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Cytology

The Effect of High Intensity Chronic Noise on Porosome Complex Structure and Synaptic Vesicle Size in Auditory Regions of the Cat Brain

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(Presented by Academy Member Nodar Mitagvatia)

In the present electron microscopic study, the effects of high intensity chronic noise on porosome complex morphology and synaptic vesicles size in auditory regions of cat brain - mesencephalic colliculus inferior and diencephalic medial geniculate body were evaluated. Specifically: (i) the diameter and depth of porosome, and (ii) the size of docked and undocked synaptic vesicles were measured. In both regions, abovementioned parameters of porosome are heterogeneous and the range of their fluctuations in control and noise-exposed animals was the same. However, chronic noise produces different effects on porosome complexes in these regions. Thus, in colliculus inferior no changes were observed, while in medial geniculate body significant increase of porosome depth was revealed. In addition, in colliculus inferior of noise-exposed animals, significant decrease of the size of docked synaptic vesicles was detected. Morphological changes of porosome and synaptic vesicle size should reflect the alterations in neurotransmission in response to chronic loud noise. The data also give the possibility to suggest fractional discharge of vesicular contents via porosome-mediated kiss-and-run mechanism. *© 2023 Bull. Georg. Natl. Acad. Sci.*

high intensity white noise, porosome complex, synaptic vesicles, electron microscopic morphometry, cat

In all types of secretory cells, physiological processes are governed by the release of intracellular products stored in membrane-bound vesicles. This is possible either (i) via complete collapse of the vesicle membrane with the plasma membrane – the process termed total fusion, or (ii) transient fusion of secretory vesicles at the plasma membrane termed kiss-and-run mechanism. In absolute majority of cases, the last mechanism acts via porosome complex – molecular nano machine located at the cell plasma membrane [1, 2]. Porosome size and shape are dictated by the content, speed of release and volume of content in secretory vesicles. Neuronal porosome ranges in size from 10 to 20 nm, with 35-50 nm synaptic vesicles are found

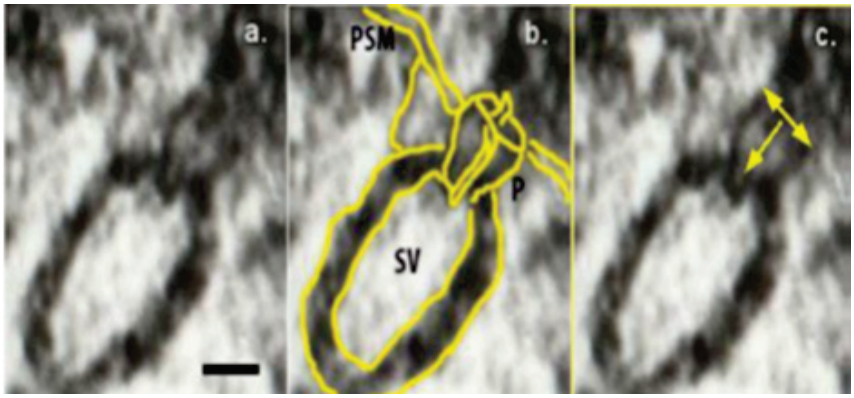

Fig. 1. Electron micrograph of synaptic vesicle docked at the base of neuronal porosome. (a) A 50 nm synaptic vesicle (SV) docked at the base of 15 nm porosome (P) at the presynaptic membrane (PSM). (b) Yellow lines outline the synaptic vesicle and porosome with the central plug. (c) Double-headed arrow shows the diameter of porosome opening; single-headed arrow pointing inward represents the depth. Scale Bar=10 nm.

to dock [2]. The peculiarity of neuronal porosome is the presence of unique substructure, central plug, which acts as a gatekeeper during neurotransmission [2]. Different positions of central plug: fully pushed outward (close conformation), halfway retracted and completely retracted (open conformation) into porosome cup have been described. Neuro-transmission takes place when porosome is in open conformation. Porosome is easily detectable in intact synaptosome and inside-out synaptosome using atomic force microscope, but it is much difficult under electron microscope (EM), due to artifacts producing by tissue processing. However, some structural particularities of porosome complex are clearly visible on EM micrographs (Fig. 1).

In our current EM studies, we described the neuronal porosome in intact brain of various mammalian species [3, 4] and mammals subjected to pathological conditions [4]. Here we evaluate the effects of high intensity chronic noise on the diameter and depth of porosome in auditory regions of cat brain - central nucleus of inferior colliculus (IC) and ventral subdivision of medial geniculate body (MGB). The size of docked and undocked vesicles was also measured.

Material and Methods

A total 8 male adult cats (P280-300) from ordinary vivarial conditions (12 h light/dark cycle, temperature 20-22°C; humidity 55–60%) were used. Experimental animals (n=4) were subjected to 100 dB noise during 20 consecutive days, 1 hour per day. For this purpose, two Paradigm Signature S1 P- Be loudspeakers (Paradigm Electronics Inc.) were used. Noise-unexposed animals (n=4) were served as a control.

Material for EM, was prepared using conventional procedures (transcardiac perfusion with paraphormaldehyde and glutaraldehyde solution, tissue postfixation in osmium tetroxide, processing in graded series of ethanol and acetone and embedding in araldite, preparation of ultrathin section, staining of sections with uranyl-acetate and lead-citrate) [5]. The sections were examined with a transmission electron microscope HF 3300 (Hitachi, Japan). For each case, 120 sections (30 sections from each animal) were studied. All procedures were performed in accordance with Ethical Guidelines for the Use of Animals in Research, given by the National Committee for Research Ethics in Science and Technology (NENT), 2018.

Morphometric analysis of porosome morphology. On EM micrographs, evaluating more than 500 synaptic terminals in each brain region, 178 porosomes in IC and 170 porosomes in MGB in control and experimental material were identified. Diameter and depth were measured with “Image J”

software (version 1.44). To define whether the noise affects porosome's diameter and depth, one-way ANOVA was performed in each region separately. In the case of significant effect, planned comparisons were carried out using *t*-test. To analyze the combined effect of noise and porosome location in brain areas, two-way ANOVA was accomplished. A *p*-value less than 0.05 was considered as significant.

Morphometric analysis of synaptic vesicle size. Large axon profiles (~2mm^2 in area), which contained >25 synaptic vesicles and made asymmetric synapses on small dendrites or spines were evaluated: In literature, such profiles are considered as the endings of auditory projections. On EM micrographs, the 200 axon terminals from control animals and 200 endings from experimental animals (50 endings from each cat) were scanned using the scan plug-in for Adobe Photoshop CS3. The vesicles in each terminal were divided as docked and undocked types, depending their localization. Docked vesicles (usually 1-2 vesicles) were in close proximity with active zone (usually 1-3 vesicles). All others composed the pool of undocked vesicles (>60nm from active zone). The diameter of docked and undocked vesicles was measured with "Image J" software (version 1.44). To evaluate whether chronic noise affects vesicle size, one-way ANOVA was accomplished. Multiple comparisons were made, using the two-sample *t*-test. A P-value less than 0.05 was considered as statistically significant.

Results and Discussion

The effect of noise on porosome complex. According to one-way ANOVA, noise does not affect the opening diameter of porosomes in both regions (F=1.955, p=0.12), however, the depth of porosome is significantly changed (F=6.434, p=0.0004). Planning comparisons of depth between intact and noise-exposed groups, revealed the difference only in MGB: here the mean value of porosome depth in noise-exposed animals was significantly higher than in control group (control: 11.42 ± 3, experiment: 13.44 ± 4, p< 0.01) (Fig.2).

To determine whether two factors – noise and porosome location – affects diameter of opening and depth porosome, the two-way ANOVA was done; no significant difference was revealed (white noise $F7,177$ = 1.03, p=0.312; location F$7, 177$ = 1.04, p=0.310).

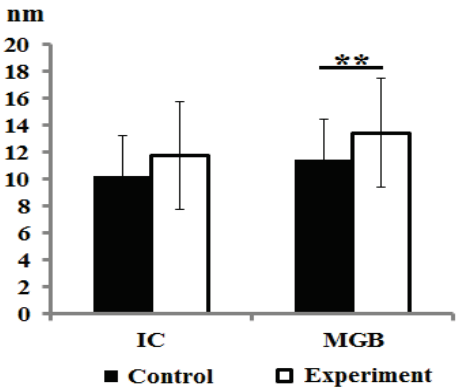

Fig. 2. Porosome depth in cat brain. IC – inferior colliculi, MGB – medial geniculate body; ** - $p < 0.01$.

The effect of noise on synaptic vesicle size. In IC of noise-exposed cats, the decreased size of docked and undocked vesicles in comparing with control animals was observed (control: 5.7%: 42.62 ± 0.68nm vs. 45.04 ± 0.35nm, p<0.001; experiment: 11.3%: 34.27 ± 0.69nm vs. 38.13 ± 0.24nm, p<0.001). Significant difference was also detected when comparing the diameters of docked and undocked vesicles in animals of control and experimental groups. Specifically, in experimental animals there was a 19.6% drop in diameter in the docked synaptic vesicles over those in control (control: 42.62 ± 0.68nm; experiment: 34.27 ± 0.69nm, p<0.001) and only 15.3% decrease in undocked vesicles diameter in experimental animals over control (control: 45.04 ± 0.35nm; experiment: 38.13 ± 0.24nm, p<0.001) (Fig. 3). Therefore, in both groups of animals, docked synaptic vesicles show more prominent decrease in size than undocked synaptic vesicles.

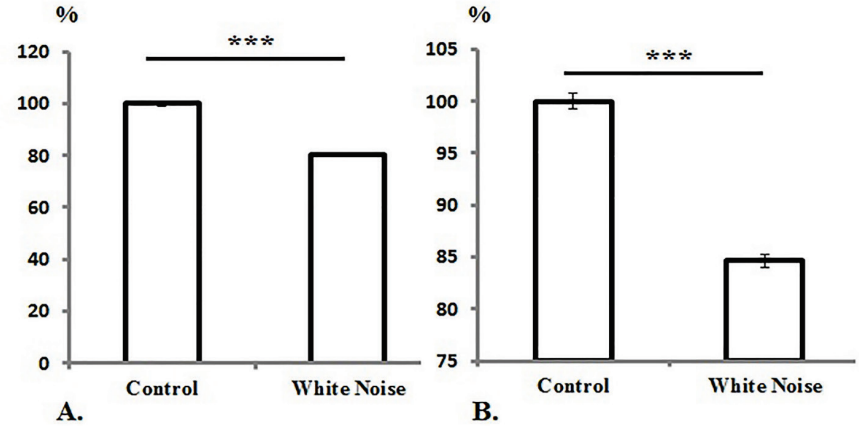

Fig. 3. Size of docked (A) and undocked synaptic vesicles (B) in inferior colliculus of control and noise-exposed cats. ***P < 0.001.

Thus, the effect of continuous loud noise on porosome structure and the size of synaptic vesicle from different pools in two main subcortical auditory areas - IC and MGB was revealed. We show significant increase of porosome depth in MGB, postero-inferior aspect of thalamus that serves as the last processing station of auditory information to the cerebral cortex and as thalamic relay between IC and auditory cortex [6]. Earlier it was shown that in response to loud noise, IC produces time-dependent increase of metabolic activity and apoptosis-related alterations [7, 8]. Because neuronal porosomes are directly associated with the neurotransmission [9, 10], we propose that altered porosome depth should reflect the increase of neurotransmission induced by noise-related sustainable stimulation. Apoptosis-related alterations may be one of the consequences of such increase. We also show that in comparing with control, in noise-exposed animals the size of docked and undocked vesicles was decreased. It was especially prominent in the mesencephalic IC, which represents the first location where inputs from cells carrying horizontal and vertical sound location data from each ear converges [11]. Secretory vesicle swelling, associated with the increase of their size, surface area and volume, is a necessary condition for secretion, including neurotransmitter release from synaptic terminals [12, 13]. Swelling of vesicles should indicate their structural changes upon loading with neurotransmitters or ion and water transport [14, 15]. Therefore, the decreased size of vesicles in experimental animals may indicate that due to continuous neurotransmission the majority of vesicles are unable to timely replenish their content. In addition, a number of studies show decreased size of trafficking synaptic vesicles following high-frequency stimulation [16]. Since the depletion of vesicle size is more prominent in experimental animals, we suggest that the fractional discharge of vesicular content via porosome-mediated kiss-and-run mechanism of synaptic vesicle fusion and neurotransmitter release at large axon terminals is interfered.

Conclusion

Present data extend existing knowledge regarding pathological effect of noise on auditory processing and fractional release of neurotransmitters. Further EM evaluation of alterations related with neurotransmission may give high-informative results.

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(წარმოდგენილია აკადემიის წევრის ნ. მითაგვარიას მიერ)

წარმოდგენილ ელექტრონულ-მიკროსკოპულ კვლევაში შეფასებულია მაღალი ინტენსივობის ქრონიკული ხმაურის ეფექტი ზრდასრული მამრი კატების თავის ტვინის სმენითი სტრუქტურების, მეზენცეფალური ქვედა ორგორაკის და დიენცეფალონის მედიალური დამუხლული სხეულის პოროსომული კომპლექსის მორფოლოგიასა და სინაფსური ვეზიკულების ზომებზე. კერძოდ, გაზომილია პოროსომას დიამეტრი და სიღრმე და პრესინაფსურ მემბრანასთან შერწყმული და თავისუფალი სინაფსური ვეზიკულების დიამეტრი. პოროსომას აღნიშნული პარამეტრები სმენის ორივე უბანში ჰეტეროგენურია, საკონტროლო და ხმაურით ექსპოზირებულ ცხოველებში ფლუქტუაციების რანჟირება მსგავსია, ამასთანავე, აღნიშნული უბნების პოროსომულ კომპლექსებზე ხმაურის ეფექტები განსხვავებულია. კერძოდ, ქვედა ორგორაკში კომპლექსის ცვლილებები არ აღინიშნა, ხოლო მედიალურ დამუხლულ სხეულში სარწმუნოდ გაიზარდა პოროსომას სიღრმე. გარდა ამისა, ხმაურით ექსპოზირებული ცხოველების ქვედა ორგორაკში სარწმუნოდ შემცირდა სინაფსურ მემბრანასთან შერწყმული სინაფსური ვეზიკულების ზომა. პოროსომული კომპლექსის და სინაფსური ვეზიკულების მორფოლოგიური ცვლილებები ქრონიკულ ხმაურზე საპასუხოდ განვითარებულ ნეიროტრანსმისიის ცვლილებებს უნდა ასახავდეს. მონაცემები ასევე იძლევა საფუძველს დაშვებისთვის, რომ ვეზიკულების შიგთავსის ფრაქციული გამოყოფა პოროსომა-მედიტირებული „აკოცე-და-გაიქეცი“ მექანიზმით მიმდინარეობს.

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