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Prenatal Inflammation Induced Alterations in Spatial Learning and Memory Abilities in Adult Offspring: Mitigated by Physical Exercise and Environmental Enrichment.

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ABSTRACT

Prenatal inflammation results in chronic cognitive deficits, including learning and memory impairments in offspring. This study investigated the effects of treadmill exercise followed by environmental enrichment in mitigating the learning and memory impairments in young adult rats that were prenatally exposed to lipopolysaccharide induced inflammation. Pregnant Wistar dams were injected intraperitoneally either saline (control group; 0.5ml; n=6) or LPS group (n=18) (0.5mg/Kg) on alternate days from embryonic day 14 till delivery. After delivery, pups were divided into following groups: (1) Control, (2) LPS, (3) LPS-exercise, (4) LPS-environmental enrichment and (5) LPS-exercise-environmental enrichment. Group 3, 4 and 5 were subjected either to the treadmill exercise or environmental enrichment or both respectively, on postnatal days 15 to 60 followed by an assessment of spatial abilities by Morris water maze test. The young adult rats exposed to prenatal inflammation and subjected to treadmill exercise followed by environmental enrichment displayed significant shorter latency to reach the target quadrant. Also, they spent longer duration in the target quadrant in probe trial compared to the age- matched groups subjected either to exercise or environmental enrichment and LPS group. The exercise and enriched environment during the adolescent period could be an alternative strategy to mitigate cognitive deficits in an individual who were exposed to prenatal inflammation.

Keywords: Prenatal inflammation, physical exercise, environmental enrichment, spatial learning and memory.

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INTRODUCTION

Maternal infection during gestation is a major cause of preterm birth resulting in the sequel of neurological deficits such as white matter injury, etc., in offspring. In addition to preterm birth, mounting evidence from epidemiological studies showed a higher association of maternal infection, local or systemic, during pregnancy with an increased incidence of neuro-psychiatric ailments, like schizophrenia, autism, etc., in offspring [1-4]. The maternal immune system produces a common response to several infectious agents, either bacterial or viral, which could affect the development of nervous system in fetus contributing to morphological, cytoarchitectural changes in brain and consequently alters the behavior of an individual[5].

In order to evoke maternal immune response during gestation, the pregnant rodents have been treated either with the viral mimic polyinosinic: polycytidylic acid (poly I:C) or bacterial endotoxin, lipopolysaccharides (LPS) [6]. In the present study, we used LPS, a widely accepted model of prenatal inflammation, to induce maternal immune activation during pregnancy. LPS, an integral component of the cell wall of Gram-negative bacteria like Escherichia coli *(E.coli)* , is the commonest causative organism of urinary tract infection in females aged between 20-40 years (approximately 90%) [7].

The innate immune response following administration of LPS includes cytokine induction, fever, inflammatory response, activation of hypothalamic–pituitary–adrenal (HPA) axis and sickness behavior[8]. LPS binds to Toll-like receptor-4 (TLR-4) on macrophages and other cells of immune system activates a cascade of signal transduction results in transcription factors activation such as nuclear factor kappa B (NFk-B) and results in encoding of genes for pro- and anti-inflammatory mediators[9]. Raised levels of pro-inflammatory cytokines, such as interleukin-1b (IL-1b), IL-6, and tumor necrosis factor-alpha, in the maternal and fetal compartment, have been associated chronic neurodevelopmental impairments in offspring[10].

'Enriched environment' (EE), is an improvised housing condition which includes combined intricate inanimate and social stimulations [11], commonly constitutes bigger housing cages, with a running wheel, tunnels and a few toys/objects that are periodically interchanged to arouse animal curiosity, exploration, and social interaction [12]. Early life events, particularly interactions of an individual with EE and physical activity can lead to beneficial neurobehavioral changes such as increasing sensory, motor, and cognitive stimulation. Studies, both molecular and cellular, have shown anatomical and physiological changes in the nervous system of animals subjected to enhanced housing conditions compared with those living in a more-standard environment [13]. Studies have shown that voluntary physical exercise (PE) enhances neuronal plasticity, promotes neurogeneration, and neuroprotection [14, 15]. PE has a wide range of beneficial functions that includes, changes in neurotransmitters, growth factors, spine density, synaptic plasticity, and vascularization[16, 17], improves spatial pattern separation [18], enhances learning and memory [19, 20]. Not only in normal/healthy animals but also but in several neurological disorder models have shown beneficial effects following exposure either to an EE or PE [21, 22]. However, little is known about the conjugated effects of environmental enrichment and physical exercise, especially during adolescences, in rarefying the prenatal inflammation induced impairment in spatial navigation and memory abilities in offspring. Thus the present study aimed to demonstrate the combined effects of the treadmill (running) exercise and enriched environment, especially during the growth spurt (adolescence) period, in attenuating the altered spatial learning and memory performance in offspring, due to exposure to inflammatory insults prenatally.

MATERIALS AND METHODS

Animals

Adult female Wistar rats (n=24), aged 3-months (250-280g) were procured from Central Animal Research Facility, Manipal University. The experimental protocol was scurtinized and approved by Institutional Animal Ethics Committee (IAEC), Manipal University. Maintenance of animals was followed according to the prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt. of India. Animals were housed in sterilized polypropylene cages with paddy husk bed, under standard environmental conditions (22 ± 2°C temperature and 50 ± 5 % humidity) with 12:12h light/dark cycle, with *ad libitum* water and food access.

A pair of adult female rats were caged overnight with an adult male rat for mating, twelve hours after mating pregnancy was confirmed by the presence of sperms in vaginal smear and considered as day '0' of pregnancy. A pair of pregnant female dams were housed in a cage with a label indicating the day of conception.

After random assignment of pregnant dams to control (n=6) and LPS group (n= 18). They were injected intraperitoneally (i.p) either with pyrogen-free, sterile saline (0.5ml) or LPS (0.5mg/Kg, E.coli serotype 0111: B4 Sigma-Aldrich) respectively, from the 14^{th} day of gestation (GD14) till delivery on alternate days. The dose selection of LPS was based on our previous study [23]. After delivery, till weaning (Postnatal day [PND] 21), all offspring were raised by their biological mother. Male offspring were used for the experiment. Pups were randomly assigned to groups (n=18/group). In addition to the control group, pups of LPS mother were 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).

Physical (Running) Exercise:

On PNDs 15 to 60, the LPS-Ex and LPS-EE-Ex animals were subjected to treadmill running exercise, 15 min/day [5 sessions, 3mins/session with an intersession interval of 4-5mins approximately]. The motor-driven treadmill equipped with 0^{o} inclined 5- parallel runways, with mild electric shock grid at one end of the runway to motivate the animal (IIITC Life Science, CA, USA. Model 805, Series 800). From PNDs 15 to 25, in order to avoid the stressful condition, running speed was gradually incremented from 1.5meter/min (on PND 15) to 10.9meters/min (on PND 25). Thereafter till PND 60, running speed was constantly set as 10.9meters/min.

Environmental enrichment

EE was administered for 45 days [PNDs 15 to 60]. The LPS-EE and LPS-EE Ex animals were housed 4h/day in a large sterile plastic cage (120cm x 100cm x 100cm)) containing a collection of tunnels (plastic), raised platform with ladder, various size metal balls, toys, and steel swing. These objects were changed on alternate days.

SPATIAL LEARNING AND MEMORY ASSESSMENT

Morris Water Maze test:

On PND 61 to 66, rats were subjected to Morris water maze test (MWM), a standard test for hippocampal- dependent task [24, 25]. The apparatus is made of galvanized steel circular pool (1.5m in diameter) filled with water (22 ± 2°C) to a depth of about 50cm, conceptually divided into four quadrants. An escape platform (4''x4'' size) was submerged 1cm (approx.) below the surface of the water in the target quadrant. Prior to the commencement of testing, the water was rendered opaque by the addition of powdered milk. To facilitate spatial orientation of the animal, distinct visible cues were positioned constantly throughout testing days. All animals were trained 4trials/day on five consecutive days, each trial consists of 60sec duration, with an approximate inter-trial interval of 6-7min. Each rat was released gently into the pool with its snout facing the wall of the pool. After reaching the escape platform, the rat was allowed to remain on the platform for 15 seconds. The training was terminated after 60sec, if the rat was unable to find the platform and a maximum score of 61sec were considered.The memory retention (probe trial) test (30sec duration) with the removal of the hidden platform was administered 24h after the last trial. Animal movements were monitered and recorded by a video camera connected to a computer installed with software (Any-Maze Version 4.82, Stoelting, UGO Basale). The latency duration and distance travelled to reach the escape platform in each trial and duration spent in the platform quadrant in search of the platform during the memory retention test were considered and analyzed. More the time taken to reach the escape platform and lesser the time spent in the target quadrant are suggestive of memory deficits [24]. Data were processed and analyzed by the individual blind to experiments.

Statistical analysis:

Data were analyzed by using One-way Analysis Of Variance (ANOVA) followed by Tukey's post-hoc test [SPSS 16.0, IBM]. A value of $p<0.05$ was considered as statistically significant. The data are expressed as the mean ± SEM.

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RESULTS

Escape latency analysis :

In the five days acquisition (training) phase, except day 2, the overall escape latency analysis showed significant difference between the groups, in MWM (day 1, ANOVA F (4, 85) = 1.356, P >0.05; day2 (ANOVA F (4, 85) = 1.128, P >0.05) ; day 3, ANOVA F (4, 85) =10.074, P <0.001; day 4, ANOVA F (4, 85) = 10.685, P <0.001; day 5, ANOVA F (4, 85) = 19.282, P <0.001). Post-hoc analysis showed there were no significant differences observed among control, LPS-Ex, LPS-EE and LPS-EE-Ex groups during the five days acquisition phase of MWM. (Fig. 1)

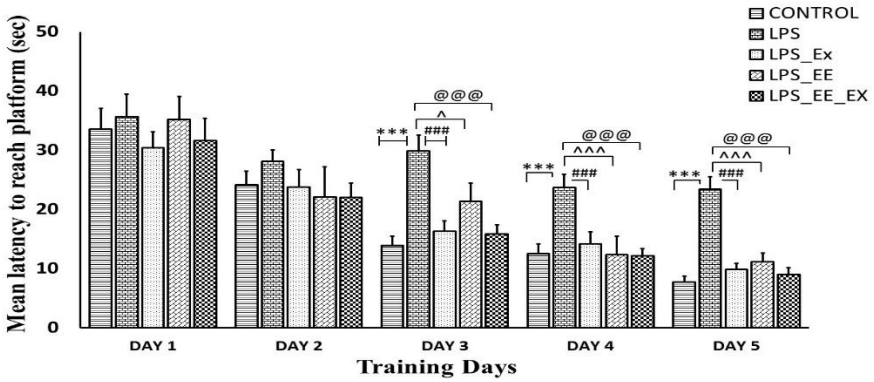

Figure 1: Effects of prenatal exposure to LPS and adolescent running exercise or housing in an enriched environment (EE) or both i.e., exercise and EE on spatial learning and memory performance in the Morris water maze test. Each bar represents mean (± SEM) latency (sec) to reach the hidden escape platform during the acquisition phase of MWM. ***p< 0.001; ### p< 0.001; ^^^ p< 0.001 (^p<0.05) and @@@ p< 0.001 LPS compared to control, LPS-Ex, LPS-EE and LPS-EE-Ex respectively.

Cumulative distance analysis :

The cumulative mean distance analysis, showed significant difference between the groups on day 3 (ANOVA F (4, 85) =4.350, P <0.01), day 4 (ANOVA F (4, 85) = 3.785, P <0.01) and day 5 (ANOVA F (4, 85) = 3.016, P <0.05). However, the difference between groups were non-significant on day 1, ANOVA F (4, 85) = 0.034, P >0.05 and day 2 (ANOVA F (4, 85) =0.548, P >0.05) of the five days acquisition phase of MWM. Post-hoc analysis showed, there was a significant difference observed on day 3, 4 and 5 between control and LPS. In addition, on day 4 significance (p <0.05) was observed between LPS and LPS-EE-Ex groups. There were no significant differences observed among control, LPS-Ex, LPS-EE and LPS-EE-Ex groups during the five days training period of MWM. (Fig. 2)

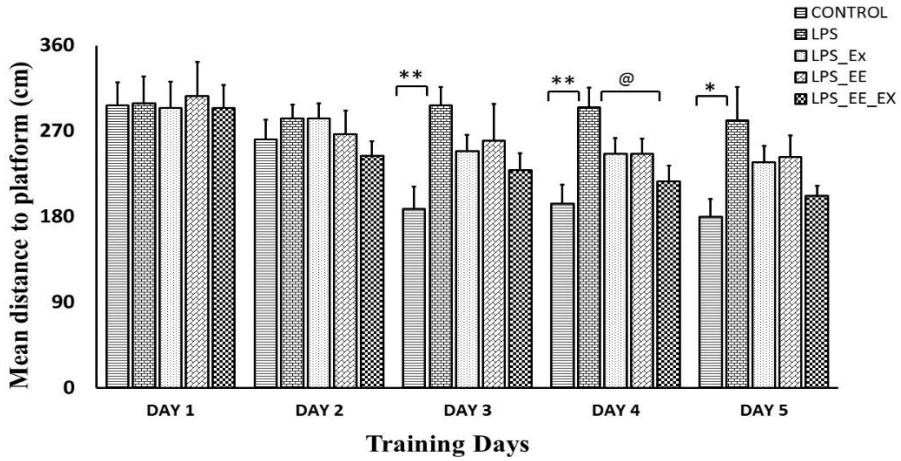

Figure 2: Effects of prenatal exposure to LPS and adolescent running exercise or housing in an enriched environment (EE) or both, i.e., exercise and EE on spatial learning and memory performance in the Morris water maze test. Each bar represents mean (± SEM) distance (cm) to reach the hidden escape platform during the acquisition phase of MWM. **p< 0.01, *p< 0.05; and @ p< 0.05- LPS compared to control and LPS-EE-Ex respectively.

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Memory Retention (Probe) test analysis:

In the retention probe trial with the hidden platform removed, the time taken to reach the target quadrant was significant between the groups (ANOVA F (4, 85) = 10.054, P <0.001). The Post-hoc analysis of the time to reach target quadrant revealed, the difference between LPS with Control (P <0.001), LPS-Ex (P <0.05) and LPS-EE-Ex (P <0.01) were significant (Fig. 3).

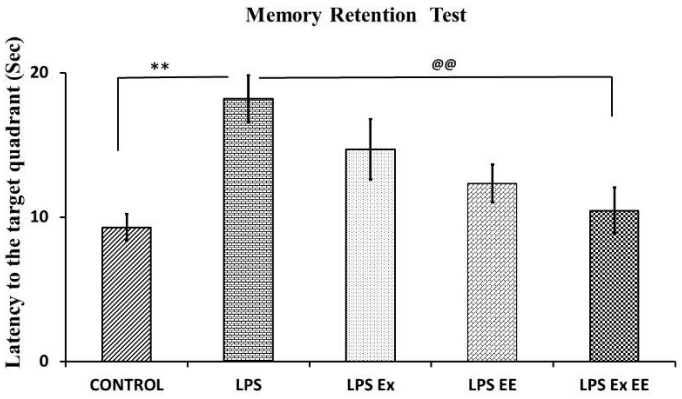

Figure 3: Effects of prenatal inflammation by LPS and adolescent running exercise or housing in an enriched environment (EE) or both, i.e., exercise and EE on latency to reach the target quadrant in memory retention test. The LPS-EE-Ex ($^{@@}$p<0.01) and control (p<0.01) groups have shown significantly shorter latency to reach the target quadrant in the probe trial compared with LPS group. A non-significant lesser latency was displayed by LPS-Ex and LPS-EE groups in comparison with LPS group.**

There was a significant difference, noted in time spent in the target quadrant in search of platform location, between the groups (ANOVA F (4, 85) = 5.311, P <0.01) (Fig.4).The post-hoc analysis, however, showed there was a non-significant difference among the LPS, LPS-EX, and LPS-EE. Whereas, a significant difference was observed with LPS versus Control (P <0.01), and LPS-EE-Ex (P <0.05).

Spatial learning and memory deficits in offspring exposed to prenatal LPS induced inflammation during pregnancy

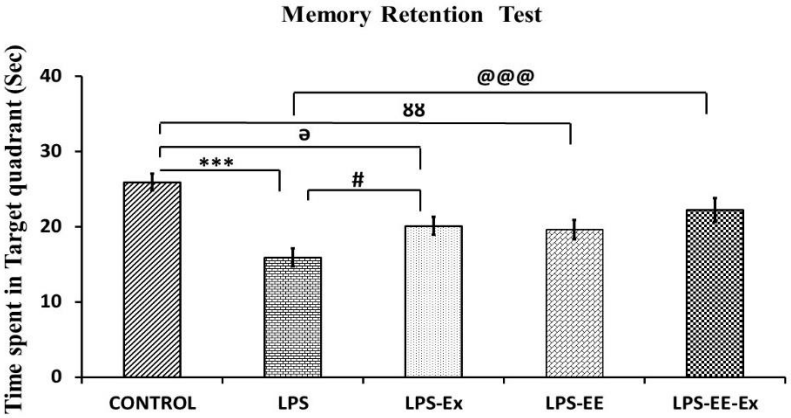

Figure 4: Effects of prenatal inflammation by LPS and adolescent running exercise or housing in an enriched environment (EE) or both, i.e., exercise and EE on time spent in the target quadrant in search of the platform location in the memory retention test. In the probe trial, LPS-EE-Ex ($^{@@@}$p<0.001) and LPS-Ex ($^{\#}$p<0.05) groups have spent significantly longer duration in the target quadrant compared with LPS. Also, the significant difference was noted between LPS (*p<0.001), LPS-Ex (^{ə}p<0.05), LPS- EE (^{ȣ} p<0.05) with the control group.**

In the five days acquisition phase of MWM, the young adult rats in the LPS group displayed significantly longer escape latencies i.e. longer duration to reach the hidden platform in comparison with the age-matched young adults of the other groups. However, the LPS group exhibited improvement in the performance, i.e., time

taken to reach the hidden platform decresead gradually during the course of five days of the acquisition phase, but this betterment was as not equivalent to the performance improvement displayed by the other groups (Fig. 1). In the memory retention test, the young adult rats of the LPS group were unable to remember the platform quadrant. Hence LPS group displayed longer latency to reach target quadrant (Fig. 3) and spent significantly lesser duration in the target quadrant in search of hidden platform (Fig. 4).

The environmental enrichment and running exercise mitigates spatial learning and memory deficits caused by LPS exposure prenatally.

The significant main effects of either treadmill running exercise or environmental enrichment or both were noticed in the acquisition phase of MWM. The LPS-Ex, LPS-EE, and LPS-EE-EX groups showed an improved performance, i.e., shorter latency to find the escape platform in comparison with LPS group (Fig.1). During the memory retention test (24hr after the last trial of acquisition phase) without the escape platform, the young adult rats of LPS-EE-Ex were able to recall the platform location thus showed significant shorter latency to reach the platform quadrant (Fig. 3) and spent significant longer duration in the traget quadrant (Fig. 4) compared with the age-matched LPS group. Though the LPS-Ex and LPS-EE groups showed improved spatial ability, but the improvement was notably higher in the performance of the young adult rats exposed prenatally to LPS induced inflammation and subjected to running exercise and environmental enrichment in their postnatal life, i.e., LPS-EE-Ex group.

DISCUSSION

This study shows that the prenatal LPS exposure induces spatial learning and memory deficits in the young adult rat. PE combined with EE during adolescent period effectively mitigates the prenatal LPS exposure induced cognitive deficits. Thus providing a supporting imperative evidence that PE with EE could be a putative non-pharmacological therapeutic tool for behavioral deficits induced by early life insults. Earlier, we reported that the treadmill running exercise during the adolescent age ameliorates cognitive deficits induced by LPS exposure prenatally in young adult rats [23].

Exposure to various insults during late prenatal and early postnatal life is deleterious to offspring since these are the sensitive and critical durations where the neuronal circuitry formation in the brain associated with early development [26]. Stimulation of maternal immune system by repeated LPS injection does not alter basal hormonal level, but affected the behavior and dopamine level in brain, in adult offspring. Prenatal administration of single-dose of viral mimic polyinosinic: polycytidylic acid (Poly I:C) on gestation day (GD) 9 led to reduced spatial exploration in the open field, whereas resulted in perseverative behavior on GD17[27]. The maternal immune challenge with LPS on GD15–16 or GD18–19 significantly reduced prepulse inhibition of acoustic startle (PPI) in adult male offspring, whereas exposure to LPS on GD10–11 had shown minimal or marginal effects [6]. The findings of Graciarena et al., study showed that prenatal LPS exposure during pregnancy leads to impairment in novel object recognition (NOR) task in 60 days-old offspring [28]. A study by Li et al., reported impaired NOR task in 2-months-aged offspring that were prenatally exposed to Poly I:C induced inflammation [29]. A study by Wang et al., demonstrated the age- and sex- dependent impairment of spatial abilities in 13-month old male and 6-month old female adult rats whose mothers were subjected to LPS exposure from GD8 to GD15 [30].The behavioral results of the present study are consistent with the observations reported in these studies. Thus, the specificity of vulnerability mainly depends on the nature and intensity of prenatal stimulus as well as gestation time window at which maternal host is exposed to the immune challenge critically influences the resultant effects on the adult brain.

Several studies documented that housing in an enriched environment can effectively antagonize cognitive impairments induced by early life insults in animals[31, 32], reverses unnatural behaviors such as alteration in emotional reaction, motor skills and spatial abilities induced by prenatal stressors[33]. Postnatal enriched environment treatment reverses exaggerated anxiety-like behavior like escape tendency associated with increased secretion of corticosterone in response to maternal stress during gestation [26, 34, 35]. In contrast with the study by Vallee et al. that showed enriched environmental experience during the early life periods did not influence the cognitive abilities in adult offspring [36]. The results of our study are in agreement with the studies by Koo et al.,(2003) and Ahmadalipour and Rashidy-Pour (2015). The study by Koo et al. demonstrated that early environmental enrichment enhanced the learning and memory abilities in Morris water maze and Y-Maze tasks, also facilitates cell proliferation and synaptic density in the hippocampus of rats exposed

to prenatal insults [37]. EE in 2-months-old adult rats leads to reversal of alterations in behavioral and biochemical levels induced by prenatal morphine exposure, a study by Ahmadalipour and Rashidy-Pour [38].

Accumulating evidence shows that physical exercise not only attenuates the ailments from various insults affecting brain but also slow down the progression of neurodegeneration in ageing. The mechanisms by which the physical exercise appears to exert both therapeutic and prophylactic effects on the cognitive deficits in prenatal-inflammation exposed adult offspring are not clearly understood. The treadmill running exercise during adolescent life period alleviates behavioral alterations induced by prenatal morphine exposure in rats [39]. Numerous studies have shown that enhancing effects of physical exercise on the spatial abilities in aged rats [40-44]. Our results showed adolescence treadmill running exercise enhances learning and memory abilities in comparison with the non-exercise group in young adult rats. The results of our study are in-line with these studies. A study by Darkhah et al., showed aerobic exercise facilitates retention phase rather than acquisition phase in MWM task, in a rat model of sleep-deprived stress[45]. Exercise effectively facilitates the acquisition phase of eight-arm radial maze test for spatial learning reported by Anderson et al.,[46].

Although numerous studies have demonstrated the independent beneficial role of the either EE or PE, but the effects of EE in combination with PE on enhancing the spatial learning and memory are scanty. Thus the present study provides important evidence to existing literature that combined effect of adolescent PE and EE are highly beneficial rather than EE or PE alone.

CONCLUSION

The relevance of these findings is in the therapeutic possibilities of instituting the physical exercise with enriched environment especially during the adolescent age helps to maintain physical and mental health. However, this study is not definitive about the reorganization of hippocampal cytoarchitecture in response either to physical exercise or environmental enrichment or both, following prenatal immune challenge. More experiments are warranted to analyse and correlate the histological, biochemical and behavioral outcomes in response to prenatal LPS induced inflammation and conjugated effects of physical exercise and environmental enrichment during the adloscent age.

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