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Original article

Stereological analysis of vascular network of subcortical auditory centers

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Summary

Introduction. Subcortical auditory centers have several sources of blood supply. Cochlear nuclei are vascularized by the anterior inferior cerebellar artery. Superior cerebellar and posterior cerebral arteries supply the inferior colliculus nucleus, while the medial geniculate body nucleus is supplied by several posterior cerebral artery branches. The aim of the research was to quantify the vascular network of subcortical auditory centers.

Methods. Twelve adult brains, both sexes, aged 21 to 78 years, without signs of neurological diseases were analyzed in this study. Samples for histological sections, stained by the Mallory method, were obtained from strata cut at levels: the junction of the middle and rostral thirds of the oliva, the inferior colliculi, and the thalamic pulvinar. Volume, surface, and length density of the vascular network of subcortical auditory centers were analyzed stereological parameters.

Results. All parameters had the highest values in the medial geniculate body nucleus. Statistically significant difference was found in the volume, surface, and length density between vascular networks of the cochlear nuclei and medial geniculate body nucleus, and between inferior colliculus nuclei and medial geniculate body. Vessels in cochlear nuclei and inferior colliculus nuclei differed only in length density.

Conclusion. Cochlear nuclei and inferior colliculi nuclei blood vessels have a similar density and diameter, but vessels in inferior colliculi nuclei are more tortuous. In the medial geniculate body nucleus vessels are larger, denser and more tortuous compared to the other two subcortical auditory centers.

Keywords: cochlear nuclei, inferior colliculi, medial geniculate body, blood vessels

Introduction

The most important nuclei of the subcortical auditory system include two cochlear (CNc), ventral (VCNc) and dorsal (DCNc) nuclei, the nucleus of the midbrain inferior colliculi (ICNc) and the nucleus of the medial geniculate body (MGBNc). There are several sources of blood supply for these structures.

Lateral branches of the anterior inferior cerebellar artery (AICA) supply DCNc and VCNc, as well as adjacent parts of the vestibular complex [1], while in a small percentage both cochlear nuclei are vascularized by the posterior inferior cerebellar artery (PICA) [2]. Duvernoy [3], due to their vascularization, placed cochlear nuclei outside the three vascular zones of the medulla oblongata. The results of his research show that the vascular network of the DCNc is not particularly dense in humans and that it is nourished by small blood vessels, while the VCNc is slightly less vascularized than the dorsal one.

ICNc is an important relay nucleus of the auditory system, which integrates information about the frequency and the location of the sound [4]. It is located within the inferior colliculi, which are localized below the tentorial tip in the quadrigeminal cistern on the posterior surface of the midbrain [5]. The rostral tree of the superior cerebellar artery (SCA) and its long circumflex branches vascularize the inferior colliculi [1, 5]. ICNc is also supplied by collicular artery branches, from the posterior cerebral artery (PCA). This network is especially developed in the area of the rostral parts and on the peripheral edge of the inferior colliculi. Some vessels of this network also supply the intercollicular commissure [1]. The inferior colliculus is considered as one of the most densely vascularized regions of the brainstem [3].

MGBNc plays a central role in the auditory process. It transmits auditory information for higher-order processing to the primary auditory cortex, however, a reciprocal link from the primary auditory cortex back to MGBNc is also important [6, 7]. MGBNc is supplied by several sources: thalamogeniculate artery, posterior medial choroidal artery, mesencephalothalamic artery and branches of the calcarine artery, which originates from PCA [1]. P2 segment of the PCA provides several lateral branches, including five to ten inferolateral branches (thalamogeniculate arteries), which supply inferolateral thalamic territory. They enter the thalamus after passing between the MGB and LGB, where they give the medial and inferolateral pulvinar branches. Medial branches supply MGB [1, 8, 9]. PCA branch, posterior choroidal artery, arises either from the distal part of the P1 or proximal part of the P2 segment of PCA and supplies the posterior thalamic territory [10]. Its medial branches pass below the corpus callosum to the choroid plexus, while the lateral branches reach the cerebral crus, thalamus, and choroid plexus. The vascular territory of this artery, besides the pulvinar and intralaminar region, are both geniculate bodies [1, 8, 11]. Some branches for MGB also arise from mesencephalothalamic artery, long circumflex artery, and calcarine artery (PCA branches) [1].

The aim of this study was to perform a stereological analysis of the vascular network of subcortical auditory centers and to determine: volume, surface, and length density of the vascular network of the mentioned structures, and to determine whether there are differences in these parameters among examined structures.

Methods

The study was performed with the permission of the Ethics Committee of the University Clinical Center of Republic of Srpska, Banja Luka, Bosnia and Herzegovina, on samples of 12 brains of adults of both sexes, aged 31 to 75 years (average age 57.07 years), who died without diagnosed neurological diseases. With the usual autopsy technique, the brains were removed from the cranial cavity and then immersion-fixed in 10% formalin. After fixation, the brainstems were separated by cutting the brain masses at the level of the posterior edge of the mammillary bodies of the hypothalamus and from the cerebellum, by cutting the cerebellar pedicles. The brain stems were then cut into three strata in the transverse plane (5 mm thick: stratified sampling). The caudal stratum for analyses of CNc was formed by cutting the brainstem at the junction of the middle and rostral thirds of the olivar bulge and 5 mm rostrally, and the rostral stratum, which contains ICNc, was taken from the midbrain tissue from the caudal to the rostral border of the ICN. By cutting the corpus callosum and the structures of the midbrain, we separated the right from the left hemisphere of the brain. With a frontal section at the level of the pulvinar thalami, MGB, and LGB, and a cross- section of 5 mm rostrally, we formed the third stratum, representing the sample for analyzing MGBNc parameters [12]. The resulting strata were molded into paraffin blocks and cut in the transverse plane into 4μm thick sections and stained with the Mallory method.

From each section, images of visual fields were taken using a Leica EC3 camera (Leica Microsystems CMS GmbH, Wetzlar, Germany), TIFF format, resolution 2048 x 1536 pixels, at magnification x400 of the light microscope Leica DM 1000 (Leica Microsystems CMS GmbH, Wetzlar, Germany) and x0.7 magnification of the c-mount of camera. During sampling, visual fields were randomly selected. The number of visual fields was determined according to De Hoff's formula:

$$n = (200 / y \bullet s / x)2$$

n - number of fields to be analyzed; x - mean of the orientation sample; s - standard deviation of the orientation sample; y - allowed deviation of the results from the arithmetic mean

ImageJ, version 1.53a (National Institutes of Health, Bethesda, USA) was used for stereological analysis. After spatial calibration with an object micrometer, the parameters of the test system A 100 were determined, based on which, using grid option in ImageJ, a network test system A 100 was formed (Table 1).

$$Lt = Pt \cdot d \cdot 2 \qquad At = Pt \cdot d2$$

Table 1. Basic parameters of the test system A 100

Test system parameter	Value under x400 magnification
Pt	100
d	0.020386 mm
Lt	4.0772 mm
At	0.04156 mm2

Pt - number of points in the test system; d - length of one line of the test system; Lt - length of all test lines; At - surface of the test area

All images were analyzed with the cell counter tool. The following stereological parameters of the vascular network of subcortical auditory centers were determined:

The volume density (Vv) was calculated using the formula: Vv (mm0) = Pf / Pt (Pf - number of hits of test points falling on the studied phase; Pt - total number of points within the reference A-100 system [13].

Surface density (Sv) was determined based on the formula: Sv (mm-1) = 2 • If / Lt (If – the number of cross-sections of the test phase with test lines; Lt - total length of test lines) [14].

Length density (Lv) was calculated according to the formula: Lv = 2xQf / At (mm-2), where Qf is the number of punctures of the examined structure in the test area, At - the surface of the test area [13].

The number of examined test fields (total research sample) for each variable is shown in the table 2.

Table 2. Number of analyzed test fields for each tested variable

Parameter	Number of analyzed test fields		
	CNc	ICNc	MGBNc
Vv	1308	734	507
Sv	792	767	389
Lv	570	636	365

Vv - volume density; Sv - surface density; Lv - length density; CNc - cochlear nuclei; ICNc - inferior colliculus nucleus; MGBNc - medial geniculate body nucleus

Statistical analysis was performed with SPSS software, version 16.0, using descriptive statistics methods and Student's t-test. The value of $p< 0.05$ was considered statistically significant.

Results

The mean value of Vv was highest in MGBNc and lowest in ICNc (Table 3).

Statistical analysis using Student's t-test showed that there was no statistically significant difference ($p = 0.548$) in the Vv of the vascular network CNc and ICNc, but that there was a highly statistically significant difference between CNc and MGBNc ($p<0.001$) as well as between Vv of the vascular network of ICNc and MGBNc ($p<0.001$).

Table 3. Average values of Vv of the vascular network of the examined structures

Structure	Vv (mm0) ± SD
CNc	0.02650 ± 0.00295
ICNc	0.02587 ± 0.00196
MGBNc	0.03386 ± 0.00183

CNc - cochlear nuclei; ICNc - inferior colliculus nucleus; MGBNc - medial geniculate body nucleus

When it comes to the Sv of the vascular network of subcortical auditory centers, the highest values were also seen in MGBN (Table 4).

Similar to Vv, comparison of values of Sv showed no statistically significant difference ($p=0.398$) between CNc and ICNc, but that there was a highly statistically significant difference between CNc and MGBNc ($p<0.001$) as well as between ICNc and MGBNc ($p<0.001$).

The third parameter (Lv) increased from the caudal to the rostral subcortical auditory center (Table 5).

There was a highly statistical difference ($p<0.001$) in the Lv of the vascular networks between all three examined structures.

Table 4. Values of the average Sv of the vascular network of the examined structures

Structure	Sv (mm-1) ± SD
CNc	8.0037 ± 0.111998
ICNc	7.8325 ± 0.622
MGBNc	12.4815 ± 1.0358

CNc - cochlear nuclei; ICNc - inferior colliculus nucleus; MGBNc - medial geniculate body nucleus

Table 5. Values of Lv of subcortical auditory center

Structure	Lv (mm-2) ± SD
CNc	231.4247 ± 3.1177
ICNc	328.4663 ± 2.2304
MGBNc	446.4475 ± 2.0654

CNc - cochlear nuclei; ICNc - inferior colliculus nucleus; MGBNc - medial geniculate body nucleus

Discussion

There is a wide distribution of changes in the blood vessels of the brain, which are not only a consequence of degenerative changes caused by age, but also of the modern way of life, and numerous predisposing factors. Although they have the same sources of vascularization and topographic position, the symptoms of the auditory system in cases of cerebral ischemia are much rarer than the vestibular symptoms. Hearing loss often becomes clinically evident only after successive bilateral infarctions occur. Tinnitus is probably the most common auditory symptom, reported spontaneously by patients with stroke [15]. Hearing loss or tinnitus may be the first symptom of a stroke or occur with latency for up to a few days. They are most often the result of a stroke in the territory of AICA [16, 17], which leads to acute loss of audiovestibular function, affecting other structures in the vascular field of AICA - the facial and abducens nucleus, pyramid tract, and sensitive pathways [18, 19].

In cases of lateral lower pons syndrome, symptoms such as deafness and tinnitus also occur, due to the involvement of the auditory nerve or its nuclei [20]. Although ICNc belongs to the vascular field of SCA, there are rare cases of hearing problems after a stroke in the field of this blood vessel. Similarly, ischemia in the PCA field may involve inferior brachium or MGBNc, but auditory symptoms have not been reported [15, 21].

The results of this study show that there is no statistically significant difference in the volume and surface densities of the vascular network CNc and ICNc, but there is a difference in length density. All three stereological parameters of MGBNc were larger than the same parameters of CNc and ICNc, and the difference was highly statistically significant. This indicates the difference in the density and diameter of the blood vessels of these structures. MGBNc has a significantly larger vascular network than two other examined structures. Therefore, the volume of the MGBNc vascular bed is higher due to the diameter of the blood vessels. Also, blood vessels are more tortuous in ICNc compared to CNc, but even more in MGBNc. Larger diameter and more tortuous blood vessels predispose MGBNc to a slower blood flow, and therefore greater susceptibility to vascular incidents.

Häusler and Levine [15] explain that significantly lower manifestation of auditory symptoms, compared to vestibular in cases of ischemia, is caused by a lower spreading of the auditory pathway, which makes the probability of ischemia affecting the auditory pathway on this basis lower. Also, a contributing factor is that parts of the auditory pathway, such as ICNc and MGBNc, have multiple sources of blood supply, with the vascular bed of MGB alone being significantly larger, indicating the absence of symptoms during changes in individual blood vessels [21]. The third factor is the abundance of the central auditory system and its strong representation above the level of the cochlear nuclei [15]. Therefore, rostral of cochlear nuclei, severe hearing deficits occur only if the damage is bilateral, and patients with widespread bilateral impairments of the auditory system are unable to respond or the disorder is incompatible with life.

Conclusion

The CNc and ICNc are supplied by vessels of similar density and diameter, but vessels in ICNc are more tortous. Blood vessels in MGBNc are significantly larger and more tortous than vessels in CNc and ICNc.

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Ethical approval. The Ethics Committee of the University Clinical Center of Republic of Srpska, Banja Luka, Bosnia and Herzegovina approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Stereološka analiza vaskularne mreže subkortikalnih auditivnih centara

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Uvod. Subkortikalni auditivni centri imaju nekoliko izvora vaskularizacije. Kohlearna jedra vaskularizuju grane a. cerebelli anterior inferior. A. cerebelli superior i a. cerebri posterior vaskularizuju jedro donjih kolikula, dok jedro unutrašnjeg koljenastog tijela vaskularizuje nekoliko grana a. cerebri posterior. Cilj istraživanja je kvantifikacija vaskularne mreže subkortikalnih auditivnih centara.

Metode. Dvanaest mozgova odraslih osoba, oba pola, životne dobi od 21 do 78 godina, bez znakova neuroloških oboljenja analizirano je u ovoj studiji. Uzorci za histološke preparate, bojeni su Mallory metodom, dobijeni su iz stratuma rezanih u nivoima: spoja srednje i rostralne trećine olivarnog ispupčenja, donjih kvržica srednjeg mozga i pulvinar-a thalami. Analizirani su stereološki parametri: volumenska, površinska i dužinska gustina vaskularne mreže subkortikalnih auditivnih centara.

Rezultati. Svi ispitivani stereološki parametri su imali najveće vrijednosti kod jedra unutrašnjeg koljenastog tijela. Statistički značajna razlika postojala je u volumenskoj, površinskoj i dužinskoj gustini između vaskularnih mreža kohlearnog jedra i jedra medijalnog koljenastog tijela, kao i između jedra donjih kolikula i jedra unutrašnjeg koljenastog tijela. Krvni sudovi kohlearnog jedra i jedra donjih kolikula su se razlikovali samo u dužinskoj gustini.

Zaključak. Krvni sudovi kohlearnog jedra i jedra donjih kolikula imaju sličnu gustinu i prečnik, ali su sudovi jedra donjih kolikula tortuozniji. U jedru unutrašnjeg koljenastog tijela krvni sudovi su veći, gušći i tortuozniji u odnosu na ostala dva subkortikalna auditivna centra.

Ključne riječi: kohlearna jedra, donji kolikuli, medijalno koljenasto tijelo, krvni sudovi