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Effects of stress-induced acute depression and antidepressant drugs on CA3 region of hippocampus of albino rats

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Abstract

Depressive disorders are amongst the most common life threatening disorders and despite of extensive research work its etiology and mode of action of antidepressant drugs remains elusive. The present study was conducted on 15 adult albino rats (200-250gm) divided into control and experimental groups. First group was control, second group received acute depression (4 weeks) by immobilization method using rat immobilizer and third group received standard 4 week treatment (using Flouxetine 1mg/kg body wt. orally) following acute depression. At the end of the experiment animals were sacrificed and 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. Neuronal density was markedly reduced (100.3 cells/cubic mm) after acute depression, as compared to control (144.5 cells/cubic mm) and after standard treatment it improved to 121.1 cells /cubic mm. These results suggested that effect of short-term stress-induced depression on hippocampus is partly reversed by the pharmacological intervention of known antidepressants and warrants longer term study.

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

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Introduction

Depressive disorders are among the most common and life-threatening illnesses and represent a significant public health problem [1]. Despite extensive preclinical and clinical investigations, the exact neurobiological processes leading to depression and the mechanisms responsible for the therapeutic effects of antidepressant drugs are not completely understood. And since the 1950s with the advent of the first generation of antidepressants, it has been apparent that depression is a biological disorder. In depression atrophy is centered in a brain region called the hippocampus (figure-1A), the structure which plays a critical role in learning and memory, and the magnitude of its volumetric loss of helps explain cognitive deficits that accompany depression. The HC is a part of limbic system and hippocampal formation includes the DG, CA1, CA2, CA3, CA4, subiculum, and EC.

Usually neurogenesis is a prenatal phenomenon and it was believed that there is no postnatal neurogenesis. This belief is based on the absence of neurons with mitotic figures in the brains of adult birds and mammals, and also absence of signs of regenerative neuronal proliferation following brain lesions. In rats, approx 80% granule cells are produced postnatally, whereas in the rhesus monkey, most are produced prenatally [2]. Using thymidine H^3 Altman, [3, 4] demonstrated that the granule layer of the hippocampus of adult rats and cats show mitotic activity. Previous works have shown neuronal loss [5], loss of pre-existent hippocampal neurons [6] as well as that of frontal cortex of depressed patients [7]. Glucocorticoids (GCC) with the human version being cortisol have also been implicated in and retraction of dendritic processes, inhibition of neurogenesis and neurotoxicity [6]. Therefore, present study was primarily aimed to note the changes in the neuronal density of CA3 area of hippocampus both after depression and after treatment.

Material and Methods

After approval from the Institutional Animal Ethics Committee, present study was carried out on 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 temperature. The animals were weighed, marked and divided into three groups of five rats each as under:

Group 1- Control (received neither immobilization nor treatment).
Group 2- Acute Depression (4 week immobilization).
Group 3- Acute group treatment (4 week Treatment after immobilization)

The cage was an indigenous one which was designed to suit the experiment. 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 (Fig.1A).

Method

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. Flouxetine 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. X5 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 o 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 (Fig. 1B, 1C, 1D).

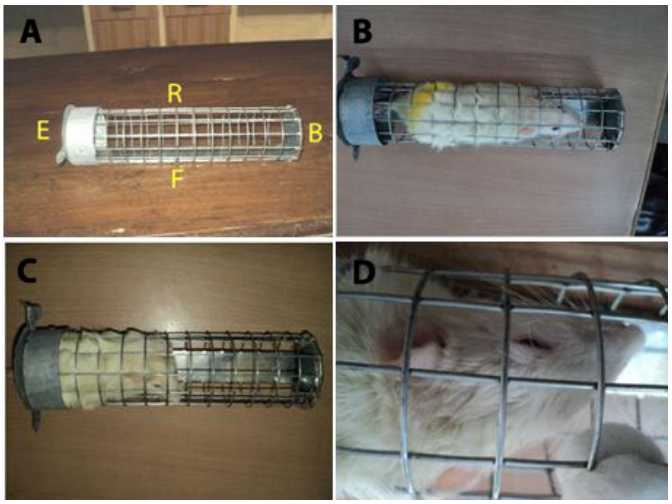

Figure 1*. Immobilization cage used in the experiment (A), rat under experiment just after entry (B), trying to push the door of cage with its back (C) and depressed (D).*

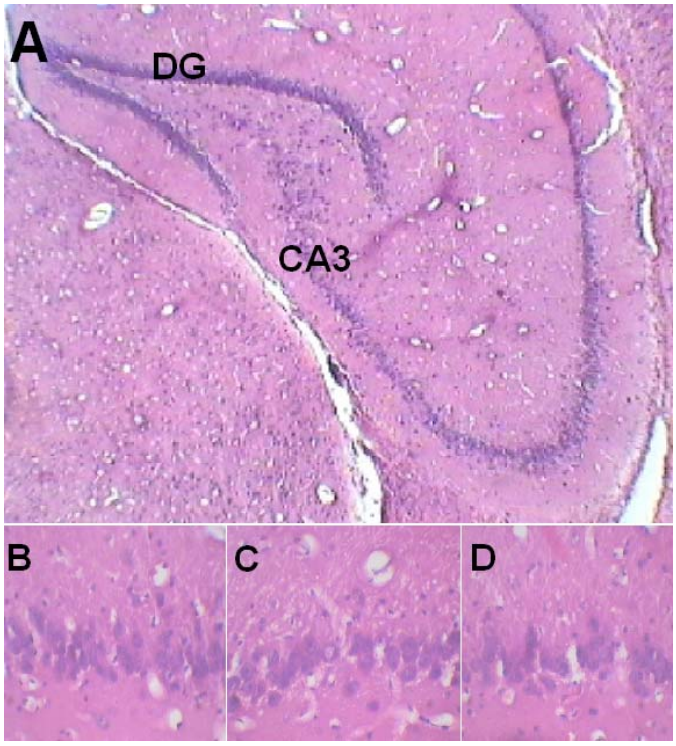

Figure 2*. Photomicrogarhs showing different parts of hippocampus including dentate gyrus (DG) and CA3 region (A) at low magnification, and neuronal samples from CA3 region of control (B), depressed (C) and treated group (D) at higher magnification. H &E stain.*

Microscopic

The CA3 region is located outside the hilum of dentate gyrus (Fig 2A) and qualitative observation revealed reduction of neuronal number in depressed rats (Fig 2C) as

compared to control (Fig 2B) and treated group (Fig 2D). Quantitative estimate of neuronal density per unit area as compared to control group (Ctrl) the acute depression (Ac.Dp) showed significant fall in neuronal density (Table-1 and Fig 3A). 4-weeks of treatment with antidepressants after induction of depression revealed improvement of neuronal density in the treatment group (Ac.Tt) towards control (Table -1 and Fig 3B) 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.

Table1. *Showing Neuronal density in control and experimental group*

Region	**Comparison of neuronal density of different groups** (cells/mm^2±S.D.)		
	Ctrl	Ac.Dp	Ac.Tt
CA3	144.5±22.1	100.3±11.9	121.1±23.7

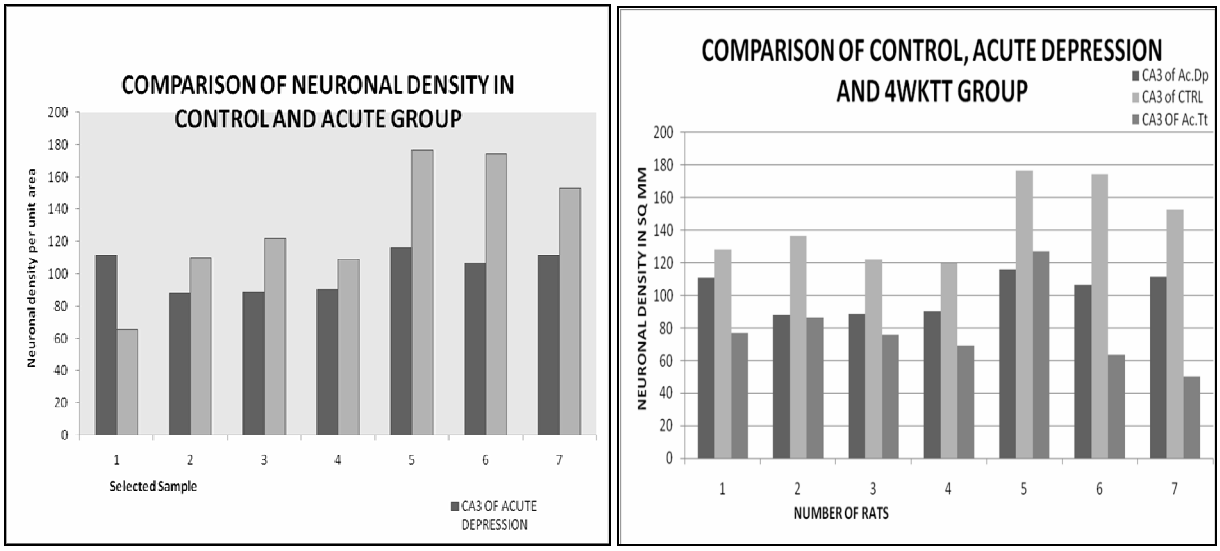

Figure 3 *Depicts effect of induction of depression on the neuronal density of CA3 region (A) and that of intervention by antidepressant drug after induction of depression (B).*

Discussion

The present study was undertaken to elucidate the effects of acute depression and role of antidepressant drugs over the neuronal density of CA3 area of the hippocampus. The results of the study showed reduced motor activity, reduced appetite, and increased sleep of the depressed rats. Rats under stress have been shown to reveal anhedonia, and reduced locomotor activity [8] altered hormone levels [9] and abnormal circadian cycles [10]. One study has shown that hippocampal volume decreases after depression [11] which can be reflected in the fall of neuronal densities as shown by the present study. Based on this background it seems reasonable to suppose that neuronal loss might be responsible for the deficits in hippocampal-dependent learning and memory associated with advanced age [12]. Results of a substantial body of research conducted over the last two decades in rats, monkeys, and humans indicate that the hippocampus is particularly susceptible to neuronal degeneration during normal aging [13]. Fall in neuronal density and behavioral changes find support from the studies of many workers [14] suggesting that this neuronal loss may be responsible for memory impairment. Thus, fall in neuronal density in the present study after immobilization stress leading to acute depression appear to be interdependent. However, reports of some other investigators [15] are not in full agreement with the present findings [16] as well.

The first evidence of neurogenesis was reported in the cerebral cortex [17] and then came a series of evidences showing neurogenesis in different parts of the brain [3]. It is believed that neurogenesis predominantly occurs in the sub ventricular zone (SVZ) of the lateral ventricles and the sub granular zone (SGZ) of the dentate gyrus of the hippocampus [18]. We now know that 50 to 80 percent of the neural precursor cells in the SGZ can mature into neurons that migrate to the appropriate cell layer and form connections with other neurons [19]. In the present study the groups of depressed rats when treated with known

antidepressants showed significant improvement in the neuronal density which possibly reflected the effect of neurogenesis and therefore induction of neurogenesis is believed to be an important mechanism of action of many antidepressants [20]. It has also been shown that antidepressants protect against stress-induced decrease in neurogenesis with preservation of hippocampal volume during a social stress paradigm [21]. Results of the present study also suggest that, antidepressants might have similar neuroprotective effects in humans. Nevertheless, depression-related volume loss does appear to be cumulative, suggesting that immediate recognition and treatment of depressive episodes is important in order to prevent possible damage due to repeated episodes of depression [22]. Thus it was concluded that antidepressants do have important role in the treatment of acute depression but warranting further long term study.

References

1. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020; Global Burden of Disease Study. Lancet 1997; 349: 1498-1504.
2. Rakic P, Nowakowski RS. The time of origin of neurons in the hippocampal region of the rhesus monkey. J Comp Neurol 1981; 196: 99-128.
3. Altman J. "Auto radiographic investigation of cell proliferation in the brains of rats and cats". The Anatomical record 1963; 145: 573-591.
4. Altman J and Chorover SL: Auto radiographic investigation of the distribution and utilization of intraventricular injected Adenine-3h, uracil-3h and thymidine-3h in the brains of cats. J. Physiol.1963; 169:770-779.
5. Reagen L and McEwen B : Controversies surrounding glucocorticoid-mediated cell death in the hippocampus. J Chem Neuroanat.1997; 13:149–167.
6. Sapolsky R: Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. Exp Gerontol.1999; 34:721–729.
7. Rajkowaska G and Miguel, Hindalgo J, Wei J, Pittman S, Dilley G, Overhoiser J,Meltzer H , Stockmeier C: Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiat.1999; 45:1085–1094.
8. Grippo AJ, Beltz TG, Johnson AK: Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol Behav.2003;* 78: 703–710.
9. Katz R, Roth A, and Carroll J: Acute and chronic Stress effects on open field activity in the rat: implications for a model of depression. Neurosci. Biobehav. Rev.1981a; 5: 247-251.
10. Cahill A and Ehret F: α-methyl-p-tyrosine shifts circadian temperature rhythms. Am. J. Phvsiol.1982; 243: 218-221.
11. Watanabe Y, Gould E, McEwen B: Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res.1992c; 588:341-345.
12. McEwen BS, Swaab D, Hoffman M, Mirmiran M, Ravid R, van Leeuwen F : Re-examination of the glucocorticoids hypothesis of stress and aging. In: Prog. Brain Res.1992; 93:365-383.
13. Coleman PD and Flood DG: Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. Neurobiol. Aging.1987; 8:521-545.
14. Kim JJ and Diamond DM: The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci.2002; 3: 453-462.
15. Volmann-Honsdorf GK, Flugge G and Fuchs E: "Chronic psychosocial stress does not affect the number of pyramidal neurons in tree shrew hippocampus," Neurosci. Lett.1997; 233:121–124.
16. Bremner JD: "Hypotheses and controversies related to effects of stress on hippocampus: an argument for stress-induced damage of the hippocampus in patient with posttraumatic stress disorder." Hippocampus.2001; 11: 121-124.
17. Altman J: "Are new neurons formed in the brains of adult mammals?" Science.1962; 135: 1127–1128.
18. Inna K, Yael B, Eldad M, Daniel O: Mesenchymal Stem Cells Stimulate Endogenous Neurogenesis in the Subventricular zone of Adult Mice. Springer Science.2010; 10.1007/s12015-010-9190-x: 1-9.
19. Barlow C: Hippocampal neurogenesis: Can it be a Marker for New Antidepressants? Psychiatry.2007; 4: 18-20.
20. Watanabe Y, Gould E, Cameron H, Daniels D, McEwen B: Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. Hippocampus.1992a; 2:431– 435.
21. Czeh B, Michaelis T, Watnabe T, Frahm J, de Biurrun G, and van Kampen M, Bartolomucci A, Fuchs E: Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci USA.2001; 98: 12796-12801.
22. Yvette IS, Milan S, Mark AM, Mokhtar HG: Depression Duration But Not Age Predicts Hippocampal Volume Loss in Medically Healthy Women with Recurrent Major Depression the Journal of Neuroscience.1999; 19:5034-5043.

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