

## Exposure to Chronic Noise Reduces the Volume of Hippocampal Subregions in Rats

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### Abstract

#### Objective

The hippocampal circuit integrity is crucial for learning and memory. Despite the existing reports on hippocampal-dependent memory impairment due to noise stress, there are only a few studies on the effect of noise stress on anatomical structure of hippocampus. The present study is aimed to investigate the likely effects of chronic noise exposure on the volume of rat hippocampus.

#### Materials and Methods

Two-month male Wistar rats were randomly divided into three groups (n=10 in each group). In the control group rats were maintained under standard laboratory conditions (150 days). In the noise-exposed group: Rats were exposed to 40 dB unmodulated sinusoidal noise with a frequency of 1100 Hz for 20 mins, three times per day for 90 days. The recovery group rats were exposed to noise for 90 days and allowed to survive without further treatment until the day of sacrifice (180th day). The right hemispheres were selected for stereological study. Twenty five  $\mu\text{m}$  thick sections were cut along the entire extent of the hippocampus. Using systematic uniformly random sampling, one section from every twenty sections was analyzed. Volume estimation was performed using Cavalieri principle.

#### Results

Statistical analysis revealed that noise stress induces a significant reduction in volume of all layers of hippocampal subdivisions, except CA1 hippocampal field. In addition, we found that rats which were allowed to recover from noise displayed larger volume of dentate gyrus and CA3 hippocampal field in comparison to noise-exposed rats.

#### Conclusion

Reduced volume of hippocampal layers most probably reflects structural alterations in the neurites of related neurons. These results provide a neuroanatomical basis that may be relevant to the reported memory disturbances in human and animals following noise stress.

**Keywords:** Hippocampus, Noise pollution, Volume estimation

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## Introduction

All living organisms experience numerous stressful events during lifetime. Nowadays, noise pollution is a stressful stimulus in industrialized communities. Exposure to noise can trigger a cascade of neuroendocrine events reminiscent of a stress response, including activation of the hypothalamic-pituitary-adrenocortical axis causing secretion of glucocorticoids (1). A growing body of evidence has shown that exposure to noise adversely affects the nervous system (2). Hippocampus is a neuroanatomically well-defined region of the brain involving in learning and memory (3). The hippocampal circuit integrity is crucial for spatial learning and memory (4). It is also well known that the hippocampal neurons contain the highest concentration of corticosteroid receptors of all cerebral neurons (5).

There are several studies showing that exposure to noise induces hippocampal-dependent memory deficits (6-8). We have previously reported that the noise exposed rats had learning impairment associated with smaller hippocampal formation than controls (9). In the literature there are reports indicating that noise stress induces alterations in the hippocampus structure, but no previous stereological study was found, examining the effects of noise on the hippocampus. The present study aimed to investigate effects of chronic noise exposure on the volumes of the layers in hippocampal subregions, where the respective cell bodies or its processes were located. In addition, a group of rats being exposed to noise and subsequently withdrawn from the noise pollution, in order to detect possible signs of structural reorganization, included in this study. A stereological technique, Cavalieri principle (10), used to estimate the volumes of the different layers of each component of hippocampal formation.

## Materials and Methods

### *Animals*

Two-month male Wistar rats (from animal house of Isfahan medical school, Iran) weighing 200 – 220 g were used in this study. They were housed in plastic breeding cages in

a temperature-controlled room ( $23 \pm 2$  °C) on a 12 hrs light/ dark cycle (light on at 07.00–19.00 o'clock) with free access to rat chow (Khorak-Dam Pars, Iran) and tap water. Animal care and handling was performed in accordance with the rules approved by the local research council at Isfahan Medical School of Iran. Each group was formed by randomly pooling ten animals, which were treated as follows:

**Control group:** Rats were kept in the animal room, without being transferred to the noise room. They were maintained under standard laboratory conditions until the day of sacrifice (150th day).

**Noise-exposed group:** Rats were exposed to 40 dB unmodulated sinusoidal noise with a frequency of 1100 Hz for 20 mins, three times per day (10 am, 1 pm and 4 pm) for 90 days. The noise was produced by using a function generator and was amplified.

**Recovery group:** Rats were exposed to noise for 90 days as previously described, and allowed to survive without further treatment until the day of sacrifice (180th day).

### *Histological procedure*

Rats were deeply anesthetized with urethan (Merck -Germany) and transcardially perfused with a phosphate-buffered solution (pH=7.2, M=0.12) of 4% formaldehyde. The brain was removed and placed in the same fixative for 2 hrs. Each brain was labelled and cerebellum and olfactory bulb were removed. The cerebral hemispheres were separated by a longitudinal cut in the midsagittal plane. Each hemisphere was then placed in a chilled slicing box and 6 mm slices were cut from the frontal pole and the posterior part, containing hippocampus, was taken. The right hippocampal formations were selected and postfixed in the fresh fixative for 5 days. They were then dehydrated in ethanol, cleared in xylene, and embedded in paraffin and serially sectioned in 25  $\mu$ m at room temperature. Using systematic uniformly random sampling, starting at random position, every 20th section (with an interval of 500  $\mu$ m) was used for analysis.

The sections were mounted on gelatinized slides, deparaffinized, hydrated, and stained with hematoxylin and eosin.

### Estimation of tissue shrinkage

Shrinkage influences all stereological size estimator including volume. Measurements were made, separately for each group, to quantify shrinkage caused by fixation and histological procedures.

A section was obtained on a vibratome (Bio-Rad Polaron H1200, UK) from the surface facing the site of transection, the remaining block were then embedded in paraffin. The first section obtained from each block, immediately adjacent to the vibratome section, was taken. The areas of each section was estimated with the principle of Cavalieri and point counting. The areal shrinkage was then calculated from the ratio of the areas of the section obtained before and after embedding.

### Estimation of volume

The volumes of the different regions of the hippocampal formation were estimated on the basis of the Cavalieri principle (10).

Using a developed special projection microscope (Olympus BHS, Japan), the image of the section was projected on a test system placed on a table at final magnification of  $\times 96$ . The cross-sectional areas of the constituent layers of the different regions of the hippocampal formation were estimated by point counting. The number of points hitting hippocampal layers,  $\Sigma P$ , was multiplied with the area associated with each point,  $a(P)$ , to obtain an unbiased estimate of sectional area of each profile. The sum of sectional areas of the subdivisions was used to estimate reference volume,  $V(ref)$ , from the following relationship, where  $t$  represents the distance between sections:

$$V(ref) = t \cdot \Sigma P \cdot a(p)$$

Discrimination between the different subdivisions of the hippocampal formation (Figure 1) was made according to cell morphology (11).

### Statistical analysis

The Coefficient of Error (CE) of the individual estimates was calculated according to Gundersen and Jensen (12). The Coefficient of Variation (CV) was determined as described by West and Gundersen (13). Analysis of

variance and post hoc Bonferroni's multiple comparison test were used to ascertain differences among groups. Differences were considered to be significant if  $P < 0.05$ .

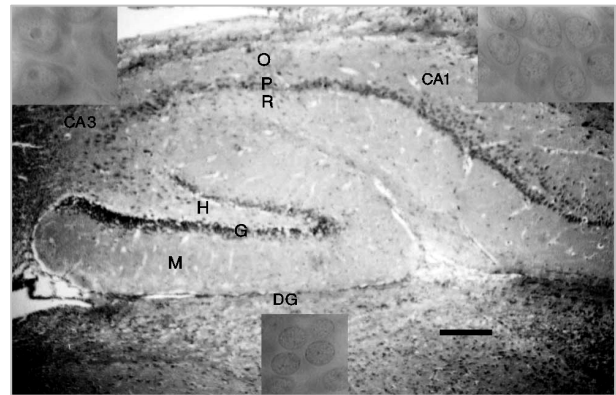


Figure 1. Light micrograph of 25- $\mu$ m thick section of the hippocampal formation. CA1 and CA3 are fields of Cornu Ammonis; DG, dentate gyrus; M; Molecular layer; G, Granular layer; H, Hilus, O, Oriens layer; P, Pyramidal layer; R, Radiatum layer. Hematoxylin stain, Scale bar, 400  $\mu$ m. Higher magnification ( $\times 1730$ ) of dentate granule, CA1 and CA3 pyramidal cells shown in boxes (granule cells in the lower box, CA1 and CA3 in the right and left upper boxes, respectively).

### Results

No areal shrinkage correction was used in the study because no difference in shrinkage was found between groups.

The volumes of the different layers of the hippocampal formation are shown in Table 1. Statistical analysis revealed a significant effect of noise exposure on the volume of the layers of the hippocampal formation, except CA1 hippocampal field. Comparison between groups revealed that noise-exposed rats had lower volumes than control and recovery animals in the molecular layer ( $P < 0.05$ ), Granular layer ( $P < 0.05$ ) and hilus ( $P < 0.05$ ) of dentate gyrus. The noised-exposed animals had lower volumes than control and recovery animals in the oriens, pyramidal and radiatum layers of CA3 hippocampal field ( $P < 0.05$ ). This study also showed that the volume of the whole hippocampal formation was significantly larger in control and recovery group than noised- exposed rats (Table 1). Moreover, we found that the rats in control group and those animals allowed to recover from the noise stress had no significant difference in the volume of hippocampal layers.

Table 1. The volumes of the layers of the hippocampal formation (mm<sup>3</sup>) in noise-exposed, recovery and control rats. Values are expressed as means (\*CV).

Hippocampal Subregions	Control		Noise-exposed		Recovery	
	Volume mean (CV)	**CE	Volume mean (CV)	CE	Volume mean (CV)	CE
<b>Dentate gyrus</b>						
Molecular layer	4.74 (0.10)	0.04	4.15 (0.06)	0.03	4.68 (0.10)	0.04
Granular layer	1.69 (0.08)	0.03	1.53 (0.12)	0.06	1.71 (0.08)	0.03
Hilus	1.75 (0.12)	0.05	1.52 (0.13)	0.06	1.72 (0.11)	0.05
<b>Hippocampal CA3 field</b>						
Stratum Oriens	2.5 (0.12)	0.06	2.2 (0.10)	0.05	2.47 (0.11)	0.05
Stratum Pyramidale	1.71 (0.10)	0.05	1.46 (0.13)	0.06	1.68 (0.10)	0.06
Stratum Radiatum + lacunosum molecular	5.95 (0.03)	0.01	5.54 (0.05)	0.03	5.88 (0.04)	0.02
<b>Hippocampal CA1 field</b>						
Stratum Oriens	2.65 (0.06)	0.03	2.55 (0.09)	0.05	2.58 (0.09)	0.05
Stratum Pyramidale	1.65 (0.12)	0.06	1.52 (0.16)	0.07	1.58 (0.15)	0.08
Stratum Radiatum + lacunosum molecular	5.07 (0.04)	0.02	4.9 (0.06)	0.03	5.05 (0.04)	0.02
<b>Hippocampal formation</b>	27.3 (0.03)	0.01	25.4 (0.04)	0.02	27.1 (0.03)	0.02

\*CV, Coefficient of Variation =SD/Mean

\*\*CE, Coefficient of Error

## Discussion

Our results were obtained using unbiased stereological methods. They showed that the prolonged exposure to noise induced marked reduction in the volume of the all layers of dentate gyrus and CA3 hippocampal field. In addition, we found that rats who were allowed to recover from noise displayed remarkable volumetric reorganization of altered layers and throughout the entire hippocampal formation. We nevertheless observed significant volumetric reduction in the layers of the CA1 hippocampal field.

These explanations can be given for the findings of present research: numerous studies have revealed that noise exposure induced an increase in glucocorticoids level (1, 14, 15). It have been previously reported that the length and the dose of noise exposure used in this experiment could increase cortisol in adult rat (16), although we did not measure cortisol level in this study. The hippocampal neurons are known to contain the highest concentration of corticosteroid receptors of all cerebral neurons (5).

Hippocampal neurons are therefore particularly vulnerable to prolonged stress conditions (17-19). Several studies

demonstrated that prolonged exposure to elevated glucocorticoid concentrations lowers the threshold for hippocampal neuronal degeneration (19, 20) and that glucocorticoids have a neurotoxic effect on hippocampal neurons (21). Our estimates demonstrate that among all of neurons in the hippocampal formation, CA1 pyramids are the least vulnerable to noise stress.

Although the exact mechanisms responsible for the noise-induced atrophy of hippocampus are still poorly understood. It is, at least partially, due to decreasing in the availability of neurotrophins (22). It is well established that a reduction in neurotrophin levels can lead to neuronal atrophy (23).

It has also been shown that noise stress induced an increase in the production of reactive oxygen species (ROS) in the hippocampal neurons (7).

Several lines of evidence could help to explain why granule neurons of dentate gyrus (DG) and pyramidal neurons of CA3 are more vulnerable to stress. First, granule cell of dentate gyrus express a high density of both type I and type II corticosteroid receptors (24, 25) and these neurons project heavily to CA3 pyramidal cells via mossy fibers (26, 27). The granule cells of the dentate gyrus have

been shown to be highly dependent on corticosterone (28). Thus, it seems that excess corticosterone exert a stimulatory effect on these neurons which could, in turn, hyperactive and promote damage to the pyramidal cells of the CA3 region. Bearing in mind the trophic role played by neuronal afferents in promoting the survival and maintenance of their target (29). It is also interesting to note that we observed no significant decrease following noise stress on the volume of CA1 pyramidal field, a region to which the dentate gyrus has no known projection in the rostral hippocampus of rat (30).

Although neuronal reorganization may occur even in the presence of a noxious stimulus (31), usually the structural reorganization becomes more evident after normal conditions have been restored (32). We found that the noise-induced volumetric alterations of hippocampus were restored after one month of recovery.

For the interpretation of the mechanisms involved in the structural reorganization which occurs after recovery, following factors must be considered: it seems that the reported augmentation of neurotrophin synthesis found after the termination of stress (22) may underlie the neuritic regrowth observed in animals allowed to recover. Another factor, particularly those relating to granule cell neurites, is neurogenesis which persists in the adult dentate gyrus (33). Indeed, a strong body of evidence demonstrates that the acquisition of new granule cells is inversely correlated with corticosteroid levels (34). Accordingly, the number of granule cells generated during the period of recovery from exposure to noise would be greater than that found in noise-exposed animals.

While, in our search, no study using unbiased stereological techniques was found, there are studies examining the effects of noise exposure on hippocampus structure.

Saljo *et al* reported that exposure to impulse noise of 198 dB or 202 dB induced neuronal death, and apoptosis in granule neurons of the dentate gyrus and the CA1-3 pyramidal neurons in the hippocampus (35).

Manikandan *et al* reported decreases in the dendritic count in the CA1, CA3 regions of rat hippocampus after noise-stress exposure, 100 dB/4h per day for 30 days (7).

In comparing our study to Manikandan *et al*'s research, it must be considered that they assessed the dendritic count in the CA1 and CA3 neurons of hippocampus following noise stress by a quantitative Golgi study, while we examined the volume of layers in all hippocampal subdivisions, i.e., CA1, CA3 and dentate gyrus, using unbiased stereological technique. Also there is a difference in the technical approaches used for. It should be noted that they employed an extreme dose of noise (100 dB/4h per day) to examine the likelihood of noise stress-induced changes at the hippocampus; whereas in the present study a mild dose of noise (40 dB/1h per day) was used for this purpose. Finally, we also investigated the volumetric changes in the hippocampus following withdrawal from the noise stress.

The current study is in agreement with previous reports showed; dentate gyrus and CA3 field were affected following chronic noise exposure. Contrary to above studies that reported the morphological changes in CA1 hippocampal field of noise exposed rats, our results indicated that there is no alteration in this region following noise stress. There are important factors, such as dose and length of noise exposure, which ought to be considered to explain controversy concerning noise-induced alterations in CA1 hippocampal field.

An experimental study has shown that the exposure to the noise during pregnancy caused growth retardation, decreased neurogenesis in the hippocampus, and impaired spatial learning ability in rat pups. The exposure to music during pregnancy, on the other hand, caused increased neurogenesis in the hippocampus and enhanced spatial learning ability in pups (36).

## Conclusion

The results of the present study indicate that the exposure to noise stress could induce significant volumetric reductions in all layers of the dentate gyrus and of the CA3

hippocampal field. These findings most likely reflect profound alterations in the neurites of the hippocampal cells, a hypothesis that is supported by data reported in a quantitative Golgi study (7). However, it is unclear whether the decline in volumes of hippocampal layers is due to neuronal loss or neuronal atrophy. The present study provides additional information regarding recovery capabilities as it shows that animals in which the normal condition was restored have a significantly higher volume in the hippocampus than rats kept under noise stress. The present study has shown the importance of the environmental conditions for brain development. While, effect of various stressors

agents on the structure of hippocampus had been studied extensively, effects of noise on the central nervous system are largely unexplored. There is little information on critical threshold values and time factors and deserves further investigation.

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