Two subpopulation of human Purkinje neurons: an electron microscopy study

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Abstract

Conventional electron microscopy of human cerebellar cortex has revealed two types of Purkinje neurons with different staining intensities. The light-stained type constitute the major type and both types have similar diameters. This finding was not reported in human before but in lower mammalian. Whether the different staining pattern of Purkinje neurons implies a specific histo-functional aspect a pathological one or histological artifacts is not known.

LETTER

Purkinje neurons are present in the cereberall cortex within a single layer intercepted between the inner molecular and outer granular layers. They present unique morphological feature as having a large soma with profound arborization of their dendritic tree. Purkinje synapse directly with the parallel fibers of granule cells and are influenced by granule cells which receive synapses from mossy fibers of the central nervous system.

Coronal sections of cerebral cortex taken from cadavers of 20 normal human adult were screened following conventional electron microscopy. Purkinje neurons are easily identified by their typical features of vesicular nuclei and prominent nucleoli.

Our study has revealed the presence of two heterogeneous types of Purkinje neurons; light and dark-stained types which were identified based on their staining intensities. The light-stained neurons has oval shape cell body and a smooth outline whereas the dark type has elliptical cell body and an irregular outline. The light stained type presents the major type and constitutes about 75% of the total number of Purkinje cells whereas the dark one presents about 25%. Furthermore the light and dark-stained Purkinje neurons show almost similar measurement of the diameter of their cell body, nucleui and nucleoli. On average Purkinje neurons cell body, nuclei and nucleoli of both types measures about 25–38 μ, 13–16 μ and 4–6 μ respectively.

In the literature, several histochemical and immunohistochemical studies have revealed that cerebellar Purkinje cells are histochemically heterogeneous [1–3]. This non uniformity of Purkinje cells was attributed to different protein [4–5], DNA and RNA [6–7] contents reflecting possible different functional aspects of both types. Only two investigators

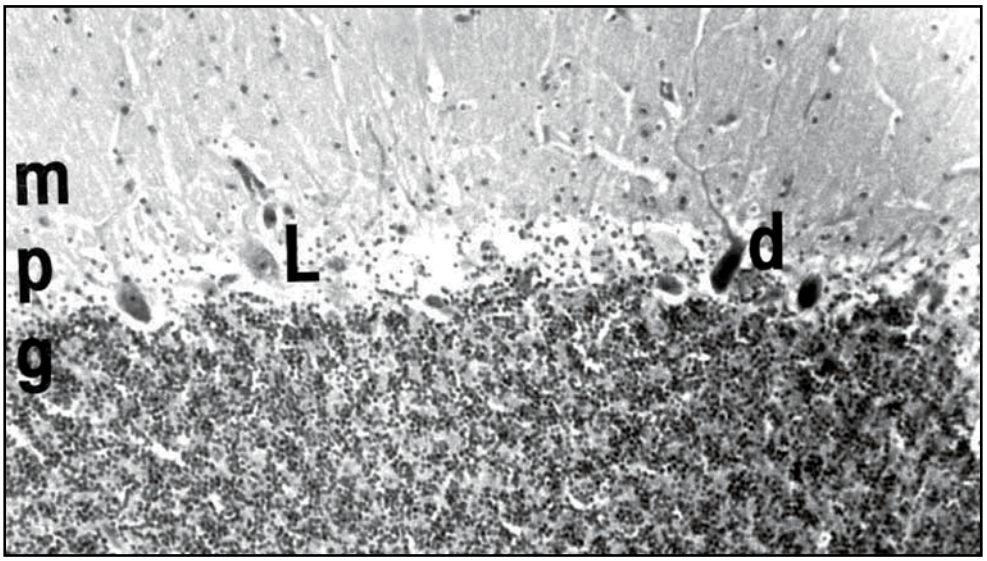

Figure 1. Coronal section of Cerebellar cortex showing Purkinje neuron layer in-between the outer molecular (M) and inner granular (G) layers of cerebellar cortex. Two distinct types of Purkinje neurons (P) with light or dark staining properties are shown.

have reported the heterogeneity of mammalian Purkinje neurons demonstrated by specific types of stains [8–9]. Other investigators have attributed the differences in staining properties due to possible degenerative changes due to old age [10]. Indeed that investigator reported that younger age groups of rats, cats and guinea pigs have less dark population of Purkinje neuron whereas older age groups of the same animals showed increased number of dark-stained Purkinje cells [10].

The presence of dark staining neurons still present a controversial issue. While some investigators consider dark neurons as a distinct class, others consider them as histological artifacts. The presence of dark stained has been reported by many investigators and have attributed to several factors including trauma to nervous tissue while collection followed by postmortem degeneration with subsequent improper fixation and other histological preparations [11,12,13]. The biochemical events that may have led to artifacts formation has been explained by several changes including mechanical depolarization, glucose deprivation and disruption of the neurons membranes [14,15]. Those artifacts has been reproduced by some investigators by inadequate fixation or exposure of the nervous tissue to certain nervous toxic chemicals as soman and sarin [14,15]. Dark neurons in most of these reports presented specific light microscopic morphological features as shrunken in size with pyknotic nuclei that merged with the surrounding perikaryal cell body and showed microvacuoles [12–15].

While we cannot role out such explanation to the presence of histological artifacts we believe that the dark staining neurons presented in our report may indeed present a distinct class of Purkinje neurons for the following obvious reasons. First, our study shows clear microscopic features at electron microscopic level and both light and dark stained neurons show similar sizes. Second, we have not been able to demonstrate the presence of pyknotic nuclei or the prersence of micovacoules in cell bodies of these neurons. Further more one investigator [8] has reported two subpopulation of Purkinje neurons in cerebelli of rats using two different types of chemical stains which selectively stained two different types of neurons.

Briefly our results may indeed demonstrate for the first time that human Purkinje neurons are heterogeneous which is in accordance with that as demonstrated in lower mammals. Finally we believe that further immunostaining and functional studies are needed to clarify this controversy.

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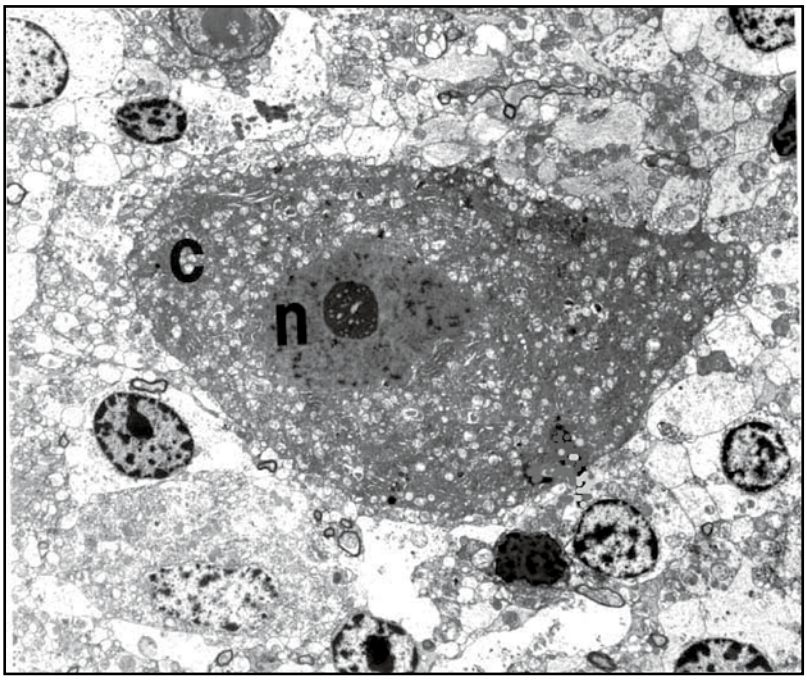

Figure 2. Dark stained Purkinje Neuron shown at higher magnification (3938). Shows irregular cell body outline (C) with marked enfolding of its cell membrane. Cell body is filled with dark stained material.

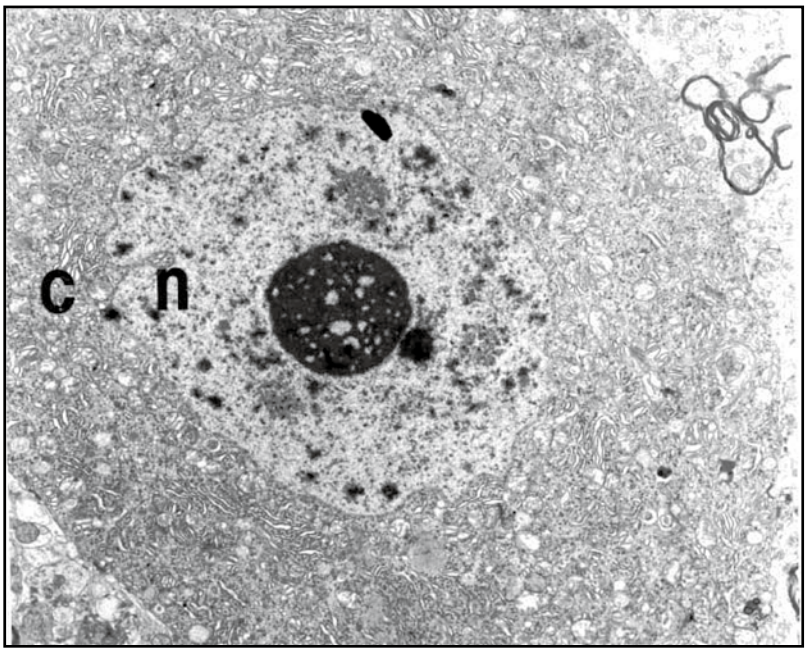

Figure 3. Light stained Purkinje Neuron shown at high magnification (2000) with oval shape cell body (C) with regular smooth outline and less staining density and Large Nucleus (N).

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