AGE RELATED CHANGES IN THE PURKINJE CELLS IN HUMAN CEREBELLAR CORTEX

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ABSTRACT: The human cerebellum is associated with smooth control of movements and memory. As age advances deterioration is noticed in the cerebellum. The Purkinje cells act as the pivot in the neuronal circuitry of the cerebellum, which initiates and maintains complex, but smooth pursuit movements and it can be identified as one of the factors which cause the decline in movement and memory in humans as age progresses. In the present study a quantitative analysis of the Purkinje cells of the human cerebellum was done using light microscopy in seventy human cerebellums. The tissue collected from post mortem specimen was subject to routine histological processing and the slides studied under light microscopy to assess the diameter and linear density of Purkinje cells. The results of the study showed that the Purkinje cells are seen as a linear interface at the junction of the molecular and granular layer. The number of Purkinje cells was observed to be more at the crests than at the troughs of each folia. The Purkinje cells showed a statistically significant decrease in number after the third decade with ballooning observed around the sixth decade. The study aims to provide information regarding quantitative changes of the Purkinje cells of the human cerebellar cortex so as to enable clinical correlation with age related cerebellar dysfunction.

KEY WORDS: Purkinje cells, number, diameter, Human cerebellar cortex

INTRODUCTION: Sherrington as early as in 1900 had coined the term 'the head ganglion of the proprioceptive system' to denote cerebellum (Pearce 2004). Degeneration and atrophy of the cerebellum increase in the elderly. Age related cerebellar studies have been mostly in Macaca nemestrina (Nandy 1981), Wistar rat (Amenta et al 1991) and 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.

The Purkinje cell bodies are arranged in a sheet, one cell thick at the interface of the molecular and granular layers. Heterotopic Purkinje cells were described in the granular layer by Julio (1971). Purkinje cells are among the largest neurons, in the central nervous system. Palkovits (1971) in his study of the cat cerebellum observed the Purkinje cell diameter to be 29µm. In the rat, according to Smolyaninov (1971), the Purkinje cell bodies are 21µm in diameter and 25µm in length. Inukai (1928) estimated Purkinje cell number in the rat as 5.5×10^5 in each cerebellum. Hall et al (1975) observed the total number of Purkinje cells in male to be 6-8% higher than that of females.

Braitenberg and Atwood (1958) gave the number of Purkinje cells in man as 15×10^6 per cerebellar hemisphere. Bell and Dow (1967) gave the number of Purkinje cells as 5×10^5 , which when compared to other studies were very low. Armstrong and Schild (1970) estimated cell counts in rat as 3.5×10^5 , which was very similar to the findings of Smolyaninov (1971) i.e. 4.5×10^5 cells.

Palkovits et al (1971) stated the Purkinje cell count in man to be $1.2-1.3 \times 10^6$. Hillmann and Chen (1983) estimated Purkinje cell count to be 2.78×10^5 in rats. The number of Purkinje cell in rat was estimated by Harvey et al (1988) as 3.38×10^5 .

Nairn et al (1989) estimated the Purkinje cell number based on nucleoli count to be 15 million per cerebellar hemisphere. Bakalian (1991) estimated Purkinje cell number to be 330350+/-35,448 cells from control animals and 299019+/-50,223 cells in old rats. Andersen et al (2003) in his study using stereoscopic methods estimated the Purkinje cell to be 28 million.

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 obtained from post mortem specimens were the material used 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 cerebellum (68 adults and two fetuses) were studied. Persons of both sexes from 2½ to 85 years were evenly distributed over seven age categories, 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61 and above. Specimens were divided into 7 groups of equal age difference. Foetuses were included in the first age group which included the age 0-10. Ten specimens each were included in the rest of the age groups. Dissected out specimens from posterior cranial fossa, were fixed in Bouin's fluid and 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 and Cresyl Fast Violet.

Mounted sections were observed under low power, high power and Oil Immersion objectives of binocular microscope with built in illumination for the position and other quantitative markers like number and diameter of the Purkinje cells.

The Purkinje cell number was counted using a linear counting technique. The Purkinje cell number and diameter of Purkinje cell was measured under X100 objective with a horizontal eye piece micrometer called "Graticule". The average value of Purkinje cell number and diameter in various fields were obtained. (Aherne 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 and diameter of Purkinje cell with age. The data were plotted on regression graphs. Correlation of above parameters with age was also worked out. (Goon 1992).

OBSERVATION: The Purkinje cell maintains its characteristic flask shaped appearance in most age groups. At around 51 years the Purkinje cell showed a slight ballooning and a rounded contour. The mean diameter of the Purkinje cell was assessed to be 10.03+/- 1.85µm up to 40 years while above 40 years it was 10.62 +/- 2.43µm. The Purkinje cell is arranged in an orderly manner as a single line at the interface of the granular and molecular layers in all age groups. Two specimens showed an uncharacteristic clump of Purkinje cells within the molecular layer (Fig 4). These Purkinje cells have morphology similar to those located at the interface. The Purkinje cells were more closely arranged at the crests than in other areas of the folia (Fig 3). The mean linear density of the Purkinje cell is observed to be 7.86 +/-2.6 cells up to 40 years while above 40 years it is 4.2 +/-1.3 cells. Special staining also revealed a decrease in the quantity of Nissl granules.

FIG. 1: 23 year old (F), Fix – Bouin's fluid, Silver methenamine staining, x 100, section shows all 3 layers cerebellum with the linear arrangement of Purkinje cells.

Fig 2: 78 year old (F), Fix – Bouin's fluid, Silver methenamine staining, x100, section shows all 3 layers of the cerebellum. Note that the number of Purkinje cells have been decreased.

FIG. 3: 32 year old (M), Fix – Bouin's fluid, H & E stain, x 100, section shows increased number of Purkinje cells at the crest than at the base of the folia.

FIG. 4: 64 year old (M), Fix – Bouin's fluid, H & E stain x 100, section shows a collection of Purkinje cells in the molecular layer.

Number of Purkinje cells: The linear density of Purkinje cells was observed to be decreasing with age. A high value of difference was observed between the first and second decades but this was not found to be statistically significant. A statistically significant decrease in the number of Purkinje cells was observed after the 3rd decade. ANOVA shows statistically significant differences between various age groups.

A 'F' test was done, the 'F' value obtained was 36.41 (P=0.000) which implied that there are statistically significant differences between at least one pair of observations.

Table 1. NUMBER OF PURKINJE CELLS IN VARIOUS AGE GROUPS

Age (in years)	Mean	Standard Deviation	Range
0-10	8.45	1.07	7-10.4
11-20	4.68	0.67	3.4-5.6
21-30	10.32	2.15	7.8-14.6
31-40	8.12	2.09	5.6-12
41-50	5.36	0.74	4.2-7
51-60	4.42	1.24	2.8-6.6
61 above	2.98	0.59	2.2-3.8

As the 'F' test was significant the data was subjected to 'Tukey's Honestly Significant Difference test (HSD) (post hoc test). This test shows statistical significance for pair wise comparison of mean values. The data was subjected to regression analysis with age as an independent variable. Simple linear equation was found and regression graphs were plotted. Regression equations revealed a steady increase in the number of Purkinje cells up to the age of 40 years and there after the number decreased gradually. Correlation coefficient of age wise distribution of number of Purkinje cells in the group up to 40 years was observed to be 0.23, which though not found to be statistically significant indicated a positive correlation with age (Table 2). In the age group of above 40 years the correlation coefficient existing between number of Purkinje cells and age was -0.82, which implied a statistically significant negative correlation with age (Table 2).

The mean linear density of Purkinje cell was observed to be 7.86+/-2.6 up to 40 years while above 40 years it was 4.2+/-1.3 cells /mm.

Table 2. CORRELATION MATRIX OF VARIOUS PARAMETERS

	Below 40 years		Above 40 years	
	Correlation coefficient	Significance (P)	Correlation coefficient	Significance (P)
Number of Purkinje cells	0.23	0.175	-0.82	0.000

Diameter of Purkinje cells

The diameter of Purkinje cells showed noticeable variations between the groups but not between adjacent groups (Table 3).

'F' test done on the obtained data gave an 'F' value of 10.08 (P=0.000) which implied statistically significant difference between at least one pair of the various age groups.

Table 3. DIAMETER OF PURKINJE CELLS IN VARIOUS AGE GROUPS

Age (in years)	Mean diameter (in μm)	Standard deviation	Range
0-10	6.53	0.89	5.6-8.2
11-20	7.22	1.57	5.6-10.4
21-30	8.56	1.07	7-10.6
31-40	7.24	1.09	5-8.4
41-50	7.48	0.75	6.6-8.6
51-60	9.78	1.16	8.6-11.4
61 above	6.36	1.44	3.2-8.4

'Tukey's Honestly Significant Difference' test shows statistically significant increase in diameter of Purkinje cells between the first and second decades (Table 3). Statistically significant decrease in diameter of Purkinje cells was noticed after 60 years of age (Table 3). Regression analysis was done with a simple linear equation which showed a negative correlation after the age of 40 years.

The correlation coefficient of diameter of Purkinje cells in the age groups below 40 years was found to be a statistically insignificant value of 0.23 (Table 4). The correlation coefficient of age wise distribution of diameter of Purkinje cells in the age groups above 40 years was found to be -0.40, which showed statistically significant negative correlation (Table 4). The mean diameter of Purkinje cells in age group up to 40 years was $10.03 \pm 1.85 \mu\text{m}$ while that in age group above 40 years was estimated as $10.62 \pm 2.43 \mu\text{m}$.

Table 4. CORRELATION MATRIX OF VARIOUS PARAMETERS:

	Below 40 years		Above 40 years	
	Correlation coefficient	Significance (P)	Correlation coefficient	Significance (P)
Diameter of Purkinje cell	0.23	0.173	-0.40	0.029

DISCUSSION: Though the cerebellum was identified to be an important component in the 'Theory of memory' (David Marr, 1969) not many studies have emphasized the age related changes of the cerebellum and associated memory loss. The present work was done to identify age related changes in the cerebellum at a cellular level. The Purkinje cells are observed as a distinct layer of flask shaped cells at the interface of the molecular and granular layer. This finding is in accordance with the findings of most researchers (Fox and Barnard, 1957, Braitenberg and Atwood, 1958, Armstrong and Schild, 1970, Smolyaninov, 1971).

In two sections a collection of Purkinje cells was observed in the molecular layer. The Purkinje cells were arranged as a distinct clump within the molecular layer. They were of normal morphology. Estable (Julio. MS 1971) called the Purkinje cells within the granular layer as hypocytomorphic because of their incomplete morphological differentiation. Sosa (1971) observed such heterotopic Purkinje cells and commented that these cells arise due to subnormal migratory ability of cells of external granular layer during histogenesis. The Purkinje cells observed in this study were of normal morphology and thus could not be classified as hypocytomorphic.

Though Hall T.C observed sexual dimorphism in the number of Purkinje cell with a male preponderance, other researchers did not support this. The present study did not reveal any sexual dimorphism and was thus in conformity with the findings of Nandy, that there is no significant differences in the number of Purkinje cells in both sexes.

The Purkinje cell had characteristic flask shaped appearance with a well-defined nucleus and single nucleolus. The present study did not reveal any binucleolated Purkinje cell, as was observed by Hall. Many researchers have applied the fact that a single cell has a single nucleolus in estimating the Purkinje cell number by counting the number of nucleoli.

No cell organelles could be visualized using routine Haematoxylin and Eosin staining. Special staining technique using Cresyl Fast Violet demonstrated the Nissl granules. The Nissl granules tend to decrease progressively with age.

The Purkinje cells were seen to be more closely arranged at the crests than in other areas of the folia. This supports the findings of Henle (Braitenberg and Atwood, 1958) who stated that the cells are close, together at the summits of the folia than at the depth of the sulci.

The mean linear density of the Purkinje cell was observed to be 7.86 ± 2.6 cells up to 40 years while above 40 years it is 4.2 ± 1.3 cells. Though a decrease in the mean linear density was observed after 40 years there were significant variations within the group. The mean linear density of Purkinje cell in the second decade is 4.68 ± 0.67 cells, which was low when compared to the first decade where the Purkinje cell count was 8.45 ± 1.07 cells. This can be attributed to the increase in cerebellar surface area. Lange (1975) observed that cell density decreases from mammals with a low brain weight and lesser surface area to those with a higher brain weight. This can be applied to the sudden decrease in number of Purkinje cells in the second decade where the surface area shows the changes as mentioned by Lange. Torvik (1986) observed linear cell densities in adults to vary from 4.4 cells/mm to 1 cell/mm, which was very low when compared to the findings from the present study. Hall (1975) observed a mean reduction of 2.5% per decade after the age of sixty years. In our study the decrease was noticeable after the age of 40 years but the percentage of decrease was less and varied between the decades. This finding was considerably different from that of Torvik. Torvik observed noticeable age dependent decrease of Purkinje cell number in certain cases but approximately one half of the cases above 80 years did not show any change from

those in the sixties. He observed a severe decrease in Purkinje cell density with increasing age in superior cerebellar vermis but not in the inferior cerebellar vermis.

The diameter of the Purkinje cell was studied. Literature did not reveal much information regarding variations of diameter. The present study showed a ballooning of the Purkinje cell with the mean diameter being $9.77 \pm 1.16 \mu\text{m}$ during the fifth decade. Nandy (1981) observed increased lipofuscin accumulation within the Purkinje cell as age advances. Dowson (1998) observed an increase in the mean total area of discrete regions of lipopigment in a Purkinje cell perikaryon as age advances. These findings aid in substantiating our observation that there is an increase in the size of Purkinje cell as age advances due to lipopigment accumulation. Ogata (1984) reported a contradictory finding that though the degree of lipofuscin accumulation in Purkinje cell increased with age the cellular volume decreases. Mann (1978) observed small amounts of finely distributed lipofuscin granules intracellularly and large clusters of pigment extracellularly in the Purkinje cell layer. Nandy 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; Rogers, 1984; Ogata 1984; Andersen, 2003). Although the precise functional significance of the change in Purkinje cells is not clear their vulnerability may be related to changes in motor function in old age.

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 fetuses 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 number and diameter of the Purkinje cell of the cerebellar cortex with respect to age were studied.

The Purkinje cell number showed a sharp decline in the second decade which can be attributed to the sudden increase in cerebellar surface area. The Purkinje cell number remained almost constant up to the fourth decade after which marked decrease in linear density was noted. The decrease was more marked as age advanced. The Purkinje cell maintained its characteristic flask shape up to the 5th decade after which slight ballooning of the cell was noticed.

The data obtained were analysed statistically. The number of Purkinje cells showed statistically significant negative correlation with age. The diameter of Purkinje cells showed no significant correlation with age.

The present work provides an opportunity to define the normal histological variations at different ages. There is further scope for functional studies based on the qualitative and quantitative changes in the normal histology of the cerebellum.

The present study will serve as a basis for further studies regarding correlation of the changes in cerebellar morphology with ageing, leading to motor in-coordination and loss of memory.

In the light of increase in incidence of geriatric diseases like Alzheimer's, this study may be used as a stepping stone to functional studies by future researchers.

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