

LOSS OF GRANULE NEURONS IN THE AGING HUMAN CEREBELLAR CORTEX

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A number of age-related morphological changes in the brain, including loss of brain weight and volume, enlargement of ventricles and subarachnoid space, and alterations in cell numbers, among other, have attracted the attention of neurobiologists for many years. Of all of these changes, however, the phenomenon of neuron loss and possible glial increase with age has probably excited the greatest interest. The cerebellar cortex provides a relatively simple model system for the study of effects of aging on neurons in the CNS. In this study, the number and volume of granule neurons (granule cell, GC) in the cerebellar cortex was measured to determine if in the human cerebellum could exist age-dependent changes in GC.

The human cerebellum consists of two hemispheres, between which is a midline, worm-like structure called the vermis. In the rostrocaudal direction, the cerebellum is divided by two fissures into three major lobes: anterior (paleocerebellum), posterior (neocerebellum) and flocculonodular lobe (archicerebellum). The posterior lobe contains the cerebellar tonsils (amygdala). The cerebellar surface is highly foliated with numerous parallel folds, called folia. A layer of grey matter, the cerebellar cortex, forms the outer surface portion of each fold (folium). The histology of the cortex consist of three layers: the outermost molecular layer, a middle Purkinje cell layer, and the innermost granular layer.

A number of studies have suggested that human cerebellar Purkinje cells (PC) decrease in number during aging (Hall et al., 1975; Torvik et al., 1986), however, the factors responsible for a loss of PC in the human cerebellum are unknown. Whether human cerebellar granule neurons (GC) undergo similar changes in number during aging are also not known (Dlugos & Pentney, 1994). In animals, no significant age-related changes in the number of GC have been found (Sturrock, 1989). It is widely accepted that extensive neuronal loss in the cerebellar cortex during aging may provide the morphological substrate for age-associated changes in cerebellar function, as are the loss of muscular tone, loss of automatism, or a decrease of the equilibrium.

The granule neurons in the cerebellar cortex were studied in male humans from 17 to 80 years. We have quantitated with the stereological technique the number and volume of granule cells in the human cerebellar cortex (amygdala area). Twenty-six brain from neurologically and psychiatrically intact autopsy cases aged between 17 and 80 years were investigated in 3 age groups: I, 17-40 years, II, 43-60 years, and III, 65-80 years. The brain was removed, and the cerebellum was separated from the cerebrum by a transverse incision through the inferior colliculus. The right cerebellar amygdala was further separated from the cerebellar hemispheres by two parasagittal incisions along its lateral boundaries. Of the amygdala and in lateral direction have extracted five not consecutive pieces (2 mm³) from each one from the subjects. These cerebellum pieces was then dehydrated in alcohols and embedded for parasagittal sectioning in Epon 812. All embedded blocks were coded to eliminate investigator bias. Each tissue block was sectioned completely with a glass knife on a LKB microtome at 1 μ m. Ten serial sections were collected at each of 10 intervals along the longitudinal axis. Two random numbers, selected from a random numbers table, were used. The first number determined the first section to be collected for the first series of 10 sections, and the second number defined the interval between successive series of 10 sections (Pakkenberg & Gundersen, 1988). A total of 10 pairs of sections per human were used for measurements of GC. Sections were aligned on glass slides, stained with 0.5% toluidine, dehydrated, and cover-slipped with Permount. The disector stereological method (Sterio, 1984; West, 1993) was used for determinate the total numbers of GC in cerebellar cortex. This method provided an unbiased estimate of the number of GC through use of a three-dimensional probe, a pair of tissue sections that provided counts of particles that were independent of particle size and shape (Dlugos & Pentney, 1994). With respect to the stereological method used in this study, we have selected an area relatively small of the cerebellar cortex, but well delimited, as is the cerebellar amygdala. In this area have been obtained samples from a great number of folias that assure an excellent representation of this structure.

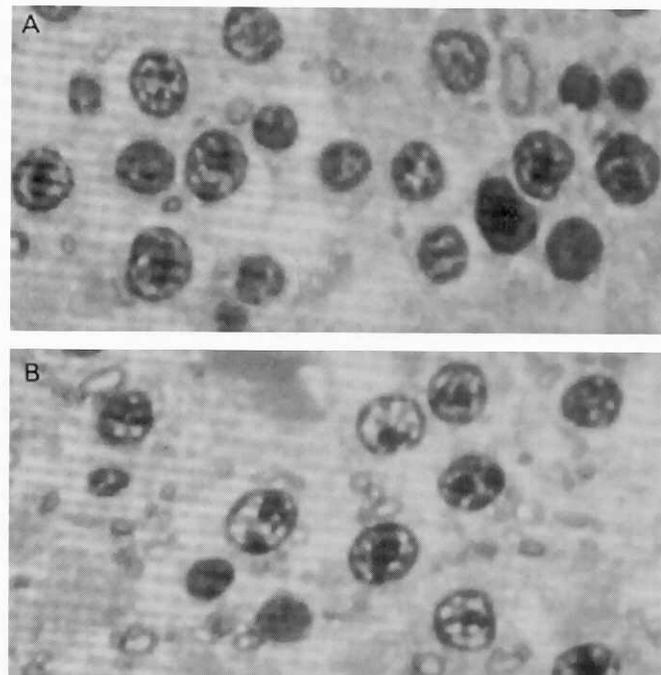


Figure 1. Section of a human cerebellar cortex (granule cell layer):
(a) 19 years, (b) 80 years

The analysis of the results of the cerebellar cortex (amygdala area) suggests cell differences according to the age (figure 1). The total number of GC decline significantly during the aging process ($r = -0.601$; $p < 0.001$). The human cerebellum aging involve a decrease of the number of GC in the granular layer ($p < 0.001$). In this study we have established 3 age groups: I of 17 to 40 years, II of 43 to 60 years and III of 65 to 80 years. The group I shows a GC densities of $388 \pm 103 \times 10^3 \text{ mm}^{-3}$. While advances the age in the cerebellar amygdala reduces the number of GC ($p < 0.001$); in the group II (43-60 years), this cellular type reduces a 23% ($299 \pm 130 \times 10^3 \text{ mm}^{-3}$) and in the group III (65-80 years) the decrease is greater (38%; $241 \pm 66 \times 10^3 \text{ mm}^{-3}$) respect to the group I. Also the volume of GC is smaller in the group III. We have found that they exist significant differences in GC between the groups II and III ($p < 0.004$). The figure 2 shows the distribution of the number of GC in each one of the groups of age selected. The profile change in the distribution of GC during the human aging is notable from 40 years. Furthermore we have demonstrated that it exists a great variability in the GC densities in the II group (43% coeff. variation, as compared to a 26% in the group I and 27% in the group III); It is possible that the premature cerebellum aging this conditioned above all in this period of maturity.

Data presented above showed that the cerebellar GC in the amygdala did change in number in age-function. These results would be in disagreement with the obtained in rodents where it has been indicated that they do not exist changes with the age in GC (Druge et al., 1986; Quackenbush et al., 1990). It is possible that these differences could be explained by a greater evolutionary degree of the cerebellar cortex in the human that makes it most vulnerable to environmental and educational effects. Recently Dlugos and Pentney (1994) shown that the numbers of PC and GC and the ratio PC/GC were stable with advancing age in F344 rats. A number of studies have suggested that human cerebellar Purkinje cells decrease during aging (Hall et al., 1975; Torvik et al., 1986), however the factors responsible for a loss of PC and GC (described in this paper) in the human cerebellar cortex are unknown.

Even though has been indicated that neuronal loss is not a requirement for disruption of normal cerebellar function, our results demonstrate that in the human aging exists a notable loss quantitative (38%) as well as qualitative (cells are smaller) of the GC. In the granule layer the cell is very numerous, with axons that extend up toward the molecular layer, where they bifurcate, pass along the length of the folium (as parallel fibres) and synapse with the hair-like dendritic trees of the Purkinje cells. This important decrease in GC must of be translated in alterations in the function of human cerebellum. In general the cerebellar dysfunction includes: disequilibrium, muscle tone disturbance, movement disorders (as incoordination and decomposition of movements, dysmetria, intention tremor...), speech deficits and cerebellar nystagmus. All these functions go being deteriorated with the age, and an explanation of this phenomenon could have their base in the decrease of the GC.

Measurements of the molecular layer, however, showed that this layer, the site of synaptic contact between GC and PC, decreased in volume with age (Dlugos and Pentney, 1994). The depletion of the synaptic interactions between the GC axons and the PC dendrites in the molecular layer of the cerebellar cortex, may also alter functions of cerebellum. The volume of the molecular layer decreased significantly at 18-27 months of age in the rats. These decrease in volume imply that the axonal processes of the GC and/or the dendritic process of the PC were less extensive in the older rats. Possibly the decrease of the PC that it has been described previously in human cerebellum could be explained also with the decrease of the granular synapses. It appears that partial regression of neurons through axonal or dendritic reductions does occur in the human cerebellar cortex. The depletion of dendritic and/or axonal processes in the molecular layer may affect the functioning of the cerebellum as a motor integration center.

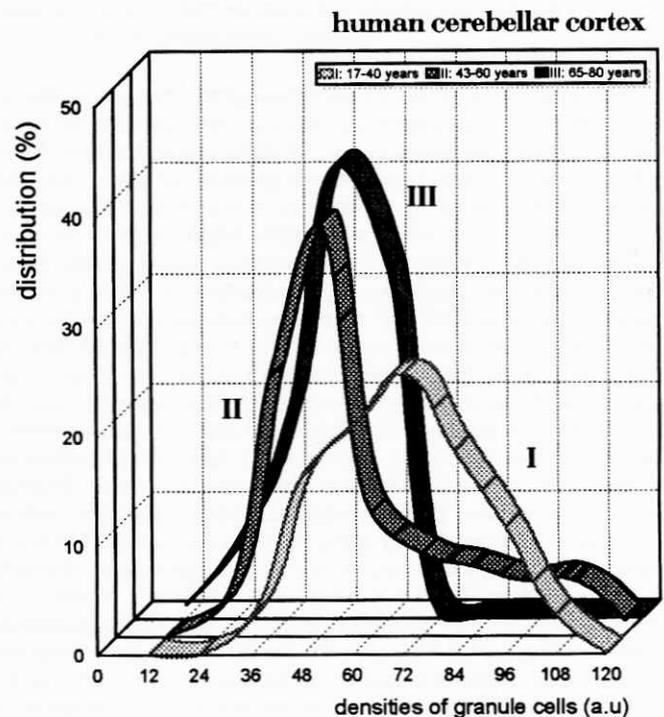


Figure 2. Distribution (%) of densities (arbitrary units) of granule cells in human cerebellar cortex (amygdala area)

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