AGING AND THE HUMAN VESTIBULAR NUCLEUS

Ivan Lopez,* Vicente Honrubia,* and Robert W. Baloh,*†

UCLA School of Medicine, *Division of Head and Neck Surgery,
†Department of Neurology, Los Angeles, California

Abstract—Degenerative changes during aging have been identified in the inner ear and in the vestibular nerve, but not in the human vestibular nuclear complex (VNC). Therefore, the purpose of this study was to document quantitative morphometric changes within the VNC in humans as a function of age. The VNC of normal human subjects was examined for age-related changes using computer-based microscopy. Neuronal counts, nuclear volume, neuronal density, and nuclear length of the 4 vestibular nuclei were determined in 15 normal people, age 40 to 93 years. Based on a linear model, there was approximately a 3% neuronal loss per decade from age forty to ninety. VNC volume and neuronal density also decreased significantly with age, although to a lesser degree than did the number of neurons. Neuronal loss as a percentage of the total number of neurons was greatest in the superior vestibular nucleus and least in the medial vestibular nucleus. Despite the overall loss of neurons, the number of giant neurons (>500 μm²) increased in older people. This increase in giant neurons could be traced to the accumulation of lipofuscin deposits in the cell somata. The overall rate of neuronal loss with aging in the VNC is comparable to that previously observed in hair cells, primary vestibular neurons, and cerebellar Purkinje cells, but is in contrast to prior reports of no age-related loss of neurons in other brain stem nuclei.

Keywords—quantitative morphometry; aging; vestibular nucleus.

Introduction

Complaints of dizziness and disequilibrium are extremely common in older people (1). Associated falls are a major cause of morbidity and mortality (2,3). Age-related morphological changes occur in all of the sensory systems essential for maintaining balance during locomotion. Neuroanatomical studies of the peripheral vestibular system have documented a significant loss of hair cells and primary vestibular neurons as a function of age (4,5). Since the vestibular nuclear complex (VNC) is a major visual–vestibular interaction center, age-related neuronal loss in the VNC could have particularly important functional implications.

There is general agreement that neuronal cell density in the cerebral cortex decreases with age, although the rate of decrease varies with the location (6–8). An age-related loss in the total number of Purkinje cells within the cerebellum is also well documented in humans (9). There is an average of 2.5% Purkinje-cell loss per decade over the age span of 0 to 100 years (9). By contrast, quantitative studies of human brain stem nuclei have found relatively little age-related neuronal loss (7,10–14). There have been no prior studies of age-related effects on the human VNC, but Sturrock (30) observed a significant decrease in the total number of neurons in the lateral vestibular nucleus of the aging mouse.

The purpose of this study was to document quantitative morphometric changes within the
VNC in humans as a function of age. These measurements will serve as normative data for a study on dizziness and disequilibrium in older people, sponsored by the National Institutes of Aging. Preliminary results have been reported in abstract form (15).

Materials and Methods

Fifteen human brain specimens (7 female, 8 male; ages 40 to 93 years) were obtained within 6 to 24 hours postmortem from subjects who had no chronic medical illnesses or known symptoms of nervous system or ear disease prior to death. At postmortem examination, the brains showed no gross evidence of pathology; the cause of death in each case was not neurological. The specimens were fixed for 2 weeks in 10% buffered formalin, embedded in paraffin, and serially sectioned at 20 micrometers. Every tenth section was stained with a modification of a formaldehyde-thionin technique (16).

The boundaries of the vestibular nuclei were outlined according to the criteria of Sadjadpour and Brodal (Figure 1) (17). The number of neurons in each vestibular nucleus was determined by using a computer-based video microscopy and image analysis system (NIH, 1.44, public domain). An image from each brain stem section in the area of the vestibular nucleus was digitized using a Macintosh II computer equipped with a frame grabber attached to a video camera mounted on a photomicroscope. The area of each individual vestibular nucleus was manually selected and recorded using the cursor. Neurons within this area were identified on the video screen. The number of neurons and the area of each neuron were automatically determined by the program. The diameter was determined by calculating the diameter of a circle of equal area to the neuron using the equation \( d = \sqrt{\frac{4 \times \text{area}}{\pi}} \). Cells with areas greater than or equal to 40 \( \mu \text{m}^2 \) were counted as neurons. All cells less than 40 \( \mu \text{m}^2 \) were considered glia and excluded from the analysis (19). To confirm this assumption, glial cells in brain stem sections of both young and older subjects were identified by using monoclonal antibodies against glial fibrilar acidic protein (GFAP) and myelin basic protein (MBP). The immunoreaction product was only found in cells less than 40 \( \mu \text{m}^2 \). Furthermore, specimens were immunoreacted with antibodies against synaptophysin, which stains axodendritic and axosomatic terminals. Rarely were cells of less than 40 \( \mu \text{m}^2 \) surrounded by immunoreactive terminals in young or older subjects.

Although the counting and measuring were done automatically, the operator viewed each field on a video screen and performed a variety of automatic editing functions. The NIH Image program allows the removal of vascular elements and artifacts, separation of contiguous cell images, and increased focal detection when part of a cell, although visible on the video screen, was not stained with sufficient intensity to be detected automatically for measurement. Among these functions, "threshold" is used to segment an image into objects of interest and background on the basis of gray level. Additional editing functions like "sharpen" and "subtract background" are used to increase contrast and accentuate details of the cells (NIH Image 1.44 manual, 1992). This process was done on every 20th section, that is, 400 \( \mu \text{m} \) apart. To compute the number of neurons in each 20-section volume, the estimated number of neurons in each analyzed section was multiplied by 20. To avoid multiple counting of cells larger in diameter than the section thickness, a correction factor was used. It was implemented by dividing the total number of neurons with a diameter between 1- and 2-section thickness (that is 20 to 40 \( \mu \text{m} \)) by 2, those between 2- and 3-section thickness (40 to 60 \( \mu \text{m} \)) by 3, and so on. The number of neurons in the vestibular nuclear complex was determined by adding the number of neurons of the 4 vestibular nuclei.

The volume of each vestibular nucleus was computed by multiplying the area of each section by the thickness of the section (20 \( \mu \text{m} \)) and by 10 (periodicity of the analyzed sections). The volume of the vestibular nuclear complex was estimated by adding together the partial volumes of each block. Neuronal density was calculated by dividing the number of neurons by the volume in cubic millimeters.

The length of each individual vestibular nucleus was obtained by multiplying the thickness...
of the section by the number of sections that demonstrated the morphometric characteristics of that nucleus. The length of the VNC was determined by identifying the beginning of the caudal portion of the descending vestibular nucleus and the rostral end of the superior vestibular nucleus. The number of slides between these levels was multiplied by the section thickness.

Results

Regression analysis documented a highly significant ($p < 0.001$) decrease with age in the number of neurons in the VNC (Figure 2A). The slope of the regression line was similar in men and women ($r = 0.86$ and 0.90, respectively). The neuronal loss in the VNC was approximately 3% per decade between 40 and 90 years of age. There was also a significant ($p < 0.05$) age-related decrease in volume and neuronal density in the VNC, but not in the length (Figure 2, panels B through D).

Each vestibular subnucleus also showed a significant ($p < 0.05$) decrease in number of neurons as a function of age. The highest rate of neuronal loss (6% per decade) occurred in the superior vestibular nucleus, and the lowest rate of neuronal loss (3% per decade) occurred in the medial vestibular nucleus (Table 1). Volume and neuronal density also decreased with age in each subnucleus, but the decrease was significant ($p < 0.05$) in only 2 of the 4 nuclei. Only the length of the descending nucleus decreased significantly ($p < 0.05$) with age.

To assess the relationship between neuronal size and age-related neuronal loss, we subdivided neurons into small (40 to 99 $\mu$m$^2$), medium (100 to 199 $\mu$m$^2$), large (200 to 499 $\mu$m$^2$), and giant (>500 $\mu$m$^2$) (Figure 3). Small and medium neurons represented the largest subgroup in each nucleus and also exhibited the greatest age-related loss. Most of the giant neurons were located in the lateral vestibular nucleus (where they represented 12% of the neuronal population) (8). The number of giant
Figure 2. Correlation between number of neurons (A), volume (B), density (C), and length (D) of the vestibular nuclear complex (VNC) versus age in 15 normal human subjects.
neurons significantly increased ($p < 0.01$) rather than decreased with age. To explain this apparent contradiction, we examined giant neurons in each of the vestibular nuclei in young and older subjects. Abundant giant neurons in older people were filled with lipofuscin deposits (Figure 4, panels A and C), whereas less frequent giant cells in younger subjects were smaller and did not contain lipofuscin (Figure 4, panels B and D). Presumably, there was loss of some giant neurons with aging (as with other neurons), but the total number of giant neurons increased due to enlargement of smaller cells because of lipofuscin deposits.

**Discussion**

In this first study of the effect of aging on the VNC in humans, we found a highly significant decrease in the number of neurons as a function of age. The volume and neuronal density also decreased significantly with age, although to a lesser degree. Most prior studies of age-related loss of neurons have estimated neuronal density rather than the total number of neurons because it can be difficult to count all neurons in a region, particularly when the borders are not well defined (6,7). Brain stem structures such as the vestibular VNC, however, have reasonably well defined borders so that the total number of neurons can be estimated (17,18). An important procedural point in our study was that we arbitrarily defined cells with areas greater than 40 $\mu$m$^2$ as neurons, assuming that the cells smaller than 40 $\mu$m$^2$ were glial cells. Using similar automated techniques for counting cerebral cortical neurons, Terry and colleagues (19) showed that almost all glial cells had cross-sectional areas $<40$ $\mu$m$^2$, whereas nearly all neurons were larger than 40 $\mu$m$^2$. Furthermore, Blinkov and Ponomarev (20) counted neurons and glial cells in the VNC with an oil immersion lens with the aid of an ocular net micrometer in 2 normal human subjects, ages 25 and 48. They counted an average number of 245,000 neurons in the VNC of these subjects. By comparison, we found an average of 250,000 neurons in the VNC of our 2 youngest subjects, age 40 and 43. Diaz and colleagues (21) defined neurons as somatic profiles with a nucleus and nucleolus and found a similar number of neurons in the VNC of young normal human subjects.

Are neurons dying out or are they simply getting smaller as a function of age? If all were getting smaller, then one would expect that the greatest loss would be in the largest neurons, with medium-sized and small-sized neurons either showing no decrease in number or possibly increasing in number. We found just the reverse. The greatest age-related loss occurred in small and medium neurons. Also there was no evidence that large numbers of neurons were shrinking below the 40 $\mu$m$^2$ detection limit in older subjects, because neurons with areas less than 40 $\mu$m$^2$, identified with immunohistochemical stains, were rare in younger and older subjects. The number of large and giant neurons actually increased with age. DeLacalle and colleagues (22) found similar age-related changes in the human nucleus basalis of Meynert. The total num-

### Table 1. Effect of Aging on the Human Vestibular System

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Approximate number age 40</th>
<th>% Loss/decade age 40 to 90*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vestibular nucleus neurons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>superior</td>
<td>260,000</td>
<td>3</td>
<td>Lopez et al. (38)</td>
</tr>
<tr>
<td>lateral</td>
<td>31,000</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>medial</td>
<td>139,000</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>descending</td>
<td>60,000</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hair cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 cristae</td>
<td>73,000</td>
<td>6</td>
<td>Rosenhall (4)</td>
</tr>
<tr>
<td>2 maculae</td>
<td>22,000</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Vestibular ganglion neurons</td>
<td>51,000</td>
<td>5</td>
<td>Richter (5)</td>
</tr>
<tr>
<td>Vestibular nerve fibers</td>
<td>18,000</td>
<td>5.5</td>
<td>Bergström (39)</td>
</tr>
<tr>
<td>Vestibular ganglion neurons</td>
<td>16,000</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>

*Assuming a linear rate of loss.
Figure 3. Number of neurons of different sizes in the VNC versus age. (A) 40–99 μm², (B) 100–199 μm², (C) 200–499 μm², (D) > 500 μm².

The number of neurons decreased with age, but the average size of remaining neurons increased. For example, in subjects aged 16 to 29 years, 54% of the neurons had areas greater than 400 μm², whereas in subjects aged 80 to 100 years, 62% had areas greater than 400 μm². The authors speculated that this hypertrophy of remaining neurons occurred as non-degenerating neurons assumed some of the function of the disappearing population. Our data suggest that deposition of lipofuscin could account for the hypertrophy of some remaining neurons.
Aging in the Vestibular Nucleus

Figure 4. Photomicrographs of VNC neurons in the lateral vestibular nucleus (A and B) and the medial vestibular nucleus (C and D), of an older (A and C) and a younger subject (B and D). Arrowheads in photomicrographs A and C demonstrate the presence of lipofuscin in the cell somata of a typical giant size Dieter's neuron and three typical medial vestibular nuclear neurons. Thionin staining. Bar in A and B = 12 μm; in C and D = 10 μm.

The rate of neuronal loss in the vestibular nuclear complex with aging is comparable to the rate of loss of hair cells and primary vestibular neurons (Table 1). Rosenhall (4) observed a hair-cell loss of approximately 6% per decade in the vestibular end organs over the age span of 40 to 90 years. He noted that the hair-cell loss tended to be greatest at the center of the cristae, the area supplied by the largest diameter vestibular nerve fibers. Bergström (23) found an overall vestibular nerve fiber loss of 5.5% per decade over the same age span, but also noted that the nerve fiber dropout was greatest for the largest diameter fibers. Of note, we found that the highest rate of neuronal dropout occurred in the superior vestibular nucleus, a nucleus that receives most of its primary afferent input from the cristae, including many large diameter fibers from the center of the cristae (24,25). The medial vestibular nucleus showed the lowest rate of neuronal dropout with age; this nucleus receives almost no large fiber primary afferent input (26). Although we, and most other investigators, used a linear model to describe the neuronal loss that occurs with aging, a curvilinear model is probably more appropriate. This is particularly evident in the hair cell and primary neuron data, where it appears that age-related effects are minimal until the 5th or 6th decade (4,5). We did not study any subjects under the age of 40, but the rate of neuronal loss in the age range of 40 to 90 appears curvilinear (see Figure 2A). Larger numbers of subjects are needed, however, to adequately define the relationship.

Most prior studies on the effects of aging on neuronal populations within the human central nervous system have focused on the cerebral cortex (6,7). The number of cerebral cortical neurons decreases with age, but the loss is regional, affecting some areas to a greater extent than others. Brody (6,27) found the greatest age-related loss of neurons in the superior temporal gyrus, superior frontal gyrus, precentral gyrus, and the area of striata. Although this neuronal loss was observed in all layers of the cortex, it was particularly evident in layers 2 and 4, the external and internal granular layers. However, such studies have problems in separating neuronal loss from volume changes associated with tissue shrinkage during embedding, which is also age-related (28). Probably the most complete study of a neuronal population with aging was performed on cerebellar Purkinje cells by Hall and colleagues (9). These investigators counted the total number of Purkinje cells in the cerebellum of 90 normal human subjects. Although wide individual variations in the number of Purkinje cells were found at all ages, they found a mean reduction of 2.5% per decade over the age range of 0 to 100 years. However, as with the vestibular hair cell and primary neuron data, the relationship between the number of Purkinje cells and age appear to be curvilinear rather than linear, with relatively little change in the number of Purkinje cells up to the...
5th and 6th decade and then a clear dropoff in the number of Purkinje cells beyond that age. For comparison with our vestibular nucleus data, we estimated the rate of dropoff for the age range of 40 to 90 years using a linear model. Hall and colleagues' (9) data suggested a 5% loss of Purkinje cell per decade for that age range. Torvik and colleagues (29) performed a morphometric analysis of the cerebellar vermis in nonalcoholic men between ages 39 and 94 years and showed a significant decline in Purkinje cell density with increasing age in all parts of the cerebellar vermis, particularly in the superior vermis. Finally, Sturrock (30) found a decline in the number of Purkinje cells with age in the cerebellar nodulus of mice.

Although there have been no prior studies of age-related effects in the human VNC, Sturrock reported an age-related loss of neurons in the lateral vestibular nucleus of the mouse (31). This neuronal loss was most pronounced in the large neurons. Loss of the large neurons was preceded by an accumulation of lipofuscin in their soma and a loss of Nissl substance (31). Sturrock also noted age-related neuronal loss and similar morphologic changes in neurons from the mesencephalic and motor nuclei of the trigeminal nerve (32) and from the facial nerve nucleus (33) in the mouse. By contrast, prior studies of other brain stem nuclei in normal human subjects, including the ventral cochlear (12), abducens (13), trochlear (14), facial (34), and inferior olivary nuclei (10), have not found age-related neuronal loss. The reason for this apparent difference in age-related effects on different brain stem nuclei in the human is not clear, but larger numbers of subjects must be studied with modern quantitative techniques before a final conclusion can be reached.

One can only speculate on the functional implications of the age-related hair cell and neuronal loss in normal human subjects, summarized in Table 1. Age-related effects on the vestibulo-ocular reflex (VOR) have been studied most extensively because the reflex is relatively easy to stimulate and there are many ways to record evoked eye movements. There is a clear age-related decrease in VOR gain along with a decrease in the dominant time constant of the VOR (35–37). As with hair cell and neuronal loss, the decrease in VOR gain appears to be curvilinear, with relatively little decrease in gain until the 5th or 6th decade, followed by a rapid dropoff in VOR gain. The age-related changes in the canal ocular reflex may be explained by the prominent age-related loss of hair cells at the center of the cristae, the relatively selective loss of large diameter primary vestibular afferents, and the neuronal loss in the superior vestibular nucleus, a major canal ocular reflex relay station. Age-related changes in the vestibulospinal reflexes are more difficult to assess because of the prominent overlap in function with other sensory motor systems. Although deterioration in balance and gait are well documented with aging, it is difficult to distinguish changes in vestibulospinal reflexes from the well-documented changes that occur in the visual and somatosensory systems (1).

Is the age-related loss of sensory cells and neurons within the vestibular system part of the normal aging process, or is it the result of an underlying pathologic process such as progressive vascular disease? None of our subjects had evidence of small vessel disease or of infarction within the brain stem. However, we did not systematically assess the status of the large vessels at the base of the brain. The age-related loss in hair cells and primary and secondary vestibular neurons, however, is so prominent and so consistent from study to study that inclusion of a few subjects with undetected vascular disease could not account for the observed findings. Hall and associates (9) systematically assessed all postmortem specimens for small and large vessel disease, and even after exclusion of all cases with vascular disease, they found a clear-cut age-related loss of Purkinje cells within the cerebellum.

Acknowledgments—This work was supported by NIH grants AG90693 and DC01404. The authors wish to thank Itsuko Aono for her technical assistance.
REFERENCES