

THE EFFECT OF NOISE EXPOSURE ON THE AVERAGE NUMBER OF TEMPORAL LOBE NEURONS IN ADULT WISTAR RATS

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ABSTRACT

Noise is an unwanted and disturbing sound, and can trigger stress and disruption in the environment. Noise covers various aspects in modern communities, one of which is the work environment. Exposure to loud and chronic noise can cause an imbalance between oxidants and antioxidants, thereby damaging cellular lipids, proteins and nucleic acids in DNA, and can impact the hearing organs. The aim of this study was to analyze the relationship between noise exposure and the number of neurons in the temporal lobe of Wistar rats. The research design used was a laboratory experimental design, specifically a pure experimental design, which was carried out using a randomized posttest-only control group design. The research sample consisted of 30 male Wistar rats who were randomized into 2 groups, namely control and treatment. The treatment group was exposed to 95dB noise for 4 hours per day for 14 days. The mice were then euthanized, and the brain tissue was fixed in 10% neutral buffered formalin. Assessment was carried out using Hematoxylin Eosin staining, and calculations were carried out blindly on the average number of neurons in the mouse temporal lobe. Data were tested for normality using the Shapiro-Wilk test and analyzed using the independent t-test for parametric comparisons. The results showed that there was a significant difference in the mean number of neurons in the temporal lobe between mice exposed to noise and the control group ($p < 0.005$). The conclusion of this research is that noise affects neuron cells in the temporal lobe.

Keywords : noise., temporal lobe., neurons.

INTRODUCTION

The problem of noise pollution has existed since the end of the 20th century along with the rapid development of human activities (such as air traffic, trains and road traffic). According to the World Health Organization, unlike many other environmental problems, noise pollution is increasing, with an increase in individuals affected by it. Noise is not only a local problem, but is a global dilemma that is disturbing for society and requires preventive action.^{1,2}

The impact of noise pollution on health needs serious attention in the field of public health. Noise can cause various health problems, such as hearing loss, sleep disorders, stress, mental health problems, and other physical impacts.^{3,5}

Excessive noise exposure can cause damage to the ears and even hearing loss. This can occur due to exposure to very loud sounds for a long time. Chronic noise exposure can also disrupt a person's sleep quality, both when starting sleep and waking up during sleep. This exposure can also cause increased stress levels in individuals, which negatively impacts a person's mental and physical health. Mental health problems that can arise include

anxiety, depression and other mental disorders. Constant noise causes physical health problems such as increased blood pressure, digestive disorders, and increased risk of cardiovascular disease.^{3,5}

Noise is defined as unwanted sound and is considered a stressor and environmental disturbance. Noise pervades many aspects of modern communities, such as workplace environments. The damaging effects of noise are mainly due to the unlimited production of free radicals in the hearing organs. The response to noise depends on the characteristics of the sound, such as intensity, frequency, sound complexity, and duration of noise exposure.^{3,6,8}

In noise research, laboratory animals have been widely used to determine how noise disrupts body and brain function, with most research focusing on hearing damage. As in humans, hearing loss in laboratory animals results from prolonged or repeated exposure to intense noise (i.e., sound pressure levels greater than 85 dB).^{9,10}

Based on the description above, this study aims to further examine the actual impact of noise on vulnerable organs, especially the effect on neuron cells related to hearing, especially in the primary auditory cortex located in the temporal lobe.^{3,6,8}

MATERIALS AND METHODS

The research design used is a laboratory experimental research design which aims to determine possible causal relationships. The research design used was a pure experimental design and was carried out using a randomized posttest-only control group design.

The research samples were white rats (*Rattus norvegicus*) Wistar strain, male, aged 60-90 weeks with a body weight of 100-150 grams. The Wistar rat was chosen as a sample because it is a mammal and is usually used as an experimental animal in research. In this study, 15 samples were used per group, so a total of 30 samples were needed for experimental research.

Rats are housed and cared for by providing food and water and maintaining cage cleanliness. Experimental animals were given standard feed and drinking water ad libitum. Experimental animals were placed in groups in cages measuring 30x40x40 cm, made of wire and well ventilated. Each cage contains 5 experimental animals, each animal separated by a divider. Once every three days, the cage is cleaned of dirt and food waste. Lighting was set on a 12-hour light cycle, 12-hour dark cycle (light cycle from 6 am to 6 pm) with room temperature maintained at 30 ± 10°C. For 1 week, the animals undergo acclimatization to get used to their environment. The physical health of the experimental animals is observed to ensure they can adapt. Healthy experimental animals are characterized by open and clean eyes, smooth and shiny fur, active behavior, and good appetite.

After acclimation, the experimental animals were divided into two groups randomly, into a control group and a treatment group, 15 animals per group. The treatment group is exposed to noise stress for 4 hours at a sound pressure level of 95 dB using Real-time analyzer software version 5.2.0 (Yoshimasa Electronic Inc., Japan) connected to a loudspeaker (Sony SRS XB30, Japan) for 14 days in a soundproof cage measuring 100 x 100 cm. The control group does not receive exposure to noise stress (Figure 1).

On day 15, the mice were euthanized by injecting peritoneal ketamine at a dose of 150 mg/kg body weight, and their brain organs were removed to examine the number of neurons in their temporal lobes. The brain was fixed in buffered formalin solution 10% in a plastic container labeled according to the experimental group and number of experimental animal samples. After 8 hours (maximum 24 hours), the brain organs are processed into histological slides using the paraffin method. Paraffin blocks were cut with a rotary microtome to a thickness of 4-5 µm and mounted on glass slides, followed by staining with hematoxylin and eosin.

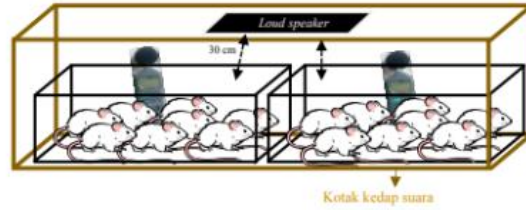


Figure 1. Illustration of noise exposure on Wistar rats with 95 dB noise. Sound intensity measurement using a sound level meter. Soundproof box constructed to dimensions of 100x100 cm.

The collected data was entered, edited, cleaned, and coded. Data is grouped according to research variables and presented in the form of tables, graphs and images. Next, the data was tested for normality using the Shapiro-Wilk test and analyzed using the comparative independent sample t test ($\alpha = 0.05$).

RESULT

The whole rat brain had been stained with hematoxylin and eosin, then the number of neurons was read. The neuron cells in the temporal lobes were counted using a microscope with two fields of view on each slide in both the control and treatment groups.

The collected data underwent entry, editing, cleaning, and coding. The data was grouped based on the research variables and presented in tables, graphs, and figures. Subsequently, the data was subjected to normality testing using the Shapiro-Wilk test and analyzed using comparative tests such as the independent samples t-test ($\alpha = 0.05$).

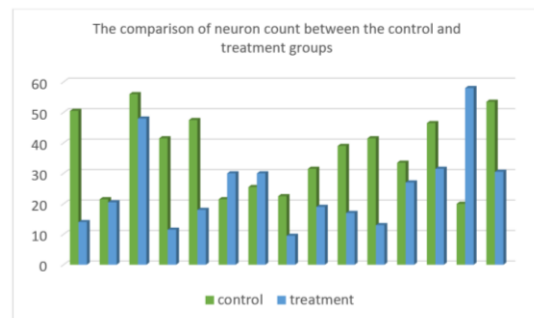


Figure 2. Comparison of neuron count between the control and treatment groups.

The results indicate a significant difference ($p=0.02$) in the neuron count of the temporal lobes between the control group and the treatment group, as shown in Figure 2.

This difference indicates that the number of neurons in the treatment group is lower compared to the control group. Presented in Figure 3.

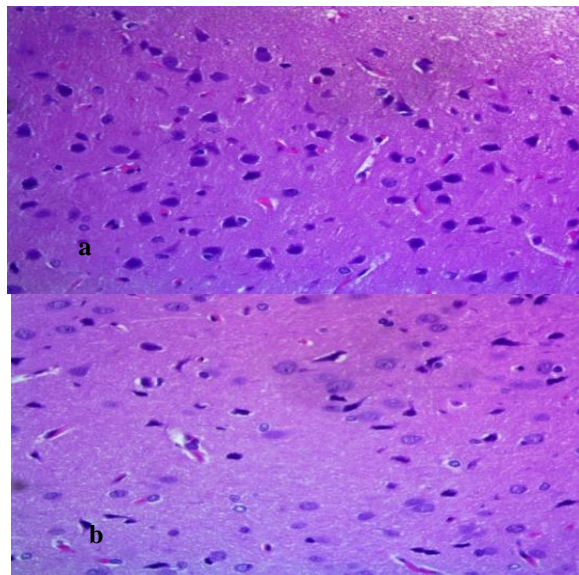


Figure 3. Comparison of hematoxylin and eosin staining results between the control and treatment groups. a) Example of a slide in the control group, showing a count of 40-60 neuron cells. b) Example of a slide in the treatment group, showing a count of 20-30 neuron cells.

DISCUSSION

In this study, there was a decrease in the number of neurons in the temporal lobe of Wistar rats that were exposed to noise of 95dB. The temporal lobe contains the main auditory functional areas that can be affected by such exposure. Chronic noise exposure can have various negative impacts on the nervous system, including reducing the number of neurons in the brain. The molecular mechanisms involved in this process may include complex biological pathways.

Environmental noise has a negative impact on health, such as decreased hearing ability caused by injury to the auditory nervous system. Exposure to noise in any form exceeding 90 dB can be a source of stressor. A study showed that memory impairment and error rates increased significantly after exposure to noise stress, especially exposure to 100 dB for 4 hours per day for 30 days, compared to control animals.¹¹⁻¹³

Acute and long-term exposure to noise can result in excessive production of free radicals. Oxygen free radicals can attack proteins, nucleic acids and lipid membranes, disrupting normal cell function and integrity. These free radicals can cause oxidative stress in brain cells by damaging cellular structures and important components such as DNA, proteins and lipids. Continuous oxidative stress can trigger the cell death pathway,

namely cell apoptosis, which can ultimately cause a decrease in the number of neurons.¹⁴⁻¹⁶

Reactive oxygen species (ROS), also known as oxygen free radicals, are a normal byproduct of cellular aerobic metabolism. If the balance of antioxidants and oxidants is disturbed, these unstable molecules can damage cellular lipids, proteins and nucleic acids in DNA. Acute and chronic exposure to loud noise produces excessive free radicals and causes disorders of extra-auditory organs such as the nervous, endocrine and cardiovascular systems.¹¹⁻¹³

Oxidative stress is a condition characterized by a significant imbalance between oxidants and antioxidants, leading to cell damage, dysfunction, or death. Under normal conditions, sufficient or excessive concentrations of endogenous antioxidants are used for protection against exposure to environmental oxidants. However, repeated exposure to environmental oxidants such as air pollution, smoking, disease conditions, noise pollution, or excessive pressure fluctuations can cause an increase in the rate of antioxidant decline, resulting in imbalance and oxidative stress.^{8,11,18-20}

Mitochondria are cell organelles that are responsible for producing energy in cells. Exposure to high levels of noise over a long period of time can cause oxidative stress in mitochondria and disrupt their function. Disruption of mitochondrial function causes disruption of energy production in nerve cells, disrupts vital processes such as metabolism and protein synthesis, and ends in nerve cell death.¹⁴⁻¹⁶

The nervous system is an organ that is vulnerable to damage caused by free radicals. Under conditions of noise exposure, neurotransmitters in brain regions increase during stress due to the exposure. Neurotransmitters in brain regions were found to increase during noise stress, even after 15 days of noise exposure.^{8,11,18-19}

Noise can affect neurotransmitter activity in the brain. For example, increased levels of glutamate (an excitatory neurotransmitter), can cause neuronal toxicity and cell death through various mechanisms. Conversely, decreased levels of inhibitory neurotransmitters such as GABA can increase nerve excitability and make them more susceptible to stress and damage.¹⁴⁻¹⁶

Apoptosis is a non-inflammatory process that involves the systematic breakdown of cells, packaging of products into membrane-bound structures, and removal via phagocytosis. Upon exposure to loud noise and subsequent activation of oxidative stress, intrinsic caspases (ROS and cytochrome C) and extrinsic pathways (TNF- α) will trigger the apoptotic pathway. A study related to neurogenesis shows the negative effect of noise on neurogenesis. This occurs because noise reduces neuroblast proliferation (Type-1 and Type-2 cells) and increases neuroblast (Type-1) apoptosis in mice.^{19,20}

Chronic noise exposure can trigger an inflammatory response in the brain. This inflammation is caused by the release of cytokines and other inflammatory mediators which can damage brain tissue and cause nerve cell death. In addition, inflammation can interfere with the nerve regeneration process and cause a decrease in the number of neurons.¹⁴⁻¹⁶

CONCLUSION

Noise exposure has a negative impact on nerve cells, especially neurons. This study shows that exposure to 95dB noise can cause a significant decrease in the number of neurons in the temporal lobe of adult Wistar rats.

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