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PURKINJE CELL NUMBER AND SIZE, COMPARISON OF HUMAN AND RAT CEREBELLA

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ABSTRACT

Estimates of total number and mean volume of Purkinje cells in five human and nine rat cerebella were obtained using stereological methods based on unbiased principles and estimators. The average total number of Purkinje cells was $30.5 \cdot 10^6$ (CV = SD/mean = 0.13) in humans and $0.61 \cdot 10^6$ (CV = 0.21) in rats. Thus the total number of Purkinje cells was 50 times higher in the human compared with rats, while numerical density per mm^3 was 13 times lower in humans ($0.81 \cdot 10^3$) compared with rats ($10.1 \cdot 10^3$). The nucleator was applied to estimate the number weighted mean volume of the large and unevenly distributed Purkinje cells. In humans the mean volume of Purkinje cell perikaryon is 14,250 μm^3 (CV = 23%). The data are compared with the mean Purkinje cell perikaryon volume in rats, 5,300 μm^3, with a CV of 5%. Expectedly, the variation in Purkinje perikaryon volume between individuals are larger in humans than the individual variation between rats.

Key words: cerebellum, human, number weighted mean volume, Purkinje cells, rat.

INTRODUCTION

The cerebellar Purkinje cells are among the largest neurons in the central nervous system and have been the object of several investigations both concerning number and size. The total number in human has been estimated to be from 14 to 26 million cells counted with earlier conventional methods (for review see Blinkov and Glezer, 1968 and Palay and Chan-Palay, 1974). The size of the large Purkinje cells has only been described with measurements of diameters. The nucleator (Gundersen, 1988) a new stereological method, is the first efficient method which makes it possible to estimate the number weighted mean volume of cells independently of their size and shape.
This study presents comparison of estimates of total number and mean volume of the large and unevenly distributed Purkinje cells in five human and nine rat cerebella using stereological methods.

MATERIAL

Five normal human male brains with no neurological disorders (average age 74 yrs) were fixed within 8 to 24 hours after death. Exclusion criteria were tumors or infection in CNS, stroke or any history of alcohol and drug abuse.

Nine cerebella from 7-month-old male Wistar rats were perfusion-fixed with formalin, removed from the cranium and fixed in formalin for 3 months. The cerebella were incubated in a plastic medium (Historesin©) and cut into sections. The sampling, cutting and counting procedure is described for human cerebellum in Andersen et al. (1992) and for rat cerebellum in Korbo et al. (1993).

METHODS

The cerebella sections were prepared as vertical sections (Baddeley et al., 1986). The unbiased principle employed for obtaining estimates of total cell number, (N), was a combination of an estimate of the respective reference volumes, V(ref), using the unbiased Cavalieri method for systematic sampling and point counting and a separately obtained estimate of 3-dimensional numerical density, N_V, using optical disectors (Gundersen, 1986). The unbiased estimate of total cell number in a region is the product of the volume of each specific layer times the numerical density of a specific cell type in that layer:

$$N = N_V \bullet V(ref) \qquad (1)$$

The nucleator method was applied to estimate the number weighted mean volume of the Purkinje cells. The Purkinje cells were sampled in optical disectors with the nucleolus as the sampling unit. The nucleator principle is illustrated in Fig. 1.

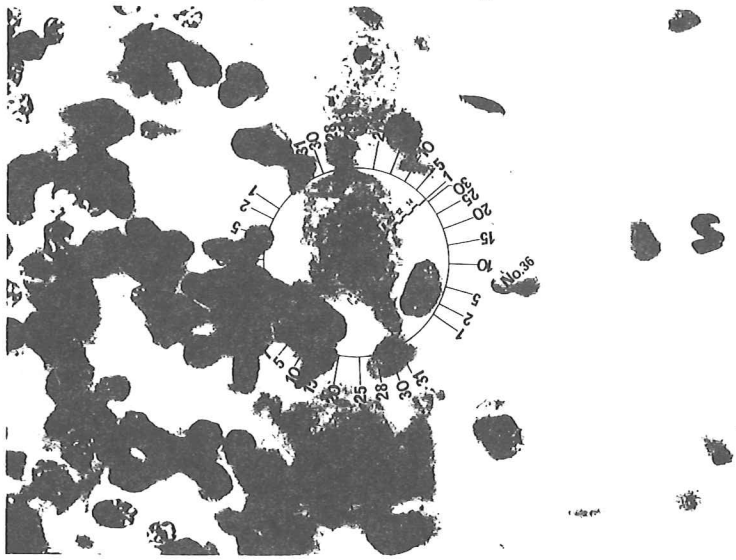

Fig.1. The center Purkinje cell of a rat was sampled with a disector. The direction of measurement was found using a random number table. Using a bidirectional ruler equidistant in length cubed (l_0^3-ruler) the class number expresed as the intersection between the nucleolus and the nucleus boundary is read off. This is done twice, the second time after the ruler is rotated roughly 90 degrees by reading off the same direction in the next quadrant. For estimating mean Purkinje cell volume from these estimates, the nucleator formula was applied:

$$\bar{v}_N = \frac{4\pi}{3} \bar{l}^3_n \qquad (2)$$

where $\bar{v}_N$ is number weighted mean volume, and $\bar{l}^3_n$ is the mean value of the ruler measurements. For details see Gundersen (1988) and Møller et al. (1990).

RESULTS

The average total number of Purkinje cells is $30.5 \cdot 10^6$ (CV = SD/mean = 0.13) in humans and $0.61 \cdot 10^6$ (CV = 0.21) in rats. Thus the total number of Purkinje cells is 50 times higher in the human cerebella compared with rats, while numerical density per mm^3 is 13 times lower in humans ($0.81 \cdot 10^3$) compared with rats ($10.1 \cdot 10^3$). In humans the mean volume of Purkinje cell perikaryon is 14,250 μm^3 (CV = 23%, Fig.2.) compared with the mean Purkinje cell perikaryon volume in rats, 5,300 μm^3, with a CV of 5%. As shown in Fig. 2, the nuclei in human Purkinje cell vary in proportion to cell size, i.e. on average the nucleus constitute a constant fraction of the cell volume. This is also the case for neocortical neurons in rat, see Møller et al., 1990, Fig. 7, but it need not be a general phenomenon. For further details see Korbo and Andersen (in preparation).

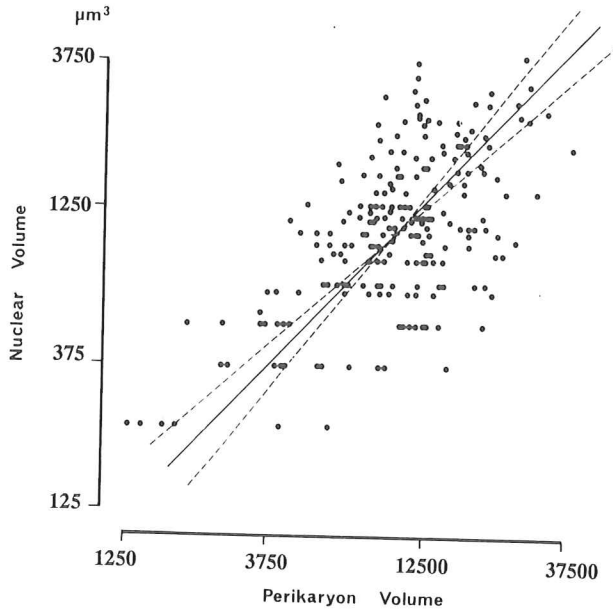

Fig. 2. The relationship between the nuclear volume (μm^3) and the perikaryon volume (μm^3) estimated in one human cerebellum. The orthogonal regression line (full drawn) and the two slopes (dashed lines) corresponding to the 95% confidence interval is shown. The slope is 1.04.

DISCUSSION

The nucleator method when used in isotropic or vertical sections provides an unbiased

estimate of mean volume of a particle independent of the particles size and shape. The mean cellular volume, however, might be influenced by unpredictable factors such as swelling or shrinkage during the fixation procedure, so the results on cell volumes presented in this study are the volume of the cells in the fully fixed and proccessed tissue.

It is a well known phenomenon that smaller brains have higher density than large ones and we found, as expected, Purkinje cell density in rats higher than Purkinje cell density in humans. It may be noted that the biological variation in Purkinje perikaryon volume between individuals are much larger in humans than the individual variation between rats.

Our results are in accordance with previous results, where an approximated diameter for the Purkinje cells has been found to be between 35-65 μm in humans and 21 μm in the rat (Palay and Chan-Palay, 1974). The approximated diameter of the cells in our study is 29.2 μm in humans and 20.8 μm in rats.

Compared with earlier conventional methods, the presented method is first of all more efficient and secondly based on an unbiased estimate. Total number and mean volume of Purkinje can be estimated in one rat cerebellum within three hours.

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