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MORPHOLOGICAL CHANGES IN ASTROCYTES OF AGING MICE FED NORMAL OR CALORIC RESTRICTED DIETS

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ABSTRACT

Astrocyte hypertrophy, associated with neuronal degeneration, has been reported in aging rat hippocampus. Dietary restriction throughout life (restriction of calories without decrease in essential nutrients) increases average and maximal lifespans in rodents. In the present study, the effect of caloric restriction on age-related changes in the maximal diameters of mouse astrocytes was measured. Specific pathogen free C57BL6 mice maintained on either *ad libitum* (NIH 31 rodent diet) or restricted (60% *ad libitum* with vitamin supplementation) diets, ages 0.6 (weaning), 6, 19, and 24 months were obtained from the NCTR aging rodent facility in Jefferson, AK. Their brains were stained by a modified Golgi method and astrocyte diameters were determined with a Zeiss Videoplan image analyzer. Astrocytes in the hippocampus and dentate gyrus of *ad libitum*-fed mice were of similar diameter from 6 to 24 months of age, and were larger than those of weanlings. However, astrocytes were significantly smaller in the hippocampus and dentate gyrus of caloric restricted mice at 19 and 24 months, suggesting the prevention or reversal of age-related astroglial enlargement. Astroglia were also significantly smaller in the frontal and parietal cortices of caloric-restricted mice at six months of age than those of *ad libitum*-fed mice. At older ages, however, no significant differences were seen between the two groups. This finding may indicate a delay in neocortical astrocyte development by caloric restriction.

INTRODUCTION

Caloric restriction throughout life without decrease in essential nutrients increases average and maximal lifespans in rodents (1). In addition, food restriction in specific pathogen-free rats has been shown to delay age-related changes in tissue structure and function, such as the development of renal lesions and loss of muscle mass. These findings suggest that food restriction prolongs life by decreasing the rate of aging (2).

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Several studies of the aging brain have shown changes in neuroglia. In the hippocampus of rats, astrocytes in aged animals (24-25 months old) have been shown to be enlarged compared to those of young animals (2-3 months old). However, this change was not found in other forebrain regions (3). A study of the dentate gyrus noted increased volume and number of astrocytic processes in 25-month-old rats compared to 3-month-old rats (4). Both of these changes were postulated to be associated with neuronal degeneration or de-afferentation.

In the present study we examined the effects of caloric restriction on age-related changes in the morphology of Golgi-stained astrocytes. We compared mice fed *ad libitum* with mice given the same quantity of nutrients and vitamins but restricted in calories to 60% of the *ad libitum* control group diet.

RESULTS

Effect of Diet on Body Weight

Figure 1 shows the body weights of mice given *ad libitum* vs. caloric-restricted diets. The latter diet significantly reduced body weight.

Morphometry of Astrocytes

Figure 2 and 3 illustrate typical protoplasmic astrocytes as they appear in the Golgi-stained material studied in these experiments. These cells possess extensively branching processes which end in numerous delicate curling lamellar structures. The branching of these processes is often so intense as to make clear visualization of the cell body difficult.

Hippocampus–Dentate Gyrus

Sizes of protoplasmic astrocytes in the hippocampus and dentate gyrus in all age and treatment groups are summarized in Table 1. The mean diameters of astrocytes showed a significant ($p<0.01$) increase from weaning to 6 months of age. No difference was found between *ad libitum* and caloric restricted groups at six months. However, at 19 months, the sizes of astrocytes in the two groups were seen to diverge, and this change persisted through 24 months of age. Cells in the *ad*

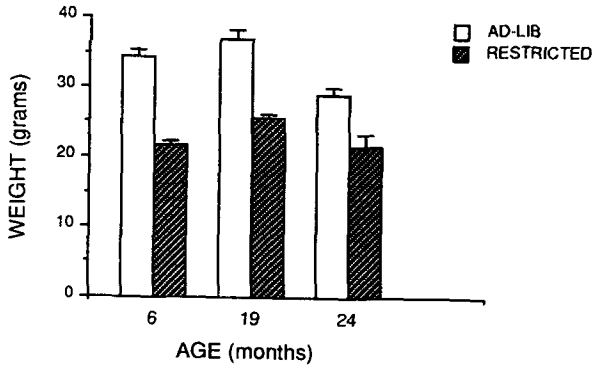

Figure 1. Body weights of *ad libitum*-fed (open bars) and caloric-restricted (striped bars) mice. Caloric restriction significantly reduced body weights at all post-weanling ages studied. For each datum, n=5. Error bars represent sample standard deviations.

Neocortex

The effects of diet on neocortical astrocyte diameter differed from those seen in the hippocampus-dentate gyrus, as shown in Table 2. In the cortex, astrocytes from weanling mice had a mean diameter of 49.5 μm. This value was increased in all age groups of mice fed *ad libitum*. In the caloric restricted mice, there was a significantly ($p<0.01$) decreased astrocyte diameter at 6 months, which was not seen at 19 months or 24 months. At these older ages, astrocyte diameters were similar to those in the *ad libitum* group.

DISCUSSION

The major finding of this study is that cortical and hippocampal-dentate astrocytes of mice fed a nutritionally complete but reduced calorie diet

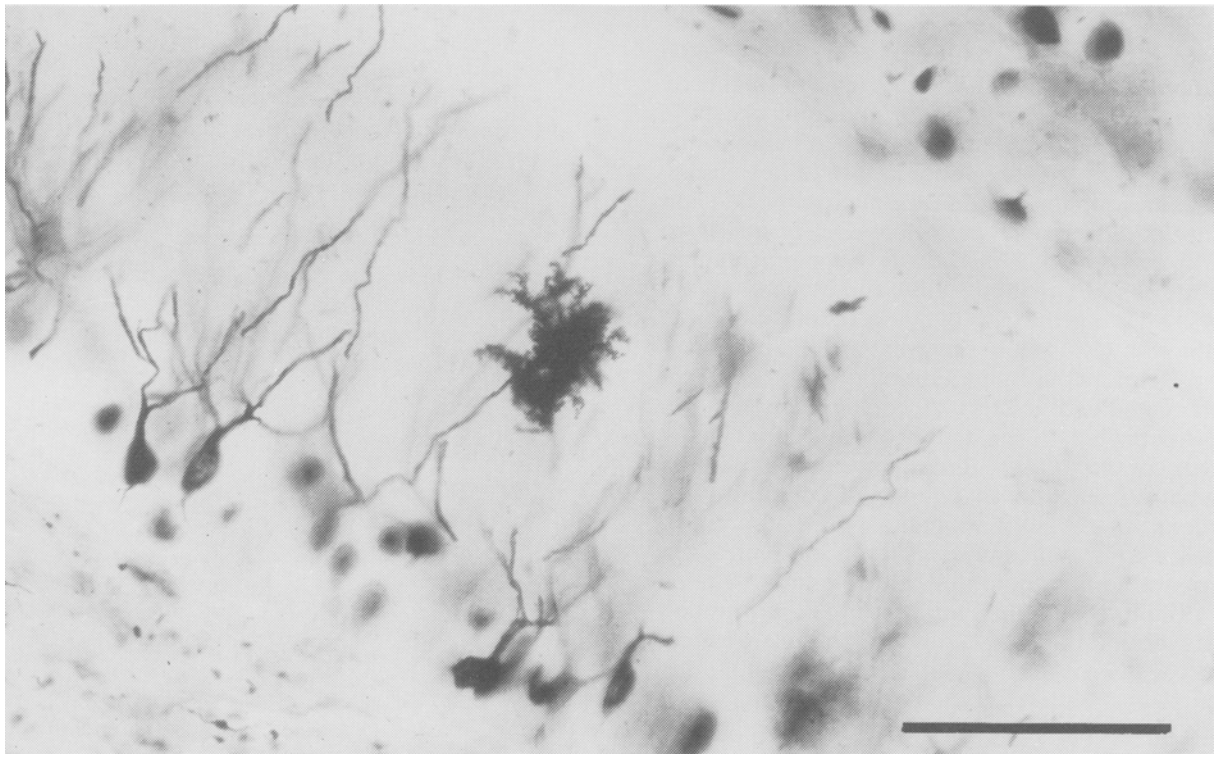

Figure 2. Thick section of hippocampus-dentate astrocyte in one plane of focus, control animal. The necessity of taking a thick section in order to measure the entire astrocyte in three dimensions precludes a photograph that is "in focus" in all planes of the cell. 25X. Bar = 100 microns.

libitum group remained enlarged compared to the weanling size. At 19 months, astroglia in the caloric-restricted group were significantly smaller than those of the *ad libitum* group, according to both types of statistical analysis (i.e., where each animal constituted one observation, as well as where each cell constituted one observation). Cells remained smaller at 24 months in the restricted group, according to the analysis wherein each cell is one sample.

developed differently from astrocytes of mice fed the same diet *ad libitum*.

Previous studies with diverse morphological techniques have shown that astrocytes in the hippocampus and dentate gyrus are larger in aged vs. very young male Fischer rats fed *ad-libitum* diets. Landfield *et al.* (1977), using Cajal's gold chloride stain modified for astrocytes, showed that hippocampal astrocytes in the aged rats (24-25 months old) were qualitatively larger

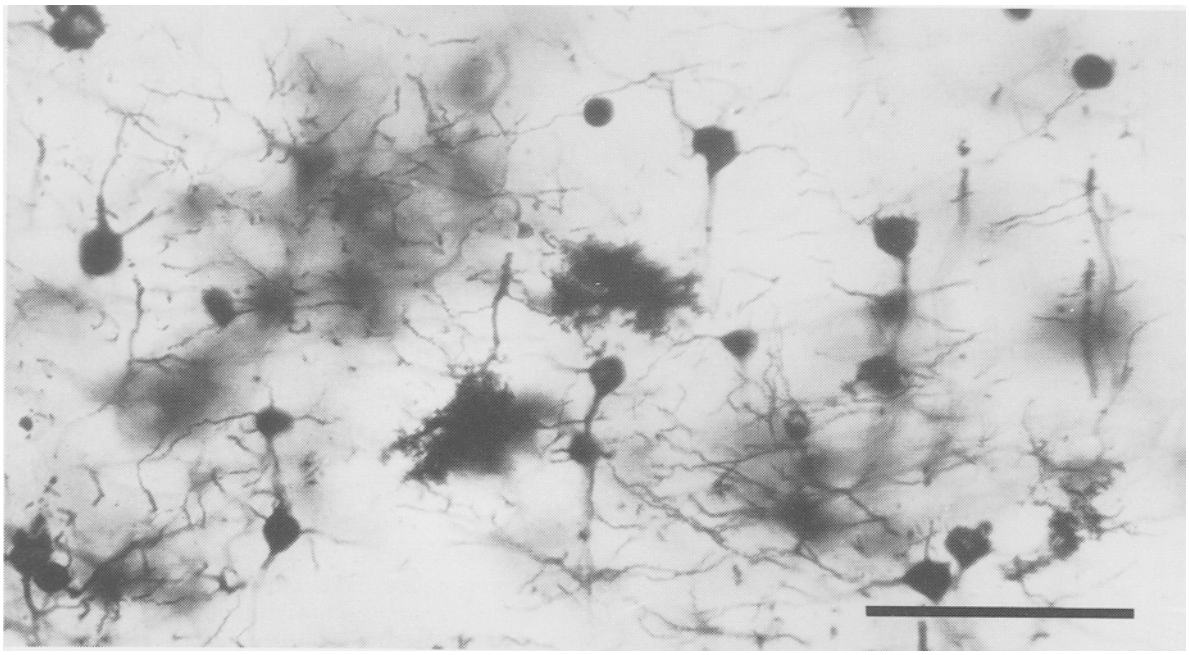

Figure 3. Thick section of a frontoparietal astrocyte in one plane of focus, caloric-restricted animal. 25X. Bar = 100 microns.

Table 1. Mean diameter of fibrous astrocytes in *hippocampus/dentate gyrus*

	x ± SD (n) *(significance, test value, df)*	
6 mo.		
ad libitum	50.10 ± 2.23 (3)	49.80 191 9.10 (152)
	(not sig., 0.6912, 3)	(not sig., 0.192, 50)
19 mo.		
ad libitum	51.30 ± 2.55 (3)	51.30 ± 10.1 (95)
cal restricted	45.11 ± 1.63 (5)	4a5.80 191 9.00 (134)
24 mo.		
ad libitum	50.75 ± 1.58 (3)	50.80 ± 9.60 (168)
cal restricted	47.56 ± 2.94 (4)	47.80 ± 8.70 (195)
	(not sig., 1.677, 5)	(sig. at 99%, 3.20, 336)*

* – significant difference in variance at 90% level

Table 2. Mean diameter of protoplasmic astrocytes in frontal and parietal cortex

	x ± SD (n) (significance, test value, df)	
Age Group	A. 1 observation = 1 animal	B. 1 observation = 1 cell
6 mo.		
ad libitum	52.19 ± 0.83 (2)	52.19 ± 8.47 (320)
cal restricted	45.81 ± 1.37 (4)	45.20 ± 8.05 (320)
	(p<0.01, 5.861, 4)	(sig. at 99%, 10.9, 638)
19 mo.		
ad libitum	52.60 ± 1.46 (3)	51.78 ± 8.68 (320)
cal restricted	51.64 ± 1.29 (4)	52.98 ± 8.94 (320)
	(not sig., 0.942, 5)	(not sig., 1.90, 638)
24 mo.		
ad libitum	51.47 ± 1.23 (2)	51.48 ± 9.12 (321)
cal restricted (51.98 ± 0.51 (2)	51.97 ± 7.95 (320)	
	(not sig., 0.547, 2)	(not sig., 0.746, 639)

than those in rats 2-3 months old. In some cases, hypertrophied astrocytes tended to be clustered, possibly near areas of neuronal degeneration. Geinisman *et al.* (1978) provided further evidence by quantitative electron microscopic analysis for a significant increase in the number or volume of astroglial processes in the dentate gyrus of rats between the ages of 3 and 25 months.

The present study is in agreement with the foregoing studies in that a large increase in hippocampal-dentate astrocyte diameters was noted between weanling and aged animals. However, our study also examined astroglia at intermediate ages, and demonstrated that the age-related increase in astroglial size was well-established by six months of age, with little subsequent hypertrophy. This developmental pattern is in good agreement with that reported by Bjorklund *et al.* (1985) for male rats, who measured cell area and perimeter of glial fibrillary acidic protein-positive cell in smears of fresh brain. When the developmental pattern we obtained was compared with that of caloric-restricted mice, it could be seen that astrocytes were significantly smaller in the restricted group than in the *ad libitum* group at 19 and 24 months of age. Thus caloric restriction ameliorated or partially reversed a characteristic pattern of astroglial maturation or aging.

Although changes induced by dietary restriction in

hippocampal-dentate astrocytes persisted into old age in the present study, those found in cortical astrocytes did not. Cortical astrocytes of *ad libitum*-fed mice showed an increase in maximum diameter between 0.6 and 6 months, and no change thereafter. A similar increase in the area and perimeter of cortical astrocytes between 6 weeks and 18 months of age has been reported previously for female rats (8). When the developmental pattern we obtained for cortical astrocytes was compared between the two dietary groups, a large reduction in astrocyte size was noted in animals on the restricted diet only at 6 months of age.

Several explanations may be given for this transient decrease. The reduced caloric diet may have favored the formation of larger numbers of astrocytes with smaller processes. Alternatively, dietary and growth factors may have encouraged additional neuronal development and elaboration of more extensive dendrites in this group compared to the *ad libitum* group, thereby leaving less space available in the tissue for expansion of astrocyte processes. Finally, these variations in astrocyte size may reflect deficient nutritional states at this point in the life cycle. Mice in this study were fed the same dietary formula from weaning until death. It has been long recognized that nutritional needs during adult life may differ from those needs during other life phases, such as rapid growth periods (5). Currently there are few data to define the "normal" age-related changes in astrocytes, either morphological or biochemical. Therefore, there is no benchmark for determining whether the alterations induced by a caloric restricted diet are deleterious or beneficial.

Previous authors have suggested that caloric restriction simply delays the aging process (5). The present results would indicate otherwise, because the age-related changes in astrocytes in both neocortical and hippocampal-dentate regions of caloric restricted mice are fundamentally different from those of *ad libitum*-fed mice, rather than simply the same curve shifted in time.

EXPERIMENTAL PROCEDURES

Animals and Diet

Male C57BL/6 mice used in this study were reared in the Specific Pathogen Free Barrier Facility operated in Jefferson, Arkansas, by the National Center for Toxicological Research (NCTR). Animals were held at 23°C on a 12/12 hr light/dark cycle with lights on from 0600 hr daily. Nursing pups maintained with dams fed *ad libitum* (AL) on the NIH-31 rodent diet continued to be fed AL to 14 weeks of age, at which time they were divided into two groups. One group of animals was fed NIH-31 AL and the other a diet identical to NIH-31 AL in vitamins and minerals but restricted to 60% of the total calories (CR). Both groups received AL water. Animals were shipped by air to College Station, TX, and were killed the following morning. Animals were studied at 6, 19, and 24 months of age, as well as at weaning. Brains from 35 animals (five animals in each age and treatment group) were used in this study. The number of brain hemispheres from which usable brain sections were obtained varied from two to four per age and treatment group.

Morphologic analysis of Astrocytes

All mice were killed by cervical dislocation and decapitated, and the brains immediately removed and immersed in 10% formalin. After at least two weeks fixation in formalin, the brains were blocked along the mid-sagittal plane. The left half of each brain (except weanlings, where both halves were used) was stained by a Rapid Golgi method optimized to demonstrate astrocytes (4). Stained tissue was then dehydrated in an alcohol series and encased in paraffin. All brains were processed simultaneously to minimize variation in histological technique. The brains were then cut into 200 micron-thick parasagittal sections, dewaxed in xylene and mounted on glass slides with Permount. Slides were coded in order to not bias data acquisition. All measurements were obtained utilizing a set procedure by a single investigator to minimize variables as much as possible. Stained astrocytes in the hippocampus-dentate gyrus complex and the fronto-parietal neocortex were measured with a drawing tube attached to a Zeiss microscope and Zeiss videoplan digitizing table. The maximum diameter of the area occupied by the processes of each atrocyte was measured. Astrocytes were chosen only if the perimeter was clearly visualized and free of overlapping cells; every complete stained astrocyte located in the cortices studied was measured in order not to bias data selection.

Statistical Analysis

Data from the Golgi study were analyzed in two ways. First, all measurements from each brain region of a single animal were averaged to constitute one observation. Seven to 92 cells were measured per animal in the hippocampus-dentate and 24 to 161 cells in the neocortex. The means of the two dietary groups were compared by a two-sided t-test for each age (6, 19, 24 months). In the case of weanlings, only one brain provided suitably stained cells for analysis. In this brain the left and right hemispheres were compared. Differences were considered to be significant if $P<0.025$. In the second method of analysis, each cell measured was treated as a separate sample, rather than as a sub-sample. The means of the two dietary groups were compared by a two-sided t-test within each age group examined. The first method of analysis gives a conservative estimate of treatment effects, whereas the second greatly increases the sample size so that smaller differences between groups can be detected (Castiglioni *et al.*, 1990). In order to compare astroglial diameters between various ages, an Analysis of Variance was performed for each dietary group, where one cell constituted one observation.

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