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Effect of White Noise and Light Exposure on Some Enzymes in Rat Testicular Tissue

Beyaz Gürültü ve Işık Maruziyetinin Sıçan Testis Dokusundaki Bazı Enzimler Üzerine Etkisi

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ABSTRACT

By definition, white noise (WN) is a type of sound that consists of a combination of various frequencies. Noise is an important source of environmental stress for humans and other living things and has a strong stimulating effect on light, circadian rhythm, hormonal, and neuro-behavioral levels. The objective of this research is to examine how environmental stressors like WN and light impact the activities of enzymes involved in testicular carbonic anhydrase, catalase, and glutathione peroxidase. A total of 18 male Sprague-Dawley rats were divided into three groups. The first was the control group (not exposed to stress, kept at 12/12 light-dark cycle and 24±2°C normal ambient temperature). The second group, known as the WN 60' group, experienced 60 minutes of white noise exposure per day for 14 consecutive days at 90 dB. The third group, referred to as the WN 60' + non daylight group, underwent 60 minutes of white noise exposure at 90 dB combined with 24 hours of continuous light exposure every other day. At the conclusion of the study, the testicular tissues were homogenized and centrifuged, followed by the spectrophotometric measurement of enzyme activities. As a result, when we compared the control group with both WN and WN + light exposure; A decrease was observed for CA enzyme in both applications, and an increase was observed in CAT and GPx enzymes in both applications.
Keywords: White Noise, Enzyme, Testicle.

ÖZ

Tanım olarak beyaz gürültü (BG), çeşitli frekansların birleşiminden oluşan bir ses türüdür. Gürültü insanlar ve diğer canlılar için önemli bir çevresel stres kaynağıdır ve ışık, sirkadiyen ritim, hormonal ve nöro-davranış düzeylerinde güçlü bir uyarıcı etkisi vardır. Bu çalışmanın amacı, beyaz gürültü ve ışık gibi çevresel stres faktörlerinin testis karbonik anhidraz (CA), katalaz (CAT) ve glutatyon peroksidaz (GPx) enzimlerinin aktiviteleri üzerindeki etkisini belirlemektir. 18 Sprag Dawley erkek rat 3 gruba ayrıldı. Birinci grup, Kontrol grubu (strese maruz kalmadan, 12/12 aydınlık-karanlık döngüsü, 24±2°C normal ortam sıcaklığı), ikinci grup BG 60' grubu (14 gün boyunca günde 60 dakika 90 dB'de BG)) ve üçüncü grup BG 60' + gün aşırı ışık grubu (90 dB'de 60 dakika/gün BG maruziyeti ve gün aşırı 24 saat sürekli ışık maruziyeti). Çalışma sonunda testis dokuları homojenize edilerek santrifüj edildi. Enzim aktiviteleri spektrofotometrik olarak ölçüldü. Sonuç olarak hem BG hem de BG + ışık maruziyeti uygulanan gruplarda kontrol ile kıyasladığımız zaman; CA enzimi için her iki uygulamada da düşüş, CAT ve GPx enzimlerinde ise her iki uygulamada da atrış olduğu gözlenmiştir.

Anahtar kelimeler: Beyaz Gürültü, Enzim, Testis.

Ethical approval (09.12.2019 Date, Decision No: 88012460-804) was obtained from the Scientific Research Ethics Committee of Atatürk University, Faculty of Veterinary Medicine.

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INTRODUCTION

White noise (WN) is a constant, monophonic type of noise that consists of an environment of different sound frequencies that are its enemies and includes all the irritating sounds from the outside world.[1,2] Sound ratios of the normal level, sound pressure level (SPL) > 105 dB are controlled as traumatic noise, and sound levels with SPL level > 80 dB are considered noise threatening. Intense sound cells, including many things done on humans and animals, have caused temporary or permanent hearing impairment. A high level of environmental protection may cause irreversible damage to the cochlea, sensitize the auditory sensation, and prolongation of auditory results may be excessive.[3] It has been determined by scientific studies for a long time that the environment has negative effects on human health. In many scientific studies, it has been determined that the environment acts as a stress source in the living body and causes function in many tissues and organs.[4,5]

The negative effect of noise is not only observed on the hearing system. In addition, it shows many different negative effects in living things. For example, it has been determined that people living in areas with noise pollution cause an increase in the rate of hypertension and cardiovascular diseases due to noise pollution.[4] It has been determined that noise pollution increases the risk of developing cancer. It has been determined that noise pollution causes a decrease in performance, disrupts sleep patterns, and causes an increase in the levels of adrenaline, noradrenaline and cortisol hormones. It has been determined that it causes anxiety, headache and nausea. It has been determined that it causes a decrease in the learning level and physical and cognitive abilities of children. It has also been observed that it reduces motivation in people and causes some psychological disorders. In experiments with rats, it was determined that noise has a reducing effect on neurotransmitters in the brain.[6]

Light plays a significant role in governing the circadian rhythms, neuroendocrine functions, and neurobehavioral processes of living organisms, and has a profound impact on the health and well-being of all mammals, including laboratory animals.[7] When confronted with stress, living organisms generate a substantial quantity of free radicals, leading to alterations within the oxidant system. The CAT enzyme is one of the important enzymes in the center of the antioxidant defense system in living things. GPx is an enzyme found in the cytoplasm that facilitates the conversion of hydrogen peroxide into water and oxygen, while also aiding in the conversion of peroxide radicals into alcohols and oxygen.[8] CA plays a crucial role in causing carbon dioxide and bicarbonate to be converted into each other, then facilitating the removal of water from the bicarbonate, leading to the replenishment of CO_2 in an acidic environment.[9] Furthermore, various other forms of CA have been discovered in different tissues, playing important roles in essential biological functions such as maintaining the balance of acids and bases, facilitating respiration, transporting CO_2 and ions, promoting bone resorption, enabling urea production, supporting glucose synthesis, facilitating fat formation, and aiding in electrolyte secretion. The wide array of CA isoforms involved in these intricate processes holds significant therapeutic potential, as they present promising targets for modulation in the treatment of various conditions including edema, glaucoma, obesity, cancer, and epilepsy, either through inhibition or activation.[10,11]

The fact that tinnitus, having sleep problems for various reasons or colic, which is a common condition in babies younger than 3 months, are exposed to white noise application by mobile phone in a bright environment by parents suggests that exposure to loud noise and light at night may have harmful effects. While noise exposure treatments, such as white noise, may provide temporary relief, they can actually expedite the aging process of the brain over time. This is due to their detrimental effects on the

functional and structural integrity of both the central auditory system and the broader brain.[12] A previous study by Sharma and Goyal found that prolonged light exposure triggers oxidative stress in rodents.[13] In another study by Ogo et al., they obtained results showing that the exchange between ROS and antioxidant enzymes also occurs in offspring with prolonged light exposure of mother rats during pregnancy.[14]

Although sound pollution and light exposure have effects on brain tissue and oxidative stress in the literature, the effects of both conditions have not been found. The aim of this study was to determine the effects of environmental stressors such as WN and light on rat testis CA, CAT and GPx enzymes.

MATERIAL AND METHOD

Studies on Experimental Animals

In our study, 18 male Spraque Dawley rats, weighing 220-260 g, obtained from Atatürk University, ATADEM, were used. The rats, which were properly cared for and fed under physiological conditions, were not given water and free food the night before they were dissacrated. After the application of anesthesia, the thorax region of the rats euthanized by cervical dislocation was opened and their intracardiac blood was taken. Tissues were placed in petri dishes sterile and stored at appropriate temperatures until the time of the study. Experimental area white noise ratio was determined with Mastech Ms6700 Digital Sound Meter. This study was carried out with the decision of the Animal Experiments Local Ethics Committee, which is under the Rectorate of Atatürk University.

Experimental Groups

The rats were divided into 3 different groups: Group 1 (Control Group) (n=6): Normal diet and standard conditions were applied as the control group. Group 2 (WN (85dB) 60' exposure) (n=6): from cell phones placed in cages for two weeks to rats at 60' HP per day, 23 hours in stand-by (217-Hz pulse rate, 2-W maximum peak power, SAR) 0.95 w/kg) was exposed to mobile phone application. This application was carried out in a separate room in order not to affect the control group. Group 3 (WN (85dB) 60' exposure + Everyday Light Exposure) (n=6): In addition to the Group 2 application, they were exposed to 24 hours of light every other day throughout the experimental protocol.

Measurement of CA Activity

Total CA activity was determined by measuring the change in absorbance at 348 nm using a 4-nitrophenyl acetate (NPA) substrate for 3 minutes at 25°C using a spectrophotometer.[15] The reaction mixture consisted of 0.4 mL of 50 mM Tris-SO_4 buffer at pH 7.4, 0.33 mL of 3 mM NPA, 0.26 mL of distilled water, 0.01 mL of enzyme solution and, for a total volume of 1.0 mL.[16]

Measured of GPx Activity

GPx activity was assessed using a modified version of the method developed by Paglia and Valentine.[17] Activity was measured spectrophotometrically for 3 minutes at 340 nM, monitoring the oxidation of glutathione and NADPH using GR as coupling agent. In brief, a 1 mL assay mixture was prepared, containing optimized concentrations of the following components: 0.5 M K_2HPO_4 (pH 7.0), 2.5 mM EDTA, 0.18 U/mL GR, 100 mM glutathione, and 10 mM reduced NADPH. To initiate the reaction, 0.5 mL of tissue extract was added to the spectrophotometer cuvette, followed by the addition of 0.1 mL of 60 mM cumene hydroperoxide, a suitable substrate for GPx.

Measured of CAT Activity

CAT activity was assessed using spectrophotometric analysis, which involved directly measuring the reduction in light absorption at 240 nm resulting from the breakdown of hydrogen peroxide by CAT.[18] The reaction mixture consisted of 0.01 M H_2O_2 and 0.05 M potassium phosphate buffer at pH 7.0, with a total volume of 1 mL. The

enzymatic reaction was initiated by adding 20 µL of the enzyme, and the rate of hydrogen peroxide utilization was measured for the initial 60 seconds by monitoring the decrease in absorbance at 240 nm.[19]

RESULTS AND DISCUSSION

According to the findings, when the literature is examined, this study is original research since it is the first time that the effects of WN and light applications on the organism have been studied together. An increase in the activity of rat testis CAT and GPx enzymes was observed after exposure to stressors such as WN and light. However, it was determined that there was a slight decrease in the activity of the CA enzyme. The obtained results are shown in Table 1-2, and Figure 1-3.

Table 1. Values Determined for WN and Light Exposure (LE) With the Enzymes Tested

Enzyme	Control	60' WN	WN + LE
CA	0.12 ± 0.005	0.107 ± 0.004	0.095 ± 0.004
CAT	14.6 ± 1.6	19.4 ± 1.8	22.3 ± 2.1
GPx	26.1 ± 2.8	32.7 ± 3.1	35.7 ± 3.8

Table 2. Change Values of the Enzymes Tested Compared to the Control Group

Enzyme	60' WN	WN + LE
CA	10.83 ↓	20.83 ↓
CAT	32.87 ↑	52.74 ↑
GPx	25.28 ↑	36.78 ↑

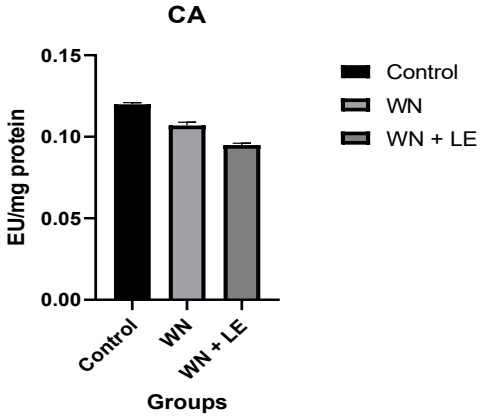

Figure 1. CA Activity Graph

When we look at Table 1, the mean total CA activity in testicular homogenate was 0.12 ± 0.005 EU/mg prt in control rats, while this value was calculated as 0.107 ± 0.004 EU/mg prt in the WN group and 0.095 ± 0.004 EU/mg prt in WN + light exposed rats. This result showed that the mean testicular CA activity of male rats exposed to both WN and continuous illumination decreased by 10.83% in the WN-only group compared to the control rats, while the WN + LE group decreased by 20.83%.

In the same table, while the mean CAT enzyme activity in testicular homogenate was 14.6 ± 1.6 EU/mg prt in control rats, this value was 19.4 ± 1.8 EU/mg prt in the WN group and 22.3 ± 2.1 EU/mg prt in WN + light exposed rats. This result showed that the mean testicular CAT activity in male rats exposed to both WN, and continuous illumination increased by 32.87% in the WN-only group, while it increased by 52.74% in the WN + LE group compared to the control rats.

According to Table 1, the mean GPx activity in testicular homogenate was 26.1 ± 2.8 EU/mg prt in control rats, while this value was 32.7 ± 3.1 EU/mg prt in the WN group and 35.7 ± 3.8 EU/mg prt in WN + light exposed rats. This result showed that the mean testicular GPx activity in male rats exposed to both WN, and continuous illumination increased by 25.28% in the WN-only group, while the WN + LE group increased by 36.78% compared to the control rats.

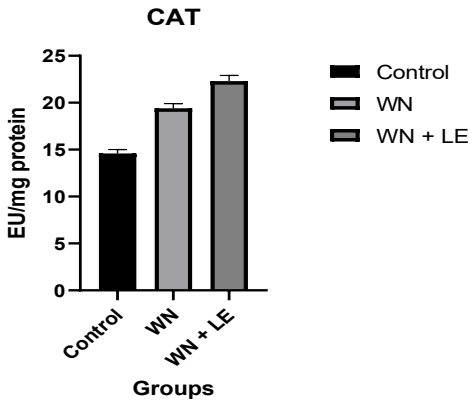

Figure 2. CAT Activity Graph.

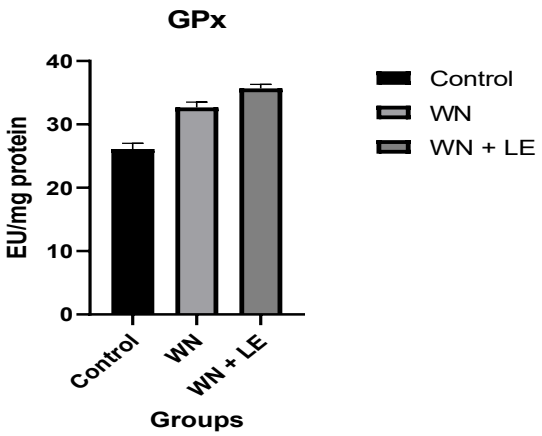

Figure 3. GPx Activity Graph.

CONCLUSION

The testis is a vital reproductive organ in males, serving both as an endocrine gland.[16] However, various environmental factors, including physical and chemical agents, can adversely impact testicular function.[20] Among these environmental pollutants, noise stands out as a prevalent natural pollutant, present both at home and in the workplace.[21] Numerous studies have highlighted the potential detrimental effects of noise-induced stress on the structure and function of testicular tissue.[22]

The alternating patterns of light and darkness within the circadian rhythm have a notable impact on endocrine and reproductive processes. In male rats, subjecting them to continuous exposure to light led to a substantial reduction in body weight compared to the control group.[23]

The presence of noise stress has a notable impact on the functioning of neuropeptides in both hypothalamic and extrahypothalamic regions, resulting in a significant increase in the secretion of hypothalamic hormones like CRH and ACTH.[24] Furthermore, these hormones not only affect areas beyond the hypothalamus but also heighten the activity of the HTPA axis and trigger the release of corticosterone, such as cortisol, from the adrenal cortex.[25] Prolonged exposure to chronic stress caused by noise can disrupt the balance of hormone levels, enzymes, and their metabolism, thereby potentially weakening the functioning of certain systems, such as the reproductive system.[26,27]

Considering the results of this study, the increase in the activities of CAT and GPx enzymes, which neutralize the radical molecules formed in the body of living things, may have emerged in response to the increase in oxidative damage. The decrease in the activity of the CA enzyme may also be due to the negative effects of stress-induced oxidant radicals.

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