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Age-related morphometric changes occurring in the somata of astrocytes of the granular layer of rat neocerebellar cortex (Crus I and Crus II)

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Summary. A quantitative study concerning the age-related changes occurring in the cell body and somatic organelles of neocerebellar astrocytes is carried out, using rats aged 2 to 24 months. Manual stereological techniques are used to determine the following parameters on electron micrographs: the somatic volume, the volume density and the absolute volume of the protoplasmic structures as well as the mean surface of the rough endoplasmic reticulum cisternae per cell.None of the parameters reveals any clear-cut general temporal pattern. The soma and the cell components show statistically significant differences in the parameters with ageing, excepting the dense bodies (relative and absolute volumes) and the Golgi apparatus (relative volume). There are significant positive linear trends between, on the one hand, the somatic volume and, on the other hand, the absolute volume of either of the following structures: nucleus, glial filaments, ground substance and dense bodies. Some linear correlations between the absolute volumes of organelles are also found. Despite the ability for karyokinesis, it is concluded that astrocytes do undergo changes with ageing.

Key words: Morphometry, Astrocytes, Cerebellar cortex, Age-changes

Introduction

Cells originated from the neuroectoderm, the spongioblasts, proliferate and differentiate to astroblasts in order to become astrocytes. This macroglial category of glial cell was considered as a distinct population since Ramón y Cajal (1913), when he used his gold chloride sublimate method. The studies in the last two decades have had a new look at the astrocytes, stressing their capabilities and thus ruling out what was previously thought about these glial cells as merely passive structural and metabolic support entities for the nerve cells. Astrocytes deserve to be duly dignified, when it is remembered that, besides neurons, astrocytes are the most abundant cell type in the central nervous system (CNS); they are here a ubiquitous population forming in higher mammals up to 50% of the cell totality of the cerebral cortex (Hansson and Ronnback, 1989). Besides, as they are described in fishes (Carrato et al., 1981), in amphibians (Stensaas, 1977), in reptiles (Kruger and Maxwell, 1967) as well as in birds (Somogyi et al., 1990), astrocytes are potentially a universal population among all species of vertebrates.

In contrast to the large quantity of publications dealing with mammalian astrogliogenesis (Sturrock, 1974, 1982; Kaplan and Hinds, 1980; Fedoroff, 1986; Cameron and Rakic, 1991; Goldman and Vaysse, 1991), relatively fewer studies in connection with ultrastructural modifications occurring in organelles of astrocytes with ageing have been carried out. Moreover, these age-related changes have been chiefly examined regarding qualitative aspects (Hasan and Glees, 1973a; Ong and Garey, 1991).

As is common knowledge, the nerve cells of cerebellar cortex, as well as nerve cells elsewhere, are fixed postmitotic cells. As they cannot dilute by karyokinesis, the accumulation of apparently useless products, e.g., lipofuscin, they are actually more prone to be modified with ageing. Nonetheless, the cortical zone also possessens cells able to undergo division, as it happens with the astrocytes, a population which exists in all ages. Therefore, this study was undertaken to seek whether or not this population with mitotic power might be somewhat immune to senility. Bearing in mind the distress and apparent immutability displayed by astrocytes when they are qualitatively observed, these alterations have to be morphometrically analysed. Indeed, to the best of our knowledge,no ultrastructural analysis has thus far been made concerning quantitative age-changes in rat cerebellar astrocytes.

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Materials and methods

Preparation of tissue for transmission electron microscopy

Female Sprague-Dawley rats 2, 6, 9, 12, 15, 18, 21 and 24 months (M) old (n = 5 per age) were perfused through the heart with 2.5% glutaraldehyde in 0.1 M Sørensen's phosphate buffer (pH 7.3) containing 0.1% sucrose, after being anaesthetized with 3.5% aqueous solution of chloral hydrate (35 mg per 100 g of body weight, intraperitoneally). Samples from Crus I and Crus II were removed and processed for conventional electron microscopy as described in a previous study (Monteiro, 1986). The grids were observed under a JEOL JEM 100CXII electron microscope, operated at 60 Kv.

Morphometric analysis

A total of 40 negatives per age (8 per specimen) of astrocytes of the granular layer were obtained at a primary magnification of x 4,000, 5,300 or 8,000. Only a single cell soma, chosen at random from each block, was photographed; the cell processes were not included in this quantitative analysis. Prints were obtained and the individual final magnification was determined for calculation purposes.

1) A multipurpose test system, widely known as a standard morphometric technique (Weibel and Bolender, 1973), with 84 lines, each 1.35 cm long, enabled the determination of the volume density or fractional volume (Vv-%) of the intracellular structures (excluding the rough endoplasmic reticulum-RER), by using the formula:

$$Vv = (Pi.\ 100)/Pt$$
(Weibel and Bolender, 1973)

in which Pi is the number of points within the component profiles and Pt is the total number of test points lying within the cell profile.

2) The nuclear absolute volume (v) was obtained with the formula:

$$v = (\pi/6).L.B^2$$
(Palkovits and Fischer, 1968)

in which L is the major diameter of the nucleus and B is the length of the perpendicular that crosses L through its middle point, measured within the organelle outline. Out of the initial 40 electron micrographs per age utilized for the multiporpose test system, only those with profiles of astrocytes considered to be cut through the middle point were used. The remaining ones were replaced, to achieve the same total, with other micrographs cut as mentioned. To calculate the somatic volume (V), the absolute volume of the nucleus was multiplied by 100 and the product was divided by the volume density of the nucleus. The multiplication of the mean value of V by the individual density of the organelles gives their absolute volumes.

3) A grid consisting of 17 parallel lines spaced 1 cm apart was used to calculate the membrane surface concentration (MSC), or surface density (Sv) of RER, i.e., the average number of square micrometres of RER per cubic micrometre of cytoplasm, by using the formula:

$$MSC = (C.M)/(L.1000)$$
(Loud, 1962)

where C is the number of crossing between the profiles of RER and the grid lines, M is the individual print magnification and L is the total line length that lies over the total cytoplasm. Finally, the mean surface of RER per cell (RER-S) was calculated by multiplying the MSC by the mean total volume of the cytoplasm.

4) Because the RER cisternae are very tiny elements, to obviate, among a series of disadvantages, the parallax errors, an indirect way to calculate their Vv was carried out. Thus, the parameters MSC, the mean width of RER cisternae (w), the absolute volume of total cytoplasm (vc) and V integrate a formula to calculate the Vv of RER (in relation to V) which may be easily and linearly deduced and presented in a simplified form as follows:

$$Vv\ (RER) = (MSC.w.vc.50)/V$$

The mean width of the cisternae was achieved by averaging the results of the direct measurement of a large number of units with a Leitz eyepiece.Owing to the elongated and flat pattern exhibited by the cisternae, it was previously assumed that they are feasible parallelepipeds.

For practical purpose, the «ground substance» component includes the endoplasmic reticulum, and owing to their scarcity, the multivesicular bodies (MVB), the centrioles, as well as both the rare and unusual structures to be described (the «hyalin globules» and the «granular deposits»).

As the objective of this study was to compare astrocyte profiles belonging to rats with different ages, stereological corrections were ignored, since the error in the various age groups is in the same direction. This evidence has been stated by Abercrombie (1946) and was more recently emphasized by Loud (1987).

Statistical analysis

The data were studied with standard statistical tests and presented as mean ± SE (standard error); percentages were condensed in a table and absolute values were presented in graphical form. Statistical significance of

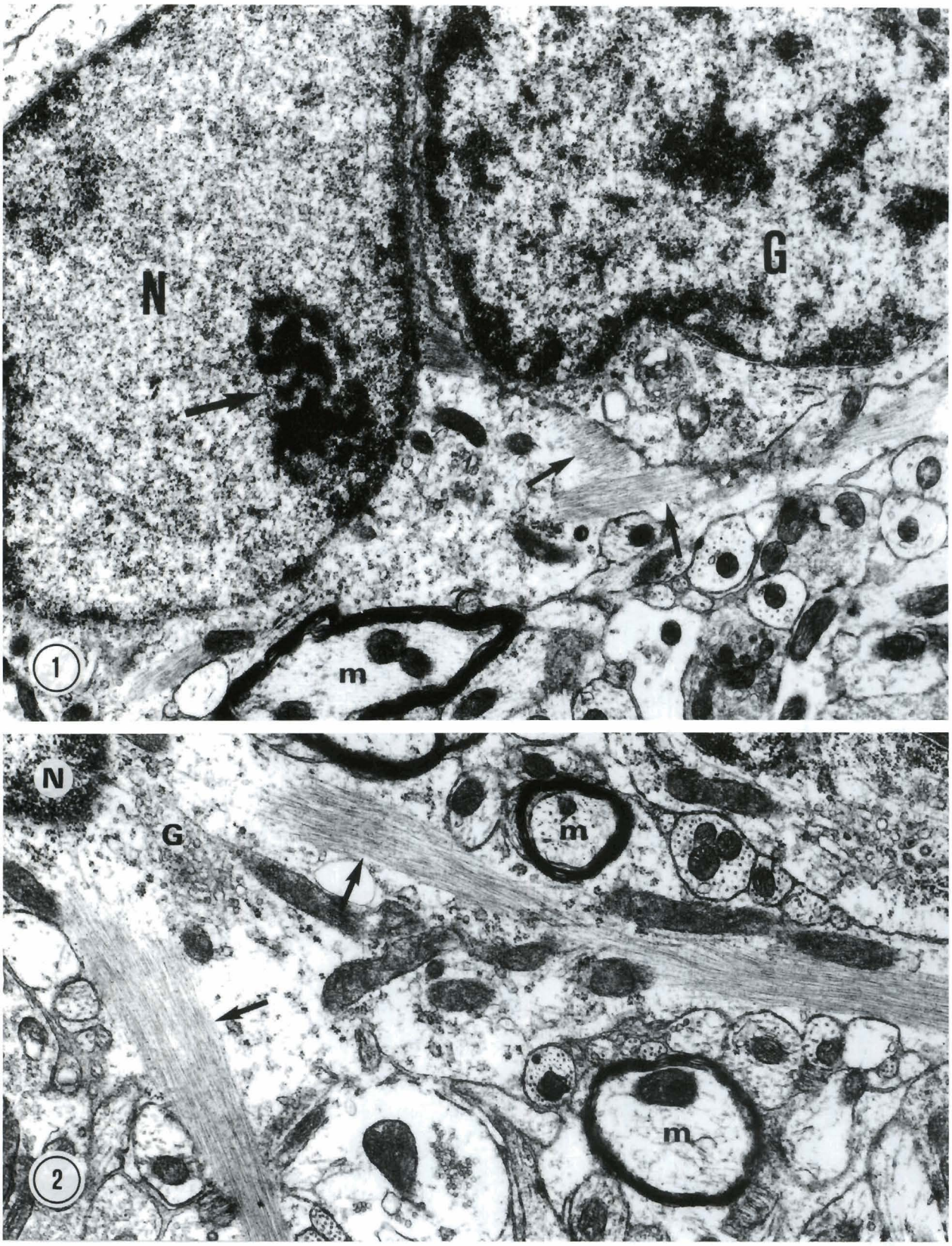


Fig. 1. An astrocyte exhibiting a clear nucleus (N) with a clearly patent eccentric, irregular nucleolus (big arrow). The also clear cytoplasm shows bundles of glial filaments (small arrows); G - nucleus of an element of the prolific population of common granule cells; m - myelinated axon of the adjacent neuropil. x 15,900. (Rat age = 6 M).

Fig. 2 An astrocyte (N - nucleus) displaying the Golgi apparatus (G) and two relatively thick bundles of glial filaments (arrows) which cross the soma towards the processes; m - myelinated axons of the adjacent neurophil. x 18,000. (Rat age - 12 M).

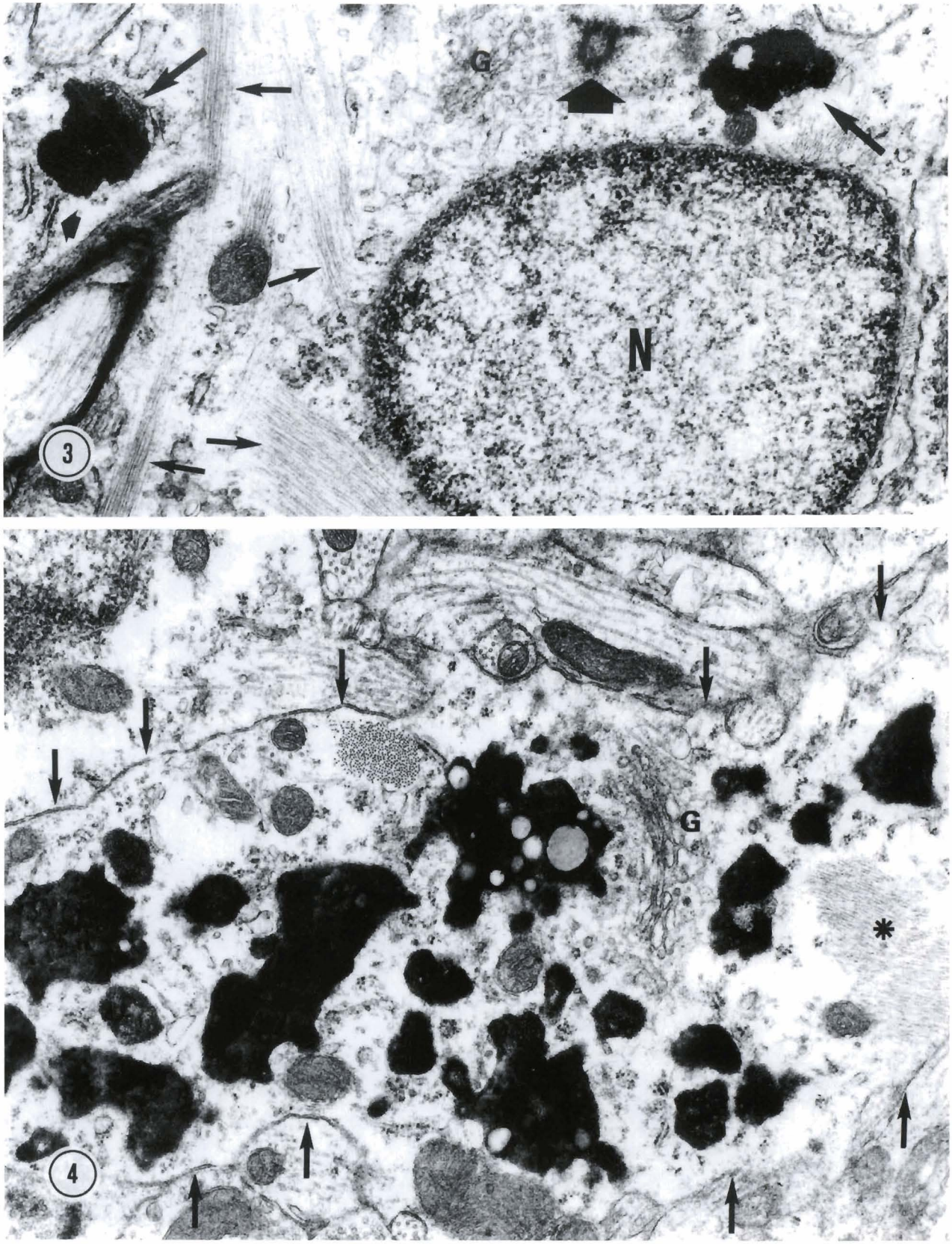
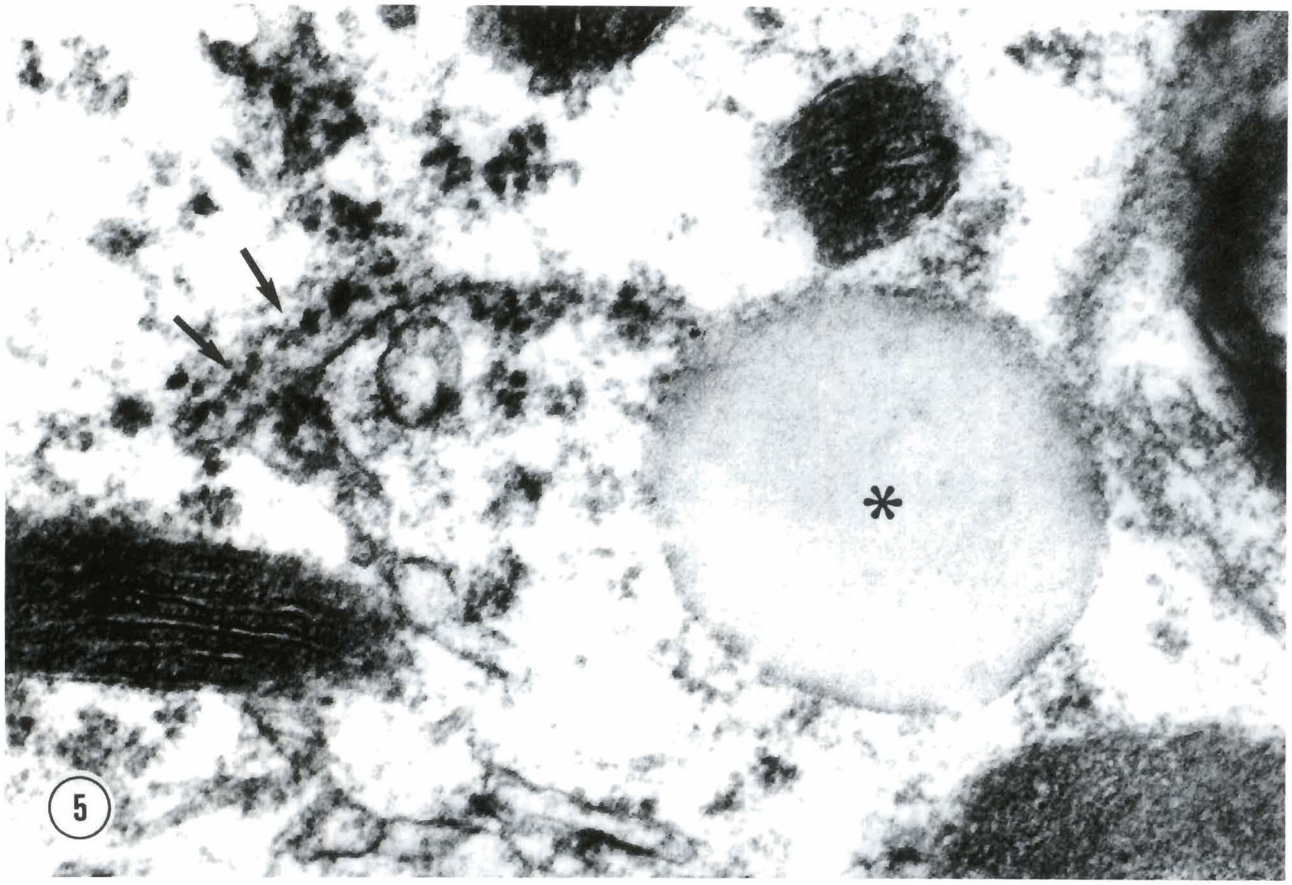


Fig. 3. An astrocyte (N - nucleus) exhibiting the Golgi apparatus (G), a centriole (big block arrow), two dense bodies (bigt hin arrows), bundles of glial filaments (small thin arrows) and cisternae of RER (small block arrow). x 24,000. (Rat age = 18 M).

Fig. 4. An astrocyte process (demarcated with arrows) showing a heavy congestion with dense bodies; asterisk-glial filaments; G - Golgi apparatus. x 24,000 (Rat age = 24 M).



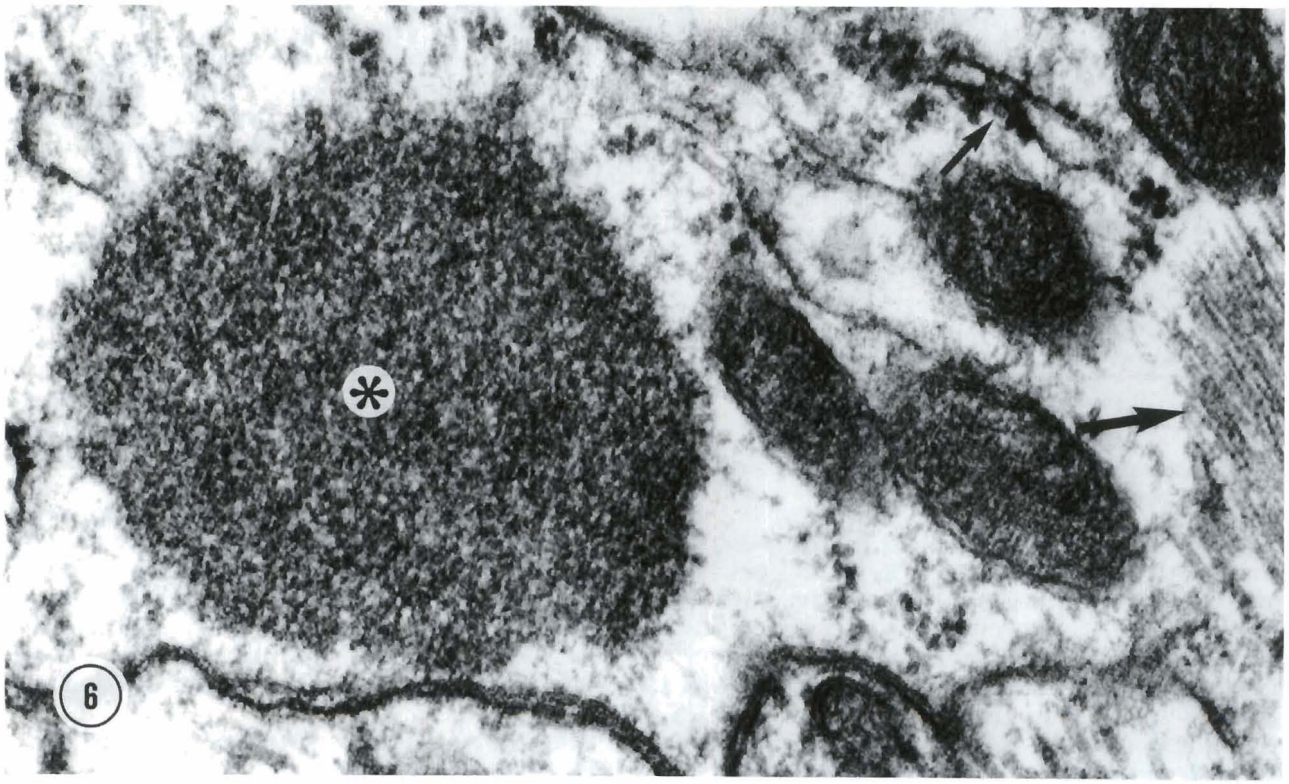


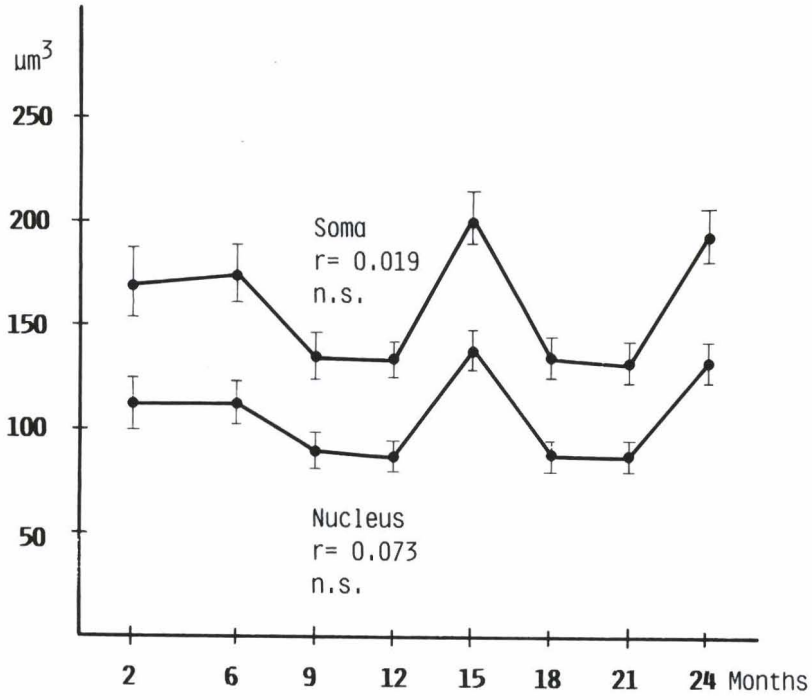
Fig. 5. Astrocyte cytoplasm revealing a cisterna of RER (arrows) with irregularly-spaced attached ribosomes. A «hyaline globule» (asterisk) with a rather homogeneous, fine, electron lucent matrix is displayed; the structure is not bound with a unit membrane. x 90,000. (Rat age = 21 M).

Fig. 6. Astrocyte cytoplasm showing a cisterna of RER (small arrow) and glial filaments (big arrow). A «granular deposit» (asterisk) is also seen, exhibiting a heterogeneous electrodense, coarse matrix; the structure is not bound with a unit membrane. x 75,000. (Rat age = 24 M).

Volume densities (Vv-%) of organelles of astrocytes of rat neocerebellum (Crus I and Crus II) from 2 to 24 months old (± SE).

AGE	2	6	9	12	15	18	21	24
Nucleus	66.30 ± 0.77	64.39 ± 0.90	66.44 ± 0.91	65.79 ± 0.95	68.37 ± 0.90	64.53 ± 0.86	66.22 ± 0.84	68.39 ± 0.80
Nucleolus (*)	0.69 ± 0.17	0.44 ± 0.15	0.34 ± 0.14	0.24 ± 0.12	0.22 ± 0.11	0.25 ± 0.11	1.24 ± 0.24	1.03 ± 0.21
Mitochondria	4.00 ± 0.32	4.35 ± 0.38	4.69 ± 0.41	4.30 ± 0.40	3.62 ± 0.36	4.15 ± 0.36	4.03 ± 0.35	2.81 ± 0.28
Golgi apparatus	0.64 ± 0.13	0.35 ± 0.11	0.52 ± 0.14	0.44 ± 0.13	0.49 ± 0.14	0.39 ± 0.11	0.66 ± 0.14	0.67 ± 0.14
Dense bodies	0.54 ± 0.12	0.74 ± 0.16	0.71 ± 0.16	0.60 ± 0.15	0.60 ± 0.15	0.64 ± 0.14	0.54 ± 0.13	0.56 ± 0.13
Glial filaments	5.56 ± 0.38	4.49 ± 0.39	4.09 ± 0.38	4.38 ± 0.41	5.59 ± 0.45	3.03 ± 0.31	4.13 ± 0.35	4.60 ± 0.36
Ground substance	22.96 ± 0.69	25.68 ± 0.82	23.55 ± 0.82	24.49 ± 0.86	21.33 ± 0.80	27.26 ± 0.80	24.42 ± 0.76	22.97 ± 0.72
RER cisternae	0.12 ± 0.03	0.29 ± 0.05	0.07 ± 0.02	0.28 ± 0.05	0.18 ± 0.03	0.19 ± 0.05	0.20 ± 0.04	0.23 ± 0.03

M = 40 profiles per age.
(*) in relation to the nucleus.



Graph 1. Age changes in the somatic volume and in the absolute volume of the nucleus of astrocytes.

pair comparisons was carried out by analysing the standard error of the difference. The critical ratio was then examined by a «two-tailed» test with a Z probability table. This analysis is summarized in hemi-step pyramid tables (Statistical Tables no. 1 to no. 5).

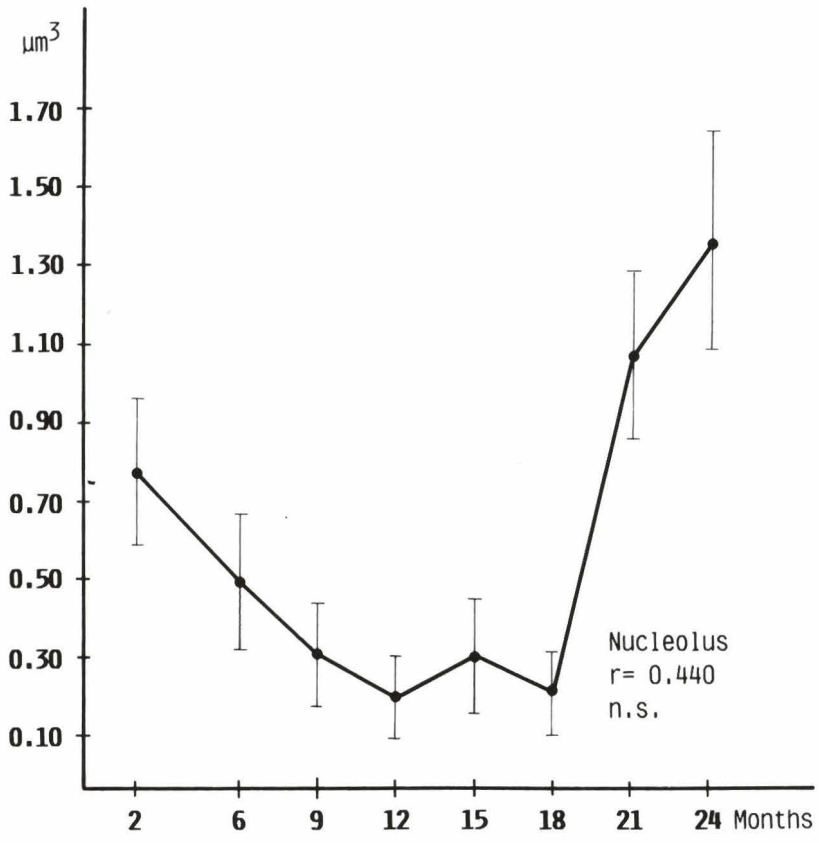
Simple linear regression analysis was achieved with a computer-assisted programme (STATGRAPHICS, version 3.0) in order to determine the correlation coefficient (Pearson) in connection with the pairs of observations (n = 8). Estimation of intercept and slope parameters were only considered for significant results and for graphical representation. Confidence and prediction limits at 95%. The statistical results were considered not significant for P >0.05 (n.s. = not significant, in Graphs and Statistical Tables).

Results

Qualitative analysis

The somata of astrocytes of neocerebellar cortex exhibited somewhat the same dimensions as the common granule cells (Fig. 1). The round to oval nucleus was clear and presented rather homogeneous chromatin pattern (Figs. 1, 3). A fibrous lamina was lacking, but a delicate ring of heterochromatin outlined the organelle (Figs. 1, 3). The nucleolus was prominent (Fig. 1). The somata also had very clear cytoplasm (Figs. 1 - 3). The mitochondria were small and very polymorphic (Figs. 1, 2) and the Golgi apparatus (Figs. 2, 3) was poorly developed. The RER displayed short cisternae being scanty and not organized (Figs. 3, 5, 6); a few free ribosomes were seen. No glycogen particles were found. Pleomorphic dense bodies showing vacuoles frequently appeared (Fig. 3); in some places, namely in the processes (Fig. 4), heavy accumulations could be seen. Some centrioles (Fig. 3) were seen. Some microtubules could be visualized; yet, they were not characteristic of this glial type. On the contrary, the most striking features was another kind of cytoskeleton structure: the glial filaments (Figs. 1, 2, 3, 6). They aggregated into bundles which ran throughout the cell processes (Fig. 2) after crossing the soma without any particular orientation.

Besides the evidence of some clear «hyalin globules» (Fig. 5), some electron dense «granular deposits» seen throughout the astrocytic cytoplasm (Fig. 6) of relatively aged rats (18 M onwards) and the above



Graph 2. Age changes in the absolute volume of the nucleolus of astrocytes.

referred dense body accumulation (Fig. 4), no apparent qualitative modifications with ageing are worth mentioning.

Quantitative analysis

The volume densities of the protoplasmic components are grouped in Table 1. The respective absolute volumes, the somatic volume as well as the RER-S are displayed in Graphs (no. 1 to no. 8). None of them revealed any of the three clear-cut general temporal pattern defined by Peters and Vaughan (1981); nevertheless, the soma and almost all the intracellular structures apparently underwent modifications in their parameters with ageing. According to the considered component, the maximum absolute value was achieved at 6, 15 or 24 M. With ageing, the nucleus contributed with the highest observed percentage; about 66% (on average), followed by the ground substance; about 24%. Minor contributions involved, in order of numerical importance as approximate average: the glial filaments (4.5%), the mitochondria (4%), the dense bodies, which included lipofuscin (0.6%) and finally the Golgi apparatus (0.5%). The estimated regression analysis indicates that neither of the relative nor of the absolute values shows any significant linear correlation with age.

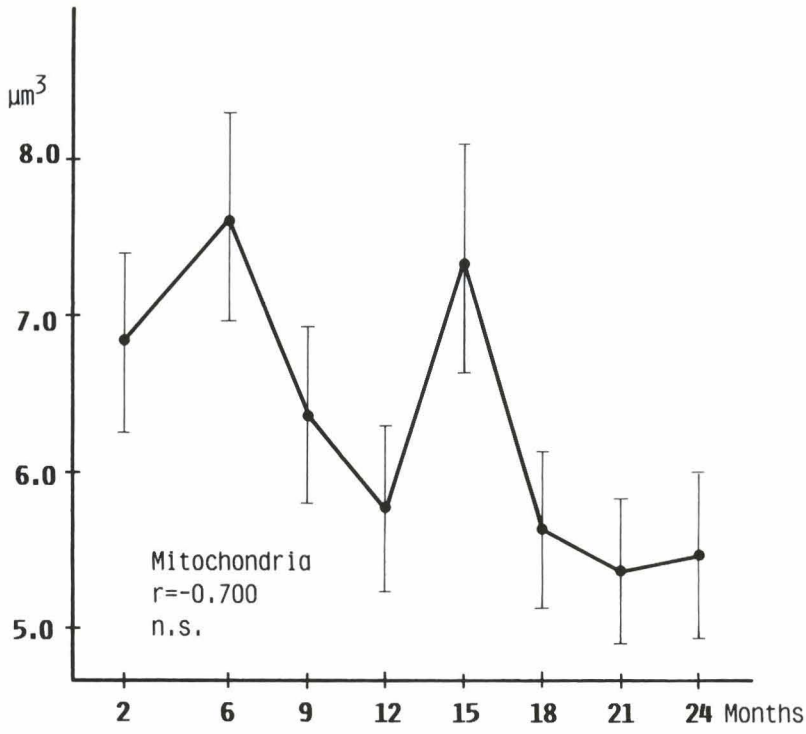
The evolution of the somatic volume (Graph no. 1) revealed a maximum peak at 15 M and an increase between 21 and 24 M; here, the second highest value was achieved. The topmost values did not significantly differ from each other; the opposite may be stated between any of them and the adjacent values. Scattergrams with significant linear correlations were detected when the mean somatic volume was plotted against the mean absolute volume of either of the following structures: nucleus (Graph no. 9), glial filaments (Graph no. 10), ground substance (Graph no. 11) and dense bodies (Graph no. 12).

Graph no. 1 also exhibits the progression of the absolute volume of the nucleus. The graphical inflexions almost perfectly matched those of the somatic volume; this is in accordance with the high degree of correlation found in the above cited Graph no. 9. In the fractional grounds, the maximum value was found at 15 M as well. Additionally, it must be stressed that from 2 to 12 M no statistical significant differences were perceived. There were statistically significant linear trends between the absolute volume of the nucleus and the absolute volume of either of the following structures: Golgi apparatus [$r = 0.72$, $F(1,6) = 6.524$, $P = 0.043$], ground substrate [$r = 0.87$, $F(1,6) = 18.167$, $P = 0.005$] and glial filaments [$r = 0.93$, $F(1,6) = 40.120$, $P = 0.0001$].

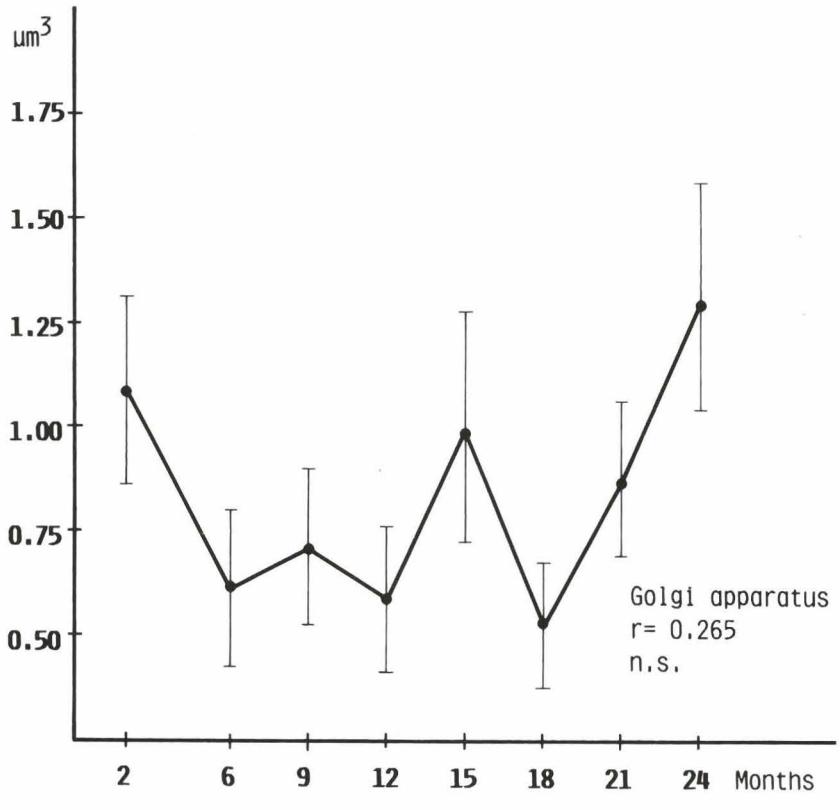
The evolution of the absolute volume of the nucleolus (Graph no. 2) indicated a decrease from 2 to 18 M, with a slight intercalary increase at 15 M which was not, however, significant when compared with its adjacent values. Between the values at 2 M, on the one hand, and the values at 9, 12 or 18 M, on the other hand, significant differences were noticed. From 18 to 24 M an abrupt increase in nucleolar volume was detected; this sloping change was corroborated in the highly significant differences noticed when pairs 18/21 M and 18/24 M were compared. Between the evolution of absolute and relative volumes an almost perfect match was observed.

The absolute volume of mitochondria (Graph no. 3) was highest at 6 M (maximum value) and at 15 M. Nonetheless, the adjacent values did not statistically differ from the values observed in each of the peaks, the comparison 15/18 M being an exception. As far as the relative volume is concerned there was a maximum at 9M; this value was significantly greater than those found at 15 or 24 M.

At 24 M, the maximum absolute volume for Golgi



Graph 3. Age changes in the absolute volume of the mitochondria of astrocytes.



Graph 4. Age changes in the absolute volume of the Golgi apparatus of astrocytes.

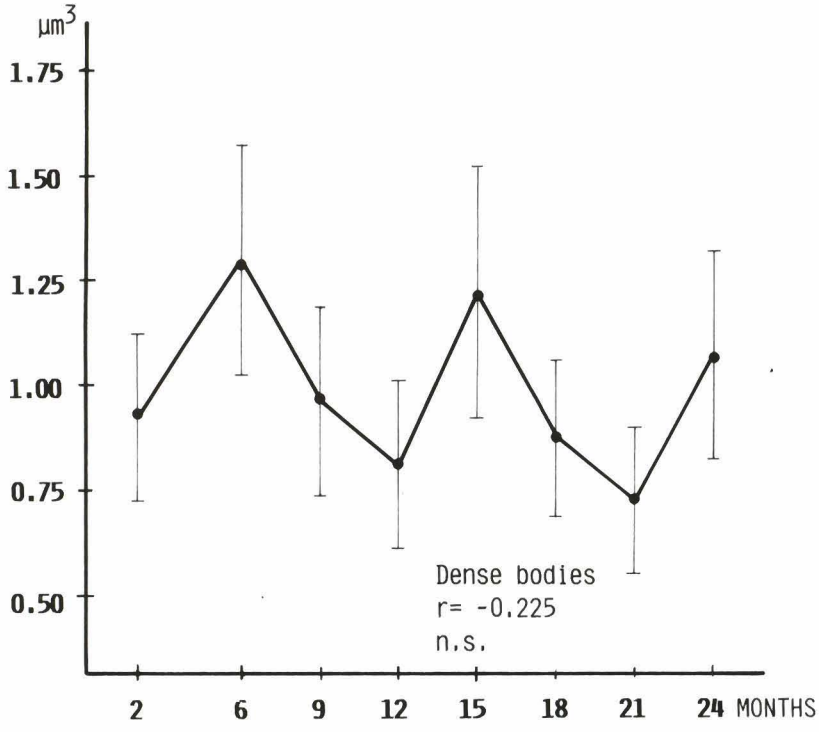
apparatus was attained (Graph no. 4). This values was statistically different from those achieved at 6, 12 or 18 M. When the relative volume was under analysis, no apparent significant differences between any pair of ages was found. Between the absolute volumes of the Golgi apparatus and of the nucleolus a significant positive linear correlation was patent [r = 0.77, F (1,6) = 8.850, P = 0.025].

The absolute volume of dense bodies (Graph no. 5) presented two peaks: one at 6 M (maximum value) and the other at 15 M. Nonetheless, when any pair from the full spectrum of ages were compared no statistically significant result was seen. The variation from 2 to 12 and from 12 to 21 M had a similar graphical pattern; in addition, it must be emphasized that a duplication, although less perfect, but with the same morphology, was noticed in the graph obtained for mitochondria (Graph no. 3). The relative volume presented two peaks as well, but at 6 and 18 M. As happened with the absolute volume, no significant comparison for any pair of ages was found. Dense bodies had their absolute volume significantly correlated with that of mitochondria [r = 0.78, F (1,6) = 9.287, P = 0.02259], of nucleus [r = 0.75, F (1,6) = 7.723, P = 0.03204] and of ground substance [r = 0.85, F (1,6) = 15.401, P = 0.008].

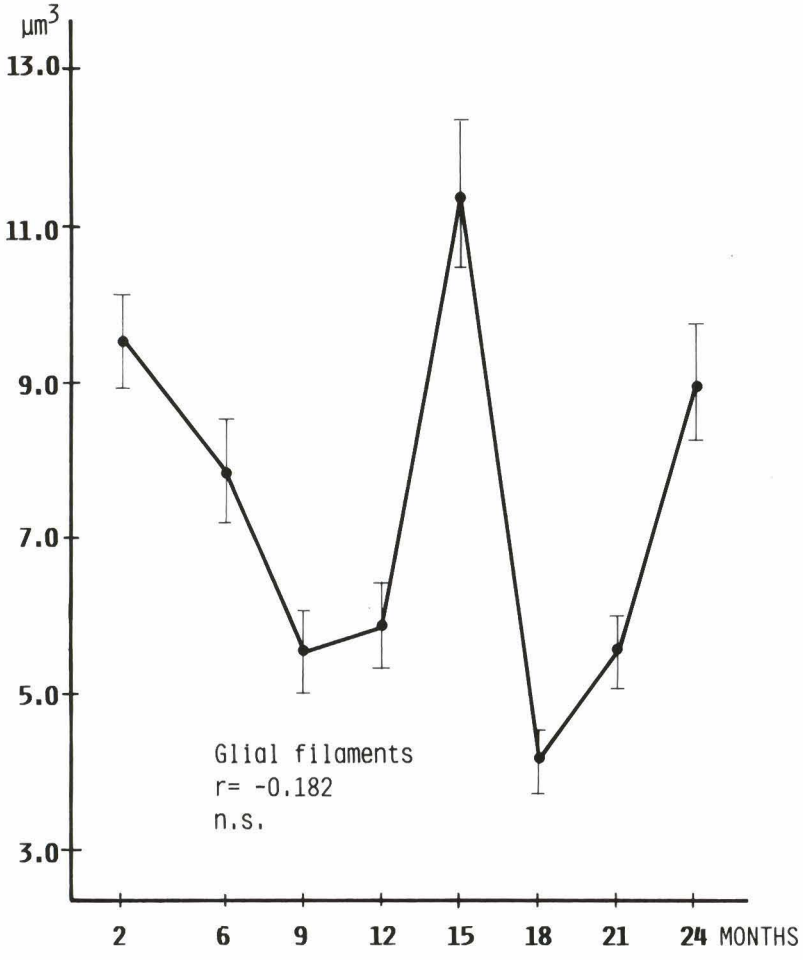
The absolute volume of glial filaments (Graph no. 6) showed a distinct peak at 15 M; only the value for 2 M did not significantly differ from this one. The relative volume also exhibited a peak at 15 M, statistically significant from the values seen for 9, 12, 18 and 21 M. Between the absolute volumes of the glial filaments and of the ground substance, a positive significant linear trend existed [r = 0.73, F (1,6) = 6.805, P = 0.040].

Concerning the absolute volume of ground substance (Graph no. 7), at 6 M (maximum) and at 15 M two peaks were displayed; in addition, from 21 to 24 M (where the second highest value was detected) an increase was patent. These three extreme values did not significantly differ from each other, as otherwise happens between any of them and all the remaining ones. As to the relative volume, at 18 M the maximum value was found; when compared with all the others it was significantly greater, the value for 6 M being an exception.

At 6 M, the variation of the RER-S (Graph no. 8) exhibited a maximum value



Graph 5. Age changes in the absolute volume of the dense bodies of astrocytes.



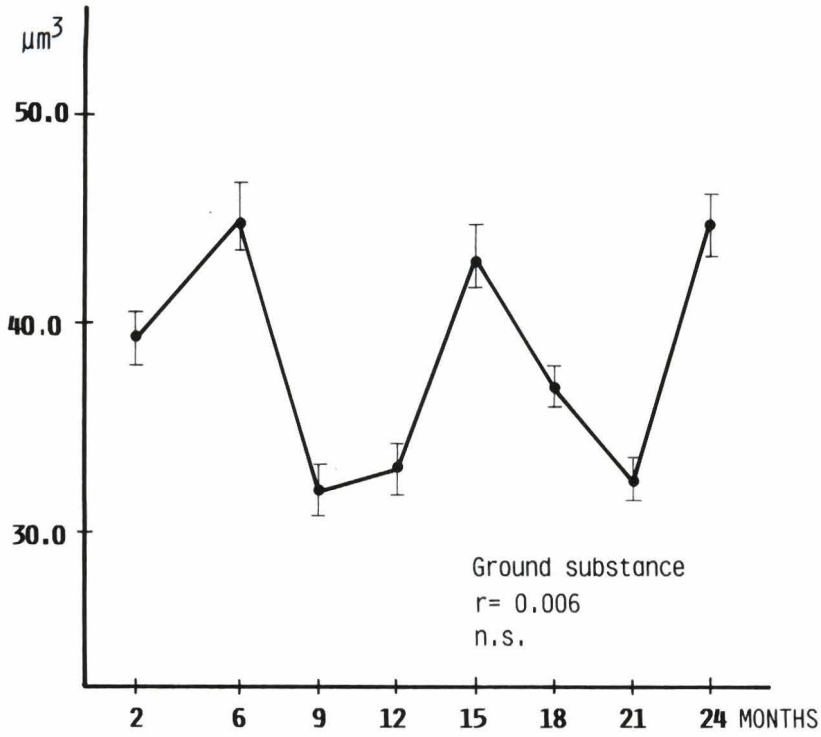
which was significantly distinct from the adjacent values. Between the lapse from 12 to 21 M the graphical inflexions did not reveal statistically significant variations. The increase observed from 21 to 24 M was significant. At 6 M, the relative volume of RER also showed a maximum value; from the statistical point of view, in the interval from 12 to 24 M the parameter did not change.

Discussion

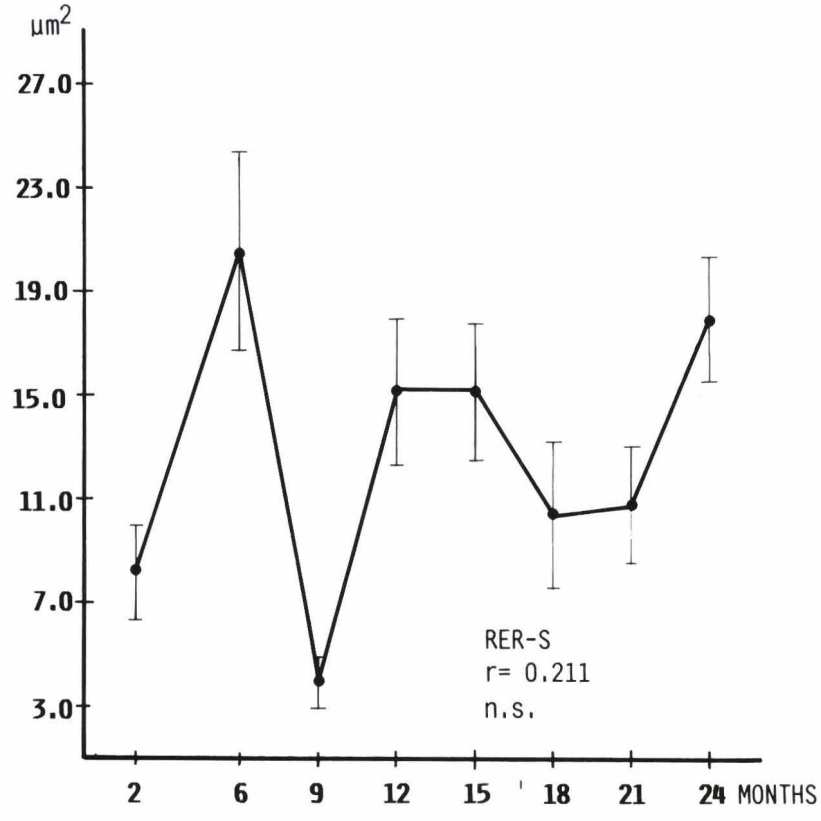
Astroglia may be traditionally subdivided into two main categories: the protoplasmic astrocytes, mostly seen in grey matter; and the fibrous astrocytes, chiefly found in white matter. Owing to a full set of intermediate forms between the two classical types found in this study, to trace a demarcation line would be very hypothetical; that is why, despite their distinction on biochemical and developmental grounds (Miller and Raff, 1984) as well as morphologically (Raff et al., 1983), the astrocytes were randomly collected in the granular layer. The majority of astrocytes we observed, however, in the neocerebellar cortical areas do really belong to the protoplasmic variety, because of their location and structure; typical fibrous astrocytes were not detected. In addition, it should be stressed that the existence of the described morphological subpopulations of astrocytes in the cerebellar cortex (Chan-Palay and Palay, 1972; Palay and Chan-Palay, 1974) was disregarded. The Bergmann cells, i.e., the special regional astrocytes situated in the Purkinje cell layer, were not considered either.

Because this study was essentially based on morphometric results and because, in general, our observations are in accordance with the descriptions concerning the cerebellum (Mugnaini, 1972; Palay and Chan-Palay, 1974) or even with some other nervous structures; e.g., the corpus callosum (Mori and Leblond, 1969), only a short account on qualitative ultrastructural aspects was carried out. Notwithstanding, two distinct apparenty abnormal features observed in rats aged over 18 M, particularly at 24 M, deserve special attention: two types of structures which we call, on account of their general aspect, «hyaline globules» and «granular deposits». As happens with the centrioles and the MVB, their scarcity and

Graph 6. Age changes in the absolute volume of the glial filaments of astrocytes.



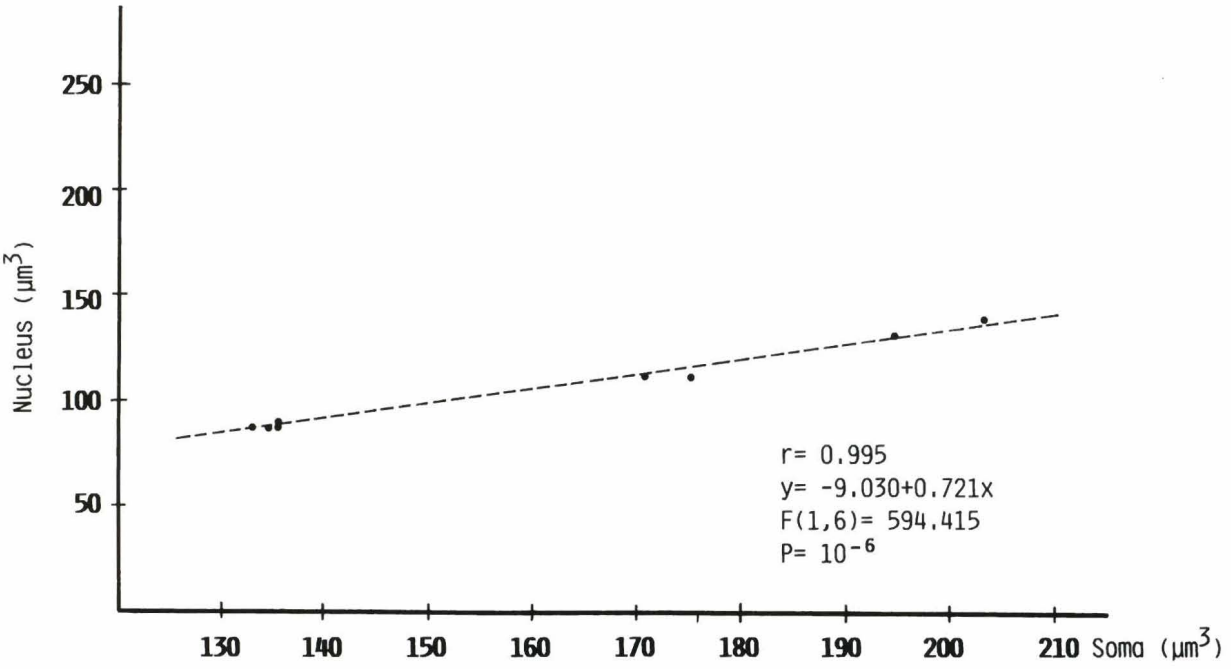
Graph 7. Age changes in the absolute volume of the ground substance of astrocytes.



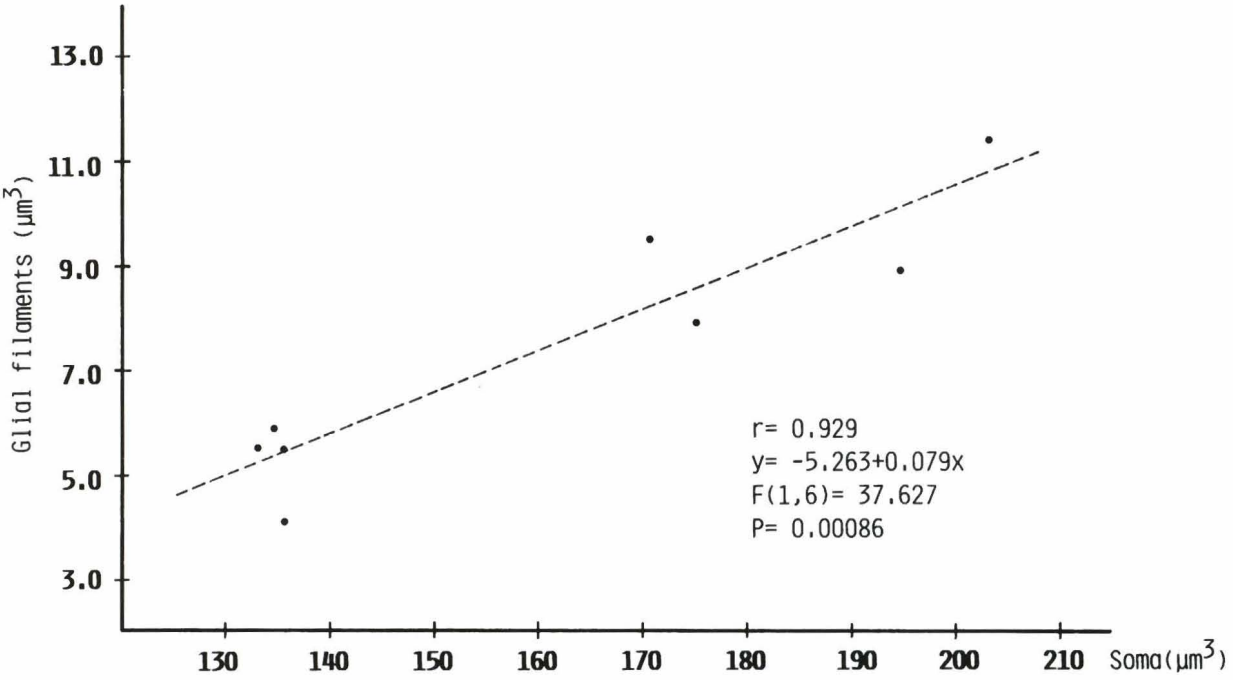
Graph 8. Age changes in the mean surface of RER of astrocytes.

erratic occurrence exempted us from a separate quantitative analysis; instead, for practical purposes, they were included in the «ground substance». The «hyaline globules» resembled the lipid droplets seen in the lipofuscin granules of the monkey pyramidal cells of the frontal cortex (Mervis, 1981) as well as the lipid vacuoles described in rat astrocytes by Ferrer and Sarmiento (1981). Here, in contrast, they were present not in aged animals but in developing ones. The «granular deposits» (which must not be confused with cross sections of filament bundles in micrographs of low magnification) to some extent seem the corpora amylacea-like structures observed throughout the CNS: in humans (Rees, 1976), in monkeys (Mervis et al., 1979; Mervis, 1981) and in dogs (Mervis, 1981). If these are truly age-related changes, both odd occurrences may signify age-impaired metabolic pathways, leading to accumulations the biochemical and/or the «physical» toxicity of which awaits to be settled. In our opinion, their scarcity in the rat indicates that they do not greatly attempt, at least physically, upon the vital space of the cell.

Taking into account the more conventional components of the cytoplasm, only the accumulation of dense bodies (including lipofuscin) seems to stand out with ageing. This congestion has been seen by a number of researchers (Sturrock, 1977; Wisniewsky and Wen, 1988); others (Hasan and Glees, 1973a) did not mention any obvious ultrastructural modification with ageing occurring in hippocampal astrocytes. Nevertheless, as far as our results are concerned, it should be stressed that zones of lipopigment congestion were much more common in the astrocytic processes than in the somata. Perhaps a special mechanism is involved in the preservation of the vital space of the soma (the cell trophic centre), taking full advantage of the stout dimensions of the processes which, for that reason, allow the uptake of large quantities of dense bodies. Despite the apparent sharp graphical inflexions, this eventual phenomenon may explain why, with ageing, the absolute volume of dense bodies does not significantly change at the level of the somata. In addition, the possibility that astrocytic processes



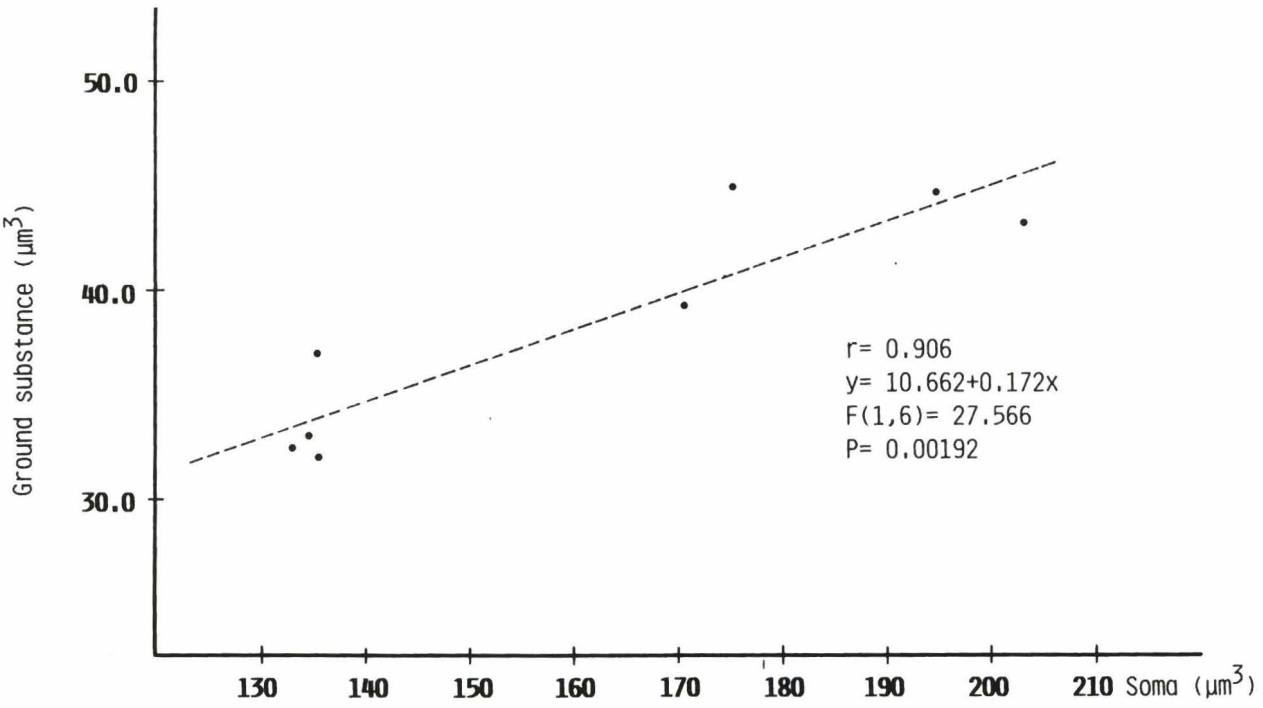
Graph 9. Simple linear regression analysis with ageing between the somatic volume and the absolute volume of the nucleus of astrocytes.



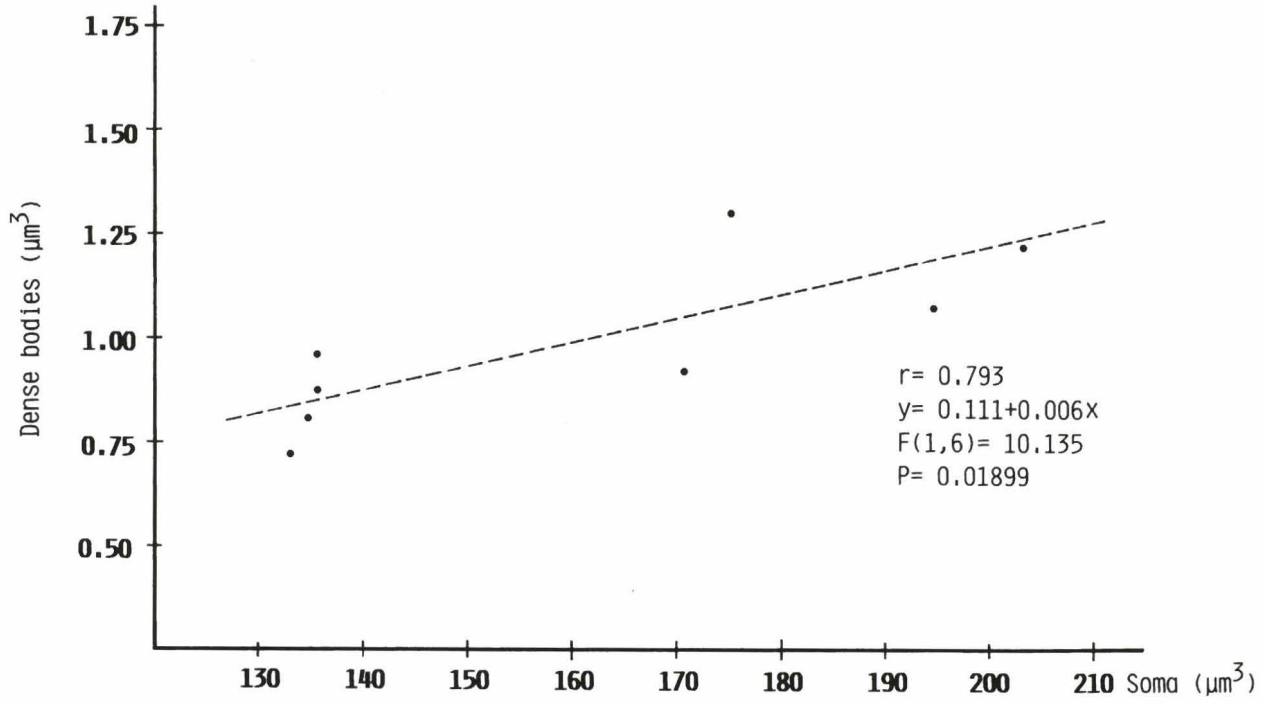
Graph 10. Simple linear regression analysis with ageing between the somatic volume and the absolute volume of the glial filaments of astrocytes.

transport dense bodies towards endothelia cannot yet be ruled out (Spoerri and Glees, 1974, 1975; El-Ghazzavi and Malaty, 1975; Lamar et al., 1980; Casey and Feldman, 1985; Totaro et al., 1985; Glees et al., 1986; Monteiro, 1991b).

These mechanisms altogether may explain why the absolute volume of dense bodies in the somata was not linearly correlated with age. On the contrary, in fixed postmitotic cells this relationship was well patent, as seen in nerve cells (Samorajski et al., 1968; Mann and Yates, 1974; Hinds and McNelly, 1979; Vaughan and Vincent, 1979; Monteiro, 1991a).



Graph 11. Simple linear regression analysis with ageing between the somatic volume and the absolute volume of the ground substance of astrocytes.



Graph 12. Simple linear regression analysis with ageing between the somatic volume and the absolute volume of the dense bodies of astrocytes.

A moot question is whether or not astrocytes play a role as recipients for the neuronal dense bodies. A number of researchers uphold this hypothesis (Brizzee, 1974; Knox et al., 1980; Davies et al., 1983; Monteiro, 1991b). Whatever the degree of significance and credibility this hypothesis may have, our observations give the impression that those glial cells do not possess any special power to become specific satellites to a particular kind of neuron. Because of the great deal of chance for fortuitous contacts, this aspect among the prolific accumulation of the common granule cells (Fig. 1) may not be detected; the lack of specific relationship

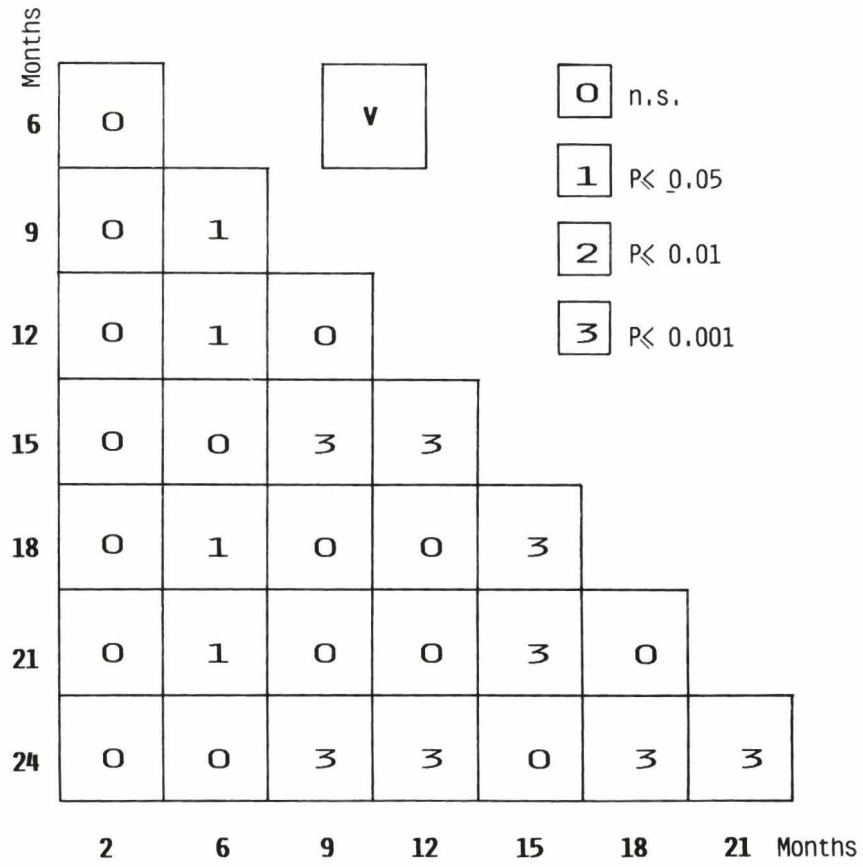


Table 1. Significance level of pair comparisons concerning the evolution with ageing of the somatic volume of astrocytes.

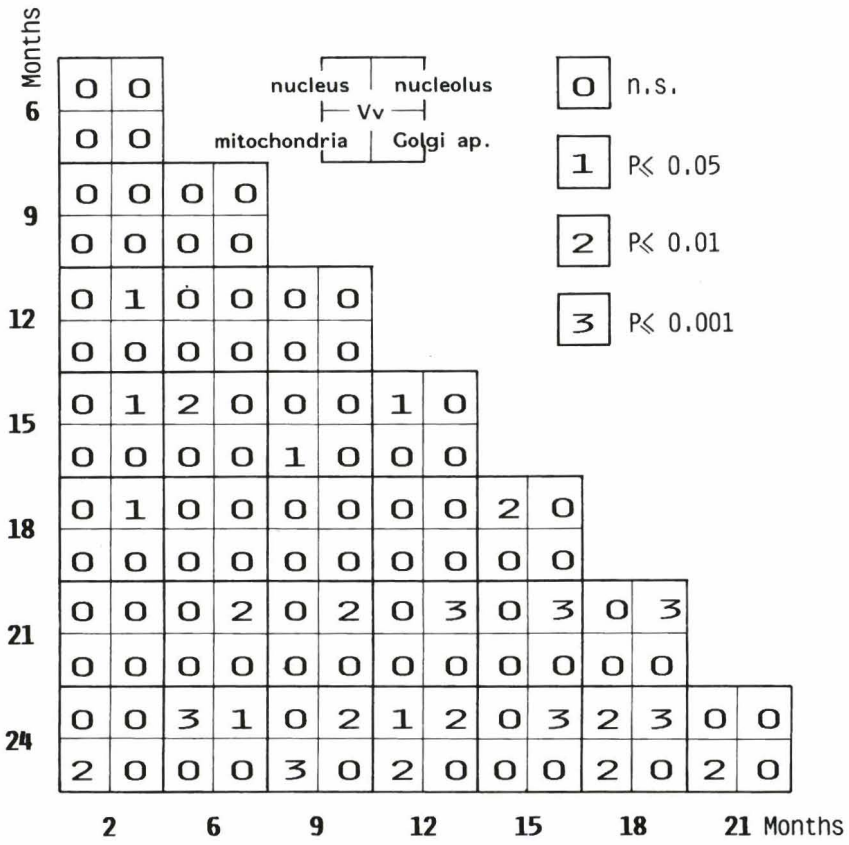
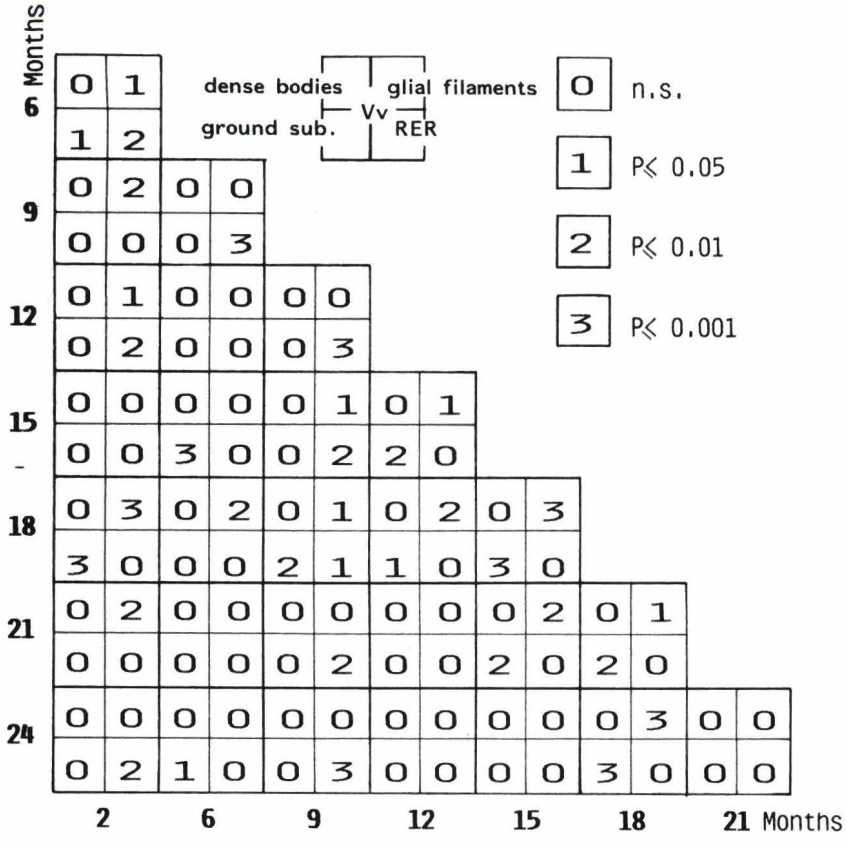


Table 2. Significance levels of pair comparisons concerning the evolution with ageing of the volume densities of the nucleus, of the nucleolus, of the mitochondria and of the Golgi apparatus of astrocytes.

is, however, well patent in connection with the neurons of intermediate size; i.e., the Golgi cells and the Lugaro cells. On the contrary, the Bergmann cells are special satellites for Purkinje cells. This same relationship for a type of oligodendrocyte has also been suggested (Monteiro, 1983).

Dense bodies had their absolute volume significantly correlated with that of mitochondria; this may eventually support the mitochondrial origin of lipofuscin, a hypothesis sustained by many authors (Glees and Gopinath, 1973; Hasan and Glees, 1973b; Gopinath and Glees, 1974; Vanneste and van den Bosch de Aguilar, 1981; Glees et al., 1986; Monteiro, 1991b). Indeed, the graphical variations of the absolute volume of both organelles are very alike (Graphs no. 3 and no. 5). Another school of thought, however, assumes that lipofuscin derives from lysosomes (Samorajski et al., 1965; Sekhon et al., 1969; Sekhon and Maxwell, 1974; Nosal, 1979; Artiukhina et al., 1981; Lippman et al., 1981). If the «physical toxicity» of dense bodies is a genuine concern (Monteiro, 1991b), the positive correlation affecting the absolute volumes of the binary dense bodies/ground substance may perhaps reveal a mechanism of increasing the space of the latter which prevents, to a certain extent, that incovenience. Finally, the somatic volume and the absolute volume of the dense bodies are also correlated (Graph no. 12). As the absolute volume of dense bodies, however did not significantly alter with ageing, it does not seem seriously engaged in causing significant variations on the somatic volume; in fact, its volumetric density was rather low in all studied ages. Nonetheless, as the relative volume of dense bodies did not modify either and, bearing in mind the expressive value of the correlation, it is suggested, as above, that a mechanism of reciprocal self-regulation in the binary dense body/cell volume could credibly exist. Indeed, as rightly emphasized by



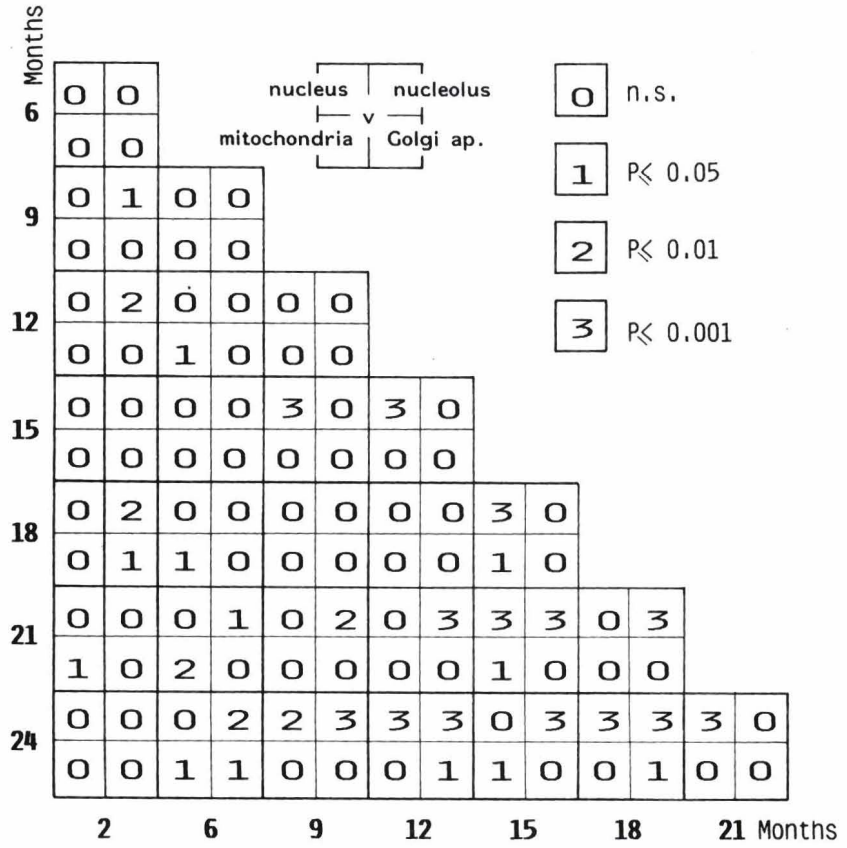
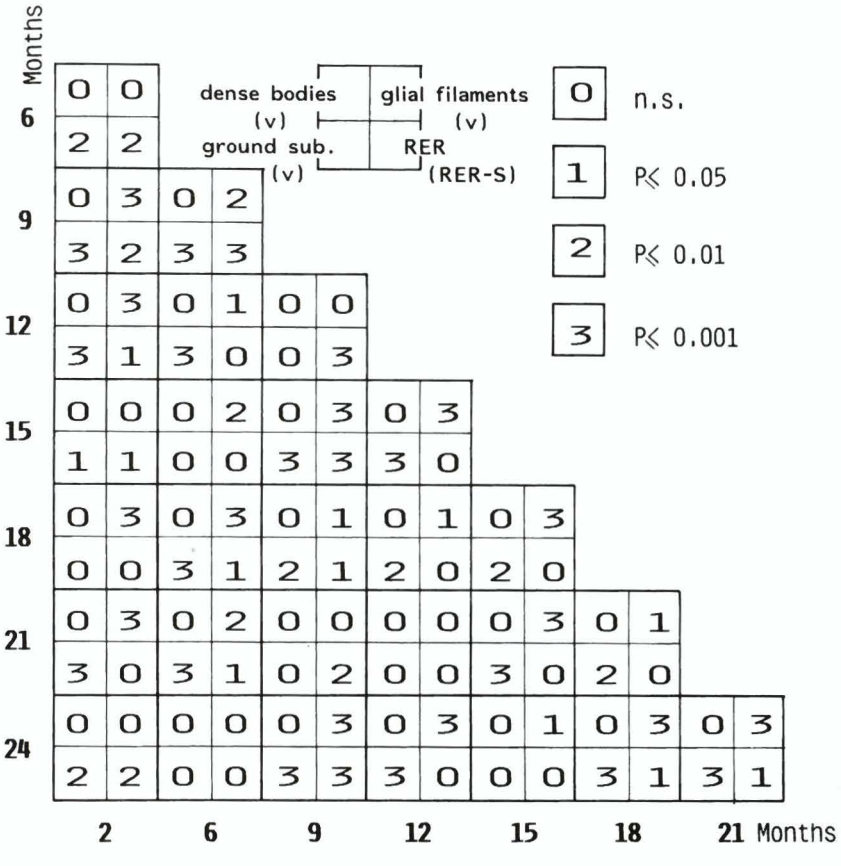


Table 3. Significance levels of pair comparisons concerning the evolution with ageing of the volume densities of the dense bodies, of the glial filaments, of the ground substance and of the RER of astrocytes.

Maslinska et al. (1984) and Ikeda et al. (1985), lipofuscin must not be simply regarded as a pigment related to ageing process. Furthermore, Collins and Thaw (1983) vindicate that the lipopigment is not lethal to gliacytes.

With ageing, the volumetric analysis of the soma revealed that there were significant changes. In an overall view, between the lowest values (at 9, 12, 18 and 21 M) and the highest ones (at 15 and 24 M) an increase of about 48% (as an average) is perceived. Furthermore, the actual graphical sequence of the somatic volume indicates periods of cell hypertrophy; if the studied age spectrum had been larger, perhaps the pattern would be recurrent. We are tempted to speculate that the phenomenon has some connection with the periods of death of the irreplaceable neurons, in an attemp to help, by scarring, the compensatory hypertrophy of the remaining ones. This neuron hypertrophy with ageing has been reported several times (Hinds and McNelly, 1977; Frolkis and Bezrukov, 1979). Indeed, the neuronal fall-out has been defended for decades as an inexorable ageing reality observed either in the cerebellar cortex (Ellis, 1920; Hall et al., 1975; Nandy, 1981) or elsewhere (Maleci, 1934; Hinds and McNelly, 1977; Sabel and Stein, 1981). An interesting finding completes this supposition: between the somatic volume and the absolute volume of glial filaments a significant positive linear correlation with age is found (Graph no. 10); this relationship had been previously suggested by O'Callaghan and Miller (1991). In fact, papers enforcing the importance of gliofibrillogenesis upon the mechanisms of healing by hypertrophy have been published (Eng and DeArmond, 1981; Trimmer and Wunderlich, 1990). If there is a truly a mutual relationship between healing activities and hypertrophic phenomena, then at 15 M a special critical period is perhaps attained. At this age significant peaks in the Graphs of absolute volume

Table 4. Significance levels of pair comparisons concerning the evolution with ageing of the absolute volume of the nucleus, of the nucleolus, of the mitochondria and of the Golgi apparatus of astrocytes..



relative to soma, glial filaments and ground substance are exhibited.

Between the somatic volume and the absolute volume of the nucleus a positive linear correlation is also displayed (Graph no. 9). Taking into account the high volumetric density of the nucleus and the high level of the correlation found (see also Graph no. 1, for realizing the match between the graphical desingns), the organelle seems to be the chief modulator of the volumetric plasticity of astrocytes with ageing. In Purkinje cells, for instance, this activity depends mainly on the ground substance fluctuations (Monteiro, 1991a). In astrocytes, however, the absolute volumes of the ground substance and of the nucleus are positively and linearly correlated. The ground substance would then have some influence in what accounts for the significant changes in somatic volume seen with ageing; so much between the absolute volume of the ground substance and the cell volume a positive and linear significant correlation is observed.

The glial fibrillary acidic protein (GFAP) is the major protein constituent of glial filaments in differentiated astrocytes (Eng and DeArmond, 1982; Eng, 1985) and is very important for preserving astrocyte shape either under normal conditions or during reactions to injuries (Eng and Shiurba, 1988). In fact, during the healing process of traumatic injuries (Bignami and Dahl, 1976; Dahl et al., 1981; Hozumi et al., 1990) or inflammatory diseases (Aquino et al., 1988) a biochemical increase in GFAP content is detected.

Table 5. Significance levels of pair comparisons concerning the evolution with ageing of the absolute volumes of the dense bodies, of the glial filaments and of the ground substance as well as of the mean surface of the RER of astrocytes.

Alterations in its production can be deduced when it is realized that the absolute volume of glial filaments significantly changes with ageing, revealing different levels of astrocytic plasticity. A significant linear trend, however, is not found, which contrasts with the biochemical findings of Goss et al. (1991) in astrocytes of mouse brain.

Because astrocytes can undergo karyokinesis, they may apparently resist senility better than neurons can do on account of the biological waste dilution that they can perform. As stated above, in brain injuries astrocytic proliferation is an expected reaction. Under normal conditions, however, the labelling index derived from autoradiographs seems to reveal a slow turnover; e.g., 0.08% in the cortical grey matter (Kaplan and Hinds, 1980). In fact, no unequivocal mitotic figure occurring in astroglia out of the 800 grids scanned in our study was found. In contrast, a recent publication (Schipper and Wang, 1990) stresses the considerable proliferative ability of glial cells (about 34%) either in young rats or in very old ones. Whatever is the degree of proliferative power, however, it must be remembered that even in dividing cells, it is assumed that the waste accumulation, apportioned between the daughter cells in successive divisions, may attain a critical level which impairs further mitoses (Hirsch, 1978). Blomquist et al. (1980) are suggested that the mitotic potential of glial cells decreased according to the number of completed cell cycles. Nevertheless, under normal conditions and whatever the level of the mitotic index, the general view that upholds the astroglia as a population capable of undergoing proliferation throughout the lifespan of animals (Korr, 1980) almost seems to be as a neurobiological law.

Thus, as a summary, we should like to underscore that cerebellar cortex astrocytes exhibit, indeed, quantitative age-related changes. There are some obvious objections, however, in extrapolating data from cerebellar astrocytes to other astrocytes elsewhere to determine universal aspects throughout the CNS. Regional differentiation of rodent astrocytes, for instance, concerning either enzymes (Horiike et al., 1987) or structural proteins, such as GFAP and vimentin (Diefenbach et al., 1991) has been proved. In addition, the regional heterogeneity in relation to morphology and surface properties (Hansson, 1990) is a well stated fact; even inside the same nervous structure regional diversities are expected, as it happens in the

cortex under study (Lange, 1974a b, 1976; Heinsen, 1981). Furthermore, some authors quoted in this paper have worked on cultured cells rather than on cells directly removed from fixed tissue in situ, not to mention the different species and strains used. As an overall view, however, it is tempting to believe that these modifications may plausibly exert influences upon the already known functions reported in the literature by so many authors: support and insulation (Palay and Chan-Palay, 1974; Miller et al., 1989); uptake of excess of K^+ from the extracellular space (Walz, 1987; Wuttke, 1990); neurotransmitter metabolism (Battaglioli and Martin, 1991; Bull and Blomqvist, 1991); production of growth factors (Frei et al., 1986; Gadient et al., 1990); production of scars in the CNS (Trimmer, 1985; Reier, 1986); response of astrocytes as targets for hormones (Juurlink et al., 1981; Krisch et al., 1991) and for neurotoxins (Hansson and Ronnback, 1989); and production of prostaglandins (Jaiswal et al., 1991; Tallant et al., 1991) and of angiotensinogen (Intebi et al., 1990).

As a concluding remark, it must be stressed that, despite the use of quantitative tools, and the security displayed by the statistical levels of probability, we are dealing with biological matter and not with pure mathematical models. So, to find logical linear explanations for every morphometric result obtained from the study of astrocytes, or any other sort of cell, without colliding with discrepancies and ambiquities is simply impossible. Moreover, we are well aware that some biochemical pathways still remain to be elucidated; so a complete comprehensive analysis about the role of astrocytes in the nervous homeostasis cannot as yet be achieved.

It is concluded that, despite the mitotic capabilities, astrocytes do undergo changes with ageing; so, apparently, they are not immune to senility.

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