

IMPACT OF CHRONIC NOISE ON HIPPOCAMPAL MORPHOLOGY AND ITS FUNCTIONS IN WISTAR ALBINO RATS

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ABSTRACT**Objective:** The study aims to investigate the effect of chronic noise stress on hippocampal morphology and its functions in male Wistar albino rats.**Methods:** Adult albino rats were randomly divided into two groups. Each group contained six animals. Rats exposed to chronic noise stress (100 dB/4 h-30 days) were compared with control animal and assessed for behavior using hole-board test, marble burying test, and morphology of hippocampus by histology.**Results:** The rats exposed to chronic noise stress showed significance ($P < 0.05$) of behavioral alterations such as increased fear and anxiety, obsessive-compulsive behavior, enlarged lateral ventricle, and reduced hippocampal volume.**Conclusion:** The results reported that chronic noise stress affects neurobehavioral due to reduced hippocampal volume.**Keywords:** Anxiety, Fear, Hippocampus, Lateral ventricle, Noise, Obsessive-compulsive, Stress.© 2021 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2021v14i7.39944>. Journal homepage: <https://innovareacademics.in/journals/index.php/ajpcr>**INTRODUCTION**

All living creatures depend on their environments for energy and materials that help in sustaining life. Although the environment sustains human life, it can also cause diseases [1]. The health impacts caused by excessive environmental noise are a growing concern. Noise exposure has been linked to a variety of health effects, including hearing loss, cardiovascular damage, cognitive impairment, and sleep disturbance, and annoyance [2]. Enormous studies have shown that noise above 90 dB increases the stress hormones and can cause a significant impact on human health. The brain is the target organ for corticosteroids [3] and the hippocampus is vulnerable to a number of insults and is prone to excitotoxic activity [4]. Receptors for glucocorticoids are found in the hippocampus, amygdala and frontal cortex, these three brain regions are involved in-memory processing, and emotional regulation [3,5]. From this, it was planned to study the effect of chronic noise on hippocampal functions in a noise-induced animal model.

METHODS**Animals**

Experimental animals were all healthy and weighed about 140-180 g. The animals were reared in the Animal House of the Institute, University of Madras, Taramani, Chennai, India, and all the animals were maintained under standard laboratory conditions housed at 3 per cage (29 cm × 22 cm × 14 cm) and constant ambient temperature with 12-h dark photoperiod; the rats were allowed free access to food and water. Appropriate ethical clearance was obtained for this work from the Institutional Animal Ethical Committee (IAEC no. 02/06/2019 dated 12/03/2019) prior to the experiments. Animals were divided into two groups, with six animals in each group.

- Group I - Control
- Group II - Chronic noise stress (100 d BA-4 h/30 days).

Noise stress induction

When noise exposure exceeds 100 dBA, it becomes a stressor. Noise was produced by two loudspeakers (15 W) driven by a white noise

generator (0-26 kHz), and they were installed 30 cm above the cage. The noise level was set at 100 dB uniformly throughout the cage and monitored by a sound-level meter D2023 (S.NO-F02199: Cygnet Systems, Gurgaon, Haryana, India). Animals were then exposed for 4 h/30 days. To avoid the influence of handling stress on evaluation, the effects of noise exposure in control animals were kept in the above-described cage during the corresponding period of time, without noise stimulation [6].

Marble burying test

Marble burying test is used to assess the Obsessive-Compulsive behavior and is done using polycarbonate rat cages (26 cm × 48 cm × 20 cm) with fitted filter top covers, fresh, unscented mouse bedding material to each cage to a depth of 5 cm and level bedding surface by inserting another cage of the same size onto the surface of the bedding, and placed standard glass toy marbles (assorted styles and colors, 15 mm diameter, 5.2 g in weight) gently on the surface of the bedding in five rows of four marbles. Rats were placed into a corner of the cage containing marbles, with care taken to place the mouse on bedding as far from marbles as possible, and then the filter top was placed on top to cover the cage. Food and water were withheld during the test. The rats were allowed to remain undisturbed for 30 min. Scoring was done when marble was buried and two-thirds of its surface area was covered by bedding. Average scores for the number of marbles buried for each rat were calculated [7].

Hole board test

The hole-board apparatus consisted of a wooden, gray box, measuring 68 cm × 68 cm. The walls were 40 cm high, and the box was raised 28 cm above the ground on a metal stand. Four holes (4 cm in diameter) were cut into the floor of the apparatus; each hole was 28 cm from a corner of the box along the diagonal from the corner to the center. The stand of the apparatus was open on all sides, allowing the floor or objects to be dimly lit. At the beginning of each trial, a patient was placed in one corner of the apparatus (always the corner closest to the door of the room), facing the center of the arena. Each trial lasted 10 min. At the end of the trial, the patients was immediately placed into a carrying box

and returned to the home cage. Between each trial, the floor and walls of the apparatus were cleaned using 70% alcohol solution. During each 10-min trial, head-dip: the animal places its head into one of the holes, to a minimum depth such that the ears were in level with the floor of the apparatus (a new bout of head-dipping was recorded if the animal raised its head fully out of the hole before resuming); grooming, rearing, and fecal pellets' behavior patterns were recorded [8].

Histology

The rats were sacrificed by cervical dislocation. After sacrifice, the brain was rapidly removed, and the hippocampus was dissected on an ice-cold plate and the weight of the discrete region of the brain was measured. Another set of rats were subjected to transcardiac perfusion and fixed in 10% neutral formalin for 48 h solution followed by dehydration in ascending grades of alcohol, cleared, and then embedded in paraffin wax. Paraffin block 5 μ m thick coronal sections were obtained using a rotary microtome, and mounted on slides, followed by silver nitrate staining [9]. For Immunohistochemistry, the brains were postfixed with 10% formalin, embedded in paraffin, and cut into 10-mm-thick sections. The sections were deparaffinized in xylene twice for 5 min, rehydrated in a descending series of ethanol (99%, 96%, and 70%), and followed by washes in distilled water. Antigen retrieval was achieved by heating the samples in a retrieval buffer with low pH of 6.2 at 110°C for 15 min. Then, the sections were washed in wash buffer. The slides were incubated overnight with primary antibody (Wnt-3a [3A6]: sc-136163, Santa Cruz Biotechnology, Inc (1: 100 Dilutions). After a rinse in PBS, the sections were incubated with rabbit biotinylated anti-goat IgG (1:200 Dilutions) for 1 h, washed again with PBS, and incubated. All subsequent incubations were performed at room temperature and with DAB for 1 h. The slides were counterstained with Mayer's Hematoxyline and mounted with DPX for examination. Photomicrographs were obtained using a Nikon Camera (Japan).

Statistical analysis

Data was presented in the form of the bar diagram with mean \pm SD. All data were analyzed with the Graphpad Prism 8. Statistical significance between the three groups was determined by *t*-test and $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Chronic stress induces reduction of the whole brain (Fig. 1) and hippocampal weight (Fig. 2) of the animals significantly when compared to the control animals. The number of marbles buried (Fig. 3) in the case of animals exposed to chronic noise were significantly greater when compared to the control animals. The number of head dipping (Fig. 4) reduced in chronic stress animals when compared to the control animals. In histological studies, sections obtained from the brain of chronic noise stress animals exhibited shrinking and enlargement of lateral ventricle when compared to the control brain (Figs. 5 and 6). Silver-staining methods are helpful for the histological identification of pathological deposits. In the control specimens, the brain neurons were stained yellowbrown, and in case of chronic stress exposed specimens, they were dark brown to black colored, the axon, cell body, neurofibrillary tangles, and neuropil threads were more readily apparent (Fig. 7). Hosseini-Sharifabad and Sabahi (2008) reported that the exposure of noise stress significantly reduced the hippocampal volume in all the layers of dentate gyrus and CA regions [10]. The hippocampal cells express the glucocorticoid receptors, they are the principal target sites for glucocorticoids which are the adrenocortical hormones secreted during stress [11]. Activation of GC receptors leads to the overproduction of ROS [12], and also increases oxidative damage to the protein due to the inhibiting activities of mitochondrial complex I and antioxidant enzyme SOD [13]. Conrad reported that chronic stress or prolonged exposure to glucocorticoids can compromise the hippocampus by producing dendritic retraction, a reversible form of plasticity that includes dendritic restructuring without irreversible cell death [14]. Reduced hippocampal volume causes behavioral changes such as obsessive-compulsive and increased fear and anxiety

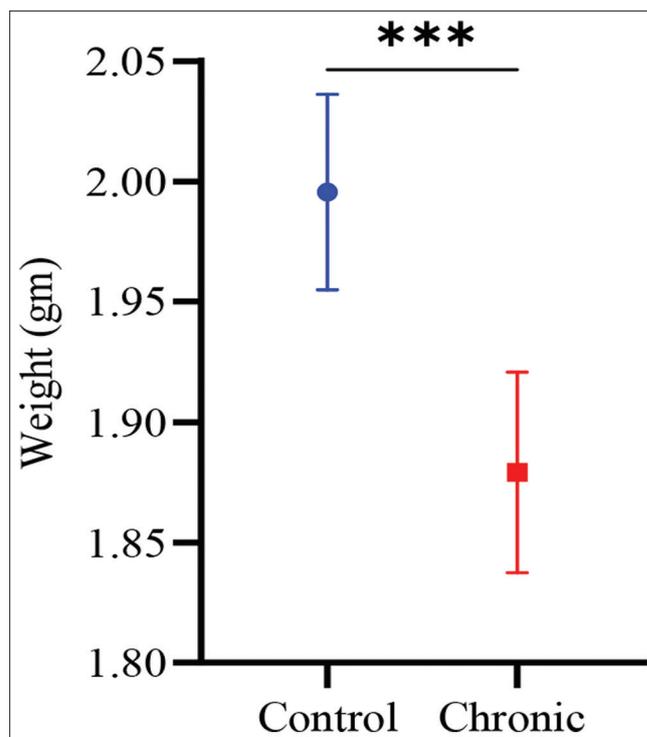


Fig. 1: Weight of the whole brain

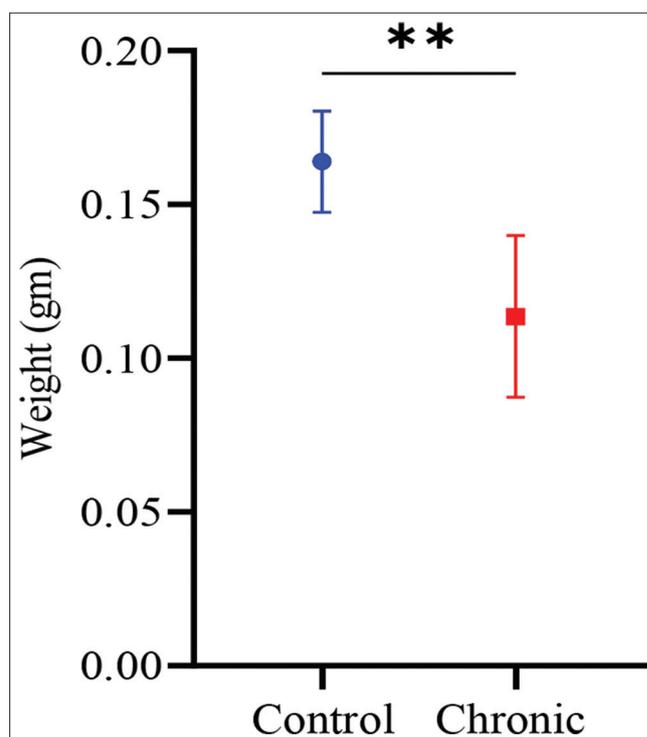


Fig. 2: Weight of hippocampus

in animals. The hippocampus has recently been identified to play a key role in the pathophysiology of adult obsessive-compulsive disorder (OCD) [15] and also Atamaca *et al.* (2008) reported that hippocampus, anterior cingulate, and basolateral amygdala interrelated together because of the fact that these structures have connections with the orbitofrontal cortex which may have a role in the pathophysiology of OCD and reduction of hippocampal volume to 12% in AD patients (Atmaca *et al.*, 2008).

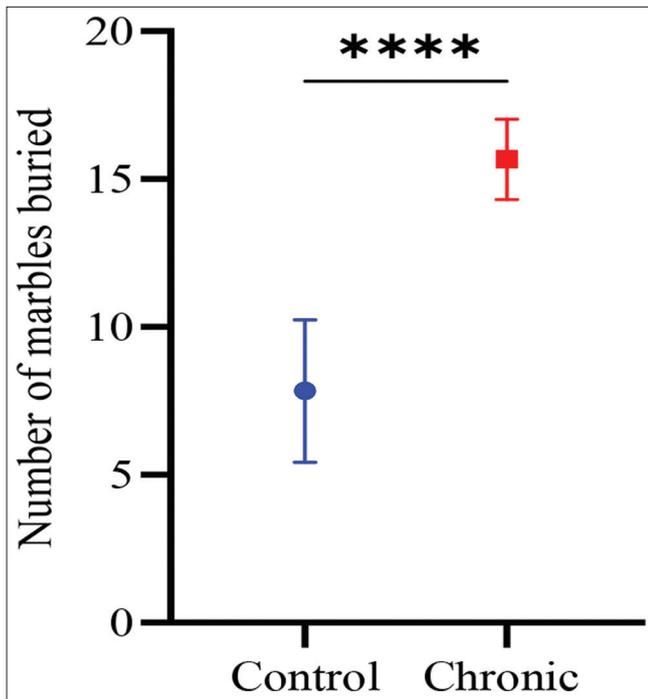


Fig. 3: Marble burying test

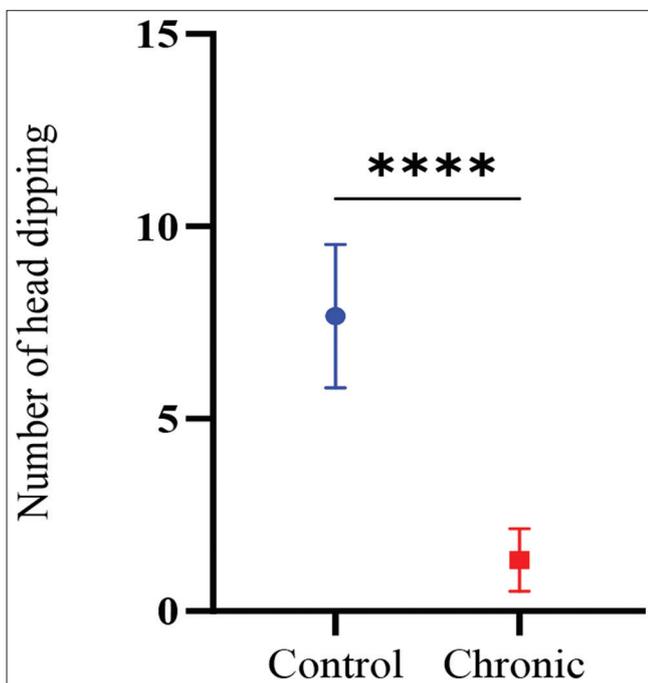


Fig. 4: Hole board test

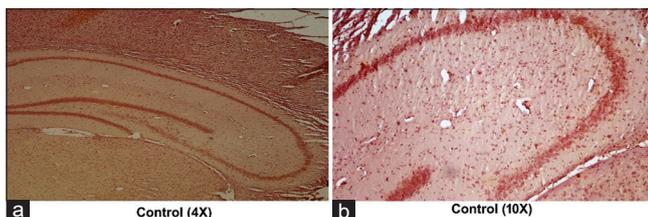


Fig. 5: (a and b) Shown control Hippocampus ×4 and ×10 magnification

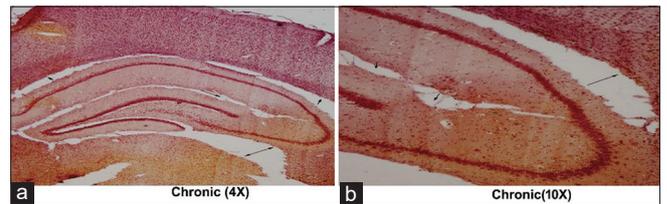


Fig. 6: (a and b) Shown chronic noise stress-induced rat Hippocampus ×4 and ×10 magnifications. (ii) Shown shrunken hippocampus and enlarged space of lateral ventricle

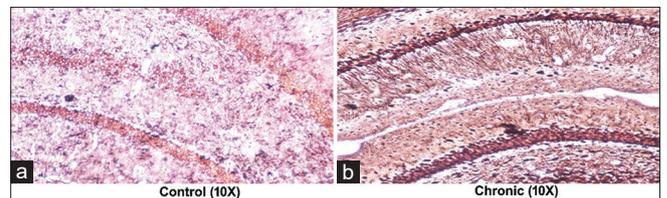


Fig. 7: (a and b) Shown silver nitrate staining of control and chronic noise with ×10 magnification. (ii) Shown black brown cell bodies and axons

CONCLUSION

Noise beyond the bearable limit would make us feel upset and even get frustrated. When sound exceeds 90 dB, it leads to deterioration of the health of an individual. The term “Noise stress” is a highly prevalent concept in the challenging and highly demanding fashionable society currently. In conclusion, our results confirmed that chronic noise stress led to a negative impact on the hippocampus and its functions in albino rats.

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AUTHORS CONTRIBUTION

The corresponding author has designed the work and critical revision of the manuscript. The first author carried out behavioral assessment and paper writing.

CONFLICT OF INTEREST

The authors declare that they no conflicts of interest concerning this research article.

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