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Morphology of NADPH-Diaphorase Reactive Neurons in the Human Thalamic Reticular Nucleus

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Nitric oxide (NO) has recently emerged as an important factor in neural signaling and synaptic plasticity. By using the histochemical procedure for NADPH-diaphorase (NADPH-d) the morphology of neurons and fibers containing NADPH-diaphorase activity in the human thalamic reticular nucleus (TRN) was examined. The morphological differences in the TRN neurons could contribute to the better understanding of the different functions in these cells associated with psychiatric disorders, epileptic seizures, and also the regulation of thalamocortical and corticothalamic inputs or outputs.

Key words: thalamic reticular nucleus, morphology, NADPH-diaphorase, nitric oxide (NO).

Introduction

The thalamic reticular nucleus (TRN) is a slender sheet of GABAergic cells, with colocalization of parvalbumin, situated around the external medullar lamina of the thalamus and medially to the internal capsule. This nucleus is considered as a pacemaker, indirectly regulating the activity of the cerebral cortex by thalamocortical and corticothalamic collaterals. On the other hand, it has direct connections with different subcortical regions and the other thalamic nuclei. The dendrites of its neurons form a local inhibitory network [1]. The TRN has been subdivided into specific sectors containing somatotopically organized projections of different parts of the body and head [1, 21].

Nitric oxide (NO) has the unconventional characteristics of being a gaseous neurotransmitter [2]. Nitric oxide synthase (NOS), the enzyme required for NO production, is localized within the GABAergic TRN neurons [3]. There is a big interest to identify the neurons producing nitric oxide (NO) with the involvement of NADPH-diaphorase (NADPH-d) [4, 5].

The structural features of NADPH-d-positive neurons in the rat [6, 7] and human brain have been well described in cortical cells [8, 9]. There is a small number of studies on them in other human brain areas. NADPH-d positive cells have been observed in the amygdaloid nucleus, the putamen [9, 10] and besides, we were only able to find few studies on NADPH-d-positive cells in the nuclei of the dorsal thalamus and the

adjacent reticular nucleus in the human brain [11, 12]. The aim of the present study was to determine the morphology and distribution of NADPH-diaphorase positive neurons in the TRN of the human brain.

Material and Methods

TRN samples were obtained from the brains of two females (40 and 45 years of age) and two males (43 and 52 years of age) at autopsy. The brains did not show any overt signs of pathology or trauma. The time from death until fixation was up to 12 hours. The brains were cut into slabs with a thickness of 1-2 cm in the coronal plane. The blocks were fixed for two days under gentle agitation in a mixture of 4% paraformaldehyde, 1% picric acid and 10 % glucose. Thereafter, the blocks were sectioned in the coronal plane and washed repeatedly in 0.1 M phosphate buffer, pH 7.4. Serial coronal sections of 40 µm were cut on a freezing microtome and collected in the same phosphate buffer. In brief, all slices, prepared as described above, were treated with sodium borohydride for 45 min followed by three consecutive rinses in 0,01 M PBS, each for 2 min. The sections were stained with the NADPHd-technique using 0.1-0.2 mg/ml nitro blue tetrazolium and 1 mg/ml b-NADPH and 0.3-0.5% Triton X100 in 0.1 M TRIS-HC1 buffer (pH 7.4) at 37°C for 30-60 min in a thermostat. Afterwards, the sections were rinsed for 5 min, 3 times in the same phosphate buffer and mounted on gelatin-coated glass slides. The slides were air dried for 24 hours, then washed in distilled water for 5 min, 3 times, air dried again and cover-slipped with Entellan (Merck, Germany) and examined using a light microscope (Olympus, Tokyo, Japan).

Results

Each of the 4 human brains showed numerous intensely or moderately stained NADPH-d positive cells in the TRN. The light-microscopic analysis of the TRN showed that the nucleus contained islets of neurons or scattered single NADPH-d-positive cells with different shape along the rostro-caudal and dorso-ventral axis and a different arrangement of their outgrowths. The staining of some of the cells was so intense that it resembled Golgi-impregnated neurons. The reaction product diffusely filled the cytoplasm of the positive neurons and their branches. At times, the non-stained nuclei of the neurons were distinguishable. The NADPH-d positive neurons were found in all sections from rostral to caudal extension of the human TRN, but their distribution and density were irregular. A high number of stained cells were observed first in the anterior portion, i.e. limbic and motor sector of the TRN. In shape, the NADPH-d positive neurons varied from fusiform, oval, or triangular to multipolar (**Fig.1**). Two to

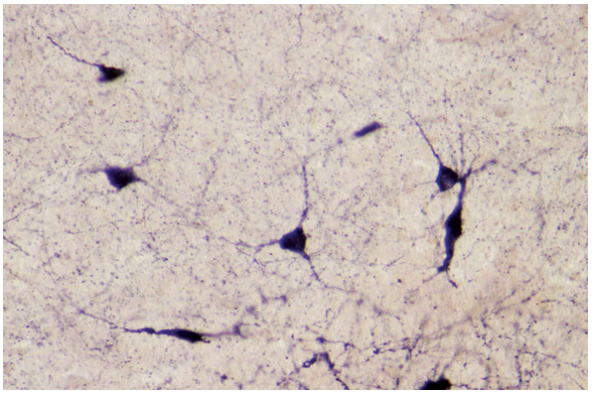

Fig. 1. Histochemical demonstration of NADPH-d reactive group of cells with fusiform, triangular and multipolar shape from the anterior part of the human TRN. The branches of these cells are short and can be followed in different directions (×400).

several outgrowths arose from each perikaryon, and then they branched dichotomically or threechotomically to secondary ones. Some of them had sparse branches that were covered with few spines. The axons of the cells could not be followed thoroughly although some of them were fork-bifurcated.

We observed clusters of positive cells only in the anterior part of the sagittal section of the TRN. In the intermediate part, a large number of neurons were typically fusiform in shape on the lateral and medial borders of the nucleus (**Fig. 2**). Others were oval and triangular in the central subdivision of the intermediate part of the TRN (**Fig. 3**) with almost straight, sparse, and very long, branched dendrites. Some of the dendrites could be followed from the cell bodies (**Fig. 4; Fig. 5**). The perikarya and dendrites of NADPH-d-positive cells of the TRN usually extended parallel to the internal capsule.

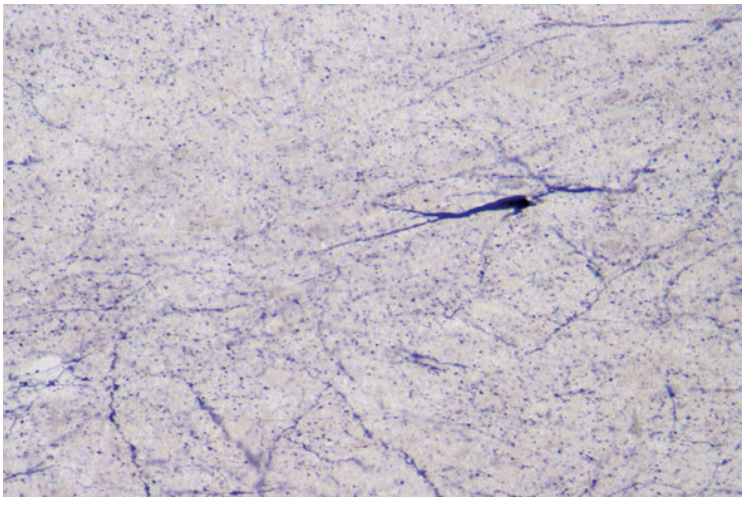

Fig. 2. NADPH-d reactive fusiform neuron from the dorsal portion of TRN with long dendrites with beads along their length (× 400).

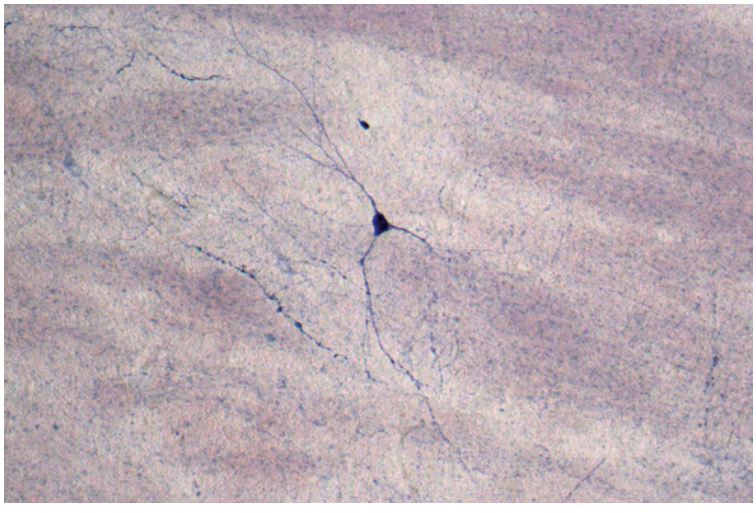

Fig. 3. A typical NADPH-d reactive triangular neuron from the dorsal part of the TRN with long, thin, straight and smooth fork-like branches (× 400).

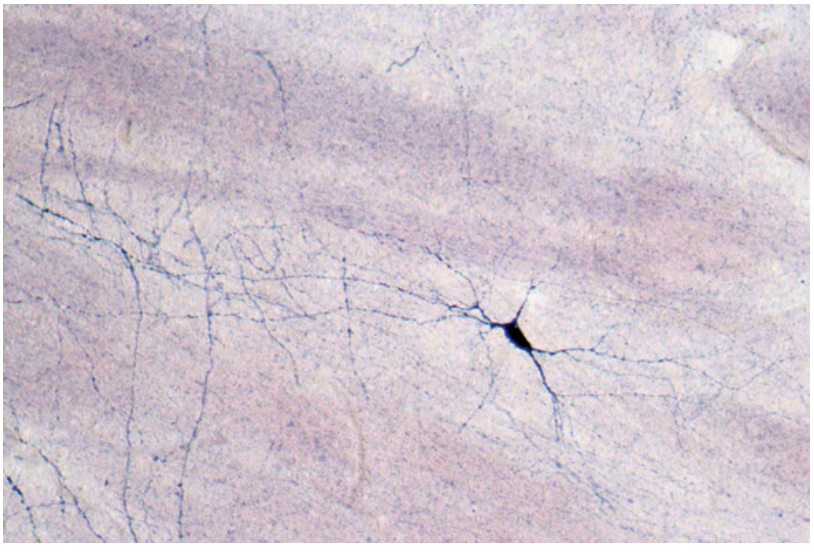

Fig. 4. NADPH-d reactive large, sparsely-branched, long-dendrite neuron of the TRN in the human (× 400).

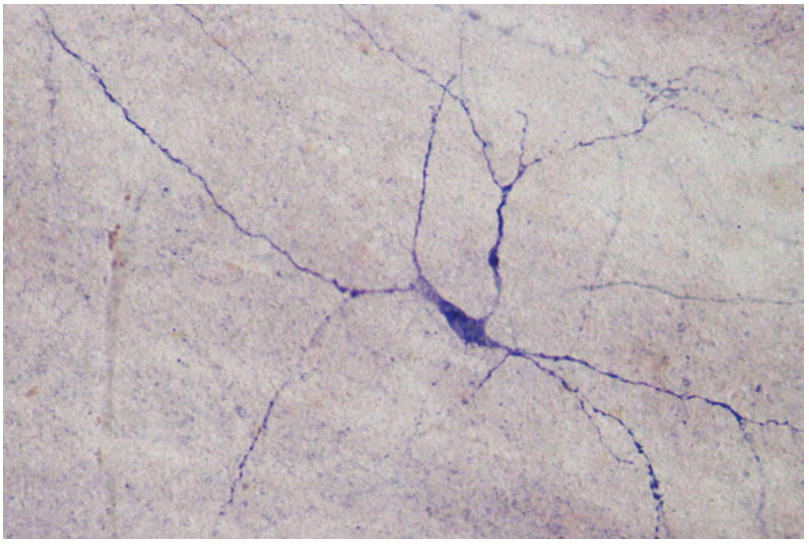

Fig. 5. A large neuron of the human TRN stained with NADPH-d with long dendrites significantly, extending from the cell body (× 400).

The posterior portion consisted of single, predominantly fusiform cells with forked dendrites parallel to the internal capsule with a lot of well visible varicosites (**Fig. 6**).

It should be noted that the neuropil everywhere in the nucleus contained a lot of unidentified NADPH-d- positive fibers with a different course and they contained beads with irregular shape and size.

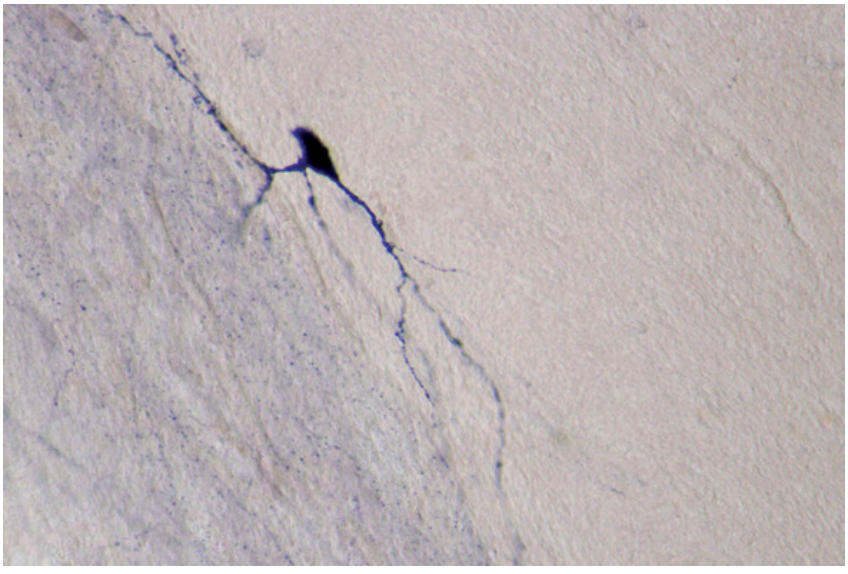

Fig. 6. An oval NADPH-d reactive neuron close to the internal capsule with thick bulbous dendrites, extending longitudinally (× 400).

Discussion

Studies on TRN in the human brain demonstrate the presence of NADPH-d-positive cells. This method also allows visualization of the structural details of neurons, which shows them to be reticular and short-axonal [12]. Our results indicate the existence of NADPH-d positive neurons in all the subdivisions of the TRN. In some cases the staining is so intense that it resembles Golgi impregnation with its axonal and dendritic arborizations [13]. We find a higher number of positive neurons in the rostral part of the nucleus and they are of a different shape. The neurons of the TRN are classified according to their perikaryal size into large, medium and small [11, 13-15]. They are not only isolated [11], but are also organized in groups or clusters, though only in the rostral part.

Previous data have shown that in the TRN there are two types of neurons. The first type is characterized by fusiform or angular neuronal profiles of large and intermediate size, with almost straight, sparse, and very long, rarely branched dendrites located on the margins of the nucleus [11]. Long collaterals with beads along their length make small-area contacts with two or three diagonal vessels. Besides, NADPH-d-positive cells of the second type possess short smooth dendrites. The bodies of these neurons vary from small to intermediate in size. The proximal parts of the cell dendrites tend to be rather thicker than the distal parts. The dendrite stems are straight or slightly tortuous [11]. Our data indicate the existence of NADPH-d positive neurons in all the subdivisions of the TRN. Recent studies have indicated that the increase in the NO levels potentiates inhibitory activity, presumably increasing the GABA release from presynaptic terminals by a cGMP-dependent process. Such an action is further

potentiated by the depolarization of the TRN neurons, thereby increasing the inhibitory tone in thalamocortical neurons [16]. NO is involved in different functions of the central nervous system, i.e. neurotransmitter release, regulation of neuronal electrical activity, and synaptic plasticity [17]. It plays a significant role in the normal aging and neurodegenerative processes in the CNS [18]. The TRN is particularly prone to oxidative stress [19] and also acts as a key player in the local sleep control [20], as well as in the temporal epileptic seizures [22].

Conclusion

Our results enlarge the existing data concerning the morphology and distribution of NADPH-d positive neurons in the human TRN. Moreover, NADPH-d positive neurons have both toxic and protective effects on the different parts of the CNS.

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