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THE EFFECTS OF DIAGNOSTIC ULTRASOUND ON WISTAR RATS DURING PREGNANCY AND THE BEHAVIOR OF THEIR OFFSPRING IN THE OPEN FIELD

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ორსულობის დროს ვისტარის ვირთაგვებზე დიაგნოსტიკური ულტრაბგერის ეფექტი და მათი შთამომავლობის ქცევა ღია ველში

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რეზიუმე

მიღებული მონაცემები მიუთითებს იმაზე, რომ ვირთაგვებზე ულტრაბგერის გავლენა საშვილოსნოში ყოფნისას იწვევს შფოთვისა და დეპრესიის განვითარებას, რაც აშკარად ჩანს ჩვენი დაკვირვების შედეგად. შფოთვის შემცირება განპირობებულია არა მხოლოდ ტვინის სტრუქტურებში ორგანული ცვლილებების ნარჩენი ეფექტებით, რომლებიც დაკავშირებულია ქცევის ორგანიზებასთან, კერძოდ, სპეციფიკურ გლუტამატერგიულ ნეირონებთან, ჰიპოთალამუსის „შეტევის ცენტრთან“, არამედ მთელი ადაპტაციურ-კომპენსატორული სისტემის გააქტიურებასთან, რომელიც ხორციელდება ხანგრძლივი ულტრაბგერითი ზემოქმედების შედეგად. ეს ჩანს ჩვენ მიერ ჩატარებული ქცევითი კომპონენტებიდან, კერძოდ, „ღია ველზე“ მიღებული შედეგებიდან. ამ საკითხების გარკვევა გააღრმავებს საშვილოსნოში მყოფი ვირთაგვების 20-30 წუთიანი ულტრაბგერითი ზემოქმედების შედეგად მიღებული ეფექტების გაანალიზებას და მომავალში ცხოველთა ნერვული სისტემის დისფუნქციის კვლევებს.

The incidence of diagnosed autism spectrum disorder (ASD) worldwide has increased over the past decade and is currently approximately at over 1% (ADDM). As emphasized by the Coordinating Committee, an important role in these processes is played by environmental risk factors [2,6,13], as well as a possible genetic contribution to the risk of ASD, which is especially evident in the early stages of human embryonic development. Among the possible risk factors, which have recently received much attention, is ultrasound diagnostics [3,16,19].

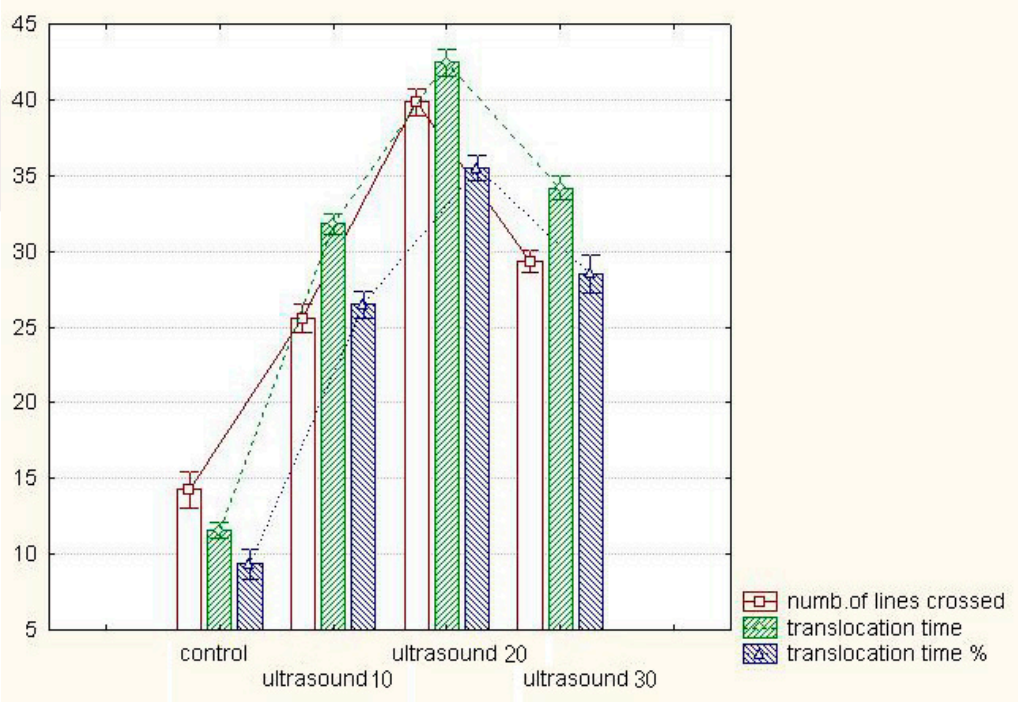
Diagnostic ultrasound (US) during pregnancy has been part of standard obstetric care for decades [1,5,9]. Diagnostic ultrasound imaging has proven clinically useful in the first trimester to screen for fetal abnormalities, locate and track multiple embryos, especially during in vitro fertilization, and, for example, determine the likelihood of miscarriage [6,18]. In the second and third trimesters, sex determination using ultrasound is possible, as well as assessment of fetal growth and analysis of potential problems that may arise during fetal development [18,20]. Occupational physicians also use pulsed Doppler ultrasound (DUS) to check for the presence and quality of a fetal heartbeat, which also begins in the first trimester. An adequate balance of benefits and risk determines the medical use of DUS during pregnancy. However, this appropriate use is increased with concomitant factors that are also associated with an increased risk of autism when combined with other risk factors such as maternal age [14,16], maternal metabolic processes [12,15], and complications during pregnancy [4,13]. All together significantly increases the risk of developing autism.

Along with the medical reassurance that expectant parents usually receive, they enjoy the ultrasound procedure because they can see the baby before birth, which gives them even more peace of mind, depending on the baby's normal condition. Currently, entrepreneurs sell ultrasound machines that give them so-called baby pictures taken with the help of ultrasound machines „as a memory or memento„ [9,18], where 30 minutes of ultrasonic exposure produces DVD images. Despite its increased use, there are currently few or no regulatory tools specifically designed to assess the general risks associated with new medical imaging and diagnostic devices. Physicians recommend that pregnant women avoid routine ultrasounds, although the practice remains unregulated in most US states [17,20].

While these ultrasound devices comply with FDA marketing requirements, their unlicensed medical use is a concern. In addition to the growing popularity of commemorative ultrasound photographs due to easy access and low cost (less than US$100), Doppler ultrasound heart rate monitors have become increasingly used in the home. It is still important to ensure the safety of the procedure and inform users and future parents of any potential side effects. This ongoing safety review also remains relevant as there are scientific studies showing that prolonged but clinically significant exposure to DUS in utero in rodents can alter their neurological function [16]. Studies have shown that mice exposed to at least 10 minutes of ultrasound in utero on the 14th day of embryonic development (E14), experience impaired learning and memory [17]. Mice exposed to ultrasound in utero also showed significantly reduced levels of dopamine, norepinephrine and serotonin in the hippocampus compared to controls, and finally, it is reported in the literature [2,3] that mice exposed to ultrasound for 30 minutes or more per day (E14.5), there was significant impairment of cortical neuron migration compared with the control group. It is worth noting that there are negative results [11] and that some scientists did not find changes in the memory and anxiety levels of rats even during 35 minutes of exposure to ultrasound in utero.

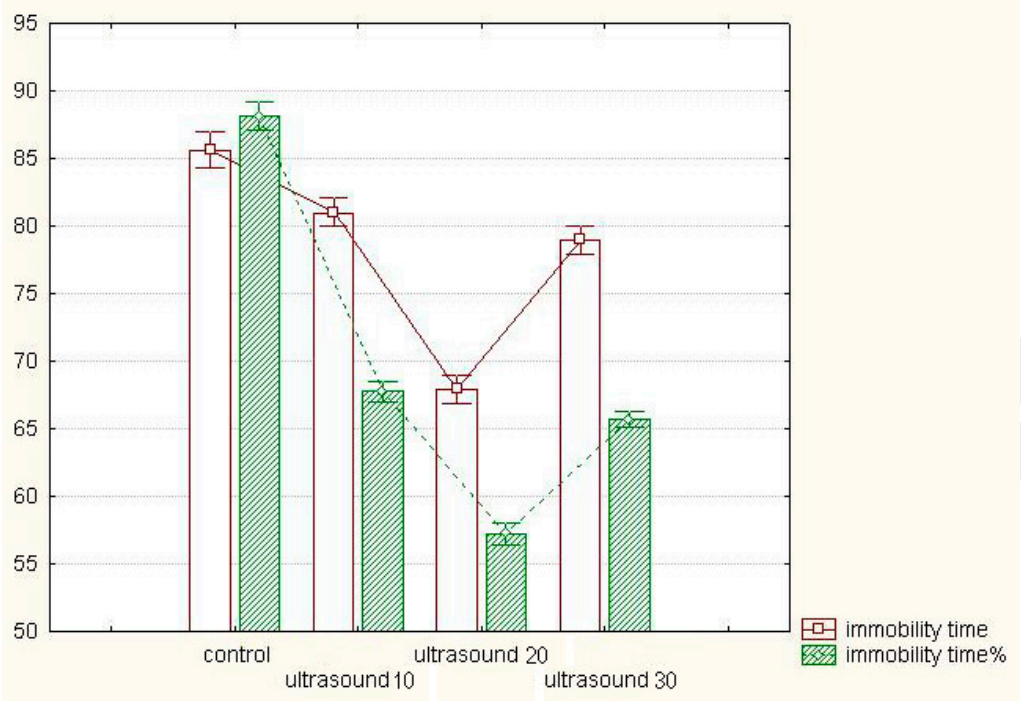
Methods. We carried out diagnostic procedures: we selected 4 groups, one of which was a control group, and the remaining three were experimental. The first experimental group was exposed to ultrasound for 10 minutes, the second group – for 20 minutes and the third experimental group – for 30 minutes, this procedure was carried out daily until the offspring appeared. After 23 days of pregnancy, we received offspring, the number of which was 9 mice in each of the four groups. 2 months after birth, we studied the behavior of the offspring in an open field.

Results and Discussion. As a result of locomotion in the open field, it was found that the latent period of exit from the central circle in control rats was shorter (1.97 ± 0.7) compared with rats that received ultrasonic exposure during their stay in utero (15 min, 25 min, 30 min) (2.1±0.6, 3.2±0.7, 3.9±0.7). As for motor activity in the open field, we saw that the number of crossed cells in control rats was (14.53±0.06), 10, 20, and 30 min after exposure to US (25.6±0.07, 39 .9±0.08, and 29.3±0.06). As for the time spent on crossed cells, it was in control rats (11.6±0.06), at 10, 20, and 30 min (31.9±0.05, 42.4±0.05, and after 30 min 34.1±0.05). And the percentage of time spent on translocation in control rats (9.31±0.05), and in rats exposed to ultrasound was 10 min, 20 min, and 30 min, respectively (26.5±0.06), (35.4±0.07), (28.46±0.06).

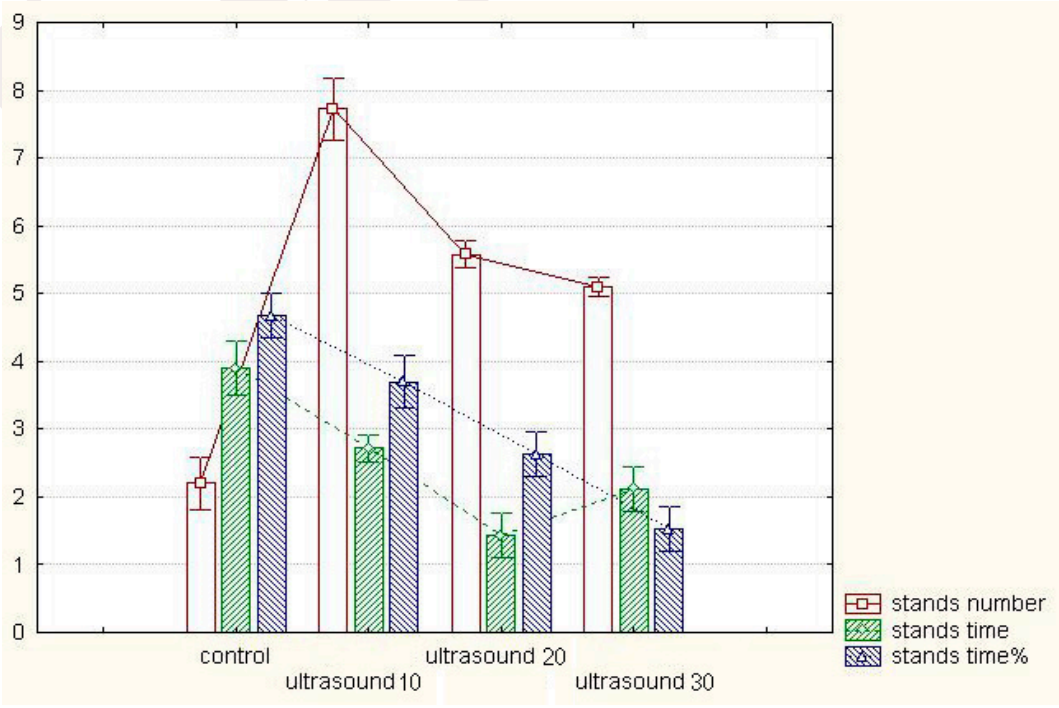


The difference is significant both in the time spent on translocation and in the percentage of time spent on translocation relative to the total time spent on the experiment. Therefore, it can be said that control rats move more slowly and have fewer crossed cages, which can be explained by grooming. The

number of times they stand against the wall and in the center, trying to correctly perceive the environment in which they are, indicates their perception of the social environment since they live in families. In this way, they are trying to make the right decision for their family members, which indicates their correct perception of the social environment, which is not the case in rats exposed to ultrasound. Compared to control rats, they move faster in the open field and cross more cages, and spend less time than control rats - (88.2±7.2%) rats 10 min, 20 min, 30 min.

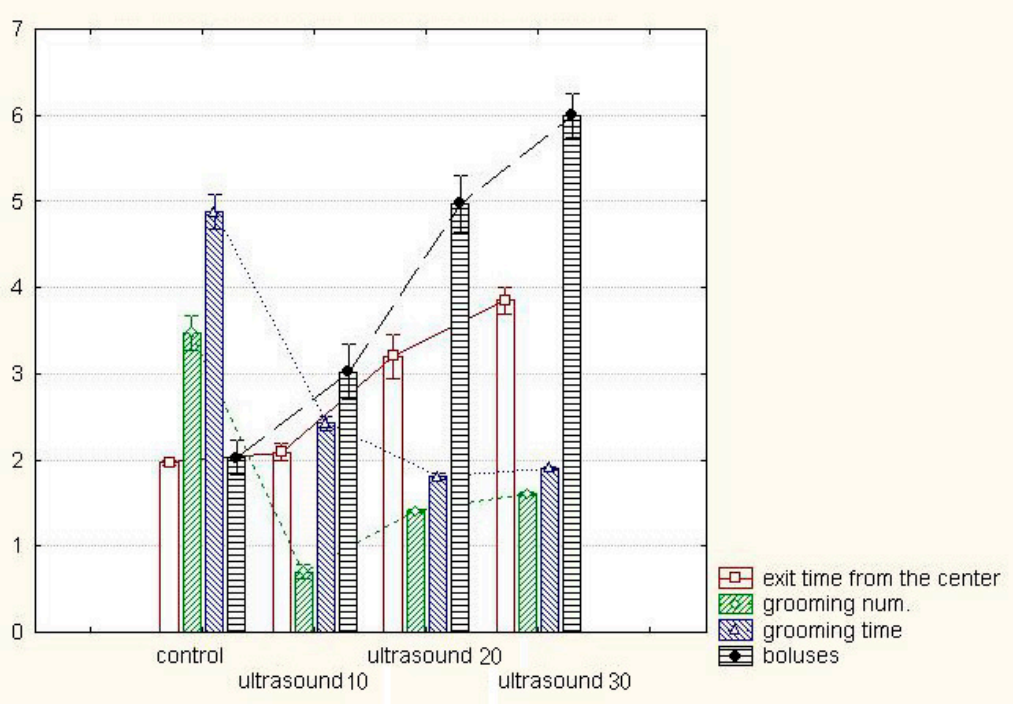


Ultrasonic exposure (67.8±5.2%) (57.6±4.0%), (and 65.8±4.0%), and this conclusion is confirmed by the number of immobility cycles. The table shows that in rats of the control group, these indicators are higher (85.9±0.2 s) than in animals of the group of rats exposed to ultrasound (79.1±0.5 s). In addition, A (control) and B (rats exposed to ultrasound radiation) also differed in orienting activity in the open field. The number of vertical rises in rats of group A is (1.5±0.01), and in group B rats, exposed to ultrasound radiation, it is (7.7±0.03), (5.6±0.01) and (5.1 ± 0.01), (but the spent % time required for A in this study is (5.7 ± 0.01), and for group B rats it is (2.4 ± 0.01), (3 .4 ± 0.01), (4.1 ± 0.02).



This means that they are more likely to stand against the wall and spend less time perceiving the environment, which is an indicator of the presence of fear and anxiety factor in it. An increase in the

number of standing positions of the control rats and a significant increase in the time spent on orientation while in one or another compartment means that the rats explore the area in which they are placed, i.e., participate in the "exploration of the open field". A comparison of the stereotypic activity of rats in groups A and B in the open field showed that, although the number of grooming cycles among animals differed, the time spent by control rats in standing, both vertical and centered, and the number and duration of grooming was higher in control rats than in ultrasound-exposed rats. That indicates their excessive emotion and anxiety.



Conclusions. The obtained data indicate that ultrasound exposure to rats in utero causes the development of anxiety and depression, which is clearly visible as a result of our observations.

The development of anxiety is due not only to the residual effects of organic changes in brain structures related to the organization of behavior, in particular, to specific glutamatergic neurons, the "center of attack" in the hypothalamus, but also to the activation of the entire adaptive-compensation system. Moreover, the development of depression and anxiety can be clearly seen as a result of the study of behavior in the open field. In particular, with the results obtained in the "open field". Elucidation of these issues will deepen the current understanding of the effects of prolonged 20-30 min ultrasound exposure in rats in utero, and future studies of animal nervous system dysfunction.

Tab 1: The effect of ultrasound on rats during pregnancy

Open field	Control A	The effect of ultrasound		
		15	25	30
exit time from the center (min)	1,97±0,7	2.1±0,6	3.2±0.7	3.9±0,7
Number of crossed lines	14.5±0,06	25,6±0,07	39.9±0,08	29.3±0,06
Time for translocation	11.6±0,06	31.9±0,05	42.4±0,05	34.1±0,05
Translocation time %	9.31±0,05	26.5±0,06	35.4±0,07	28.46±0,06
Immobility time %	88.2±7,2%	67,8±4,2%	57.4±4,0%	65,8±4,0%
Immobility time	85,9±7,2	81±4,2	68±4,1	79±4,0
The number of vertical rises	2.2±0,01	7,7±0,02	5.6±0,01	5.1±0,01
The time of the vertical rises	3.9±0,01	2.7±0,01	1.4±0,01	2,1±0,02
The time of the vertical rises %	4.7±0,01	3.6±0,01	2,6±0,01	1.5±0,01
The number of grooming	3.5±0,01	0.7±0,01	1.4	1.6±0,01
The time of grooming	4.9±0,02	2.4±0,02	1.8±0,01	1.9±0,01
Boluses	2	3	5	6

References:

1. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators; Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders--Autism and Developmental Disabilities Monitoring Network, 14 sites, United States 2008. MMWR Surveillance Summaries. 2012; 61(3):1–19.
2. Grether JK, Li SX, Yoshida CK, Croen LA. Antenatal ultrasound and risk of autism spectrum disorders. Journal of Autism and Developmental Disorders. 2010; 40(2):238–45.
3. Lyall K, Pauls DL, Spiegelman D, Ascherio A, Santangelo SL. Pregnancy complications and obstetric suboptimality in association with autism spectrum disorders in children of the Nurses' Health Study II. Autism Research. 2012; 5(1):21–30.
4. Lin CS, Tao PL, Jong YJ, Chen WF, Yang CH, Huang LT, Chao CF, Yang SN. Prenatal morphine alters the synaptic complex of postsynaptic density 95 with N-methyl-D-aspartate receptor subunit in hippocampal CA1 subregion of rat offspring leading to long-term cognitive deficits. Neuroscience. 2009 Feb 18;158(4): 1326-37.
5. Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, Barbaro RP, et al. Mouse behavioral tasks relevant to autism: Phenotypes of 10 inbred strains. Behavioral Brain Research. 2007; 176:4–2.
6. Miller DL. Safety assurance in obstetrical ultrasound. Semin Ultrasound CT MR 2008; 29:156–164.
7. Miller MW, Nyborg WL, Dewey WC, Edwards MJ, Abramowicz JS, Brayman AA. Hyperthermic teratogenicity, thermal dose and diagnostic ultrasound during pregnancy: implications of new standards on tissue heating. Int J Hyperthermia. 2002;18:361–384.
8. Maeda K, Ohkura S, Tsukamura H. Physiology and reproduction, In: Krinke GJ. The laboratory rat London (UK): Academic Press. 2000; 145–176.
9. Stalberg K, Axelsson O, Haglund B, Hultman CM, Lambe M, Kieler H. Prenatal ultrasound exposure and children's school performance at age 15-16: follow-up of a randomized controlled trial. Ultrasound in Obstetrics and Gynecology. 2009; 34(3):297–303.
10. Sikov, M.R., B.P. Hildebrand, and J.D. Stearns. Postnatal sequelae of ultrasound exposure at 15 days of gestation in the rat. In: Ultrasound in Medicine. D. White and R.E. Brown, eds. Plenum Press, NY, 1977; 2017-23.
11. Spurney CF, Leatherbury L, Lo CW. High-frequency ultrasound database profiling growth, development, and cardiovascular function in C57BL/6J mouse fetuses. J Am Soc Echocardiogr. 2004;17:893–900.
12. Tarantal AF, O'Brien WD, Hendrickx AG. Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (Macaca fascicularis): III. Developmental and hematologic studies. Teratology. 1993 Feb;47(2):159-70. doi: 10.1002/tera.1420470208. PMID: 8446929.
13. Vorhees, CV. Principles of Behavioral Teratology. In: Riley, RP.; Vorhees, CV., editors. Handbook of Behavioral Teratology. New York: Plenum Press; 1986; 23-48.
14. Williams EL, Casanova MF. Reassessment of teratogenic risk from antenatal ultrasound. Translation Neuroscience. 2013; 4(1):81–87.
15. Verlohren S, Niehoff M, Hering L, Geusens N, Herse F, Tintu AN, Plagemann A, LeNoble F, Pijnenborg R, Muller DN, Luft FC, Dudenhausen JW, Gollasch M, Dechend R. Uterine vascular function in a transgenic preeclampsia rat model. Hypertension. 2008; 51:547–553
16. Warkany J. Teratogen update: hyperthermia. Teratology. 1986; 33:365–371.
17. Weiss J, Taylor GR, Zimmermann F, Nebendahl K. Collection of body fluids, In: Krinke GJ. The laboratory rat London (UK): Academic Press. 2000; 485–510.
18. Zagzebski, J.A. Acoustic output of ultrasound equipment: Summary of data reported to the A41UM Ultrasound Med. Biol., 1989; 15(l):55.
19. Yu Q, Leatherbury L, Tian X, Lo CW. Cardiovascular assessment of fetal mice by in utero echocardiography. Ultrasound Med Biol. 2008; 34:741–752.
20. Zhou YQ, Foster FS, Qu DW, Zhang M, Harasiewicz KA, Adamson SL. Applications for multifrequency ultrasound biomicroscopy in mice from implantation to adulthood. Physiol Genomics. 2002;10:113–126.

АЛЕКСАНДР ТАРХНИШВИЛИ[1,2], МАРИНА НИКОЛАИШВИЛИ[1]

ВЛИЯНИЕ ДИАГНОСТИЧЕСКОГО УЛЬТРАЗВУКА НА КРЫС ВИСТАР В ПЕРИОД БЕРЕМЕННОСТИ И ПОВЕДЕНИЕ ИХ ПОТОМСТВА В ОТКРЫТОМ ПОЛЕ

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РЕЗЮМЕ

Полученные данные свидетельствуют о том, что воздействие ультразвука на крыс во время их пребывания в утробе матери вызывает развитие тревоги и депрессии, что хорошо видно в результате наших наблюдений. Снижение тревожности обусловлено не только остаточными явлениями органических изменений структур головного мозга, связанных с организацией поведения, в частности, специфическими глутаматергическими нейронами, «центром атаки» гипоталамуса, но и активация всей адаптационно-компенсаторной системы, которая осуществляется в результате длительного ультразвукового воздействия. Это видно по проведенным нами поведенческим компонентам, а именно по результатам, полученным в «чистом поле». Выяснение этих вопросов позволит углубить текущее понимание последствий длительного 20-30-минутного ультразвукового воздействия на крыс в утробе матери и будущие исследования дисфункции нервной системы животных.

ALEXANDER TARKHNISHVILI [1,2], MARINA NIKOLAISHVILI [1]

THE EFFECTS OF DIAGNOSTIC ULTRASOUND ON WISTAR RATS DURING PREGNANCY AND THE BEHAVIOR OF THEIR OFFSPRING IN THE OPEN FIELD

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SUMMARY

The data obtained indicate that the impact of ultrasound on rats during their stay in the womb causes the development of anxiety and depression, which is clearly seen as a result of our observations. The decrease in anxiety is due not only to residual effects of organic changes in brain structures associated with the organization of behavior, in particular, specific glutamatergic neurons, the "attack center" of the hypothalamus, but also to the activation of the entire adaptive-compensatory system, which is carried out as a result of prolonged ultrasonic exposure. It is seen from the behavioral components we conducted, namely, from the results obtained in the "open field". The clarification of these issues will deepen the current understanding of the effects of prolonged 20-30 min ultrasound exposure in rats in utero, and future studies of animal nervous system dysfunction.

Keywords: diagnostic ultrasound, wistar rats, pregnancy, behavior, offspring