აქტიური მონომერული ალკალოიდების ფრაქციების გამოყოფისათვის გამოყენებული იყო სითხე - სითხოვანი ექსტრაქცია, დალექვა პეტროლეინის ეთერით (I), პოლიბუფერული დაყოფის ხერხი (II) და 10% ძმარმჟავას ექსტრაქცია (III). LC-MS/MS და GC/MS მეთოდებით იდენტიფიცირებულია 14 ცნობილი შენაერთი: დაბალმოლეკულური (M+226, M+202, №1,2, M+168) და ალკალოიდები: *Ajmalicine, Tetragidroalstonine, C20-dihydrovallesiahotamine, C19-C20 Vallesiahotaminole, Vallesiahota mine lacton, Polyneuridine, Pericyclivine, Lochnerame, Norharmane, Vidorosine, Vindolinine, Isovindolinine, Akuammicine.* მონომერული ალკალოიდების ციტოტოქსიკურობა შეფასდა A-549 (ფილტვის კიბოს ხაზოვანი უჯრედები), DLD-1 (სწორი ნაწლავის ადენოკარცინომის ხაზოვანი უჯრედები) და W-1 (ადამიანის ნორმალური ფიბრობლასტების ხაზოვანი უჯრედები). სამივე (I, II, III) სუბსტანცია ამჟღავნებს მკვეთრად გამოხატულ ციტოტოქსიკურ აქტივობას.

THE EFFECT OF HIGH INTENSITY WHITE NOISE ON THE ULTRASTRUCTURE OF AXO-DENDRITIC SYNAPSES IN COLLICULUS INFERIOR OF ADULT MALE CATS. QUANTITATIVE ELECTRON MICROSCOPIC STUDY

[1]Gogokhia N., [1,2]Pochkhidze N., [2]Japaridze N., [2]Bikashvili T., [1,2]Zhvania M.

[1]Ilia State University, Tbilisi, Georgia. [2]Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

Every day, people are exposed to various types of undesirable or harmful sounds created by various sources, including transport, household machines, recreational or industrial activities [1,2]. Chronic loud noise is known to produce numerous adverse effects on different levels of the organism. In addition to behavioral changes, the involvement of different auditory and non-auditory regions of the brain were described. Thus, structural and molecular modifications in subcortical auditory structures, and some "non-auditory" regions (the hippocampus, cerebellum, reticular formation, amygdala nuclei, others), involved in the processing of auditory information were detected [3-5]. The analysis of such modifications revealed that as a result of chronic noise exposure the alterations in neurotransmission take place. Therefore, of special interest should be the elucidation of the effects of chronic noise on the fine structure of synapses. Earlier, we show that high intensity white noise provokes ultrastructural alterations in porosome complex of auditory regions of cat brain [6]. Porosomes are the universal neurotransmitter-release or secretory machinery in cell plasma membrane – special site, where synaptic vesicles transiently dock to expel their content [7,8]. Each type of secretory cell porosome is characterized with specific shape and size, which is dictated by vesicle unique content, speed of release and volume of content. In neurons (fast secretory cells) porosome range in size from 10 to 20 nm, where 35-50 nm synaptic vesicles are found to dock [6,9,10]. Neuronal porosome has central plug - unique structure, atypical gatekeeper during neurotransmission, which is absent in other types of secretory cells. Using atomic force microscopy, electron microscopy, solution X-ray, 3D contour mapping and some other modern approaches, three conformational states of porosome plug – fully pushed outward, halfway retracted and completely retracted into porosome cup has been described [8,11]. The process of neurotransmission closely depends from such positions of central plug. Describing ultrastructural changes in porosome complex as a result of noise, we have found modifications in the position of porosome plug also. Such data indicate that the alterations in neurotransmission provoked by white noise may be reflected on the finest structural level of synapses.

In the present study, we continue our experimental electron microscopic studies of the effects of loud noise on fine morphology of the brain. In particular, we describe the consequences of high intensity prolonged noise on the morphology of axo-dendritic synapses, and size and diameter of synaptic vesicles in such types of synapses of adult male cats. We are focused on subcortical auditory area – a central part of inferior colliculus, the region of midbrain, which performs one of the key roles in auditory signal integration, frequency recognition, pitch discrimination. In addition, this region is actively involved in transfer of auditory sensory signals to and from the superior colliculus [12,13].

Material and methods. *Animals and Noise exposure***.** Adult male cats (14-16 months old) were used. The animals were housed 1/cage, in a wire cages (38 × 30 × 25 cm) that ensured acoustic transparence. The room was well controlled (a light/dark cycle of 12:12 h; the temperature − 20°C −22°C, humidity − 55-60%). The animals had free access to food and water. Experimental animals were exposed to 100 dB (5-20 kHz) white noise in their home cage for one hour per day, for 10 consecutive days. The noise was provided by two Paradigm Signature S1 P- Be loudspeakers (Paradigm Electronics Inc., Canada), which were mounted 55 cm above the floor of the cages. The same approach was used in our early studies performed on rats [14,15]. Sound levels were constantly monitored using the microphone, suspended in a line 45 cm above the cage. On the next day after the last noise exposure the brains for electron microscopy were taken. Control animals were not exposed to noise. The animal maintenance and other procedures were conducted in accordance with European Union Directive on the protection of animals used for scientific research (Regulation (EU)2019/1010, adopted by the European Parliament, on 5 June 2019). The Committee of Animal Care at Ivane Beritashvili Center of Ex-

perimental Biomedicine and Committee on Ethics at Ilia State University approved the protocols.

For electron microscopic studies, conventional techniques, described in our early studies was used [15,16]. Briefly, under pentobarbital injection, animals underwent cardiac perfusion with ice cold heparinized 0.9% NaCl, followed by 500 mL of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at a perfusion pressure of 120 mm Hg. The left hemispheric brain tissue blocks containing the areas of interest were post-fixed in 1% osmium tetroxide and cut into 400-micron thick coronal slices using a cryostat. The inferior colliculus and medial geniculate body were identified with an optical microscope Leica MM AF, cut out from the coronal slices, dehydrated in graded alcohols and acetone and embedded in araldite. Blocks were trimmed and 70–75 nm thick sections were cut with an ultra-microtome Reichert, picked up on 200-mesh copper grids, double-stained with uranyl-acetate and lead-citrate, and examined with a JEM 100 C (JEOL, Japan) and HF 3300 (Hitachi, Japan) transmission electron microscopes. For each case, 120 sections were observed. On EM micrographs, taken from these sections, we were focused on large axon profiles (~ 2mm2 in area), which made asymmetric junctions and contained 25-40 spherical synaptic vesicles. Thus, the 250 axon endings from control animals and 250 endings from noise-exposed animals (50 endings from each cat) were randomly selected and the diameter of synaptic vesicles were measured. For this purpose, the tracings of axon terminals were scanned, using the scan plug-in for Adobe Photoshop CS3 and saved as 150 dpi tiff files. The scans were imported into ImageJ software (version 1.44, The National Institute of Mental Health). The images of the axon terminals were enlarged onto the computer screen and each vesicle was sequentially marked, using the brush tool. The diameter of docked and undocked spherical synaptic vesicles were measured with "Image J" software (version 1.44, The National Institute of Mental Health). To determine whether white noise impacted vesicle size, one-way ANOVA was performed. Multiple comparisons were made using the two-sample ttest. A P-value less than 0.05 was considered to be statistically significant. In addition to quantitative analysis, qualitative description of the ultrastructure of axo-dendritic synapses in abovementioned brain areas was made.

Results and discussion. *Ultrastructure of central part of colliculus inferior.* In this subcortical auditory region, a number of ultrastructural modifications of synapses were observed. In particular, in ~ 15% large synaptic terminals the clustering of synaptic vesicles, as well as swelling, partial vacuolization or degeneration of presynaptic mitochondria were observed. In some cases, partially destructed/moderately vacuolated mitochondria and/or vacuoles of identified origin were detected in some postsynaptic regions. In comparing with control material, comparatively often large presynaptic terminals contained only few or even single synaptic vesicles in parallel with relatively large and highly osmiopilic active zone. In addition to such changes, some axons were slightly demyelinated; a number of large dendrites contained vacuoles; in some cells moderate chromatolysis is observed (Fig. 1A,B,C).

Such data indicate that chronic auditory stimulation provokes in inferior colliculus the depletion of some synapses, which should be related with their hyperactivity as a result of noise. We suggest that the biggest part of such synapses are made by projections from cochlear nucleus and lateral lemniscus, since they represent major afferents of this regions; moreover, ~60% of these projection have large terminals, contain spherical synaptic vesicles and made asymmetric excitatory axo-dendritic synapses [17,18]. At the same time, absolute majority of small presynaptic terminals with spherical vesicles (probably terminals of interneurons), remained unchanged. Therefore, we show that different by origin axonal projections of colliculus inferior are differentially vulnerable to white noise.

The size of synaptic vesicles. The results of morphometric analysis demonstrate that in both control and experimental animals, the size of docked vesicles in comparing with undocked vesicles is lesser. In experimental animals such difference is especially prominent. Thus, in control cats, the loss of the diameter of docked synaptic vesicles over undocked vesicles is 5.7% (42.62±0.68 nm vs. 45.04±0.35 nm, $P < 0.001$), while in noise-exposed cats, such decrease constitutes 11.3% (34.27±0.69 nm vs. 38.13±0.24nm, $P<0.001$). Significant difference was also detected when comparing the diameters of docked and undocked synaptic vesicles in control and noise-exposed cats. Thus, in experimental animals there was a 19.6% drop in diameter in the docked synaptic vesicles over those in control (42.62±0.68 nm vs. 34.27±0.69 nm, $P<0.001$), while a 15.3% decrease in undocked synaptic vesicles diameter was observed in experimental animals over control (45.04±0.35nm vs. 38.13±0.24 nm $P<0.001$), Fig. 2.

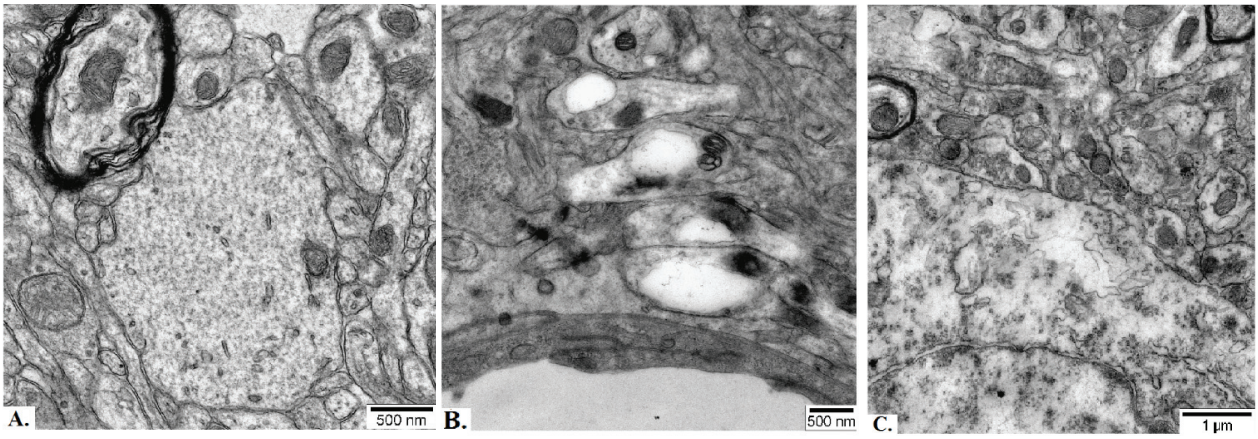

Fig. 1. Ultrastructural changes in the colliculus inferior of noise-exposed cat.
A – In neuropil slightly demyelinated large axon is seen.
B – The part of neuropil a number of dendrites contain large vacuoles and destructed mitochondria.
C – The part of neuron with signs of moderate chromatolysis

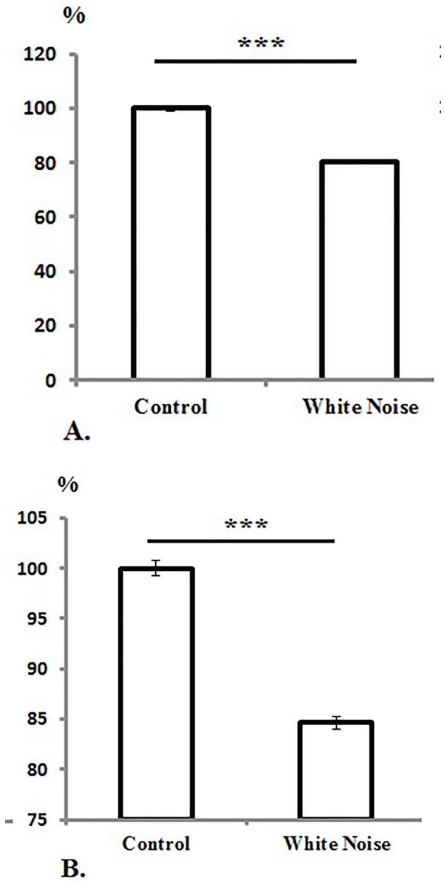

*Fig. 2. Size of docked and undocked synaptic vesicles in the inferior colliculus of control and noise-exposed cats, demonstrating the decrease in size of both docked and undocked vesicles as a result of white noise exposure. A - Bars represent percent difference in mean values of docked vesicles' diameters in control animals vs. noise-exposed animals; B - Percent difference in mean values of undocked vesicles in control animals vs. noise-exposed animals. ***P<0.001*

Therefore, in both groups of animals, docked synaptic vesicles show more prominent decrease in size/diameter than undocked synaptic vesicles (Figure 2). Such results suggest that due to continuous transmission, the majority of vesicles are unable to replenish their cargo via transporters. On the other hand, since both control and experimental animals show the decreased size of docked vesicles in comparing to undocked vesicles, the fractional discharge of vesicular content via porosome-mediated kiss-and-run mechanism of synaptic vesicle fusion and neurotransmitter release at large axon terminal is interfered [17,18]. Such data are reminiscent with our earlier studies, demonstrating that chronic noise-exposure in cats alters the main structural parameters of porosome complex – diameter and depth [6].

It is well established that secretory vesicle swelling is required for the process of secretion, including neurotransmission [19,20] recent studies using fluorescence correlation spectroscopy and cryogenic electron microscopy, show that glutamatergic synaptic vesicles reversibly increase their size upon filling with glutamate [21-23] The increase in diameter usually corresponds to an increase in surface area and in volume [20] The large size increase implies a large structural change in vesicles upon loading with neurotransmitters, and or ion and water transport. Other studies report the changes in both number and size of trafficking synaptic vesicles following stimulation [21, 22, 24]. Our data reminiscent with these studies.

In summary: the results of our electron microscopic morphometric study revealed that high intensity chronic loud noise affects the ultrastructure of subcortical auditory regions – inferior colliculus. In addition to ultrastructural changes in a number of presynaptic regions, we show the depletion of synaptic vesicles in some large terminals forming axo-dendritic synapses.

Evaluation of synaptic vesicles size undertaken in the current electron microscopic study has advanced the understanding of the pathophysiology of white noise exposure on auditory brain processing regions, in addition to our understanding of fractional neurotransmitter release at the nerve terminal and on overall brain function.

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SUMMARY

THE EFFECT OF HIGH INTENSITY WHITE NOISE ON THE ULTRASTRUCTURE OF AXO-DENDRITIC SYNAPSES IN COLLICULUS INFERIOR OF ADULT MALE CATS. QUANTITATIVE ELECTRON MICROSCOPIC STUDY

[1]Gogokhia N., [1,2]Pochkhidze N., [2]Japaridze N., [2]Bikashvili T., [1,2]Zhvania M.

[1]Ilia State University, Tbilisi, Georgia. [2]Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

Environmental noise is a serious problem for the society and medicine. Chronic loud noise is known to produce numerous adverse effects on different levels of the organism. In addition to behavioral changes, the involvement of different regions of the brain was documented. The analysis of structural modifications provoked by noise in brain give the possibility to suggest that one of the effects of noise may be the alterations in neurotransmission. Therefore, of special interest should be the elucidation of the effects of chronic noise on the fine structure of synapses of brain areas participating in the processing of auditory information. In the present study, using transmission electron microscope. We elucidate the effects of high intensity chronic white noise on the morphology of axo-dendritic synapses, and size and diameter of synaptic vesicles in auditory region, inferior colliculus of adult male cats. Experimental animals were exposed to 100 dB (5-20 kHz) white noise for one hour per day, for 10 consecutive days. On 11th day, after special procedures, the area of interest was examined under electron microscope. In ~ 15% large synaptic terminals the clustering of synaptic vesicles, as well as swelling, partial vacuolization or degeneration of presynaptic mitochondria were detected. Morphometric analysis of docked (with presynaptic membrane) and undocked synaptic vesicle size revealed that docked vesicles are smaller than undocked vesicles. It was observed in both control and experimental animals, however, in experimental animals, such difference was more significant. Such results suggest that due to continuous transmission, the majority of vesicles are unable to replenish their cargo via transporters. Evaluation of synaptic vesicles size undertaken in the current electron microscopic study has advanced the understanding of the pathophysiology of white noise exposure on auditory brain processing regions, in addition to our understanding of fractional neurotransmitter release at the nerve terminal and on overall brain function.

Keywords: high intensity chronic white noise. transmission electron microscope. colliculus inferior. descriptive and morphometric analysis of synapses. cat.

РЕЗЮМЕ

ЭФФЕКТ БЕЛОГО ШУМА ВЫСОКОЙ ИНТЕНСИВНОСТИ НА УЛЬТРАСТУКТУРУ АКСО-ДЕНДРИТНЫХ СИНАПСОВ НИЖНИХ БУГРОВ ДВУХОЛМИЯ ВЗРОСЛЫХ КОШЕК-САМЦОВ. КОЛИЧЕСТВЕННОЕ ЭЛЕКТРОННОЕ МИКРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ

[1]Гогохия Н.Г., [1,2]Почхидзе Н.О., [2]Джапаридзе Н.Д., [2]Бикашвили Т.З., [1,2]Жвания М.Г.

[1]Государственный университет им. Ильи, Тбилиси; [2]Центр экспериментальной биомедицины им. Иване Бериташвили, Тбилиси, Грузия

Экологический шум - серьезная проблема общества и медицины. Хронический экологический шум вызывает многочисленные негативные эффекты на разных уровнях организма. Описаны как изменения в поведении, так и вовлечение разных областей головного мозга. Анализ структурных модификаций указывает на возможные изменения в процессах трансмисии. Соответственно, большой интерес представляет изучение эффектов экологического шума на ультраструктуру областей мозга, участвующих в обработке слуховой информации.

В исследовании с использованием трансмиссионного электронного микроскопа описан эффект хронического шума на морфологию аксо-дендритных синапсов и размер синаптических везикул в слуховом отделе – нижнем двухолмии взрослых котов. Экспериментальные животные в течение 10 дней, 1 час каждый день подвергались воздействию белого шума - 100 dB (5-20 kHz). Животных выводили из опыта перфузией на 11 день. В 15% широких синаптических терминалей отмечались кластеры синаптических везикул и набухшие, частично вакуолизированные или разрушенные пресинаптические и постстинаптические митохондрии. Морфометрический анализ синаптических везикул выявил,

что как у контрольных, так и экспериментальных животных размеры стыкованных с пресинаптической мембраной везикул меньше, чем размеры свободных везикул. Такое отличие более выражено у экспериментальных животных. Таким образом, следует предположить, что ввиду продолжительной трансмисии некоторые везикулы неспособны полностью загрузиться с помощью транспортеров. Электронномикроскопическое исследование размеров синаптических везикул расширяет знание о патофизиологических механизмах воздействия белого шума на слуховые области мозга, участвующие в переработке слуховой информации, а также понимание фракционного выделения нейротрансмиттеров из нервных терминалей.

რეზიუმე

მაღალი ინტენსიობის თეთრი ხმაურის ეფექტი ზრდასრული მამრი კატების ქვედა ორგორაკის აქსო-დენდრიტული სინაფსების ულტრასტრუქტურაზე. რაოდენობრივი ელექტრონულ-მიკროსკოპული ანალიზი

[1]ნ.გოგოხია, [1,2]ნ.ფოჩხიძე, [2]ნ.ჯაფარიძე, [2]თ.ბიკაშვილი, [1,2]მ.ჟვანია

[1]ილიას სახელმწიფო უნივერსიტეტი, თბილისი; [2]ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო

გარემოს ხმაური საზოგადოების და მედიცინის უმნიშვნელოვანესი პრობლემაა. ქრონიკული ხმაური ორგანიზმის სხვადასხვა დონეებზე მრავალრიცხოვან უარყოფით ეფექტებს იწვევს. ქცევითი ალტერაციების გარდა, ცვლილებები თავის ტვინის სხვადასხვა დანაყოფებში ვლინდება. ასეთი მოდიფიკაციების ანალიზი იძლევა საშუალებას დაშვებისთვის, რომ ხმაურის ერთ-ერთი ეფექტი ნეიროტრასნსმიის ცვლილებებს წარმოადგენს. ამგვარად, საინტერესოა ქრონიკული ხმაურის ეფექტების შესწავლა სმენითი ინფორმაციის გადამუშავებაში ჩართული თავის ტვინის უბნების სინაფსების აღნაგობაზე. წარმოდგენილ კვლევაში, ტრანსმისიული ელექტრონული მიკროსკოპის გამოყენებით, შესწავლილია მაღალი ინტენსიობის თეთრი ხმაურის გავლენა მამრი ზრდასრული კატების ქვედა ორგორაკის სინაფსების აღნაგობასა და სინაფსური ვეზიკულების დიამეტრზე. ექსპერიმენტული ცხოველები 10 დღის განმავლობაში, ყოველდღიურად 1 საათი, იმყოფებოდნენ 100 dB (5-20 kHz) თეთრი ხმაურის ზემოქმედების ქვეშ. დიდი ზომის სინაფსური ტერმინალების ~15%-ში გამოვლინდა სინაფსური ვეზიკულების კლასტერიზაცია, პრესინაფსური და პოსტსინაფსური მიტოქონდრიების გაჯირჯვება, მათი ნაწილობრივი ვაკუოლიზაცია და/ან დეგენერაცია. მორფომეტრული ანალიზის მიხედვით, ექსპერიმენტულ და საკონტროლო ცხოველებში პრესინაფსურ მემბრანასთან უშუალოდ მიახლოებული ვეზიკულების ზომები თავისუფალი ვეზიკულების ზომებთან შედარებით, სარწმუნოდ მცირეა. განსხვავება განსაკუთრებით გამოხატული იყო ექსპერიმენტულ ცხოველებში. არ არის გამორიცხული, რომ გახანგრძლივებული ტრანსმისიის გამო, ვეზიკულების ნაწილი ვერ ახერხებს კარგად შევსებას ტრანსპორტიორების მეშვეობით. სინაფსური ვეზიკულების ზომების ანალიზი ხელს უწყობს ხმაურის შედეგად განვითარებული უჯრედული ცვლილებების პათფიზიოლოგიის გაშუქებას, ასევე ნერვულ ტერმინალებში ნეიროტრანმსიტერების ფრაქციულ გამოყოფასთან დაკავშირებული მექამიზმების შესწავლას.

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