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Direct Projections from the Mesencephalic Trigeminal Neurons to the Trigeminal Motoneurons and Interneurons in the Rat

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The afferent projections of the mesencephalic trigeminal nucleus (MTN), which is a unique structure in the CNS and composed of primary sensory neurons, are well known although the efferent projections still remain unclarified and controversial. Descending projections from the MTN were studied using biotinylated dextran amine as an anterograde tracer. The tracer was injected under pressure unilaterally into the pontine part of the nucleus. The anterograde tracing experiments show the presence of direct projections from mesencephalic trigeminal neurons, located in the caudal part of the MTN, to the motor trigeminal nucleus and to interneurons of the supratrigeminal, intertrigeminal and juxtatrigeminal areas. Our results show that through all these projections the mandibular movement is facilitated.

Key words: mesencephalic trigeminal nucleus, trigeminal motoneurons, anterograde tracing, biotinylated dextran amine, rat.

Introduction

The mesencephalic trigeminal nucleus (MTN) is a unique structure in the CNS, composed of primary sensory neurons. At the pontine level, MTN neurons are situated in the triangle between the locus coeruleus and the medial parabrachial nucleus. At the level of the mesencephalon they border laterally the central periaqeductal gray. Mesencephalic trigeminal neurons innervate the jaw-closing muscles, periodontal ligament and a subset of the extraocular muscles [1]. While the MTN afferent projections in the rat are well studied, little is known about the afferent connections of the nucleus with other brain regions. The presence of direct projections from the MTN to the motoneurons, located in the motor trigeminal nucleus (MoTN) has been studied by different researchers using various tracing techniques [3, 5]. It has been demonstrated that projecting fibers reach the supratigeminal nucleus [2], and also the intertrigeminal and juxtatrigeminal areas [6].

The aim of this study is to describe the efferent projections of mesencephalic trigeminal neurons to the trigeminal motoneurons and interneurons in the rat. After

injecting biotinylated dextran amine (BDA) into the caudal part of the MTN it is possible to follow intensely marked axons from the MTN neuronal perykaria to their peripheral target areas.

Material and Methods

Ten adult Wistar rats of both sexes weighing 280-350 g were used for this study. The animals were anesthetized with Thiopental (Biochemie, GmbH, Kundl, Austria; 25mg/kg b.w.) and then mounted in a stereotaxic frame. Under aseptic conditions small craniotomies were performed. The location of the injection site was precised to the following coordinates according to the rat brain stereotaxic atlas of P a x i n o s and W a t s o n [4]: 0.68 mm posterior to the interaural line and 1.4 mm lateral to the midline. A 10% solution of BDA (m.w. 10,000; Molecular Probes Europe BV, Leiden, The Netherlands) dissolved in phosphate buffer (PB; 0,1M, pH 7.2) was injected under pressure unilaterally with a Hamilton microsyringe (Hamilton Co, Reno, Nevada, USA), while the contralateral side remained intact to serve as a control. At the end of injecting, the microsyringe was held in place for 2 min to insure that the injected BDA had been absorbed into the tissue. Following 6-8 days of survival, the animals were perfused transcardially, first with 100 ml of 0.9 % saline, followed by 400 ml of 4% paraformaldehyde in PB (Merck, Darmstadt, Germany).

The brains were quickly removed and then placed into the same fixative at 4°C for 4 hours. Frozen sections (40 μm of thickness) were prepared on a freezing microtome Cryocut E (Reichert — Jung, Austria) and collected in PB in a free-floating state. The sections were reacted by using the avidin-biotin complex (Vectastain ABC Kit, Vector Laboratories Inc., Burlingame, USA) in PB and then the peroxidase activity was developed in 0,05 M Tris-HCl buffer, pH 7,6 containing 0,012% 3,3 diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0,01% H_2O_2 for up to 15 min. All the sections were mounted onto gelatin-coated glass slides, air-dried and counterstained with Cresylviolet. The slides were viewed with a Zeiss Axioplan 2 light microscope and photographed with an Axiocam MRc digital camera.

Results

After leaving the MTN a bundle of well-marked fibers goes ventrolaterally to the region, situated above the MoTN, called supratrigeminal nucleus, where intensely labeled axons are clearly observed. The fibers pass in the vicinity of the somata of trigeminal interneurons located here (Fig. 1). In the MoTN well marked fibers are seen, spread in its dorsolateral portion, whose collaterals are in direct contacts with the perikarya of large-sized trigeminal motoneurons (Fig. 2). Intensely labelled fibers and terminals are also found in the ipsilateral juxtatrigeminal area, situated below the ventral pole of the MoTN. Furthermore, marked fibers reach the intertrigeminal region, located between the MoTN and the principal trigeminal nucleus.

To verify these projections, we injected BDA in the MoTN and were able to see that all mesencephalic trigeminal neurons along the whole rostrocaudal length of the MTN were labeled. This projection is invariably unilateral (Fig. 3).

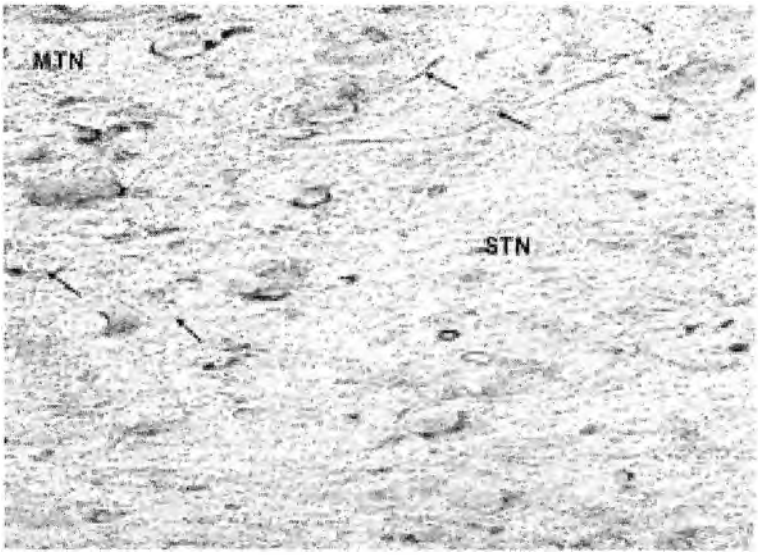

Fig. 1. Microphotograph illustrating the labeled projection fibers (arrows), passing through the supratrigeminal nucleus (STN). × 160

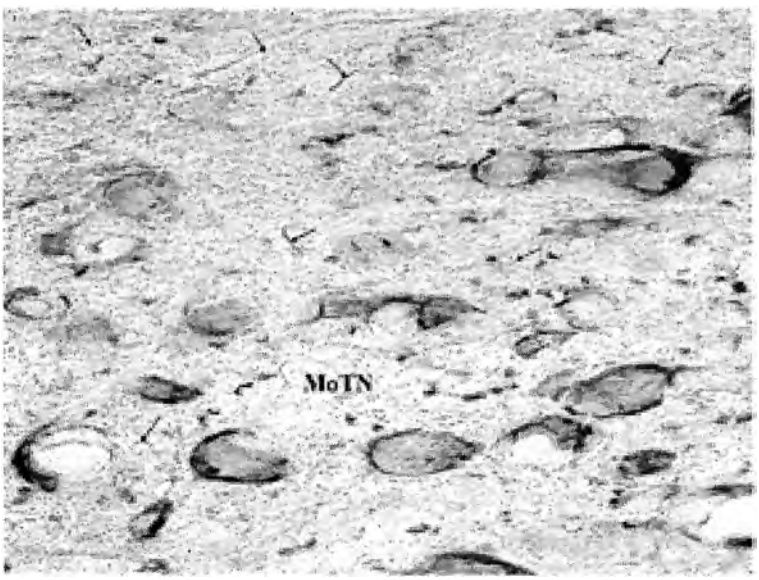

Fig. 2. Anterogradely BDA-labeled fibers (arrows) traveling toward large-sized perikarya in the motor trigeminal nucleus (MoTN). × 400

Discussion

Our results clearly show the presence of a direct projection from the MTN to the MoTN, especially to its dorsolateral part. This portion of the latter is known to contain the cell bodies of motor neurons innervating masticatory and suprahyioid muscles.

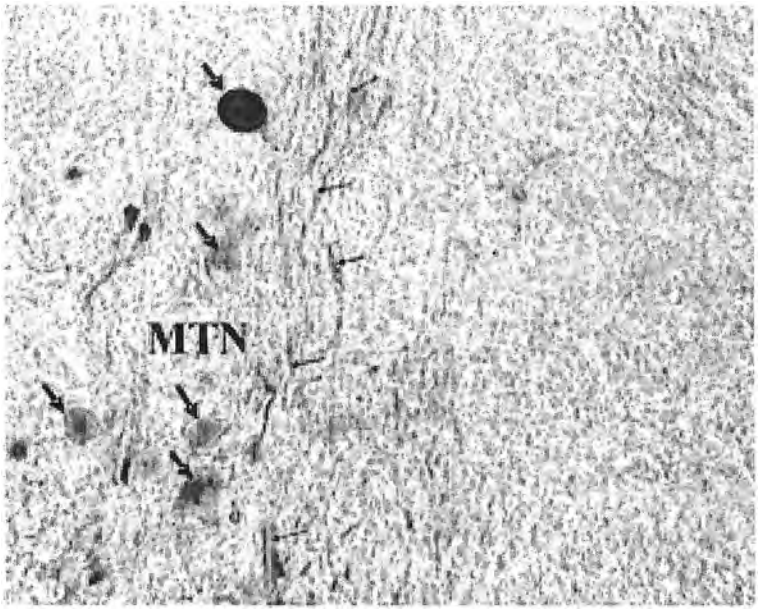

Fig. 3. Retrogradely labeled cell bodies (large arrows) and fibers (small arrows) in the MTN after injecting BDA into the motor trigeminal nucleus. × 400

The masticatory muscle spindle afferents send, via the central axons of the mesencephalic trigeminal neurons, their projections to jaw-closing motoneurons located in the MoTN. In this study we were able to observe numerous intensely labelled projection fibers to the dorsolateral part of the MoTN, where the majority of jaw-closing motoneurons are located. These data correlate well with previous studies, which confirm the presence of labelled fibers also in the supratrigeminal nucleus [3, 5]. It can be inferred from our findings that mesencephalic trigeminal neurons, supplying the jaw-closing muscles, may during mastication activate supratrigeminal neurons, which in turn inhibit trigeminal motoneurons innervating the masticatory muscles.

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