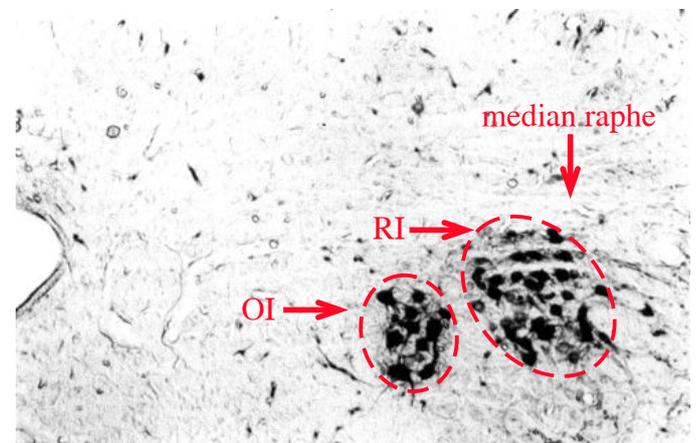


Figure 4 Photograph showing the localization of the inferior rectus (RI) and inferior oblique (OI) muscle subgroups at the mid-level of the oculomotor nerve nucleus.

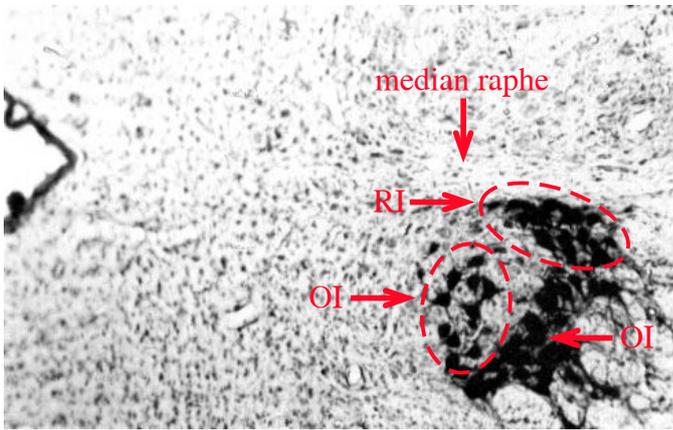


Figure 5 Photograph showing the localization of the medial rectus (RM), inferior rectus (RI) and inferior oblique muscle (OI) subgroups at the mid-level of the oculomotor nerve nucleus. The localization of the contralateral superior rectus subgroup is the area between these three subgroups and as a reverse letter "V" in shape.

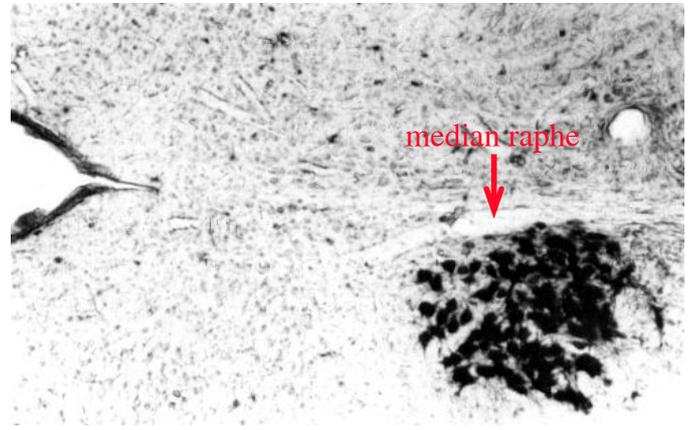


Figure 6 Photograph showing the localization of all subgroups.

Discussion

In the detection of the somatic cell nuclei of the oculomotor nuclear complex by using HRP, various results were found on the same species by different authors. In some of the previous studies, it is reported that the HRP injections were applied into one point of the extraocular muscle [3, 4] or only into one nerve fiber innervating it [7]. Because of the limited spreading activity of the tracer, it is obvious that, HRP has to be applied into more than one point of the muscle or into more than one nerve fiber in order to see the entire nucleus. Also in some of the previous studies on rats, the tracer was applied into only one muscle and then sections were estimated. Minimal changes on the section plane may cause false assessment of the position of the nucleus. No simultaneous injections into more than one muscle group were reported in these studies. So to reviewuante the relative position of the subgroups by single muscle injection is difficult. Finally, there may be false results due to the contamination of the tracer to other muscles. All these would be the reasons of various results. Here in this study our aim was to show the organization of the oculomotor nuclear complex by multiple HRP injections into more than one muscle simultaneously in order to define the precise organization of the nucleus. Multiple injections of HRP into more than one muscle at once enable us to reviewuante the position of the subgroups. In the study of Labandeira Garcia [6], it is claimed that there is overlapping between the subgroups. We think that it is because of the absence of simultaneous injections, or the minimal changes of the section plane. Our results present no prominent overlapping between the subgroups. In our study, at first we applied HRP into the medial rectus muscle and detected the position of the medial rectus subgroup within the nucleus. Then, differently from the previous studies, we applied HRP both into the medial rectus muscle and into another muscle simultaneously. So that we had the chance to detect the positions of two subgroups according to each other. This procedure was done for all muscles (Figs. 3, 4). At the next step, again differently from the previous studies, we applied HRP into three extraocular muscles at once and found the positions of the subgroups according to each other (Fig. 5). Hence, the possible overlapping between the subgroups on the same plane was detected better.

Studies using tracers revealed different results about the innervation of the extraocular muscles. First studies about the subject was performed on monkeys by Warwick [1].

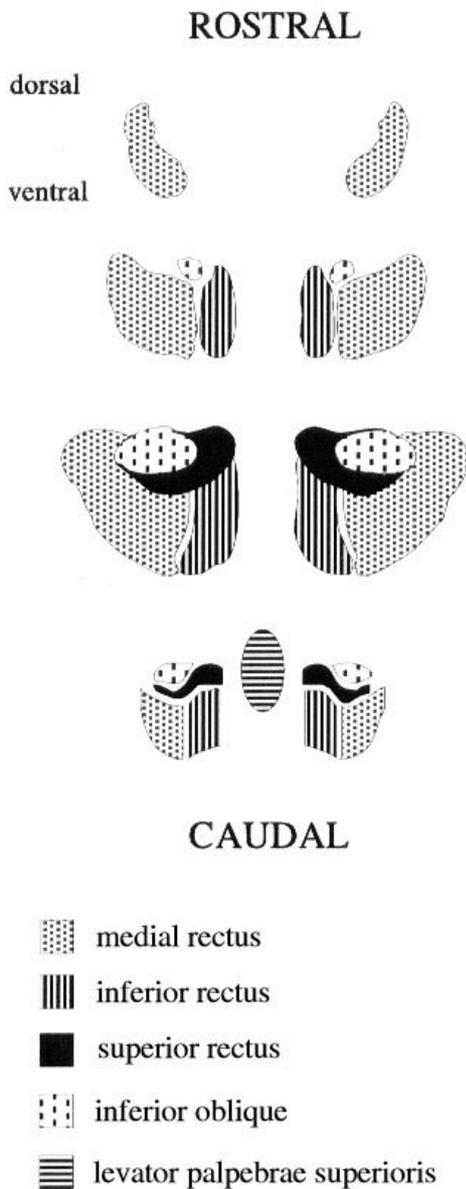


Figure 7 Schematic arrangement of all subgroups from rostral to caudal.

Then Tarlov et al. [2] studied on kittens by using retrograde degeneration methods, but their results were different from that of Warwick's. This is possibly because of the difference in the organization of the nucleus in different species. Researchers show different schematic organizations of the oculomotor nuclear complex so far. The first generally accepted drawings, that were based on the observation of the chromatolysis technique in monkeys and pathologic studies in humans, were performed by Bernheimer [8] and Brouwer [9] respectively. The authors concluded that the motor columns innervating the extraocular muscles were rostro-caudally arranged. Later Abd-el-Malek [10], Szentagothai [11] and Danis [12] accepted this rostro-caudal organization for cats and dogs but with a quite different order. Bach [13] and van Biervliet [14] mentioned that the subgroups innervating the extraocular muscles in rabbits have a dorso-ventral organization. That makes us think the different organizations in different animals. According to the study of Glicksmann [5] the organization of the oculomotor nuclear complex in rat and rabbit are similar. In his study with HRP, he found that neurons of the inferior and medial rectus subgroups were located ipsilaterally in the rostral 2/3 of the nucleus, whereas the neurons of the inferior oblique subgroup was located ipsilaterally in the middle 1/3 of it. He defined the position of the superior rectus subgroup contralaterally in the caudal 2/3 of the nucleus. Additionally, he noted that there were neurons within the MLF related to the medial rectus subgroup. In our study we also observed neurons within the MLF related with the medial rectus subgroup (Figs. 1, 5, 6, 7). Unlike the results of Glicksmann [5] we found that the medial rectus subgroup was extending throughout its rostro-caudal length. The superior rectus subgroup was located contralaterally in the inferior half of the nucleus whereas the inferior rectus subgroup was located ipsilaterally at all levels except the rostral end (Figs. 2, 3, 4, 5, 6, 7). Labandeira Garcia [6] stated that the motoneurons within the oculomotor nuclear complex of rats were not found as separate subgroups, but overlap one another. Furthermore he claimed that all the extraocular muscles were innervated by neurons within the MLF. But in our study, confirming the studies of Glicksmann [5], we found that there are only the medial rectus subgroup neurons scattered within the MLF. Labandeira Garcia [6] also stated that the medial and inferior rectus subgroups were found throughout its

rostro-caudal length, but 90% of the neurons of the medial rectus subgroup were concentrated in the rostral 2/3 of the nucleus and 50-55% of the neurons of the inferior rectus subgroup were concentrated in the rostral 1/3 of the nucleus. He also observed the medial and inferior rectus subgroups as merged in the rostral levels. In our study we also observed the medial rectus subgroup throughout the ventrolateral length as Labandeira Garcia [6] but found no overlapping with the inferior rectus subgroup. Also we found the inferior rectus subgroup located only medially and in the inferior 3/4 of the nucleus.

Gomez-Segade [15] studied only the superior rectus subgroup in rats and observed that the neurons of this subgroup was located contralaterally in the caudal 2/3 of the nucleus that is compatible with our results. Contrary to ours, he stated that there were neurons of this subgroup within the MLF.

In accordance with the results of Labandeira Garcia [6], we found that the inferior oblique subgroup was located dorsally throughout the nucleus except the most rostral levels (Figs. 4, 5, 7). But we did not observe any neuron within the median raphe belonging to the inferior oblique subgroup. Labandeira Garcia [6] reported that the neurons of the superior rectus subgroup were located throughout the nucleus except for the most rostral levels, but the majority were in the caudal two thirds. In our study we observed the neurons belonging to this subgroup located contralaterally in the caudal two thirds of the nucleus. He also noted that in the caudal half of the nucleus there were neurons within the MLF. Moreover it is reported that 2-3% of the neurons were found in the ipsilateral nucleus. We found no neurons of the superior rectus subgroup neither within the MLF nor within the ipsilateral nucleus. As a result, in this study we detected the organization of the somatic cell nuclei within the oculomotor nuclear complex by using a different technique which we found more reliable. We believe that most of the discrepancies about this subject in literature are because of the inadequacy in technique. It is possible to clarify the organization of the oculomotor nuclear complex in other species by using this technique.

Acknowledgements

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