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RESEARCH ARTICLE

Attenuation of Restraint Stress-Induced Behavioral Deficits by Environmental Enrichment in Male Rats

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A B S T R A C T

Post-exposing the rats to enriched environment has shown to attenuate the psycho-neurological and behavioral deficits produced as a result of chronic stress in rats. But here we studied the possibility whether the pre-exposure of enriched environment might affect the behavioral deficits produced as a post-treatment to chronic restraint stress. Behavioral parameters, associated with chronic stress such as depression, anxiety and memory were monitored before and after stress treatment, in socially enriched and socially non-enriched conditions. According to the results the rats that were pre-exposed to socially enriched conditions indicated an anti-depressive and anxiolytic activity after repeated stress procedure as compared to the rats of socially non-enriched environment. Furthermore, improvement and retention of short term and long term memory was observed in enriched rats before and after restraint stress procedure. This study can be related to the beneficial role of earlier exposure to enriched environment that may affect the behaviors related to response of brain to stress stimuli and prevention of behavioral deficits that may lead to various psychological abnormalities that are related to prolonged stress exposures.

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INTRODUCTION

Environmental factors play an important role in neuropsychology and behavior. It has been observed that from an early age, growth in a healthy and stimulating environment has shown improved performance in physical and mental endeavors (Ramey and Ramey, 2004; Schneiderman *et al.*, 2005). On the other hand mild to severe forms of stress, depression and other psychological disorders are linked to stressful and non-stimulating past (McEwen, 2012).

Earlier studies demonstrate that enriched environment consisting of social endeavors and other physical activities, helps in development and improvement of brain functions (Donnelly *et al*., 2016). Enriched environment is designed as an often-changing, colorfully manipulating and socially integrated environment that allows the continuous exercise of the brain in response to changes that occurs in the surroundings and helps in adaptation to the novel stimulus more effectively. All this stimulation and manipulation promotes the process of neurogenesis in brain that results in the formation of new and healthy brain cells and connections that improve the response and adaptation of an individual in various stress inducing situations (Hu *et al*., 2010; Ali *et al*., 2015; Garthe *et al*., 2016). Biologically stress activates the central nervous system to initiate the mechanism involving certain array of hormones and neurotransmitters such as corticosterone, adrenocorticotropic hormone (ACTH) and serotonin that produces a targeted physical and psychological response to cope-up with the sudden changing situation, a phenomena also known as activation of HPA axis of the brain (McEwen, 2007; Spanakis *et al*., 2016).

If stress persists for longer duration, it may affect the functioning of the specific parts of brain that impairs the performance and results in production of some behavioral despairs such as agitation, aggression, anger, anxiety, lack of attention and impairment of learning and memory. Untreated stress may also lead to mild to severe depression, drug dependence and other neuropsycho-logical disorders that require proper medical care and treatment that are both expensive and time consuming

(Keyes *et al*., 2011; Galvan and Rahdar, 2013). The interaction between enriched environment and stressful condition has been identified by many researchers. Animals reared in enriched environment exhibited an improvement in mild stress-induced memory impairment in Morris water maze. This improved performance was not showed by the animals that were raised in impoverished environment. There are studies which report that poor environmental conditions contribute to the development of anxiety and depression due to stress. Rats raised in socially enriched conditions showed better performance in spatial memory task as compared to rats that were raised only in socially interacted environment (Lambert *et al*., 2005; Fuchs *et al*., 2016; Mahmood *et al*., 2016; Melani *et al*., 2017).

Previous studies mainly target the effects of enriched environment in treatment of abnormalities produced as a result of stress, addiction and other neurological disorders. However, the current study emphasizes the contributions of pre-exposure of socially enriched conditions on stress induced behaviors such as depression, anxiety, and disturbance in short term and long-term memory retention. It is hypothesized in this study that early exposure to socially enriched environment may provide potential to cope or adapt with stress in a much better manner as compared to socially non-enriched conditions.

MATERIALS AND METHODS

Animals: Twenty-four locally bred adolescent Albino Wistar rats weighing about 200 g were used in the study. All rats were kept in 12 h light dark cycle and given standard laboratory diet and free access to water during the entire phase of study.

Housing conditions: Animals were divided into four equal groups; control, stress, EE (enriched environment) and EE+stress group (n=6 each), control and stress group were kept socially in standard cages having (59×38×20 cm) dimensions in a group of 3 rats/cage. While EE and EE+stress group were kept socially in a group of 3 rats/ cage in enriched condition that consisted of housing capacity of (120×100×60 cm) and equipped with three to four colorful synthetic toys, tunnels and running wheels. Toys were replaced every twice per week while tunnels and running wheels were kept constant for entire phase of study.

Experimental protocol: Rats were kept in the respective environmental conditions for four weeks. All rats were subjected to behavioral tests using forced swim test (FST), light/dark transition (LTD) test and Morris water maze (MWM) to monitor the depression, anxiety and memory functions, respectively, after 2 weeks and 4 weeks of environmental exposure. After four weeks; rats of stress and EE+stress group were subjected to restraint stress procedure which was followed by same behavioral assessment as done before in the previous weeks. All treatment and behavioral monitoring were done in a balanced design to avoid order and time effect.

Stress procedure: Animals of stress and EE+stress groups were given 2 h/day restraint stress for seven consecutive days in ventilated closed plastic tubes that allowed only mild lateral movements (Delaney *et al*., 2012).

Behavioral procedures:

Forced swim test (FST): Expression for depression was monitored in glass tank with 56 cm height and 30 cm width. The apparatus is usually filled up with water to a suitable height of 22-24 cm so that the rat is forced to swim but cannot escape the surface of the water. The procedure creates a behavioral despair in rat when it fails to escapes and creates an immobilization posture which is considered as an indicator of behavioral depression (Castagné *et al*., 2011). Total cutoff time in this procedure is 300 s during which total immobilization time for each rat is recorded in seconds.

Light/dark transition (LTD) test: The apparatus consists of two compartments connected with a central wall with a guillotine door. One compartment is dark while the other is bright one. If the rats experience anxiety they tend to stay more in the darker side of the box and enter into light box less often (Bourin and Hascoët, 2003). Hence increase in time spent in light box is considered as an index of anxiolytic activity and recorded during a total cutoff time of 300s for each rat.

Elevated plus maze test (EPM): The dimensions of the apparatus and procedure were same as described previously (Haider *et al*., 2015). The test protocol consisted of training and test sessions during which the transfer latency to reach one of the closed arm with all four paws considered as one entry, was noted for each rat. Short term memory (STM) and long-term memory (LTM) were evaluated during test phase conducted after 60 min and 24 h after training sessions, respectively. The cutoff time was 2 min. Shortened transfer latency during test sessions was taken as an indicator of improvement in memory retention.

Statistical analysis: Statistical analysis for results was done using Bonferrroni test after performing three-way ANOVA using repeated measure design. Values of $P<0.05$ were considered significant.

RESULTS

Effect of environment and stress on depression: Effects of social and enriched pre-exposure before and after restraint stress procedure on swimming activity are shown in Fig. 1. Analysis of data by three-way ANOVA using repeated measure design indicated significant effect of weeks [$F(2,40)=3.824$, $P<0.05$], environmental condition [$F(1,20)=507.159$, $P<0.01$], stress [$F(1,20)=7.539$, $P<0.05$] and significant interaction between weeks × environment [$F(2,40)=69.281$, $P<0.01$], week × stress [$F(2,20)=10.629$, $P<0.01$], environment × stress [$F(1,20)= 6.244$, $P<0.05$], and weeks × environment × stress [$F(2,40)=5.082$, $P<0.05$]. Bonferroni post-hoc test showed that the rats kept earlier in enriched environment exhibited significant and gradual decrease in immobility time after 2 weeks ($P<0.01$) and 4 weeks ($P<0.01$) of enriched exposure as compared to control group. After Restraint stress treatment, a significantly increased immobilization time was observed in stress rats ($P<0.01$) as compared to controls. Whereas, the rats that were kept earlier in enriched environment demonstrated significantly reduced

(P<0.01) depression-like behavior as compared to stress group of rats and control group that were both kept earlier in social environment without enrichment.

Effect of environment and stress on anxiety: Effect of social and enriched pre-exposure and stress treatment on anxiety is shown in Fig. 2. Analyzing with three-way ANOVA with repeated measure indicated significant effect of weeks [F(2,40)=129.349, P<0.01], environmental condition [F(1,20)=49.710, P<0.01], stress [F(1,20)= 18.482, P<0.01] and significant interaction between weeks × environment [F(2,40)=35.188, P<0.01] and weeks × stress [F(2,40)=5.704, P<0.01]. Other interactions were not statistically significant. Bonferroni post-hoc test showed that the restraint stress procedure produced a significant anxiogenic activity in stress group (P<0.05) as compared to control group. Whereas, the rats that were pre-exposed to enriched environment showed significant anxiolytic activity as compared to stressed rats (P<0.01) and control group (P<0.05) that were both kept in social non-enriched environment.

Effect of environment and stress on short term memory (STM) retention: Effect of environment and stress on STM retention is shown in Fig. 3. Analyzing with three-way repeated measure ANOVA indicated significant effect of weeks [F(2,40)=12.748, P<0.01], environment [F(1,20)=191.240, P<0.01], stress [F(1,20)=24.898, P<0.01] and significant interaction between weeks × environments [F(2,40)=47.74, P<0.01], week × stress [F(2,20)=8.107, P<0.01], environments × stress [F(1,20)= 52.866, P<0.01], and weeks × environment × stress [F(2,40)=7.705, P<0.01]. According to Bonferroni post-hoc test, EE rats exhibited a significant improvement in STM retention as compared to control group on week 4 (P<0.01) and week 6 (P<0.05) of environmental exposure. Restraint stress however induced a significant impairment of memory in stress group as compared to controls (P<0.05). Pre-exposure to enriched environment significantly reduced stress induced STM impairment as compared to stress rats (P<0.01) and control rats (P<0.05) that were kept in social environment throughout the experiment.

Effect of environment upon stress treatment and Long-term memory (LTM) retention: Effect of environment and stress on LTM retention is shown in Fig. 4. Analyzing with three-way ANOVA using repeated measure indicated significant effect of weeks [F(2,40)= 12.952, P<0.01], environment [F(1,20)=261.520, P<0.01], stress [F(1,20)=78.741, P<0.01], and significant interaction between weeks × stress [F(2,40)= 10.688, P<0.01]. Other interactions were not statistically significant. According to Bonferroni post-hoc test, EE rats exhibited a significant improvement in LTM as compared to controls on week 2 (P<0.01), week 4 (P<0.01) and week 6 (P<0.01) of environmental exposure. After restraint stress procedure, a significant impairment of LTM was observed in stress group as compared to control group (P<0.05). Meanwhile a significant improvement of LTM retention was observed in EE rats as compared to that of stressed rats after the stress treatment (P<0.05).

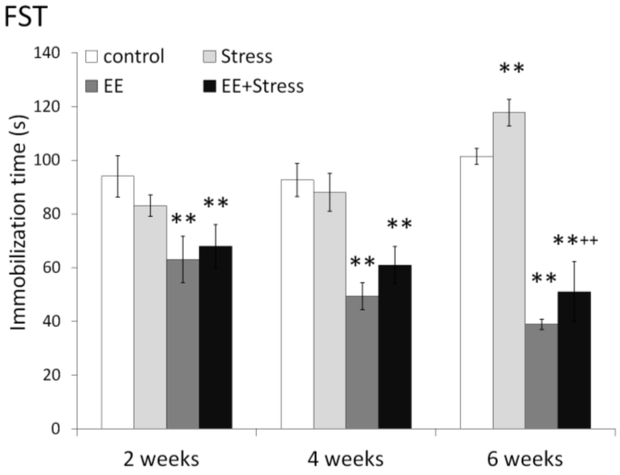

Fig. 1: Effects of environmental enrichment before and after subjecting to restraint stress on forced swim test activity. Values are means±SD (n=6). Data was analyzed by three-way ANOVA with repeated measure design. Significant differences by Bonferroni test: **P<0.01 from respective control animals; $^{++}$P<0.01 from stressed animals.

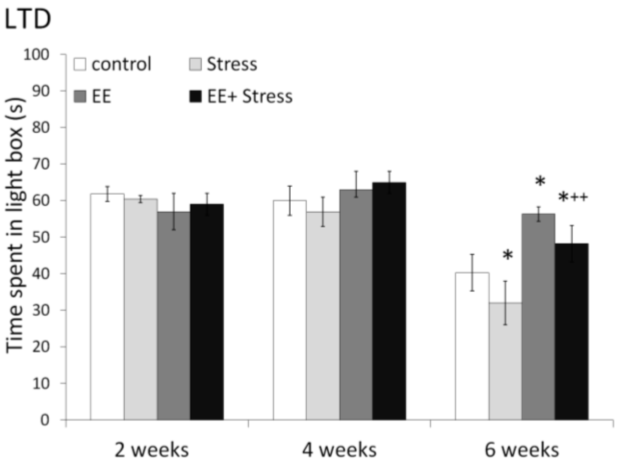

Fig. 2: Effects of environmental enrichment before and after subjecting to restraint stress on anxiety. Values are means±SD (n=6). Data was analyzed by three-way ANOVA with repeated measure design. Significant differences by Bonferroni test: *P<0.05 from respective control animals; $^{++}$P<0.01 from stressed animals.

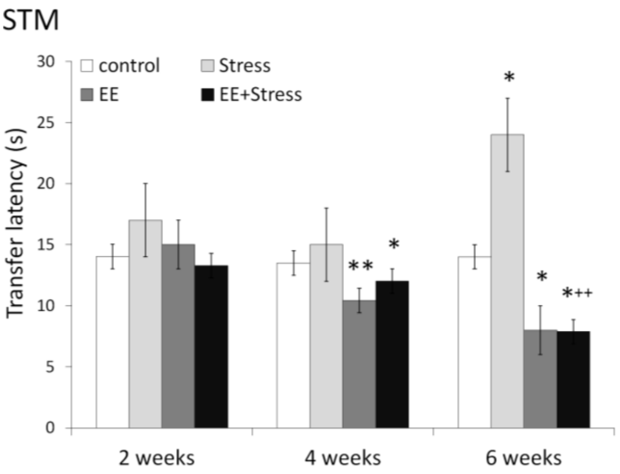

Fig. 3: Effects of environmental enrichment before and after subjecting to restraint stress on short term memory retention. Values are means±SD (n=6). Data was analyzed by three-way ANOVA with repeated measure design. Significant differences by Bonferroni test: **P<0.01 and *P<0.05 from respective control animals; $^{++}$P<0.01 from stressed animals.

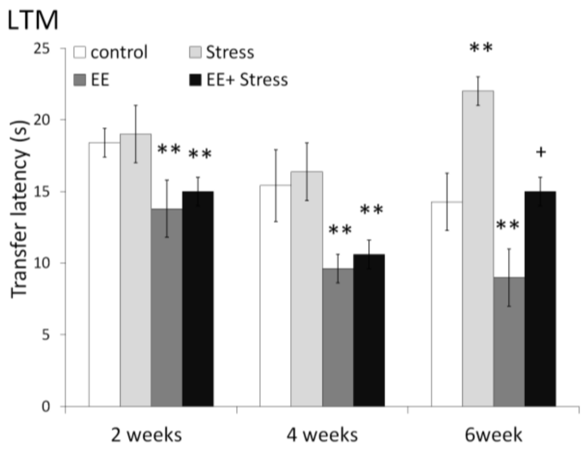

Fig. 4: Effects of environmental enrichment before and after subjecting to restraint stress on long term memory retention. Values are means±SD (n=6). Data was analyzed by three-way ANOVA with repeated measure design. Significant differences by Bonferroni test: **P<0.01 from respective control animals; +P<0.05 from stressed animals.

DISCUSSION

The social and physical factors that make up any environment can influence the individual to behave in certain manners that are different from others. In human and animal studies, different environments have shown to impart some specific properties in brain morphology on genetic and molecular levels (Raymond *et al*., 2010; Mehta-Raghavan *et al*., 2016). Animals that have suffered from some sort of chronic stress, addiction, injury, and trauma and then exposed to enriched environment for sometimes have shown improvement in recovery from these conditions when placed in enriched environment rather than standard or improvised environment (Hoffman *et al*., 2008; Pang and Hannan, 2013).

Depression is considered as the most characterized behavioral deficit produced as a result of prolonged stress. Previous studies suggested that depression is associated with low hippocampal volume. A low functioning and low concentrations of serotonin neurotransmitter either due to insufficient serotonin production or/and down regulation of serotonin post synaptic receptors in brain has also been shown to create a neuropsychological situation as a common cause of depression (Lokuge *et al*., 2011). Our results indicated that pre-environmental exposure of enriched condition produced a significant anti-depressive activity in EE rats as compared to control rats that were pre-exposed to social environment without enrichment before as well as after restraint stress procedure. Enriched environment on the other hand has previously shown to increase both the volume and diversity of the neurons in hippocampus as well as increased the concentration of serotonin on receptor binding site thus acting as an effective anti-depressant therapy in stress model (Brenes *et al*., 2008). Our results here also indicated a significant anxiolytic effect in socially enriched rats when exposed to LTD test for anxiety after the exposure to stress, which is in accordance with previous findings that environmental enrichment has previously shown to help in attenuation of stress induced anxiety mainly by mediating the limbic system of brain (Namburi *et al*., 2015) Hence this can be suggested in light of literature review that the rats that are raised in social along with physical enrichment helps in reducing depression and anxiety produced as a behavioral deficit of restraint stress procedure.

Majority of researchers agree that rearing the animals in enriched conditions improves the cognitive potential and restores memory deficits via activating and enhancing the process of neurogenesis and increased neuronal plasticity of the specific brain cells that are particularly involved in improvement of spatial learning and memory such as pre-frontal cortex, hippocampus and adult rat dentate gyrus (Wright and Conrad, 2008; Clemenson *et al*., 2015). The studies have also shown that enriched environment also helps in restoration of memory deficits produced as a result of aging and neurodegenration due to brain injury, trauma such as psychiatric disorders or chronic stress (Segovia *et al*., 2009; Kovesdi *et al*., 2011). Enriched environment has shown to enhance new cell growth, reduce the stress induced lesions in the brain and increasing the expression of brain derived neurotropic factor mRNA in hippocampus that is associated with improved learning and memory (Cui *et al*., 2006; Fan *et al*., 2016). Our results here also manifested the results of previous findings that exposure of socially and enriched conditions helped the rats in improvement of short term and long-term memory before as well as after restraint stress procedure as compared to controls and stressed rats that were kept in socially non-enriched environment. It can also be emphasized here that physical and manipulative enrichment is necessary in socialized enclosure to extract the maximum benefits to cope up with startle emotional responses related to sudden stress stimulus such as fear and/or anxiety, restoration of normal behavioral and physiological response related to prolonged stress, such as depression and lastly the improvement and restoration of memory processes in adult brain.

Conclusions: Hence, in the light of present study it can be suggested that the introduction of enriched environment from the early life may have significant effects in the attenuation of stress-induced behavioral deficits. This study also emphasizes the significance of socially and physically enriched environment over the social surroundings only. Human populations are exposed to ever increasing, unpredictable and sudden stressors in daily routine life. General health of animals may also be affected due to rearing in impoverished environment. Thus, it can be concluded from the current study that the early exposure to socially interactive and physically enriched environment may produce a significant effect in attenuation of depression, anxiety and impairment of spatial learning and memory produced as a result of psychoneurological behavioral deficits related to stress experienced in later life. A healthy and stimulating environment like environmental enrichment can allow the brain perception to explore new domains and give the strength to manage and deal with sudden adverse stimuli such as stress in a more natural and effective way.

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Authors contribution: ANK was involved in designing of research protocol, conducted all aspects of the laboratory work and participated in writing of various versions of the manuscript. ZB, SA performed detailed statistical analyses and participated in drafting of various papers' version. SA, SK and IS participated in all aspect of laboratory work and various steps of manuscript preparation. LA involved in preparation of various versions of document. HS was the Primary Investigator and conceived, designed and supervised all aspects of the research. All authors contributed to the study and have approved the final draft of the manuscript.

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