

Age-related Decrease of the Perineuronal Satellite Cell Number in the Rabbit Spinal Ganglia

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Abstract: This study was undertaken to establish whether a change in the perineuronal satellite cell number contributes to the age-related reduction of the volume ratio between the perineuronal glial sheaths and their associated nerve cell bodies, observed to occur in rabbit spinal ganglia. The volumes of the nerve cell bodies and the numbers of the related satellite cell nuclei were estimated on serial semithin sections from young adult and old rabbits. As satellite cells are mononucleate, the number of the nuclei corresponds to that of these cells. The satellite cell sheaths in both age groups were also examined under the electron microscope. The mean number of satellite cells was significantly smaller in the aged animals than in the young adults although the mean volume of the nerve cell bodies was significantly larger in the former. Cytoplasmic vacuoles, invaginations of the connective tissue and autophagic vacuoles were more frequent in the old rabbits. Satellite cells with pyknotic nuclei and remnants of degenerated satellite cells were only found in aged animals, although rather rarely. The decrease in the satellite cell number is one of the mechanisms by which the age-related reduction of the volume ratio between the perineuronal glial sheaths and their associated nerve cell bodies takes place. The decrease in the satellite cell number seems to occur, at least in part, through cell degeneration. However, other mechanisms (e.g., detachment of satellite cells from the perineuronal sheaths) cannot be excluded. Since satellite cells play a role in neuronal support, the significant decrease in their number probably has negative consequences for neuronal activity.

Keywords: Peripheral neuroglia, Sensory neurones, Aging, Dorsal root ganglia, *Oryctolagus cuniculus*.

INTRODUCTION

In an earlier work we showed that the volume ratio between the satellite cell sheaths and the related nerve cell bodies was significantly smaller in the spinal ganglia of aged rabbits than in those of young adults [1]. In the present research we have sought to establish whether a change in the perineuronal satellite cell number contributes to the above age-related reduction. To this end, we have compared the number of the perineuronal satellite cells in the spinal ganglia of aged rabbits with those of young adults.

MATERIALS AND METHODS

Five rabbits (*Oryctolagus cuniculus*), whose dates of birth were well documented, were used: two animals (one male and one female, 3.4-3.5 kg body weight) were 12 months old, three animals (one male and two females, 4-4.2 kg body weight) were 60-79 months old. Since the life span of the normal healthy *Oryctolagus* is 60-72 months [2] or 84-96 months [3], the 12-month-old rabbits we studied were young adults and the 60-79-month-old animals were aged rabbits. Furthermore, the end of fertility is usually considered to mark the onset of senescence and female rabbits are not normally fertile after 60 months, so that the 60-79-month-old animals are to be considered aged also from this point of view.

The animals were perfused transcardially with a solution containing 2% formaldehyde and 2% glutaral-

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dehyde in 0.1 M sodium cacodylate buffer (pH 7.3) under deep anaesthesia with Nembutal (80 mg kg⁻¹). After fixation for about 3h, the thoracic spinal ganglia were removed, washed in cacodylate buffer (0.2 M, pH 7.3) for 2h and then postfixed at 0°C for 1.5h in 2% OsO₄, buffered with 0.1 M sodium cacodylate. The specimens were washed in distilled water, stained with 2% aqueous uranyl acetate, dehydrated in alcohol and embedded in Epon-Araldite resin.

Light microscopy

Since serial sectioning provides (for all practical purposes) an unbiased estimate of the volume of an object of arbitrary shape, e.g., a cell [4], we chose this procedure even though it is laborious and time-consuming. Five ganglia (one from each animal), selected according to the quality of fixation, were used. Each ganglion was trimmed to obtain a block with cutting surface of about 0.55 × 0.35 mm. Serial semithin sections 1 µm thick were cut from each block with a diamond knife; both the location and orientation of the sections were always random. The sections were stained with 0.5% toluidine blue in 1% sodium borate. The number of sections in each series varied from 110 to 135. Each section was photographed in its entirety at a magnification of 350× and the micrographs printed to a final magnification of 1000×; a montage of several prints was necessary for each section. The profile of each nerve cell body present in the first section of each series was numbered and given the same number in the subsequent serial micrographs until its full extent was delineated. The unnumbered profiles in the subsequent section were also labelled and followed as before until all profiles corresponding to each nerve cell body were identified. We found that 115 nerve cell bodies in young adult rabbits and 121 in old rabbits were entirely contained within the series of sections. The volume of each of these nerve cell bodies was determined by measuring, with the aid of a digitizing tablet connected to a computer, the surface area of each of its profiles within the series of sections, multiplying these values by the section thickness and summing the results obtained. Fur-

thermore, for each nerve cell body the number of the related satellite cell nuclei was counted. As these cells have only one nucleus [5,6], this number corresponds to the number of satellite cells enveloping that nerve cell body.

Each measurement was performed by two independent observers. The difference between the two values was consistently small, and the average of both was used for the calculations. For each age group the following were calculated: (1) the mean volume of the nerve cell bodies, (2) the mean number of the related satellite cell nuclei, and (3) the mean volume of nerve cell body corresponding to each satellite cell. The values obtained for the young adults were then compared with the values for the aged animals.

The statistical comparisons employed the 2-tailed Student's *t*-test (differences with *p* < 0.05 were considered significant). All data analyses were carried out using a statistical graphics program (Statgraphics) on a Compaq 386/s computer.

Electron microscopy

Twenty ganglia (four from each animal) were used for the electron microscope study. The thin sections, cut at random location and orientation from these ganglia, were examined under a Siemens Elmiskop 101 electron microscope.

RESULTS

Light microscopy. As shown in Table 1, the mean number of satellite cells was about 44% smaller in the aged rabbits than in the young adults, although the mean volume of the nerve cell bodies was approximately 35% larger in the aged animals. Consequently the mean volume of nerve cell body corresponding to each satellite cell was significantly greater (*p* < 0.05) in the old rabbits than in young adults.

The relations between the volumes of the nerve cell bodies and the numbers of the related satellite cells are shown in Fig. 1 for both age groups. Both in young adult and old rabbits the mean number of satellite

Table 1. Relations between the volumes of the nerve cell bodies and the numbers of the related satellite cells in young adult and old rabbits.

Age group	Total number of nerve cell bodies examined	Mean volume of the nerve cell bodies (µm ³)	Mean number (±SEM) of satellite cells related to each nerve cell body	Mean volume of nerve cell body corresponding to each satellite cell (µm ³)
Young adult	115	16796	9.77 ± 0.47	1719
Old	121	22873	5.50 ± 0.12	4159

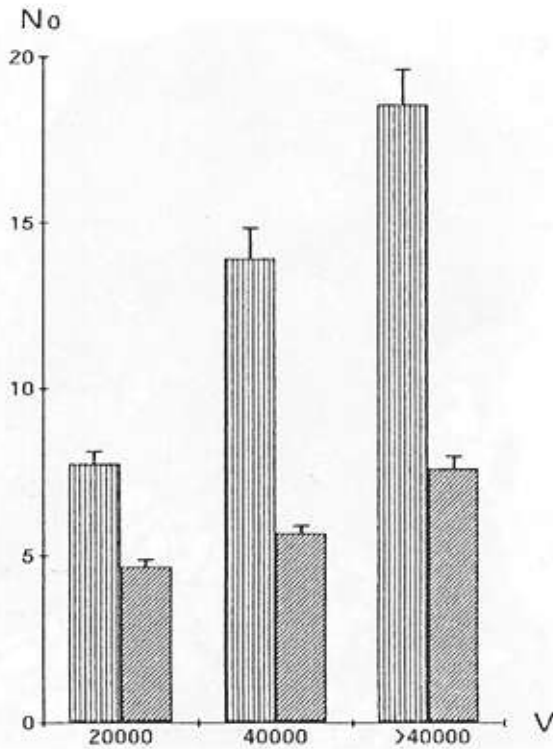


Figure 1. Histogram showing the relations between the volumes of the nerve cell bodies (V, abscissa) and the numbers of the satellite cells (No, ordinate) in young adult (▨) and old (▧) rabbits. The vertical bars indicate the standard error above the mean value.

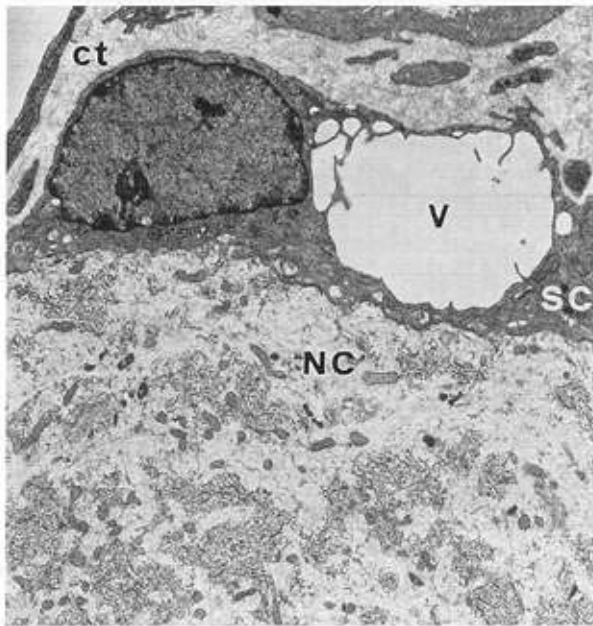


Figure 2. A vacuole (V) can be seen in the cytoplasm of a satellite cell (SC) of an aged rabbit; slender projections protruded into the vacuolar space; ct = connective tissue space; NC = nerve cell body. Spinal ganglion of a rabbit aged 60 months. $\times 7000$.

cells increased with increase in the volume of the nerve cell body. For each nerve cell body class the mean number of satellite cells was significantly ($p < 0.05$) smaller in the aged animals than in the young adults.

Electron microscopy. We limit ourselves to describing features of satellite cell sheaths which could plausibly be related to the observed decrease in the number of these cells. Membrane-bounded vacuoles of varying size were found in the cytoplasm of satellite cells (Fig. 2) more frequently in the old rabbits than in the young adults. Slender projections often protruded into the vacuolar space, which contained an electron-transparent material. A continuity between the vacuolar space and the perineuronal connective tissue space was never found.

Large invaginations of the connective tissue space (Fig. 3) were encountered much more often in the satellite cell sheaths of the old rabbits than young adults.

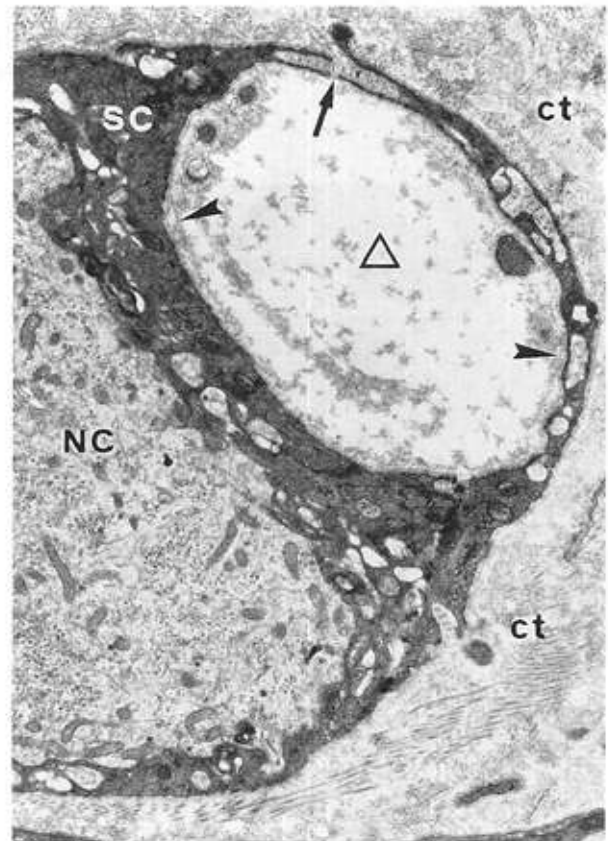


Figure 3. An invagination (Δ) of the connective tissue space (ct) can be seen in a satellite cell sheath (SC) of an aged rabbit. The invagination is lined by a basal lamina and contains a granular material and fibrils (arrowheads). The arrow indicates the continuity of the invagination with the connective tissue space. NC = nerve cell body. Spinal ganglion of a rabbit aged 79 months. $\times 9200$.

These invaginations, which were lined by a basal lamina, usually contained a moderately dense, granular material, fibrils of varying thickness and occasionally membrane ghosts. These invaginations were continuous with the connective tissue space by means of narrow channels.

Autophagic vacuoles, enclosing cytoplasmic organelles, were present within satellite cells, more often in old than in young adult rabbits.

Finally, the following were found exclusively in old rabbits, although rather rarely: (1) satellite cells with pyknotic nuclei (Fig. 4), and (2) satellite cell sheaths containing masses of dense material (Figure 5), in which membrane fragments could be recognized. These masses were probably remnants of degenerated cells.

DISCUSSION

The present study was undertaken to establish whether a change in the perineuronal satellite cell number plays a role in the age-related reduction of the volume ratio between the perineuronal glial sheaths and their associated nerve cell bodies. Our results clearly showed that the decrease in the perineuronal satellite

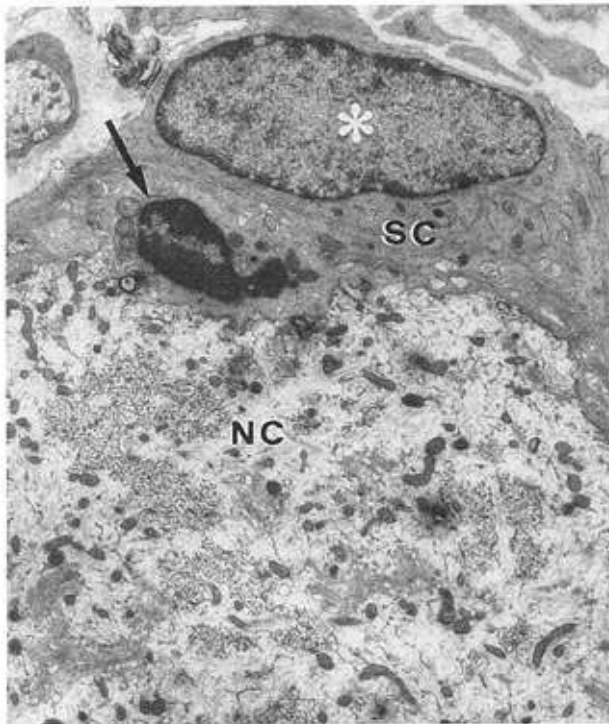


Figure 4. A pyknotic nucleus (arrow) can be seen close to a nucleus of normal appearance (*) in a satellite cell sheath (SC) of an aged rabbit. NC = nerve cell body. Spinal ganglion of a rabbit aged 60 months. $\times 8000$.

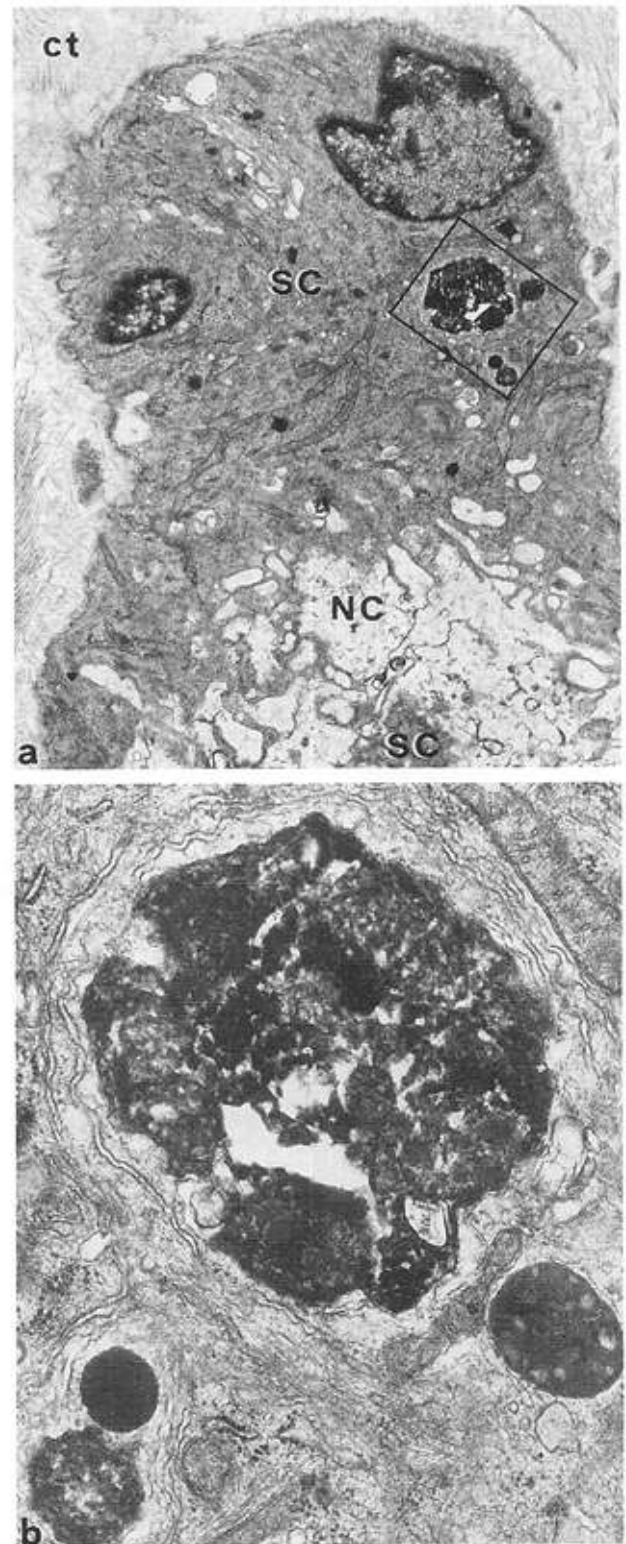


Figure 5 a,b. Masses of dense material, probably remnants of a degenerated cell, appear enclosed in cytoplasmic vacuoles of the satellite cell sheath (SC) in a section grazing the surface of a nerve cell body (NC). The boxed area of Fig. a is shown at greater magnification in Fig. b. ct = connective tissue space. Spinal ganglion of a rabbit aged 60 months. a: $\times 7600$; b: $\times 30000$.

cell number is one of the mechanisms by which this age-related reduction takes place. However, it cannot be excluded that a decrease in the size of individual satellite cells contributes to the above age-related reduction. Satellite cells have extremely complicated shapes [5,6], so it is not possible even in electron microscope sections to distinguish profiles belonging to one cell from those belonging to others. Therefore, at present it is not possible to estimate the volume of individual satellite cells and hence completely clarify this aspect of the problem.

As regards the mechanisms which could be involved in the decrease of the total number of perineuronal satellite cells, this decrease may occur (1) through cell degeneration or (2) by some satellite cells becoming detached from the perineuronal sheaths. The pyknotic nuclei and the degenerated or degenerating satellite cells found in the old rabbits seem to indicate that cell degeneration is indeed involved in this process. In reality, however, altered cells were observed rather rarely in the satellite sheaths of aged rabbits, but this could be due to rapid cell degeneration, so that a significant number of altered cells escaped observation. In this regard it may be remembered that perisomatic satellite cells undergoing degeneration have been reported in the vestibular ganglion of old rats [7]. Concerning the possibility that some cells become detached from the perineuronal sheaths, this is difficult to test with the static images obtained by electron microscopy.

Whatever the mechanism by which satellite cells decrease in number, it is important to emphasize that in young adult rabbits each satellite cell is related to about $1700 \mu\text{m}^3$ of nerve cell body, whereas in old animals each satellite cell corresponds to about $4900 \mu\text{m}^3$ of nerve cell body. If, as suggested by a number of experimental findings [8–14], satellite cells play a role in neuronal support [see 6 for a review], it is likely that the significant decrease in their number has negative consequences for neuronal activity.

Finally, we noted that in both young adult and aged rabbits the number of the satellite cells enveloping each nerve cell body was directly proportional to the volume of the latter. This result, which is in agreement with observations in the rat and the lizard [15,16], supports the idea that a quantitative balance between satellite cell number and nerve cell body volume is a general rule in spinal ganglia.

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