Stereological Age-Related Changes in Neurons of the Rat Dorsal Lateral Geniculate Nucleus

FLORENTINA DIAZ,1* ALICIA VILLENA,1 PILAR GONZALEZ,1 VIRGINIA REQUENA,1 FRANCISCA RIUS,2 AND IGNACIO PEREZ DE VARGAS1

1Department of Normal and Pathological Morphology, Faculty of Medicine, University of Málaga, 29080 Málaga, Spain
2Department Public Health, Faculty of Medicine, University of Málaga, 29080 Málaga, Spain

ABSTRACT
Quantitative methods were used to compare the changes taking place in the volume of the dorsal lateral geniculate nucleus (dLGN) and corresponding neurons of young, adult and old rats. The study was carried out on male albino rats aged 3, 18, 24 and 28 months. In order to estimate the volume of the dLGN, neuronal volume density, numerical density and total number of neurons, we used serial sections stained according to the Klüver-Barrera technique and stereological methods. We found that dorsal lateral geniculate nucleus volume increases between 3 and 28 months, with a larger increase between 24 and 28 months. Neuronal volume density and numerical density of neurons are greater at 3 months and undergo a significant decrease between 24 and 28 months. Finally, the total number of neurons is shown to be smaller in adult and old animals than in younger ones, even though no significant variations are found between 18 and 28 months. Furthermore, this study confirms the need to analyze the total number of neurons and not just neuronal density if we want to correctly evaluate some of the microscopic changes occurring during senescence. Anat Rec 255:396–400, 1999.

Key words: dorsal lateral geniculate nucleus; stereological study; aging

The morphological variations that occur in selected brain regions during aging have been analyzed from different points of view, e.g. size, density and number of neurons, dendritic extent and number of synapses per cell (Curcio and Hinds, 1983; Coleman and Flood, 1986; Markus et al., 1987; Flood and Coleman, 1988; DeLaCalle et al., 1991; Crespo et al., 1992; Stroessner-Johnson et al., 1992; Flood and Coleman, 1993; Rance et al., 1993; Peinado et al., 1993, 1997). However, the data are controversial; some studies have demonstrated neuronal hypertrophy during senescence (Flood and Coleman, 1988; DeLaCalle et al., 1991; Stroessner-Johnson et al., 1992; Ahmad and Spear, 1993; Rance et al., 1993; Villena et al., 1997), stability or a shrinkage in size (Peinado et al., 1993, 1997; Requena et al., 1996), decreases in density and number of neurons (Brizzee et al., 1980; Henderson et al., 1980; Nandy, 1981; Knox, 1982; Terry et al., 1987) or stability of these parameters (Curcio and Coleman, 1982). In some cases the differences observed in similar regions might be due to variations in the methodology, the species and the strains used (Flood and Coleman, 1988).

The behaviour of the centers closely related to the visual system shows that over the lifetime of humans and rodents a reduction in neuron density occurs in the visual cortex with aging (Brody, 1955; Ordy et al., 1978; Knox, 1982; Leuba and Garey, 1987). A similar decrease in neuronal density, with no significant change in the number of neurons, has been observed in the dLGN of rats and monkeys (Satorre et al., 1985; Ahmad and Spear, 1993). Recently, we reported an increase in neuronal density in the thalamic reticular nucleus (TRN) of rats once these rodents have fully entered old age (Ramos et al., 1995).

*Correspondence to: F. Díaz, Department of Normal and Pathological Morphology, Faculty of Medicine, University of Málaga, 29080-Málaga, Spain. E-mail: fdiaz@uma.es

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In a previous study (Villena et al., 1997) we have demonstrated that dLGN neurons undergo hypertrophy in the soma and nucleus between 24 and 30 months as compared to those from 3-month-old animals. In this work we have evaluated the changes in the total volume of the dLGN, as well as the density and the total number of neurons, with the aim of knowing whether or not neuronal loss occurs during old age. We selected Wistar rats as experimental animals in order to find out whether we would encounter differences in the behaviour of the dLGN throughout their lifetime in comparison with other breeds of rodents.

**MATERIALS AND METHODS**

**Animals**

Twenty male Wistar rats aged 3, 18, 24 and 28 months (five per age group) were used in this study with weights of 250 ± 16 g (3 months), 365 ± 30 g (18 months), 407 ± 27 g (24 months) and 370 ± 31 g (28 months). The animals were kept at a day/night cycle using artificial light, with a room temperature of 20–22°C and with water and standard food pellets freely available. They had never paired and were cared for in accordance with the Venice Conference (Rogers, 1991) and European Union guidelines.

**Tissue Preparation**

The animals were fully anaesthetised with 8% chloral hydrate (0.1 ml/30 g weight) and perfused transcardiacally with a saline solution followed by buffered 4% formaldehyde. The skulls were opened, the brains removed and immersed in the same fixative for 48 hr, and finally embedded in paraffin. For location of the dLGN the stereotaxic criteria of Pellegrino et al. (1979), Sherwood and Timiras (1970) and Paxinos and Watson (1986) were followed using serial 8µm coronal sections. These sections were systematically selected at 56µm intervals along the dLGN in all the brains included in the study and were stained according to the Klüver and Barrera technique (1953).

**Stereological Study**

**dLGN volume.** In order to estimate the total volume (Vt) of the dLGN we first traced the profile of each section chosen using a camera lucida at a magnification of 4×. The actual area of each section, expressed in mm², was obtained with an IBAS I Image Analysis System. We then proceeded to calculate the volume using the following formulae as in previous publication (Villena et al., 1989),

\[
V_t = (S_1 + S_2) \times \frac{d}{2} + (S_2 + S_3) \times \frac{d}{2} + \ldots + \ldots + (S_{n-1} + S_n) \times \frac{d}{2}
\]

where S₁ ... Sₙ represent the areas of the sections and d (56µm) is the distance between two consecutive sections. The volume was obtained without correction for shrinkage and expressed in mm³.

**Neuronal volume fraction.** Neuronal volume fraction (Vv), volume occupied by neuronal bodies per unit volume of tissue, was calculated using a point-counting method (Hilliard and Cahn, 1961; Weibel et al., 1966). For this purpose, we designed a grid where the distance d between every two points was 1 cm and the total number of points was 225. Each tissue section was analysed with a camera lucida using a ×100 objective. Depending on the size of the dLGN the grid was superimposed two or three times over the projected image of each section and the cell bodies of the neurons that presented a fully visible nucleolus were traced onto the grid to determine neuronal volume fraction. Neuron cell bodies were easily distinguished from glia by the following characteristics: the neuronal nuclei are paler and have a granular appearance, they are larger and often have a well-developed nucleolus as well as an easily visible cytoplasmic edge (Leuba and Kraftsik, 1994).

**Neuronal density.** Neuronal density (Nₐ) (cells/mm³) was calculated by applying the method of Weibel and Gomez (1962), which relates this parameter to both the number of neurons per surface unit (Nₐ) and the volume fraction (Vv).

\[
N_v = K (N_a)^{3/2} / \beta (V_v)^{1/2}
\]

Nₐ was obtained from the number of cells observed in an actual area of 0.0362 mm². The size distribution coefficient (K) was estimated by K = (D₃/D₁)³/², the ratio of third to first moment of the distribution of diameters. D₁ represent the mean diameter of the neurons from the dLGN, and D₃ the moment of the third order with respect to the mean. Shape coefficient, β, was calculated by determining the relationship between the highest and lowest axis of each neuron and the mean of the set (Weibel, 1979, 1980). K (size coefficient) and β (shape coefficient) values were 1.012 and 1.40, respectively.

**Total neuron number.** The total neuron number (Nₗₚ) was calculated as the product of the dLGN volume at each
age (mm³) and the corresponding numerical density (neurons/mm³). It was expressed as the mean ± SEM.

**Statistical Analysis**

The results obtained were evaluated using an analysis of variance for the total volume and the Kruskal-Wallis non-parametric test for the rest of the parameters. Finally, a Least Significant Difference (LSD) multiple comparisons test was carried out to detect significant differences in each parameter between ages (P < 0.05).

**RESULTS**

We observed that the volume of the dorsal lateral geniculate nucleus increases between 3 and 28 months from 1.24 ± 0.05 mm³ to 1.87 ± 0.06 mm³. This growth takes place in two stages: the first up to 24 months, with a slow increase of 13.7%, and the second, between 24 and 28 months, with the maximum increase in volume (32.62%) (P < 0.05) (Fig. 1, Table 1).

Neuronal volume fraction reaches its maximum at 3 months (0.077 ± 0.003), undergoes a nonsignificant decrease between 3 and 24 months and reaches its lowest value at 28 months (0.059 ± 0.002) (Fig. 2, Table 1; P < 0.05).

Neuronal density is greatest in the 3-month old animals (32,034 ± 827 cells/mm³), and it significantly decreases (P < 0.05) (28.86%) up to 18 months (22,786 ± 930 cells/mm³). It does not change between 18 and 24 months but decreases again between 24 months (21,993 ± 416 cells/mm³) and 28 months (16,666 ± 413 cells/mm³) with a 24.22% loss (P < 0.05) (Fig. 3, Table 1).

Neuron numbers undergo a significant decrease (23.94%; P < 0.05) between 3 months (39,607 ± 1,023) and 18 months (30,123 ± 1,230) but are stable from this age onwards with no differences observed in the 24- and 28-month-old animals (Fig. 4, Table 1).

**DISCUSSION**

It is well known that the effects of age vary in different brain regions (Haug et al., 1984; Rogers and Bloom, 1985), species (Morgan et al., 1987), breeds (Hinds and McNelly, 1979, 1981) and individuals (Leuba and Kraftsik, 1994).

Our investigation was carried out on dLGN from albino rats. The aim was to know the effects of age in this region of the visual system to complete others studies in which we had analysed some others aspects, such as the activity of neuronal cytochrome oxidase (Diaz et al., 1996), the content in nuclear and cytoplasmic RNA in single neuronal cells (Villena et al., 1998), and changes in size and shape of neurons in these stages of life (Villena et al., 1997).

**TABLE 1. Values of the different parameters analyzed in the dLGN of young, adult, and old rats**

<table>
<thead>
<tr>
<th>Months</th>
<th>Volume</th>
<th>Volume fraction</th>
<th>Neuronal density</th>
<th>Neuron number</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–18</td>
<td>1.24 ± 0.05</td>
<td>0.077 ± 0.003</td>
<td>32034 ± 827</td>
<td>39607 ± 1023</td>
</tr>
<tr>
<td>18–24</td>
<td>1.32 ± 0.02</td>
<td>0.067 ± 0.002</td>
<td>22786 ± 930</td>
<td>30123 ± 1230</td>
</tr>
<tr>
<td>18</td>
<td>1.41 ± 0.07</td>
<td>0.071 ± 0.001</td>
<td>21993 ± 416</td>
<td>30989 ± 586</td>
</tr>
<tr>
<td>24–28</td>
<td>1.87 ± 0.06</td>
<td>0.059 ± 0.002</td>
<td>16666 ± 413</td>
<td>31178 ± 773</td>
</tr>
</tbody>
</table>

Values, mean ± SEM, and increase and decrease in percentages. Volume density in mm³ per mm³. Neuronal density in cell per mm³. *Change is significant (P ≤ 0.05).

**Fig. 2.** Volume fraction of the dLGN. Vertical lines = standard error.

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*3** P < 0.05 between 3 and 28 months old; ** P < 0.05 between 18 and 28 months old; *** P < 0.05 between 24 and 28 months old.

We evaluated several stereological parameters in this nucleus at different ages. The ages we chose for our study coincide with the different stages of life: young (3 months), mature adulthood (18 months), first signs of old age (24 months) and fully into senescence (28 months). In this way we were able to study any changes occurring in a sequential manner.

We observed that the volume of the dLGN undergoes two growth stages: the first, slow and gradual until 24 months and the second, with an important increase (32.62%) between 24 and 28 months. This behaviour is similar to the one observed in the suprachiasmatic nucleus of the rat (Woods et al., 1993) and the dLGN of the Sprague-Dawley rat (Satorre et al., 1985), although the increase in the latter breed is not so pronounced. In a previous study (Villena et al., 1997) we also showed that dLGN neurons between 24 and 30 months present hypertrophy of the soma and nucleus (33%) as compared with young rats (3
months), due perhaps to a compensatory mechanism to maintain the same total somatic volume in spite of increases in the total volume in the dLGN. It is equally probable that glia, neuropil and blood vessels undergo an increase in volume (Satorre et al., 1985; Ahmad and Spear, 1993) all of which would account for the increase in the total dLGN volume.

As regards neuronal volume fraction we have not found any other studies related to this parameter. Our results show a decrease as the animal’s age advances, of 16.90 % between 24 and 28 months. This event can be partly explained by the increase in volume observed in this study, independently of the neuronal hypertrophy found in this stage.

The results concerning neuronal density allow us to distinguish several stages during the life period analyzed. The first stage, between 3 and 18 months, is characterized by a significant decrease in the number of neurons/mm³ from 32,034 ± 827 to 22,786 ± 930 (28.86%). In the second stage, from 18 to 24 months, no changes in neuronal density are registered. Finally, in the third stage, from 24 to 28 months, another significant decrease (24.22%) is found which coincides with the increase in dLGN volume and the decrease in neuronal volume fraction. Ordy et al. (1978) reported a 18% loss in neuronal density in the visual cortex of F344 rats between the ages of 17 and 29 months, while Knox et al. (1982) found in Wistar Kyoto rats reductions of 30% between 3 and 23–25 months. However, in the visual cortex of monkeys (Peters, 1993) and Sprague-Dawley rats (Peters et al., 1983) this parameter is stable. On the other hand a significant increase was observed in the thalamic reticular nucleus between 24 and 30 months of Wistar rats (Ramos et al., 1995). Studies carried out on the dLGN of Sprague-Dawley rats (Satorre et al., 1985) and monkeys (Ahmad and Spear, 1993) have shown a decrease in numerical density with age, although the number of neurons/mm³ reported by Satorre et al. (1985) is smaller than the one we have observed. It is possible that differences in the methodology used for counting and/or the different breed of the experimental animals employed could well account for this (Flood and Coleman, 1988).

Regarding the total neuron number our observations reveal that the number of neurons is lower in adulthood (18 months) and old age (24 and 28 months) than at an earlier age (3 months). The evolution of this parameter differs from the data obtained in the dLGN of Sprague-Dawley rats (Satorre et al., 1985), where no changes were found between 3 and 29 months. Comparisons of these data to those of neuronal density clearly show that there has been no significant loss of neurons in the dLGN of the Wistar rat, as might be thought if only the results concerning neuronal density were considered. In fact, the number of neurons remains fairly stable from 18 months onwards. These results are consistent with the behaviour observed by other researches regarding different brain regions, especially in rodents, where there is little evidence pointing to relevant neuronal loss in the last stages of life (Curcio and Coleman, 1982; Hinds and McNelly, 1979; Morgan et al., 1987). Nevertheless, in another study also carried out on this nucleus, we detected a decrease in the activity of neuronal cytochrome oxidase between 24 and 28 months which was interpreted as reflecting a decrease in the bioenergetic competence of the neurons of the dLGN in old age (Díaz et al., 1996). It is likely that although the

![Fig. 3. Neuronal density of the dLGN. Vertical lines = standard error. *P < 0.05 for comparison between 3 and 18 months old; **P < 0.05 between 3 and 24 months old; ***P < 0.05 between 3 and 28 months old; +P < 0.05 between 18 and 28 months old; ++P < 0.05 between 24 and 28 months old.](image)

![Fig. 4. Numbers of neurons of the dLGN. Vertical lines = standard error. *P < 0.05 for comparison between 3 and 18 months old.](image)
number of neurons does not vary in the Wistar breed from 18 months onwards, certain neuronal functions could be affected upon the arrival of old-age. In conclusion, we think that these results establish the importance of determining the total number of cells and not only neuronal density if some of the microscopic changes that occur during senescence are to be properly evaluated.

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LITERATURE CITED


