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# Purkinje cells pathology in schizophrenia. A morphometric approach

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## Abstract

*Objectives*: Schizophrenia is a brain disorder that affects more than 21 million people worldwide. Ventricle enlargement and reduction in the volume of the temporal lobe overall and in medial temporal structures constitutes the main macroscopic findings, whilst synaptic and spinal changes as well as gliosis in the hippocampal formation, the prefrontal and the entorhinal cortex stand among cardinal microscopic findings in the schizophrenic brains. In recent years, accumulated evidence comes to light about the role of cerebellum in the pathophysiology of schizophrenia. *Materials and Methods*: The present study is based on the morphological analysis and 3D neuronal reconstruction of the Purkinje cells from 10 schizophrenic brains and 10 normal controls. *Results*: Significant morphological alterations such as loss of distal and terminal dendritic branches and decrease of the density of the dendritic spines constitute the main morphological findings found in the present study. *Conclusions*: The present findings may be added to accumulated evidence on macroscopic and microscopic pathology of the cerebellum in schizophrenia. Morphological alterations of Purkinje cells seem to be a central feature of neuropathology of schizophrenia, reflecting to impairment of neuronal connectivity and functionality, and related to motor and cognitive symptoms.

***Keywords***: Golgi method, schizophrenia, Purkinje cells, 3D neuronal reconstruction.

## Introduction

Schizophrenia is a brain disorder that affects more than 21 million people worldwide. Schizophrenia typically begins in late adolescence or early adulthood and is clinically characterized by positive symptoms, which include delusions, hallucinations and thought disorder, as well as negative symptoms such as avolition, alogia and affective flattening [1, 2]. In addition to the aforementioned symptoms, schizophrenic patients also usually develop cognitive deficits and neurological signs such as dysdiadochokinesis or motor coordination impairment and smooth pursuit eye movements' disorder [3–7].

Ventricle enlargement and reduction in the volume of the temporal lobe overall and in medial temporal structures constitutes the main macroscopic findings, whilst synaptic and spinal changes as well as gliosis in the hippocampal formation, the prefrontal and the entorhinal cortex stand among cardinal microscopic findings in the schizophrenic brains [8–13]. In recent years, accumulated evidence comes to light about the role of cerebellum in the pathophysiology of schizophrenia [14–17]. Cerebellar impairment is thought to be implicated in dyscoordination, abnormal posture, eye blink conditioning, procedural learning deficits and poor cognitive performance [18–23]. Structural magnetic resonance imaging (MRI), functional MRI and positron emission tomography (PET) studies have shown a significant decrease of the cerebellar volume and decreased activation of certain cerebellar regions in schizophrenia [14, 16, 17, 20, 23, 24]. Furthermore, Tran *et al.* (1998) using Nissl cresyl violet method revealed a significant decrease of Purkinje cells soma area, while Maloku *et al.* (2009) demonstrated lower number of Purkinje cells in the cerebellar cortex of schizophrenic brains, associated with reduced reelin expression [25, 26].

Despite the fact that there are numerous studies on the cerebellar volume loss and a few studies about Purkinje cells density, there is no morphological study of the Purkinje cells in schizophrenia. In previous studies, we demonstrated morphological changes of Purkinje cells from the upper surface of the cerebellar hemispheres and the cerebellar vermis in Alzheimer's disease [27, 28].

In the present study, we tried to figure out the dendritic and spinal alterations of the Purkinje cells of the upper surface of the right cerebellar hemisphere in schizophrenic brains.

## Materials and Methods

### Subjects

Tissue samples were obtained from 10 neurologically normal individuals' post-mortem, and 10 suffered from schizophrenia, all of them aged between 40 and 62 years (mean 49.4±5.3 years). A written informed consent was obtained from the relatives of the deceased for each one of the brain, in which it was clearly defined that the tissue would be used for research purposes. The research was carried out with full respect to the appropriate legislation of the Greek Democracy (v. 2472/1997, 2819/2000, 2915/2001, 3235/2004, 3471 /2006), as is clearly stated by the Committee for Research Deontology Principles of the Aristotle University of Thessaloniki [29]. The average autolysis time for all subjects was 11±3.5 hours. All brains, after their excision from the skull were immersed in 10% neutral buffered formalin for at least 25 days. All possible information on each subject, concerning their previous physical and illness history, was obtained from autopsy reports as well as medical records. All the brains were examined by an independent neuropathologist for gross and microscopic signs of pathology. The brains did not exhibit trauma, edema or chronic illness. The diagnosis for the schizophrenic patients was set by independent psychiatrists according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders Text Revision* [30].

### Tissue selection and processing

A tissue block from the upper surface of the right cerebellar hemisphere was excised. The tissue blocks were coded in order to prevent experimental bias and were used for Golgi method and Nissl staining [31].

### Cell selection criteria

For each one of the brains, five Purkinje cells were selected. Neurons examined for quantitative alterations met the criteria set forth by Jacobs *et al.* (1997) that request uniform staining of neuronal processes, absence of precipitated debris, good contrast between cells and background and relatively uniform tissue thickness [10]. For purposes of randomization, all the cells that met the selection criteria were randomly pooled and every third neuron in the series was chosen.

### Golgi method

For silver impregnation, the specimens were immediately immersed in a dilution of potassium dichromate (7 g of potassium dichromate and 20 mL of 37% formaldehyde solution in 300 mL of tap water), at room temperature. They remained in that solution for one week, and then they were immersed in an aqueous solution of 1% silver nitrate, where they remained for one more week, at a temperature of $15^0$C, in a photoprotected environment. After fixation, the specimens were embedded in low-melting-point paraffin and cut with a slicing microtome in thick sections at a range of 120 μm and after rapid differentiation, they were covered with entellan.

### Nissl staining

Adjacent sections were cut in a range of 20 μm and used for Nissl staining [31], in order to evaluate the neuronal population, and to define the depth of molecular layer. The depth of the molecular layer was measured in Nissl-stained slices using the Image J software. Every 50 μm of horizontal distance, the vertical distance of the molecular layer was taken and all the measurements were used to give the average thickness of the molecular layer.

### Neuronal tracing and dendritic quantification

For every cell, we took a 30-second video at a magnification of 400× while the microscope table was moving at the standard velocity of 20 μm/s. The microscope stage was moving using a motorized *XYZ* microscope stage system (MLS203/MZS500-E-ThorLabs), with the movement on the *Z*-axis being controlled by the MZS500-E – *Z*-Axis Piezo Stage and Controller Kit, with the aim of the APC software provided by Thorlabs with a JogStep of 1 μm a Travel Range of 250 μm. The videos were analyzed in digital image sequences of 200 serial pictures, which were ultimately imported in Neuromantic application to trace the cells, quantifying them along *x*-, *y*- and z-coordinates [32]. Each one of the selected cells was traced using the Neuromantic application. Neuronal tracing was carried out in the semi-automatic form by two different investigators, and the average of these measurements was used for statistical analysis. The neuronal tracing started with the cell soma and moved onto the basilar dendrites and the apical shaft. Dendritic trees were quantitatively evaluated in a centrifugal manner for apical dendrites and basal dendrites according to Uylings *et al.* (1990) [33]. Dendrites arising from the cell soma are considered first-order segments, up to their first symmetrical bifurcation. Dendritic branches arising from first-order segments are considered second-order segments, in turn, up to their symmetrical bifurcation into third-order segments, and so on. When asymmetric branching is met during the neuronal tracing, the offspring dendritic branch, recognized by a qualitatively thinner diameter, is classified as a next-order branch, whereas the parent dendrite would retain its order level past the branching point.

### Dendritic measures and Sholl analysis

The parameters measured were: soma size, total dendritic length, cell contraction, dendritic field asymmetry, total number of dendritic segments and bifurcations, number of terminal branches, local and remote bifurcation angles, as well as the length and number of dendritic segments per order. Furthermore, the tracing was quantitatively analyzed with Fiji and Simple Neurite Tracer plugin based on Sholl's (1954) method of concentric spheres [34]. Concentric spheres were drawn, at intervals of 10 μm centered on the cell bodies, and dendritic intersections within each sphere were counted [32].

### Spine counts

Spine counts were carried out at 360 pictures, which were taken with an AxioCam HR, at the standard magnification of 1000×, on an Axiostar Plus photomicroscope. Visible spines were counted on three segments of the dendritic field. The first segment, 20–30 μm in length, was located in a distance of 50 μm of cells soma, the second segment, 20–30 μm in length in 150 μm and the third one, 20–30 μm in 250 μm from cells soma.

### Neuronal density

The linear density of the Purkinje cells was estimated on 30 pictures from each brain, using the cell counter function on Image J based on the method described by Maloku *et al*. (2010) [26].

### Statistical analysis

Individual cellular measurements were averaged for each of the study groups. Statistical analysis was based on the Student's *t*-test based on 100 cells in SPSS ver. 17.0. Significance was taken as $p<0.05$. To ensure that autolysis time did not affect neuronal density and dendritic measurements, two-tailed Pearson's product correlations were performed between all dependent measures and autolysis time [32].

## Results

### Dendritic changes

Purkinje cells of both groups did not exhibit autolytic changes described by Williams *et al.* (1978) [35]. Purkinje cells from the schizophrenic brains showed substantial changes of dendritic tree complexity. Primary and secondary dendritic branches did not exhibit significant difference between the two groups, however the total dendritic length was significantly decreased [$p<0.005$] (Figure 1A), even as a severe loss of terminal and distal dendritic branches was also noticed [$p<0.01$] (Figure 1B). The daughter ratio was reduced in the schizophrenic group [$p<0.001$] (Figure 1C), while the ratio between remote bifurcation tilt to local bifurcation tilt was likewise affected [$p<0.01$] (Figure 1D). Dendritic tree area and dendritic tree volume were also decreased in the schizophrenic group [$p<0.001$] (Figure 2, A and B). Sholl analysis revealed a restriction of the dendritic field due to the loss of distal branches, at distance 150 µm from cell soma (Figure 2C). The peak of the development of the dendritic arborization occurred at 90 µm from cell soma for schizophrenic brains and at 130 µm for the normal controls.

### Spinal changes

The density of dendritic spines was significantly lower in the schizophrenic group [$p<0.001$] (Figure 3, C and D). Spinal loss seemed to be more prominent in distal branches (Figure 2D). Besides spinal loss, significant morphological alterations of dendritic spines were also revealed while the majority of the remaining spines on schizophrenic brains were of the short-stubby type (Figure 2B), while Purkinje cells of normal controls bear spines of the long-neck type and only a few from the short-stubby type. Moreover, dystrophic and giant spines were noticed in the schizophrenic brains.

### Neuronal density and cortical thickness

The depth of the molecular layer was severely lower in the schizophrenic brains. Although there was loss of Purkinje cells in schizophrenic group, this was not statistically significant. Empty baskets, which are visible due to loss of Purkinje cells in schizophrenic brains, were also noticed (Figure 3E).

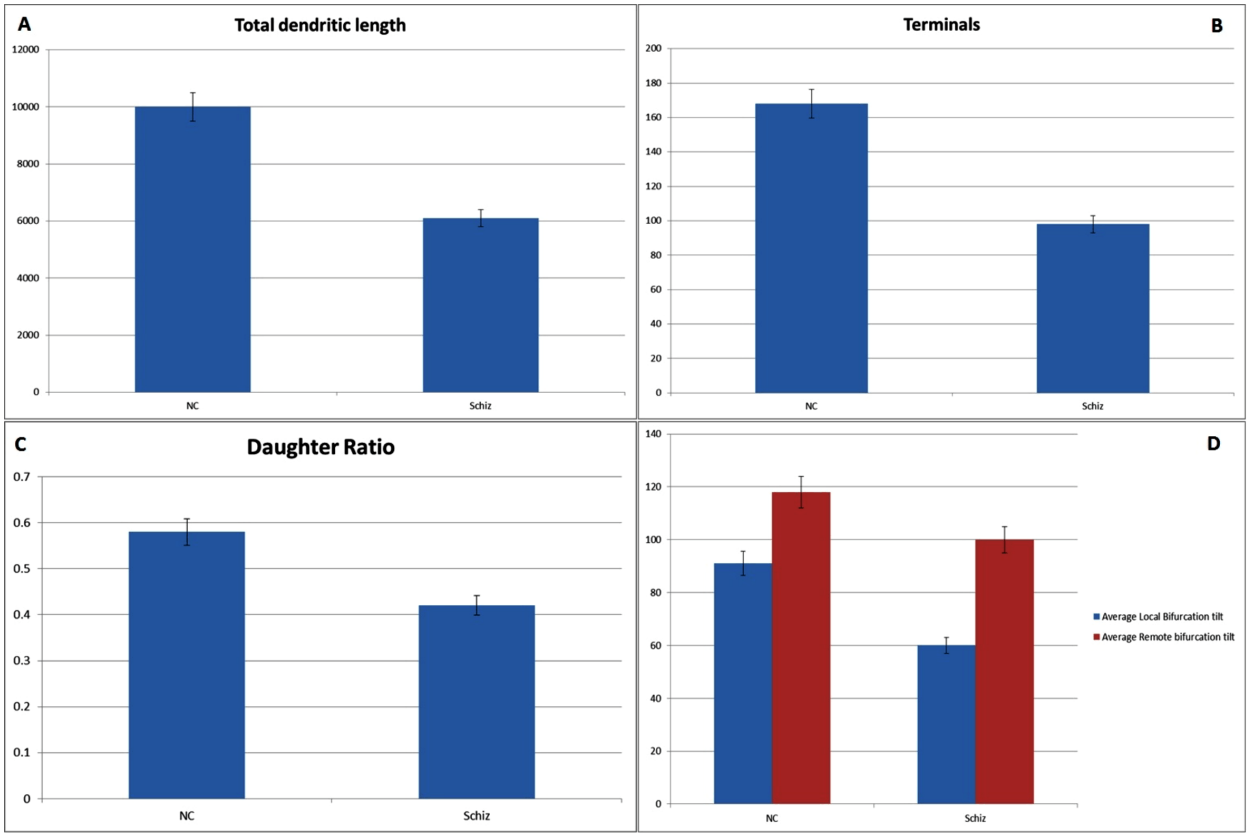


**Figure 1** – ***Total dendritic length in µm (A), number of terminal branches (B), daughter ratio (C) and average bifurcation tilt (D) of the Purkinje cells from normal controls and schizophrenic brains. Error bars indicate standard deviation.***

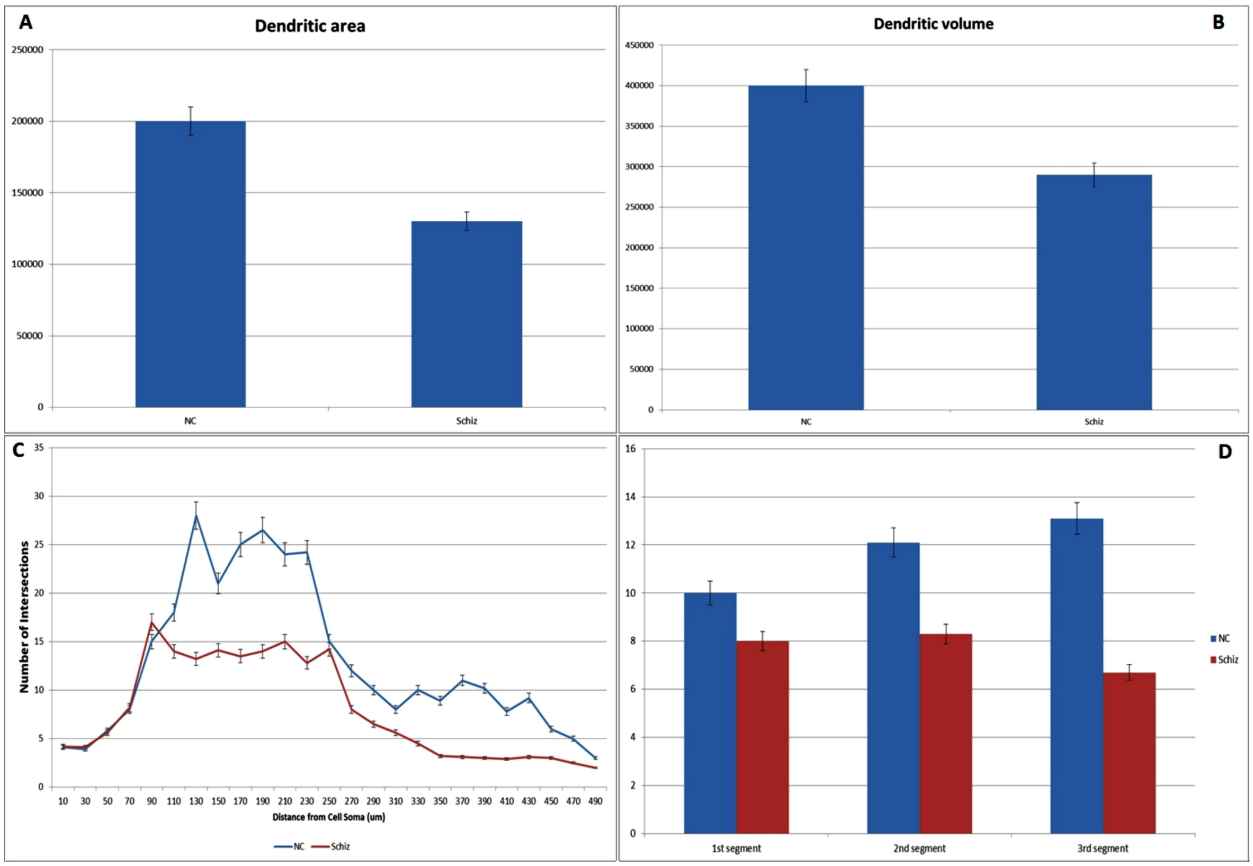


**Figure 2** – ***Dendritic area in µm$^2$ (A), dendritic volume in µm$^3$ (B), Sholl analysis (C) and density of dendritic spines which refers to the number of spines per 10 µm (D) of the Purkinje cells from normal controls and schizophrenic brains. Error bars indicate standard deviation.***

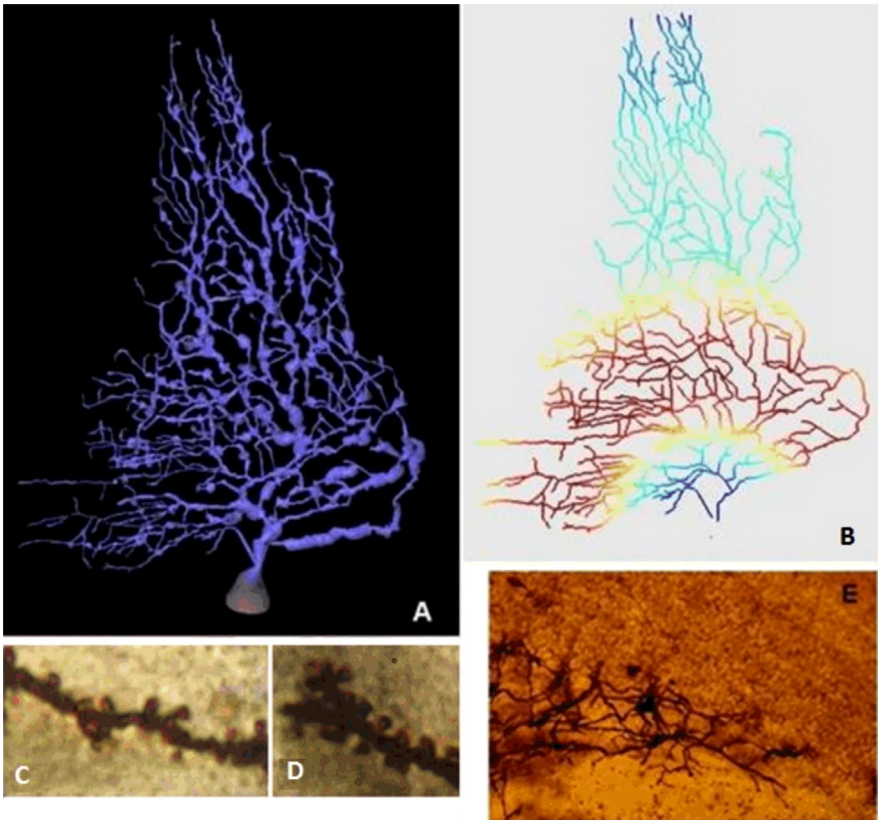


**Figure 3** – ***3D reconstruction of a Purkinje cells (A), example of color masks after Sholl analysis of a Purkinje cells (B), where different colors corresponds to different dendritic density, dendritic segment from Purkinje cells from schizophrenic (C) and normal control brains (D). Empty baskets are visible due to loss of Purkinje cells in schizophrenic brains (E). Golgi method staining: ×1000 (C and D); ×400 (E).***

## Discussion

There have been only a few morphometric studies about Purkinje cells in schizophrenia, however to our knowledge this is the first study to reveal the morphological changes of the Purkinje cells dendritic fields and spines in schizophrenia. Lingärde *et al.* (2000) and Tran *et al.* (1998) found no significant difference in linear density in schizophrenia, while the latter found significantly smaller cross-sectional areas of Purkinje cells and significant correlations between Purkinje cell size and scores on the *Mini-Mental State*, the *Brief Psychiatric Rating Scale* and the dose of the antipsychotic drug that patients were on [25, 36].

In the present study, we revealed significant morphological alterations in schizophrenic brains in comparison to normal controls using Golgi method and 3D reconstruction of Purkinje cells.

Schizophrenic brains revealed a severe loss of distal and terminal dendritic branches and an overall reduction of total dendritic length. Purkinje cells from the schizophrenic group showed a significant decrease of spinal density, as well as certain morphological alterations of the remaining dendritic spines. Besides dendritic and spinal loss, specific changes were also noticed at the level of Purkinje cells dendritic tree formation and orientation in schizophrenic brains. Although the thickness of the molecular layer was severely decreased in schizophrenic brains, there was no statistical significance in the linear Purkinje cell density, a finding that confirms the findings of previous studies of Lingärde *et al.* (2000) and Tran *et al.* (1998) [25, 36].

The dendritic and spinal changes described in the present study are not specific to schizophrenia, since they have been noted in Purkinje cells in patients with hereditary ataxias [37], in chronic alcoholics [38], in Alzheimer's disease [27] and in essential tremor [39]. Furthermore, the reduction in dendritic arborization is a structural change associated with dysfunction, and is thought to precede neuronal death [38].

The morphological changes found in here may be related to molecular and gene abnormalities described by previous studies in schizophrenia, and might be the morphological background of a number of symptoms in schizophrenic patients, while the loss of distal dendrites and dendritic spines seen in thick sections of Golgi stained material, leads to a substantial decrease of the synaptic area and synaptic contacts of the Purkinje cells and this could contribute to motor and cognitive symptoms of schizophrenia.

## Conclusions

This study is the first about morphological changes of the Purkinje cells in schizophrenia. The present findings may be added to accumulated evidence on macroscopic and microscopic pathology of the cerebellum in schizophrenia. Morphological alterations of Purkinje cells seem to be a central feature of neuropathology of schizophrenia, reflecting to impairment of neuronal connectivity and functionality, and related to motor and cognitive symptoms.

**Conflict of interests**

There is no conflict of interests.

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