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

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Abstract

The present study was conducted using 15 albino rats (150-200 gm) and was divided equally into one control and two experimental groups. First group was control, second group received chronic depression (7 weeks) by immobilization method using rat immobilizer and third group received standard 4 week treatment (using Flouxetine 1mg/kg body wt. orally) following chronic depression. The animals were sacrificed after the experiment, perfused with 10% formaldehyde, brains were dissected and tissue blocks were processed for paraffin embedding. Observations were made between control, experimental and among experimental groups with equal duration of survival on 10 micron thick H & E stained sections. Estimation of neuronal density of CA3 regions was performed using Motic images plus 2.0 Software. Neuronal density was markedly reduced (93.4 cells/cubic mm) after chronic depression, as compared to control (144.5 cells/cubic mm) and after standard treatment it was enhanced to 159.3 cells /cubic mm. Statistical analysis of these results suggested that effect of long-term stress-induced depression on hippocampus may significantly be reversed by the pharmacological intervention with known antidepressants.

Key words: Hippocampus, Chronic, Stress, Depression, Antidepressants, Neurogenesis

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Introduction

Throughout human history, it has been apparent that few medical maladies are as devastating in their effects as major depression. Buried within the depths of the cerebrum are several large aggregates of limbic structures and nuclei which are preeminent in the control and mediation of memory, emotion, learning, dreaming, attention, and arousal, and the perception and expression of emotional, motivational, sexual, and social behavior including the formation of loving attachments. Indeed, the limbic system not only controls the capacity to experience love and sorrow, but it governs and monitors internal homeostasis and basic needs such as hunger and thirst [1,2,3] including even the cravings for pleasure-inducing drugs [4].

The hippocampus is most usually associated with learning and memory encoding, e.g. long term storage and retrieval of newly learned information [5] particularly the anterior regions. Hence, if the hippocampus has been damaged the ability to convert short term memories into long term memories (i.e. anterograde amnesia), becomes significantly impaired in humans [6] as well as primates [7]. In humans, memory for words, passages, conversations, and written material is also significantly impacted, particularly with left hippocampal destruction [5].

And since the 1950s, with the advent of the first generation of antidepressants, it has been apparent that depression is a biological disorder. This has generated tremendous intellectual challenge of how to understand the material, reductive bases of a disease of malignant sadness. Both the tragic components and the intellectual challenge of depression have deepened in the last decade with a series of high-visibility reports that indicate prolonged, ma-
Major depression is associated with atrophy within the central nervous system. Hippocampus is the area of interest which is involved and shows changes in depression. Newborn neurons are detected in the dentate gyrus of the hippocampus [8] and olfactory bulb [9] of adult mammals, including monkeys [10]. Hippocampal cells greatly alter their activity in response to certain spatial correlates, particularly as an animal moves about in its environment [11].

**Material and methods**

After approval from the Institutional Animal Ethics Committee, present study was carried out on 15 adult albino rats weighing between 150-200 gms. The animals were provided with standard pellet laboratory diet (Lipton India Limited) and water ad-libitum. They were housed under identical diurnal conditions and

Group 1- Control (Ctrl)
Group 2- Chronic Depression
[7 week immobilization] (ChDp)
Group 3- Treatment group (ChTt)

The cage was an indigenous one which was designed to suit the experiment as described and depicted previously [12]. It was framed to provide adequate immobilization without giving any physical harm to the animal. It was small, made up of steel wire, measuring 9”x 2.75”, of light weight and easy to carry, with no maintenance cost.

**Method**

15 Adult Albino rats weighing 150-200gm. Experimental animals were grouped and housed under controlled conditions in rat immobilizer for 30 minute per turn, 3 times a day cycle for 4 weeks. Experimental rats were handled daily for 7 days before experiments to minimize the stress of handling.

All experiments were performed between 10 and 11 a.m. to minimize any influence of the circadian rhythm. After repeated immobilization gross behavioral changes were observed, in terms of alteration in food intake and struggling period. Fluoxetine was given to the treatment group in dose of 1mg/kg body weight, orally for 4 weeks. The animals were sacrificed after the experiment, perfused with 10% formaldehyde, brains were dissected and tissue blocks were processed for paraffin embedding. Observations were made on 10 micron thick H & E stained sections. Estimation of neuronal density of CA3 regions was performed using Motic images plus 2.0 software. A number of Observations of the hippocampus were taken. 5 x objective was used to take the topography whereas 10x, and 40x objective were used to take representative pictures from CA3 region of the hippocampus. Neuron was identified by the presence of Nissl substance in the cell body, prominent nucleus and nucleolus and these features were better appreciated at 40x objective so all readings were taken from different areas at 40x objective.

The area of the pyramidal cell layer of CA3 was estimated using Motic images plus 2.0 Software. Different groups were compared to check the alteration in the densities of neurons per unit area due to depression and depression followed by drugs. Students’ T-test was applied to know the significance observations.

**Observations**

**Behavioral**

The rats became less active when released from the cage. The rat tried to bite the cage for a longer duration in the beginning but with repeated immobilization the rat gave it up early. The rat is adapted to the cage as they used to go inside on their own in latter days. Total general activity was reduced markedly in depressed rats.

**Microscopic**

The CA3 region is located outside the hilum of dentate gyrus and qualitative observation revealed reduction of neuronal number in depressed rats as compared to control (Fig and treated group. Quantitative estimate of neuronal density per unit area as compared to control group (Ctrl) the chronic depression (Ch.Dp) showed significant fall in neuronal density (Table 1).

4-weeks of treatment with antidepressants after induction of depression revealed improvement of neuronal density in the treatment group (Ch.Tt) towards control (Table -1) increase in neuronal density was seen. From all the observations it was found that depression affects HC in a way it caused fall in neuronal density which can be somehow reversed by the use of antidepressants.
Stress-induced chronic depression and antidepressant drugs on CA3

Figure 1. Sample photomicrographs from the CA3 region of hippocampus of control rat (A), after chronic immobilization (B), and after post-immobilization treatment (C & D). x400, H&E stain

Table 1. Showing neuronal density of different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Ctrl</th>
<th>Ch.Dp</th>
<th>Ch.Tt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal Density</td>
<td>144.5±9.0</td>
<td>93.4±3.8</td>
<td>159±13.3</td>
</tr>
</tbody>
</table>

Discussion

The present study was undertaken to know the long term effects of depression over the CA3 region of hippocampus and its response to standard treatment by Flouxetine. At first glance, one exciting implication of this study is the suggestion that the hippocampal volume loss in prolonged depression arises from loss of neurons, and that antidepressant treatment normalizes the former by preventing the latter. Prolonged stress causes reduction in neuropil and volume of hippocampus [13]. Chronic stress has been shown to lead to degenerative changes affecting the apical dendrites of pyramidal neurons in field CA3 in rats, tree shrews, and monkeys [14]. Prolonged immobilization stress also leads to decreases in the number of neurons in hippocampal field CA3 in castrated rats [15]. As in present study chronic stress leads to highly significant fall in neuronal density. Fall in neuronal density & behavior changes finds support from [16, 17] suggesting that this neuronal loss may be responsible for memory impairment. However, studies reported by other investigators [18] do not support these results, and the question remains unresolved [19]. According to one study there is no convincing data demonstrating that stress has a neurotoxic action on the nervous system [19]. Neurogenesis in the adult hippocampus is restricted to the sub granular zone, and newborn neurons appear to migrate only as far as the nearby dentate granule layer. For hippocampal neuroanatomy neophytes, this means that the revolution in adult neurogenesis occurs entirely in a fairly small sub-section of the hippocampus; there has been some debate over just how much adult neurogenesis occurs and how much turnover there is in adult dentate gyrus neurons [21]. Thus, if changes in overall hippocampal volume are
secondary to changes in cell proliferation, one would predict that (i) psychosocial stress would lead to a marked reduction in the volume of the dentate granule layer, and (ii) this would be prevented by Fluoxetine. It is not immediately obvious how much these findings generalize to other antidepressants. The vast majority of antidepressants in clinical use work by increasing the synaptic availability of monoamine neurotransmitters. Fluoxetine increases serotonin and to nicely commensurate with the involvement of serotonin, there is some evidence that increased serotonin availability can stimulate cell proliferation in the hippocampus [22, 23]. To conclude further research is needed to know the molecular mechanism and factors affecting neurogenesis/degeneration.

References


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