ACTA STEREOL 1998; 17/3: 353-356
ORIGINAL SCIENTIFIC PAPER

# GRANULE CELL AND PURKINJE CELL NUMBER IN HUMAN CEREBELLA FROM YOUNG AND OLD MEN: A stereological study

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## ABSTRACT

Cerebella from three young men (mean age 20.3 yrs) and three old men (mean age 76.6 yrs) with no neurological disorders were examined postmortem with stereological methods. The Cavalieri principle was used to provide unbiased estimates of volumes, the optical disector to obtain estimates of numerical densities of granule and Purkinje cells in the cerebellar cortex, and a combination of these two to get total cell numbers. A significant difference was found in the total volume of cerebellum in the two age groups, where the volume in the young males was 138.3 $cm^3$ (CV = SD/mean = 0.04) and in the older group 113.7 $cm^3$ (0.11), p = 0.04. In the anterior lobe, a significant Purkinje cell loss was found in the old men compared to the young ($2.23 \times 10^6$ (0.34) vs. $3.9 \times 10^6$ (0.08) p = 0.03). No cell loss was found in granule cell number, neither in the whole cerebellum nor in any of the subregions. The differences between young and old brains are only rough estimates, since this study is based on a very small material.

## INTRODUCTION

The aim of this pilot study was to apply modern stereological methods to provide unbiased estimates to show any cell loss occurring in the human cerebellum during aging. The total number of Purkinje and granule cells in cerebella from three elderly men and three young men was found using unbiased estimates of the major volumes of the cerebellum and the numerical density of Purkinje and granule cells. The cerebellum coordinates motor functions and balance, but recent research indicates that the cerebellum also plays a role in higher cortical functions (Leiner et al., 1993).

## MATERIAL

Cerebella from three older males with no neurological disorders (mean age 76.6 yrs) and three younger men (mean age 20.3 yrs) were obtained postmortem in accordance with the laws of Denmark regarding autopsied human tissue.
The brains were included if fixated within 12 to 48 hours after death. Exclusion criteria were presence of tumours or infection in the central nervous system, strokes, and any history of alcohol or drug abuse. To avoid influence of sex differences, only male brains were included (Pakkenberg & Gundersen, 1997).

The cerebellum is divided into four regions: a midline structure (vermis), two lateral hemispheres (anterior and posterior lobe), and the flocculonodular lobe (FlocNo). The cerebellar cortex is made up of three layers, the outer molecular layer, the middle Purkinje cell layer, and the inner granular layer. The Purkinje cells are the major output cells from the cerebellar cortex.

## METHOD

The Cavalieri principle, consisting of systematic random sampling and point counting, was applied on consecutive slices to provide unbiased estimates of the volumes of the human cerebellum. The optical disector was used to obtain estimates of the numerical density of the Purkinje and granule cells, and a combination of these two measures for total cell numbers (Gundersen et al., 1988a+b).

The cerebellum was dissected from the brain stem, and the surface stained with waterproof ink in different colours to distinguish between the different regions. After removing the flocculonodular lobe, the cerebellum was embedded in 7% agar and cut in a systematic random manner into 4.1-mm slices. Starting randomly, the cerebellar slices were cut systematically into 4.0-mm-wide columns or rods, and every n'th rod was sampled to provide approximately six to eight rods from each of the four regions. Large areas of white matter were removed and the rods rotated around their longitudinal axis and embedded in agar. The FlocNo, divided into three parts, was rotated clockwise, the first part randomly, the other two 90° and 180° to the first, respectively. All were embedded in 7% agar and cut into 2-mm-thick slabs. For more details on the vertical section design, see Baddeley et al. (1986), and for practical details on the cerebellum design, see Andersen et al. (1992). The sampled rods were dehydrated and embedded in glycolmethacrylate (Historesin®) for sectioning. From each block, a central 40-$\mu$m-thick section was cut parallel to the vertical axis and stained with a modified Giemsa stain.

The optical disector equipment consists of a microscope with a high numerical aperture (NA = 1.40) oil immersion (60 or 100x) objective, which allows focusing on a thin focal plane inside a thick section. A TV-camera transmits the image to a videoscreen superimposed with a counting frame. The microscope stage is driven by a pair of stepping motors with preset steps of a known length in the x- and y-direction. A microcator is used to measure stage movements on the z-axis. For further description of the method, see Andersen et al. (1992).

## RESULTS

The mean total volume of three young cerebella was 138.3 $cm^3$ (0.04) compared to 113.7 $cm^3$ (0.11) in the old men, which is statistically significant at the 5% level, $p = 0.04$. There were no statistical differences in the total Purkinje cell number, $29.9 \times 10^6$ (young) compared to $25.6 \times 10^6$ (old), but the Purkinje cell number in the anterior lobe was $3.9 \times 10^6$ (0.10) in the young brains and $2.23 \times 10^6$ in the old, which is statistically significant, $p = 0.03$. The volume and cell numbers are given in Table 1 and 2.

**Table 1.** Cerebellar volume ($cm^3$) in young and old men.

| | Young | Old |
|---|---|---|
| WHOLE CEREBELLUM ($cm^3$) | 138.3 (0.04)* | 113.7 (0.11) |
| CORTEX ($cm^3$) | 95.9 (0.10) | 85.1 (0.10) |
| Pars anterior | 17.6 (0.16) | 11.9 (0.32) |
| Pars posterior | 108.8 (0.03)* | 90.3 (0.12) |
| Vermis | 8.7 (0.12) | 8.7 (0.03) |
| Floccolonodular lobe | 0.8 (0.21) | 0.9 (0.26) |
| WHITE MATTER | 40.1 (0.10) | 26.5 (0.35) |

Coefficient of variation (CV = SD/mean) is shown in parenthesis.
*Statistically significant at the 0.05 level.

**Table 2.** Total mean number of granule ($10^9$) and Purkinje cells ($10^6$) in the four different regions of cerebellum in three old and three young men

| | | pars anterior | pars posterior | vermis | lobus flocculo-nodularis | total number |
|---|---|---|---|---|---|---|
| Young | Purkinje cells | 3.9 (0.10) | 23.4 (0.10) | 2.4 (0.24) | 0.22 (0.31) | 29.9 (0.04) |
| | granule cells | 15.59 (0.34) | 96.04 (0.15) | 7.76 (0.26) | 0.60 (0.20) | 120.00 (0.20) |
| Old | Purkinje cells | 2.23 (0.34)* | 21.1 (0.35) | 2.0 (0.12) | 0.22 (0.14) | 25.6 (0.35) |
| | granule cells | 9.41 (0.27) | 96.75 (0.19) | 6.79 (0.15) | 0.59 (0.24) | 113.54 (0.14) |

Coefficient of variation is shown in parenthesis after mean cell number.
*Statistically significant at the 0.05 level.

## CONCLUSION

The study presents the first unbiased estimates of the total number of Purkinje and granule cells in the human cerebellum of old and young men. This pilot study shows

a total volume loss with age, mostly due to a white matter reduction, but it is not significant in this small material.
In the anterior lobe a significant Purkinje cell loss was found in the old men, but no significant reduction of total Purkinje cell number in the whole cerebellum. Previous studies (Hall et al., 1975; Blinkov & Glezer, 1968) have reported volume or/and Purkinje cell loss with age especially in vermis. In this region we do not find any significant loss. No cell loss was found in the granule cell number, neither in the total number nor in any subregion.

## ACKNOWLEDGEMENTS

The author thanks H.J.G. Gundersen for his statistical help and Lykke Steffensen, Susanne Primdahl, Hans Jørgen Jensen and Ulla Vonger for their skilful technical assistance. The economic support given by the Hartmann Brothers Foundation, the Stanley Foundation, the Ferd. and Ellen Hindsgaul Foundation, Direktør Emil C.Hertz and wife Inger Hertz' Foundation and IMK Almene Fond is highly appreciated.

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*Presented at the 7th European Congress for Stereology, Amsterdam, April 20th to 23rd, 1998.*