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Original paper

The protective role of ADAC and noise stress effects on Wistar rats behaviour

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Abstract

Noise, a main component of modern society, has become an important environmental problem. Noise can be not only an irritating element, but also a stress factor leading to serious health problems.

With this experiment we study the negative effects of noise on pregnant Wistarrats females, examining gestation duration and pups'evolution in terms of birth weight, growth and performance testing in anxiety and depression causing environments. We also demonstrated the benefit of Adenosine Amine Congener in preventing damages induced by noise pollution.

Our data proves noise stress is harmful to pregnancy evolution and is a risk factor for both anxiety and depression and should be avoided.

Keywords Noise stress, adenosine amine congener, Wistar rats, pregnancy.

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Introduction

Noise is a widespread stress resource that may have negative effects on the health. However, the molecular basis of the stress response caused by noise remains elusive.

The vast majority of people live and work more or less willingly in sound-polluted environments. Sound stress can cause serious damage not only to the hearing but also to the entire body (Aguilera, G [1]).

Traffic noise is experienced as a negative environmental factor particularly when it disturbs human activities (e.g. communication, concentration, recreation etc.) (Babisch, W. [2]). This causes mental stress reactions, which are usually accompanied by biochemical and circulatory stress effects (Eggermont, J.J. [3]).

It is known that living in a noise-polluted environment may cause premature birth, newborns' lower weight that is harder to compensate on the long run.

Exposure to stress usually triggers the endocrine, nervous and behavioral systems to activate in order to adapt the human body to the stress and maintain homeostasis (Gitanjali [4]).

The main stress response is cortisol and corticosterone production and through them a regulation of the whole body begins (Grissom, N. [5]).

Due to using rats as animal models in this study, all the procedures and methodology followed were according to the national and international legislation, specific to work with experimental animals (Masini, [6]).

Based on scientific literature results, we aimed to find a substance that would protect both the inner ear and the brain (Uygur, E. [7]).

Adenosine is an endogen nucleotide made of one molecule of adenine attached to a molecule of ribose and it is found in every cell of the human body. We chose one of its synthetic forms called ADAC (adenosine amine congener) because the effects of adenosine as a cerebral protector against ischemia are already known. The study intended to examine if ADAC had a protective effect both at the inner ear and at the cerebral neuronal level. ADAC was obtained from Sigma Aldrich, and was prepared as follows: 6 mg of ADAC were dissolved in 200 µl of 1N HCl and then 120 ml of PBS 0.1 M and pH = 7 were added. The final solution had a concentration of 50 µg / ml. The vehicle solution was prepared using 200µl of 1N HCl and 120 ml of PBS 0.1 M, pH = 7.4. The pH of the final solutions was 7.3-7.4 in both cases. The solutions thus obtained were stored in micro-containers at a temperature of -20°C. Before each injection, the solutions were brought to 37°C. For this we used several batches of rats that were exposed to loud white noise with and without ADAC protection to see how they will perform to tests of anxiety and depression. Because lately more people choose to listen to white noise at a pleasant volume for relaxation purposes, we also chose to have a batch stimulated with white noise at 50dB to study the effect of this type of noise (Schell LM [8]). Studies show that people who already have hearing impairments and continue to work in a noisy environment are more affected than people with normal hearing (World Health Organization), [9]). They have a reduced response to attention and focus tests (the tests consisted of mathematical calculations, the ability to memorize words, the ability to read and understand a text), and have increased levels of catecholamine and salivary cortisone (Miller JD [10]).

Material and Method

For the first part of the study we used a batch of 18 Wistar rats that were divided as follows:

- 6 females were the control group;
- 6 females were physically stressed with a loud white noise of 100dB, 2 hours a day between day 14 and day 21 of pregnancy;
- 6 females were exposed to white noise at 50 dB, 2 hours a day, between day 14 and day 21 of pregnancy.

All females were monitored throughout the pregnancy, weighed before they were pregnant, on the 14th day and then daily until the day they gave birth, taking into account their weight on the last day before pregnancy ended. We aimed to observe if noise stress could interfere with the duration of pregnancy of Wistar rats. We were also interested in the pups' weight, delivered from stressed and non-stressed mothers. The pups were weighed daily, considering the weight on the day they were born and then on the 14th day postpartum. Only male rats were then selected and studied.

For the second part of the study, the effect of noise stress on rats and the benefits of ADAC treatment were examined. At 12 weeks of age, tests for anxiety and depression (Open Field Test and Elevated Plus Maze test) were performed on every rat from the two lots we decided to study (work group and control group), the rats being randomly assigned to each of them (n = 10 per lot).

Each Wistar rat from these lots was exposed to a white noise of 100 decibels for 2 hours a day, 7 days.

Every day for 7 days at an interval of 24 hours apart, starting 6 hours after the first exposure to noise the Wistar rats were injected intra-peritoneally with a dose adjusted to the rat's weight (200 µl/100 g body weight). This dose was

described in the literature to be effective in protecting the brain. We administered the substances every day for 7 days.

Body weight and intra-rectal temperature were monitored to highlight potential side effects such as weight gain or loss or hypo/hyperthermia. The rectal temperature was monitored before and after the ADAC administration at 30 and 60 minutes. The body weight was monitored daily for the first 7 days and then on day 14. On the seventh and twenty-first day they were subjected to anxiety and depression tests (OFT, EPM) once again in order to compare the results with the ones we got at the beginning of our study. For the second part of the study we tried to demonstrate the effect of noise stress on rats and the benefits of ADAC treatment. At the age of 12 weeks, tests for anxiety and depression (Open Field Test and Elevated Plus Maze test) were performed on every rat from the two lots we decided to study (work group and control group), the rats being randomly assigned to each of them (n = 10 per lot).

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Results

This research has produced a number of results that clearly show the harmful effect of noise stress on gestation and on the quality of life. A working protocol has been standardized for noise stimulation of laboratory animals.

In the first part, data on the effects of gestational stress were processed. The pregnant females were exposed to noise stress during the last period of pregnancy, because during that period it was proven to have the greatest effect on the fetuses. Mothers exposed to sound stress gained significantly less weight, compared to those not exposed to noise.

We also compared a batch of mothers who were exposed to white noise at an intensity used for relaxation with the group of mothers who were stressed. They gained weight almost the same as mothers that were not stressed, some of them even exceeding the weight of those in the non-stressed group.

Figure 1. The noise stimulation of different rat lots.

Thus, we can argue that listening to relaxing sounds during pregnancy does not negatively affect its evolution.

Regarding the duration of pregnancy, the present study shows a decrease in gestation duration in animals exposed to noise stress. Mothers exposed to 100 dB of noise gave birth earlier than the other two groups, and the pups had lower weights at birth. Of the stressed mothers, two dead pups were born.

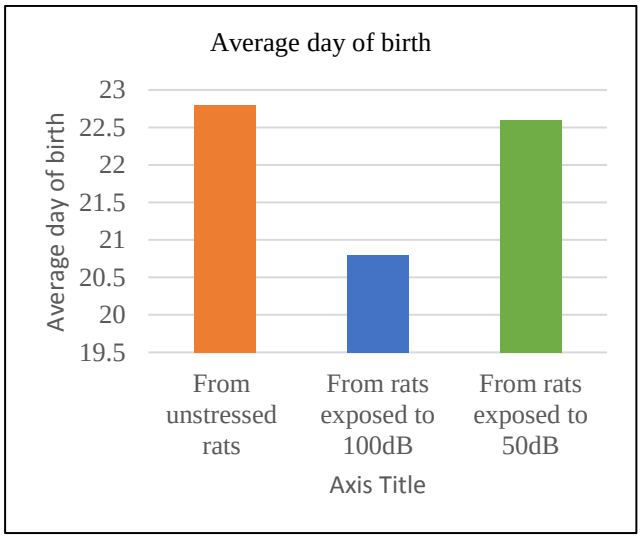

Figure 2. The day the mothers gave birth.

At 4 weeks of age the pups still had significant differences between their weights, those born from mothers

Tabel 1. The day the rats gave birth (ANOVA)

The day they gave birth (ANOVA)	Sum of Squares	df (degrees of freedom)	Mean Square	F-test	Sig. (statistical significance)
Between Groups	14,778	2	7,389	6,520	0,009
Within Groups	17,000	15	1,133		
Total	31,778	17			

exposed to noise stress weighting significantly less than the others.

At 12 weeks of age, there were no more significant differences between the weights of the pups. During this time the stress factor has been removed, so we can say that if the newborn is taken out of the stressful environment, it can recover the deficits. Although differences still existed between the pups, those born of stressed mothers, however, had slightly lower weights and slightly worse results on tests performed but they were no longer statistically significant.

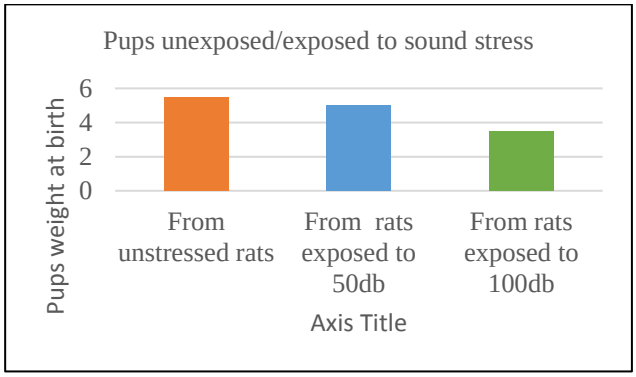

Figure 3. Pups weight (in grams) at birth.

The results obtained after 7 days of phonic stimulation show changes in batches of rats. All batches exposed to 100 dB for 2 hours a day had longer reaction times in the EPM and OFT tests than those not exposed to stress.

The distance they spent exploring the arena, the time spent in the open area of arenas or in the dark area – factors that assess the anxiety of the subjects, were significantly altered in the case of those stressed, regardless of the group of mothers they came from. It has been noticed that ADAC-injected lots have statistically significant better results than those injected with serum, thus potentially supporting the protective effect of ADAC.

At 14 days after cessation of phonic stimulation and ADAC administration, we repeated the tests to see if the effects were sustained over time. Thus, the rats in the groups exposed to sound stress have still spent smaller distances exploring arenas and preferred dark areas to the detriment of the bright ones. ADAC-injected consignments still had better outcomes in OFT and EPM tests than those injected with serum. We can say in this case that the stressful effects of exposure to noise have been maintained over time and the protective effect of ADAC continues 14 days after the end of the sound stress.

Conclusion

The study's outcome is significant for the lives of human subjects living and working in sound stressful environments, as premature birth and inappropriate growth in the first few weeks of life can have important long-term consequences.

It is well known that premature birth has an important negative effect on the fetus. In this study, we were able to demonstrate that stressed mothers gave birth earlier than other groups, so stress is a risk factor for premature delivery. Noise stress causes a decrease in gestation time and lower weights of the pups at birth. Without having the possibility to quantify the results we concluded that listening to relaxing sounds has a beneficial effect on the pregnancy.

Rats born from mothers exposed to sound stress have worse results in tests that reveal anxiety and depression than those delivered by non-stressed rat females. So we can conclude that noise stress has a negative effect in normal

Tabel 2. Pups weight at birth - ANOVA

Pups weight at birth (ANOVA)	Sum of Squares	df (degrees of freedom)	Mean Square	F-test	Sig. (statistical significance)
Between Groups	72,036	2	36,018	85,223	0,000
Within Groups	44,799	106	0,423		
Total	116,835	108			

development of fetus during gestation, especially in the last trimester of pregnancy.

After the age of 12 weeks, exposure to 100dB white noise increases anxiety in the studied groups. We demonstrated once again the harmful effect of loud noise especially in youngsters that may be associated to similar effects on teenagers and young humans.

The use of adenosine amine congener decreases the anxiety of sound-stricken rats.

The effects of the sound stress continue after its cessation.

References

1. AGUILERA, G. Corticotropin releasing hormone, receptor regulation and the stress response. *Trends Endocrinol. Metab. 9*, 329-336 (1998).
2. BABISCH, W. Stress hormones in the research on cardiovascular effects of noise. *Noise Heal*. 5, 1-11 (2003).
3. EGGERMONT, J.J. Nonauditory effects of noise. In: Noise and the Brain: Experience *Dependent Developmental and Adult Plasticity*, pp. 266-295, Academic Press, Elsevier (2014).
4. GITANJALI, B. ANANTH, R. Effect of acute exposure to loud occupational noise during daytime on the nocturnal sleep architecture, heart rate, and cortisol secretion in healthy volunteers. *J. Occup. Health* 45, 146-152 (2003).
5. GRISSOM, N., BHATNAGAR, S. Habituation to repeated stress: get used to it. *Neurobiol. Learn. Mem*. 92, 215-224 (2009).
6. MASINI, C.V, DAY, H.E. W., CAMPEAU, S. Long-term habituation to repeated loud noise is impaired by relatively short interstressor intervals in rats. *Behav. Neurosci.* 122, 210-223 (2008).
7. UYGUR, E.E., ARSLAN, M. Effects of chronic stress on cognitive functions and anxiety related behaviors in rats. *Acta Physiol. Hung*. 97, 297-306 (2010).
8. SCHELL LM, D.M. Environmental pollution in urban environments and human biology. *Amm Rev. Anthropo*l, 111-134 (2003).
9. WORLD HEALTH ORGANIZATION, Deafness and hearing impairment, Fact sheet no. 300 (2006).
10. MILLER JD. Effects of noise on people. *J Acoust Soc Am;* 56:729-64 (1974).