



Original Contribution

AN ANTEROGRADE TRACER STUDY OF A DIRECT TRIGEMINO-NIGRAL PROJECTION IN THE RAT BRAIN USING BIOTINYLATED DEXTRAN AMIDE (BDA)

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ABSTRACT

The mesencephalic trigeminal nucleus (MTN) is a unique structure in the central nervous system (CNS). It is composed of primary sensory neurons, which are mainly typical of dorsal root ganglia. The afferent projections of the MTN neurons are relatively well studied, although the data about the efferents are still controversial. The goal of the present study was to verify the MTN projections to the substantia nigra (SN) in the rat brain via anterograde tracing using the marker BDA. Anterograde tracing experiments showed the presence of a direct projection arising from the MTN neurons, located in the caudal portion of the nucleus, to the SN. Single ipsilateral projection fibres were found in the SN pars compacta dorsal part, and then penetrated its pars reticulata. It can be inferred from the study results that the MTN is bi-directionally linked with the SN, which probably in a way determines the mandibular motor activity and oro-facial dyskinesias.

Key Words: Mesencephalic trigeminal nucleus; Substantia nigra; Anterograde tracing; Biotinylated dextran amine; Rat Brain

INTRODUCTION

The mesencephalic trigeminal nucleus (MTN) is a unique structure within the central nervous system (CNS). It comprises the primary sensory neurons₁. In the rat it extends over 4 mm along the rostral pons and the whole length of the midbrain (1). About 70-80% of MTN neurons are situated in the large caudal part of the nucleus within the rostral pons (2).

While the afferent projections of the rat MTN neurons have been studied in detail (for a recent review see 1, and references therein), little is known about the efferent connections of the nucleus with other CNS structures (3). It is well established that the MTN neurons innervate spindles in the jaw-closing and extraocular muscles, as well as a subset of mechanoreceptors in the periodontal ligament and the tooth pulp (4,5,6,7). It is also worth noting that the cell bodies of the MTN neurons display differential topographical distribution: masticatory afferents

are dispersed throughout the whole rostrocaudal extent of the MTN, while periodontal receptor MTN afferents are mainly situated in the caudal part of the nucleus (4). The MTN is functionally homogenous as its neurons are involved, without exception, in the proprioceptive information processing.

On the other hand, the chewing movements are attributed to the substantia nigra (SN). It is a large midbrain nucleus composed of neurons containing neuromelanin and dopamine. Morphologically, the SN is composed of two parts: pars compacta, which is located dorsally and pars reticularis, situated ventrally. Pars reticularis extends rostrally to the subthalamic area and globus pallidus.

The probable connection between the trigeminal nuclei and SN has provoked a great interest. For example, in an anterograde and retrograde tracing study, Yasui et al. (8) showed that output signals from the SN pars reticulata are transmitted disynaptically to the trigeminal motor nucleus via the parvocellular reticular formation premotoneurons. More recently, an anterograde tracing study of ours (9) showed the presence of a direct bilateral projection from the SN pars compacta to the MTN in rats.

Using BDA as a very high sensitive

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anterograde tracer we set it as a goal of this study to show the existence of a bidirectional connection between the SN and the MTN in order to re-examine the efferent projections of the latter.

MATERIALS AND METHODS

Ten adult Wistar rats of both sexes weighing 280–350g were used for this study. The animals were anaesthetised with Thiopental (Biochemie, GmbH, Kundl, Austria; 25mg/kg b.w.) and then mounted on a stereotaxic frame. Under aseptic conditions small craniotomies were performed. The location of the injection site was stipulated in the following coordinates according to the rat brain stereotaxic atlas of Paxinos and Watson (10): 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 control. At the end of the injection procedure the microsyringe was held in place for 2 min to ensure 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 (Reichert – Jung, Austria) and collected in PB in a free-floating state. The sections were processed 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% H₂O₂ 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

We found that in three of the 10 BDA-injected animals there had been a precise application

of the tracer in the MTN (**Figure 1**). Therefore, the results reported are based mainly on these animals. In the rest of the animals the injection site was in the MTN, but adjacent structures, such as the locus coeruleus (LC) and the supratrigeminal region were also partially labelled (**Figure 2**). The intact contralateral side of the brain was used as a control.

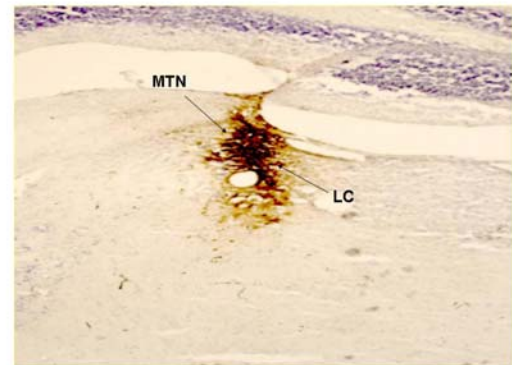


Figure 1. BDA injection site within the caudal part of the MTN. Magnification x50

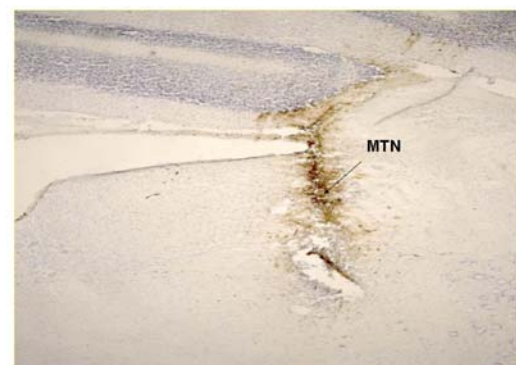


Figure 2. The injection site is in the MTN but the adjacent locus coeruleus (LC) and the supratrigeminal region are also partially labelled. Magnification x50

Following the injecting of BDA within the caudal part of the MTN, it is found that the fibres travel rostrally and cross the nigral pars compacta in its dorsal part. The most intense anterograde labelling was present in the median part of the SN while both the medial and lateral parts of the nucleus were devoid of labelling. The labelling was oriented parallel to the plane of section and the anterogradely labelled fibres passed adjacently to the nigral neurons and then penetrated its pars reticulata (**Figures 3, 4**). The labelling is obviously unilateral since the anterogradely labelled fibres were seen only in the ipsilateral to the injection site SN, while in the contralateral side they were absent.

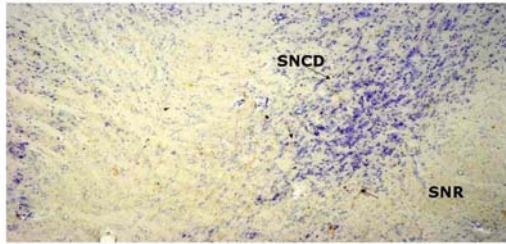


Figure 3. The labelled fibres cross the nigral pars compacta in its dorsal part. Magnification x50

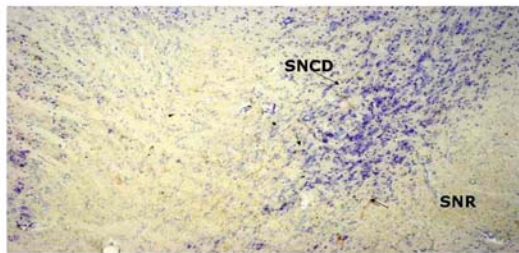


Figure 4. The anterograde labelled fibres pass adjacently the SNCD neurons. Magnification x100

DISCUSSION

The present study reveals for the first time the existence of unilateral direct projections from MTN neurons to the SN, predominantly to the dorsal part of the SN pars compacta. Through BDA intracellular application we were able to show extensive and abundant anterograde labelling of MTN axons and terminals.

The presence of a direct projection from the SN to the MTN was reported some years ago (9). According to the authors this relationship is topographically organised as neurons situated in the lateral part of the SN pars compacta that project to the MTN caudal portion. By injecting the caudal part of the nucleus, we established that MTN neurons send projections to the nigral neurons, situated in the dorsal part of the SN pars compacta. According to Lakke et al. (9) such a projection is bilateral, while the one we have found is unilateral.

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 (4). Proprioceptive information from these muscles may influence the activity of neurons in the SN and thus may act in the oral chewing movement.

It is known that SN contains two different neuronal populations: dopaminergic

cells located in SN pars compacta, and GABAergic neurons located in SN pars reticulata (9). On the other hand dopaminergic innervation of MTN has already been shown (2).

It is well known that MTN does not contain dopaminergic neurons, so it is unlikely the projection would be dopaminergic. It could, however, be GABAergic since a population of small-sized GABA-ergic neurons has been described in the MTN (11). These neurons are interneurons, so they could be part of a neuronal chain connecting the SN and motor trigeminal nucleus.

Further experiments in the future are needed to establish the exact neurochemical nature of the projection discussed in the present study.

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