Original Article

Effects of Stress-induced Depression and Antidepressant Drugs on CA3 Region of Hippocampus in Adult Albino Rats

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Abstract

Introduction: Hippocampus is the most extensively studied part of the brain in recent years. The connections of the hippocampus are extensive and very complicated, covering lots of functions in the body. The aim of the study is to find out the effect of stress-induced depression in the CA3 region of the hippocampus and also to see the effect of antidepressants for the reversal of changes in the similar area. **Material and Methods:** The study conducted on adult albino rats weighing 200–250 g. The study involved 50 albino rats and divided into five groups. The first group was control of ten rats and received water and food ad libitum, and the second group was experimental having two subtypes E1 and E2 receiving 4 weeks and 7 weeks immobilization with ten rats each, respectively. The third is treatment group which has two subtypes T1 and T2 for 4- and 7-week treatment by fluoxetine drug (1 mg/kg body weight orally) with ten rats each, respectively. The animals sacrificed after the experiment, perfused with 10% formaldehyde, brains dissected, and tissue blocks processed for paraffin embedding. Observations were made on 5-μ thick H and E-stained sections. Estimation of neuronal density of CA3 regions performed using Motic Images Plus 2.0 software (Hong Kong China). **Results:** Neuronal density was markedly reduced (98.7 ± 6.1 cells/cubic mm) in acute depression and 66.3 ± 4.8 cells/cubic mm in chronic depression group, respectively, as compared to control (124.5 ± 7.2 cells/cubic mm). The density improved after giving drug treatment. Neuronal density was 111.2 ± 9.6 cells/cubic mm and 92.3 ± 5.5 cells/cubic mm in 4- and 7-weeks treatment, respectively. **Discussion and Conclusion:** These results suggested that neurodegenerative effects of depression on the hippocampus, which is reversed by giving antidepressant drug.

Keywords: *Albino rats, CA3, depression, hippocampus, loss of neurons, neurodegeneration*

Introduction

The hippocampus is a small sea horse-shaped organ located within the brain's medial temporal lobe. It is an important part of the limbic system. It regulates emotions, memory, and spatial navigation. Studies showed that damage to the hippocampus might lead to confusion, loss of memory, and navigation.[1-3] In 1960s, neuroscientist John O' Keefe and Psychology Professor Lynn Nadel published a book titled "The Hippocampus as a Cognitive map," which supports the role of the hippocampus in the formation of cognitive maps.

The parts of hippocampus are the dentate gyrus, subiculum, and entorhinal cortex. Further, the areas of dentate gyrus which is shaped like "cornu ammonis" are classified into four regions named as CA1, CA2, CA3, and CA4. Among these areas, CA3 is known for its role in memory processing, susceptibility to seizures, and neurodegeneration. CA3 is connected to various areas of the hippocampus giving a very dense picture of its neurons. CA3 region also receives mossy fibers from the entorhinal cortex.[4] Recent studies suggest its role in processing afferent activity from the dentate gyrus.[5] Memories are formed by the hippocampus.[6] Furthermore, long-term memories are stored in the hippocampus.[7] Depression can affect person's thoughts, behavior, feelings, and physical well-being.[8] Magnetic resonance imaging scans of patients with depression had smaller hippocampal volume in comparison to normal individuals[9] and increased numbers of hyperintensive lesions.[10] There may be a link between depression and neurogenesis of the hippocampus.[11] Drugs may increase serotonin levels in the brain, stimulating neurogenesis and thus increasing the total mass of the hippocampus.[12] Brain-derived

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neurotrophic factor (BDNF) is drastically reduced (more than three-fold) in depressed individuals as compared to the normal. Antidepressant treatment increases the blood level of BDNF. It is the mechanism of action of antidepressants.[13]

Aims and objectives

The aim of the study is to observe the effects of stress-induced depression on the CA3 region of the hippocampus, and also to assess the role of antidepressant drugs in the reversal of these effects.

The objectives of the study are as follows:

1. To observe the effect of stress-induced depression in the CA3 region of the hippocampus in adult albino rat
2. To observe the effect of antidepressant drugs for reversal of these changes in CA3 region of the hippocampus in adult albino rat
3. To note down behavioral changes after the experiment in adult albino rat.

Material and Methods

Animal model

Type of the study: Experimental

limitations of the study

1. The use of light microscopy is not very accurate
2. Neuronal density calculation is software based
3. Sample size is medium.

The study was undertaken after ethical clearance from the Institutional Animal Ethical Committee. Fifty adult albino rats of either sex weighing 250–300 g were maintained at 21°C and given access to food and water *ad libitum*. Animals were randomly assigned to five equal groups:

1. Control Group (C)
2. Experimental Group (E)
   i. Experiment 4 weeks (E1)
   ii. Experiment 7 weeks (E2).
3. Treatment Group (T)
   i. Treatment 4 weeks (T1)
   ii. Treatment 7 weeks (T2).

Instrument of research

The dimensions of the cage were as follows: measuring 9"× 2.75", made of steel. It was designed to suit the experiment model as described and depicted previously.[14] It was framed to provide adequate immobilization without giving any physical harm to the animal. It was also useful for drug delivery.

Experimental procedure

Before the experiment, animals were handled manually for 1 week to remove handling stress. The Control group (C) received food and water. The rat immobilizer immobilized the Experimental group (E) three times (30 min per session) a day for 4 and 7 weeks, respectively. The experiment was conducted between 10 and 11 am. The treatment group (T) received fluoxetine 1 mg/kg body weight once a day for 4 and 7 weeks after being immobilized for 4 and 7 weeks, respectively. Animals were anesthetized by diethyl ether and perfused intracardially with 10% formaldehyde. Brains were removed, and the hippocampus was dissected. Tissues were processed with alcohol, xylene, and paraffin embedding was done. Blocks were made, and 5-μ thin sections of identical regions were taken of different groups. Observations were made under × 40 resolution by compound microscope after H and E staining. CA3 region was identified in the hilum of dentate gyrus, and neuronal density was compared in different groups using Motic 2.0 software (Hong Kong China).

Results

Behavioral

General activity of the rat was markedly reduced. Moreover, the struggle duration was also affected following prolonged immobilization for 7 weeks. Female rats were more active as compared to male rats following immobilization procedure.

Microscopic

CA3 region was identified in the hilum of the dentate gyrus. The observations were made by a compound microscope at ×40 for CA3 region. The neuronal density was markedly reduced in whole of dentate gyrus in experimental and increased in treatment group as shown in Figure 1. Neuronal density/unit area was calculated and compared as shown in Table 1.

Discussion

This study showed significant decrease in neuronal density in the selected area of the hippocampus. The decrease was more after 7 weeks of immobilization in comparison to 4 weeks. The antidepressant drug showed significant neurogenesis but never reached to normal levels. The effect was more pronounced with 4-week treatment. Treatment with 7 weeks does not show any increase in density. Results of different research conducted over the past two decades in rats, monkeys, and humans indicate that the hippocampus is particularly susceptible to neuronal degeneration during normal aging.[15] Neuronal loss might be responsible for deficits in hippocampal-dependent learning and memory associated with advanced age.[16] Hippocampal neuron loss is widely viewed as a hallmark of normal aging. Many studies have shown that hippocampal volume decreases after depression,[17] which can be reflected in the fall of neuronal densities as shown by the present study. By taking advantage of improved methods for quantifying neuron number, the present study assessed the changes in the neuronal density due to depression and treatment by antidepressants. Chronic stress has been shown to lead degenerative changes affecting the apical dendrites of

Table 1: Neuronal density of different groups

Group	Control	Acute depression	Chronic depression	4-week treatment	7-week treatment
Neuronal density	124.5±7.2	98.7±6.1	63.3±4.8	111.2±9.6	92.3±5.5

Comparison of neuronal density in the CA3 region of hippocampus in different groups (cells/mm2 ±S.E). SE: Standard error

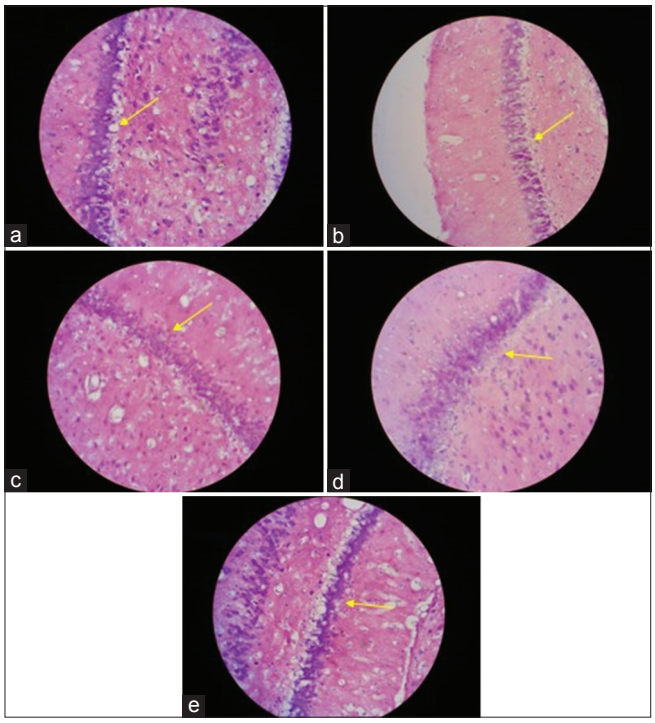

Figure 1: Sample photomicrographs CA3 region of control group (a) 4-week immobilization (b), 7 week immobilization (c), after 4 week treatment (d), and after 7-week treatment (e) x400, H and E stain

pyramidal neurons in field CA3 in rats, tree shrews, and monkeys.[18] Prolonged immobilization stress also leads to decreases in the number of neurons in hippocampal field CA3 in castrated rats.[19] As in the present study, chronic stress leads to highly significant fall in neuronal density. Fall in neuronal density and behavior changes find support from the study,[20] suggesting that this neuronal loss may be responsible for memory impairment. However, studies reported by other investigators[21] do not support these results, and the question remains unresolved.[22] According to one study, there are no convincing data demonstrating that stress has a neurotoxic action on the nervous system.[23]

Conclusion

Further research is needed to know the molecular mechanism and factors affecting neurogenesis/degeneration. Neurodegeneration and neurogenesis are known phenomenon which finds support from the previous researches. The current study supports this view and finds evidence of neurogenesis and neurodegeneration.

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Conflicts of interest

There are no conflicts of interest.

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