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Exposure to an enriched environment promotes dendritic remodelling in hippocampal neurons affected by endogenous depression

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Neuronal plasticity is enhanced in an enriched environment (EE) with more sensory and social interaction. In an animal model of endogenous depression, we have previously shown that EE has positive effects on spatial memory and hippocampus synaptic plasticity. However, nothing is known about how EE influences dendritic remodelling in hippocampal neurons affected by endogenous depression. In depressed rats, the impact of EE on hippocampus neuronal morphology was examined. Neonatal clomipramine exposure from postnatal days (PND) 8-21 days induced endogenous depression. The depressed-like rats were exposed to an enriched environment for two weeks in adulthood. Brains were then collected, stained with a modified Golgi-cox technique and, the hippocampal CA1 dendritic arborisation was evaluated using the Neurolucida software. Depression resulted in the atrophy of CA1 hippocampal neurons. The number of branching points and the overall number of dendritic intersections were reduced in depressed rats,. Exposure to an enriched environment significantly increased dendritic branching and the total number of dendritic intersections in hippocampal CA1 pyramidal neurons. The hippocampal pyramidal neuronal morphology of depressed rats improved after exposure to environmental enrichment. Neuronal plasticity and the development of novel therapeutic strategy will be improved by a greater understanding of how the environment affects neuronal morphology in depressed states.

Keywords: Environamental enrichment, Memory, Neuronal morphology, Psychiatric disorders

Depression is a mental disorder that has been related to increased mortality, morbidity, and a low quality of life. Approximately, 9% of persons experience depression at least once in their lifetime[1]. Anomalies in corticolimbic brain regions' neuronal plasticity are the root cause of depression[2].

The hippocampus is an important part of learning and memory, and previous research has demonstrated that early environmental factors have a significant impact on field CA1 hippocampal pyramidal neurons[3]. Additionally, increasing data points to a critical connection between spatial memory and CA1 hippocampal atroph[4,5]. Stress and depression have been linked to decreased hippocampal neurogenesis, dendritic spines, dendritic atrophy, and synaptic loss[6,7]. Previous neuroimaging research suggests that depressive patients may have a shrunken hippocampus[8,9]. Reduced spine number and dendritic complexity, significant hippocampus neuronal death, and atrophy in depressed people may be involved in the decreased hippocampal volume[2,8].

An enriched environment (EE) activates cellular and molecular pathways linked to neural plasticity, resulting in morphological alterations such neurogenesis, gliogenesis, dendritic arborization, and the development of new synapses[6,7]. Additionally, Schaffer collateral synapses in the hippocampus region exhibit improved LTP following exposure to EE[4]. Earlier, we demonstrated that exposure to an enriched environment ameliorated endogenous depression-induced spatial learning deficits and hippocampal LTP and volume[10]. Therefore, we investigated the impact of an enriched environment on morphological alterations linked to endogenous depression in the hippocampus CA1 pyramidal neurons.

Materials and Methods

All experiments followed the National Institute of Health Guidelines for the Care and Use of Mammals

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in Neuroscience and Behavioral Research (National Research Council (US) Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research., 2003), and experimental protocols were approved by the institutional animal ethics committee (AEC/51/316/N.P.). The number of animals utilised was kept to a minimum, and the suffering of experimental animals was minimised.

Experimental animals

The Central Animal Research Facility (CARF) of the National Institute of Mental Health and Neuro Sciences (NIMHANS) provided pregnant Wistar rats, and male pups were divided into three groups: normal control (NC), depressive-like (DEP), and vehicle control (SA). From postnatal days (PND) 8-21, pups were administered with clomipramine subcutaneously (15 mg/kg body weight, Sigma-Aldrich, St. Louis, MO, USA) or saline (0.9% NaCl); twice daily as vehicle control [10-13]. Male pups were left with the mother until they were weaned (21st day). After that, three rats were housed in one polypropylene cage (29 × 22 × 14 cm) under standard conditions. Food (standard laboratory chow, Hindustan Lever Ltd) and water were provided *ad libitum*, and animals were maintained in a well-ventilated room with 12 h light-dark cycle at room temperature (25 ± 2°C) and normal humidity (50-55%).

On a postnatal day (PND) 76, a sub-set of clomipramine administered rats were subjected to 6h of environmental enrichment (DEP + EE) for 14 days (*i.e.*, PND 76-89; Fig. 1A). In addition, we also subjected control animals to 6h of environmental enrichment (EE *per se*) for 14 days to test for the effects of EE on naïve animals. Subsequently, on PND 90, all groups of animals were sacrificed and subjected to morphological evaluation[11-13].

Exposure to an enriched environment

Between PND 76 and 89, adult male Wistar rats were exposed to EE for 14 days. 8-12 rats were put in a large cage (108 cm × 65 cm × 65 cm) containing toys, plastic tunnels, and metal ladders of different shapes, sizes, and textures. Playthings were made of plastic, wood, and coconut shells. Tunnels and ladders were rearranged every day, and toys were changed every two days. Rats were exposed to EE every day for 6 h (between 10 am and 4 pm) and returned to their respective home cages. EE cages were designed to provide an opportunity for social interaction with a larger group of animals and also to provide scope for physical activity and somatosensory, motor, visual and cognitive stimulation[4,10,14].

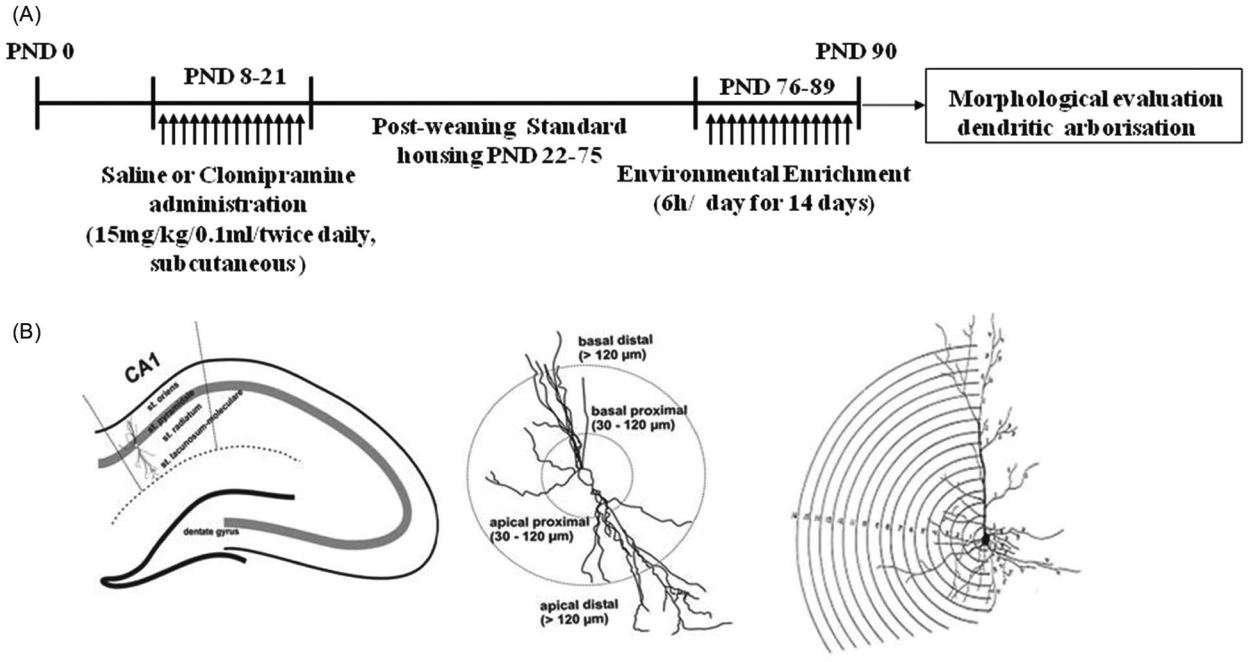

Fig. 1 — (A) Study design- duration of clomipramine administration and exposure to the enriched environment in male rats. PND: postnatal day; and (B) Schematic representation of CA1 hippocampal region and dendritic regions. Dendrites extending toward the corpus callosum were classified into basal, proximal dendrites (30-120 µM from the soma), and basal distal dendrites (120 µM from the soma) in pyramidal neurons in the dorsal half of CA1. Apical dendrites projecting into the dentate gyrus were divided into two groups: proximal to the soma (30-120 µM) and distal to the soma (120 µM)

Tissue preparation and Golgi-Cox staining

On a postnatal day of 90, animals were anaesthetised with halothane and euthanised by decapitation. Brains were immediately removed and placed in Golgi-Cox solution (5% *w/v* potassium dichromate, 5% *w/v* mercuric chloride, 5% *w/v* potassium chromate solutions prepared in distilled water; final stain contains 5 volumes of both potassium dichromate and mercuric chloride, 4 volumes of potassium chromate and 10 volumes of distilled water) for 14 days, followed by 3 days in 30% sucrose solution. Coronal sections of the 150 µM thickness of the hippocampal region were obtained using a Vibratome™ (Leica, Wetzlar, Germany). Sections were arranged serially on gelatine coated slides and treated with freshly prepared 75% ammonia solution for 10 minutes in the dark. Then, sections were washed thoroughly 4-5 times in distilled water. After that, sections were immersed in 1% sodium thiosulphate solution prepared freshly. Following this, slides were washed with double distilled water and dehydrated in increasing grades of ethanol (70%, 90%, and 100%). Slides were blot dried, cleared with xylene, mounted with DPX, and finally, coverslipped. The slides were air-dried for a week in the dark and stored for analysis[15].

Morphological quantification analysis

Golgi-impregnated pyramidal neurons of the CA1 subfield of the hippocampus (Plates 27-36) were selected in this study. Three-dimensional dendritic trees of 6-8 neurons were randomly selected in 6 animals per group from both hemispheres. Each neuron was reconstructed at a magnification of 40X with a digital camera (Olympus BX51, Olympus, Denmark) using Neurolucida software system (MBF Bioscience, Microbrightfield, Inc., USA). Pyramidal neurons were identified by triangular shape cell body with apical dendrites extending towards the pial surface. Therefore, the following criteria were used to select a neuron for analysis: (1) relatively isolated neurons from nearby neurons to evade indistinct dendritic arbours; (2) completely stained neurons; (3) fully impregnated neurons without any truncated branches[15-16].

Several aspects of dendritic morphology were examined. The cell body, apical and basal dendrites were traced in three dimensions using the Neurolucida program (Micro Bright Field Inc., Williston, VT, USA) interfaced with an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan). Neurolucida Explorer software was used to analyse the reconstruction of the neuron with built-in Sholl's analysis (Fig. 1B)[16]. A grid with concentric circles spaced 50 µM apart was overlayed on the dendritic tracing, and the number of intersections was estimated. In addition, the total apical dendritic length was calculated by multiplying the total number of interactions of each ring per 50 µM. The total number of apical dendritic branches (branches indicating bifurcations) counted at each order away from the cell body was also estimated. The traced neurons were chosen before the commencement of the analysis by an independent investigator blind to the experimental conditions to minimise any bias.

Statistical analysis

Data are expressed as mean ± SEM. The number of dendritic branching points, intersections, total branching points, and dendritic length were statistically analysed using One-way ANOVA followed by Tukey's multiple comparisons test or repeated measures two-way ANOVA followed by Bonferroni multiple comparisons test. A probability level of $P < 0.05$ was considered to be statistically significant.

Results

Exposure to an enriched environment restores depression-induced morphological deficits in apical dendrites

Apical dendritic branching points

Repeated measures two-way ANOVA showed a significant difference among groups studied ($F_{(4,167)} = 136.1$; $P < 0.0001$). The number of apical branching points was significantly lower in all segments (0-300 µM) in depressed rats compared to the normal control group. Interestingly, the number of branching points was restored to normal in DEP + EE group ($F_{(4,167)} = 136.1$; $P < 0.0001$). In EE *per se* rats, a significant increment ($F_{(4,167)} = 136.1$; $P < 0.0001$) was observed in the number of apical branching points compared to the normal control group ($P < 0.001$; Fig. 2A).

Apical dendritic intersections

Two-way ANOVA revealed significant difference among groups ($F_{(4,167)} = 261.5$; $P < 0.0001$). Detailed segmental analysis showed a drastic decrease in apical dendritic intersections in a depressed group compared to the control group. Exposure to an enriched environment resulted in highly significant restoration of dendritic intersections in depressed rats ($F_{(4,167)} = 261.5$; $P < 0.0001$). Naïve animals subjected to enrichment also demonstrated a significant increase in the number of dendritic intersections compared to the control group ($P < 0.01$) (Fig. 2B).

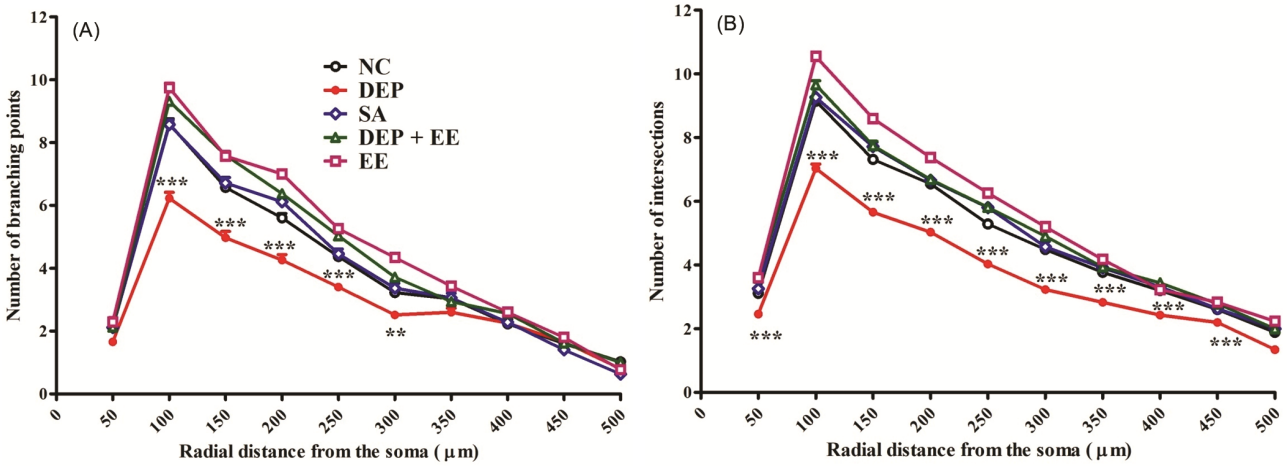

Fig. 2 — Effect of enriched environment on depression-induced hippocampal CA1 apical dendritic atrophy. Depressive-like rats exposed to 6 h EE for 14 days show complete recovery of dendritic atrophy in CA1 pyramidal neurons. Data expressed as Mean ± SEM. (A) The number of dendritic branching points at various segments and (B) intersections of CA1 pyramidal neurons of the hippocampus. NC = Normal control (N=5 animals, n = 35 neurons), DEP = Depressive-like rats (N=5 animals, n = 35 neurons), SA = Saline administered (neonatal) (N=5 animals, n = 35 neurons), DEP + EE = Depressed rats exposed to 6h of EE for 14 days (N=5 animals, n = 32 neurons), EE *per se* = Normal rats exposed to 6h of EE for 14 days (N=5 animals, n = 35 neurons). ***P <0.001, **P < 0.01, *vs* NC; Two-way ANOVA Followed by Bonferroni's post hoc test

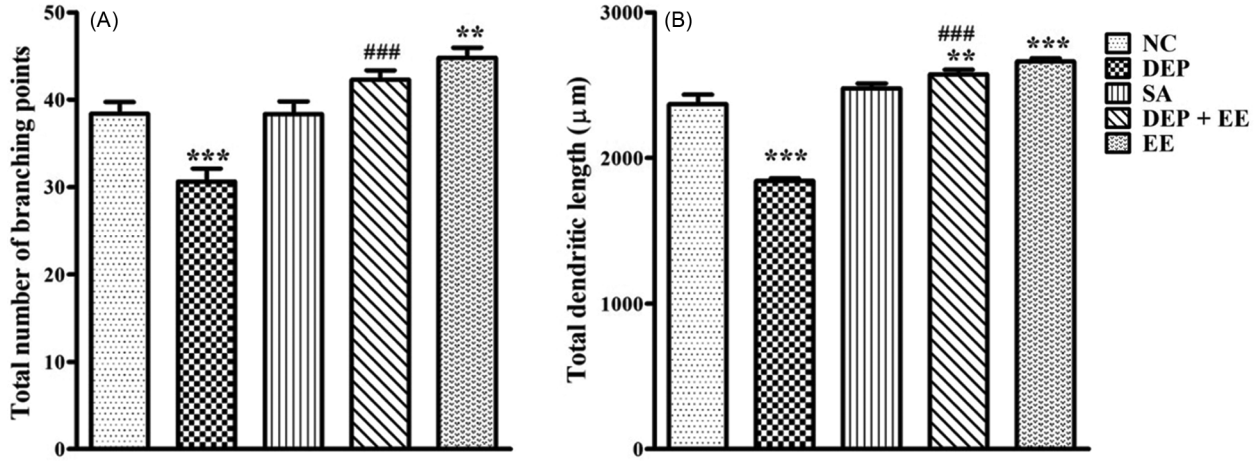

Fig. 3 — Effect of enrichment on depression-induced alterations in the total number of branching points and dendritic length of CA1 apical dendrites. DEP + EE group showed complete restoration of the number of branching points and dendritic length. Data expressed as Mean ± SEM. (A) Total number of dendritic branching points and (B) Total dendritic length (µM) from NC = Normal control (N=5 animals, n = 35 neurons), DEP = Depressive-like rats (N=5 animals, n = 35 neurons), SA = Saline administered (neonatal) (N=5 animals, n = 35 neurons), DEP + EE = Depressed rats exposed to 6h of EE for 14 days (N=5 animals, n = 32 neurons), EE *per se* = Normal rats exposed to 6h of EE for 14 days (N=5 animals, n = 35 neurons). ***P <0.001, **P < 0.01 *vs*. NC; ###P <0.001 *vs*. DEP; One-way ANOVA followed by Tukey's post hoc test

Effect of enriched environment on the total number of branching points and dendritic length of apical CA1 pyramidal neurons

Depression resulted in a decrement in the total number of branches and total dendritic length compared to control animals. When DEP rats were exposed to 6h of enriched environment for 14 days, persistent increment was observed in both number of branches ($F_{(4,167)}$ = 16.63, P <0.0001; Fig. 3A) and dendritic length ($F_{(4,167)}$ = 73.08, P <0.0001; Fig. 3B). EE *per se* group showed an enhanced number of branches (P <0.001) and total dendritic length in comparison with the control group (P <0.001).

Effect of enriched environmental exposure on the morphology of hippocampal basal CA1 neuronal dendritic arborisation

Depressed rats showed reduced basal dendrites in segments (0-200 µM) studied. This morphological

deficit in depressed (DEP) rats was restored when subjected to 6h of enriched environment for 14 days. In DEP + EE rats, a significant increment ($F_{(4,167)}$ = 130.8; P <0.0001) was observed in the number of basal dendritic branching points (Fig. 4A). Similarly, depressed rats, when exposed to enrichment, showed an increased ($F_{(4,167)}$ = 254.5; P <0.0001) number of basal dendritic intersections (Fig. 4B).

Exposure to enriched environment (DEP + EE) for 14 days significantly increased the both number of basal dendritic branches ($F_{(4,167)}$ = 31.6, P <0.0001; Fig. 5A) and dendritic length ($F_{(4,167)}$ = 37.42, P <0.0001; Fig. 5B) relative to DEP group. The normal and saline groups did not differ significantly. The length of dendrites was significantly increased in the enrichment *per se* group compared to the control group (P <0.001).

Discussion

In the present study, we observed hippocampal dendritic atrophy in depressive-like rats. Interestingly, an enriched environment facilitated dendritic remodelling in depressed rats (Fig. 6). For the first time, we demonstrated that the endogenous depression model causes quantitative neuronal reorganisation in the hippocampus CA1 area. Previous research has shown that olfactory bulbectomy causes dendritic degeneration in the CA1 region of the hippocampus, which is consistent with these findings. The removal of the olfactory bulb resulted in CA1 and CA3 neuronal degeneration[17]. Different animal models of depression and corticosterone diminish the spine density of the CA1 pyramidal neurons or cause neuronal atrophy[18-19]. Previous studies reported that the hippocampal CA1 neuronal degeneration in learned helplessness and maternal deprivation animal models of depression[19-20]. Growing evidence indicates a crucial connection between spatial memory and CA1 hippocampal shrinkage. Previous work in our group has shown that endogenous depression is associated with reduced spatial learning and memory[10-13]. Together, these investigations suggest that CA1 hippocampal neurons are vulnerable to a range of stresses or compromised mental states like depression.

In our research, endogenous depression drastically decreased the dendritic complexity of CA1 pyramidal neurons. The manifestation of depressive-like symptoms has been connected to the hippocampus CA1 area, which is important in learning and memory[19]. In this study, CA1 spines are negatively correlated with escape latency in the learned helplessness model. Additionally, nighttime exposure to low light increased immobility during forced swim tests and lowered sucrose intake, which is linked to a reduction in spine density in the CA1 region[21]. Clinically, patients with significant depression displayed smaller somata in the CA1, CA2, and CA3 regions[22]. In depressive patients, a structural MRI research revealed significant shrinkage in the hippocampus's subregions, including the subiculum and CA1 subfield spreading into CA2-3 subfields[23-24].

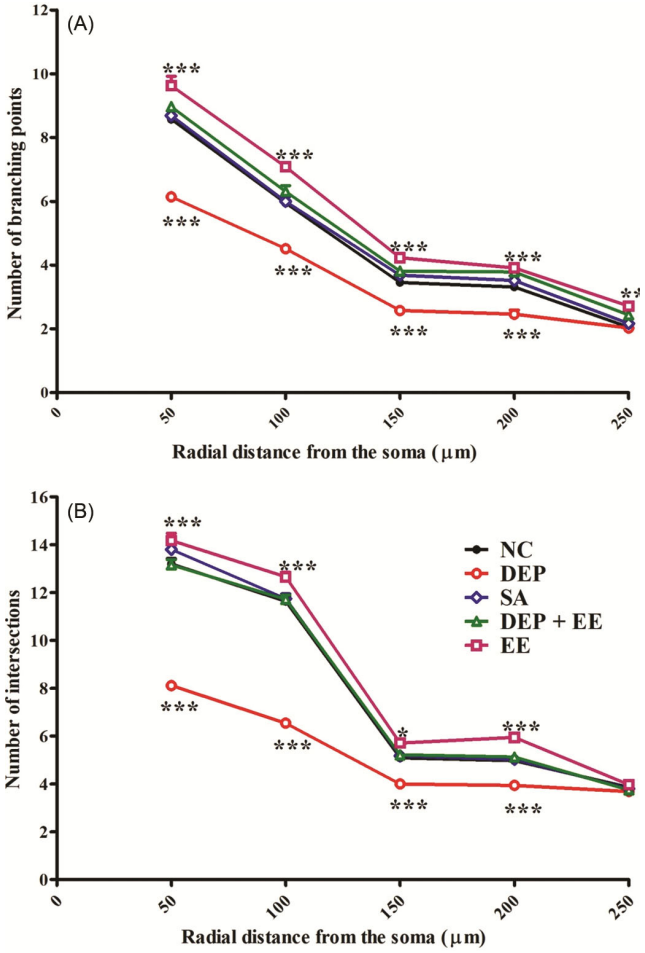

Fig. 4 — Effect of enriched environment on depression-induced hippocampal CA1 basal dendritic atrophy. Depressive-like rats exposed to 6h EE for 14 days show complete recovery of dendritic atrophy in CA1 pyramidal neurons. Data expressed as Mean ± SEM. (A) The number of dendritic branching points at various segments and (B) intersections of CA1 pyramidal neurons of the hippocampus. NC = Normal control (N=5 animals, n = 35 neurons), DEP = Depressive-like rats (N=5 animals, n = 35 neurons), SA = Saline administered (neonatal) (N=5 animals, n = 35 neurons), DEP + EE = Depressed rats exposed to 6h of EE for 14 days (N=5 animals, n = 32 neurons), EE *per se* = Normal rats exposed to 6h of EE for 14 days (N=5 animals, n = 35 neurons). *P < 0.05, **P < 0.01, ***P <0.001, *vs*. NC; Two-way ANOVA followed by Bonferroni's post hoc test

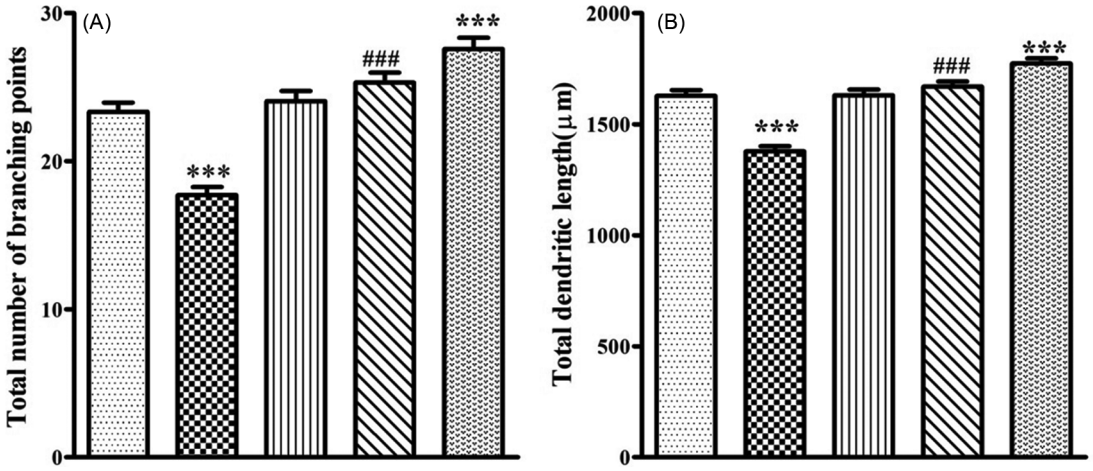

Fig. 5 — Effect of enrichment on depression-induced alterations in the total number of branching points and dendritic length of CA1 basal dendrites. DEP + EE group showed complete restoration of the number of branching points and dendritic length. Data expressed as Mean ± SEM. (A) Total number of dendritic branching points and (B) Total dendritic length (μM) from NC = Normal control (N=5 animals, n = 35 neurons), DEP = Depressive-like rats (N=5 animals, n = 35 neurons), SA = Saline administered (neonatal) (N=5 animals, n = 35 neurons), DEP + EE = Depressed rats exposed to 6h of EE for 14 days (N=5 animals, n = 32 neurons), EE *per se* = Normal rats exposed to 6h of EE for 14 days (N=5 animals, n = 35 neurons). ***P <0.001, **P < 0.01 *vs*. NC; ###P <0.001 *vs*. DEP; One-way ANOVA followed by Tukey's post hoc test

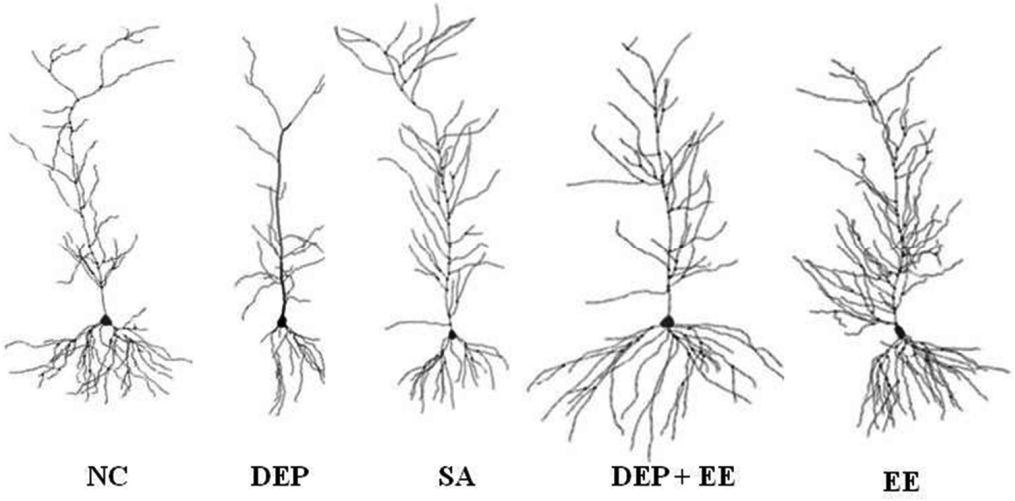

Fig. 6 — Representative CA1 pyramidal Neurolucida traced neurons of the hippocampus from normal control (NC), Neonatal saline administered (SA), depressed (DEP), depressed rats exposed to enrichment (DEP + EE) and Naïve animals exposed to enriched environment (EE), Note a decrease in the number of branching points in DEP group compared to control. However, depressed rats exposed to enrichment restored dendritic atrophy. Scale bar in image = 100 μM and applies to all the frames

Neonatal antidepressant drug administration produces a reduction of monoaminergic neurotransmitters, cognitive deficits, and alterations in synaptic plasticity in adulthood[12,13]. Additionally, it has been reported that the hippocampus contains lower levels of brain-derived neurotrophic factor (BDNF), cortical and medullary weight, total protein, and DNA. This shows that the decrease in serotonin during the neonatal period causes long-term changes to the brain's structure and cognitive capacities[25].

An enriched environment restored depression-induced dendritic atrophy in hippocampal CA1 pyramidal neurons. This is the first work to show CA1 dendritic atrophy in an animal model of endogenous depression and its subsequent amelioration by exposure to an enriched environment. Previous studies have shown that EE causes progressive structural plasticity. EE increased the volume of the DG[10] by inducing neuronal synaptic changes such as an increase in the hippocampal long-term potentiation[4], dendritic

arborisation of hippocampal neurons, and a decrease in the number of microglial cells[26-28]. In CA1 pyramidal neurons of the hippocampus, EE has also been found to increase dendritic spine density[29] and the number of newly born cells in the hilus and the subventricular zone of the DG[6]. Also, EE enhanced the thickness of postsynaptic densities[30], increased BDNF, and increased expression of the NR2A, NR2B, and AMPA receptor subunits[14,31-33].

Conclusion

Overall, our findings show that depressed rats exposed to an enriched environment exhibited neuroprotective benefits and increased dendritic arbours in CA1 pyramidal neurons of the hippocampus. Results of the current study offer the neuroanatomical basis of the beneficial effect of an enriched environment in improving cognitive functions in depressed rats.

Conflicts of interest

All authors declare no conflicts of interest.

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