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Aging in the vestibular nuclear complex of the male golden hamster (*Mesocricetus auratus*): anatomic and morphometric study

J.A. Fernández[1], C. Suárez[2], A. Navarro[3], C. Díaz[4], J.C. Alvarez[5], C. González del Rey[6] and J. Tolivia[3]

[1]Service of Otorrinolaringology of the Burela Coast Hospital, Lugo, Spain, [2]Service of Otorrinolaringology of the Asturias Central Hospital, Oviedo, Asturias, Spain, [3]Department of Morphology and Cellular Biology, University of Oviedo, Asturias, Spain, [4]Service of Otorrinolaringology of the San Agustín Hospital, Avilés, Asturias, Spain, [5]Service of Otorrinolaringology of the Cabueñes Hospital, Gijón, Asturias, Spain and [6]Service of Anatomy Pathologic of the Virgin of the Shell Hospital, Zamora, Spain

Summary. To study the effects of senescence on the vestibular nuclear complex twenty brainstems from male golden hamsters between 3 and 27 months-old were used and the possible variations in the number of neurons, neuronal morphology and nuclear volume were studied. The neuron profiles were drawn with a camera lucida and Abercrombie's method was used to estimate the total number of neurons. The test of Kolmogorov-Smirnov with the correction of Lilliefors was used to evaluate the fit of our data to a normal distribution and a regression analysis was done to decide if the variation of our data with age was statistically significant. The results of the present study are relevant only for male animals and the effect of senescence could be different in female vestibular nuclear complex. Aging affects the volume of the superior and lateral vestibular nuclei, as well as the nuclear neuronal diameter of the medial vestibular nucleus, but no significant neuronal loss has been appreciated in vestibular nuclear complex related with age. During the aging process we have observed that the distribution of neurons within the vestibular nuclei of the golden hamster does not show important changes and most of their morphometric parameters do not vary significantly.

Key words: Vestibular nuclei, Neuroanatomy, Neuron, Morphometry, Aging

Offprint requests to: Dr. Jorge Tolivia Fernández, Dpto. Morfología y Biología Celular, 8ª Planta Facultad de Medicina, Universidad de Oviedo, c/ Julián Clavería s/n, 33006 Oviedo, Spain. e-mail: jtolivia@correo.uniovi.es

Introduction

The vestibular nuclear complex (VNC) is found in the lateral part of the floor of the IV ventricle, and it extends from the caudal pole of the motor nucleus of the trigeminal nerve to the rostral pole of the external cuneate nucleus. It consists of four principal vestibular nuclei, the interstitial nucleus of the vestibular nerve, and other small neuronal subgroups that are not always present in the mammals studied. The main nuclei are classically named superior (SVN), medial (MVN), lateral (LVN) and descending or inferior (DVN) vestibular nucleus. These nuclei are centres of integration of the information that comes from the peripheral vestibular system (PVS) and of the proprioceptive and visual information. The VNC has been an object of study from the beginning of the twentieth century, and the first studies were carried out by Ramón y Cajal (1878) and by Lorente de Nó (1933) in human. Subsequently, many studies have been carried out in humans (Olszewski and Baxter, 1954; Sadjadpour and Brodal, 1968; Ruberton and Haines 1982; Diaz et al., 1993, 1996; Suárez et al., 1997; Álvarez et al., 1998, 2000; Tang et al., 2002) and also in other mammals such as rabbit (Meessen and Olzsewski 1949; Sugawara, 1978), guinea pig (Gstoettner and Burian, 1987), opossum (Henkel and Martin, 1977), cat (Brodal and Pompeiano; 1957; Nyberg-Hansen and Mascitti, 1964; Brodal and Angaut, 1967; Brodal, 1972), chinchilla (Suárez et al., 1989; Gómez et al., 1990; Newman et al., 1992), rat (Suárez et al., 1993), macaque monkey (Brodal, 1984), gorilla (Noback, 1959) and golden hamster (Fernández et al., 2000). The distribution of the VNC and its relationship with neighbouring structures are similar in all of them. However, effects of aging in the different nuclei of the brainstem are different. Some

nuclei show a selective loss of neurons (Sturrock 1990c), others do not present such a selective loss (Vijayashankar and Brody, 1979; Wree et al., 1980; Casey and Feldman, 1982; Sturrock, 1987, 1988b; Marcyniuk et al., 1989; Sturrock, 1989c, 1990a,b) and finally some nuclei do not suffer any loss of neurons with aging (Moatamed, 1966; Königsmark and Murphy, 1972; Monagle and Brody, 1974; Vijayashankar and Brody, 1977a,b; Sturrock, 1989a; Casey, 1990).

Several studies have been done to determine the effects of aging in the peripheral vestibular system. Age-dependent changes in the peripheral vestibular system include loss of hair cells, progressive decrease of primary afferent fibres, degeneration and loss of calcium of otoliths, ruptures of the saccular membrane and accumulation of lipofuscin in the cellular elements of the vestibular system (Babin, 1982; Nakayama et al., 1994; Takumida and Zhang, 1997). Some authors have described small changes in the number of vestibular fibres (Reske-Nielsen and Hansen, 1963; Stuknecht, 1964), while others find a significant reduction of hair cells and nerve fibres of the vestibular receptors (Johnson and Hawkins, 1972; Bergström, 1973; Engström et al., 1974; Rosenhall and Rubin, 1975). The morphologic effects of aging in the VNC have been studied in different mammals (Whiteford and Getty, 1966; Nanda and Getty, 1971, 1973; Jhonson and Miquel, 1974; Sturrock, 1989b), including man (López et al., 1997; Álvarez et al., 1998, 2000, Tang et al., 2002).

The aim of the present work is to achieve a cytoarchitectonic and morphometric study of the four main vestibular nuclei in male hamsters at different ages during lifetime. The analysis of the data obtained in VNC during aging could contribute to the knowledge of neuronal loss pattern and it would also allow establishing the effects that senescence originates in its different regions.

Materials and methods

Male syriam hamsters (*Mesocricetus auratus*) were used in the present study. Animal groups of 3, 12, 18 and 27 months-old were studied. Five specimens were used from each group, comprising a total of 20 hamsters. They were housed in a temperature-controlled room (20±2°C) with a photoperiod of 14/10 h light/dark cycles and free access to laboratory chow and water. Any animals with evidence of macroscopic or microscopic pathology were excluded from the study. The average life span for our golden hamsters is 27 months and the sexual maturity of the males was reached at 6- 8 weeks.

Animals were anaesthetized with sodium pentobarbital (10.5 mg/100 gm body weight) and intracardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.2. Brains were removed and postfixed in the same fixative for about 18 h at 4°C. The brainstem was dissected out, dehydrated and embedded in paraffin. Axial sections 20 µm in thickness were obtained and attached to gelatine-covered slides, dried at 36°C, deparaffined, hydrated and stained with a modification of formaldehyde-thionin method (Tolivia et al., 1994) dehydrated cleared in eucalyptol and mounted with Eukitt. The staining method was applied and commented in previous papers (Díaz et al., 1993, 1996; Navarro et al., 1994; Suárez et al., 1997; Álvarez et al., 1998, 2000).

To study the neuronal morphometric parameters, all neurons of the principal vestibular nuclei were drawn in sections separated 80 mm with the aid of a camera lucida under x200 magnification. Only the neurons in which the nucleus was visible were considered, and special care was taken to discard glial cells. With the staining method used (formaldehyde-thionin), neurons and glial cells show appreciable differences in cytoplasmatic and nuclear coloration, which permits the distinction between the two types of nerve cells. When this method is applied, the neurons show a dark blue cytoplasm and a pale blue nucleus, whereas the cytoplasm of the glial cells does not appear colored. We also drew the profiles of the neuronal nuclei at high magnification (x600) to calculate the equivalent diameter (the diameter of a circle of the same area as the one we have drawn) to obtain the correction factor. To calculate the volume of the vestibular nuclei we drew the profile of each section of every nucleus and, according to the Cavalieri principle, the volume is the sum of the products of the each section area multiplied the distance between two adjacent sections.

The total number of neurons was estimated by multiplying the mean number of neurons counted in the sections by the number of sections that spanned the region. These data were corrected with a factor reported by Abercrombie (1946).

The neurons of the vestibular nuclei were separated according to their cross-sectional area in three arbitrary groups, small cells (<200 mm^2), medium cells (200-500 mm2) and large cells (>500 mm^2), to establish the neuronal distribution. These neuronal groups were similar to that established in previously related papers (Álvarez et al., 1998, Díaz et al., 1993, 1996; Suárez et al., 1993, 1997).

The study of morphometric parameters and the volume of the vestibular nuclear complex were accomplished with Image Tool for Windows (Version 3.0). Data obtained from morphometric study were statistically tested to estimate the significance of the results. The test of Kolmogorov-Smirnov with the correction of Lilliefors was used to evaluate the fit of the data to a normal distribution. A regression analysis was done to study the correlation between age and the variation of the number and size of neurons. The level of significance was established in $P<0.05$. Statistical study was accomplished with the program SPSS 8.0. Throughout this study, values are expressed as mean±SEM. The methodology used in the present study was applied previously by us to study morphometric characteristics of the nucleus supraopticus of the hamster

(Navarro et al., 1994) and the human vestibular nuclei (Díaz et al., 1993, 1996; Suárez et al., 1997) and the effects of aging on the human vestibular nuclei (Álvarez et al., 1998, 2000). The validity of this methodology to evaluate the morphometric aging variations is supported by the study of Tang et al. (2002). These authors use modern unbiased stereological methods to study the effects of aging on the human medial vestibular nucleus and found the same results previously obtained by them (López et al., 1997) and by our group (Álvarez et al., 1998) using the same stereological method applied in the present study.

Results

Superior vestibular nucleus (SVN)

The SVN shows rostrocaudally an ovoid morphology in transverse sections. The first nucleus of the vestibular area, in rostrocaudal direction, is the SVN. Throughout its length, the SVN is located in the ventral part of the VNC, in the floor of the fourth ventricle. Its rostral pole appears at the caudal level of both the sensory trigeminal nucleus and the *locus coeruleus*, and ends at the level of the rostral third of the abducens nucleus. The nucleus is bounded ventrally by the mesencephalic trigeminal nucleus during 60-120 micrometers and medially by the beginning of the rostral level of the *locus coeruleus* (Fig. 1a). Rostrally, the nucleus intrudes posteriomedially between the *brachium conjunctivum* (dorsally located) and both the locus coeruleus and the mesencephalic trigeminal nucleus (medially located). In the intermediate level the SNV is ventromedially bounded by the medial vestibular nucleus (MVN). Caudally the SVN appears ventrally related with the most rostral level of the lateral vestibular nucleus (LVN) and ends between the LVN and MVN (Fig. 1b).

The average volume of the SVN is 0.40 + 0.03 mm^3. This value ranges from 0.5 mm^3 in the 3-month-old specimens to 0.32 mm^3 in the 27-month-old specimens (Table 1). The volume decrease correlates negatively with age (r=-0.550, P=0.032), which is statistically significant. The average length of the SVN is 0.55±0.01 mm. This value ranges from 0.57 mm in the 12-month-old specimens to 0.53 mm in the 27-month-old specimens. The variations with age are not significant.

The neuronal morphology shows an oval or rounded shape (Fig. 1e,f) and it does not change with age. The medium-sized neurons constitute the most abundant group (50%), followed by small (42%) and giant neurons (8%) in the 3-month-old specimens (Fig. 1c, d). With aging, an increase in the small neurons (68% at 27-month) and a decrease of the other groups are found (Table 2). These changes are not statistically significant.

The neuronal nucleus is oval or rounded and occupies a central position, with a prominent nucleolus (Fig. 1e,f). The value of its diameter ranges from 10.5 mm at 3 month-old to 11.1 mm in the 18 month-old specimens (Table 3). Age-related variations are not significant.

The average number of neurons in the SVN is 3137±27, this value ranges from 3873±33 neurons in the 3-month-old specimens to 2660±23 neurons in the 18-month-old specimens (Table 4; Fig. 5). The change in the number of neurons is not significant.

Medial vestibular nucleus (MVN)

The MVN shows, in transverse sections, two regions with different morphology. The rostral third is rounded and the two caudal thirds are fusiform or oval shaped. The MVN extends from the caudal half of the SVN to the rostral pole of the external cuneate nucleus. This

Table 1. Volume and length of the vestibular nuclei.

Age (months)	SVN	MVN	LVN	DVN
Volume (mm^3)				
3	0.50±0.02	0.94±0.11	0.94±0.05	0.88±0.05
12	0.37±0.02	1.04±0.11	0.81±0.06	0.75±0.03
18	0.40±0.06	0.84±0.04	0.75±0.04	0.75±0.06
27	0.32±0.09	0.96±0.14	0.74±0.06	0.78±0.09
Length (mm)				
3	0.55±0.01	1.17±0.01	0.65±0.01	0.74±0.01
12	0.57±0.01	1.23±0.03	0.63±0.01	0.74±0.01
18	0.53±0.01	1.22±0.02	0.62±0.01	0.72±0.01
27	0.53±0.02	1.22±0.05	0.63±0.02	0.72±0.01

(value±S.E.M.).

Table 2. Percentage of the different types of neurons in the vestibular nuclei.

Age (months)	<200 mm^2	200-500 mm^2	>500 mm^2
SVN			
3	42	50	8
12	76	23	1
18	84	16	0
27	68	31	1
MVN			
3	85	15	0
12	87	13	0
18	92	8	0
27	84	16	0
LVN			
3	55	32	13
12	52	33	15
18	67	22	11
27	52	35	13
DVN			
3	60	36	4
12	72	26	2
18	81	18	1
27	73	26	1

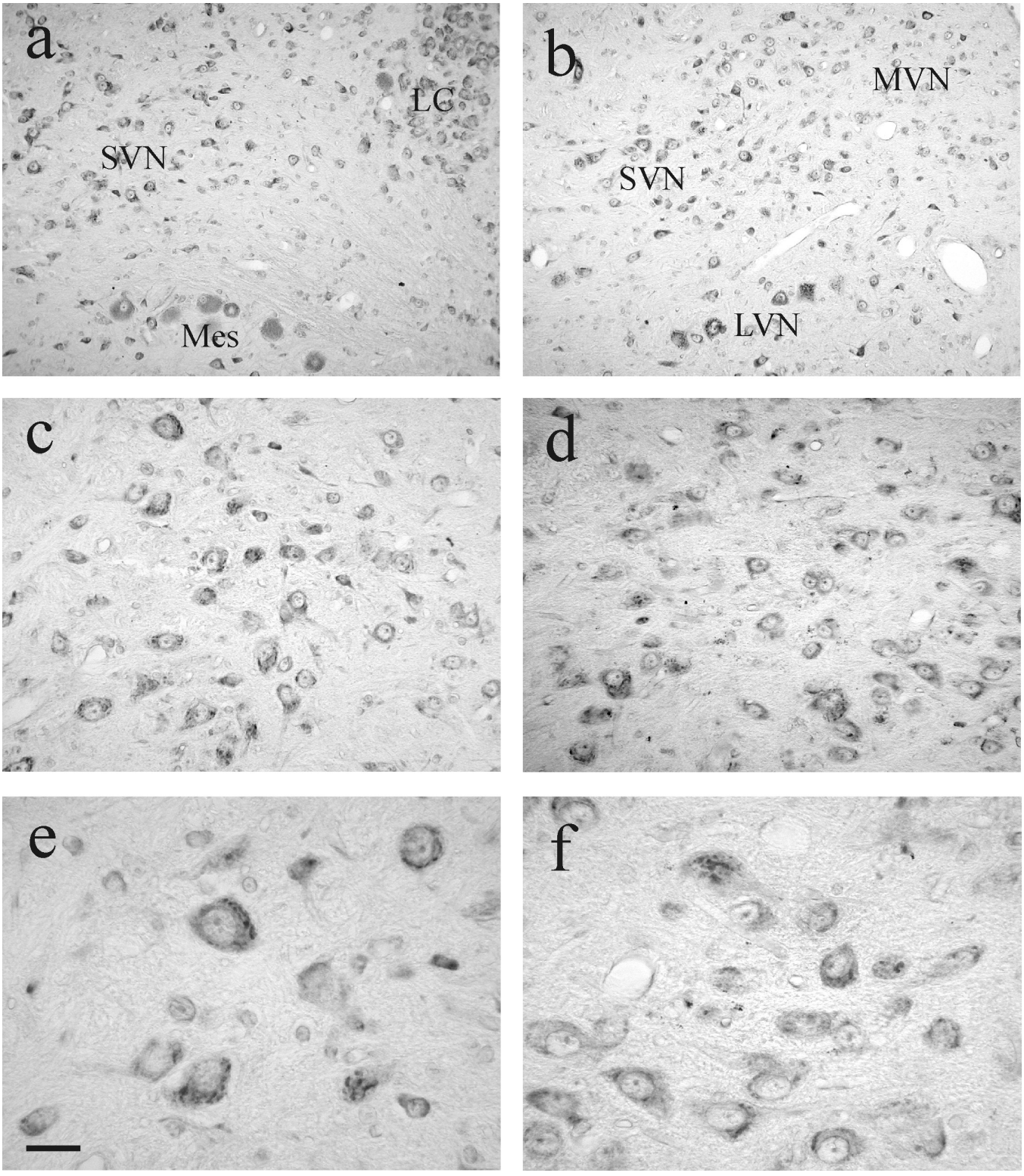

Fig. 1. Location of SVN and comparison of neuronal population in normal and aging subjects. **a.** The nucleus is bounded ventrally by the mesencephalic trigeminal nucleus (Mes) and medially by the beginning of the rostral level of the locus coeruleus (LC). **b.** Caudally it appears related ventrally with the most rostral level of the LVN and ends between the LVN and the MVN. **c-d.** The medium-sized neurons constitute the most abundant neuronal group. **e-f.** The neuronal morphology shows an oval or rounded shape and does not change with age. (a, b, c, e) SVN 3 month. (d, f) SVN 27 month. Bar: a,b, 72 µm; c,d, 36 µm; e,f, 18 µm.

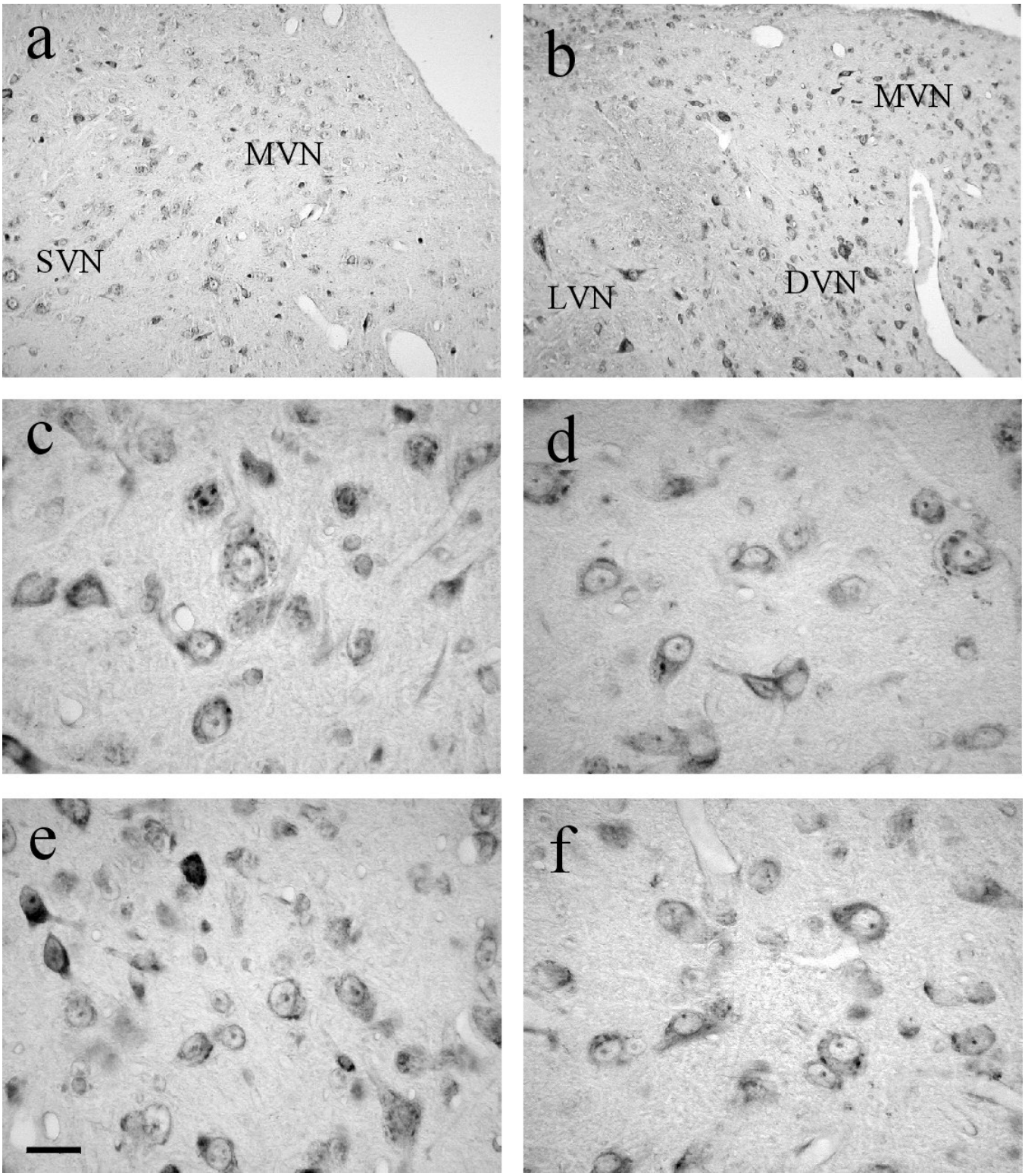

Fig. 2. Location of MVN and comparison of neuronal population in normal and aging subjects. **a.** The MVN appears limited laterally by the SVN in its more rostral region, and caudally by the multipolar neurons of the LVN. **b.** Caudally the MVN appears laterally related with the DVN. The delimitation between these two nuclei is less clear than with the LVN. The MVN shows a regional distribution of its neurons: the medium cells appear preferentially in the rostral region **(c, d)** while in the caudal region are more numerous the small neurons **(e, f). (a, b, c, e)** MVN 3 month. **(d, f)** MVN 27 month. Bar: a,b, 72 µm; c-f, 18 µm.

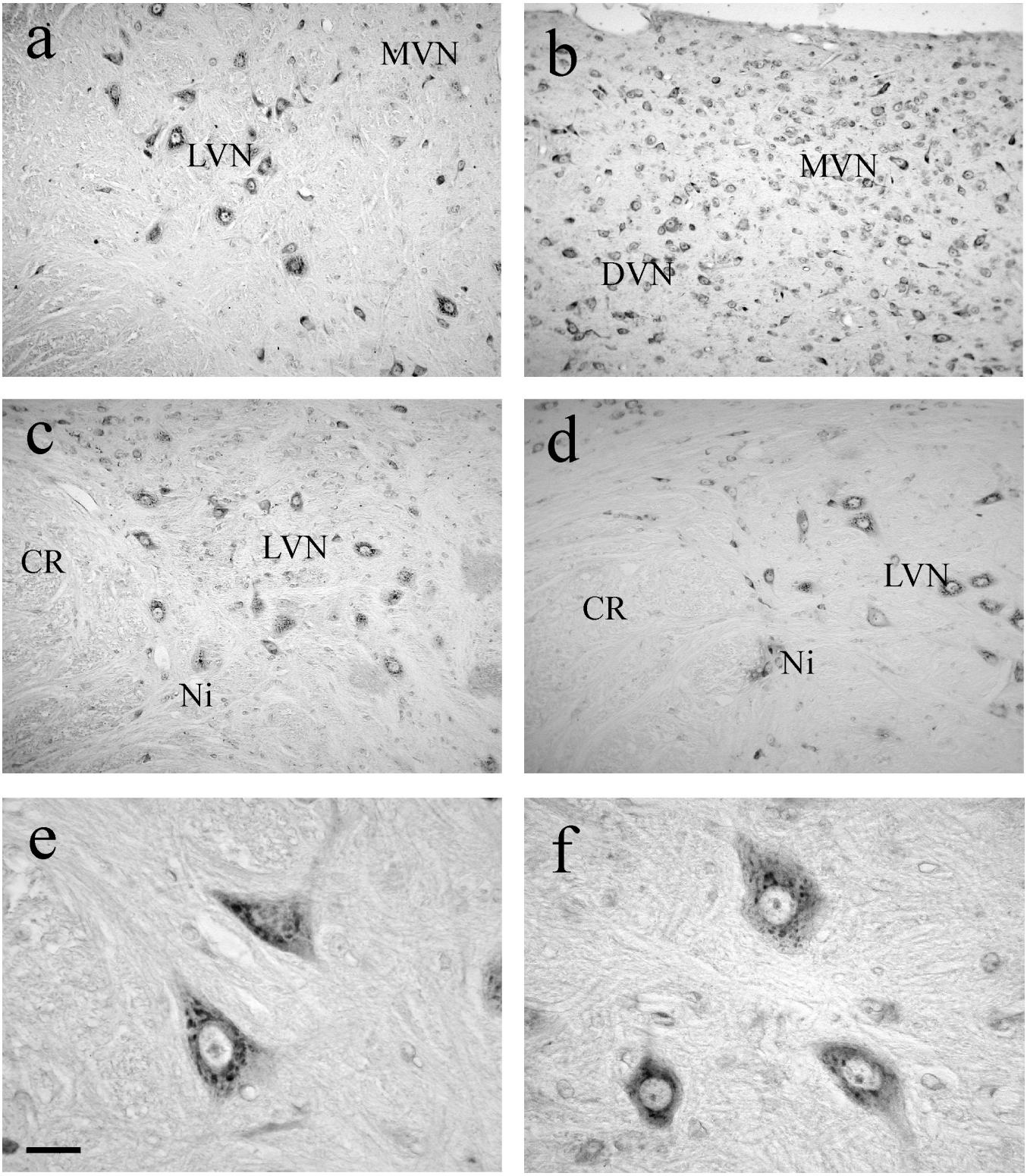

Fig. 3. Location of LVN and comparison of neuronal population in normal and aging subjects. **a.** The LVN appears limited by the MVN at its mediocaudal level. **b.** Its caudal pole is replaced gradually by the DVN in a limit not very established. **c, d.** Laterally the nucleus is related with the restiform body (CR) and the interstitial nucleus (Ni) associate group described by Cajal. **e, f.** These neurons generally show a pyramidal or elongated shape and their morphology does not change with age. **(a, b, e)** LVN 3 month. **(c)** LVN 12 month. **d, f.** LVN 27 month. Bar: a-d, 72 µm; e,f 18 µm.

nucleus is sited beneath the floor of the IV ventricle and appears medially limited by the *nucleus praepositus hipoglossi*. The MVN appears limited laterally by the SVN in its rostral region, and by the multipolar neurons of the LVN in its caudal region (Fig. 2a). The MVN is easy to distinguish from the SVN and the LVN because there are noteworthy differences in the fibroarchitecture and neuronal morphology. In the caudal region, the MVN appears related with the DVN (Fig. 2b). The boundaries between both nuclei are very clear, because many myelinic fibres cross the DVN.

The MVN is the biggest vestibular nucleus with an

Table 3. Diameter of neuronal nucleus (mm).

Age (months)	SVN	MVN	LVN	DVN
3	10.5±0.2	10.6±0.2	11.4±0.2	10.1±0.2
12	10.9±0.2	10.2±0.2	12.8±0.2	11.5±0.2
18	11.1±0.2	9.7±0.2	13.3±0.2	10.9±0.2
27	10.7±0.2	10.1±0.2	12.1±0.2	10.7±0.1

Table 4. Number of neurons in the vestibular nuclei.

Age (months)	SVN	MVN	LVN	DVN
3	3873±33	11819±110	2945±26	4129±39
12	3153±28	11092±105	2876±24	4158±36
18	2660±23	9848±48	2944±24	4859±46
27	2862±25	11476±54	2925±27	3988±23

(number±S.E.M.).

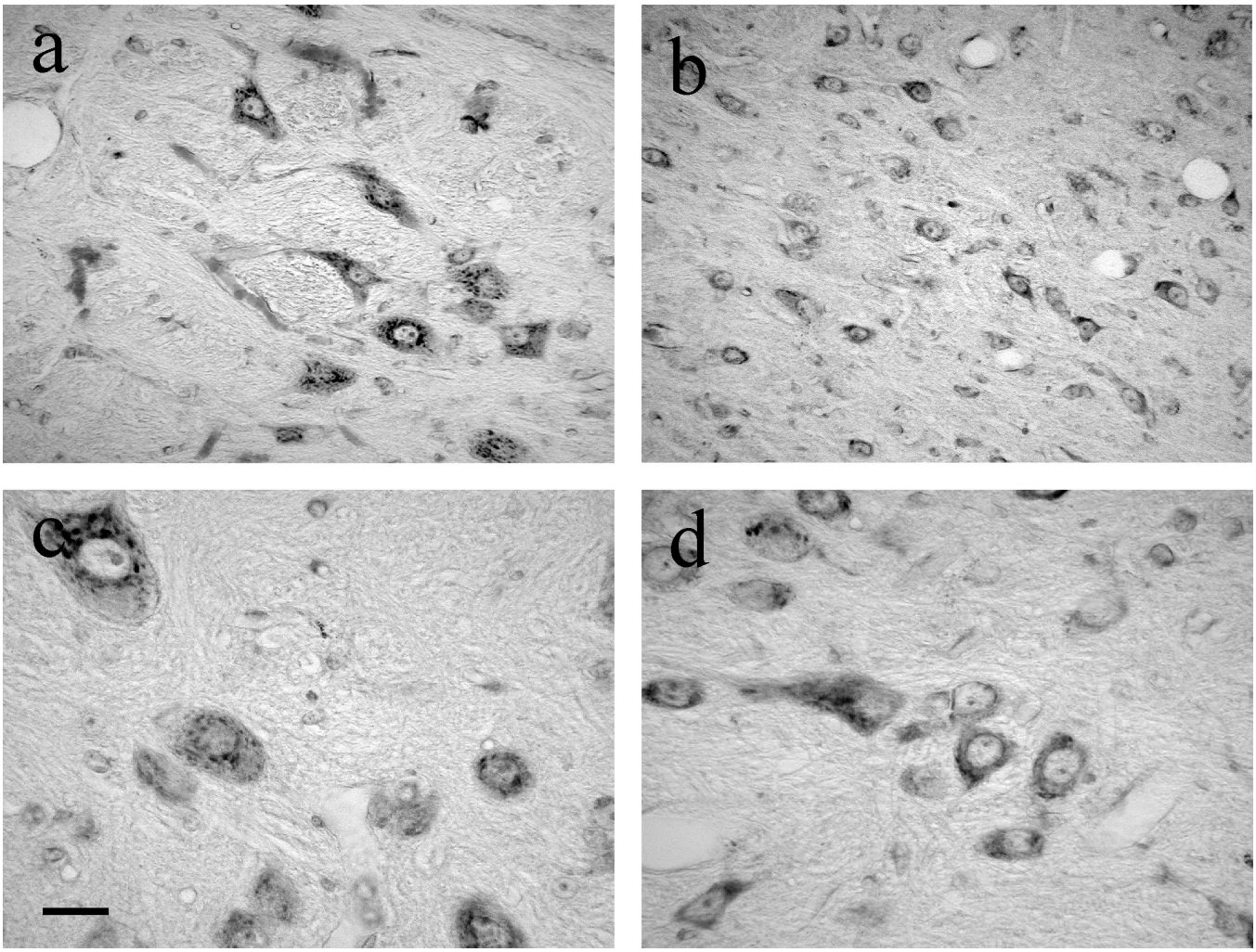

Fig. 4. Comparison of neuronal population of DVN in normal and aging subjects. **a, b.** The neurons are scattered due to the presence of nervous fascicles. The neuronal morphology of medium and large neurons shows a multipolar or fusiform shape while the small neurons are oval in morphology **(c, d)**. **(a, b, d)** DVN 27 month. **(c)** DVN 12 month. Bar: a, b, 36 µm; c, d, 18 µm.

average volume of 0.95±0.05 mm^3 (Table 1). This value ranges from 0.84 mm^3 in the 18-month-old specimens to 1.04 mm^3 in the 12-month-old specimens (Table 1). The average length of the MVN is 1.21±0.03 mm. This value ranges from 1.17 mm in the 3-month-old hamsters to 1.23 mm in the 12-month-old hamsters. The variations of length and volume are not significant.

The neurons of the MVN adopt the most compact arrangement in the VNC. Its neurons show an oval or rounded shape and it does not change with age. The MVN shows regional differences in the distribution of its neurons, the medium cells appear preferentially in the rostral region while small neurons are the main type of cell in the caudal region (Fig. 2c-f). The small-sized neurons constitute the most abundant neuronal group (85%), followed by medium-sized neurons (15%) in the youngest specimens (Table 2). The variation with age is not significant.

The neuronal nucleus is oval or rounded and it is located in a central or slightly eccentric position, with only one apparent nucleolus (Fig. 2c-f). The value of its diameter ranges from 10.6 mm in the 3 month-old to 9.7 µm in the 18 month-old specimens (Table 3). The decrease of nuclear diameter with age is statistically significant (r=-0.448, P=0.010).

The MVN has the largest number of neurons of VNC, with an average number of 11059±79. This value ranges from 11819 neurons in the 3-month-old specimens to 9848 neurons in the 18-month-old specimens (Table 4, Fig. 5). The analysis of data does not show any statistical difference in the variation of the number of neurons.

Lateral vestibular nucleus (LVN)

This nucleus shows a triangular shape in transverse sections. The LVN is located in the pons, in a lateroventral position with respect to the MVN, caudal to the SVN and rostral to the DVN. The LVN appears dorsolaterally limited by the SVN and rostrally by the MVN at its mediocaudal level (Fig. 3a). Its caudal pole is replaced gradually by the DVN. The caudal two thirds of the DVN are ventrally related with the small neurons of the nucleus of the solitary fascicle, and laterally with the restiform body and the root of the vestibular nerve where the interstitial nucleus of Cajal can be observed (Fig. 3c,d).

The average volume of the LVN was 0.81±0.03

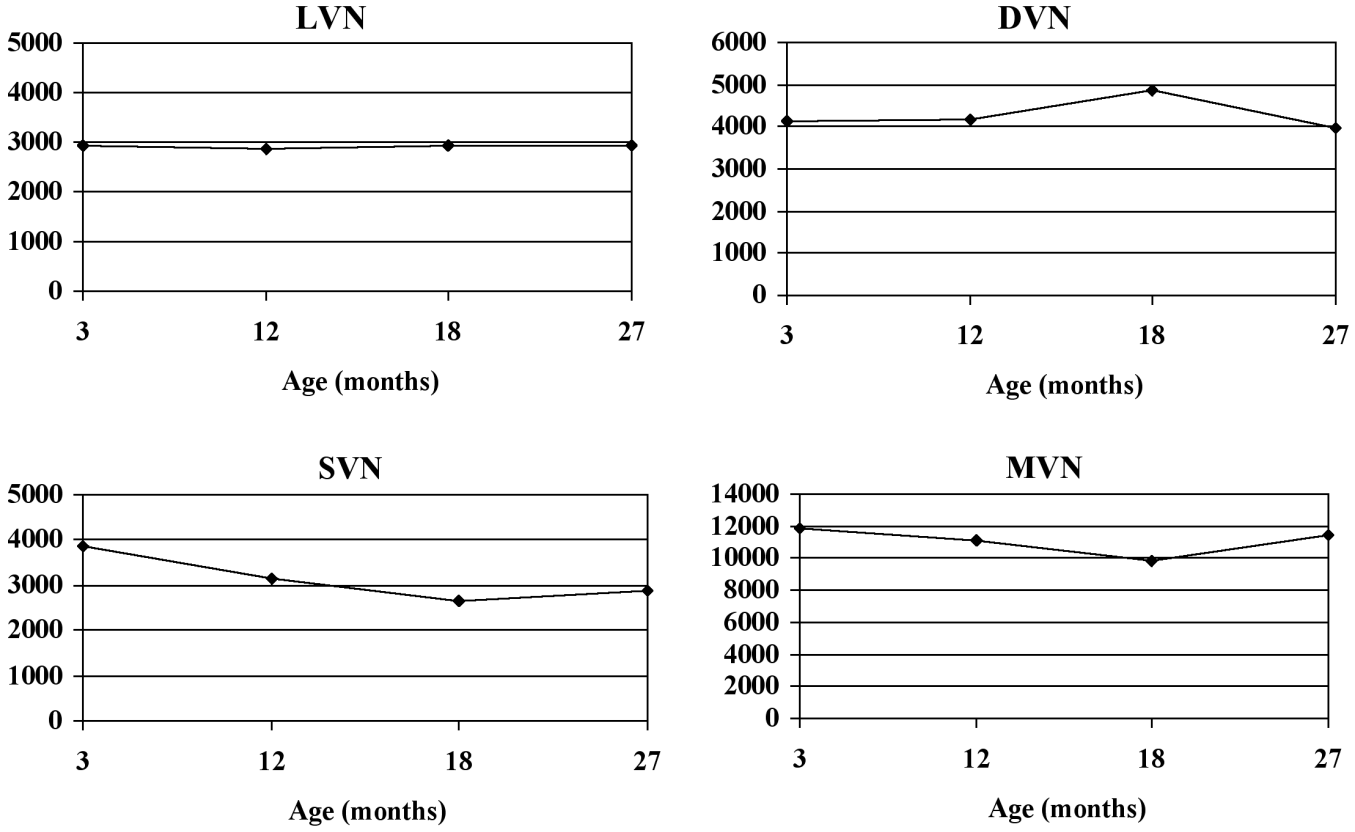

Fig. 5. Variations in the number of neurons of the vestibular nuclei during aging.

mm^3. This value ranges from 0.94 mm^3 in the 3-month-old to 0.74 mm^3 in the 27-month-old hamsters (Table 1). The decrease of volume, related with aging, is statistically significant (r=-0.654, P=0.010). The average length of the SVN is 0.63±0.01 mm (Table 1), this value ranges from 0.65 mm in the 3-month-old specimens to 0.62 mm in the 18-month-old specimens. The variation with age is not significant.

The neurons of the LVN do not differ from those of the rest of the vestibular nuclear complex, except for the presence of Deiters' cells (giant neurons). These neurons generally show a pyramidal or elongated shape. The neuronal morphology does not change with age (Fig. 3e,f). The LVN also shows regional differences in the distribution of its neurons. The large-sized neurons and Deiters' cells appear located preferentially in the dorsocaudal region of the nucleus. The small-sized neurons constitute the most abundant group (55%), followed by medium (42%) and large neurons (13%) in the 3-month-old specimens (Table 2).

The neuronal nucleus is rounded and located in a central position, with a prominent nucleolus. Its diameter ranges from 11.4 mm in the 3 month-old to 13.3 mm in the 18 month-old specimens (Table 3). The nuclear diameter increases slightly with aging, but this variation is not significant.

The LVN has fewer neurons than the other nuclei, with an average number of 2923±25; this value ranges from 2945±26 neurons in the 3-month-old specimens to 2925±27 neurons in the 27-month-old specimens (Table 4; Fig. 5). There are not significant differences in the number of neurons among groups.

Descending vestibular nucleus (DVN)

This nucleus presents an oval-shaped morphology in transverse sections. Its rostral pole replaces progressively the LVN. Their rostral limit is complicated to define and appears constituted by multipolar and pyramidal neurons of medium size. The DVN is limited dorsolaterally by the MVN (Fig. 3b). The inferior third of the DVN is limited laterally by the restiform body and the external cuneate nucleus and ventrally by the nucleus of the solitary fascicle.

The average volume of the DVN is 0.79±0.03 mm^3. This value ranges from 0.88 mm^3 in the 3-month-old to 0.75 mm^3 in the 18-month-old groups (Table 1).The variation of volume with age is not significant. The average length of the DVN is 0.73±0.01 mm. The length of nucleus ranges from 0.74 mm in the 3-month-old specimens to 0.72 mm in the 27-month-old specimens. The variation of length with age was statistically significant (r= -0.7353, P=0.048).

The neurons of the DVN show a disperse arrangement due to the presence of nervous myelinated fascicles. The neuronal morphology of medium and large neurons shows a multipolar or fusiform shape while the small neurons are oval in morphology (Fig. 4a-d). The neuronal morphology does not change with age (Fig. 4c,d). The small-sized neurons constitute the most abundant neuronal group (60%), followed by medium neurons (36%) and large neurons (4%) in the youngest group (Table 2). The variations with age are not significant.

The neuronal nucleus is oval or rounded and occupies a central or slightly eccentric position, with only one nucleolus. The diameter ranges from 10.1 µm in the 3 month-old to 11.5 µm in the 12 month-old specimens (Table 3). The variations related to age are not significant.

The average number of neurons in the DVN is 4284±36. This value ranges from 4859±46 neurons in the 18-month-old to 3988±23 neurons in the 27-month-old specimens (Table 4; Fig. 5). The correlation analysis does not show any significant differences in the number of neurons.

Discussion

Many authors have studied the age changes in several regions of the central nervous system (CNS), but only few of published works deal with the effect of aging in the vestibular nuclei. Some encephalic regions of the human, like the prosencephalon (Brody, 1955; Colon, 1972; Shefer, 1973; Bugiani et al., 1978; Henderson et al., 1980; Mann et al., 1984; Lacalle et al., 1991), the cerebellum (Ellis, 1919; Hall et al., 1975) and some nuclei of the brainstem (Vijayashanhar and Brody, 1979; Wree et al., 1980; Marcyniuk et al., 1989) present a clear reduction in their neuronal populations. However, the studies realized on the VNC show contradictory results. Sturrock (1989b) found a significant loss of large neurons in the LVN of the mouse. López et al. (1997) described a significant reduction of neuron number in all the vestibular nuclei of man, being particularly important in the SVN. Thus, Álvarez et al. (1998, 2000) also found a significant loss of neurons in the LVN, the MVN and the DVN while the neuronal population of the SVN was not affected by aging.

We are going to discuss the results from our cytoarchitectonic and morphometric study in the VNC of the hamster by comparing them with those found in the literature.

Superior vestibular nucleus

The ventromedial region of the SVN in the hamster appears related with the *nucleus mesencephalicus* of the trigeminal nerve as occurs in human (Sadjadpour and Brodal, 1968; Suárez et al., 1997; Álvarez et al., 2000) and in chinchilla (Suárez et al. 1989; Gómez et al., 1990; Niewman et al., 1992), while in rat (González del Rey et al. 1991; Suárez et al., 1993), cat (Brodal and Pompeiano, 1957; Hauglie-Hanssen, 1968) and rabbit (Sugawara, 1978) this nucleus is related with the dorsomedial region of the SVN. We have also seen that the locus coeruleus appears related with the ventromedial region of the SVN in the golden hamster,

while in the rat and rabbit it is found to be associated with the dorsomedial region.

Concerning the cytoarchitecture of the SVN, there is not uniform description either. A neuronal heterogeneous population has been described for shape, size and dendrite orientation, prevailing small and medium-sized neurons. The medium-sized neurons are more abundant in the central region in rat (Suárez et al., 1993), cat (Brodal and Pompeiano, 1957; Hauglie-Hanssen, 1968), opossum (Henkel and Martin, 1977) and galago (Rubertone and Haines, 1982). Conversely, in human (Sadjadpour and Brodal, 1968; Suárez et al., 1997; Álvarez et al., 2000) and guinea pig (Gstoettner and Burian, 1987) medium neurons are more numerous in the caudal region.

The diameter of the neuronal nuclei of the SVN does not show significant differences among the different groups of age. These results are similar to that described in human SVN (Álvarez et al., 2000) in the small neurons of the mouse LVN (Sturrock, 1989b) and in other neuronal nuclei of the brainstem such as superior olivary complex (Casey, 1990), nucleus retrofacialis of the mouse (Sturrock, 1988b) and external cuneatus nucleus (Sturrock, 1989c). Other studies found significant increments in the diameter of the neuronal nucleus with aging in some nuclei of the brainstem: mesencephalic and motor trigeminal nuclei (Sturrock, 1987), motor facial nucleus (Sturrock, 1988a), abducens nucleus (Sturrock, 1989b), red nucleus (Sturrock, 1990c), ventral and lateral pontine nuclei (Sturrock, 1990b).

The length of the SVN scarcely varies with aging, while its volume suffers an important reduction. These data agree with those obtained in human (López et al., 1997; Álvarez et al., 2000). We have not found significant differences in the number of neurons with aging according to Álvarez et al. (2000) in human, however, López et al. (1997) find a significant decrease.

We have observed in the SVN of the golden hamster a notable reduction in the percentage of the large neurons with aging. This agrees with the studies done in other animals, such as red nucleus and LVN of the mouse (Sturrock, 1989a, 1990c). The selective loss of neurons could be caused by the retrograde transneuronal degeneration secondary to the loss of spinal motor neurons, or could be due to the anterograde transneuronal degeneration secondary to the loss of a large number of Purkinje cells in the anterior region of the cerebellum. On the other hand, the ventral portion of the LVN of the mouse, where the small neurons are more abundant, is the region where primary vestibular fibres end. This fact could be a preventive factor, according to Sturrock (Sturrock, 1989a), for the loss of the small neurons, which is observed with aging, because these fibres do not suffer a significant decrease with age. In human, Álvarez et al. (2000) also found a reduction in the percentage of large neurons of the SVN. Our results are consistent with the functional alterations in the integration of the vestibulo-ocular reflexes, in which this nucleus plays an important role.

Medial vestibular nucleus

It is the largest of the main vestibular nuclei in the golden hamster, and in all animals studied, with the exception of the chinchilla (Suárez et al., 1989; Gómez et al., 1990; Newman et al., 1992). As in most of the mammals studied, the limits with the SVN and the nucleus praepositus hypoglossi in hamsters are imprecise, due partly to the presence of neurons similar in size and morphology in the region of transition between these nuclei and hindering sometimes their correct delimitation (Sadjadpour and Brodal, 1968; Brodal, 1984; Suárez et al., 1989, 1993, 1997; Gómez et al., 1990; Díaz et al., 1996; Álvarez et al., 2000). The relationships of MVN with the rest of the nuclei of the VNC and with other brainstem structures are similar to those described in the rat (González et al., 1991; Suárez et al., 1993), the chinchilla (Newman et al., 1992; Gómez et al., 1990; Suárez et al., 1989) and man (Sadjadpour and Brodal, 1968; Díaz et al., 1996; Suárez et al., 1997; Álvarez et al., 1998, 2000).

The MVN shows a uniform neuronal morphology. The medium size neurons are more abundant in the rostral zone, and the small neurons appear concentrated in the caudal half. In some species, but not in hamsters, giant neurons can be found in its superior third as was described in the rat (González et al., 1991; Suárez et al. 1993), the guinea pig (Gstoettner and Burian, 1987), the macaque (Brodal, 1984) and man (Díaz et al., 1996; Suárez et al., 1997).

The length and volume of the MVN nucleus in the golden hamster do not change with aging, which agrees with the data obtained in human (López et al., 1997; Álvarez et al., 1998, 2000). The nuclei of MVN neurons diminish progressively with aging showing significant differences. These results differ from the studies in different brainstem nuclei of rodents and human (Moatamed, 1966; Sturrock, 1987, 1988a, 1989a, 1989b, 1990b, 1990c), and also in the MVN of the man (Álvarez et al., 1998, 2000) where a significant increment of the neuronal nuclear diameter during aging was described.

The neuronal population of the MVN in the golden hamster does not vary with aging. These results are different from those obtained in human where a reduction in the number of neurons with aging was reported (López et al., 1997; Álvarez et al., 1998, 2000, Tang et al., 2002). The small size neurons prevail in all ages and we have not found significant differences with aging in the percentage of neurons according to their size. These data contrast with those obtained in the MVN of man (Álvarez et al., 1998, 2000), where a significant increment in the number of neurons of medium size has been reported.

The MVN is, with the SVN and the DVN, the main source of the commissural connections, which coordinate the function of the vestibular nuclei of both

sides (Carleton and Carpenter, 1983). The MVN seems to be a coordination centre of eyes and head movement, and can be very important for vestibular compensation after labyrinth lesions (Baloh and Honrubia, 1990). The alterations in the postural control that appear with age and the failure in their compensation could have a relationship with the variations in size of its neuronal nuclei.

Lateral vestibular nucleus

Some authors have divided the LVN of mammals into a rostroventral and a dorsocaudal region, excepting the gorilla (Noback and Goss, 1959) and the human (Sadjadpour and Brodal, 1968; Díaz et al., 1993; Suárez et al., 1997; Álvarez et al., 2000), in which a medial and a lateral region are established. On the other hand, most of the authors describe the existence of a major concentration of giant cells in the dorsocaudal region of the LVN (Brodal and Pompeiano, 1957; Brodal, 1984; Gstoettner and Burian, 1987; Gómez et al., 1990; Newman et al., 1992; Suárez et al., 1997). In human, the LVN shows a different distribution and a progressive magnification of the neuronal size in the caudorostral direction (Díaz et al., 1996; Suárez et al., 1997), with the biggest percentage of giant neurons in the rostral region. We have observed in the golden hamster a topographical distribution regarding the size of the neurons, with the larger cells located mainly in the dorsocaudal region of the LVN. We have not appreciated the existence of two different regions within the LVN, as were described in other species.

The cytoarchitecture of the LVN is conspicuous for the presence of Deiters´ giant pyramidal cells. These neurons constitute not more than 15% of the neuronal population of the LVN in the golden hamster. Rubertone and Haines (1982), in the galago, found that Deiters´ cells represent 47% of the neurons of the LVN. The rest of the neurons are of small and medium size.

The diameter of neuronal nuclei does not show significant differences with aging, which agrees with the studies made in humans (Álvarez et al., 2000). These results differ from the studies of Moatamed (1966) in the inferior olivary nuclear complex and Sturrock in different brainstem nuclei, like the mesencephalic and motor nuclei of the trigeminal nerve, motor facial nucleus, abducens nucleus, lateral vestibular nucleus, red nucleus or ventral and lateral pontine nuclei (Sturrock, 1987, 1988a,b, 1989b, 1990b,c).

The LVN of the golden hamster does not show significant variations of length with aging as occurs in the LVN of man (López et al., 1997; Álvarez et al., 2000). The volume of the LVN suffers an important reduction with aging, which agrees with the results of López et al. (1997) in the LVN of man. Álvarez et al. (2000), however, do not find any significant change in this parameter with aging.

The neuronal population of the LVN hardly suffers variation with aging in contrast to the LVN of the mouse (Sturrock, 1989b), which shows a significant reduction in the number of large neurons. In addition, a significant loss of neurons has been reported in the LVN of man (López et al., 1997; Álvarez et al., 2000). We have not found significant differences with aging in the percentage of neuron size in the LVN, which differs from the results reported by Álvarez et al. (2000) in the LVN of man, where a significant increase of the medium-sized neurons was found.

According to the hypothesis of Sturrock (1989b), aging and the related muscular atrophy would originate a loss of motor neurons of the spine due to retrograde degeneration, which causes a reduction in the number of neurons in the LVN. In addition, the neuronal loss could be due to anterograde transneuronal degeneration secondary to the loss of Purkinje cells of the anterior region of the cerebellum. We have not observed a significant reduction in the number of neurons so we hypothesize that other alterations at neuronal cytoplasmatic level could interfere with spine-vestibular reflexes and finally alter the balance in the old animals.

Descending vestibular nucleus

The neuroanatomic relationships of the DVN the golden hamster are similar to in the rat (González et al., 1991, Suárez et al., 1993), the chinchilla (Suárez et al., 1989; Gómez et al., 1990; Newman et al., 1992) and man (Suarez et al., 1997). However, some accessory groups (named “z" and "g "in human and "g" in the chinchilla) were not found in our study of the hamster VNC.

Small and medium-sized are the most abundant neurons but there are also few number of large neurons and Deiters´ cells as occurs in other species. This morphologic pattern is similar to the rat, the chinchilla and man. The neuronal population is also characterized by its distribution in small neuronal groups, separated by many fibres. We have observed a regional distribution of the neurons in the golden hamster similar to that described in the rat (González et al., 1991, Suárez et al., 1993), the guinea pig (Gstoettner and Burian, 1987), the macaque (Brodal 1984) and man (Díaz et al. 1993; Suarez et al., 1997; Álvarez et al., 2000). The large and Deiters´ neurons are concentrated in the rostral pole of the nucleus and a progressive decrease of neuronal size in caudal direction is observed. These findings support the existence of an anatomic-functional unit of the arch of the vestibulo-ocular reflexes cited previously.

The neuronal nuclear diameter does not change with age in hamster, which is similar to the results obtained in other brainstem nuclei (Casey, 1990; Sturrock, 1988b, 1989a,c), and differ from some studies that show significant increments of the nuclear diameter (Moatmed, 1966; Sturrock, 1987, 1988, 1989a,b, 1990b,c).

The length of the DVN decreases with aging, being the only nucleus of the VNC of the golden hamster that shows statistically significant differences. Our data are

similar to those found by López et al. (1997), but different to those reported by Álvarez et al. (2000) in humans. The volume of DVN in the hamster does not suffer significant changes, which agrees with the results of Álvarez et al. (2000).

The number of neurons in our series does not reduce with aging. This result differs from those found in human where a significant reduction in the number of their neurons was observed (López et al., 1997; Álvarez et al., 2000).

In the DVN a reduction of medium and large-sized neurons is observed which produces an increase in the percentage of the small-sized neurons. Similar results have been described in the LVN and the red nucleus of the mouse (Sturrock, 1989b, 1990c). Álvarez et al. (2000) found a reduction of the small neurons in the DVN of man and an increase of the rest of neuronal types. The relative increment of the giant neurons with aging was statistically significant in humans. López et al. (1997) consider change as the consequence of an increment in the size of the small neurons produced by accumulation of lipofuscin granules during aging.

The DVN have a large number of afferents from the peripheral vestibular system that come from the *cristae ampullaris* and the maculas (Stein and Carpenter, 1967). On the other hand, the spinal afferences are scarce and they reach only the caudal region of the DVN (Angaut and Brodal, 1967; Rubertone and Haines, 1982). The cerebellar afferences are distributed throughout all nuclear regions intermingled with the vestibular afferences (Baloh and Honrubia, 1990). We think that the reduction in the number of large neurons with aging could generate an alteration in the DVN as integrator of labyrinthic and cerebellar signals and contribute to the postural alterations of the equilibrium. These changes, according to the hypothesis of Sturrock (1989b), could deal with a transneuronal retrograde degeneration, caused by a loss in the neurosensitive epithelium of cristae ampullaris and maculas of the vestibular peripheral system or could be caused by anterograde transneuronal degeneration secondary to the loss of Purkinje cells.

In conclusion, we have observed that the distribution of the neurons within the vestibular nuclei of the golden hamster does not suffer important changes with the aging process and that the morphometric parameters measured vary slightly. Probably, VNC of the golden hamster constitutes an area of the CNS particularly protected from neuronal changes induced by aging as was previously described in other brainstem nuclei of different species. The results of the present study are relevant only for male animals and the effect of senescence could be different in female vestibular nuclear complex.

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