

Increase in number of the gap junctions between satellite neuroglial cells during lifetime: An ultrastructural study in rabbit spinal ganglia from youth to extremely advanced age

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Abstract

This study investigated quantitative aspects of the gap junctions between satellite neuroglial cells that envelope the spinal ganglion neurons in rabbits aged 1 year (young), 3.6 years (adult), 6.7 years (old), and 8.8 years (very old). Both the total number of gap junctions present in 30,000 μm^2 of surface area occupied by perineuronal satellite cells, and the density of these junctions increased throughout life, including the extremely advanced age. By contrast, the mean length of individual gap junctions did not change with age. Thus, the junctional system which provides morphological support for the metabolic cooperation between satellite cells in rabbit spinal ganglia becomes more extensive as the age of the animal increases. These results support the hypothesis that the gap junctions between perineuronal satellite cells are involved in the spatial buffering of extracellular K^+ and in neuroprotection.

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1. Introduction

Neuroglial cells were long believed to simply provide structural support for neurons. However, evidence has now accumulated to show that these cells closely and actively interact with neurons in most functions of the nervous system (e.g. see [17]). As a result, interest in neuroglial cells and their interactions with neurons has greatly increased.

We have been studying the neuroglial cells that envelope the neurons in sensory ganglia for many years (for reviews, see [26,27]). These cells are usually called satellite cells and will be referred to as such in what follows. The satellite cells comprising a given perineuronal sheath are coupled to each other via gap junctions [11,19,25,26,28,29]. Gap junctions are important because they make possible metabolic cooperation between cells (e.g. see [2,39]) which in turn enhances

the efficiency of satellite cells in their role of meeting the demands of the neurons with which they are associated.

Information on age-related changes of the gap junctions between perineuronal satellite cells is still incomplete. In this study, we report our findings on gap junctions in the spinal ganglia of rabbits from the young to the extremely advanced age.

2. Materials and methods

The present study was carried out on rabbits (*Oryctolagus cuniculus*) of both sexes. Rabbits aged 1 year (three animals, 3.4–3.5 kg body weight), 3.6 years (three animals, 3.6–3.8 kg body weight), 6.7 years (three animals, 4.0–4.2 kg body weight), and 8.8 years (three animals, 4.2–4.5 kg body weight) were used. The rabbits were cared for according to the European Community Council Directive (86/609/EEC) on the use of laboratory animals. The dates of birth of all animals were documented; all had been raised by a specialist

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rabbit breeder with particular attention to hygiene and regular veterinary inspections and had been fed an unrestricted diet. Because the mean life span of the normal healthy *Oryctolagus* is approximately 5.6 years [12] and the maximal life span is approximately 8 years [38], the 1-year-old rabbits were young, the 3.6-year-old rabbits were adult, the 6.7-year-old rabbits were old, and the 8.8-year-old animals were very old.

The animals were perfused transcardially with a solution containing 2% formaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) under deep anaesthesia with Nembutal i.p. (80 mg/kg). After fixation for about 3 h, the thoracic spinal ganglia were removed, washed in cacodylate buffer (0.2 M, pH 7.3) for 2 h and then postfixed on ice for 1.5 h 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.

As even during optimum fixation, dehydration and embedding there is some degree of cellular swelling or shrinkage, to study the surface area occupied by perineuronal satellite cells and the length of each gap junction a basic assumption was that any artifactual surface area and length changes were about the same in all four age groups. This assumption seems justified by the fact that all the ganglia used for the study satisfied the following conditions: (a) the interval between the nerve cell body and the enveloping satellite cell sheath was of uniform width; (b) the clefts between the satellite cells were of constant width; (c) neither nerve cell bodies nor perineuronal satellite cells showed signs of swelling or shrinkage; (d) neither empty areas nor clumping were observed in the connective tissue space surrounding the satellite cell sheaths. Overall, 96 ganglia (8 for each animal) were used for this study.

Isotropic uniform random (IUR) sections were obtained following the orientator procedure [20]. For each ganglion, a single IUR thin section (about 0.15 mm × 0.10 mm) was photographed under the electron microscope. Each section was photographed in its entirety at a magnification of 8000× and the negatives printed to a final magnification of 32,000×. A montage of 60–70 prints was necessary to reconstruct each section. The following were determined in each section: (1) the total number of gap junctions occurring between perineuronal satellite cells; (2) the length of each gap junction; (3) the total surface area occupied by perineuronal satellite cells. This area was measured with the aid of a digitizing tablet connected to a computer. Subsequently, the mean number of gap junctions per unit of surface area (100 μm²) occupied by satellite cells was calculated for each rabbit (eight ganglia). The mean length of gap junctions for each rabbit was also calculated.

The values obtained for the three rabbits in each age group were compared by one-way ANOVA to establish whether they differed significantly. Subsequently, the values obtained for each age group were compared by one-way ANOVA. When ANOVA revealed significant differences, the post hoc Tukey test for multiple comparisons was used to iden-

tify differences between individual age groups. Values were expressed as means ± S.E.M. Both for ANOVA and post hoc Tukey test, differences were considered significant for *P*-values < 0.01.

3. Results

The morphological relationships between nerve cell body and satellite cells did not change with advancing age. Gap junctions were never observed at the neuron-satellite cell boundary, but in all age groups occurred between the satellite cells comprising a single perineuronal sheath (Fig. 1). As described in the literature (e.g. see [4,30]), at these junctions, the normal intercellular space was abruptly reduced to about 2 nm. The width of this gap remained constant throughout the junctional area. Most gap junctions occurred singly; occasionally they were found close to adhering junctions. Sometimes mitochondria were close to gap junctions (Fig. 1), but it was not clear whether this association had some functional significance or was due to chance.

The mean densities of gap junctions (numbers of junctions per unit of surface area (100 μm²) occupied by perineuronal satellite cells) did not differ significantly between the three rabbits of each age group. In each age group we examined a total surface area occupied by perineuronal satellite cells of 30,000 μm². Both the total number of gap junctions found in this area, and the density of these junctions increased progressively with age. Fig. 2A shows the density of gap junctions in the four age groups. The differences between the density of gap junctions in very old rabbits and those in young, adult, and old animals were significant (*P* < 0.01, in all cases).

The mean length of individual gap junctions did not differ between the three rabbits of each age group, or between the four age groups (Fig. 2B).

4. Discussion

In the rabbit, both the total number of gap junctions present in a surface area of 30,000 μm² and the mean density of these junctions increased progressively throughout life, including the extremely advanced age. By contrast, the mean size of individual gap junctions did not change with age. These findings indicate that the total area of this system of junctions increases throughout life.

To our knowledge, the relationships between aging and number or total area of gap junctions, studied in the electron microscope using strict criteria for identifying these junctions [4,30], have not been investigated in other regions of the nervous system. However, the literature contains reports on the relationships between aging and gap junctions that are based on other approaches. For example, Cotrina et al. [7] revealed connexins immunohistochemically and observed that the coupling between astrocytes in the mouse brain did not change significantly with age. Further studies are required

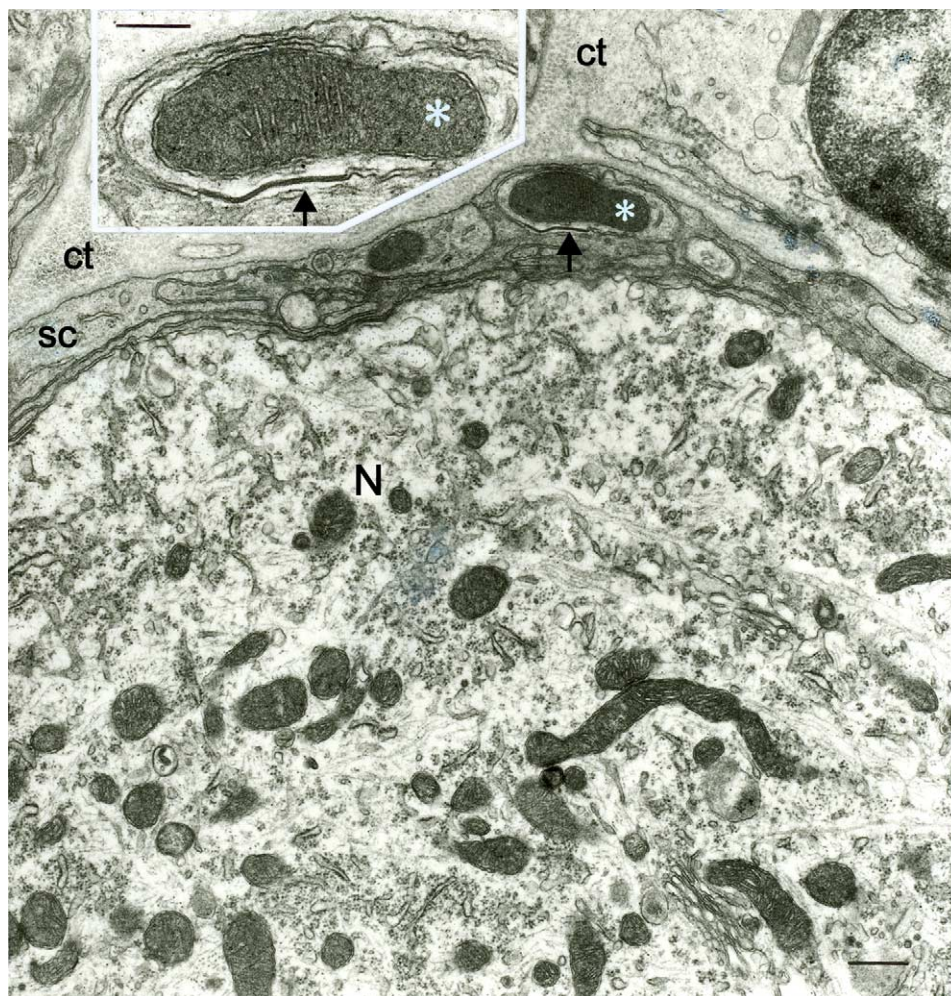


Fig. 1. Electron micrograph showing a gap junction (arrow) within a perineuronal satellite cell sheath (sc); ct: connective tissue, N: nerve cell body of a sensory neuron. The asterisk indicates a mitochondrion close to the gap junction. The latter is shown at greater enlargement in the inset. Spinal ganglion from a very old rabbit (8.8 years). Scale bar: 0.5 μm . *Inset*: scale bar: 0.25 μm .

to determine whether the number and total area of gap junctions, and gap junction coupling change with age in different species and in different regions of the nervous system. Studies on relationships between age and density, and age and size of gap junctions have been carried out in pure fibroblast cultures [16]. The authors reported that gap junctions were more sparsely distributed and distinctly smaller in old than young cultures. Our findings, that gap junctions are more numerous in very advanced age and that the mean size of individual gap junctions remains constant with age, are in sharp contrast to these *in vitro* findings. This discrepancy could be due to the difference in cell type or to differences between pure cell cultures and ganglia. The cultured fibroblasts of Kelley et al. [16] were only in contact with each other and thus gap junction formation between them depended exclusively on their intrinsic properties. By contrast, in the ganglia we studied, satellite cells were under the influence of the sensory neurons they surrounded. It is known, for example, that central nervous system neurons may influence the formation of astrocyte gap junctions (for review, see [32]).

With regard to the functions of the gap junctions between satellite cells, the following hypotheses seem the most plausible. (1) K^+ concentration increases in the perineuronal environment as a result of neuronal activity and a rapid removal of this excess K^+ is required to maintain neuronal excitability (for review, see [33]). It is widely accepted that astrocytes play a major role in the spatial buffering of extracellular K^+ within the central nervous system (e.g. see [8,13,18,24]). Satellite cells are believed to perform the same function in sensory and autonomic ganglia (for reviews, see [26,27]). The satellite cells closest to the enclosed neuron may take up the extracellular K^+ into their cytoplasm and redistribute it to the other satellite cells within the sheath. K^+ would eventually be discharged into the connective tissue space or returned to the neuron. Studies on the central nervous system have shown that gap junctions between neuroglial cells improve the buffering capacity of these cells (e.g. see [14,22]). It is likely that the gap junctions between satellite cells in sensory ganglia have the same function. (2) Neurons cultured *in vitro* are less vulnerable to various types of insult

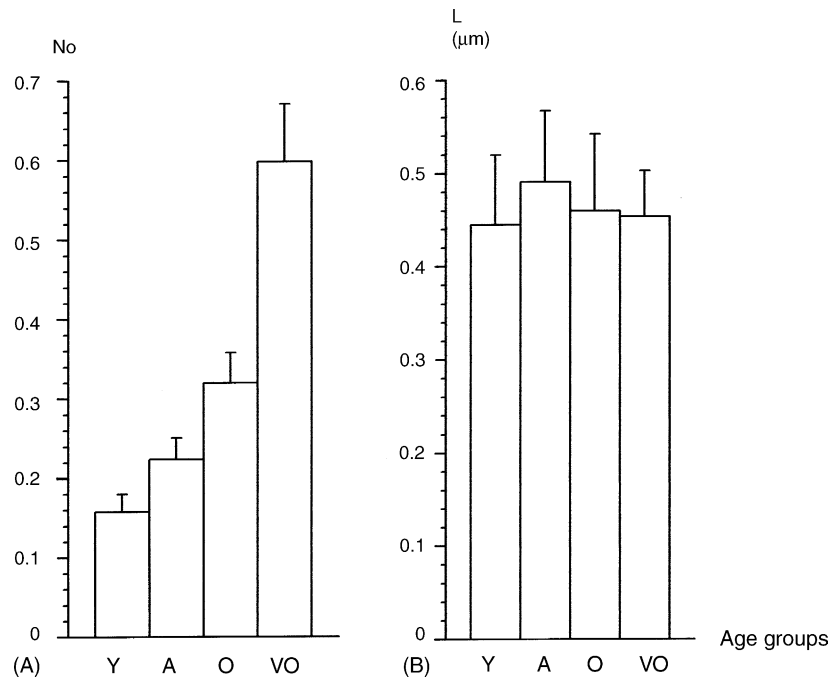


Fig. 2. (A) number (No) of gap junctions per unit of surface area ($100 \mu\text{m}^2$) of perineuronal satellite cells in rabbits aged 1 year (young, Y), 3.6 years (adult, A), 6.7 years (old, O), and 8.8 years (very old, VO). Values are means \pm S.E.M.: Y = 0.158 ± 0.022 , A = 0.224 ± 0.027 , O = 0.320 ± 0.038 , and VO = 0.598 ± 0.073 . The differences between Y and A, and between A and O are not significant ($P < 0.01$, Tukey test), whereas the differences between Y and O, between Y and VO, between A and VO, and between O and VO are significant ($P < 0.01$, Tukey test). (B) length (L) of individual gap junctions in perineuronal satellite cells of rabbits aged 1 year (young, Y), 3.6 years (adult, A), 6.7 years (old, O), and 8.8 years (very old, VO). Values are means \pm S.E.M.: Y = 0.445 ± 0.075 , A = 0.487 ± 0.076 , O = 0.460 ± 0.082 , and VO = 0.454 ± 0.049 . These values did not differ significantly (ANOVA).

if they are cocultured with astrocytes (e.g. see [21]). This and other data indicate that astrocytes play a neuroprotective role. In sensory ganglia, each neuron is usually enveloped by an individual satellite cell sheath whose outer contour faces the interstitial connective tissue containing capillaries. Thus, all substances from the blood must pass through the satellite cell sheath to reach the neuron. Since neurons in sensory ganglia lack the protection provided to central nervous system neurons by the blood–brain barrier, only the satellite cell sheath controls the traffic of substances to the ganglionic neuron. Therefore, the satellite cells in these ganglia are well placed to perform a neuroprotective function. Recent findings [37] support this hypothesis. That gap junctions participate in the neuroprotection carried out by astrocytes, is indicated by the finding that the blockade of gap junctional communication between these cells results in a markedly enhanced neuronal vulnerability to oxidative damage [3]. Gap junctions between perineuronal satellite cells are likely to have a similar role.

The permeability of gap junctions in non-nervous tissues [9,34,36], in the central nervous system [31], and in spinal ganglia [15] is regulated by a number of conditions. In the absence of data on the gap junction permeability in satellite cells of various ages, it is not clear what influence the age changes in number and density of gap junctions have on the functions of perineuronal satellite cells. Nevertheless, our results indicate that the junctional system which provides morphological support for the metabolic cooperation

between satellite cells in rabbit spinal ganglia becomes more extensive with advancing age.

Gap junctions consist of connexins, a family of closely related proteins, whose members are identified according to their predicted molecular mass in kDa (for review, see [1]). Several connexins have been detected in the nervous system. Among neuroglial cells, the vast majority of astrocytes express connexin43, oligodendrocytes express mainly connexin32, while Schwann cells express a variety of connexins but mainly 32 and 46 (for reviews, see [5,8,10,23,35,39]). However, little information is available on the connexins expressed by satellite cells in sensory ganglia. The only study we are aware of was carried out on the rat petrosal ganglion, where immunostaining for connexin43 was present in satellite cells enveloping large neurons, but absent from those enveloping other neurons [6]. Future studies should aim to establish: (1) what connexins are expressed by perineuronal satellite cells in spinal ganglia, and (2) whether these connexins remain the same throughout life or change with age.

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