

Conference Paper

Effects of Enriched Environment (EE) on Depressive-Like Behavior and Hippocampal Structure in Rat Model of Chronic Stress

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Abstract

Chronic stress is associated with the development of depression. It can trigger structural and neurobehavioral changes in the brain and has been shown to induce depressive-like behavior in animals. An enriched environment can modulate the structure and function of the brain by altering the expression of various genes and proteins as well as affecting neurotransmitters' activity. The hippocampus plays an important role in controlling the networks for mood regulation and has been implicated in the course of depression. This study aimed to investigate the effect of an enriched environment on the depressive-like behavior and hippocampal structure in rats after unpredictable chronic mild stress (UCMS) exposure. Male Wistar rats (*Rattus norvegicus*) were divided into three groups, each consisting of 6 rats including the control, UCMS and UCMS+EE group. Unpredictable chronic mild stress and EE were given for 21 days. Body weight gain, depressive-like behavior, and hippocampal structure were evaluated at the end of the experiment. Depressive-like behavior was assessed with Forced Swim Test (FST) and Sucrose Preference Test (SPT). Thickness of the pyramidal layer of CA1 and CA3 area were measured with histologic examination to see changes in the hippocampal structure. Data were analyzed using One-Way ANOVA or Kruskal-Wallis followed by multiple comparison post hoc test. The enriched environment could significantly maintain body weight gain ($p = 0.036$) and rat's preference to sucrose solution ($p = 0.001$) in a stressful condition. Enriched environment reduced immobility time in FST but it was not statistically significant ($p = 0.177$). There was a significant difference in the thickness of CA1 and CA3 pyramidal layer of the hippocampus among groups ($p=0.015$ and $p=0.019$ respectively). Stress markedly decreased the thickness of CA1 and CA3 pyramidal layer ($p=0.014$ and 0.011 respectively). The enriched environment can ameliorate stress-induced depressive-like behavior and alteration in hippocampal structure in rats.

Keywords: Environmental enrichment, depression, stress, hippocampus

1. Introduction

Stress is one of the main causes of depression [1]. Various kinds of stressor such as physical, psychological and social stressors can induce changes in the neurobiology

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and neurochemistry of the brain which then gives rise to the occurrence of several psychiatric disorders including depression [2-4]. Depression is one of the most commonly found mood disorders. Multiple factors contribute to the development of depression but frequently a stress-related mechanism initiates the process [5, 6].

Hippocampus has been shown to play an important role in cognitive function and mood regulation. In particular, it is reported to be vulnerable to chronic stress and mental disorders [7, 8]. Factors that decrease neurogenesis in the hippocampus such as stress, aging and inflammation were reported to induce anxiety-like behaviour as well as depression like behaviour. Wilner (2005) showed that UCMS led to a significant reduction in the number of newborn neurons in rodents' hippocampus which caused anhedonia and anxiety-like behavior [9]. In addition, several other studies also confirmed that stress in the form of either physical or psychosocial stress and either acute or chronic stress reduced hippocampal neurogenesis which was associated with anxiety- and depression-like phenotype [10]. While Watanabe et al. (1992) [11] and Woolley et al. (1990) [12] demonstrated that atrophy of hippocampal neurons occurred during stress, Sheline et al. (1996) reported that decreased hippocampal volume was found in depressed patients [13].

Interestingly, stress impact occurs most prominently in the ventral pole of the hippocampus which forms a connection to the hypothalamus and the amygdala, two structures responsible for neuroendocrine function and mood regulation. It has also been shown that lesions in the ventral hippocampus alter emotional behavior and stress response. This supports the hypothesis that hippocampus plays an important role in controlling the networks for mood regulation. Together, these results indicated that hippocampus may play a role in the course of depression [10].

A number of pharmacological agents have been used to treat depression. However, the treatments are not fully effective and produce side effects [14]. Goldberg et al (1998) [15] and Fava, M (2003) [16] reported that approximately 50-60% of depressed patients did not fully respond to the therapy. Arroll et al. (2005) [17] also showed that the proportion of patients who did not respond completely to first-line anti-depressant namely selective serotonin reuptake inhibitors (SSRIs) such as paroxetine (PRX) and fluoxetine (FLX) was relatively high. In addition, the side effects also limit the usage of anti-depressant. A study revealed that only 7% of patients remained taking anti-depressant while most of the patients stopped the medication due to the occurrence of side effects [18]. These reports suggest that there is a need to find a new strategy for treating depression. Therefore, researchers have tried to identify non-pharmacological

approach such as enriched environment (EE) in an attempt to find a more effective therapy for depression.

An enriched environment is a modified housing condition created by incorporating social and physical stimuli in which animals receive a variety of novel stimuli every 2-6 days [19]. It can modulate the brain's structure and function by altering the expression of various genes and proteins as well as affecting neurotransmitters' activity [19, 20]. In the field of neuropsychiatry, research about EE has been well documented. It is reported that EE can slow the onset of dementia, improve cognition and have a potential to be used as anti-depressant, anti-epilepsy as well as anti-oxidant [21-23]. This is due to the fact that EE can influence physical and psychological condition by altering the biochemical, physiological and behavioral response [24]. Therefore, EE can be an alternative for treating neuropsychiatric disorders [25]. Considering this fact, we aimed to investigate the effect of EE on the depressive-like behavior and hippocampal structure in rats after UCMS exposure.

2. Methods

2.1. Animal and grouping

Eighteen male Wistar rats (*Rattus norvegicus*) aged approximately 6 weeks and weighed around 100 grams were used in this study. Rats were put in standard cages with free access to water and food. They were randomly divided into three groups including the control group (C), stress group receiving unpredictable chronic mild stress (UCMS) and stress group receiving UCMS along with EE treatment (UCMS+EE). Each group consisted of 6 rats. The Medical Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia had approved all experimental procedures used in this study (Ethical Clearance number 508/VI/HREC/2016). We made every effort needed to minimize the suffering of the animals.

2.2. Stress model

Various kinds of stressor were used in this study including swimming in cold water (10°C) for 4 minutes, predator noise (30 minutes), cage tilting at 45° (4 hours), continuous cage shaking (10 minutes), overnight illumination, damp sawdust (5 hours), tail pinch (2 minutes), food deprivation (24 hours) and water deprivation (24 hours). Two kinds of stressor were given at different time each day for a period of 21 consecutive days. For

example, on day 1, rats were exposed to cold swimming at 8 a.m and predator noise at 2 p.m while on day 2, 45° cage tilting was given at 10 a.m followed by overnight illumination starting at 6 p.m. The type and time of exposure varied day by day over the period of the experiment thus making the stress exposure chronic and unpredictable.

2.3. Enriched environment

The procedure for EE was modified from a previous study [19]. Various kinds of enrichment were applied in the enriched cage. As compared to standard cages, the enriched cage had a larger width (80 cm x 55 cm x 45 cm). The cage was equipped with a variety of toys such as a small ball, toy car, slide, mirror, and tunnels that were frequently changed day after day. Running wheel and ladder step were provided to stimulate the voluntary physical activity of the rats. To facilitate more social interaction, the rats were put in a larger group. The environment was also made more complex with aromatherapy and more varied food including, fruits, vegetables and certain kinds of Indonesian traditional foods.

2.4. Experimental procedure

Prior to treatment, rats were adapted to the new environment for 7 days. On day 8, rats' body weight was measured. Then, stress was given to UCMS and UCMS+EE group for 21 days from day 8 to day 28. EE was given along with the stress exposure. Upon completion of the treatment procedure, body weight measurement was repeated and then the behavioral test was performed. Forced swim test was conducted on day 29 while the sucrose preference test was done the following day. Finally, rats were sacrificed using cervical dislocation technique and then, the brain was collected for histologic examination.

2.5. Hippocampal structure

The hippocampal structure was assessed by comparing the thickness of the pyramidal layer of *Cornu Ammonis* 1 (CA1) and CA3 area of hippocampus in each group. After necropsy, the brain was prepared for histologic examination. Staining was performed using Hematoxylin and Eosin (HE). OLYMPUS CX21 light microscope with 400 X magnifications was used to capture the images. Image Optilab Pro6.1 software was used to assess the images.

2.6. Behavioral test

2.6.1. Force swim test (FST)

A transparent cylinder (30 cm diameter, 35 cm height) was filled with water (25°C) to a depth of 20 cm. Rats were put into the cylinder for 6 min. Immobility time was measured during the last 4 minutes of the test period. Immobility time was defined as the period during which rats floated without any motion and only made small movements required to maintain their heads above the water.

2.6.2. Sucrose preference test (SPT)

Rats were habituated to two similar bottles. One bottle was filled with water while the other was filled with 1% sucrose solution. On day 30, SPT was conducted where the rats were free to choose between the two bottles for 24 hours. There was no food or water deprivation prior to SPT. Sucrose preference was determined by comparing the volume of water and sucrose consumed.

2.7. Statistical analysis

Data were reported as a mean \pm standard error of the mean (SEM). Data on FST and SPT were analysed using One-Way ANOVA followed by Bonferroni Test; CA1 thickness with One-Way ANOVA followed by Tukey HSD Test while CA3 thickness and body weight gain were analysed with Kruskal-Wallis Test followed by Post Hoc Mann Whitney test with SPSS for Windows Release 22.0. $p < 0.05$ was set as the significance level.

3. Results and Discussion

3.1. Depressive like behaviour and body weight gain

Effect of chronic stress on the depressive-like behavior was assessed with FST and SPT. Force swim test is one of the most common assays to assess depressive-like behaviors in rodents. The behavioral despair is reflected by the immobility time during the test [26]. Anhedonia, one of the core symptoms in depression, can be defined in rats by assessing sucrose consumption in the SPT [26]. In this study, the stress group showed the longest immobility time in the FST test as compared to other groups. EE tend to decrease the duration of immobility time. However, this reduction is not statistically significant ($p =$

0.177). In the SPT test, a statistically significant difference was found among groups ($p = 0.001$). Subsequent Bonferroni Post Hoc test revealed that, as compared to the control group, stress exposure significantly reduced sucrose preference ($p=0.000$) and EE could maintain sucrose preference markedly ($p=0.027$). This result is consistent with previous finding showing that EE could ameliorate stress-induced depressive-like behaviors in rats [27]. In addition, other studies also reported that EE could maintain rat's preference to sucrose [25, 28]

The Kruskal-Wallis test showed that there was a significant difference in the body weight gain among groups ($p = 0.036$). Body weight gain was significantly lower in the stress group as compared to control group ($p=0.027$) and EE could markedly recover the weight loss ($p=0.048$). Body weight gain was measured to see physical changes induced by stress [29]. Our finding was consistent with a previous report showing that rats exposed to chronic stress had significantly lower body weight as compared to control [5]. Stress can affect body weight gain due to the fact that in a stressful condition, there was increased activity of the Hypothalamic Pituitary Adrenal (HPA) axis and the sympathetic nervous system. The raised activity subsequently influenced the activity of certain hormones that regulate appetite such as ghrelin and leptin [29]. Stress-induced over-activity of HPA axis also elevates the level of adrenocorticotrophin-releasing hormone and cortisol so that glucose metabolism rate becomes higher. This hormone also increases protein catabolism in skeletal muscles and bone leading to reduced body mass [30]. Our study showed that EE attenuated weight loss caused by stress exposure. This is in line with a previous finding by Zeeni et al. (2015) which reported that in an enriched environment, rats could gain more body weight as compared to those living in standard cages [29].

TABLE 1: Results of FST, SPT and Body Weight Gain Measurement in each group. Data are expressed as mean \pm SEM.

Group	FST (second)	p	SPT (ml)	p	Body Weight Gain (gr)	p
C	8.50 \pm 2.790	0.354 ^b	57.00 \pm 8.075	0.000 ^b	32.50 \pm 7.500	0.000 ^b
UCMS	16.83 \pm 3.331	0.081 ^a	17.67 \pm 3.353	0.000 ^a	10.83 \pm 3.745	0.027 ^a
UCMS+EE	12.83 \pm 2.810	0.057 ^a	33.33 \pm 4.145	0.027 ^a	20.83 \pm 2.007	0.048 ^a
^a as compared to control group						
^b as compared to stress group						

3.2. Hippocampal structure

There was a significant difference in the thickness of CA1 and CA3 pyramidal layer of hippocampus among groups ($p=0.015$ and $p=0.019$ respectively). Stress markedly decreased the thickness of CA1 and CA3 pyramidal layer ($p=0.014$ and 0.011 respectively). Enriched environment tended to restore the reduction in the thickness of hippocampal CA1 and CA3 pyramidal layer. Data on the measurement of CA1 and CA3 pyramidal layer thickness are presented in Table 2.

TABLE 2: Thickness of CA1 and CA3 pyramidal layer of the hippocampus in each group. Data are expressed as mean \pm SEM.

Group	CA1 pyramidal layer (um)	<i>p</i>	CA3 pyramidal layer (um)	<i>p</i>
C	3.671 \pm 0.274	0.014 ^b	5.908 \pm 0.065	0.011 ^b
UCMS	2.783 \pm 0.135	0.014 ^a	4.638 \pm 0.286	0.011 ^a
UCMS+EE	3.170 \pm 0.129	0.093 ^a	5.770 \pm 0.357	0.929 ^a
^a as compared to control group				
^b as compared to stress group				

Several brain regions are the most vulnerable area to stress, including the hippocampus [31]. Stress can alter the structure and function of the hippocampus [32]. The hippocampal damage occurs through several mechanisms such as neuronal remodeling and decreased neurogenesis [33]. Structural changes in the hippocampus may play a role in the development of depression as shown in a report that depressed patients have reduced hippocampal volume [13]. EE has been shown to attenuate structural damage in the CA3 area of the hippocampus as seen from the degree of dendritic retraction [20]. Our study showed that stress exposure caused a marked reduction in the thickness of CA1 and CA3 pyramidal layer of the hippocampus. This structural change can be restored with EE. As can be seen in Table 2, the thickness of CA1 and CA3 pyramidal layer of EE group had increased reaching almost the same level as the control group.

4. Conclusion

EE could ameliorate depressive-like behavior and reduction in the thickness of hippocampal CA1 and CA3 pyramidal layer induced by unpredictable chronic mild stress in rats.

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