Journal of Theoretical Biology 404 (2016) 273–284

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Classification of adult human dentate nucleus border neurons: Artificial neural networks and multidimensional approach

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HIGHLIGHTS

- Border neurons from adult human dentate nucleus can be divided into EBNs and IBNs, according to their topology and based on morphological parameters.
- The differences are subtle but significant.
- This has potentially significant neurofunctional implications but further studies are needed to elucidate that.
- Multimethodological approach is shown as the best for finding the solution closest to reality.

ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form
5 June 2016
Accepted 9 June 2016
Available online 15 June 2016

Keywords:
Border neurons
Classification
Multidimensionality

ABSTRACT

Aims: Primary aim in this study is to investigate whether external and internal border neurons of adult human dentate nucleus express the same neuromorphological features or belong to a different morphological types i.e. whether can be classified not only by way of their topology as external and internal, but also based on their morphological features or in addition to their topology also by way of their morphology. Secondary aim is to determine and compare various methodologies in order to perform the first aim in a more accurate and efficient manner.

Material and Methods: Blocks of tissue were cut out from the adult human cerebellum and stained according to the Kopsch-Bubenaite method. Border neurons of the dentate nucleus were investigated and digitized under the light microscope and processed thereafter. Seventeen parameters quantifying various aspects of neuron morphology are then measured. They can be categorized as shape, magnitude, complexity, length and branching parameters. Analyzes used are neural networks, separate unifactor, cluster, principal component, discriminant and correlation–comparison analysis.

Results: The external and internal border neurons differ significantly in six of the seventeen parameters investigated, mainly concerning dendritic ramification patterns, overall shape of dendritic tree and dendritic length. All six methodological approaches are in accordance showing slight clustering of data. Classification is based on six parameters: neuron (field) area, dendritic (field) area, total dendrite length, and position of maximal dendritic arborization density. Cluster analysis shows two data clusters. Separate unifactor analysis demonstrates inter–cluster differences with statistical significance ($p < 0.05$) for all six parameters separately. Principal component, discriminant and correlation–comparison analysis further prove the result on a more factor integrate manner and explain it, respectively. Thus, these neurons can be classified, not only according to their location but also according to some morphological features. Also, the group if internal border neurons is more homogeneous in itself than the other group of external border neurons.

Conclusion: Border neurons from adult human dentate nucleus can be divided to external and internal according to its topology and based on neuromorphological computational parameters. This has potentially significant neurofunctional implications but further studies are needed to elucidate that. Multimethodological approach is shown as the best for finding the solution closest to reality. The possible functional meaning of these morphological differences for cerebellar network structure and function are discussed.

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http://dx.doi.org/10.1016/j.jtbi.2016.06.011
0022-5193/

1. Introduction

The dentate nucleus occupies a central position in the cerebellar white matter, serving as a relay center for fibers coming from the cerebellar cortex, namely, from the axons of Purkinje cells (Chan-Palay, 1977). It represents the largest and phylogenetically most recent of the cerebellar white matter nuclei and plays an important role as major relay center between the cerebral cortex and the other parts of the brain. It receives afferents from the premotor cortex and supplementary motor cortex (via the pontocerebellar system), Its efferents project 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).

According to literature, neurons of adult human dentate nucleus can be divided, based on histological criterion, to small and large ones (Maric, 2010). Small neurons have a small cell body and short dendrites and belong to the functional class of interneurons. Perikaryodendritic compartments of small interneurons are synaptically coupled with numerous excitatory and inhibitory input neural systems as their afferents and represent pivots of very important neural integrated circuits responsible for motor learning (Maric, 2010). As been said, most of them make local projections, thus are interneurons, while some of them as projecting neurons generate output systems into the inferior olivar complex. Eventually, some of them belong to a special class of projecting neurons, so called inter–nuclear inhibitory neurons with ipsilateral and contralateral inter–nuclear connections (Chan-Palay, 1977).

Large neurons on the other hand have large somata and long dendrites (Ristanovic et al., 2006, 2009, 2010, 2011, 2012; Milosevic et al., 2007, 2010a, 2010b). They are targets of a large number of various extracerebellar systems. Topologically, large neurons can be classified as central and border neurons (BNs). Central neurons occupy the central grey nuclear mass, surrounded by BNs. BNs as the outer neuron cluster are predominantly distributed along the medial nucleus edge in the rostrolateral columnar zone, in the zone of intermediate curvature and the caudolateral columnar zone. Thus, they occupy external and internal quarter of grey matter lamina of the dentate nucleus while central ones are distributed in central two quarters of nucleus lamina (Maric, 2010). Qualitative and quantitative analysis of large dentate nucleus neurons morphology and dendritic tree orientation revealed that in contrast to the central neuron type which is characterized by radial symmetry of the dendritic tree, BNs have an asymmetric dendritic arborization. This feature is tightly related to the sources of information inputs to a specific neuron type (Jansen, 1972, Chan-Palay, 1977, Schmahmann and Pandya, 1995, Hoover and Strick, 1999, Horn et al., 2002, Manto and Pandolfo, 2002, Kelly and Strick, 2003, Pastor et al., 2004, Ramnani et al., 2006).

BNs can be further subdivided into two topological subclusters, namely external border neurons (EBNs), located on the external surface of the nucleus and internal BNs (IBNs) located on the internal nuclear surface enveloping that way the bottom of the hilar region (Fig. 1). Current literature (Braak and Braak, 1983, Maric, 2010), histologically, doesn't differentiate BNs within each other i.e. they represent the same neuromorphological histological type. According to it, BNs are predominantly aspinous multipolar neurons characterized by elongated cell body with asymmetrical dendritic tree. In the monkey BNs are spinous neurons (Maric, 2010).

It is a known fact that output cerebellar systems in huge majority originate from dentate nucleus neurons projecting with their axons outside the cerebellum. However, some nuclear outputs remain local ones connecting that way dentate nucleus with cerebellar cortex. EBNs project with their axons to the cerebellar cortex and represent the so–called dentatocortical system. IBNs together with central axons represent the majority of output extracerebellary projecting neurons. The variety of these connections determines dentate nucleus functions and the morphology of the dentate neurons may play a role in control of these functions, but the nature of these morpho–functional relations remains to be determined by other studies (Maric, 2010).

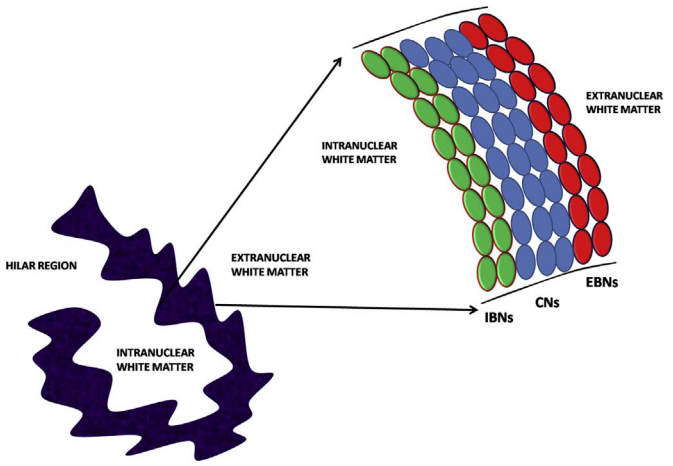

Fig. 1. Scheme of a longitudinal cross–section through the dentate nucleus and positions of BN neuron types in it. IBNs with their pericaryodendritic compartments are oriented toward to the internal surface of the nucleus while EBNs are directed to the outer side.

Our previous study (Grbatinic et al., 2015) showed that there are no significant morphological differences between central neurons and BNs and that they only differ in their topology. However, in contrast to our previous results, there are indications that there might be some differences, in size and in shape complexity. For example, Maric (2010) showed that internal BNs are significantly larger than central neurons and that central neurons have much lower complexity than BNs.

Having this in mind and taking into account known topological and functional differences (in terms of the different connections (Maric, 2010) between EBNs and IBNs and in view of the interdependence between morphology and function, we wanted to further deepen the dentate nucleus neurons classification problem by trying to classify BNs accordingly. In addition to the difference in localization, potential differences in the neuromorphological properties of two neuron clusters which are, however, subtle and difficult to grasp due to the variations within the two groups require application of multivariate classification methods. Thus, the first aim of this study is to investigate if and to what degree there are systematic differences between the morphology of external and internal border neurons of the dentate nucleus. As our previous study was the first one which applied neural networks to this kind of problem, we wanted now to perform some kind of methodology–comparison analysis by including other methods of multidimensional statistics to the neuron classification problem, especially Fisher's linear discriminant analysis. The second aim was to provide and compare methods suited for the classification of neurons based on morphological properties, such as soma size, dendritic ramification pattern, dendritic length etc. So we hope to be better able to describe and quantify these potential inter–cluster differences if there is any, by way of the classification methods we use. And we do this because we expect the morphological differences to be relevant with respect to neural circuitry enrolled. Just to emphasize, our analysis is based on cell bodies and dendrites, not on axons.