ORIGINAL ARTICLE

AGE RELATED CHANGES IN THE GRANULE CELL NUMBER IN THE HUMAN CEREBELLAR CORTEX

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ABSTRACT: The number of Granule cells in the human cerebellar cortex and its quantitative variation with respect to age was studied in seventy human cerebellums using light microscopy. The study revealed a progressive decrease in number of granule cells with increase in the fibre components. The number of granule cells showed statistically significant negative correlation with age. This study provides more information regarding the quantitative histological structure of human cerebellar cortex.

KEY WORDS: Granule cells, number, Human cerebellar cortex

INTRODUCTION; The cerebellar cortex has become the focus of particularly intense research because it is presumed to be responsible for planning movement and adapting to special conditions, and is also involved in storing memories over various time-periods (Attwell *et al* 2002). In fact, aspects of structural configuration, neuronal elements, fibre communications and neurotransmitters in the cerebellar cortex have been extensively investigated (Zhang 2006). Despite numerous anatomical and physiological studies devoted to the cerebellum we do not yet properly understand its function and its co-ordination with other parts of the brain. Sherrington as early as in 1900 had coined the term 'the head ganglion of the proprioceptive system' to denote cerebellum(Pearce JMS 2004). The cortex has become the focus of particularly intense research because it is presumed to be responsible for planning movement and adapting to special conditions, and is also involved in storing memories over various time periods(Attwell *et al* 2002). It has been documented that cerebellar hemispheres undergo age-related morphological changes. These studies have been mostly in Macaca nemestrina (Nandy 1981), Wistar rat (Amenta *et al* 1991), and the humans (Jernigan *et al* 2001). The results of these studies show that in senile individuals there is a decline in the thickness of the cerebellar cortex as well as loss of neurons, and hypertrophy and hyperplasia of the astrocytes. Purkinje (1837) described numerous 'fig' shaped corpuscles arranged in a row with their rounded ends directed towards the white centre of the folium and their tail like apical process directed towards the surface and disappearing in the gray matter. Nandy (1981) observed that the Purkinje cells are more prone to age changes than the granule cells of the cerebellum regarding both lipofuscin formation and cell loss. This age related loss of Purkinje cell was substantiated by most other researchers (Hall, 1975; Ogata 1984; Andersen, 2003).

MATERIALS AND METHODS: Human cerebellum was the material for the present study. The light microscopic structure of human cerebellum, with special reference to their age related changes, was studied in detail. Specimens from seventy persons of different age groups and two foetuses were studied. Persons of both sexes from 2½ to 85 years were categorized into different age groups. Foetuses were included in the first age group. TABLE 1. Age-wise distribution of the material studied

Age group Age in years	0-10	11-20	21-30	31-40	41-50	51-60	61 and above
Number of specimens	10	10	10	10	10	10	10

METHODOLOGY: Human cerebellum was obtained from the autopsies. Entire cerebellar hemispheres were dissected out of the posterior cranial fossae.

Dissected out specimens were subjected to routine histological processing (Mc Manus and Mowry 1960).The paraffin blocks thus obtained were serially sectioned at a thickness of 10μm for staining with Haematoxylin and Eosin and 20-25μm thickness for special stains like Silver Methenamine staining to demonstrate the nerve fibres.

Mounted sections were observed under low power, high power and Oil Immersion objectives of binocular microscope with built in illumination. The number of Granule cells was quantitatively estimated.

Counting of the granule cells was done under oil immersion lens (x100 objective andx10 eyepiece). The number of granule cells was counted using a net micrometer with 10 mm x 10 mm square grid composed of 100 squares of 1mm size. A selected section of the cerebellum was viewed under oil immersion and placed under the grid. The cells in each 1mm square area were counted and recorded until the area of 100 squares (100 mm2 area) was evaluated. The cells were counted in five such different areas and the average value per 100 mm2 area was calculated. (Aherne, W. 1975)

The data obtained from morphometric measurements of cerebellum were subjected to ANOVA *(Analysis of Variance).* Regression analysis was done as the appropriate statistical technique for observing the dependence of number of granule cell with age. The data were plotted in regression graphs. Correlation of above parameters with age was also worked out.(Goon1992)

OBSERVATION: Each granule cell was spherical in shape with a prominent nucleus. The nucleus almost completely occupied the cell leaving a very thin rim of cytoplasm. No cell organelles could be discerned. The dendritic and axonal patterns of the granule cell could not be visualized. In younger age groups the granular layer was observed to be closely packed with granule cells (Fig. 1) while as age advanced fibre elements became more prominent (Fig. 3). The mean number of granule cells in the age group below 40 years was estimated to be 240.5+/- 33.26-cells/100 mm^2 while that above 40 years was 166.37 +/- 46.73-cells/100 mm^2. The number of granule cell was seen to decrease progressively with age. This observation was statistically significant.

The number of granule cells showed a gradual decrease from childhood to old age. ANOVA shows significant differences in various age groups.

'*F*' test done on observed data, estimated a '*F*' value of 67.204 (P=0.000) which was found to be statistically significant.

TABLE 2: NUMBER OF GRANULE CELLS IN VARIOUS AGE GROUPS

Age (in years)	Mean	Standard Deviation	Range
0-10	134.88	9.73	123-150
11-20	127.80	14.62	108-148
21-30	103.24	7.07	94.4-116
31-40	109.00	7.88	99.6-122
41-50	99.82	4.28	95.6-107.4
51-60	92.04	8.38	84-110.6
60 above	52.78	14.22	36.8-87.4

The post hoc pair wise comparison test showed statistically significant decrease in the number of granule cells from first decade onwards

Regression analysis was done with simple linear equation, which showed a varied amount of decrease in the 2 age groups i.e. up to 40 years and above 40 years. Regression graphs were plotted which revealed a steady decrease in the number of granule cells

Correlation coefficient of age wise distribution of number of granule cells in the group upto 40 years was observed to be –0.66 and that above 40 years was –0.76. Both these coefficients indicate a statistically significantly negative correlation with age (Table 2).

The mean number of granule cells in the age group below 40 years was estimated to be 240.57 +/- 33.26 cells/100mm2 while that above 40 years was 166.37+/-46.73 cells per 100mm2.

Table 3: CORRELATION MATRIX OF VARIOUS PARAMETERS

	Below 40 years		Above 40 years	
	Correlation coefficient	Significance (P)	Correlation coefficient	Significance (P)
Number of granule cells	-0.66	0.000	-0.76	0.000

DISCUSSION: The granule cells were seen to be arranged without any definite pattern within the internal granular layer. E.G. Gray (1961) opined that the granule cell bodies form a layer that extends from the sub cortical white matter to the Purkinje cell layer. Its thickness varies, being only 30 μm in the trough but exceeding 200 μm in the summit of the folia.

The mean number of granule cells in the age group below 40 years was estimated to be 240.5 +/-33.26 cells/100mm2 while that above 40 years was 166.37+/-46.73cells/100mm2. These absolute values could not be compared with those values obtained from literature as these researchers have utilized stereological techniques and fractionator methods to assess the cell number in either a cubic millimeter of the cerebellum or the cerebellum as a whole.

The quantitative study has helped us to understand that the cell number decreases with age and this sets in by about 40 years. This finding is consistent with that of Arsenio Renovell (1996) and Andersen (2003). Arsenio Renovell et al (1996) in their study of human cerebellum identified that the total number of granule cell declined significantly during the ageing process. Druge et al (1986) Quackenbush et al (1990) and Dlugos et al (1994) observed that the granule cell number was stable with advancing age in rodents. These differences as observed in the human and rodent cerebellum could be due to greater evolutionary degree of human cerebellar cortex, making it more vulnerable to environmental effects.

Some researchers reported no age-related changes in the number of Granule cells in aged Macaca nemestrina (Nandy 1981) and rat (Bakalian et al 1991), while other workers in the field found significant changes in aged rats (Amenta et al 1991) and humans (Renovell et al 1996). Zhang et al 2006 showed a significant decrease of 22.57% in the density of granular cells and only a slight increase in thickness (7.82%) of the granule cell layer in the ageing cat (anterior lobe). Zhang et al 2006 found a large loss of neuron density (22.57%). The discrepancies of the observations noticed among the different groups of workers may be interpreted as due to the differences in methodologies adopted by them.

The axons of the granular cells bifurcate into parallel fibres. These fibres are known to send excitatory project into the Purkinje cells (Attwell *et al* 2002). Granule cells have also been known to be concerned with storage of memories over different period of time. Consequently, these events tend to regulate the dynamics of movements and allow bidirectional changes in movement amplitude (Boyden 2004). If this is true, the age-related loss of granule cells may directly lessen the excitatory inputs to the Purkinje Cells in ageing animals, which in turn, brings about reduction in the functional role of these neurons in storage of memory, thereby affecting motor learning in elderly individuals. The exact mode of interaction of granule cells and the Purkinje Cells in this dynamic activity requires further elucidation.

SUMMARY: The present work is a light microscopic study of human cerebellum with special emphasis to age related changes. The cerebellum of sixty-eight autopsies of different age groups and two foetuses were studied.

Specimens procured from autopsies were subjected to routine histological processing. A detailed histological study was done on serial sections of the cerebellum stained with Haematoxylin and Eosin as well as special stains. The changes occurring in the granule cell of the cerebellar cortex with respect to age were studied.

Age related change associated with the granular layer was decrease in the granule cell number with increase in the fibre element. The granule cells were spherical cells wholly occupied by the nucleus with a very little perinuclear cytoplasm. This characteristic appearance was constant throughout life.

The data obtained were analysed statistically. The number of granule cells showed statistically significant negative correlation with age.

The study shows that as age advances there is an increase in the fibres element of the granular layer. The cerebellum is considered to be the seat of memory and the decrease in the number of cells can explain the loss of memory as age advances. The increase in fibre element can also be used to explain the clinical findings in Alzheimer's disease with characteristic neuro fibrillary tangles

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FIG. 1. 14 year old (F), Fix – Bouin's fluid, H & E stain, x 400, section shows the closely packed granule cells.

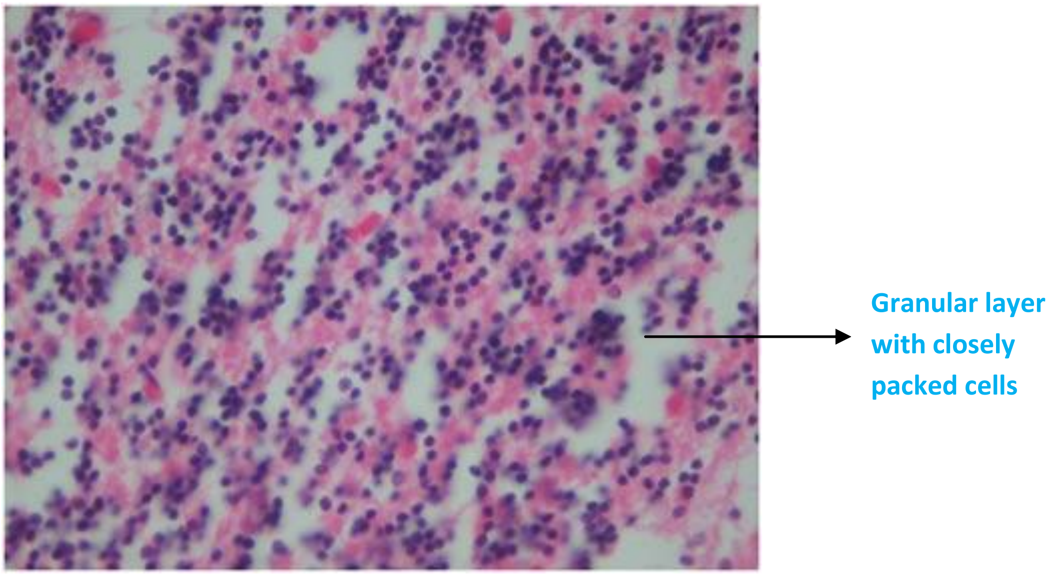

FIG. 2 14 year old (F), Fix – Bouin's fluid, silver methenamine staining, x 400, section shows closely packed granule cells.

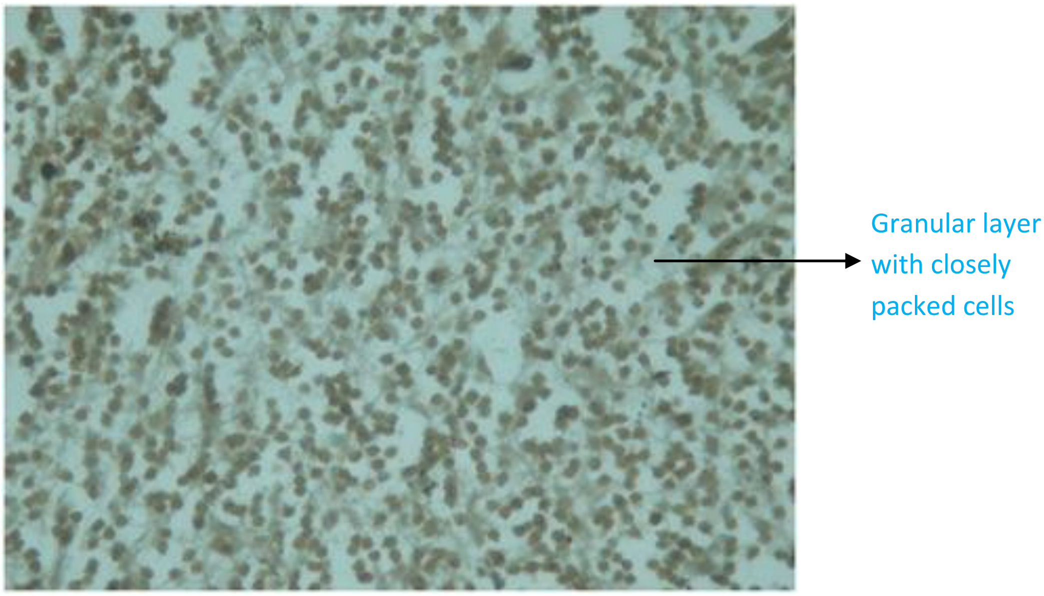

FIG. 3 78 year old (F), Fix – Bouin's fluid, H & E stain, x 400, section shows the granule cells to be decreased in number with increase in the fibrous element

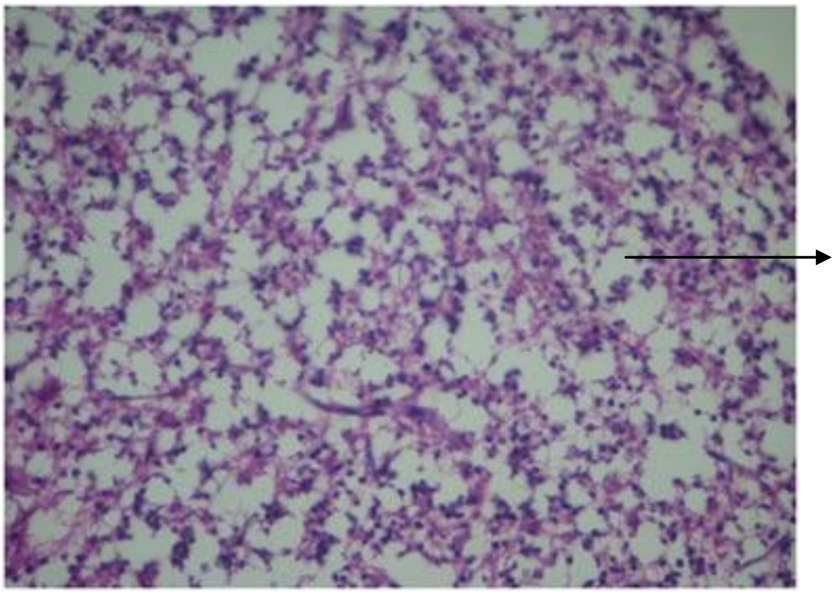

FIG. 4 78 year old (F), Fix – Bouin's fluid, silver methenamine staining, x 400, section shows sparse distribution of granule cells with increase in quantity of fibrous elements
Number of granule cells