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Descending Projections from the Mesencephalic Trigeminal Nucleus to the Caudal Brainstem in the Rat: an Anterograde Tracer Study Using Biotinylated Dextran Amine

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The afferent projections of the mesencephalic trigeminal nucleus (MTN), a unique one in the CNS comprising primary sensory neurons, are well known, while the efferent projections are still controversial and arguable. Descending projections from the MTN were studied using biotinylated dextran amine. The tracer was injected under pressure unilaterally into the pontine portion of the nucleus. Anterograde tracing studies demonstrated the presence of direct projections from the MTN neurons located in its caudal part to the medial, spinal, lateral and superior vestibular nuclei, and also to the parvocellular, dorsal paragigantocellular and intermediate reticular nuclei. The results suggest that these connections relay signals playing a role in the eye-head coordination. The present study shows a descending projection to the hypoglossal nucleus, thus affirming anew that periodontal MTN afferent neurons also terminate in this nucleus.

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

Introduction

The mesencephalic trigeminal nucleus (MTN) is a unique structure within the central nervous system (CNS) composed of primary sensory neurons. At the pontine level, MTN neurons are situated in the triangle between the locus coeruleus and the ventral parabrachial nucleus. At the mesencephalic level, they are located laterally, confined by the central gray around the aqueductus cerebri.

The MTN neurons innervate muscles of mastication, the tooth pulp, periodontal ligament and extraocular muscles [1, 2, 5, 7, 14]. It is known that the masticatory sensory neurons are distributed throughout the whole rostrocaudal extent of MTN, while

periodontal and extraocular muscle sensory neurons are situated mainly in the caudal part of the nucleus [1, 10].

While the afferent projections of the rat MTN neurons have more or less been studied in detail, little is still known about the efferent connections of the nucleus with other CNS structures [8]. By using biocytin as an anterograde tracer some neurons located in the caudal part of MTN have been found to project to the medial (MVN), spinal (SpVN), lateral (LVN) and superior vestibular nuclei (SVN) [12]. On the other hand, the existence of direct trigemino-cerebellar efferents is controversial. Such projections have been accepted [4] or denied [9]. Using the same tracer, for instance, it has been documented that MTN projections reach the ipsilateral sagittal zone X in the anterior lobe, lobule VI and lobule IX of the cerebellum [3].

Direct descending projections from the MTN in the rat have also been demonstrated to reach the brainstem reticular formation by autoradiography [15] and anterograde transport tracing [13]. By applying horseradish peroxidase (HRP), Ruggiero et al. [13] reveal descending projections from the MTN to the medullary raphe nuclei, as well as to nucleus tractus solitarii, facial and hypoglossal nuclei. Connections to the hypoglossal nucleus have also been studied by using biotinylated dextran amine (BDA) [16, 17].

The aim of the present study is to provide an accurate description of the efferent projections of MTN neurons to the caudal brainstem in the rat. Following application of BDA into the caudal part of the MTN, it is possible to trace the intensely labelled axons from the MTN neuronal perikarya to the peripheral target field areas.

Materials and Methods

Ten adult Wistar rats of both sexes weighing 280-350g 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 Paxinos and Watson [11]: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 was 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 section (40 μm of thickness) were cut with a freezing microtome Cryocut E (Reinchert – 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 up to 15 min. All the sections were mounted

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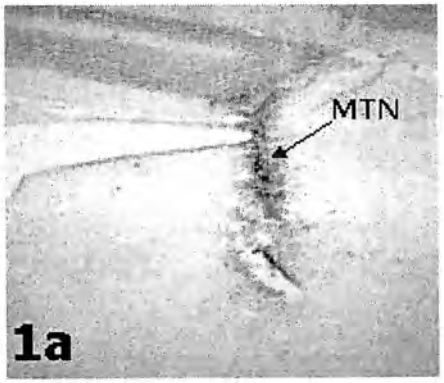
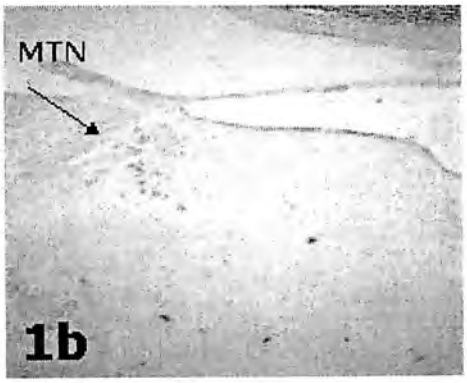
Fig. 1. BDA injection site within the caudal part of the MTN **a** – Site of injecting the tracer; **b** – Contralateral intact side (× 50)

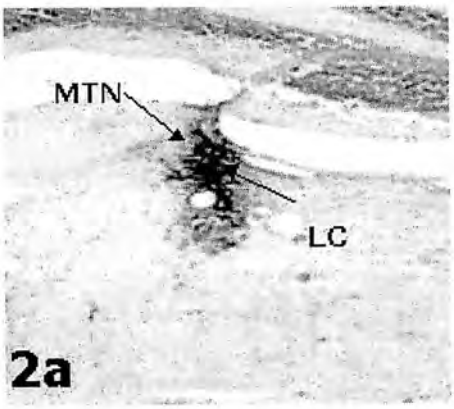
Fig. 2. The injection site is in the MTN but the adjacent locus coeruleus (LC) is also partially labelled (a). Contralateral intact side (b) (× 50)

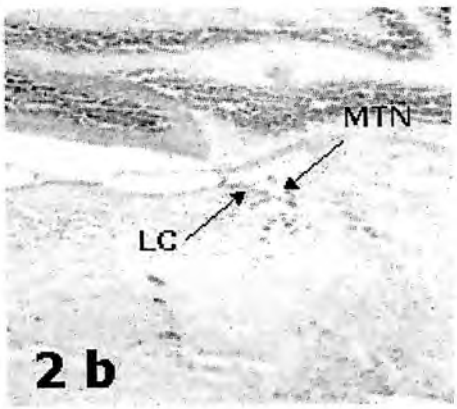
Fig. 3. BDA-labelled fibers in the MVN and the SpVN (a) (× 50); Larger magnification of labelled neurons in the SpVN (b) (× 250)

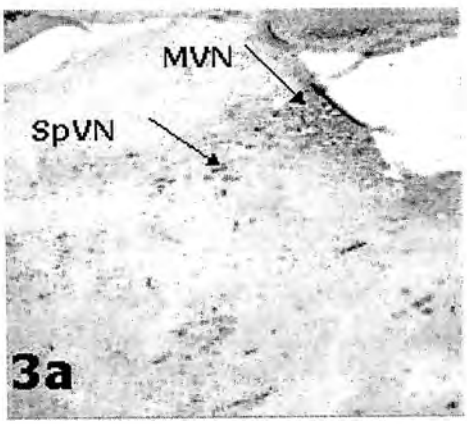
onto gelatin-coated glass slides, air-dried and counterstained with Cresylviolet. The slides were viewed with a Zeiss Axioplan 2 light microscope and photographed with Axiocam MRc digital camera.

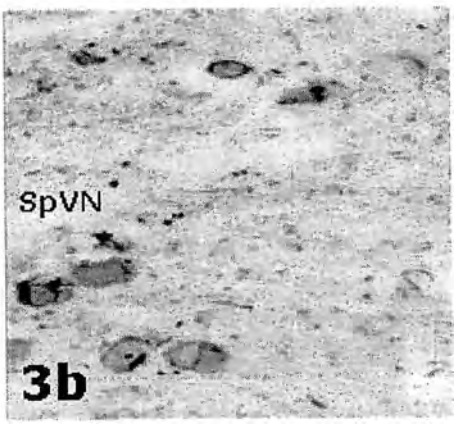












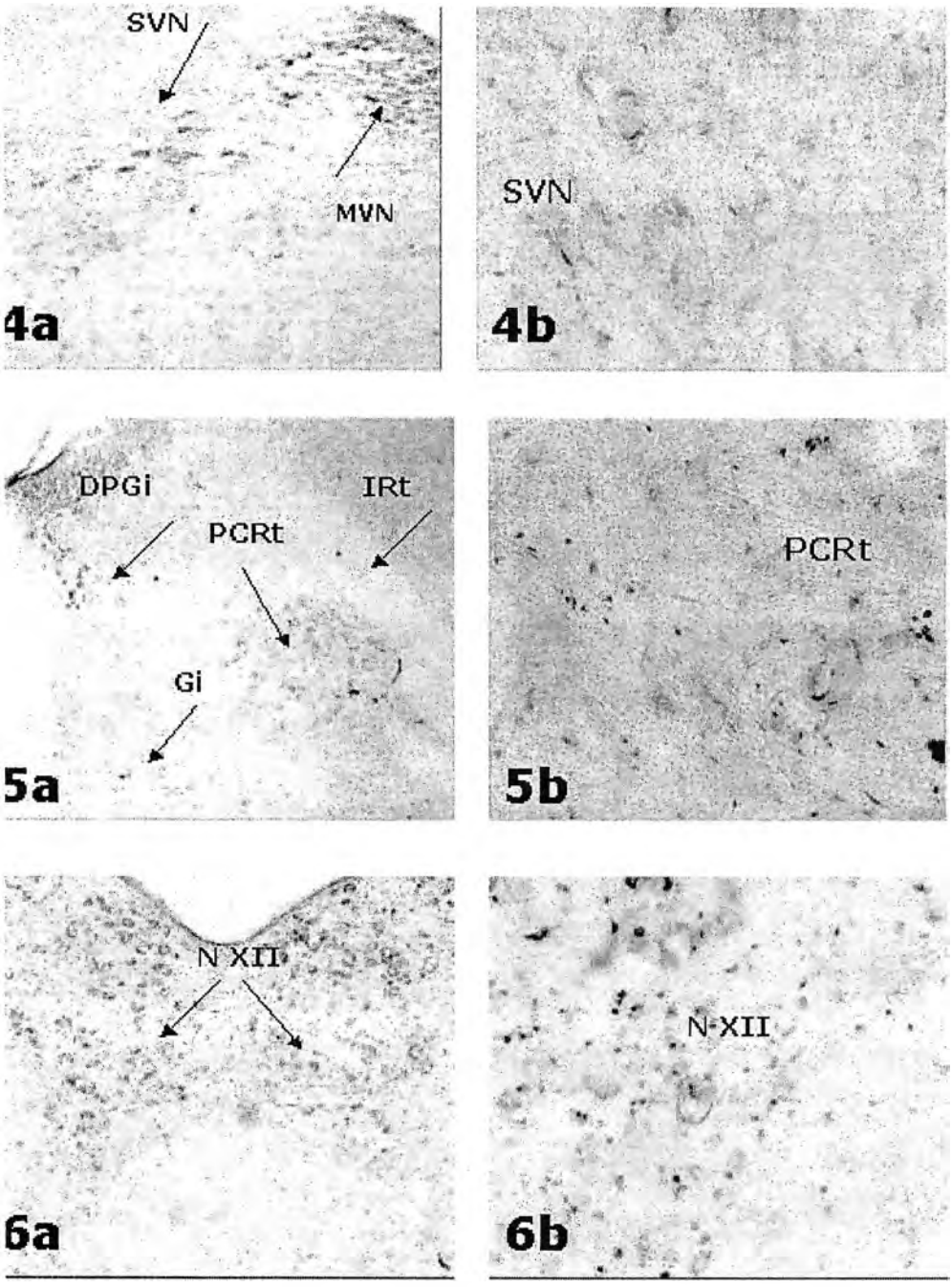


Fig. 4. Fibers with small terminal boutons in the peripheral part of the SVN (a) (× 50); Larger magnification showing labelled perikarya in the SVN (b) (× 250)
Fig. 5. BDA labelled fibers descending via the Probst's tract to the parvocellular, dorsal paragigantocellular, gigantocellular and intermediate reticular nuclei (a) (× 50); Larger magnification of labeled PCRt neurons (b) (× 250)
Fig. 6. Anterograde labelled fibers, predominantly in the dorsal part of the hypoglossal nucleus (a) (× 50); Larger magnification of labeled neurons in the motor hypoglossal nucleus (b) (× 250)

Results

In three of the 10 BDA-injected animals, the tracer was injected accurately into the MTN and was confined within the nucleus (Fig. 1). It were indeed these cases that were used for the present study. In the rest of the animals the injection site was in the MTN, albeit adjacent structures, such as locus coeruleus (LC) and the supratrigeminal region, were also partially labeled (Fig. 2).

Following injections of BDA within the caudal part of the MTN, fibers and terminals were anterogradely labeled in the MVN, LVN and SpVN (Fig. 3). A few fibers with small terminal boutons were also seen in the peripheral part of the SVN (Fig. 4).

BDA-labeled fibers were found to descend via the Probst' tract to the parvocellular, dorsal paragigantocellular, and intermediate reticular nuclei (Fig. 5).

It was found that MTN neurons send projections along the Probst' tract to the caudal brainstem. The labelling of their terminals was most intense in the dorsal part of the ipsilateral hypoglossal nucleus (Fig. 6).

Discussion

This study reveals the existence of collateral projections from MTN neurons to the vestibular nuclei in the brainstem, mainly to the MVN, SpVN, and LVN. It also provides information about MTN projections to the reticular formation and the hypoglossal nucleus. Our findings about the existence of trigeminal-vestibular connections are in concordance with the results reported by Buisseret's research group, obtained by using HRP and fluorescent tracing methods [12].

Depending on its molecular weight BDA can be used as either an anterograde or a retrograde tracer. Through its intraneuronal application, we are able to give an extensive and detailed anterograde labeling of MTN axons and terminals, and trace them down to the caudal brainstem. Thus, we are in a position to prove definitively the existence of a direct projection from the MTN neurons to hypoglossal motoneurons in the rat. Such a monosynaptic connection has also been shown at the electronmicroscopic level [16, 17]. By using the advantages of BDA as a very sensitive tracer, we reevaluated the MTN descending projections to the medullar reticular formation and confirm the classical finding of R u g g i e r o et al. [13] about the descending connections to the parvocellular, dorsal paragigantocellular, and intermediate reticular nuclei.

The presence of a direct projection to the vestibular nuclei, together with previous electrophysiological data [6], reveals a pathway possibly contributing to the control of gaze orientation. Most of the neurons, located in the MVN and LVN are involved in the control of horizontal eye movements via vestibulo-abducens pathways [12]. It is well known that the cell bodies of neurons innervating extraocular muscles and the periodontal ligament are located in the caudal part of the MTN [1]. Proprioceptive information from these muscles may determine the activity of neurons in the vestibular nuclei and thus may act in the associated eye and head movement for gaze stabilization.

As already mentioned, the MTN neurons send their axons predominantly to the dorsal part of the hypoglossal nucleus, where motoneurons innervating the tongue retractor muscle are located. Therefore, the presence of a direct trigemino-hypoglossal projection serves to protect the tongue from damage during mastication [16].

The descending pathways from the MTN to the brainstem reticular formation might serve as a link between the MTN and some reticular nuclei, regarding its role in the regulation of salivation, and movements of the jaw, face and tongue.

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