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Additive Effects of Physical Exercise and Environmental Enrichment in Attenuating Alterations in the Hippocampal Neuronal Morphology of Adult Wistar Rats Induced by Prenatal Inflammations

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Abstract

The present study is aimed at elucidating the additive effects of treadmill running exercises and environmental enrichment (E.E) during adolescence in ameliorating the alterations in the dendritic morphology of hippocampal CA3 neurons induced by prenatal inflammations. Pregnant Wistar dams were injected intraperitoneally with either 0.5ml of saline (control group) or lipopolysaccharide (LPS group) (0.5mg/kg), from the embryonic day fourteen till delivery, on alternate days. Following parturition, pups were allocated into following groups [n=6/group]: (1) Control, (2) LPS, (3) LPS-exercise, (4) LPS-environmental enrichment and (5) LPS-exercise-environmental enrichment. On postnatal days fifteen to sixty, rats of the groups three, four and five were subjected either to treadmill running exercises or environmental enrichment or the combination of the two, respectively. On PND (postnatal day) sixty-seven, animals were euthanized, brains were carefully dissected out, and impregnated with modified Golgi-cox stain. Dendritic arborization of CA3 neurons in hippocampus was traced by camera lucida and analysed by Sholl's method. The young adult rats of LPS- environmental enrichment -exercise group showed a significant enhancement in dendritic arborization of CA3 hippocampal neurons, compared with other groups. Being reared in an intricate and enriched environment supported by treadmill exercises during adolescence enhances the dendritic arborization of hippocampal CA3 neurons that were exposed to LPS-induced prenatal inflammations. This study investigates the effects of using these methods in combination rather than administering either treadmill exercise, or environmental enrichment each on its own.

KeyWords: Prenatal inflammation, Lipopolysaccharide, Physical exercise, Enriched environment, dendritic arbor.

1. Introduction

Exposure to various insults during gestation such as stress, infection, malnutrition, etc. may cause adverse chronic changes in behavior, neuroendocrine responses, and cognition of the offspring (Kohman *et al.,* 2008). Early life experience can profoundly impact the shaping of the physiological and psychological health of an individual (Kohman *et al.,* 2008). Epidemiological evidence shows an increased risk of developing several neuropsychiatric disorders such as schizophrenia (Brown, 2012), autism (Ciaranello and Ciaranello, 1995), mental retardation (McDermott *et al.,* 2000), and cerebral palsy (Hermansen and Hermansen, 2006), in addition to preterm birth, following prenatal inflammations induced by infections. Studies have investigated the effects of prenatal stress or immune challenge on the alterations of neuronal cytoarchitexture and on behavior or on both.

Asymptomatic infections, either bacterial or viral, during pregnancy that remain undiagnosed can lead to severe deleterious complications even intrauterine fetal death. Systemic administration of lipopolysaccharide (LPS), a cell-membrane component of gram negative bacteria, is a commonly accepted model to challenge the immune system during gestation leading to induction of inflammatory chemical mediators-the cytokines (Cui *et al.,* 2009). Maternal infection during gestation is thought to affect the developing fetal brain by inducing the proinflammatory cytokines in both compartments i.e. fetal and maternal compartments (Golan *et al*., 2005, Ashdown *et al*., 2006). Pregnant rats injected with LPS showed

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induction of interleukin-1 beta (IL-1β), interleukin- 6 (IL6) and Tumor-necrosis factor- alpha (TNFα) in the amniotic fluid, maternal and fetal sera. In humans, the prenatal exposure to lipopolysaccharide during gestation takes place as part of bacterial vaginitis (Ling *et al.*, 2009). In women aged between 25-40 years, *E.coli* is the most common (95 % approximately) etiological factor resulting in urinary tract infections (Faro and Fenner, 1998).

Early postnatal life events and experiences during the adolescent period play a crucial part in the behavioral development of an individual during adulthood. In the past decade, studies have documented the abilities of physical exercise (PE) in alleviating cognitive decline due to senescence, increasing hippocampal volume, promoting the hippocampal neurogenesis, reducing apoptosis in the hippocampus and preventing the neurodegenerative diseases (García-Capdevila *et al*., 2009, van Praag *et al*., 2005, Kirk-Sanchez and McGough, 2014, Kim *et al*., 2010, Bherer *et al.*, 2013), as well as enhancing learning and memory processes, for example, the abilites in Morris water Maze (Creer *et al.*, 2010), radial arm maze (Anderson *et al.*, 2000) in addition to facilitating long-term potentiation (van Praag *et al*., 1999b).

Enriched Environment (EE) includes housing the animals in a complex variety of sensory-motor stimuli like a tunnel, swings, toys, running wheels, and social interaction with cage-mates (Lambert *et al.*, 2005). Studies have demonstrated that EE can increase dendritic arborization (both number of spines and dendritic branching), the size of the neuronal cell body and the level of neurotransmitters (Ickes *et al*., 2000, Rampon *et al*., 2000b). Middle- and old-aged rats and mice housed in intricate enriched conditions showed a reduction in age-related impairments in various types of learning and memory performance *(*Frick and Fernandez, *2003*, Kempermann *et al.*, 1998). Evidence from several studies confirms the beneficial role of PE and EE in various models of disease in rodents. However, the beneficial effects of PE and EE during the adolescent period in prenatal immune challenge models still need to be thoroughly studied. Similarly, studies of the additive effects of treadmill exercise along with the enriched housing conditions in reversing the alterations in neuronal morphology induced by prenatal LPS exposure are scarce. Thus, this study was conducted to investigate the additive effects of treadmill running exercises followed by enriched housing conditions in reversing the alterations in the neurons hippocampal region induced by prenatal LPS inflammations.

2. Materials and Methods

2.1. Animals

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Manipal Academy of Higher Education, prior to the commencement of the experiment (No: IAEC/ KMC/ 01/2015). Maintenance of animals was performed according to the prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt. of India. Adult female Wistar rats (n=24), aged three-months were housed in sterile cages with paddy husk as bedding, under standard laboratory conditions (22 ± 2°C temperature and 50 ± 5 % humidity) with a twelve-hour light/dark cycle. Water and food access to animals were *ad libitum*.

A pair of adult nulliparous female rats were caged with an adult male rat, pregnancy positivity was confirmed by the presence of sperms in the vaginal smear examined daily, and was considered as embryonic day '0' (E0). After random allocation either to control (n=6) or LPS group (n= 18), the pregnant dams were intraperitoneally (i.p) administered either with non-pyrogenic, sterile saline (0.5mL) or LPS (0.5mg/Kg, *E.coli* serotype 0111: B4 Sigma-Aldrich) respectively, from E14 till parturition on alternate days. After parturition, the pups were randomly assigned to groups (n=6/group), all offspring were raised by their biological mother, and the male offspring were used for the experiment. The pups born to the LPS mother were further sub-grouped as follows, (a) LPS, (b) LPS Exercise (LPS-Ex), (c) LPS Environmental Enrichment (LPS- EE) and (d) LPS Environmental Enrichment and Exercise (LPS- EE-Ex), in addition to the control group pups.

2.2. Treadmill (running) Exercise

Rats of the LPS-Ex and LPS-EE-Ex groups were subjected to treadmill running exercises, fifteen minutes/ per day [five sessions, three minutes/ per session with an intersession interval of four-five minutes approximately] from PNDs fifteen till PND sixty. The treadmill was equipped with horizontal motor-driven, five-parallel runways (IIITC Life Science, CA, USA. Model 805, Series 800). To avoid stressful conditions, the running speed was gradually increased from 1.5 meter/min (on PND 15) to 10.9 meters/min (on PND 25). The running speed of 10.9 meters/min was constantly maintained from PND twenty-five till PND sixty (Toy *et al*., 2014).

2.3. Environmental Enrichment

From PNDs fifteen to sixteen, four hours daily, the LPS-EE and LPS-EE Ex rats were subjected to environmental enrichnment. They were housed in a large sterile plastic cage, (120cm x 100cm x 100cm) with husk bedding, containing hard plastic tunnels, raised metal platform, ladder, objects of various sizes (metal balls, toys) and a steel swing [the cage was not provided with a voluntary running wheel]. The objects in EE cage were changed on alternate days.

2.4. Modified Golgi-Cox Staining

On PND sixty-seven, the young adult rats were euthanized following deep anaesthesia by an intramuscular injection of ketamine (100mg/kg body weight). The whole brain was carefully removed without transcardial perfusion, and was immersed in Golgi-Cox reagent for three to four weeks. The modified Golgi-cox solution was prepared as follows: five parts of 5 % potassium dichromate mixed with five parts of 5 % mercuric chloride, to this four parts of 5 % potassium chromate diluted with five parts of distilled water was added slowly with continuous stirring (Suvarna *et al.*, 2013). This solution was left undisturbed for four hours, and was filtered before use. After three weeks, each brain tissue was mounted on microtome tissue holder chucks with fevikwik glue. The tissue was coronally sliced at a

thickness of 180µm using a sledge microtome (Spencer sledge microtome) and collected in distilled water. Following treatment with the 5 % sodium carbonate solution for fifteen–twenty minutes, the sections were subjected to dehydration in ascending grades of alcohol, and were cleared in xylene. Finally, the sections were mounted with distyrene plasticizer xylene [DPX] on gelatin pre-coated glass slides, and cover slipped.

2.5. Tracing and Analysis of Hippocampal Neuron

The current study examined the two major morphological features - dendritic branching points and dendritic intersections as an indicator of dendritic arborization of a neuron of the hippocampal CA3 region. Golgi-Cox stained CA3 pyramidal neurons (six-eight neurons/animal) of the hippocampus was observed under 100x magnification and drawn on a plain white sheet of A4 size with the assistance of camera lucida attached microscope. The Sholl's method of the concentric circle was followed to quantify dendritic arborization. The researchers have used Sholl's grid with five concentric circles drawn at a calibrated (calibration equivalent to 20µm) distance of 2cm between each circle (Figure 1) (O'Neill *et al*., 2015). For neuronal analysis, Sholl's grid drawn transparent sheet was kept on a traced neuron in a manner that the center of the soma of traced neuron coincides with the mid-point of the smallest circle. A number of dendritic branching points, at the apical and basal level, between the concentric circles were noted. The number of dendrites crossings per radius (dendritic intersections) was counted. The analysis was carried after blinding the slides with codes to minimize any bias by the experimenter.

2.6. Statistical Analysis

Statistical analysis was performed using SPSS 16.0 for windows. Mean value for all neurons of each animal was calculated and then mean group difference in dendritic branching points and dendritic intersections were analyzed. One-way Analysis Of Variance (ANOVA) followed by Tukey's post-hoc test were used to analyse the data. A statistical significant level of $p<0.05$ was considered. The data in the graph are expressed as the mean ± SEM.

3. Results

In the Golgi-cox stained sections of control, LPS, LPS-Ex, LPS-EE and LPS-EE-Ex groups, to ascertain the spatial dispersion of dendritic branches in the apical as well as the basal regions in relation to the neuronal cell body, the mean number of the dendritic branching points and dendritic intersections per concentric sphere was analyzed for each group.

3.1. Dendritic Branching Points in the Apical Region

Analysis of apical branching points of CA3 pyramidal neurons revealed that there was a significant difference in the mean dendritic branching points among the groups between the circles as shown in Table 1; Figures 1- 4.

Table 1. Apical dendritic branching points of CA3 pyramidal neurons.

Sholl's Concentric Circle level (µm)	ANOVA "F" value; level of significance	Tukey's Post-hoc comparison	
		Groups	Level of significance
20-40	F (4, 25) = 4.338;$p<0.01$	LPS Vs LPS-Ex	$p<0.01$
40-60	F (4, 25) = 7.969 ;$p<0.001$	Control Vs LPS LPS Vs LPS-EE-Ex	$p<0.001$ $p<0.01$
60-80	F (4, 25) = 6.350; $p<0.01$	Control Vs LPS LPS Vs LPS-EE LPS Vs LPS-EE-Ex	$p<0.05$ $p<0.05$ $p<0.001$
80-100	F (4, 25) = 7.614; $p<0.001$	Control Vs LPS LPS Vs LPS-Ex LPS Vs LPS-EE LPS Vs LPS-EE-Ex	$p<0.05$ $p<0.05$ $p<0.01$ $p<0.001$

The analysis indicates that young adult rats of the LPS group showed reduced dendritic branching. Whereas, the rats that were exposed prenatally to LPS and were subjected to postnatal treadmill exercises followed by environmental enrichment (LPS-EE-Ex group) during adolescent age, showed increased dendritic branching points in the apical region of CA3 pyramidal neurons.

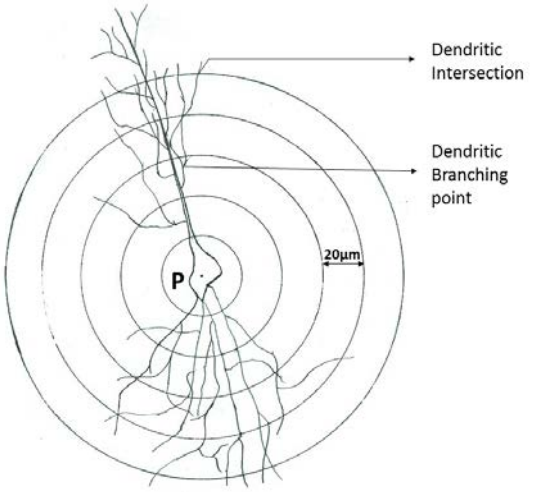

Figure 1. Representative image of camera lucida tracing of modified Golgi-Cox stained neuron of hippocampal CA3 region. The superimposed Sholl's grid containing successive concentric circles placed at an interval of 20µm from each circle. Letter 'P' indicates perikaryon.

3.2. Dendritic Intersections in the Apical Region

Analysis of apical intersections of dendrites of CA3 pyramidal neurons of groups showed that there was a significant difference in the mean number of dendritic intersections at the various radii level between the groups as shown in Table 2; Figures 1- 5.

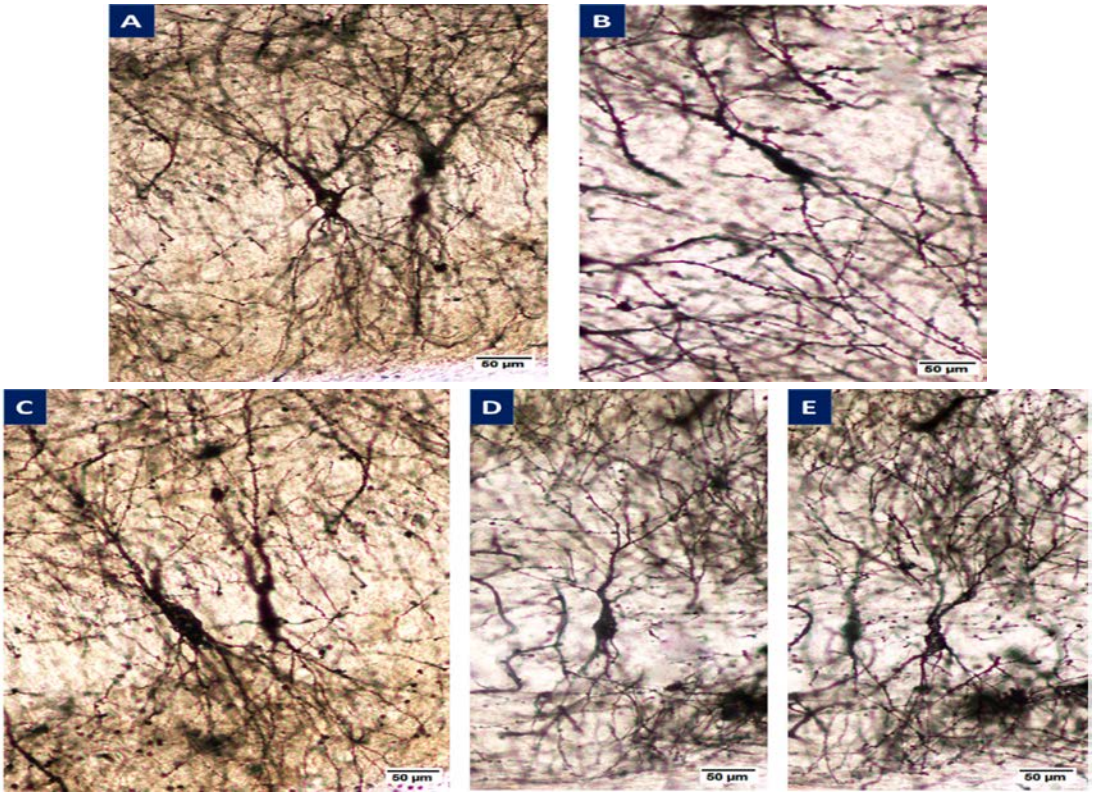

Figure 2. Representative photomicrographs of neurons of hippocampal CA3 region, stained with modified Golgi-Cox stain. Alphabet denotes: A- Control group; B- LPS group; C- LPS Ex group; D- LPS EE group and E- LPS EE Ex group. Photograph captured under 100x magnification

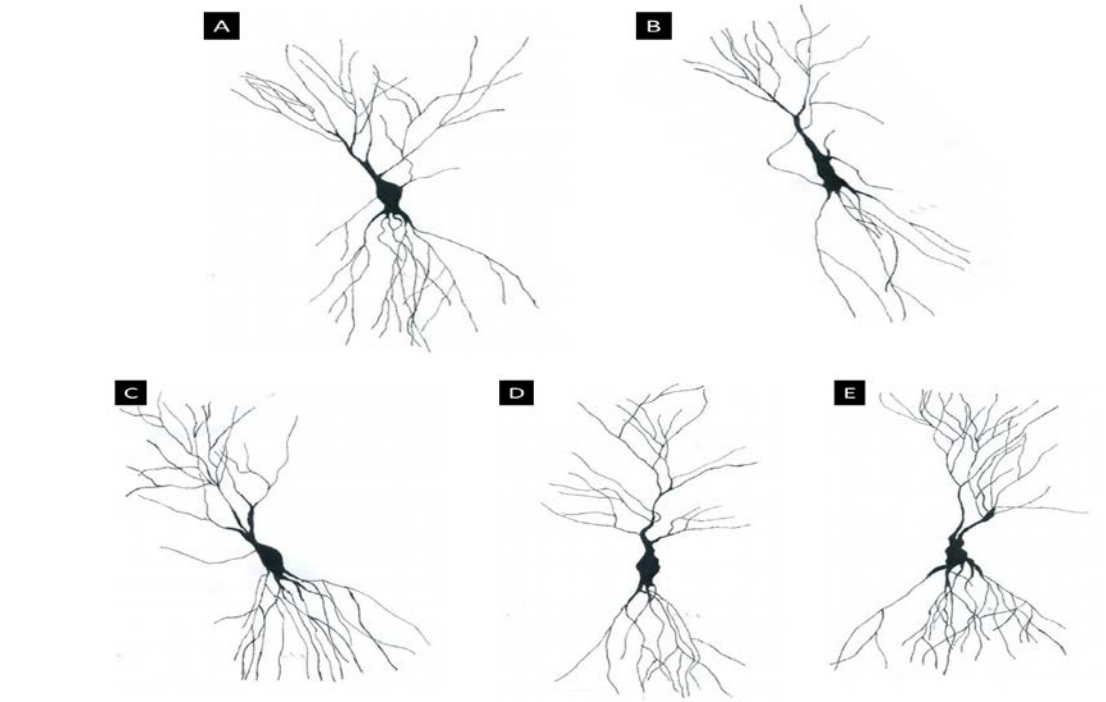

Figure 3. Representative images of camera lucida tracings of modified Golgi-Cox stained neurons of hippocampal CA3 region. Alphabet denotes: A- Control group; B- LPS group; C- LPS Ex group; D- LPS EE group and E- LPS EE Ex group. Images drawn under 100x magnification.

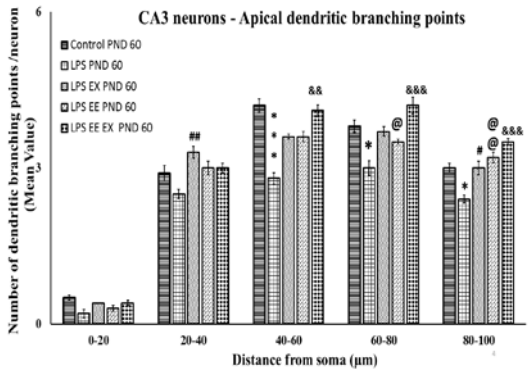

Figure 4. Effects of prenatal exposure to LPS and adolescent running exercise or being reared in a complex and enriched environment (EE) or a combination of the two methods of exercise and EE, on apical dendritic branching points of CA3 neurons. Symbols indicate levels of significance *p<0.01 and ***p<0.001- LPS Vs Control: && p<0.01 and &&& p<0.001 - LPS Vs LPS-EE-Ex; ## p<0.01 and # p<0.05- LPS Vs LPS-Ex; @ p<0.05 and @@ p<0.01- LPS Vs LPS-EE. Each bar represents mean ± SEM.

Table 2. Apical dendritic intersections of CA3 pyramidal neurons.

Sholl's Concentric Circle level (µm)	ANOVA "F" value; level of significance	Tukey's Post-hoc comparison	
		Groups	Level of significance
40	F (4, 25) = 6.413;p<0.01	Control Vs LPS	p<0.05
		LPS Vs LPS-Ex	p<0.01
		LPS Vs LPS-EE	p<0.05
		LPS Vs LPS-EE-Ex	p<0.05
60	F (4, 25) = 20.492;p<0.001	Control Vs LPS	p<0.001
		LPS Vs LPS-Ex	p<0.001
		LPS Vs LPS-EE	p<0.001
		LPS Vs LPS-EE-Ex	p<0.001
80	F (4, 25) = 10.975;p<0.001	Control Vs LPS	p<0.001
		LPS Vs LPS-Ex	p<0.01
		LPS Vs LPS-EE	p<0.05
		LPS Vs LPS-EE-Ex	p<0.001
100	F (4, 25) = 17.056;p<0.001	Control Vs LPS	p<0.001
		LPS Vs LPS-Ex	p<0.001
		LPS Vs LPS-EE	p<0.001
		LPS Vs LPS-EE-Ex	p<0.001

Analysis indicates that young adult rats of LPS group showed reduced dendritic intersections at the different radii of Sholl's grid. Whereas, the rats that were exposed prenatally to LPS and subjected to postnatal treadmill exercise followed by environmental enrichment (LPS-EE-Ex group) during adolescent age, showed increased dendritic intersection in the apical region of CA3 pyramidal neurons.

3.3. Dendritic Branching Points in the Basal Region

The basal branching points analysis of CA3 pyramidal neurons revealed that there was a significant difference in the mean dendritic branching points among the groups between the concentric circles. (Table 3; Figures 1, 2, 3 and 6).

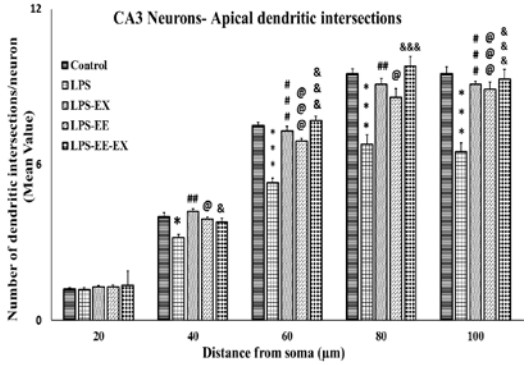

Figure 5. Effects of prenatal exposure to LPS and adolescent running exercise or being reared in a complex and enriched environment (EE) or a combination of the two methods of exercise and EE, on apical dendritic intersections of CA3 neurons. Symbols indicate levels of significance *p<0.01 and ***p<0.001- LPS Vs Control; ## p<0.01 and ### p<0.05- LPS Vs LPS-Ex; @ p<0.05 and @@@ p<0.001- LPS Vs LPS-EE; & p<0.05 and &&& p<0.001 - LPS Vs LPS-EE-Ex. Each bar represents mean ± SEM.

Table 3. Basal dendritic branching points of CA3 pyramidal neurons.

Sholl's Concentric Circle level (µm)	ANOVA "F" value; level of significance	Tukey's Post-hoc comparison	
		Groups	Level of significance
20-40	F (4, 25) = 3.625;p<0.05	Control Vs LPS	p<0.01
40-60	F (4, 25) = 6.818 ;p<0.01	Control Vs LPS	p<0.001
		LPS Vs LPS-EE-Ex	p<0.05
60-80	F (4, 25) = 4.749; p<0.01	Control Vs LPS	p<0.01
		LPS Vs LPS EE Ex	p<0.05
80-100	F (4, 25) = 5.055; p<0.01	Control Vs LPS	p<0.01
		LPS Vs LPS EE Ex	p< 0.05

Analysis indicates that prenatal exposure to LPS reduced the basal dendritic branching points of CA3 neurons of young adult rats (LPS group). Whereas, the young adult rats (PND 67) that were exposed to prenatal LPS and subjected to postnatal treadmill exercise followed by environmental enrichment (LPS-EE-Ex group) during adolescent age, showed increased dendritic branching points in the basal region of CA3 pyramidal neurons.

3.4. Dendritic Intersections in the Basal Region

The analysis of basal dendritic intersections of CA3 pyramidal neurons of PND sixty groups revealed that there was a significant difference in the mean dendritic intersections of dendrites among the groups at the different radii of the Sholl's grid. (Table 4; Figures 1, 2, 3, and 7).

The young adult rats that were exposed to LPS induced inflammation (LPS group) showed reduced basal dendritic intersections at the different radii of Sholl's grid. Whereas, the rats that were exposed prenatally to LPS, and were subjected to postnatal treadmill exercise followed by environmental enrichment (LPS-EE-Ex group) during adolescent age, showed increased dendritic intersection in the basal region of CA3 pyramidal neurons.

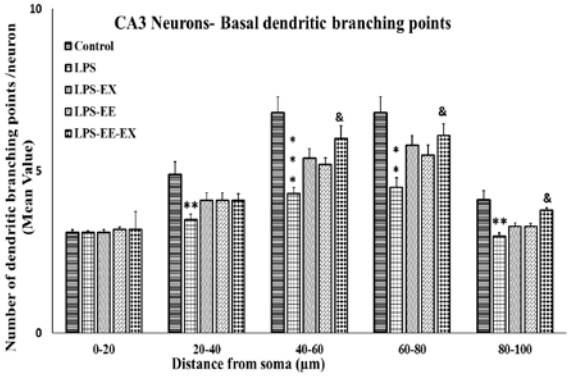

Figure 6. Effects of prenatal exposure to LPS and adolescent running exercise or being reared in a complex and enriched environment (EE) or the combination of the two, i.e., exercise and EE, on basal branching points of CA3 neurons. Symbols indicate levels of significance ***p*<0.01, ***p*<0.01 and ****p*<0.001- LPS Vs Control; & *p*<0.05 - LPS Vs LPS-EE-Ex. Each bar represents mean ± SEM.

Table 4. Basal dendritic intersections of CA3 pyramidal neurons.

Sholl's Concentric Circle level (µm)	ANOVA "F" value; level of significance	Tukey's Post-hoc comparison	
		Groups	Level of significance
40	F (4, 25) = 8.421;*p*<0.001	Control Vs LPS	*p*<0.001
		LPS Vs LPS-Ex	*p*<0.01
		LPS Vs LPS-EE	*p*<0.05
		LPS Vs LPS-EE-Ex	*p*<0.01
60	F (4, 25) = 11.703;*p*<0.001	Control Vs LPS	*p*<0.001
		LPS Vs LPS-EE-Ex	*p*<0.01
80	F (4, 25) = 17.979;*p*<0.001	Control Vs LPS	*p*<0.001
		LPS Vs LPS-Ex	*p*<0.05
		LPS Vs LPS-EE	*p*<0.01
		LPS Vs LPS-EE-Ex	*p*<0.01
100	F (4, 25) = 16.344;*p*<0.001	Control Vs LPS	*p*<0.001
		LPS Vs LPS-Ex	*p*<0.01
		LPS Vs LPS EE and	*p*<0.01
		LPS Vs LPS-EE-Ex	*p*<0.001

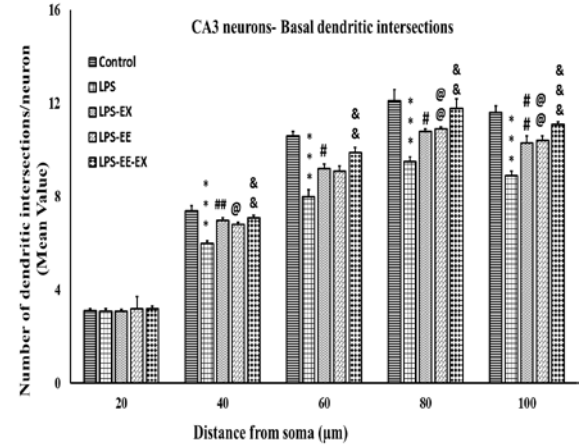

Figure 7. Effects of prenatal exposure to LPS and adolescent running exercise or being reared in a complex and enriched environment (EE) or a combination of the two, i.e., exercise and EE, on basal dendritic intersections of CA3 neurons. Symbols indicate levels of significance ****p*<0.001- LPS PND 60 Vs Control PND 60; && *p*<0.01 and &&& *p*<0.001- LPS PND 60 Vs LPS EE Ex PND 60; # *p*<0.05 and ## *p*<0.01 - LPS PND 60 Vs LPS Ex PND 60; @ *p*<0.05 and @@ *p*<0.01 LPS PND 60 Vs LPS EE PND 60. Each bar represents mean ± SEM.

4. Discussion

The results of the present study indicate that exposure to LPS induced prenatal inflammation resulted in decreased dendritic arborization of pyramidal neurons of CA3 region of the young adult hippocampus. This observations of the current study are in strong agreement with earlier reports; The prenatal LPS inflammation alters the dendritic arborization including significant reduction in the dendritic length in the medial pre-frontal cortex of PND 10 and PND 35, and in CA1 region at the age of PND sixty (Baharnoori *et al.*, 2009). Similarly, the maternal inflammation by LPS led to an altered thickness of CA1 region in young adult rats (Golan *et al*., 2005). LPS administration to pregnant rabbits during 28th day of gestation resulted in reduced dendritic arborization and spine density of thalamic neuron with decreased expression of synaptophysin in the newborn (Balakrishnan *et al.*, 2013). Although the researchers observed reduction in dendritic arborization of CA3 hippocampal neurons, the mechanisms underlying these morphological changes in the neurons are not addressed in the current study. However, evidence suggests that inflammatory cytokines, such as IL-6, IL-1β, and TNF- α, released in response to LPS exhibit deleterious effects on the neuronal development (Jeohn *et al*., 1998, Giovanoli *et al*., 2016). The inhibitory effect of TNF- α and IL-6 on the cortical and hippocampal dendritic arborization in *In vitro* neural culture cells (Gilmore *et al*., 2004, Neumann *et al*., 2002). The release of various neurotrophic factors such as brain-derived neurotrophic factor(BDNF) and nerve growth factor (NGF) in response to LPS induced prenatal inflammations affects the morphology of dendrites in cortex and hippocampus(Cohen-Cory *et al*., 2010, Ashdown *et al*., 2006). Thus, neuronal morphological alterations, or the reduced dendritic arborization of CA3 neurons, observed in the present study, may be attributed to the release of proinflammatory cytokines and altered levels of neurotrophins.

The cytoarchitecture of the neurons enables them to maintain their dynamic plasticity in response to various changes/stimulus of internal and external milieu. The ability of neurons to remodel its plasticity serves to protect against various adverse insults. The current results, also, demonstrated that the treadmill running exercise followed by housing in the complex environment during adolescent age significantly mitigates the prenatal LPS inflammation which causes morphological alterations of the dendrites. In the present study, it was noticed that the effects of treadmill exercise combined with environmental enrichment increases the dendritic arborization in the CA3 region to a greater extent, compared to effects of either the treadmill exercise only or environmental enrichment on its own.

The observations of this study are consistent with earlier studies; EE increased the brain weight of the mice that have been exposed to ethanol prenatally but not the cortical thickness (Wainwright *et al.*, 1993). The genes, downregulated by prenatal LPS exposure, that are specific for synaptic plasticity and transmission (such as EAAT2, BDNF, and TrkB) were upregulated by the enriched complex environment in early-life (Kentner *et al*., 2016). Evidence from clinical studies demonstrated the beneficial role of EE in autism (Woo *et al*., 2015), cerebral palsy

(Morgan *et al*., 2015), and schizotypical personality behavioral rehabilitation(Adrian Raine *et al.*, 2003).

A large body of growing evidence from various studies demonstrates that EE, and physical exercise improves the hippocampal neurogenesis (van Praag, 2008, van Praag *et al.*, 1999a, van Praag *et al.*, 1999b, Kempermann and Gage, 1999, Kempermann *et al*., 1997), counteracts the adverse effects of several prenatal insults like infection (Kentner *et al.*, 2016), stress (Morley-Fletcher *et al*., 2003, Lemaire *et al*., 2006), morphine(Ahmadalipour and Rashidy-Pour, 2015, Ahmadalipour *et al.*, 2015), and inhibits the progression of Alzheimer's disease (AD) - like pathology(Adlard *et al*., 2005). Also, EE, and physical exercise increase the level of nerve growth factors and neurotransmitter expression (Leggio *et al.*, 2005, Hüttenrauch *et al*., 2016, Rampon *et al*., 2000a), and improve dendritic arborization and the total length of dendrites of DG granule cells (Redila and Christie, 2006). An earlier report conducted by the researchers of this study, showed that the exposure to prenatal LPS inflammation resulted in cognitive deficits in young adult rats, whereas treadmill running exercise and being reared in a complex enriched environment attenuated the impaired memory and spatial abilities in Morris water maze (Thangarajan *et al*., 2015, Rajesh *et al*., 2016). These behavioral performances could be correlated to the neuronal morphological alterations observed in the current study.

5. Conclusion

The present study contributes to the existing literature by providing evidence that physical exercise combined with being reared in an enriched complex environment during early postnatal period can be an essential target for non-pharmacological interventions in preventing neuronal structural changes and cognitive deficits induced by prenatal LPS inflammations. However, further studies are still needed to examine and correlate the biochemical and molecular changes in response to prenatal exposure to LPS and the additive effects of treadmill exercise followed by enriched environment during the adloscent age.

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Conflict of Interest:

The authors have no conflicts of interest to disclose

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