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Original Article

Age associated changes in the human cochlear nucleus – A three-dimensional modelling and its potential application for brainstem implants

Saroj Sharma [a], *T.C. Nag* [b], *Alok Thakar* [c], *D.N. Bhardwaj* [d], *T.S. Roy* [e,*]

[a] *Assistant Professor, Department of Anatomy, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi 110029, India*

[b] *Additional Professor, Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*

[c] *Professor, Department of Otorhinolaryngology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*

[d] *Professor, Department of Forensic Medicine, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*

[e] *Professor and Head, Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India*

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ABSTRACT

Introduction: The cochlear nucleus (CN) is vulnerable to physio-pathological alterations with age that may contribute to varied degree of hearing impairment.

It was planned to study the age changes in the three-dimensional (3-D) structure of human CN.

Methods: Forty-one human brainstems were collected (birth – 90 years) from the mortuary of the All India Institute of Medical Sciences (AIIMS), New Delhi, with ethical committee permission. Tissues were fixed in 4% paraformaldehyde (pH 7.4), cryosectioned (40 μm) serially and stained with cresyl violet. A 3-D reconstruction model of the adult human CN was made using the 3-D Solid body tracing probe of the Stereo Investigator software (MBF Biosciences, VT, USA).

Results: The CN appeared as a thin, crescent-shaped protuberance along the floor and lateral recess of the 4th ventricle in relation with inferior and middle cerebellar peduncle at the level of the pontomedullary junction. The rostral tip was observed deep in the middle cerebellar peduncle and the dorsal subdivision, within the wall of lateral recess of the 4th ventricle. Microscopically, different types of neurons were observed in all the ages studied and no change in the distribution was noted. The volume of the CN did not change with ageing.

* *Corresponding author*. Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India., Tel.: +91 11 26594880, +91 11 26593216; fax: +91 11 26588663.

E-mail addresses: tarasankar@hotmail.com, tsroyaiims@gmail.com, tsroy@aiims.ac.in (T.S. Roy).

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Discussion: The 3-D model of the CN can be used as a landmark for safe surgical route to access the intraventricular surface of the 4th ventricle and to engineer brainstem implants.

1. Introduction

The cochlear nucleus (CN), also referred to as the auditory nucleus, is located on the dorsolateral aspect of the brain stem at the junction of the pons and the medulla. It is divided into a ventral and dorsal cochlear nucleus (VCN and DCN), but in humans, the boundaries between the two subdivisions are indistinct. The CN is populated by morphologically distinct neurons that have independent functions and connectivity.[1–3] These neurons respond to acoustic stimulations and produce characteristic physiological and biochemical responses[4] and contribute specifically to the formation of the complex functional networks in the CN.[2] The CN receives neural inputs from specific frequency-related region of the cochlea with a tonotopic organization (the basal and apical turns of the cochlea stimulated by high and low-frequency sounds, respectively). The acoustic stimulus determines the manner in which the different parts of the cochlea are stimulated by its characteristics of frequency and intensity. These characteristics also determine the portion of the cochlear nerve that carries the impulses and the parts of the CN to which they transmit their signals. Fibers coming from apical (low frequencies) and basal region (high frequencies) of the cochlea are located in the central and the peripheral parts of the auditory nerve respectively.[5] Cochleotopy is also maintained in the CN, where low-frequency fibers project to the ventral regions of the nucleus and high frequency fibers project to the dorsal regions.[6]

The CN receives afferent input from the cochlea and is highly susceptible to various environmental insults that lead to pathological alterations and may further contribute to varied degrees of hearing impairment. The peripheral auditory system (the cochlear nerve and cochlea) is affected in many conditions.[7] In these conditions cochlear implants are contraindicated. The alternate treatment would be auditory brainstem implants (ABI). The quality of sounds perceived with the help of the available ABI is not comparable to that with cochlear implants (CI).[8] Therefore clinicians are in search of a better ABI that can provide a quality of sound similar to CI's.

In order to construct a better ABI, an excellent understanding of the CN anatomy at various ages is required. Extensive studies have been conducted on the topographical description of human CN in relation to the neighbouring structures from a neurosurgical point of view[9–12] and its application in brainstem prosthesis in patients with sensory neural hearing loss.[13–15] The three-dimensional spatial relation of CN during advancing age have been inadequately studied. In the present study, we have prepared three-dimensional models of the CN at various ages that can provide a base line data to help scientists, engineers and clinicians to construct a more physiological and effective ABI.

2. Materials and methods

2.1. *Brainstem collection and tissue preparation*

All the brainstems used in this study were collected from human cadavers in accordance with the protocol approved by the Ethics Committee, All India Institute of Medical Sciences,

Table 1 – Particulars of the human brain stem used in the study.

Groups (years)	Age	Sex	Side
One(Birth – 20)	2 day	M	R
	26 day	M	L
	3 yrs	F	R
	5 yrs	F	R
	14 yrs	M	R
	15 yrs	F	L
	17 yrs	F	R
	18 yrs	M	R
	20 yrs	F	R
Two (21–40)	22 yrs	M	R
	23 yrs	M	L
	26 yrs	M	R
	27 yrs	M	R
	30 yrs	M	L
	31 yrs	M	R
	33 yrs	M	R
	35 yrs	M	L
	36 yrs	M	R
	39 yrs	M	R
Three (41–60)	41 yrs	M	L
	44 yrs	M	R
	45 yrs	M	L
	47 yrs	M	R
	50 yrs	M	R
	52 yrs	M	R
	54 yrs	M	R
	58 yrs	M	R
	60 yrs	M	L
	60 yrs	M	L
Four (61–90)	61 yrs	M	R
	65 yrs	M	R
	67 yrs	M	R
	69 yrs	F	R
	70 yrs	M	R
	72 yrs	M	R
	73 yrs	M	L
	75 yrs	M	R
	77 yrs	M	R
	80 yrs	M	L
	86 yrs	M	R
	90 yrs	M	L

Time interval between death and post-mortem = 6–12 h. F – female, M – male, L – left, R – right, yrs – years.

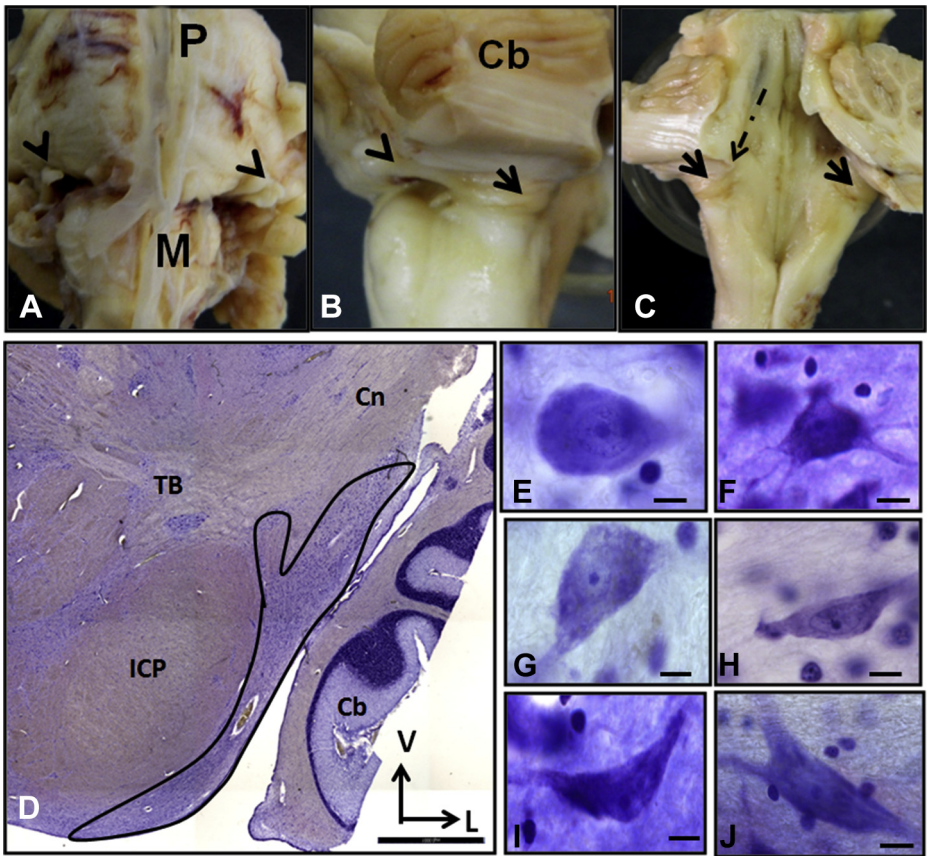

Fig. 1 – A–C: Adult Human Brainstem showing the cochlear nucleus (CN) as small bulge (Solid arrows) at the pontomedullary junction. A – Ventral view B – Lateral view C – Dorsal view. Broken Arrow in the lateral part of floor of 4th ventricle (C) indicate the position of blood vessel in the notch demarcating the dorsal part of cochlear nucleus and vestibular area, Arrow heads – Cochlear nerve, Cb – Cerebellum, M – Medulla, P – Pons. Scale Bar – 1000 μm. D: Photomicrograph of Nissl stained transverse sections of adult human brainstem. Cochlear nucleus (Area marked with black boundary) in relation with inferior cerebellar peduncle (ICP), Cb – cerebellum, Cn – Cochlear nerve, D – Dorsal, L – Lateral, TB – Trapezoid body. E–J: Different types of neurons present in the CN. Bushy neuron (E), Multipolar neuron (F), Octopus neuron (G), Small cell (H), Bipolar neuron (I) and Multipolar neuron (J). Scale bar – 50 μm.

New Delhi, India, following the guidelines of Helsinki declaration. Forty-one brainstems collected from individuals aged between 2 days to 90 years were used. All the samples were divided into four groups according to their biological age: birth to 20 years (group 1), 21–40 years (group 2), 41–60 years (group 3) and 61–90 years (group 4). Each group had ten samples, except group four where there were twelve samples. Strict exclusion criteria were used for brain stem collection. All the specimens were obtained within 6–12 h of death during winter months (chances of autolysis are minimised) and were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The tissues were preserved at 4 °C in order to minimize the post-mortem changes. Only those samples that did not show any histological features of post-mortem degeneration were utilised in this study. Particulars of the brain stem material used in the study are shown in Table 1.

The total brain weight was measured in all cases studied. The segment of the brainstem with CN and cochlear nerve was excised (Fig. 1A–C) and cut sagittally into two halves. A tissue block, with the CN, measuring 15 mm in length rostrocaudally, was cut from the brain stem. Both blocks (right and left half) were kept in the fixative for 1–2 weeks at 4 °C. One half of the tissue block was washed in 0.1 M phosphate buffer (PB, pH 7.4) and cryoprotected in 30% sucrose. Forty microns thick, serial transverse sections of the CN were cut in a caudal-to-rostral direction, using a cryomicrotome (Microm International Gmbh, Germany). The location and margins of the CN were identified histologically from the adjacent nuclear groups by using Luxol fast blue staining.[16,17] Every 12th section was stained with 1% cresyl violet acetate, dehydrated in ascending grades of alcohol, cleared in xylene and mounted with DPX. Stained sections of the CN were studied under the Olympus BX26 research microscope (Japan) attached to a motorized stage controller (LUDL, Germany) and a video camera (MBF Biosciences, CX 9000) (Fig. 3).

2.2. *Three-dimensional reconstruction of CN*

Three-dimensional reconstructions of the CN were made by alignment of live images of serial sections of the CN with the

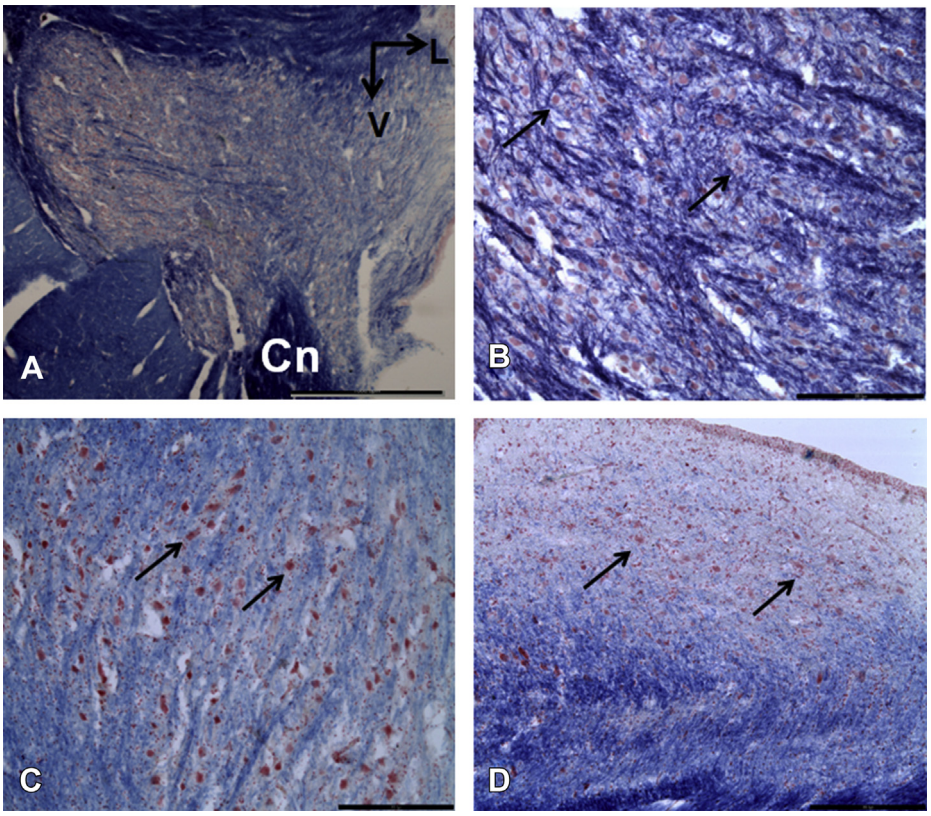

Fig. 2 – Photomicrographs of Luxol fast blue stained adult brainstem sections A. Cn- Cochlear nerve fibers passing through the VCN, Orientation arrows V – Ventral, L – Lateral. B. Homogenous distribution of large size bushy neurons (arrows) in the rostral part of the ventral part of the CN (VCN) C. Heterogenous distribution of medium to large size neurons (arrows) (Multipolar and octopus) in the caudal part of the VCN D. A portion of the dorsal part of the CN (DCN) showing scattered neurons (arrows) (Bipolar and Giant neurons) intermingled with fibers Scale bars- 300 μm.

use of a Solid Module tool of Stereo Investigator software programme (MicroBrightfield Inc. VT, USA). The software matches sections and stacks the two-dimensional contours of the CN and produces a three-dimensional model. Every 12th cresyl violet stained section was traced under a 2× objective and aligned with the help of rotation and translation such that corresponding margins of the CN, inferior cerebellar peduncle (ICP) and the cut margins of the brain stem in all the sections were superimposed. The three-dimensional geometry was reconstructed from the stacks of all contoured sections. This procedure was standardised after a pilot study performed in order to set the parameters to obtain a coefficient of error (CE) less than 0.05%. Thereafter, the total volume of the whole CN was estimated using the Cavalieri principle.[18,19]

2.3. *Statistical analysis*

Statistical analysis was carried out using Graph Pad Prism (6.0) software. The data were expressed as mean ± SD. The difference in mean volume of the whole nucleus among the groups was analysed using Kruskal–Wallis non-parametric test followed by Dunnett's post-hoc analysis for multiple comparisons. A '*p*' value of less than 0.05 was considered to be statistically significant.

3. Results

The brain weight was measured in all the cases studied and it was ranged between 1250 and 1560 g except in the 2 and 26 days old infants, where it was 370 g and 400 g, respectively. After removing the cerebellum from the rest of the brainstem, the CN was identified and it appeared as a continuous, thin, crescent-shaped bulge along the floor and lateral recess of the forth ventricle with respect to inferior and middle cerebellar peduncle at the junction of the pons and medulla. Ventral and dorsal parts of CN were recognized as a continuous prominence, located lateral and dorsolateral to the inferior cerebellar peduncle (Fig. 1A–C). The intra- and the extraventricular junctional part of the CN coincided with line of attachment of the inferior medullary velum and the terminal part of the vestibulocochlear nerve. The cochlear nerve was seen on the ventromedial surface of the VCN (Fig. 1A and B). The CN was limited dorsally by a notch that contained a blood vessel, which separated it from the vestibular area (Fig. 1C).

3.1. *Light microscopic observations*

The CN was recognised superficially under the ependymal surface (Fig. 1D). In Luxol fast blue stained sections the

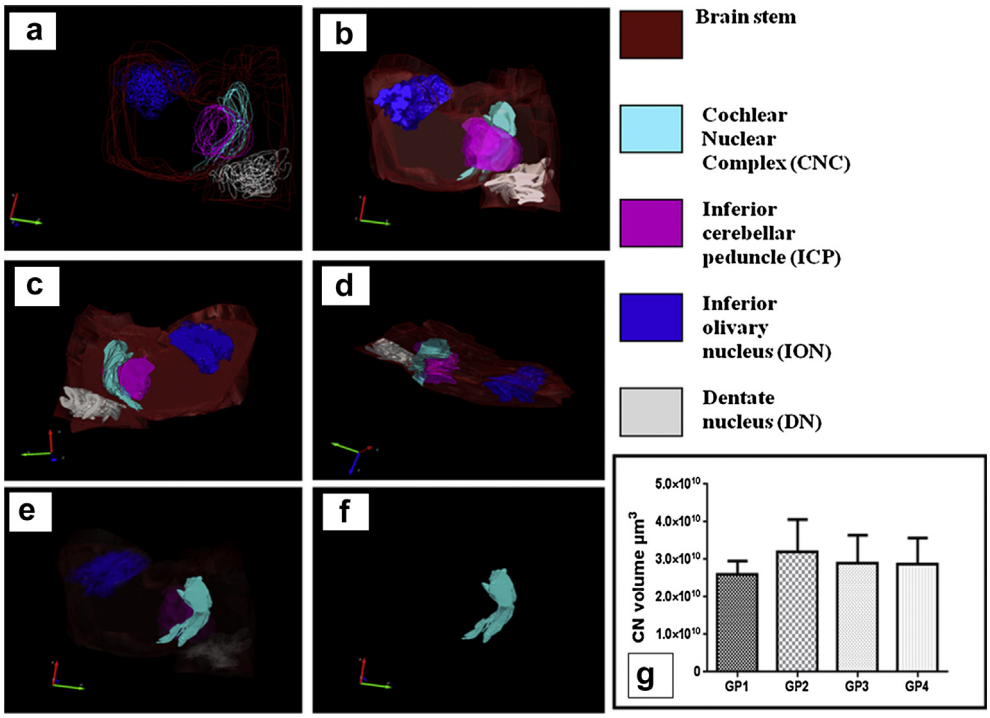

Fig. 3 – From stacks to solid model using 3-D solid module of Stereo Investigator (a) Stacks of contour lines derived from systematic uniformly randomized Nissl stained serial sections of Human Brainstem. Colour coding of the contour lines is shown on the right most panel. (b–f) – Transformation of the stacks of individual contour lines into a 3-D model of important structures in relation to cochlear nuclear complex (CNC). (g) – Bar diagram showing the distribution of the CN volume among four groups (Gp). X and Y axis represent groups and mean volume of the CN (μm^3).

cochlear nerve fibers were seen traversing through the VCN and further radiating towards the DCN (Fig. 2A). The axons were seen converging on the medial side of the VCN to form the large fiber bundle (trapezoid body) which was seen medially and rostrally across the medulla that reached the higher centres (Fig. 1D). The most rostral tip of the VCN was observed in the middle cerebellar peduncle.

Microscopically, the different subdivisions of the CN were indistinct although the regions were recognised by their predominant cell type (Fig. 1E–J) as described earlier.[16,17] Densely packed large homogeneous groups of neurons were observed rostrally and around the cochlear nerve root in the VCN and these were recognised as spherical and globular bushy neurons by their characteristic features[16,17] (Fig. 2A and B). Heterogeneous population of relatively medium to large cell bodies with round to oval nucleus were distributed caudorostrally in the VCN and identified as multipolar and octopus neurons (Fig. 2C). The dorsal portion of the CN was well developed and identified with their characteristically arranged scattered neurons (Fig. 2D). Slender bipolar or triangular shaped neurons were distributed irregularly in the middle part along with large cell body located in the deeper regions of the DCN and both were identified as fusiform and giant cells respectively. Granule and small cells have relatively small spindle shaped soma with round nucleus and were widely distributed in the CN. These observations were consistent in all the specimens studied and there were no changes in either the appearance or distribution of the neurons, among all the age groups studied.

3.2. *Three-dimensional reconstruction of the CN (Fig. 3)*

A three-dimensional (3-D) reconstruction model of the human CN had been developed using the images obtained from the histological sections of the post-mortem brains. The images of the CN was projected on a computer screen and using the Stereo Investigator programme, the CN was identified and traced serially in relation with inferior cerebellar peduncle (ICP) from caudal-to-rostral end. The CN was more complex in its spatial orientation. It was observed the largest part of the CN surface, particularly the DCN, was fully within the lateral recess of the fourth ventricle. The VCN was wider towards the rostral part and longer dorsally towards the DCN. The CN was consistent in position with respect to the ICP, inferior olivary nucleus and the dentate nucleus in all age groups. Individual variability in the volume of the CN was observed among different ages. The minimum and maximum volume of CN was observed in the 26-day infant and a 27-year-old individual, respectively and no significant change in the CN volume was observed among the groups studied (Fig. 3g). There was no significant difference noted between the right and left sides of the CN.

4. Discussion

The present study deals with the three-dimensional spatial surface modelling of the CN and the changes associated with

progressive age. The CN extended caudo-rostrally up to the middle cerebellar peduncle and no change in the CN volume and distribution and morphology of different neuron types was observed between the right and left sides.

Topographical description of human CN have been extensively studied with reference to the floor of the forth ventricle and the neighbouring structures[9–12] but to the best of our knowledge, no study has dealt with changes in the spatial orientation of the CN seen with ageing. In the present study, the different types of neurons were identified in all the cases as described in the literature.[16,17] The ability to distinguish different cell types in the CN facilitates correlations of structure and function.[2,3] Each neuron has a typical distribution pattern within the CN, and their functions have been partially characterized with respect to their role in providing information regarding tonal and temporal aspects.[20] There are contradictory reports regarding the age associated morphological changes in the CN in animal species and humans. In our study, no change in the mean CN volume was observed among all groups studied, though a decrease in human CN volume has been reported with age.[21,22] In other study, we have observed age associated neurochemical changes in the human CN.[23] The highest prevalence of hearing loss is found in people over the age of 65[24] and the 5-year incidence of developing hearing loss in normal adults aged 48 and above is 21%.[25] Even mild degrees of hearing loss can compromise the ability to process speech in the presence of background noise or multiple speakers, leading to social isolation, depression, diminished cognitive function, and poorer quality of life.[26,27] Besides ageing, there are many conditions like genetic disorders, trauma, meningitis affecting the peripheral part of the auditory system and lead to varying degree of hearing loss. Since these patients are not able to use the CI's, the probability of the restitution of the lost neurological functions has become possible with neural prosthesis.[7]

Extensive research has been conducted in order to facilitate the performance of these implants for better speech perception in the profoundly deaf cases.[28] Understanding of the spatial orientation and distribution of the different cell types in the CN is significant in designing and situating brainstem prostheses for stimulation of the CN.[29] Auditory brainstem implants (ABI) has multiple electrodes and is placed on the surface of the CN in the lateral recess of the fourth ventricle of the brain stem.[8] None of the available surface ABI electrodes stimulate the deeper frequency specific regions of the CN. The data regarding frequency specific spatial distribution of the various neurons along with ages and the consistent size of the CN noted in this study might play an important role in deciding the applicability of penetrating auditory brainstem prosthesis in age related hearing loss, especially in cases where other hearing aids fail.

5. Conclusion

The present study constructs the three-dimensional model of the CN from post-mortem brain samples ranging 2-day infant to 90 years and revealed that no change was noted in the spatial orientation of the CN and its volume.

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Conflicts of interest

All authors have none to declare.

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