Quantitative age-related changes in NADPH-diaphorase-positive neurons in the ventral lateral geniculate nucleus

Alicia Villena a,*, Florentina Díaz a, Lourdes Vidal a, Mercedes Moreno b, Ignacio Pérez de Vargas a

a Department of Histology and Pathology, School of Medicine, University of Málaga, 29071 Málaga, Spain
b Department of Ophthalmology, School of Medicine, University of Málaga, 29071 Málaga, Spain

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Abstract

Age-related changes in nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) were examined in the rat ventral lateral geniculate nucleus (vLGN) using histochemical methods. Eighteen rats aged 3, 24, and 26 months were studied using quantitative methods to investigate the number of neurons per mm², the cross-sectional area, and the orientation of dendritic processes of NADPH-d-positive neurons. We have described three types of neurons: types A and B are both located in the lateral and medial vLGN (vLGN-l and vLGN-m, respectively), and type C neurons over the optic tract. The number of NADPH-d-positive neurons was significantly reduced in the old rats (−39%) when compared with controls (3-month-old rats). The quantitative analysis of cell areas revealed a significant decrease of somatic size in type B neurons, both in the lateral and medial vLGN, and in C neurons; however, type A neurons did not show significant changes. By quantifying the orientation of dendritic processes, we observed a predominant dorsolateral orientation in type A and B neurons. During aging, there are no changes in the dendritic orientation of neurons located in the vLGN-m; however, vLGN-l neurons show an increase in dendritic processes with dorsoventral orientation. In type C neurons, our results show that 87.4% of dendritic processes are lateromedially oriented at 26 months old. Therefore, the types A and B neurons behave differently during senescence. Type A neurons do not change in size, but those located in the vLGN-l modify the orientation of their dendritic processes; however, type B neurons, reduce their size and those located in the vLGN-l also modify their dendritic process orientation. Finally, the type C neurons modify their size and dendritic process.

Keywords: Visual system; Histochemical study; Nitric oxide synthase; Number of neurons; Somatic size; Dendritic orientation

1. Introduction

The ventral lateral geniculate nucleus (vLGN) of the rat is a thalamic visual nucleus receiving direct visual input from the retina (Hichey and Spear, 1976) and the occipital cortex (Pasquier and Villar, 1982; Brauer et al., 1984) and establish reciprocal connections with a large number of subcortical nuclei, both visual and nonvisual (Pasquier and Villar, 1982). The precise function of the vLGN remains obscure, although it has been implicated in brightness discrimination and in the pupillary light reflex (Legg and Cowey, 1977), and in the visuomotor integration (Harrington, 1997). It is also involved in circadian rhythms through its connections with the suprachiasmatic nucleus (Harrington, 1997).

On the basis of its cytoarchitecture, it can be subdivided into two regions: one occupies the lateral region of the nucleus adjacent to the optic tract (vLGN-l), and the other forms the medial part of the vLGN (vLGN-m) (Niimi et al., 1963; Hichey and Spear, 1976). A third small division near the border of the dorsal lateral geniculate nucleus (dLGN) has been described as the intergeniculate leaflet (Hichey and Spear, 1976). The neurons from these regions have been described using Golgi-impregnation techniques (Mounty et al., 1977; Brauer et al., 1984) and, more recently, via immunocytochemical studies (Takatsui and Tohyama, 1989; Arai...
et al., 1992; Gabbott and Bacon, 1994; Meng et al., 1998).

These regions of the rat vLGN reflect a segregation of afferent and efferent pathways through this thalamic nucleus (Hayashi and Nagata, 1981; Pasquier and Villar, 1982; Mackay-Sim et al., 1983). Thus, the vLGN-I receives the majority of the input from retinal ganglion cells in both eyes (Hayhow et al., 1962; Hichey and Spear, 1976) and contains the cells that respond to visual stimuli (Sumimoto et al., 1979; Hayashi and Nagata, 1981).

Various studies have reported the presence of neuronal nitric oxide synthase (nNOS) in the vLGN (Mitrofanis, 1992; Vincent and Kimura, 1992; González-Hernández et al., 1993; Gabbott and Bacon, 1994; Rodrigo et al., 1994; Bertini and Bentivoglio, 1997; Iwase et al., 1998). Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) histochemical staining can be used to identify this enzyme, since many studies have shown that NADPH-d may correspond to nNOS; it is, therefore, suggested that neurons containing NADPH-d are probably capable of producing nitric oxide (Dawson et al., 1991; Bredt and Snyder, 1992; Hashikawa et al., 1994; Franca et al., 2000; Saxon and Beitz, 2000; Wang et al., 2001; Necchi et al., 2002).

Many groups have published exact mappings of NADPH-d-positive neurons in the CNS (Vincent and Kimura, 1992; Northington et al., 1997; Cork et al., 2000; Zemel et al., 2001) and their presence at all levels of the visual system from the retina to the cortex suggests that the NO-cGMP system plays an important role in the visual function (Mitrofanis, 1992; Vincent and Kimura, 1992; González-Hernández et al., 1993; Gabbott and Bacon, 1994; Meng et al., 1998). These studies have demonstrated that rat vLGN-I and vLGN-M stain differently for NADPH-d activity, with the vLGN-I containing many heavily stained neurons. However, studies on NOS/NADPH-diaphorase activity in the aging nervous system are scarce (Kawamata et al., 1990; Unger and Lange, 1992; Sobreviela and Mufson, 1995; Benzig and Mufson, 1995; Yamada et al., 1996; Lolova et al., 1996, 1997; Roufaiıl and Rees, 1997; Huh et al., 1997a,b, 1998; Necchi et al., 2002) and there are none on the vLGN.

Despite the increasing number of reports on the histochemical and immunohistochemical distribution of nNOS in the mammalian brain, the expression and functional roles of this enzyme in the thalamus have been rather neglected up to now. Thus, the main objective of this study is to understand the activity of diaphorase as a marker of the presence of nNOS during aging, which could help to gain further insight into the neuronal degenerative processes in the vLGN of old rats. In order to achieve this objective, we carried out a light-microscopic analysis of the morphology and orientation of neuronal dendritic processes displaying NADPH-d activity in young adult rat vLGN as well as an analysis of the size of diaphorase-positive neuronal soma, and their number per mm² of tissue. In addition, we investigated the effect of normal aging on different NADPH-d-positive neuronal populations which allowed us to establish the pattern of age-related changes in NOS.

2. Material and methods

2.1. Animals and tissue preparation

Eighteen male Wistar rats, 3 (n = 6) (young rats used as control), 24 (n = 6), and 26 months old (n = 6), weighing between 322.5 ± 10.3 and 522.5 ± 47.5 g were used. The experiments were carried out in accordance with the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals and were approved by the Scientific Committee of the University of Málaga regarding minimizing animal suffering and the number of animals used. All animals were raised in groups (2–4) in a specific pathogen-free and regulated environment with automatic temperature control (23 ± 0.5 °C), relative humidity (55 ± 5%), and a 12-h light:12-h dark cycle. They also had free access to food and water.

Animals were deeply anesthetized with an intraperitoneal injection of chloral hydrate (0.1 ml/30 g weight). The chest cavity was opened and a canula was inserted into the ascending aorta via the left ventricle. Perfusion was performed using 100–150 ml of 0.9% NaCl, followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Following tissue fixation with paraformaldehyde, NADPH-d activity was considered to represent NOS activity (Matsumoto et al., 1993). The brains were promptly removed from the skull, postfixed with the same fixative at 4 °C overnight and then placed in 20–30% sucrose in phosphate-buffered (PB) at 4 °C.

2.2. Staining procedures

Coronal sections, 50 μm thick, were cut in a cryostat (−21 °C). These sections were processed for NADPH-d histochemistry. Briefly, free-floating sections were incubated at 37 °C for 90 min in 0.1 M PB (pH 7.4), containing 1 mg/ml β-NADPH, 0.2 mg/ml nitro blue tetrazolium (NBT), and 0.3% Triton X-100 (all histochemical reagents were obtained from Sigma Chemical Company, St. Louis, MO). Control sections were incubated in a solution without NADPH-d or NBT and no positive staining was observed in these sections. After incubation, the sections were rinsed in PBS (pH 7.4) to stop the reaction, dehydrated in alcohol, defatted
in xylene and mounted on Entellan for examination by light microscopy.

2.3. Quantitative analysis

To assess the effects of aging on NADPH-d neurons in the vlLGN, we compared their number per mm², somatic size, and dendritic orientation in 3-, 24-, and 26-month-old rats. Quantitative analysis was performed with a computer-assisted image-analysis system consisting of a Nikon microscope, a Hitachi videocamera, a computer, and Visilog version 5 morphometry software.

2.3.1. Number and somatic size of diaphorase-positive neurons

We counted all diaphorase-positive neurons in each vlLGN section without taking into account the type of neuron or their location. In addition, we calculated the vlLGN area for each section, which allowed us to calculate the number of NADPH-d neurons expressed as neurons per mm².

To follow changes in nerve cell somata size, we measured the cross-sectional area of NADPH-d-positive neurons in several sections throughout the vlLGN. Specifically, different types of cells were projected from the microscope onto a TV monitor using a video camera system. Perikarya were circumscribed by the cursor.

2.3.2. Dendritic processes orientation

The aim of this study was to determine quantitatively the orientation of type A, B, and C neuronal dendritic processes. We based this study on previous publications by Gabbott et al. (1988) and Gabbott and Bacon (1994). The orientation of 2152 primary dendritic processes was calculated. When processes curved sharply, only the initial orientation was recorded. We studied the processes lying within a range 0°–90° in relation to the optic tract, which passes laterally over the vlLGN. Using this scheme, a value of 0° represents a process running parallel to the optic tract, while a value of 90° represents a process lying perpendicular to the optic tract (Fig. 1A). If the orientation is directed towards the optic tract, the sign is positive (from 0 to 90°), and if the orientation is away from the optic tract, the sign is negative (from 0 to −90°) (Fig. 1B–D).

We analyzed the percentage of processes falling within each 30° segment interval. When angles were between 30 and −30°, the dendritic orientation was dorsoventral (Fig. 1B); those between 30 and 60°, and −30 and −60° had an intermediate orientation (Fig. 1C); and for those between 60 and 90°, and −60 and −90°, the orientation was lateromedial (Fig. 1D).

2.4. Statistical analysis

The results obtained for the number and somatic size of NADPH-d-positive neurons were analyzed by applying the Kruskall–Wallis non-parametric test (P < 0.05). In order to study the relationship between age and dendritic orientation angles, we used Pearson’s correlation coefficient; subsequently, the percentages were grouped into intervals and the relationship between the two variables was studied using contingency tables and the $\chi^2$ statistic as the test of independence.

3. Results

3.1. Morphological description

Rat vlLGN has been shown to be highly reactive to NADPH-d. It is divided into two well-defined regions: (i) the vlLGN-l is highly diaphorase positive and it appears to be crossed by stripes of strong diaphorase activity with a dorsoventral orientation separated by other paler bands running parallel to the optic tract fibers; and, (ii) the vlLGN-m, very slightly positive, where very few stripes are observed (Fig. 2A). Surrounding the vlLGN in the lateral part, we can see a wide band corresponding to the optic tract (Fig. 2A, o.t.). We found that the distribution of NADPH-d-positive neurons was similar in the different age groups, although reactivity was lower in the 26-month-old animals (Fig. 2B). At this stage, there was an apparent decrease in the number of NADPH-d-positive neurons, although the stripes and bands were well defined.

Many NADPH-d-positive neurons were found presenting different morphology, size, and dendritic orientation. In the vlLGN-l we detected two types of neurons. The first, called type A neurons, was located both within and between the stripes of strong diaphorase activity (Fig. 2C). The somatic size and shape were highly variable; primary, secondary, and, occasionally, tertiary processes were observed (Fig. 2D). Dendrites were occasionally oriented in every spatial direction. Groups of smaller neurons were found in the ventral part of the vlLGN-l (Fig. 2A). The second class, type B neurons, are much less frequent than type A neurons. They are small in size and normally spindle-shaped, with two dendrites emerging from opposite poles of the cell body and rarely branched. They are highly reactive and mainly located within the dorsoventral stripes (Fig. 3A).

In the vlLGN-m we also found type A and B neurons. The former ones, have an oval or polygonal perikarya with a size apparently similar to neurons located in the vlLGN-l (Fig. 3B). In some instances they are located in the boundary with the vlLGN-l, and their dendrites are perpendicularly oriented to the optic tract, entering the
Occasionally, it is possible to follow the dendrite’s trajectory for a long way (Fig. 3B). Type B neurons are very similar to those found in the vLGN-l, although their number is lower.

Finally, there is a type of neuron found over the optic tract, called type C neurons. They are large, with an oval or round-shaped soma (Fig. 3C). The dendrites are parallel as well as perpendicular to the optic tract, and sometimes their length is such that they enter the vLGN. In some instances, we observed a single, thick dendritic bundle that went into the vLGN and branched out after a short way (Fig. 3C).

Similar morphological types were found in the 24- and 26-month-old animals with some of the types previously described having triangular soma and dendritic processes oriented in all directions (Fig. 3D), or which were spindle-shaped with dendrites emerging from opposite poles very similar to those detected in the 3-month-old animals.

3.2. Quantitative analysis

3.2.1. Number and somatic size of diaphorase-positive-neurons

The number of diaphorase-positive neurons per mm² of tissue in the 3-month-old rats was $292 \pm 20.5$ neurons per mm². At 24 months it significantly decreased ($185 \pm 11.5$ neurons per mm²; $-36.6\%; P < 0.05$), but no further changes were found in the 26-month-old animals ($178 \pm 12$ neurons per mm²) (Fig. 4).

In the vLGN-l, the somatic area of type A neurons was $112.3 \pm 2.8$ μm² at the age of 3 months. The changes detected from this age onwards are not statistically significant (Table 1). In the vLGN-m, this type of neurons had a somatic area of $114.5 \pm 5.3$ μm² at 3 months. At 26 months, there was a non-significant decrease in size ($-7.32\%)$ (Table 1).

In the vLGN-l the type B neurons had a somatic area of $81.2 \pm 4.2$ μm² at 3 months, and undergo a significant decrease at 24 ($69.4 \pm 3.3$ μm²; $-14.47\%; P < 0.05$) and 26 months ($67.5 \pm 4.8$ μm²; $-16.82\%; P < 0.05$) compared with the 3-month-old animals (Table 1). In the vLGN-m, the somatic area was $62.6 \pm 2.3$ μm² at 3 months, which was maintained at 24 months but, declined significantly at 26 months ($-16.12\%; P < 0.05$) (Table 1).

Finally, the neurons located over the optic tract, type C neurons, are the largest ones in the vLGN. At 3 months, their size was $129.4 \pm 5.7$ μm²; by the 26th month they had decreased significantly compared with younger animals ($96.1 \pm 4.5$ μm²; $-25.83\%; P < 0.05$) (Table 1).
3.2.2. Dendritic orientation

Regarding dendritic process orientation, 42.15% of the dendritic processes in type A neurons, located in the vLGN-l, were dorsoventrally oriented at the age of 3 months; 30.77% presented a lateromedial orientation, and 27.01% an orientation half-way between the two previous ones (Table 2). The \( \chi^2 \)-test did not reveal significant differences between these distributions. In the 24-month-old animals, the orientation was similar to the one described for the younger ones; however, at 26 months the percentage of dendritic processes with dorsoventral orientation increased (51.64%) at the expense of lateromedially oriented dendrites (19.4%) (Table 2; \( \chi^2 = 5.29; \) df = 2; \( P < 0.05 \)). Neurons located in the vLGN-m had a similar orientation to neurons located in the vLGN-l at 3 and 24 months, while at 26 months, the percentage of dendritic processes dorsoventrally oriented increased (71.3%) at the expense of lateromedial orientation (13.68%) (Table 2). However, the differences are non-significant.

Finally, the study of dendritic process orientation in type C neurons, revealed that at 3 months, no predominant orientation existed. At 24 months, the percentage of dorsoventrally oriented processes significantly decreased, and at 26 months they had disappeared, while those perpendicular to the optic tract and oriented towards the vLGN (lateromedial orientation) increased up to 87.5% (Table 2; \( \chi^2 = 5.27; \) df = 2; \( P < 0.05 \)).

4. Discussion

The aim of this investigation was to assess the effect of normal aging on the morphology, number per mm\(^2\), somatic size, and dendritic process orientation of different neuronal populations containing NADPH-d in rat vLGN.

Rat vLGN is clearly divided into two distinct regions: the vLGN-l and the vLGN-m. The vLGN-l forms an
intensely positive shell with diaphorase stripes across and aligned parallel to the outlying optic tract. These stripes are the result of dorsoventrally aligned bundles of unstained fibers travelling through regions of strong diaphorase staining (Gabbott and Bacon, 1994). In contrast, the vLGN-m is weakly to moderately stained in a more uniform manner where the stripes were barely visible. During aging, we observed that the two subdivisions of the vLGN are visible, but neuronal density is lower, and consequently, the number of diaphorase-positive neurons is also lower. Confirming several previous studies carried out by either Golgi-impregnation (Mounty et al., 1977) or NADPH-d histochemistry (Gabbott and Bacon, 1994), we have described three heavily stained neuron types: type A, B, and C neurons. Type A neurons project to different centers (Ribak and Peters, 1975; Mounty et al., 1977; Legg, 1979; Hayashi and Nagata, 1981; Mackay-Sim et al., 1983; González-Hernández et al., 1994) and type B neurons are thought to represent local circuit neurons. A subpopulation of these neurons was found to be immunopositive for GABA (Gabbott and Bacon, 1994), although an earlier

Fig. 3. (A) An elongate type B neuron with two dendrites emerging from opposite poles at 3 months. It is located over a stripe. Scale bar = 50 μm. (B) Detail of a type A neuron located in the vLGN-m with a large, triangular cell body and long dendritic processes. Scale bar = 50 μm. (C) A C neuron, over the optic tract, at 3 months with an oval soma. Note a thick and single dendritic trunk leading towards the vLGN that branches out after a short distance. Scale bar = 40 μm. (D) Some type A neurons at 26 months with triangular soma and dendritic processes with a different orientation. Scale bar = 50 μm.

Fig. 4. Neurons per mm² of labeled cell bodies in the rat vLGN using NADPH-d histochemistry at each of the ages included in the study. (*) Significant differences in relation to 3-month-old animals.
study (Mitrofanis, 1992) failed to find NADPH-d co-localized with GABA in type B neurons. 

In the vLGN-lateral and medial, we have observed type A neurons. The size and shape of their cell bodies vary as well as the number of proximal dendrites; they were frequently multipolar with round, triangular or polygonal somata. In agreement with Gabbott and Bacon (1994), we also report that they are located both within and between the stripes of strong diaphorase activity. No appendages were observed in the dendrites as is the case with Golgi-impregnated neurons (Brauer et al., 1984). Gabbott and Bacon (1994) found differences in the intensity of cytoplasmic staining, with strongly stained cells predominating in the vLGN-l, while weakly stained cells were present more medially. However, in our study, we found a similar intensity in the neurons from both regions, although in the vLGN-l, some small neurons, bundled in the ventral zone, appeared to be highly reactive.

Type B neurons, although less frequent than type A, are also located in both the vLGN-l and the vLGN-m. Their size was generally smaller and their cell body had an oval, elongated shape. The dendritic processes arose from opposite poles of the soma and rarely branched. In the vLGN-l, type B neurons were usually located in the stripes of strong diaphorase reactivity (Gabbott and Bacon, 1994). Finally, we observed another type of neuron, which had not been previously described with Golgi-impregnation techniques. Following Gabbott and Bacon’s (Gabbott and Bacon, 1994) lead, we called them C neurons which were always located over the optic tract. These are large neurons with varied morphology, whose dendrites run either parallel or perpendicular to the optic tract. Sometimes they appear as a single, thick trunk running towards the vLGN, which branches out after a short way.

The quantitative study showed that the number of NADPH-d-positive neurons per mm² significantly decreased at the age of 24 (−36.6%) and 26 months (−39%) compared with young control rats. Our data are consistent with other reports in the literature. Thus, Yamada and Nabeshima (1998) found that the number of neurons presenting NADPH-d reactivity in the cerebral cortex and striatum of aged rats was signifi-

### Table 1
Mean somatic size of NADPH-d-positive neurons in the vLGN-lateral and medial of 3-, 24-, and 26-month-old rats

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>vLGN-lateral</th>
<th>vLGN-medial</th>
<th>Optic tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type A neuron</td>
<td>Type B neuron</td>
<td>Type A neuron</td>
</tr>
<tr>
<td>3</td>
<td>112.3 ± 2.8</td>
<td>81.2 ± 4.2</td>
<td>114.5 ± 5.3</td>
</tr>
<tr>
<td>24</td>
<td>114.5 ± 2.8</td>
<td>69.4 ± 3.31a</td>
<td>117.8 ± 4.3</td>
</tr>
<tr>
<td>26</td>
<td>117.2 ± 4.5</td>
<td>67.5 ± 4.82b</td>
<td>106.2 ± 4.1</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.

a Significant differences between 3 and 24 months (P < 0.05).

b Significant differences between 3 and 26 months (P < 0.05).

c Significant differences between 24 and 26 months (P < 0.05).

### Table 2
Orientation of dendritic processes (in percentages) corresponding to labeled neurons located in the vLGN-lateral, vLGN-medial and optic tract using NADPH-diaphorase histochemistry

<table>
<thead>
<tr>
<th>Age in months (number of neurons)</th>
<th>vLGN-lateral</th>
<th>vLGN-medial</th>
<th>Optic tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DV</td>
<td>LM</td>
<td>I</td>
</tr>
<tr>
<td><strong>Type A neurons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (102)</td>
<td>42.15</td>
<td>30.77</td>
<td>27.01</td>
</tr>
<tr>
<td>24 (99)</td>
<td>45.07</td>
<td>26.12</td>
<td>28.68</td>
</tr>
<tr>
<td>26 (111)</td>
<td>51.64*,**</td>
<td>19.40*</td>
<td>28.84</td>
</tr>
<tr>
<td><strong>Type B neurons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (71)</td>
<td>62.50</td>
<td>12.30</td>
<td>25.18</td>
</tr>
<tr>
<td>24 (74)</td>
<td>70.11*,**</td>
<td>9.49</td>
<td>20.30</td>
</tr>
<tr>
<td>26 (82)</td>
<td>70.39*,**</td>
<td>8.60</td>
<td>21.40</td>
</tr>
<tr>
<td><strong>Type C neurons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (32)</td>
<td>29.42</td>
<td>41.22</td>
<td>29.34</td>
</tr>
<tr>
<td>24 (39)</td>
<td>21.63*,**</td>
<td>40.37</td>
<td>37.97</td>
</tr>
<tr>
<td>26 (41)</td>
<td>0</td>
<td>87.40***</td>
<td>12.50</td>
</tr>
</tbody>
</table>

Significant differences (P < 0.05) were observed between: dorsoventral and lateromedial orientation (*); dorsoventral and intermediate orientation (**); and lateromedial and intermediate orientation (**). DV, dorsoventral orientation; LM, lateromedial orientation; I, intermediate orientation.
cantly reduced compared with young rats, and Lolova et al. (1999) observed that NADPH-d-positive neurons of the dorsolateral column of the periaqueductal gray was significantly reduced at 26 months. However, other authors (Kawamata et al., 1990; Unger and Lange, 1992; Lolova et al., 1996; Huh et al., 1997a,b, 1998) reported no significant reduction in numbers in different centers, while Kanda (1996) found an increase in the spinal motoneurons and Reuss et al. (2000) in the superior olivary complex. We think that the reduction of NADPH-d-positive cells in the vLGN may be an indication of a reduced production of NO in the vLGN of aged rats compared with young rats, which could affect brain functioning significantly in aged rats.

In relation to somatic size, type A neurons, located in the vLGN-l, did not show changes; however, type B neurons, smaller than type A, show a reduction in size between 3- and 24-months, but no significant differences were found between the 24- and 26-month-old animals. In the vLGN-m, type A neurons did not change their size and type B neurons decreased at 26 months (−16.12%; P < 0.05). The study of type C neurons revealed that at 3 months, their size was 129.4 µm², but this decreased with age (−25.83% at 26 months).

Other researchers, such as Lolova et al. (1996) in the laterodorsal and pedunculopontine tegmental nuclei, and Kuo et al. (1997) in the striatum, reported that in 26-month-old rats, the cross-sectional areas of NADPH-d cell bodies were significantly reduced. However, Huh et al. (1997a,b), in the cerebral cortex, Roufail and Rees (1997), in the retina, and Kuo et al. (1997), in the hippocampus, found no significant differences between the control and the aged group. Finally, regarding the vLGN in the literature, we only found data regarding the diameter of the neuronal cell bodies of adult rats which was reported by Gabbott and Bacon (1994). This means that we cannot compare it with our results regarding aging. Nevertheless, these authors report that type B neurons have a smaller diameter than type A, being type C neurons the largest ones.

Thus, we have shown in this study that type A neurons, considered as projecting relay neurons (González-Hernández et al., 1994), maintain their size during aging; but type B neurons, considered as local circuit interneurons (Mitrofanis, 1992; Gabbott and Bacon, 1994), and type C neurons located over the optic tract, and whose function is unknown, decrease in size.

Finally, we have studied quantitatively, the orientation of neuronal dendritic processes during aging. The interest of our study centers on the fact that the dendrites are among the more plastic morphological elements of the brain. They respond to alterations in their microenvironment long before the cell body shows any clearly detectable change at light microscopy levels (Coleman and Flood, 1986).

Some authors have reported net dendritic growth and reorganization of the dendritic tree during normal aging of rodent and humans (Coleman and Flood, 1986; Arendt et al., 1995a,b). They assumed that dendritic growth was a compensatory reaction to the age-related atrophy of neighboring neurons. Arendt et al. (1995a,b) suggested that the neuronal loss and dendritic growth were two processes occurring simultaneously during normal aging.

In type A neurons dendritic processes mainly presented a dorsoventral orientation, i.e. the dendrites are parallel to the optic tract, in the three age groups. In the vLGM-l, the percentage of dorsoventral orientation significantly increased at 26 months, while the lateral-medial orientation decreased. This did not take place in the vLGN-m, which at 26 months presented similar percentages of the three types of orientation.

In type B neurons, it is clear that the most frequent orientation is parallel to the optic tract. These data are consistent with morphological observations describing type B neurons as fusiform and bipolar in shape. In the vLGN-l, the differences regarding the two other models of dendritic orientation under consideration are significant at the age of 24 and 26 months; in other words, in older animals there is a significant change, since the dorsoventral orientation predominate in association neurons. In the vLGN-m the increase of dendritic processes with dorsoventral orientation at 26 months is not significant. Our results are similar to those found by Gabbott and Bacon (1994) in the adult rat; but no data has been found during the aging. Therefore, we can conclude that type A and B neurons from the vLGN behave differently during senescence. Type A neurons, i.e. projecting relay neurons, do not change in size, but those located in the vLGN-l modify the orientation of their dendritic processes. Type B neurons, i.e. local circuit interneurons, reduce their size and those located in the vLGN-l also modify their dendritic process orientation.

Over the optic tract, type C neurons presented dendritic processes oriented in line with the three models under consideration at 3 months and no significant differences were found between them; a relevant fact is that at 26 months dendritic processes oriented parallel to the optic tract disappeared while perpendicularly oriented process increased (87.4%).

The results of this analysis reveal that only vLGN-l diaphorase-positive neurons show modifications in the orientation of their dendritic arbor which, together with the findings reported, i.e. a reduction of diaphorase-positive neurons could be related to plasticity or to an adaptation phenomenon of the remaining neurons during aging, perhaps in an attempt to increase synaptic effectiveness. Bearing in mind that this region receives the majority of the input from retinal ganglion cells and that the number of these cells decrease with age (Katz
and Robison, 1986; Weisse, 1995), we suggest that the change in dendritic orientation of the neurons located in the reception region is aimed at adapting to the decreasing number of afferences reaching the region.

Although the vLGN has many, well-known connections, the role of NO in these connections has not been properly elucidated. Some researchers (Meng et al., 1998) have observed that 96% of nNOS neurons in the vLGN project into the ipsilateral pretectal nuclei which suggests that these nuclei are the main, and possibly the only, target of the NOS-containing neurons of the vLGN. However, some vLGN neurons afferent to the superior colliculus in the rat are also NADPH-d-positive (González-Hernández et al., 1994). We think these neurons are mainly located in the vLGN-lateral, since, according to Gabbott and Bacon (1994), 73% of the neurons in this region are diaphorase-positive, something which is not the case in the medial region, where the percentage is much lower. The fact that NO has an influence on the functions these projections carry out makes it difficult to draw conclusions, but the reduction observed in the number of diaphorase-positive neurons during aging, together with the change in dendritic processes orientation in the projection neurons from the lateral region, could reflect alterations in the retinogeniculopretectal via related with visual reflexes and visuomotor functions. However, further research should determine the functional significance of these changes, although we believe this paper is the first study done on this issue.

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References


