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Neurons from the adult human dentate nucleus: Neural networks in the neuron classification

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HIGHLIGHTS

- The paper deals with the extended classification of dentate nucleus neurons by artificial neural network (ANN).
- A previously applied classification by Ristanovic and colleagues is tested as whether this classification succeeds in correctly determining the neuron class on central and border groups according to eight morphological parameters. The analysis is further extender to virtual neurons and shown that neurons cannot be classified on central and border depending of localization according to measured parameters.
- In conclusion, the topological criteria pose difficulties and that it is better to use morphological criteria for classification of dentate nucleus neurons.

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ABSTRACT

Objectives: Topological (central vs. border neuron type) and morphological classification of adult human dentate nucleus neurons according to their quantified histomorphological properties using neural networks on real and virtual neuron samples.

Results: In the real sample 53.1% and 14.1% of central and border neurons, respectively, are classified correctly with total of 32.8% of misclassified neurons. The most important result present 62.2% of misclassified neurons in border neurons group which is even greater than number of correctly classified neurons (37.8%) in that group, showing obvious failure of network to classify neurons correctly based on computational parameters used in our study. On the virtual sample 97.3% of misclassified neurons in border neurons group which is much greater than number of correctly classified neurons (2.7%) in that group, again confirms obvious failure of network to classify neurons correctly. Statistical analysis shows that there is no statistically significant difference in between central and border neurons for each measured parameter ($p > 0.05$). Total of 96.74% neurons are morphologically classified correctly by neural networks and each one belongs to one of the four histomorphological types: (a) neurons with small soma and short dendrites, (b) neurons with small soma and long dendrites, (c) neuron with large soma and short dendrites, (d) neurons with large soma and long dendrites. Statistical analysis supports these results ($p < 0.05$).

Conclusion: Human dentate nucleus neurons can be classified in four neuron types according to their quantitative histomorphological properties. These neuron types consist of two neuron sets, small and large ones with respect to their perykarions with subtypes differing in dendrite length i.e. neurons with short vs. long dendrites. Besides confirmation of neuron classification on small and large ones, already shown in literature, we found two new subtypes i.e. neurons with small soma and long dendrites and with large soma and short dendrites. These neurons are most probably equally distributed throughout the dentate nucleus as no significant difference in their topological distribution is observed.

1. Introduction

Dentate nucleus, the largest and phylogenetically most recent of the cerebellar white matter nuclei, plays an important role as major relay center between the cortex and the other parts of the brain. It

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receives its afferents from the cerebellar cortex through axons of Purkinje neurons, premotor cortex and supplementary motor cortex (via the pontocerebellar system) and its efferents projects via the superior cerebellar peduncle through the red nucleus to the ventrolateral thalamus (crossing over at the pontomesencephalic junction). It is responsible for the planning, initiation and control of volitional movements (Mathiak et al., 2002).

Very little information is known on the neuronal types that constitute this nucleus and mediate in its specific actions. Thus, it is very important to address the problem of dentate nucleus neuron classification. In the early gestation period, neuron precursors of human dentate nucleus are similar in shape and form, being mainly bipolar (Hayaran et al., 1992). The initial phase of neuronal maturation involves a transformation from bipolar to various forms of multipolar neurons (Wadhwa and Bijlani, 1988). Working on different aspects of cytology and prenatal organization of the human dentate nucleus neurons, Mihajlovic and Zecevic (1986) have proved that the class of large neurons of the dentate nucleus could be further subdivided into four types according to their neurosoma/perykarion size and dendrite's arborization features. A similar classification has been used by Hayaran et al. (1992) in their study of cytoarchitectural development of the human dentate nucleus. The Hayaran's classification is not strictly precise about the categorization criteria used for neuron type classification. It does not put boundaries in between topological and morphological criteria. The neurons are not classified according to their topology and their morphology separately. Thus, neurons belonging to different parts of dentate nucleus are lumped together with neurons characterized by different histomorphological features in the same classification e.g. central and fusiform neurons.

Existing histological classification classifies human dentate nucleus neurons on small and large (Braak and Braak, 1983). Small type neurons are characterized by small size perykarion, short dendrites and short axon. Oppositely, neurons of large type have large soma and long neurites. The large neuron subpopulation is further divided into four types: (a) central neurons, (b) border neurons, (c) intermediate asymmetrical neurons and (d) intermediate fusiform neuron type (Mihajlovic and Zecevic, 1986; Hayaran et al., 1992). The central neurons are located in deeper parts of the nuclear mass, away from the nuclear periphery. Their somata are usually round and 'prickly'. From every direction of the soma numerous dendritic trunks emerge and each dendrite divides and subdivides giving rise to a complex branching pattern. The dendritic fields of these neurons are usually spherical. The border neurons are concentrated at the boundary of the nuclear lamina. They have an elliptical soma. A stout axon that is directed into surrounding white matter emerges from one end of the elliptical soma, while four to six primary dendrites that branch into the nuclear mass, emerge from the other end. The dendritic fields of these neurons resemble a tetrahedron with the cell body in one corner. The intermediate asymmetrical neurons are evenly distributed throughout the nuclear mass. Typically, they have large elliptical soma and five to nine primary dendrites radiating in all directions from the cell body. One or two of these dendrites are much longer than the rest, giving an asymmetrical appearance to the dendritic field. The intermediate fusiform neurons are also scattered throughout the dentate nucleus. These neurons have elongated elliptical somata. The soma is tapered at both ends. From the apex of the soma emerge three to five primary dendrites, which divided once to twice giving a few very long dendrites. The basis of the cell body tapers and divides into two parts. The upper part turns at right angle becoming parallel to the apical dendrites. In most of the fusiform neurons the dendrites are oriented toward the nuclear core being perpendicular to the indented surface of the nucleus (Milošević et al., 2010; Ristanovic et al., 2010).

Numerous qualitative histological and computational quantitative methods through various studies are used to classify neurons of human or monkey dentate nucleus. Their methods are mostly based on mere direct measurement of various neuron structural parameters such as neuron size and neurosoma major diameter (Braak and Braak, 1983; Chan-Palay, 1977; Fix, 1975; Sultan et al., 2003) but as well on relatively recent, indirect and more complex methods i.e. computational measurements of neuron morphological features e.g. fractal dimensions (Takeda et al., 1992; Milosevic et al., 2007). Ristanovic et al. (2010) applied computational approach for quantitative method on human dentate nucleus neuron classification.

All these studies use histological, neurobiological and molecular methods to classify dentate nucleus neurons but neither of them has used neural networks to assess this problem. On the other hand, a few studies used neural networks for cell classification problem based on different structural and functional criteria but not for dentate nucleus neurons. For example, some studies analyzed functional electrophysiological parameters of hypothalamic neurons using neural networks (Sim and Forger, 2007; Diekman and Forger, 2009). Samavedam's study (Samavedam et al., 1994) used the neural networks to classify red blood cells. Thus, none of the studies has used neural networks for adult human cerebellar dentate nucleus neuron classification.

Thus, our study has four objectives, (1) in order to prove potential relationship in between neuron localization and morphology, to determine whether the neurons of human dentate nucleus can be topologically classified i.e. on central and border types depending of localization according to structural and morphological characteristics, (2) regardless to this, to verify the sustainment of their proven classification on four morphological types, namely, (a) neurons with small soma (perykarion) and short dendrites (*SS*), (b) neurons with small soma and long dendrites (*SL*), (c) neuron with large soma and short dendrites (*LS*), (d) neurons with large soma and long dendrites (*LL*), according to their histomorphological properties but taking into account other parameters as well, (3) based on the previous, if possible, to potentially confirm two relatively novel neuron types (*SL* and *LS*) including the other criteria and (4) methodological aim is to do all of this by applying neural networks to both type of neuron classification problems, topological and morphological, due to their powerful neurocybernetic properties such as parallel data processing, ability of training and learning, high processing capacity and general reliability.

2. Materials and methods

2.1. Impregnation procedure and image acquisition

Material used in this study has been collected during the period of 2011–2012 at the Department of Forensic Medicine, School of Medicine, University of Novi Sad (Serbia), with the approval of the Ethics Committee of the University of Novi Sad, School of Medicine (Serbia). The cerebella are dissected and 2.5 mm thick blocks, impregnated after Kopsch–Bubenaite method (Schierhorn et al., 1977), dehydrated in an increasing concentration of alcohol and then embedded in paraffin. Serial horizontal sections are cut in 90 μm thick slices and mounted on glass slides.

Each section is observed by a light microscope under 400× magnifications. The fully-impregnation of a sample is successful when the area of interest (or section images) showed innumerable impregnated neurons of various types throughout the series of sections. Neurons are recorded and transformed into digital images by digital camera "Leica DC 100" (Leica Microsystem Wetzlar GmbH, Wetlzar, Germany) with appropriate software package (Leica Microsystem

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