- Bengtsson AK, Ryan EJ, Giordano D, Magaletti DM, Clark EA. 17beta-estradiol (E2) modulates cytokine and chemokine expression in human monocyte-derived dendritic cells. Blood 2004; 104: 1404-1410.
- 3. *Tyagi AM, Srivastava K, Mansoori MN, Trivedi R, Chattopadhyay N, Singh D.* Estrogen deficiency induces the differentiation of IL-17 secreting Th17 cells: a new candidate in the pathogenesis of osteoporosis. PLoS One 2012; 7: 44552.
- Pioli PA, Weaver LK, Schaefer TM, Wright JA, Wira CR, Guyre PM. Lipopolysaccharideinduced IL-1 beta production by human uterine macrophages up-regulates uterine epithelial cell expression of human beta-defensin 2. J. Immunol. 2006; 176: 6647-6655.
- Pioli PA, Jensen AL, Weaver LK, Amiel E, Shen Z, Shen L, Wira CR, Guyre PM. Estradiol attenuates lipopolysaccharide-induced CXC chemokine ligand 8 production by human peripheral blood monocytes. J. Immunol. 2007; 179: 6284-6290.
- 6. *Miller AP, Feng W, Xing D, Weathington NM, Blalock JE, Chen YF, Oparil S.* Estrogen modulates inflammatory mediator expression and neutrophil chemotaxis in injured arteries. Circulation 2004; 110: 1664-1669.

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## CRONIC ACOUSTIC STRESS AND A<sub>2</sub>. ADRENOBLOCKERS EFFECT ON OPEN FIELD ACTIVITY OF THE RAT

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Open field, noise,  $\alpha_2$ -adrenoblockers,behavioral activity, stress «Բաց դաշտ», աղմուկ,  $\alpha_2$ -ադրենաբլոկատորներ, վարքային ակտիվություն, սթրեи Открытое поле, шум,  $\alpha_2$ - адрноблокаторы, поведенческая активность, стресс

It is shown that among hazardous factors of the environment the high level of industrial, transport and community ambient noise are extremely dangerous, which drastically decrease the resistibility of an organism, promote different diseases development and particularly lead to the **Cognitive disorders**, affect learning, memory, perception, and the problem solving [20,15,17]. The latteris much likely to be associated with an increase of the stress hormone release and the oxidative stress (OS) development [1]. Epidemiological research provides the possibility of an integral risk estimation of community noise based directly on the empirical data gained under the genuine conditions of exposure, considering factors potent to amplify or attenuate the noise exerted effects [21].

The data obtained serve as evidence of the structural reorganization and the functional change of bio-membranes in experimental animals under the noise action due to lipid peroxidation (LPO) process activation and  $\alpha$ -Tocopherol exhaustion in tissues and significant protective effects of antioxidants and the stress –limiting compounds, among which we consider adrenoblockers [14]. Our investigations have shown a harmful action of chronic noise on the protein oxidative modification processes intensity both in plasma and Erythrocyte Membranes and the modifying effects of  $\alpha_{2.}$ adrenoblockers on oxidative intensity, as well [14,22].

Considering all the above mentioned it is necessary to study effects related only to the noise influence in experiments, eliminating possibility of side effects.

Open field activity enables scientists evaluating locomotor behavioral activity, including the Total distance travelled, Average speed, Number of line crossings, Total

freezing episodes, Total time freezing, Average freezing score, Number of entries to the center zone, Time in the Center zone, Distance travelled in the Center zone, Corrected integrated path length for the Center zone, Time in the Periphery zone, Number of entries to the Periphery zone, Distance travelled in the Periphery zone, Time freezing in the Periphery zone, Corrected integrated path length for the Periphery zone was investigated first by Calvin Hall [9,10], who verified behavioral reaction of rodents by means of encountering defecation and urination.

The open field test nowadaysis one of the most well-known manipulation in animal psychology [3,4,5,]. The open fieldactivityof differentvariantsareavailable for application - differing in shapeof the environment (circular, square or rectangular), lighting (lighting from above with a bulb above the open field or lighting from underneath with a bulb placed under a transparent floor, usually red light is used), presence of objects within an arena such as platforms, columns, tunnels [18,19], etc. Taking into account existing data [16], the open field is a successful devicefor evaluating behavioral damage in animal under the noise. Researchesconducted earlier have revealed that pre-exposure to moderately intense light and white noise facilitated open field activity measured by initial activity, lowered defecation scores, and supplementary measures (rearing, grooming, center field penetration) [12,16]. Ultimately, in the given research we applied the following 4 parameters: Total distance travelled, Average speed, Total freezing episodes, Number of entries to the center zone,

#### Materials and methods

Investigations were carried out on the white male rats weighing 150-200 g kept in ordinary vivarium conditions. The animals were divided into 4 groups: rats of the 1<sup>st</sup> group serve as a control, rats of the  $2^{nd}$ ,  $3^{rd}$  and  $4^{rd}$  groups underwent 91 dB(A) noise influence with maximal energy in the region of average and high frequency; the duration of noise influence was 7, 30 and 60 days, each day 8hr noise exposition; animals of the  $3^{rd}$  group were injected Mesedin (10mg/kg) intraperitoneally, animals of the  $4^{rd}$  group were injected Beditin (2mg/kg) intraperitoneally, the first injection 12hr before the first exposition to noise action. Injections were repeated every 24 hr. Noise applied was obtained from the white noise generator joint with attenuator. The acoustic system supplied the reproduction in the range of 63-16000 Hz.

A square open field [6,11] is represented by the wooden square-shaped plate (60x60), in which in regular periods (6x6) 36 pits of 3.5 cm in diameter and of 2 cm in depth are done. From edges the plate was covered by the walls of 20 cm of height. The proximal 15 cm proximal to the edges of the arena were considered as "peripheral zone" and the central 30x30 cm area was considered as the "central zone". The rats were replaced in the middle of the plate, after which during 5 minutes the active dislocation time was recorded t (in seconds). The manipulation usually involved compelled confrontation of a rodent with the situation. The animal was placed in the centerand the following behavioral items are recorded for a period ranging from 5 min: horizontal locomotion (number of crossings of the lines marked on the floor), frequency of rearing or leaning (sometimes termed vertical activity), grooming (protracted washing of the coat).

In given situation, rodents spontaneously preferred the periphery of the open field, then the central part, so that the rats moved close to the walls (thigmotaxis) [13]. The central part prevailing and spending time in the central part, as well as, decrease of the latency are considered to be indications of anxiolysis [7, 8].

Each trial was recorded for latter analysis, using a video camcorder positioned above the apparatus. Rats of line cross were obtained with an automated camera-based computer tracking system on a computer with the camera fixed to the ceiling, 2.1 m above the apparatus. The research has been approved by Institutional Committee on Bioethics and corresponds to the principles of the Manual of Operation and use of the laboratory animals published by US NIH (N 85-23, reconsidered in 1985).

Statistical analysis was made using BioStat. Significance of means' difference was evaluated using paired Student Newman – Keuls test (Anova). ( $p_1$ - compare with control,  $p_2$ - compare with noise).

#### Procedure

Ratswere transferred to the test room from their home cages and were placed into the center or one of four corners of the open field and allowed to explore the apparatus for 5 minutes (Pic. 1). After the 5 minutes test, rats were returned in their home cages, after which the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests.



Picture 1. Rat is placed into the center or one of four corners.

### **Results and discussion**

Our results indicate the initial behavioral response to stress in an open field is activation. Chronic acoustic stress generally lowered open field activity [14] and prevented the activation effect, and that was restored by chronic injection of  $\alpha_2$ -adrenoblockers (Mesedin and Beditin).

Fig. 1 shows that the total distance travelledfragments in open field test under the acoustic stress conditions after 7, 30 and 60 days have different tendency of changes depending on the duration of experiment. Total distance travelled reports the total distance that the animal travelled during the test period [2].

Total distance travelled was significantly lower (p<0.001) in the 2,3 and 4<sup>th</sup> compared to control groupsafter 7 days noise action. Administration of  $\alpha_2$ -adrenoblockers Mesedin and Beditin prior to noise action to the animals produced remarkable changes on the stress elicited activity and revealed noticeable regulatory effect on the open field activity after 30 (p<0.001) and 60 (p<0.001) days compared to the noise group.





Average speed reports the average speed of the animal during a test. The result is the Total distance travelled during the period divided by the Test duration. Overall activity as well as the amount of time was measured with video-track in the open field (Pic. 2).



Fig. 2 shows average speed in open field test under the acoustic stress conditions after 7, 30 and 60 days. In rats, which were injected Mesedin the average speed was significantly higher (p<0.05) thanin the noise group, where average speed was significantly lower (p<0.001) after 7 days. Yet, under the action of noise after 30 and 60 days the average speed of noise group became more lower (p<0.001), compared to control group, but in the Beditin (p=0.001) and Mesedin injected groups (p<0.001) the average speed partially and significantly increased by influence of  $\alpha_2$ -adrenoblockers.



Fig 2. Average speed of animals under the noise action (n=6, 8hr noise /day)

Behavioral and locomotive activity was also characterized by number of entries to the center zone as well and the locomotive activity of the rats also estimated by number of entries to the center zone. By their behavior rats in the open field prefer to be near to a protective wall, rather than exposed to danger out of the open, but a rival forage instinct will stimulate them to explore.

Fig. 3 shows number of entries to the center zone in the open field test under the experimental conditions. The noise action lead to the drastic decrease of entries to the center zone. Beditin injected animals also reveal the same tendency, entered less times into the center, what was less expressed compare for noise group (p<0.001 in the noise group and

p<0.01 in the Beditin group compare with control).

Rats of the Mesedin injected group reveal non-significant (p=0.26) decrease of entries to the center zone. The same direction of changes observed after 30 and 60 days of noise action. Nevertheless the recovering effect of Mesedin was appeared after 30 and 60 days of noise action, where rats of the Mesedin group spent more time in the center(non significant,  $p_1$ =0.56),



Fig 3. Number of entries to the center zone (each group is of 6 animal, 8hr noise /day) than the control rats.

Fig 4. shows significant differences across categories in number of entries to the center zone under the influence of Mesedin and Beditin. Rats of Mesedin group spent significantly more time in the center, then noise group after 7 days ( $p_2 < 0.05$ ), 30 days ( $p_2 < 0.01$ ) and 60 days action of noise. Moreover, after 60 days action of noise, there was significant ( $p_2 < 0.05$ ) higher scoreacross Beditin and noise group.



Fig 4. number of entries to the center zone across categories (each group is of 6 animals, 8hr noise /day)

Total freezing episodes reports the number of times the animal frozen during the test. The number of freezing episodes was encountered from the starting period of time [2]. For the control animals' a number of freezing episodes increases in the time.

Fig. 5 shows that total freezing episodes in open field test under the acoustic stress conditions after 7, 30 and 60 days have different direction of changes depending on the duration of experiment. The motor activity of 2<sup>nd</sup> group animals significantly decreased after 7 days of noise influence compared with the control group. An opposite direction of activity was observed after 4 weeks action of noise. The most interesting was the fact of restoring control level of studied parameter after 8 weeks, which could be evidence of adaptation reaction. This was partially and significantly (p<0,001) restored by the Mesedin and Beditin effects after 7 days. The 30 days action of noise was of a particular interest, where chronic stress reduced basal activity and Total freezing episodes in open field was significantly (p<0.001) increased in all three experimental groups. However, after 60 day's influence of noise action Total freezing episodes of the noise group no significantly (p=0.42) decreased compared to the control group. Meanwhile a score of Total freezing episodes was significantly greater of Mesedin (p<0.001) and Beditin (p<0.05) injected groups, which was conditioned with restore effects of  $\alpha_2$  adrenoblockers. These findings were justified statistically, and are presented in Fig 6, which shows significant (p < 0.001) differences between the groups (Noise and Mesedin) after 60 days influence of noise action.



Fig 5. Total freezing episodes (each group 6 animal, 8 hr noise /day)



Fig 6. Total freezing episodes between the groups (each group 6 animal, 8hr noise /day)

#### Conclusion

In conclusion, chronic noise action decreases locomotor behavior and increases anxiety, intensity of whichdepend on the duration of noise action. Administration of  $\alpha_2$ adrenoblockers Mesedin and Beditin to the animals reveals a regulatory effect under the chronic acoustic stress conditions, the efficiency of which depends both on the duration of noise action and the studied examples.

## References

- Дубинина Е.Е. Продукты метаболизма кислорода в функциональной активности клеток (жизнь и смерть, созидание и разрушение) // Физиологические и клиникобиохимические аспекты. Из-во «Медицинская пресса». - 2006. – 400 с.
- 2. A detailed description of the ANY-maze measures. www.anymaze.com
- Belzung, C., 1999. Measuring exploratory behavior. In: Crusio, W.E., Gerlai, R.T. (Eds.), Handbook of Molecular Genetic Techniques for Brain and Behavior Research (Techniques in the Behavioral and Neural Sciences). Elsevier, Amsterdam, pp. 739–749.
- 4. *Belzung, C.,* 2001. Rodent models of anxiety-like behaviors: are they predictive for compounds acting via non-benzodiazepine mechanisms? Curr. Opin. Investig. Drugs 2, 1108–1111.
- 5. *Blanchard, D. C., Griebel, G., Blanchard, R. J.* 2001. Mouse defensive behaviors: Pharmacological and behavioral assays for anxiety and panic. Neuroscience and Biobehavioral Reviews, 25, 205-218.
- Colombel C., Lalonde R., Caston J. The effects of unilateral removal of the cerebellar hemispheres on motor functions and weight gain in rats. // Brain Res. – 2002. – Vol.950. – P.231-238.
- C. O. Nku1, G. O. Oghale1 and I. O. Ajiwhen. Locomotor Behaviour and Anxiety in the Open Field and Light/Dark Box in CD1 Mice Treated with Aspirin, Cataflam and Ethanolic Extract of Cannabis sativa // British Journal of Medicine & Medical Research 6(6): 563-572, 2015, Article no. BJMMR.2015.234 ISSN: 2231-061
- D. A. Christakis, J. S. B. Ramirez & J. M. Ramirez. Overstimulation of newborn mice leads to behavioral differences and deficits in cognitive performance, 31 July 2012, SCIENTIFIC REPORTS | 2:546| DOI:10.1038/srep00546.
- 9. *Hall, C. S.* (1934) Emotional behavior in the rat: Defecation and urination as measures of individual diferences in emotionality. J. Comp. Psychol. 18:385-403.
- Hall, C. S., E. L. Ballechey (1932) A study of teh rat's behavior in a field: A contribution to method in comparative psychology. University of California, Publ. Psychol. 6: 1-12.
- Jahkel, M., Rilke, O., Koch, R., Oehler, J. 2000. Open field locomotion and neurotransmission in mice evaluated by principal component factor analysis effects if housing condition, individual activity disposition and psychotropic drugs. Progress in Neuropsychopharmacology and Biological Psychiatry, 24, 6184.
- 12. *Kevin A. Roth, Richard J. Katz.* Stress, behavioral arousal, and open field activity A reexamination of emotionality in the rat.Neuroscience & Biobehavioral ReviewsVolume 3, Issue 4, Winter 1979, p. 247–263.
- Laetitia Prut, Catherine Belzung. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. European Journal of Pharmacology 463 (2003) 3–33.
- 14. Melkonyan M.M., Hunanyan L.S., Harutyunyan H.A., Grigoryan A.M., Tovmasyan N.V., Pogosyan G.A., Zakaryan G.V., Manukyan A.L. The effects of a2 adrenoblockers on oxidation intensity in white rats blood under high level noise chronic action. The New Armenian Medical Journal, Vol. 9 (2015), No3 Supplement, p. 26.
- 15. Proceedings. The 9th Congress of the International commission on the biological effects of Noise. Noise as a Public Health Problem (ICBEN), Foxwoods, CT (2008)
- R.J. Katz, K.A. Roth, B.J. Carroll, Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. Neuroscience & Biobehavioral ReviewsVolume 5, Issue 2, Summer 1981, p. 247–251

- 17. Stansfeld S., Crombie R., Cardiovascular effects of environmental noise: Research in the United Kingdom. Noise Health 13, 2011, pp. 229-233.
- Takahashi, L.K., Kalin, N.H., 1989. Role of corticotropin-releasing factor in mediating the expression of defensive behavior. In: Blanchard, R.J., Brain, P.F., Blanchard, D.C., Parmigiani, S. (Eds.), Ethoexperimental Approaches to the Study of Behavior. NATO ASI Series. Kluwer Academic Publishing, Kluwer, Dordrecht, Boston, London, pp. 580–594.
- Tatem K. S., Quinn, J. L., Phadke, A., Yu, Q., Gordish-Dressman, H., Nagaraju, K. Behavioral and Locomotor Measurements Using an Open Field Activity Monitoring System for Skeletal Muscle Diseases. J. Vis. Exp. (91), e 51785, doi:10.3791/51785 (2014).
- 20. W. Babish, "Health aspects of extra-aural noise research", Noise & health 6, 69-81(2004
- Wright B, Peters E, Ettinger U, Kuipers E, Kumari V.Noise Health. Understanding noise stress-induced cognitive impairment in healthy adults and its implications for schizophrenia. 2014 May-Jun; 16(70): 166-76
- 22. Xu G, Zhang H, Zhang S, Fan X, Liu X. Plasma fibrinogen is associated with cognitive decline and risk for dementia in patients with mild cognitive impairment. Int J. Clin. Pract. 2008; 62: 1070–1075.

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# BIOTECHNOLOGY OF CITRIC ACID PRODUCTION FROM TOPINAMBUR

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Citric acid is widely (75%) used in the food, drags and chemicals productions. Commercially, citric acid is produced from molasses. However plant polyfructans such as inulin are not studied for production of citric acid point of view. Inulin contains 97% fructose and 3% glucose. Considerable amount of inulin contains the tubers of topinambur (*Helianthus tuberosus*) (20-25%). The main aim of present studies is the development of a new microbiological one stage technology with application of the new isolated original strains of fungi for the production of citric acid from a new raw material – topinambur. With this purpose the active strains from *Aspergillus* genera have been isolated. It was shown that indispensable condition for citric acid biosynthesis from inulin by microorganisms is the presence of their inulinase activity. The isolated and selected strains have inulinase activity. It was established that topinambur (inulin) is perspective source for production of citric acid.

Citric acid – inulin – bioconvertion - topinambur

Կիտրոնաթթուն լայնորեն օգտագործվում է սննդի (75%), ինչպես նաև դեղագործական և քիմիական արդյունաբերության մեջ։ Դիմնականում այն արտադրվում է մելասսայից։ Սակայն բուսական արլիֆրուկտանները, օրինակ ինուլինը, գրեթե չեն ուսումնասիրվել կիտրոնաթթվի ստացման համար։ Ինուլինը 97% ֆրուկտոզա և 3% գլյուկոզա պարունակող պոլիսախարհդ է։ Ինուլինի զգալի քանակներ (20-25%) պարունակվում են գետնախնձրդի պալարներում (*Helianthus tuberosus*)։ Ուսումնասիրությունների հիմնական նպատակն է մշակել միափուլ մանրեաբանական տեխնոլոգիա՝ նոր մեկուսացված բորբոսասնկի շտամների միջոցով գետնախնձորից (ինուլին) կիտրոնաթթվի արտադրության համար։ Մեկուսացվել են *Aspergillus* ցեղին պատկանող բորբոսասնկի նոր շտամներ, որոնբ օժտված են կիտրոնաթթվի սինթեզի ունակությամբ։ Պարզվել է, որ ինուլինից կիտրոնաթթվի կենսասիլթեզի համար անհրաժեշտ պայման է միկրոօրգանիզմներում ինուլինազային ակտիվության առկայությունը։

Պարզվել է, որ գետնախնձորի պալարները (ինուլին) հեռանկարային հումք են հանդիսանում կիտրոնաթթվի ստացման համար։

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