

INFLUENCE OF THE NOISE ON THE BEHAVIOR OF FEMALE WISTAR RATS

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ABSTRACT

Noise is one of the harmful agents to homeostasis and is linked to many branches of economic activity. It may cause hearing impairment, insomnia, annoyance, mental illness, cardiovascular diseases, work inefficiency, modification of social behaviors and many other negative effects for health. If the exposure to noise is temporary, the body tends to return to its original state or to the pre-exposure in a few minutes. However, if the stimulus is maintained regularly, damages may be irreversible. The objective of this study was to evaluate the effects of noise in the behavior of female Wistar rats. Thirty animals weighting 200g-220g were divided randomly in G1: Control group that was fed water and rat food *ad libitum*; G2 (n=10): Group exposed to noise for 4 hours/day for 20 days (this exposition was performed during the light cycle); G3 (n=10): Group exposed to noise for 8 hours/day for 20 days (this exposition was performed during the light cycle). G2 and G3 were also fed water and rat food *ad libitum*. Our results showed a significant impact of sound exposure on the behavior of Wistar rats, and the introduction of a noise source even in a short period of time increases anxiety and stress, and discreetly reduces weight. It was also observed that noise removal reduced the anxiety parameters indicating that the exposure time to which the animals were subjected was not sufficient to produce definitive modifications.

KEYWORDS: noise, behavior, Wistar rats.

INTRODUCTION

The industrial revolution is related to several benefits to the modern societies, nevertheless, some basic values of health were lost. Noise is one of the harmful agents to homeostasis and most prevalent complaints reported by workers. It is directly linked to many branches of economic activity.^[1,2]

It has become increasingly common to see people exposed to excessive noise, a problem that affects underdeveloped and developed countries, and identifies itself both in health problems and in the personal life. This kind of exposition may not be perceptible and the signs go unnoticed by the patient. Furthermore, tolerance and adaptation make it difficult to identify and thus, difficult to prevent future damages.^[3,4]

Some authors have shown several kind of disturbance associated with noise exposition. Fuks et al.^[4] showed that long-term residential exposures to noise are linked with higher incidence of hypertension. Other consequences are social isolation and the induction of hearing loss. These consequences of exposition to the

noise is an important social burden for millions of people and lead to use of billions of dollars annually for the treatment.^[1,5,6]

Noise may also damage the cochlea and in intense and low frequency it may trigger the loss of outer hair cells, decreasing acoustic amplification and increasing thresholds. One consequence of this damage lead to lipid peroxidation and oxidative stress that is related to several illness such as chronic degenerative diseases that are among the main caused of death worldwide.^[1]

Besides causing hearing impairment noise may also induce to insomnia, annoyance, mental illness, cardiovascular diseases, work inefficiency, communication disability, modification of social behaviors and many other negative effects for health. If the exposure is temporary, the body tends to return to its original state or to the pre-exposure in a few minutes. However, if the stimulus is maintained regularly, damages may be irreversible.^[7-10] The objective of this study was to evaluate the effects of noise in the behavior of female Wistar rats.

METHODS

Ethical Principles

This research was approved by the Animal Research Ethics Committee of the Medical School of Marília (UNIMAR), Marília – São Paulo, Brazil. Animals were cared for according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".

Group of Animals

Thirty female Wistar rats weighting 200g-220g were housed in collective cages (5 animals/cage) under a dark/light cycle of 12 hours, room temperature of $22 \pm 2^\circ\text{C}$, and relative air humidity of $60 \pm 5\%$ at the Medical School of Marília (UNIMAR), Marília - SP, Brazil. After seven days of acclimation to laboratory conditions, the animals were divided randomly in:

G1 (n=10): Control group that was fed water and rat food *ad libitum*;

G2 (n=10): Group exposed to noise for 4 hours/day for 20 days (this exposition was performed during the light cycle). These animals also were fed water and rat food *ad libitum*;

G3 (n=10): Group exposed to noise for 8 hours/day for 20 days (this exposition was performed during the light cycle). These animals also were fed water and rat food *ad libitum*.

The weight of the animals was evaluated each three days and the consumption of the rat feed was based on the leftovers found every day.

Type of Noise

To produce the noise, there was a compilation of urban sound noises such as conversations between several people, horns, traffic noise, ambulance, church bell and etc.

Behavioral test and Elevated Plus Maze

The Elevated Plus Maze (Boerngen-Lacerda *et al.*^[11] and Blanchard *et al.*^[12] was used for behavioral assessment with the objective of evaluating the anxiety index in the animals of the three groups. This test was performed at the beginning of the experimental protocol (before starting the exposition to the noise) and after 11 days of the end of exposition.

This apparatus is constructed with wood, fits 50 cm from the ground, presents two open and opposed arms (50x10 cm), and two enclosed (50 x 10 x 40cm). There are also platforms with the same extension of the open arms that cross them perpendicularly, delimiting a central area (10 cm²). The animals were placed in the Elevated Plus Maze for 5 minutes for evaluation of the frequency and the time spent in the center, in open and in closed arms.

Statistical analysis

Data were analyzed with ANOVA, T Student and Kruskal-Wallis Test. Variables were presented as mean and standard error mean, adopting a 5% level of significance.

RESULTS AND DISCUSSION

In Figure 1 we may observe in group B that at the beginning of the exposition to sound there was a reduction in the consumption of food. Besides an exception between two days (September, 25 and 27) there was a lower consumption until the end of the sound exposure. There was not significant differences between the groups ($p > 5\%$), but there was a tendency of the groups exposed to sound to consume a lower amount of food. No significant differences were found in the consumption of water (Figure 2).

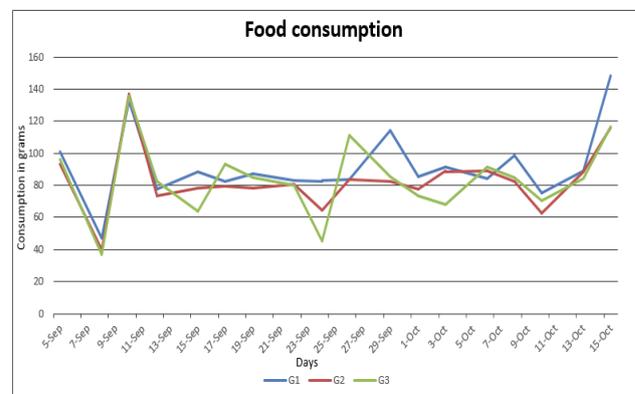


Figure 1: Comparison in the food consumption among the Control Group (G1), G2 that was exposed to noise 4 hours/day and G3 that was exposed for 8 hours/day.

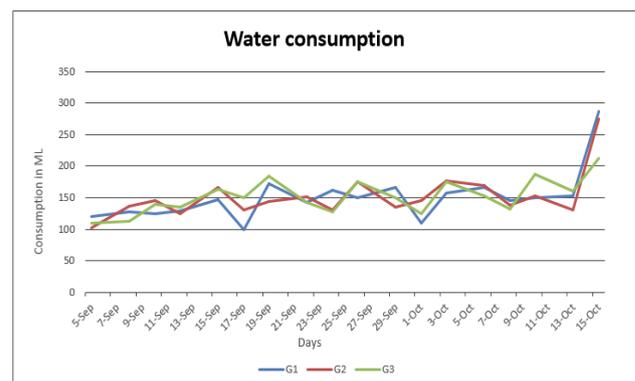


Figure 2: Comparison in the water consumption among the Control Group (G1), G2 that was exposed to noise 4 hours/day and G3 that was exposed for 8 hours/day.

The weight of the control group remains higher at all evaluations until the end of the experimental protocol when compared to the groups exposed to the noise. When analyzing the percentage of weight gain, no significant differences were observed ($p > 5\%$), however, when comparing the means of the weight gain

percentage between the groups, those who were exposed to sound for 8 hours/day showed the lowest mean (89.8%) followed by the group that was exposed for 4 hours (94.3%) (Figure 3).

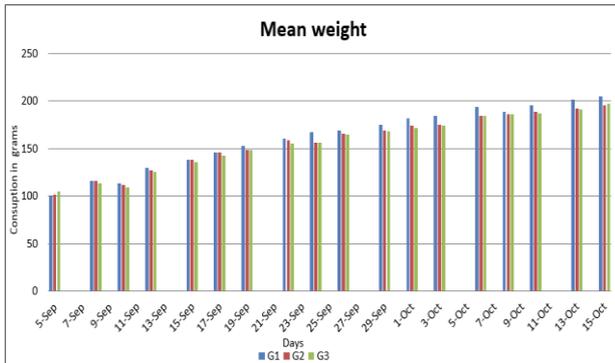


Figure 3: Comparison in the weight among the Control Group (G1), G2 that was exposed to noise 4 hours/day and G3 that was exposed for 8 hours/day.

Figure 4 shows that during the exposure period there was a significant longer stay in the open arms of groups 2 and 3. No significant differences in the frequency were observed among the groups in the open arm, but it is possible to observe that during the sound exposure the animals tended to move into the open spaces more frequently than when compared to the control group.

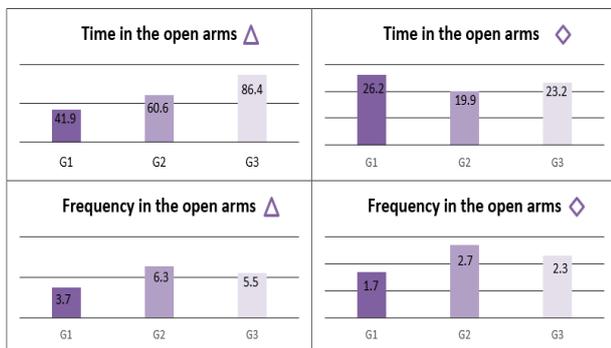


Figure 4: Comparison of time and frequency spent in the open arms among the Control Group, Group 2 and Group 3 during the exposition to the noise (Δ) and after its removal (◇).

Regarding the time spent in the center, no significant differences were observed, however it is possible to note that the longer the sound exposure time, the greater was the permanence in the center. Animals exposed to sound showed a significantly higher frequency in the center, but after the noise is removed, there was an approximation of the means between the groups. Rats decreased their locomotion, showing lower frequency values both for groups G2 and G3 (Figure 5).

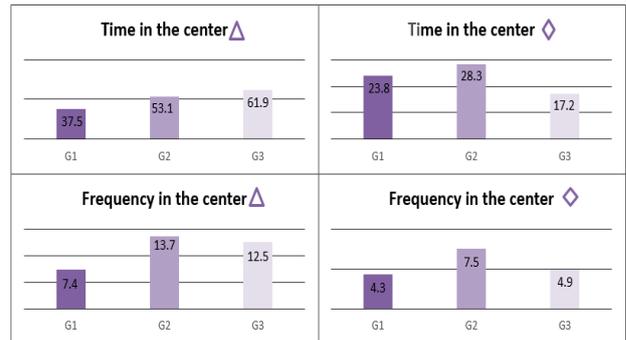


Figure 5: Comparison of the averages of the time and frequency spent in the center among the Control Group, Group 2 and Group 3 during the exposition to the noise (Δ) and after its removal (◇).

We observed a significant reduction between G1 and G3 in the time spent in the closed arm in the presence of noise. When the stimuli is removed, opposite behavior is observed. In the frequency, a significant increase was observed in the groups exposed to the noise. After its removal, an increase was observed only in G2 (Figure 6).

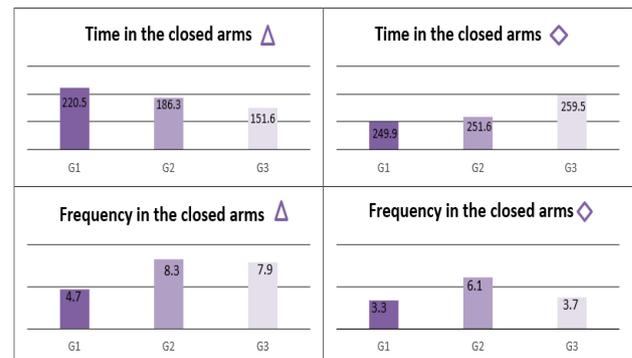


Figure 6: Comparison of the averages of the time and frequency spent in the closed arms among the Control Group, Group 2 and Group 3 during the exposition to the noise (Δ) and after its removal (◇).

By analyzing the time spent by the animals in cleaning, it was possible to see that those exposed to noise significantly spent less time in this procedure. After the experimental protocol, it was observed that in the absence of the stimuli, G2 significantly reduced cleaning time while G3 increased. No significance was observed in the frequency of cleaning but we may observe that the longer the time exposed to the sound, the lower the movement of cleaning is performed (Figure 7).

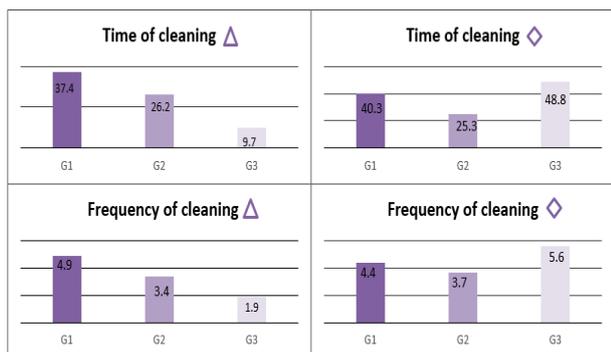


Figure 7: Comparison of the averages of the cleaning among the Control Group, Group 2 and Group 3 during the exposition to the noise (Δ) and after its removal (◇).

In addition to comparisons between time and frequency in the groups, analyzes were performed within the same group, comparing their behavior when noise was present and after noise removal. No significant differences were observed, except for the frequency in the open and closed arms (Table 1).

Table 1: Values of p obtained by analyzing the behavior in the presence or absence of noise in the same group.

| Parameters | G2 | G3 |
|-------------------------|-----------|------------|
| Time in open arm | p= 0.05% | p< 0.0001% |
| Time in center | p= 0.21% | p= 0.15% |
| Time in closed arm | p< 0.001% | p< 0.0001% |
| Time of cleaning | p= 0.94% | p< 0.0001% |
| Frequency in open arm | p= 0.16 | p< 0.0001% |
| Frequency in center | p= 0.31 | p< 0.0001% |
| Frequency in closed arm | p= 4.43% | p= 0.08% |
| Frequency of cleaning | p= 4.64% | p= 0.43% |

G2: Group exposed to noise for 4 hours/day for 20 days;
G3: Group exposed to noise for 8 hours/day for 20 days.

Comparing the behavior of G2 and G3 in the Elevated Plus Maze, a significant difference was observed for all parameters analyzed, except for the cleaning time for G2. It is shown that G3, which was exposed to noise for a longer time, showed a greater variation in the parameters when compared do Control Group or G2. It is also possible to identify the difference in the frequency at the entrance of each arm and the cleaning movement. Both groups showed significant difference in behavior with and without noise for their movement in the labyrinth. In addition, it can be observed that G3 presented the smallest p-value for all analyzed data (Table 1).

Many studies have shown that noise is predictor of damages in behavior and hearing of workers. This undesired sound is one of the major environmental pollutant and stressor for humans and is deeply related to several health outcomes. In modern population, environmental noise / noise pollution can be produced by industry, traffic, workplace and recreational activities. It

is a consensus that noise may induce or increase stress both in animals and humans models and severally interferes in the homeostasis and in the balance of life with physiological, psychological or behavioral modifications.^[13-16]

The Elevated Plus Maze apparatus is commonly performed to assess anxiety-like behavior in laboratory animals. An animal from control group (that did not receive treatment) tends to stay longer periods in closed arms that is a situation that represents facing natural threatening issues. For this reason, animals exhibit behavior of avoiding exploration the open arms. On the other hand, the closed arms represents to the animal a safer place against dangerous situations or presence of predators.^[17-20]

Normally researchers allow animals to spent and explore the Elevated Plus Maze for 5 minutes. The exploration of the open arms in 20 to 25% of the time may indicate avoidance in exploring these arms. In our work, introduction of a noise source even in a short period of time increased anxiety and stress. Other authors have shown that the noise may increase the anxiety patterns indicating it work as a stress condition.^[21-23] Haider et al.^[17] showed that 15 days of exposure to noise stress resulted in deficits in working and recognition memory in rats and related this condition to changes in dopamine and in the hydroxy-tryptamine.

Badache et al.^[24] investigated the individual and combined effects of prenatal noise and restraint stress on balance control, exploration, locomotion and anxiety behavior of adolescent rats and observed increase in terminal plasma Acetylcholine and presence of larger adrenal glands. Authors have concluded that prenatal stress cause anxiety, and loud noise exposure produces the highest effects.

Zheng et al.^[25] applied acoustic trauma to induce tinnitus in rats and evaluated the results on social interaction and anxiety in animals and observed that, when social behaviors were grouped, animals with tinnitus presented significantly more aggressive behaviors.

In another study,^[26] authors intended to evaluate the effects of noise exposure on behavior of rats and its relation to altered neurochemistry and showed that after a period of 15 days of sub-chronic exposition to noise stress lead to anxiety and depression-like behavior in male rats suggesting that modifications in brain serotonergic and dopaminergic activity can be linked to several psychological disorders.

Huygur, Arslan,^[27] found that animals exposition to noise lead to the impairment of spatial memory. Repeated stress in brain can cause adaptive plasticity inducing structural and functional modifications in neurotransmitters and systemic hormones.^[28] The time of exposure of our animals was not enough to produce such

profound changes since after the removal of the noise stimulus many behavioral characteristics were reestablished.

CONCLUSION

Our results showed a significant impact of noise exposure on the behavior of Wistar rats, and the introduction of a noise source even in a short period of time increases anxiety and stress, and discreetly reduces weight. It was also observed that noise removal reduced the anxiety parameters indicating that the exposure time to which the animals were subjected was not sufficient to produce definitive modifications.

REFERENCES

- Gilels F, Paquette ST, Beaulac HJ, Bullen A, White PM. Severe hearing loss and outer hair cell death in homozygous Foxo3 knockout mice after moderate noise exposure. *Sci Rep*, 2017 Apr 21; 7(1): 1054. doi: 10.1038/s41598-017-01142-3.
- Boger ME, Sampaio ALL, Oliveira CACP. Analysis of Hearing and Tinnitus in Workers Exposed to Occupational Noise. *Int Tinnitus J*, 2017 Apr 19; 20(2): 88-92. doi: 10.5935/0946-5448.20160017.
- Mofateh M, Karimi Q, Hosseini MH, Sharif-Zadeh GR. Effect of smoking on hearing loss in refractory's factory male worker with occupational noise exposure in Iran. *J Pak Med Assoc*, 2017 Apr; 67(4): 605-608.
- Fuks KB, Weinmayr G, Basagaña X, et al. Long-term exposure to ambient air pollution and traffic noise and incident hypertension in seven cohorts of the European study of cohorts for air pollution effects (ESCAPE). *Eur Heart J*, 2017 Apr 1; 38(13): 983-990. doi: 10.1093/eurheartj/ehw413.
- Yankaskas, K. Prelude: noise-induced tinnitus and hearing loss in the military. *Hear Res*, 2013; 295: 3-8. doi:10.1016/j.heares.2012.04.016.
- Arlinger, S. Negative consequences of uncorrected hearing loss—a review. *International journal of audiology*, 2003; 42 (Suppl 2): 2S17-20. doi:10.3109/14992020309074639.
- Min KB, Min JY. Exposure to environmental noise and risk for male infertility: A 2population-based cohort study. *Environ Pollut*, 2017 Apr 12; 226: 118-124. doi: 10.1016/j.envpol.2017.03.069.
- Park J, Chung S, Lee J, Sung JH, Cho SW, Sim CS. Noise sensitivity, rather than noise level, predicts the non-auditory effects of noise in community samples: a population-based survey. *BMC Public Health*, 2017 Apr 12; 17(1): 315. doi: 10.1186/s12889-017-4244-5.
- Basner, M., Babisch, W., Davis, A., Brink, M., Clark, C., Janssen, S., Stansfeld, S. Auditory and non-auditory effects of noise on health. *Lancet*, 2014; 383: 1325e1332.
- Ristovska, G., Laszlo, H.E., Hansell, A.L. Reproductive outcomes associated with noise exposure - a systematic review of the literature. *Int. J. Environ. Res. Public Health*, 2014; 11: 7931e7952.
- Boerngen-Lacerda RB, Souza-Formigoni ML, Souza O. Does the increase in the locomotion induced by the ethanol indicate its stimulant or anxiolytic properties? *Pharmacology, Biochemistry and Behavior*, 2000; 67: 225-232.
- Blanchard CD, Griebel G, Blanchard RJ. Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. *Neuroscience and Biobehavioral Reviews*, 2001; 25: 205- 218.
- Wada T, Sano H, Nishio SY, Kitoh R, Ikezono T, Iwasaki S, Kaga K, Matsubara A, Matsunaga T, Murata T, Naito Y, Suzuki M, Takahashi H, Tono T, Yamashita H, Hara A, Usami SI. Differences between acoustic trauma and other types of acute noise-induced hearing loss in terms of treatment and hearing prognosis. *Acta Otolaryngol*. 2017 Apr; 10: 1-5. doi: 10.1080/00016489.2017.1297899.
- Goines L, Hagler L. Noise pollution: a modern plague. *South Med J*, 2007; 100: 287e294.
- Samson J, Sheeladevi R, Ravindran R, Senthilvelan M. Stress response in rat brain after different durations of noise exposure. *Neurosci Res*, 2007; 57: 143-147. <http://dx.doi.org/10.1016/j.neures.2006.09.019>.
- Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M. Noise stress induced brain neurotransmitter changes and the effect of *Ocimum sanctum* (Linn) treatment in albino rats. *J Pharmacol Sci*, 2005; 98: 354-360. <http://dx.doi.org/10.1254/jphs.FP0050127>. [PubMed]
- Haider S, Naqvi F, Batool Z, Tabassum S, Perveen T, Saleem S, Haleem DJ. Decreased Hippocampal 5-HT and DA Levels Following Sub-Chronic Exposure to Noise Stress: Impairment in both Spatial and Recognition Memory in Male Rats. *Sci Pharm*, 2012 Oct-Dec; 80(4): 1001-11. doi: 10.3797/scipharm.1207-15.
- Rahmati B, Kiasalari Z, Roghani M, Khalili M, Ansari F. Antidepressant and anxiolytic activity of *Lavandula officinalis* aerial parts hydroalcoholic extract in scopolamine-treated rats. *Pharm Biol*, 2017 Dec; 55(1): 958-965. doi: 10.1080/13880209.2017.1285320.
- Cooney LG, Lee I, Sammel MD, Dokras A. High prevalence of moderate and severe depressive and anxiety symptoms in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod*, 2017 Mar; 9: 1-17. doi: 10.1093/humrep/dex044.
- Norouzi F, Abareshi A, Anaigoudari A, Shafei MN, Gholamnezhad Z, Saeedjalali M, Mohebbati R, Hosseini M. The effects of *Nigella sativa* on sickness behavior induced by lipopolysaccharide in

- male Wistar rats. *Avicenna J Phytomed*, 2016 Jan-Feb; 6(1): 104-16.
21. Masoumi-Ardakani Y, Mahmoudvand H, Mirzaei A, Esmailpour K, Ghazvini H, Khalifeh S, Sepehri G. The effect of *Elettaria cardamomum* extract on anxiety-like behavior in a rat model of post-traumatic stress disorder. *Biomed Pharmacother*, 2017 Mar; 87: 489-495. doi: 10.1016/j.biopha.2016.12.116. Epub 2017 Jan 7.
 22. Hosseini M, Mohammadpour T, Karami R, Rajaei Z, Sadeghnia HR, Soukhtanloo M. Effects of the hydro-alcoholic extract of *Nigella Sativa* on scopolamine-induced spatial memory impairment in rats and its possible mechanism. *Chin J Integr Med*, 2015; 21: 438-44.
 23. Ishaq H. Anxiolytic and antidepressant activity of different methanolic extracts of *Melia azedarach* Linn. *Pak J Pharm Sci*, 2016 Sep; 29(5): 1649-1655.
 24. Badache S, Bouslama S, Brahmia O, Baïri AM, Tahraoui AK, Ladjama A. Prenatal noise and restraint stress interact to alter exploratory behavior and balance in juvenile rats, and mixed stress reverses these effects. *Stress*, 2017 Apr; 21: 1-9. doi: 10.1080/10253890.2017.1307962.
 25. Zheng Y, Hamilton E, McNamara E, Smith PF, Darlington CL. The effects of chronic tinnitus caused by acoustic trauma on social behaviour and anxiety in rats. *Neuroscience*, 2011 Oct 13; 193: 143-53. doi: 10.1016/j.neuroscience.2011.07.026. Epub 2011 Jul 18.
 26. Uygur EE, Arslan M. Effects of chronic stress on cognitive functions and anxiety related behaviors in rats. *Acta Physiol Hung*, 2010; 97: 297-306.
 27. Naqvi F, Haider S, Batool Z, Perveen T, Haleem DJ. Sub-chronic exposure to noise affects locomotor activity and produces anxiogenic and depressive like behavior in rats. *Pharmacol Rep*, 2012; 64(1): 64-9.
 28. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res*, 2000; 886: 172-189. [http://dx.doi.org/10.1016/S0006-8993\(00\)02950-4](http://dx.doi.org/10.1016/S0006-8993(00)02950-4).