

Trochlear Nerve Nucleus in Albino rat

Singh A., Khanna J. & Bharihoke V.

Department of Anatomy, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi India

Abstract: A study of the trochlear nerve nucleus and its course within the brain is reported based on histological and fluorescent tract tracing techniques. Twenty inbred adult Wistar albino rats weighing between 150 to 250 gm of either sex were taken in the study. The localization of the nucleus giving rise to the nerve supplying the superior oblique muscle was done by using fluorescent dyes, Fast blue and Diamidino yellow. Fast blue was applied to the trochlear nerve of the right side and the Diamidino yellow was applied to the nerve of the left side in each animal. Animals were sacrificed after appropriate survival period. The labeled neurons were localized in the fourth nerve nucleus in the midbrain with the help of a Zeiss fluorescence microscope. The majority of the fibers of the trochlear nerve cross to the opposite side and a few fibers remain ipsilateral. Many neurons were labeled with both the dyes indicating bilateral innervation. The nucleus was studied in paraffin sections stained with Kluver Barrera and Marshland, Gleys and Erikson's stain to trace the nerve fibers within the midbrain.

Key words: Fluorescent tract tracing techniques, midbrain, superior medullary vellum, trochlear nerve nucleus, Diamidino yellow, Fast blue

I. Introduction

The basis of origin of the trochlear nerve from the posterior aspect of the midbrain and its crossing before exiting from the brain remains an enigma. Unlike the other cranial nerves its fibers cross in the superior medullary vellum. Further the proportion of fibers crossing in the vellum still needs to be clarified. This study is an attempt to elucidate the above mentioned facts. Many techniques are used in neuroanatomy to determine the neuronal pathways. These include nerve degeneration studies, neuronal tracers like HRP and Fluorescent tracers etc. [1, 2, 3, and 4]. All techniques have their limitations. Trochlear nerve nucleus has been studied with help of HRP and fluorescent tracers [2, 5, & 6]. Simultaneous use of two or more fluorescent tract tracer has opened new vistas for determining multiple connections to a single nucleus [7, 8, 9 & 10]. This study was designed to trace the trochlear nerve fibers bilaterally and determine decussation between the nerves of the two sides supplying the superior oblique muscle using double fluorescent labeling techniques, based on retrograde transport of fluorescent dyes.

II. Material and Method

20 albino rats weighing 150 to 250 gm of either sex were procured from animal house of University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi and kept under standard laboratory conditions.

The rats were divided into two groups of 10 rats each. Animals in Group I were perfused with 10% formal saline. The midbrains were removed and embedded in paraffin. 7 μ thick sections were cut and mounted on glass slides. Every fifth section was stained with Kluver Barrera stain counter stained with cresyl fast violet for neurons in the nucleus and Marsland Gleys and Erikson's stain to trace the axons of the trochlear nerve within the midbrain. Animals in Group II were used for fluorescent tract tracing study. All animals were anesthetized with ether. The procedure was passed by the ethical committee of the college.

The trochlear nerve was exposed and severed close to the superior oblique muscle in Group II animals. 3-5 μ l of a dye was applied to the proximal end of the severed nerve. 2% aqueous solutions of Fast blue was applied to the right nerve and 2% aqueous solutions of diamidino yellow to the left nerve, as the survival period of both the dyes was same. The dyes were applied in one sitting in both the orbits. The nerve was sealed with the wax to avoid spillage in the adjacent tissues. The wound was closed.

The optimal survival period for fast blue and diamidino yellow was found to be from 168 to 216 hours. The rats were perfused after 200 hours of the operation with 10% formal saline. The entire brain with the brainstem was removed and the midbrain was immersed in 10% sucrose in cacodylate buffer at 4^oC. 35 μ m serial sections were cut on a cryostat and mounted on slides and examined under Zeiss fluorescence microscope with a filter having a combination of exciter filter, 360 nm, beam splitter 395 nm and barrier filter of 397 nm. The labeled neurons were noted in the trochlear nerve nuclei. Diamidino yellow labeled the nucleus of the neuron yellow and fast blue labeled the cytoplasm blue.

III. Result

The trochlear nerve nucleus is situated in the midbrain at the level of the inferior colliculus. It appears as a cluster of multipolar neurons dorsomedial to the medial longitudinal fasciculus. It extends rostrocaudally for a mean length of $451.1\mu \pm 20.55$. The neurons in the trochlear nucleus vary from triangular, polyhedral, and fusiform to round comprising of a distinct round nucleus with a central nucleolus and clear nucleoplasm (Fig. 1) With Glees silver stain the nucleus appears light orange with a darkly stained nucleolus in the center. The neuronal processes are dark brown. (Fig.2). The fibers of the trochlear nerve are seen originating from upper part of the nucleus and travelling contralaterally around the periaqueductal gray to reach the vicinity of the mesencephalic nucleus of the trigeminal nerve. (Fig.3).. The fourth nerve fibers in the lower part of the nucleus are seen originating bilaterally from the nucleus and curving dorsally around the PAG and enter the superior medullary velum after piercing the inferior colliculus. The fourth nerve is given off from the dorsal side of the frenulum. (fig.4)

The fast blue which was applied to the right nerve was found in the cytoplasm of the neurons on left side and the nuclei showed a dark negative shadow in some of the neurons of the nucleus (fig.5). The nuclei of these cells were not labeled. The diamidino yellow was found to have concentrated more in the nuclei of the neurons on the right side. (fig.6). Some labeled neurons were seen in the ipsilateral side with both the dyes. Some neurons showed double labeling i.e., the nucleus was yellow and the cytoplasm was blue in the same neuron on both the sides. (Fig.7).The finding of the dye uptake was consistent in all the animals and did not show any individual variation.

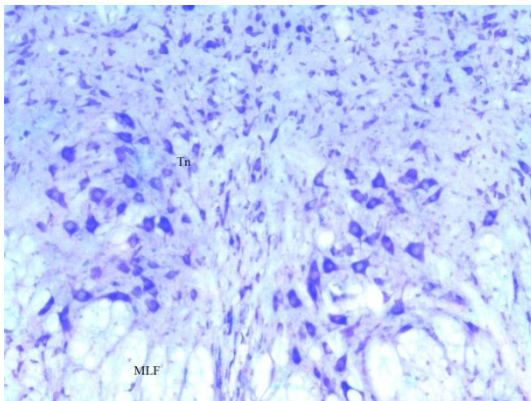


Fig.1. T.S. of midbrain through the inferior colliculus showing the position of trochlear nerve nucleus (Tn), lying dorsomedial to the medial longitudinal fasciculus (MLF), Luxol Fast Blue counter stained with cresyl violet. 400X

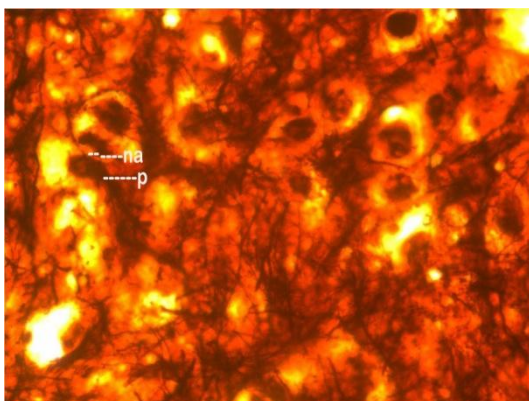


Fig.2. Photomicrograph of transverse section of midbrain at the level of inferior colliculus showing the cytoplasm and nerve processes containing a dense network of brown neurofibrils (p), with a darkly stained nucleolus (na) in the center of the light nucleus 200X

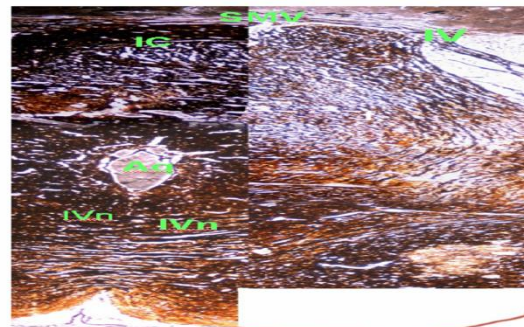


Fig. 4: A collage of photomicrographs from a section of the midbrain close to the superior medullary velum showing the fibers of the fourth nerve getting contribution from the nuclei of the right and left side and arching backwards to enter the superior medullary velum before coming out as the fourth nerve dorsally. Aqueduct (Aq), fourth nerve nucleus (IVn), inferior colliculus (IC), superior medullary velum (SMV), fourth nerve (IV). Glees stain 400X.

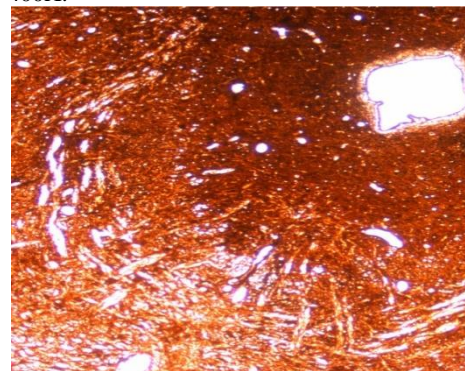


Fig 3. A photomicrographs of the trochlear nucleus (Tn) showing the nerve fibers of the fourth nerve (IVn) encircling the PAG to reach medial to the mesencephalic nucleus (Vn) of the trigeminal nerve. Glees stain (40X).

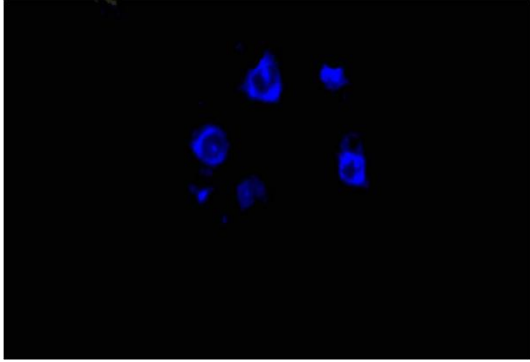


Fig. 5 Photomicrograph of T.S. of midbrain at the level of inferior colliculus showing blue fluorescence with negative nuclear shadow 400X

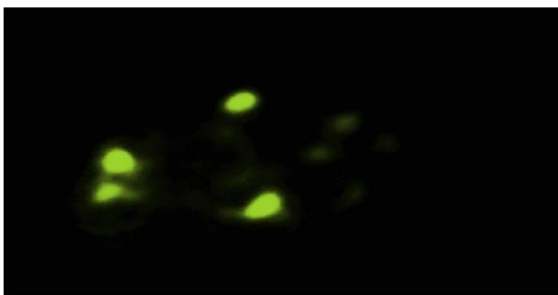


Fig.6: Photomicrograph of transverse section of the midbrain showing the nuclei of the neurons labeled yellow in the right trochlear nucleus. 400X

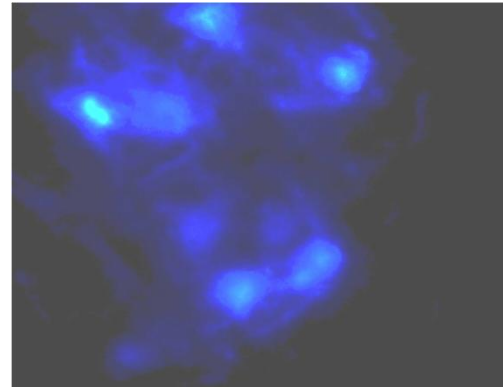


Fig. 7: Photomicrograph of T.S. of midbrain at the level of inferior colliculus showing double labeled cells. Fast blue in the cytoplasm and diamidino yellow in the nuclei of the neurons of trochlear nucleus. 400 X.

IV. Discussion

The fibers of the IV nerve descend down medial to the mesencephalic nucleus to enter the superior medullary velum where most of the fibers decussate before supplying the muscle [2].

These fibers could be traced right up to the lateral part of superior medullary velum from where they were seen entering the contra lateral trochlear nerve. The majority of the fibers of the trochlear nerve cross to the opposite side and a few fibers remain ipsilateral, this confirms the findings of different workers in rat [2], rabbit [3]. Guinea pig and rabbit [4]

The crossing fibers of the fourth nerve within the midbrain as found in the present study have not been reported by [2, 3, 4 & 5]. In addition there is some bilateral contributions from the nuclei to the nerve. This finding has not been reported previously. Bilateral contribution in the facial nerve has been reported by [10].

The presence of neurons supplying bilateral muscles in this nucleus may be responsible for the conjugate movements of the eyes during intorsion when both eyes are simultaneously intorted. Predominantly contralateral paralysis has been reported by [11-14] in which vertical diplopia, sometimes with a torsional component, marked with diplopia on looking down has been reported.

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