

## ORIGINAL PAPER

# Cerebellar atrophy – a comparative microscopic study

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### Abstract

The cerebellum is required for the coordination of fine movement. In health, it provides corrections during motion which are the basis for precision and accuracy, and it is critically involved in motor learning and reflex modification. Disease of the cerebellum or its connections leads to incoordination. The study puts forth the morphometric study of cerebellum cortex and of the modifications that occur at this level during cerebellar atrophy. Our research used routine histological methods, but also special methods, adequate to the studied cerebellar cortex (silver impregnation), in order to observe the characteristic structures of the organ. We used comparative morphometric methods in order to gather data about the structural changes that occur in the cerebellar cortex during cerebellar atrophy. In order to analyze the histological modifications we determined the following parameters: thickness of the cerebellar cortex – is a derived feature appropriate for elongated or thin structures, area fraction of the molecular layer in the cerebellar cortex, number of Purkinje neurons per microscopic field and area fraction of the blood vessels per microscopic field. The reference microscopic field has an area of 280.000  $\mu\text{m}^2$ . Observations and conclusions that arose from this study may represent a contribution to the theoretical knowledge on which the medical practice is based.

**Keywords:** cerebellum, atrophy, morphometry.

### Introduction

Cerebellar atrophy is a progressive degeneration of the cerebellum with loss of cellular elements. The cerebellar degenerations are characterized by the development of progressive ataxia together with other associated neurological signs.

The age of onset and clinical features depend on the diagnosis of the type of cerebellar degeneration. The great majority of cerebellar degenerations are heredo-familial genetically-determined conditions. Cerebellar degeneration occurs also as a complication of chronic alcoholism and may also occur as a non-metastatic complication of a distant carcinoma [1].

Our study puts forth the morphometric study of cerebellum cortex and of the modifications that occur at this level during cerebellar atrophy.

### Material and methods

This study was performed on human cerebellar specimens, necroptic pieces (cerebellum harvested during autopsy) from subjects of different ages.

We studied a total of 30 cerebellar specimens that belong to the research fund of Histology Department, Faculty of Medicine, "Ovidius" University, Constanța. The pieces were split in two groups:

- control group – 15 cerebellar specimens from subjects with no history of neurological disease;
- test group – 15 cerebellar specimens from patients with cerebellar atrophy.

In the present study, three analysts (E.Gh., S.V. and V.T.) worked independently to assess cerebellar cortex thickness, the molecular layer area fraction (%), and Purkinje neurons number per microscopic field, and no discrepant results were found.

The obtained histological specimens were fixed and then processed using paraffin embedding. Serial sections were made at 4–5  $\mu\text{m}$ , which were stained using Hematoxylin–Eosin technique in order to observe the general structure of the cerebellum, and silver impregnation technique, obtaining histological specimens in which the nerve fibers are readily identified.

The images were captured from the microscope using a videocamera, a capture board and a computer. In order to analyze the histological modifications the following parameters were determined:

- Thickness of the cerebellar cortex – is a derived feature appropriate for elongated or thin structures.
- Area fraction of the molecular layer in the cerebellar cortex.

AreaFraction value is the ratio of segmented image

area and the Measured Area (of the cerebellar cortex). It has a strong stereological interpretation: in the case of isotropic uniform random sections it is equal to the volume fraction:

$$\text{Area Fraction} = \frac{\text{Area}}{\text{Measured Area}}$$

- Number of Purkinje neurons per microscopic field – the software automatically recognize the nuclei and count them.

- Area fraction of the blood vessels per microscopic field.

The reference microscopic field has an area of 280.000  $\mu\text{m}^2$ . The obtained data were interpreted and represented in graphics.

### Statistical analysis

Descriptive statistics compared the cerebellar cortex thickness, the molecular layer area fraction (%), and Purkinje neurons number per microscopic field between control and test group.

Results are reported as mean  $\pm$  standard deviation, medians and ranges for the counts performed for each group. A P-value equal to or less than 5% was considered statistically significant.

### t-Test

Paired Two Sample for Means was used to compare the two groups in regard to the categorical data. If the *t* value that is calculated is above the threshold chosen for statistical significance (usually the 0.05 level), the null hypothesis that the two groups do not differ is rejected in favor of an alternative hypothesis, which typically states that the groups do differ.

### Results

The median cerebellar cortex thickness was 1151.58  $\mu\text{m}$  (ranges: 800.39 – 1428.5  $\mu\text{m}$ , mean  $\pm$  SD 1175.82  $\pm$  169.5657) in control group, 620.78  $\mu\text{m}$  (ranges: 562.79 – 694.65, mean  $\pm$  SD: 619.294  $\pm$  36.86318) in test group.

When the mean values of cerebellar cortex thickness in control and test group were compared, significant difference was noted ( $P = 6.22\text{E}-10$ , t-Test: Paired Two Sample for Means).

The median molecular layer area fraction (%) was 34.26 (ranges: 28.46 – 36.21, mean  $\pm$  SD: 33.52533  $\pm$  2.487442) in control group, 45.36 (ranges: 42.58 – 48.25, mean  $\pm$  SD: 45.248  $\pm$  1.667985) in test group.

When the mean values of the molecular layer area fraction (%) in control and test group were compared, significant difference was noted ( $P = 1.68\text{E}-10$ , t-Test: Paired Two Sample for Means).

The median Purkinje neurons number per microscopic field was 2.95 (ranges: 2.21 – 3.42, mean  $\pm$  SD: 2.85  $\pm$  0.367248) in control group, 1.82 (ranges: 1.13 – 2.18, mean  $\pm$  SD: 1.82  $\pm$  0.285132) in test group.

When the mean values of Purkinje neurons number per microscopic field in control and test group were compared, significant difference was noted ( $P = 1.97\text{E}-07$ , t-Test: Paired Two Sample for Means).

### Discussions

The cerebellum has two hemispheres separated by the vermis. The surface of the cerebellum has many sulci perpendicular to the vermis. These sulci divide the organ into lobules, each of which has a superficial layer of gray matter (cortex) and a core of white matter (Figure 1).

The great majority of the neurons in the cerebellar cortex are the small and extremely densely packed granule cells. Granule cells are so densely packed that the cerebellar cortex contains many more neurons than the cerebral cortex.

Unlike the cerebral cortex, which has a large number of diverse cell-types which are arranged differently in different regions, there are only a few types of cells in the cerebellar cortex and these are interconnected in a highly stereotyped way. The Purkinje cells are quite large, and their dendrites divide repeatedly in one plane, forming a sort of fan.

The most superficial layer of the cerebellum (the molecular layer) has few perikaryons and many unmyelinated nerve fibers. One region of the cerebellar cortex looks very much like another (Figure 2).

In its regular, repetitive cytoarchitecture, the cerebellar cortex lends itself to quantitative analysis.

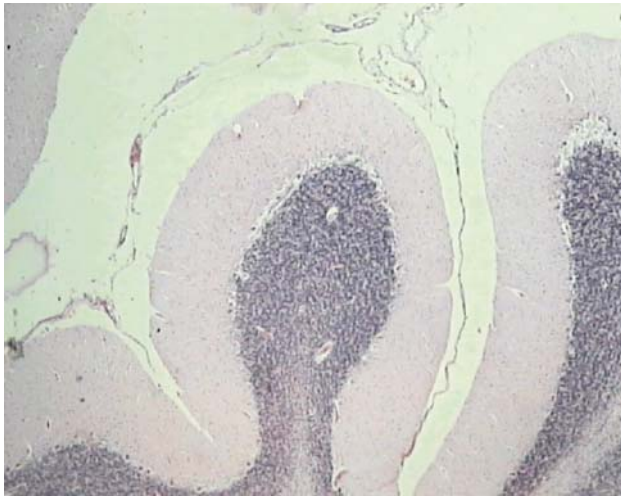
A vertical column of human cerebellar cortex, 1 mm square in area at a folia summit, contains about 500 Purkinje, 600 basket, 50 Golgi and perhaps 3 000 000 granular neurons, with about 600 000 glomeruli [2].

It is difficult to estimate the total cerebellar cortical area [3], but since its sagittal dimension is said to be over a meter, and its transverse, unrolled extent about one-sixth of a meter the total surface area would be about 200 000 square millimeters. By these calculations there would be over 1 011 granule cells and their associated circuitry in the cerebellar cortex.

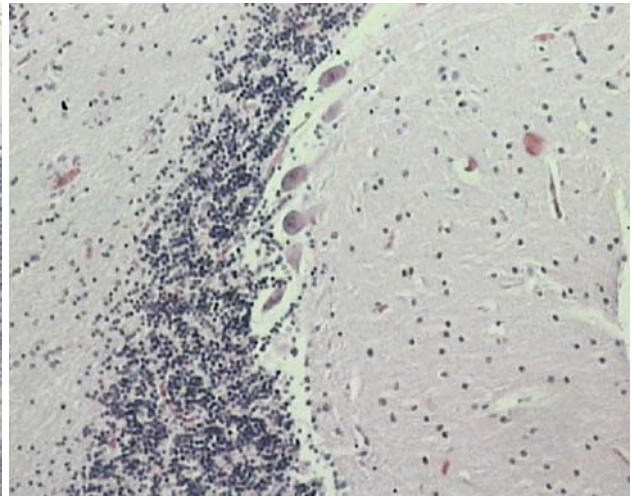
On the input side a single olivary afferent gives rise to ten climbing fibers; each climbing fiber synapses with only one Purkinje neuron, and by collaterals with an undetermined number of interneurons. In contrast, a single mossy fiber diverges greatly; synapsing with 400 or more granular neurons in one folium; if branches to adjacent folia are included, the number is probably several thousand. Conversely, each granule cell receives synapses from four or five different mossy fiber terminals (Figure 3).

Ascending axons of granule cells bifurcate into parallel fibers, synapsing with Purkinje, Golgi, basket (internal) and external stellate neurons. Thus, there is divergence from a mossy fiber through the granular neurons to perhaps hundreds of thousands of Purkinje neurons; there is uncertainty about the amount of overlap between parallel fiber territories, but enormous convergence of paths to individual Purkinje neurons exists, a dendritic tree of one Purkinje cell receiving an estimated total of 175 010 synapses from different parallel fibers [4].

The connections of the cerebellum are organized in two perpendicular planes, corresponding to the planar organization of the cerebellar cortex.



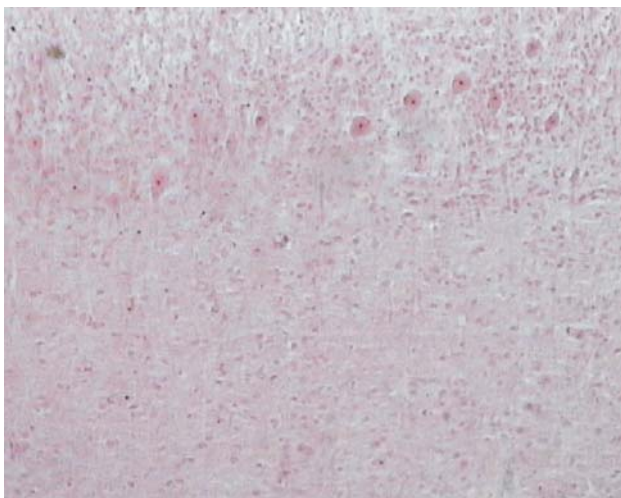
**Figure 1 – Cerebellum – control group.** Cerebellar cortex with the three distinct layers. A reduced portion of white matter is also seen in the image. The cortex is covered by the pia-mater, with small blood vessels inside it (HE staining, ob.  $\times 20$ )



**Figure 2 – Cerebellar cortex – control group.** There are clearly identified the molecular, Purkinje and granular layers. Purkinje neurons are large, pear-shaped and arranged in a single row (HE staining, ob.  $\times 100$ )



**Figure 3 – Cerebellar cortex – control group.** Blood vessels have a straight course through the thickness of the cortex. Pia-mater penetrates a sulcus and covers the cortex (Silver impregnation, ob.  $\times 100$ )



**Figure 4 – Cerebellar cortex – test group.** Purkinje neurons are small and less numerous than normal (HE staining, ob.  $\times 100$ )



**Figure 5 – Cerebellar cortex – test group.** Large area affected by atrophy, with no Purkinje cells in its structure. The blood vessels are thin and reduced in number (Silver impregnation, ob.  $\times 20$ )

Efferent connections of the cortex are disposed in parasagittal sheets or bundles. They connect longitudinal strips of Purkinje cells with certain cerebellar or vestibular nuclei [5].

Afferent connections of the cerebellum include the mossy fibers and the climbing fibers. The monoaminergic afferents were considered in the section on the chemoarchitecture of the cerebellum. The organization of the mossy fiber systems differs substantially from that of the climbing fibers. Mossy fiber systems terminate bilaterally in transversely oriented "lobular" areas.

The terminations of different mossy fiber systems overlap considerably. Climbing fibers from different subnuclei of the inferior olive terminate contra-laterally, on discrete, longitudinal strips of Purkinje cells. This longitudinal pattern closely corresponds with the zonal arrangement in the corticonuclear projection (Figure 4).

Post-mortem examination of the brain in one case showed selective symmetrical atrophy of the cerebellar hemispheres with Purkinje cell loss and Bergmann astrocytosis, and with preservation of the cerebral hemispheres and brainstem [6].

Microscopically, an elective destruction of Purkinje cells with preservation of the basket fibers was found. These changes were most marked in the superior vermis and to a less extent in the corresponding portions of the hemispheres [7, 8] (Figure 5).

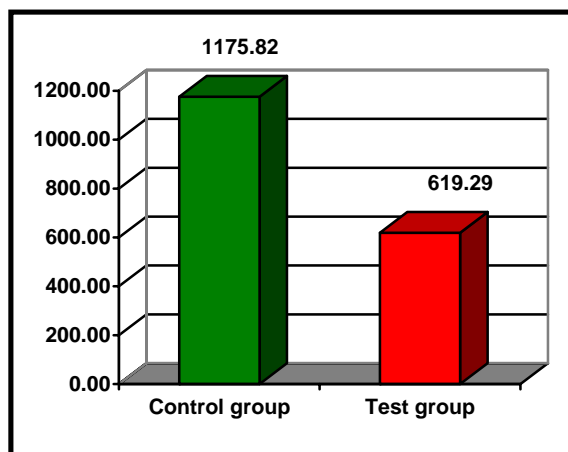
In the test group, morphometry of the cerebellum revealed significant atrophy, with a decrease in the volume of the molecular layer by 24% and of the granular layer by 22% in comparison with controls.

The 32% decrease in the total number of Purkinje cells that was observed correlates with the atrophy of the molecular layer, whereas the 30% reduction in the total number of granule cells correlates with the atrophy of the molecular and granular layers [9, 10] (Table 1, Figure 6).

In the test group it was noticed extensive degeneration not only of Purkinje cells but of granular cells and the molecular layer [9, 10] (Table 2, Figure 7).

**Table 1 – Comparative analysis of cerebellar cortex thickness ( $\mu\text{m}$ )**

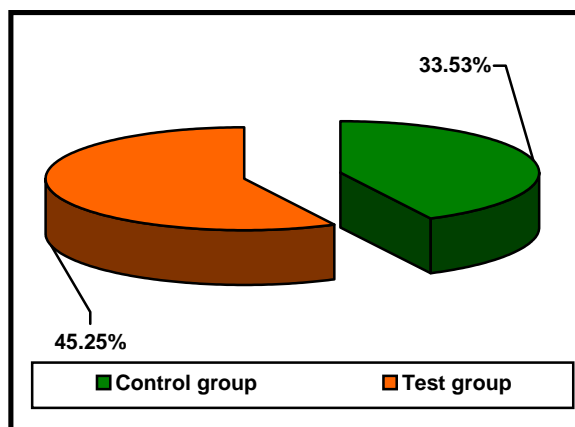
	Control group	Test group
1.	1121.95	570.65
2.	1151.58	582.44
3.	1177.78	637.81
4.	1381.14	694.65
5.	1113.19	636.41
6.	1282.20	619.88
7.	1416.29	654.58
8.	1275.91	648.61
9.	1037.77	585.12
10.	800.39	562.79
11.	1264.86	650.14
12.	1098.13	587.45
13.	1428.54	620.78
14.	1067.23	635.67
15.	1020.34	602.43
<b>Mean</b>	<b>1175.82</b>	<b>619.294</b>



**Figure 6 – Comparative analysis of cerebellar cortex thickness ( $\mu\text{m}$ )**

**Table 2 – Comparative analysis of molecular layer area fraction (%)**

	Control group	Test group
1.	35.15	45.14
2.	28.46	46.22
3.	29.63	43.12
4.	35.37	46.89
5.	33.48	47.34
6.	36.12	48.25
7.	31.56	46.24
8.	34.26	44.17
9.	35.87	42.85
10.	31.67	45.36
11.	35.27	44.27
12.	36.21	45.91
13.	31.54	42.58
14.	32.61	45.88
15.	35.68	44.50
<b>Mean</b>	<b>33.53</b>	<b>45.25</b>



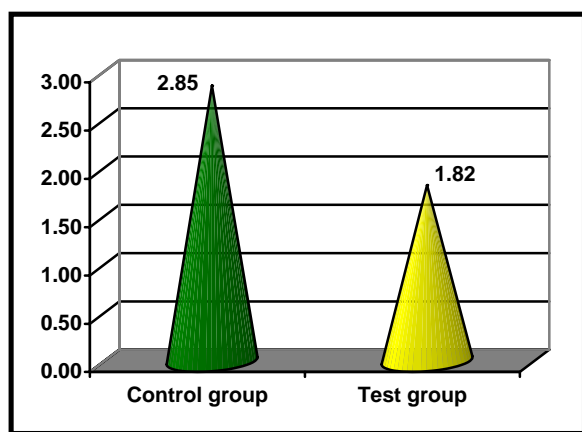
**Figure 7 – Comparative analysis of molecular layer area fraction (%)**

The average thickness of the cerebellum cortex decreased from 1175.82  $\mu\text{m}$  in the control group to 619.94  $\mu\text{m}$  in the test group, while the area fraction of the molecular layer in the cortex registered a relative increase, from 33.55% in the control group to 45.25% in the test group (Table 3, Figure 8).



**Table 3 – Comparative analysis of Purkinje neurons number per microscopic field**

	Control group	Test group
1.	3.14	1.56
2.	2.96	1.89
3.	2.56	2.15
4.	2.98	2.18
5.	3.21	1.45
6.	3.42	2.07
7.	2.77	2.12
8.	2.24	1.76
9.	2.68	1.81
10.	2.95	1.93
11.	3.26	1.67
12.	3.18	1.79
13.	2.56	1.82
14.	2.63	1.97
15.	2.21	1.13
<b>Mean</b>	<b>2.85</b>	<b>1.82</b>

**Figure 8 – Comparative analysis of Purkinje neurons number per microscopic field**

The average number of Purkinje neurons per microscopic field decreased from 2.85 in the control group to 1.82 in the test group.

The area fraction of blood vessels per microscopic field decreased from 2.45% in the control group to 2.11% in the test group.

### ☐ Conclusions

The atrophy affects mainly the cerebellar cortex, especially the vermis area and in paravermian cortex.

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The neural degeneration affects all the three layers of the cerebellum cortex mainly the molecular and ganglionic layer of the cerebellum, and Purkinje neurons disappear on large territories, while the granular cells only decrease in number.

Reduced area fraction of the blood vessels per microscopic field suggests that modifications of the blood vessels may represent an etiologic factor of cerebellar atrophy.

The atrophy of the cerebellar cortex evolves continuously in association with clinical changes.

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