Int J Clin Exp Med 2017;10(7):9963-9975
www.ijcem.com /ISSN:1940-5901/IJCEM0047256

Original Article

Enriched environment treatment remediated hippocampal monoamine neurotransmitters and emotional deficits in offspring induced by maternal chronic stress rat during pregnancy

Su-Zhen Guan[1], Wen-Jing Ji[2], Yu Jiang[3], Li Ning[3], Yu-Long Lian[4], Ji-Wen Liu[3]

Departments of [1]Social Medicine, [3]Occupational Health and Environmental Health, College of Public Health, Xinjiang Medical University, Urumqi, P. R. China; [2]Department of Pharmacy Administration and Clinical Pharmacy, School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an, Shaanxi, P. R. China; [4]Department of Occupational Health and Environmental Health, College of Public Health, College of Medical, Nantong University, Nantong, Jiangsu, P. R. China

Received September 26, 2016; Accepted March 28, 2017; Epub July 15, 2017; Published July 30, 2017

Abstract: Early life environment affect the normal development of the mood in the process of infant development and increase the risk of mental disorder diseases. The aim of current study was to investigate enriched environment can repair emotional deficits in offspring rats induced by maternal chronic stress during pregnancy, explore the molecular mechanisms from the changes of monoamine neurotransmitters, dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine; 5-HT) in the hippocampus. A rat model of maternal chronic stress during pregnancy was mating from 3rd day during subjected to chronic unpredictable mild stress (CUMS). Offsprings were weaned on day 21 and housed under either standard or enriched environment 30 days. Plasmacorticosterone levels of maternal rats and their offspring were determined by radioimmunoassay. The body weights were recorded in PND 21 and PND 50. Emotional responses were tested using open-field test (OFT), sucrose preference test (SPT) and tail of suspend test (TST). The levels of DA, NE and 5-HT in hippocampus of all experimental animals were detected using enzyme-linked immunoassay kit. Results indicated that an elevation was observed in the plasma corticosterone level of rat model of maternal chronic stress during pregnancy. Enriched environment treatment reduced offspring's plasma corticosterone level and improved their body weight, changed the emotional function, increased the level of hippocampal NE, DA and 5-HT in OPS group. Collectively, these findings disclose that increasing of hippocampal monoamine neurotransmitters and decreasing of corticosterone in offspring after enriched environment might be the possible cause for emotional dysfunction induced by maternal chronic stress rat during pregnancy.

Keywords: Enriched environment, hippocampus, monoamine neurotransmitters, emotional deficits, maternal chronic stress rat during pregnancy

Introduction

A recent study showed that 6% of pregnant women been in high levels of psychological stress, such as depression, anxiety, anger, day-to-day challenges, sudden change of environment, or social isolation [1]. Maternal stress during pregnancy has been implicated as one of the risk factors for development of affective disorders in the offspring [2].

A large number of epidemiological evidences have indicated that a variety of adverse maternal stress during pregnancy outcomes appears: the increased of the maternal plasma cortisol levels, birth rate of low birth weight babies and preterm [3-6]. It may also cause an increase in disruption of sleep patterns, reduction of deep sleep time, abnormality in sleep time, and prolongation of crying time [7-9]. In addition, it can also have an effect on children's later growth and lead to weaker attentiveness in school-age children [10], emotional and behavioral problems and poor learning and memory abilities [11]. It has been found that attention deficit and hyperactivity disorder of 9~11 years

old children were related with maternal stress during pregnancy [12, 13]. The experiments in rodents have established that prenatal stress is associated with cognitive deficits in spatial learning and memory of offspring [14-16]. Abnormal adrenal responses, some physiological changes, and higher excitability of offspring are caused by maternal prenatal stress [17, 18].

From the middle of the last century, a great effort has been made to elucidate the brain areas involved in emotion control and in the pathophysiology of mood disorders. Animal and human studies have indicated the involvement of the limbic system-including the hippocampal formation, cingulate gyrus, and anterior thalamus [19, 20]. These structures are connected in two main networks: the "orbital" and the "medial prefrontal networks". The medial network is probably more significant for mood disorders [21]. Hence, hippocampus as a part of the limbic system has a major role in mood regulation. It is innervated with serotonergic, noradrenergic and other neurotransmitter systems. However, high level of corticosteroids after stress is very easy to damage the hippocampus, the reason is that contains high concentration of adrenal cortical hormone receptor [22]. It is an important structure associated with anxiety, depression and mood, which may lead to disruption of neurotransmitters [23]. However, currently, the hippocampus loop on emotion regulation mechanism is not clear.

To our knowledge, only a few studies have investigated the effects of pregnancy stress on offspring's brain monoamine neurotransmitters involved in emotional functions. The neurotransmitters viz. catecholamines [dopamine (DA) and norepinephrine (NE)] and serotonin (5-hydroxytryptamine; 5-HT) play critical role in emotions, sleep, arousal and cognitive function [24-27].

As is known to all, the central nervous system structure is influenced by genetic factors and many external conditions. Studies have shown that early life environment can affect the normal development of the mood in the process of infant and child development and increase the risk of mental disorder diseases. Therefore, if we could choose a kind of environmental stimulation in early of life, it may improve mood change for offspring due to maternal prenatal chronic stress.

Enriched environment (EE) is defined as a combination of complex inanimate and social stimulations [28]. It involves alterations to the animal's home cage or secondary exploratory area which provide enhanced sensory, motor, cognitive and potentially social opportunities. A large space within which the animal experiences exploration and introduction to a variety of objects, varying in shape, size, weight, smell and texture, renders stimulation of visual, somatosensory, and olfactory systems. It has been demonstrated to enhance long-term potentiation in the hippocampus, as well as improve emotion performance [29]. Alterations in these monoamine neurotransmitters in brain can be correlated with emotion disturbances.

The monoamine systems are probably also very dynamic, as the modification of behaviors and emotion has to be rather rapid and able to adjust to changes in the environment. However, the underlying mechanisms of emotional alternation in animals with enriched environment treatment remain largely unknown. The purpose of the current study was to test the hypothesis that EE can repair emotional deficits of offspring rats induced by maternal chronic stress during pregnancy. Meanwhile, we aimed to further explore its underlying molecular mechanisms from the changes of monoamine neurotransmitters in the hippocampus.

Materials and methods

Animals

Twenty female adult Wistar rats weighing 240~270 g and fifteen male Wistar rats weighing 300~350 g (all supplied by the Animal Laboratory Center of Xinjiang Medical University, Urumqi, Xinjiang, China; experimental animals certificate number: SCXK (new), 2011-000), sexual maturity, were randomly allocated into seven cages (5 rats in each cage, male and female apart) after acclimatizating for a week. All rats were maintained under standard laboratory conditions (12 h light/dark cycle, temperature 21-23°C, relative humidity 45-65%, and food and water ad libitum) during one week.

Chronic unpredictable mild stress (CUMS) procedure

The CUMS procedure followed a previously described method [30] with minor modifications.

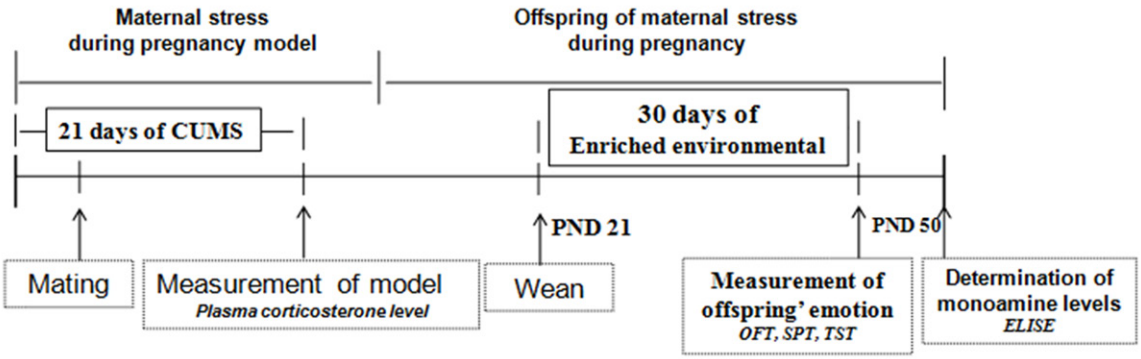

Figure 1. Experimental procedure. (CUMS: Chronic unpredictable mild stress; OFT: Open-field test; SPT: Sucrose preference test; TST: Tail of suspend test; PND 21: Postnatal day 21).

Chronic unpredictable mild stress consisted of exposure to the following stressors in a random order every day for 3 weeks: food deprivation for 24 h; water deprivation for 24 h; cage tilt (45, 7 h); noise housing (1500 Hz, 92 db, for 1 h); behavioral restriction for 4 h; forced swimming for 1 h in 31°C water; squeezing tail for 1 min; shaking stress (one-second, 30 min); hot stress in oven (42°C, 5 min); soiled cage (200 ml of water poured into the bedding, 8 h). One of nine different stressors was randomly administered each day. During the process, the model rats were moved into another room (light intensity and temperature of two room were basically the same), then back to the room after the stimulation.

Treatments of maternal chronic stress rat during pregnancy

The maternal chronic stress rat during pregnancy was mating from 3rd day during subjected to CUMS. Twenty female adult Wistar rats were randomly allocated into two groups (10 rats per group), namely a prenatal control group (PC group) and a prenatal stress model group (PS group). Before gestation, PS group was mated with a male in one cage, two rats in PC group was mated with a male in one cage. The vaginal wall of all female rats were examined every morning and pregnancy was confirmed by sperm positivity, designated gestational day 0 (GD 0), then divided male and female rate. After gestation, each group also had 10 rats, PC rats were housed 5 per cage (1 per cage after GD 18), while PS rats were housed individually (1 per cage). When the mating of PS rat, every stress factors didn't suspend. All rats were maintained under standard laboratory conditions (**Figure 1**).

Measurement of CUMS model through plasma corticosterone level

Blood samples (1 ml) from angular vein were collected from the rats on the day before stress (Baseline) and then on the 1st, 7th, and 14th day. Plasma was used for determination of plasma corticosterone level. Blood samples were centrifuged (3000 rpm for 20 min at 4°C), and the plasma obtained was stored at -35°C. Corticosterone was measured using a radioimmunoassay (RIA) kit in accordance with the manufacturer's instructions supplied by them manufacturer (Coat-A-Count, Diagnostics Products Corporation). The plasma corticosterone levels were derived from the determined cortisol values using the following conversion formula: Corticosterone concentration = Cortisol concentration × 50 [31].

Grouping of the offspring

The day that offspring were born was designated postnatal day 0 (PND 0). The offspring were weaned from their mothers at PND 21, male and female offspring separated. They remained undisturbed and were divided into four groups: two groups from offspring of PC (OPC, n=16, 8 males vs. 8 females; OPC&EE, n=16, 8 male vs. 8 female), two groups from offspring of PS (OPS, n=16, 8 male vs. 8 female; OPS&EE, n=16, 8 male vs. 8 female). All experimental offspring were reared in the same room with free access to water and food. Cages were located in a temperature and humidity con-

trolled room, under day/night cycle (lights on between 07:00 and 19:00).

Enriched environmental conditioning

OPC and OPS but none EE treatment offspring were housed in standard cages (n=4/per cage, 60 cm L × 40 cm W × 25 cm H). And the EE treatment offspring were housed in the large cages (n=8/per cage, 90 cm L × 50 cm W × 60 cm H), with one extra level constructed of galvanized wire mesh and connected by ramps of the same material to create two interconnected levels [28]. The EE cages contained wood shavings, a running wheel, a shelter, plastic color toys and small constructions such as chain, swing and tunnels. The shelter and running wheel were kept in the cages, while the toys and constructions were changed once a week. Also once a week, the feeding boxes and water bottles were moved to different cage points to encourage foraging and explorative behaviors. Each group was housed under those respectively conditions through all experiments until tested (older than 51 days of age, PND 21-PND 50).

Measurement of offspring' emotion

Open-field test (OFT): The open field test was performed at PND 51. An open-field method was used to conduct praxeological scoring in the four groups of rats. The open-field device was made of opaque materials with a 80 cm × 80 cm square, located at the bottom, which was divided equally into 25 equilateral squares. Around, there was a wall with a height of 40 cm. The rat was put in the central square, and the number of squares the rat traversed in 3 min was recorded (only the squares on which the rat landed with four legs could be numbered as the score of horizontal activity) and the duration of standing on hind limbs was noted. Each rat was measured once for 3 min. A score was given by each of the two observers and the aver-age value was taken. The percentage time spent in this central zone is considered indicative of exploratory behavior and may reflect a decrease in anxiety, although this parameter is not sensitive to all anxiolytics and may not model certain features of anxiety disorders [32].

Sucrose preference test (SPT): In the sucrose preference test, the animals were allowed to consume water and a 1% sucrose solution for 1 h after food and water deprivation for 20 h, following 48 h of exposure to both water and sucrose solution. The positions of the two bottles (right/left) were varied randomly across animal sand were reversed after 30 min. The sucrose preference was calculated according to the following ratio: sucrose preference (%) = [sucrose intake (g)/sucrose intake (g) + water intake (g)] × 100%. The results reflect mean values of daily tests over three days [33].

Tail of suspend test (TST) [34]: A computerized device (Itematic-TST) developed by Item-Labo (France) was used to measure the total sum of periods of immobility. Mice were suspended by the tail using adhesive scotch tape, to a hook connected to a strain gauge that picked up all the movements of the mouse and transmitted them to a central unit that calculated the total duration of immobility during a 6-min test. Data collected were consisted of immobility time and the number of rising. Four animals were tested simultaneously.

Brain tissue sample preparation

By intraperitoneal injection of chloral hydrate anesthesia, the hippocampus was removed quickly from brain, placed on a freezing aluminum dissection stage, bisected midway between the septal and temporal poles, then rapidly frozen in liquid nitrogen and dissected on ice-cold glass plates, frozen on dry ice and stored at -80°C.

Determination of the monoamine levels: For the measurement of DA, 5-HT and NE levels, the frozen hippocampus tissue was thawed at room temperature, then add 10 times volume of acid n-butyl alcohol to do hippocampus tissue homogenate slurry and were centrifuged (4000 rpm for 30 min), take on the clear liquid for the measurement of DA, 5-HT and NE levels. DA, 5-HT and NE were measured using a Enzyme-linked immunoassay (ELISE) kit in accordance with the manufacturer's instructions supplied by manufacturer (CSB-E08660r, CUSABIO; CSB-E08364r, CUSABIO; CSB-E08847, CUSABIO), respectively.

Statistical analysis

Data were analyzed with SPSS software (version 17.0); all graphs were constructed in GraphPad Prism 5.0. All variables expressed as

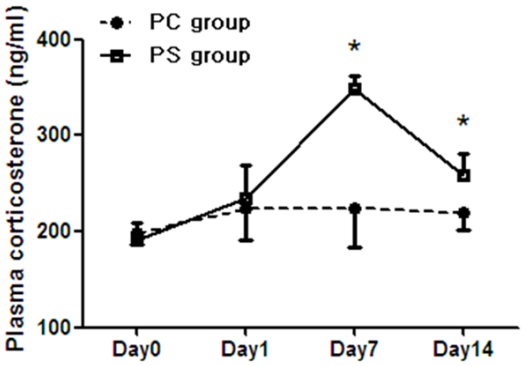

Figure 2. Comparison of plasma corticosteronelevel between CUMS and control group. The figure shows that changes in plasma corticosterone level were noted after maternal chronic stress during pregnancy model is established successfully. Data were analyzed using repeated measurement analysis of variance. Each data represents mean ± SD. Number of animals in each group =10. **P*<0.05 vs. PC group.

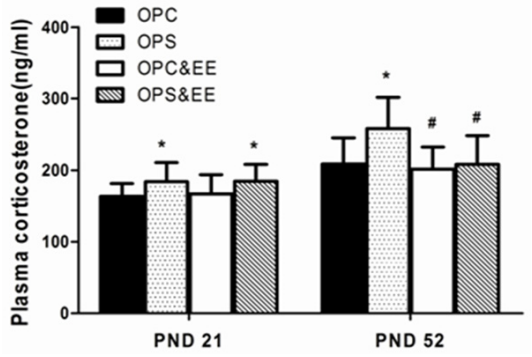

Figure 3. Comparison among OPC, OPS, OPC&EE and OPS&EE in plasma corticosterone level. Differences in corticosterone level were detected among OPC, OPS, OPC&EE and OPS&EE. The plasma corticosterone level is the highest in OPS group, the lowest in the OPC&EE group. Data were respectively analyzed using One-way ANOVA, followed by LSD-t test to make comparison at the two different groups. Each data represents mean ± SD. Number of animals in each offspring group =16 (50% male, 50% female) **P*<0.05 vs. OPC. #*P*<0.05 vs. OPS.

mean accompanied by standard deviations. Differences of plasma corticosterone level between the PS and PC group were analyzed using repeated measurement analysis of variance, if Mauchly's test of sphericity was significant, sphericity was not assumed and the Greenhouse-Geisser adjustment was used. The difference among OPS, OPC, OPS&EE and OPc&EE in OPT, SPT and TST performance and hippocampal monoamine neurotransmitters were analyzed using one-way analysis of variance (ANOVA) followed by LSD-t test to make comparison between the two different groups. *P* value less than 0.05 was considered to be statistically significant.

Results

CUMS elevates plasma corticosterone level of maternal stress during pregnancy

The repeated measurement analysis of variance showed that chronic stress had a significant impact on the circulatory corticosterone level (F=14.604, P=0.001), corticosterone level of the PS group drastically changed with stress time (F=62.609, P<0.001), additionally, there was an interaction relationship between time and stress factors (F=5.467, P=0.002). LSD-t test revealed plasma corticosterone level of the PS group rose to the peak value and higher than that of the PC group following exposure to stress for 7 days (t=3.261, P=0.004). However, the plasma corticosterone level of the PS group declined after being subjected to stress for 14 days, but still remained higher than that of the PC group (t=5.235, P<0.001), indicating that the PS group was in a stressful state. Plasma corticosterone levels did not differ between rats in the PC group and the PS group at the time of the baseline (t=0.971, P=0.344) and after exposure to stress for 1 day (t=0.734, P=0.473) (**Figure 2**).

Enriched environment treatment recovered offspring's plasma corticosterone level induced by maternal chronic stress during pregnancy

High plasma corticosterone level of offspring can be used as an index of physiological response for prenatal stress. To examine the offspring's plasma corticosterone level induced by prenatal stress in the different groups, we found a significant effect for four groups [$F_{(PND\ 21)}$=2.871, P=0.044]. Specifically, significantly less exploration of plasma corticosterone level was observed in the OPS and OPS&EE groups compared with OPC (P<0.05, respectively) using LSD-t test. After enriched environment, we found a significant effect for four groups [$F_{(PND\ 50)}$=5.336, P=0.003], LSD-t test revealed plasma corticosterone level of the OPS group was higher than that of the OPC, OPC&EE and OPS&EE group (all P<0.05). As shown in **Figure 3**, plasma corticosterone level is the highest in OPS group, the lowest in the OPC&EE group, indicating that the OPS group

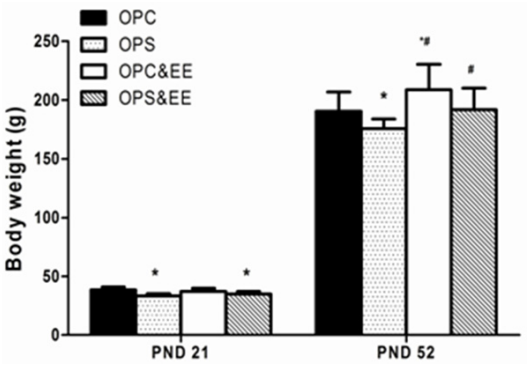

Figure 4. Comparison among OPC, OPS, OPC&EE and OPS&EE in body weight. Differences in body weight were detected among OPC, OPS, OPC&EE and OPS&EE. The body weight is the lowest in OPS group, the highest in the OPC&EE group and the OPS&EE is higher than OPS. Data were respectively analyzed using One-way ANOVA, followed by LSD-t test to make comparison at the two different groups. Each data represents mean ± SD. Number of animals in each offspring group =16 (50% male, 50% female) $^{*}P<0.05$ vs. OPC. $^{\#}P<0.05$ vs. OPS.

was in a stressful state and it will be recovered by enriched environment (**Figure 3**).

Enriched environment treatment recovered offspring's body weights induced by maternal chronic stress during pregnancy

In order to investigate if enriched environment might redress the growth retardation in offspring, we measured pups body weight at the time of PND 21 and PND 52. One-way ANOVA revealed a significant decreased in OPC and OPC&EE group [$F_{(PND\ 21)}$=4.230, P=0.009]. Our results showed that enriched environment treatment restored the changes of body weights in OPS and OPS&EE groups compared with OPC (P<0.05, respectively) using LSD-t test. After enriched environment, we found a significant effect for four groups [$F_{(PND\ 50)}$=6.492, P=0.001], LSD-t test revealed plasma corticosterone level of the OPS group was higher than that of the OPC, OPC&EE and OPS&EE group (all P<0.05). Seeing from the data, body weights is the highest in OPC&EE group, the lowest in the OPS group, indicating that the OPS group was in a stressful state and it will be increased by enriched environment (**Figure 4**).

Enriched environment treatment changes the emotional function of offspring induced by maternal chronic stress during pregnancy

Enriched environment treatment improves horizontal and vertical movements in OPS rats: Rats raised horizontal and vertical movements on this task after the enriched environment. One-way ANOVA revealed that enriched environment boosted the horizontal and vertical movements in the OPS&EE and OPC&EE group (F=24.458, 5.862; P=0.001, 0.00). Significant differences between the OPS group and the other three groups were observed at baseline and after stress (P<0.05). Both horizontal movement (**Figure 5A**) and vertical movement (**Figure 5B**) in the OPS group were lower than those of other groups (P<0.05). At the same time, the horizontal movement and vertical movement of OPS and OPC were improved by enriched environment, prompting environment enrichment increased offspring's mobility and curious about the new environment (**Figure 5**).

Enriched environment treatment improves sugar consumption in OPS rats: One-way ANOVA revealed that enriched environment significantly improved pure water consumption (**Figure 6A**), sugar water consumption (**Figure 6B**) and 1% sucrose preference (**Figure 6D**) in the OPS and OPC rats (F=4.841, 8.375, 8.620; P=0.020, 0.003, 0.003), it showed that enriched environment was good for fluid consumption index of offspring rats due to maternal stress during pregnancy. However, we did not observe a significant interaction on total liquid consumption (**Figure 6C**) between these groups (F=0.363, P=0.781). Pure water consumption, sugar water consumption and 1% sucrose preference is the lowest in OPS group, the highest in the OPC&EE group, indicating that sugar water consumption and offspring's pleasure will be recovered by enriched environment (**Figure 6**).

Enriched environment treatment changes immobility time and the number of rising in OPS rats: A significant interaction between immobility time (**Figure 7A**) and the number of rising (**Figure 7B**) was found that enriched environment significantly improved in the PS and PC rats (F=6.861, 4.059; P=0.001, 0.011), it showed that enriched environment was good for immobility time and the number of rising of offspring rats due to maternal stress during pregnancy. OPC&EE and OPS&EE group rats spent more time on immobility than the OPC and OPS group rats. At the same time, the number of rising is the fewest in OPS group. The results indicated that immobility time and the number of rising will be recovered and depression of OPS reduced by enriched environment (**Figure 7**).

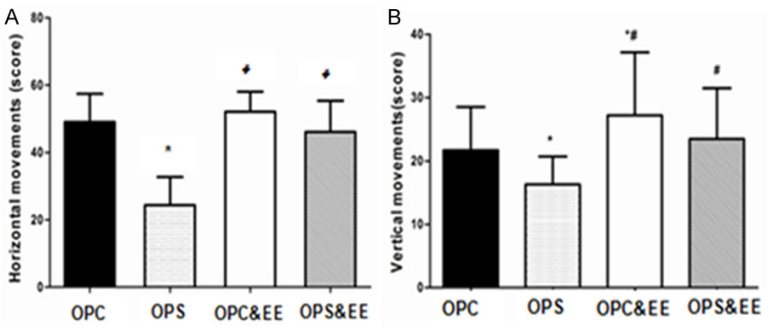

Figure 5. Comparison of behaviors in open-field test among OPC, OPS, OPC&EE and OPS&EE. Differences in horizontal movement (A) and vertical movement (B) were observed among OPC, OPS, OPC&EE and OPS&EE. The score of horizontal movement and vertical movement is the lowest in OPS group, the highest in the OPC&EE group and the OPS&EE is higher than OPS, at the same time, and the OPC&EE of vertical movement score is higher than OPC. Data were respectively analyzed using One-way ANOVA, followed by LSD-t test to make comparison at the two different groups. Each data represents mean ± SD. Number of animals in each offspring group =16 (50% male, 50% female) $^{*}P<0.05$ vs. OPC. $^{\#}P<0.05$ vs. OPS.

Enriched environment treatment improves hippocampal monoamine neurotransmitters in OPS rats: There were significant differences in concentrations of NE (**Figure 8A**), DA (**Figure 8B**) and 5-HT (**Figure 8C**) of hippocampus among four groups (F=60.550, 62.791, 68.448; all P<0.001). OPS exposure reduced the total levels of NE, DA and 5-HT, while EE treatment had a trend of increasing in concentrations of NE, DA and 5-HT of hippocampus. The OPS group had lower concentration as compared with the other 3 groups. In addition, EE treatment increased the level of NE, DA and 5-HT in OPS and OPC group (**Figure 8**).

Discussion

It is well known that many factors can lead to physical malformations or behavioral dysfunctions during pregnancy. It also can provoke dramatic developmental retardations-such as the case of maternal chronic stress during pregnancy. The newborns after birth remain very sensitive to the action of the environment. Meanwhile, pre- and post-natal environments have negative effects on behavior and emotion. Environmental factors seem to play a key role in brain and behavioral development, both in humans and animals [35].

Because of difficulties inherent in human research, the effects of prenatal stress have been examined most extensively in animal models, and especially in rat. Indeed, different types of stressful procedures applied to pregnant rodents are well documented and have been shown to produce numerous biological and behavioral dysfunctions in both dams [28] and pups [36, 37]. In all of these studies, the animals were subjected to stressors chronically, either during the entire pregnancy [38, 39]. In this study, we chose chronic unpredictable mild stress (CUMS) model, originally developed by Paul Willner and colleagues, is widely used rodent model of depression produced by stress which entail repeated exposure to an array of varying and unpredictable, mild stressors over a sustained period of time (ranging from 10 days to 8 weeks). We started mating experiment during 3rd day in 21 days period. It was shown that high secretion of corticosterone, a stress hormone which is a glucocorticoid and the primary end product of the hypothalamic-pituitary-adrenal (HPA) axis in rodents [40, 41] in response to stress. Our experimental data showed a higher plasma corticosterone level of rats in the PS group than that of PC rats, indicated that the model of maternal chronic stress during pregnancy was established successfully.

Fortunately, a number of events can act positively during the neuro-development of pups (e.g., enriched environments). The concept of Enriched Environment (EE) was first described by Hebb [42]. Typically, a group of several animals (8-12) is placed in a large cage with a number of objects that are frequently removed and replaced by others. Thus, EE consist of enhanced social interactions and of considerably more opportunities for interactions with non-social stimuli. In contrast, standard environments (SE) consist of standard laboratory cages in which 4 to 5 animals are housed together. Note that the terms “enriched” and “standard” are clearly relative, however, there are great differences between both types of environments. Indeed, animals reared in EE are almost permanently forced to explore the environment while this is not the case for animals

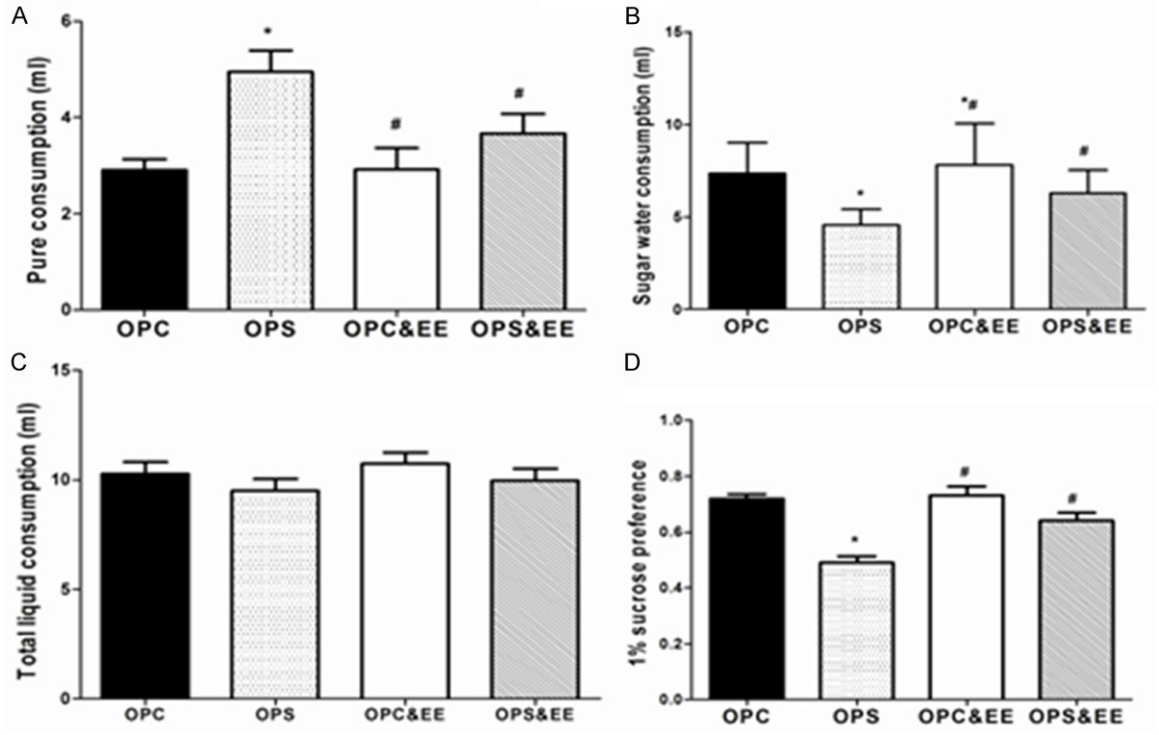

Figure 6. Comparison of behaviors of liquid consumption test among OPC, OPS, OPC&EE and OPS&EE in Body weight. Differences in pure water consumption (A), sugar water consumption (B) and 1% sucrose preference (D) were observed among OPC, OPS, OPC&EE and OPS&EE. The pure water consumption is the highest in OPS group, the lowest in the OPC&EE group and the OPS&EE is lower than OPS; the change of sugar water consumption and 1% sucrose preference among them is on contrast. There isn't a significant interaction on total liquid consumption between these groups (C). Data were respectively analyzed using One-way ANOVA, followed by LSD-t test to make comparison at the two different groups. Each data represents mean ± SD. Number of animals in each offspring group =16 (50% male, 50% female) $^{*}P<0.05$ vs. OPC. $^{\#}P<0.05$ vs. OPS.

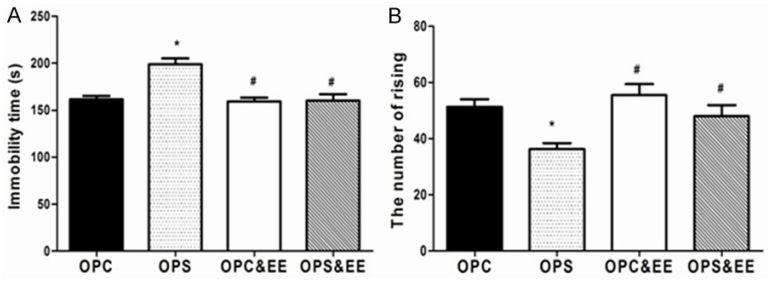

Figure 7. Comparison of behaviors in the tail of suspend test among OPC, OPS, OPC&EE and OPS&EE. Differences in immobility time (A) and the number of rising (B) were observed among OPC, OPS, OPC&EE and OPS&EE. The immobility time is the longest in OPS group and the OPS&EE is shorter than OPS, the number of rising is just the opposite. Data were respectively analyzed using One-way ANOVA, followed by LSD-t test to make comparison at the two different groups. Each data represents mean ± SD. Number of animals in each offspring group =16 (50% male, 50% female) $^{*}P<0.05$ vs. OPC. $^{\#}P<0.05$ vs. OPS.

reared in SE. Most studies on the influence of differential rearing conditions have been concerned with biological differences between animals reared in EE and SE. EE was found to rescue abnormal behaviors, such as emotional reactivity and spatial learning, as well as motor skills deficits, induced by maternal chronic stress during pregnancy [16, 43, 44].

In addition, it was shown that high secretion of corticosterone in response to stress, in the prenatal stressed animals, can be reversed by postnatal EE treatment. Specifically, how this manipulation may affect the offspring's ability to cope with a stressful experience after birth. Short- and long-term alterations in the offspring elicited by prenatal stress are usually thought to be mediated through physiological disturbances in

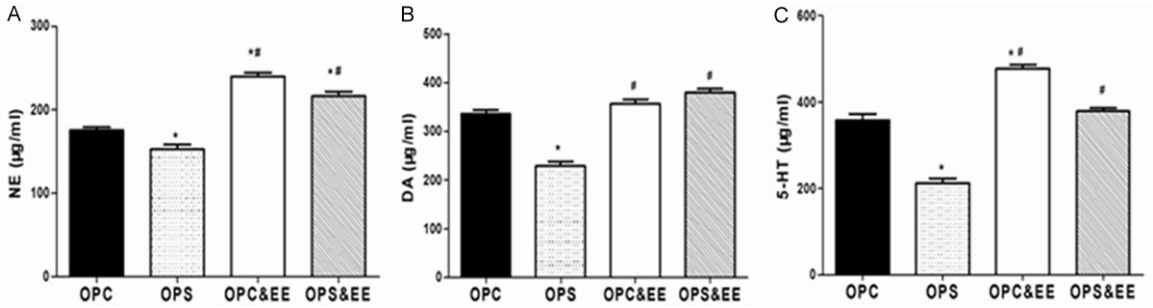

Figure 8. Comparison of hippocampal monoamine neurotransmitters among OPC, OPS, OPC&EE and OPS&EE. Differences in concentrations of NE (A), DA (B) and 5-HT (C) of hippocampus were observed among OPC, OPS, OPC&EE and OPS&EE. EE treatment increased the level of NE, DA and 5-HT in OPS and OPC group. Data were respectively analyzed using One-way ANOVA, followed by LSD-t test to make comparison at the two different groups. Each data represents mean ± SD. Number of animals in each offspring group =16 (50% male, 50% female) $^{*}P<0.05$ vs. OPC. $^{\#}P<0.05$ vs. OPS.

the dam that are likely to result in fetal distress. Indeed, it is known that corticoid secretion is increased by stress [45]. We also found that birth weight of OPS was lower than that of OPC. Therefore, it is conceivable that physiological disorders following stress of the pregnant rat greatly altered fetal growth when occurring early during the gestational period. These disorders had only mild effects on fetal growth when they occurred later. One explanation would be that increased plasma corticosterone of mothers and their offspring, which biological function can curb weight growth, accelerate protein decomposition and suppress synthesis of it [46, 47], and affect sugar and lipid metabolism, eventually leading to weight growth of OPS be slower than of OPC. More fortunately, all weight growth of OPC and OPS were improved by environmental enrichment, especially in OPS group. Concerning central nervous system anatomical changes, exposure of animals to EE leads to an increase of total brain weight.

Prenatal stress, not only affects the behavior of the animals in their physical environment, but also their social behavior mainly through increased fearfulness and more defensive behavior. This study used open field test (OFT), sucrose preference test (SPT) and tail of suspend test (TST) to observe the effect of maternal chronic stress rat during pregnancy on emotions. OFT, a procedure for measuring the activity of a rat or other small animal by placing it in an enclosed area of floor space, divided into squares, and counting the number of squares that it crosses in a specified time period, could record locomotors activity and exploratory behavior as a major tool to detect depression and anxiety [32]. SPT, since 1985 when Steru et al. introduced the immobility of the subject has been quantified either manually by a trained observer during direct observation (subjective scoring) or automatically using devices which utilize a strain gauge to detect the movements of the subject (objective scoring) [33], is thought to represent anhedonia and a core symptom of major depression. This anhedonic behavior is commonly assessed in rats. TST is also a commonly used screening depression in mice, assessment of their mood status. Their simultaneous presence represents a strong argument towards the occurrence of depressive-like phenotype in rodents and the assessment of their mood status [48].

This study showed that offspring of maternal stress during pregnancy were poor performance in these three tests on emotion, therefore, offspring of maternal stress during pregnancy will tune more depressive than of their mother who didn't have chronic stress during pregnancy. Meanwhile, our study found that depressive-like behaviors was redressed after living in enriched environment, for example, the horizontal and vertical movements of OFT, sugar water consumption of SPT, immobility time and the number of rising of TST are recovered by enriched environment in the OPS group. Together, our data highlighted that environment enrichment increased the emotional reactivity of the offspring, including mobility, curious about the new environment, and rat's pleasure. It's the same as other reports, most studies have demonstrated that prenatal stress pro-

duces behavioral changes in the offspring such as decreased ambulation in an open field and increased latency to enter the open arms of the elevated plus maze [49]. Several studies, however, have reported opposite trends-that prenatally treated animals have shorter latencies to enter an anxiogenic area and are more active in a novel situation than untreated controls. Our results are consistent with a recently published article by Rosenfeld and Weller [50], regarding an increase in anxiety- and depressive-like behaviors in the prenatal EE adult offspring. As a whole, we can assume that animals reared in EE display a lower level of emotional reactivity than those reared in standard conditions. Then, EE could differently modify, by means of neurobiological modulations, the various aspects of emotional reactivity. Studies presented here confirm the very important effects of EE on emotional behaviors.

In this study, we focused on discussing the molecular mechanism underlying emotional behaviors in the offspring of maternal stress during pregnancy after EE from the monoamine neurotransmitters. There is a growing body of evidence suggesting a crucial role for the amygdala and other limbic structures (e.g., hippocampus) in the synthesis of information and control of behaviors and emotions [21]. In humans, the important role of the monoamine systems in regulating emotions and behavior is illustrated. These structures handle the processing of emotion-eliciting information and trigger certain emotions in certain situations, and are projecting towards the monoaminergic-nuclei which serve to deliver the message, the emotion to the whole brain [19, 20]. In other words, the monoamine transmitter systems might form one final path way for the simultaneous delivery of emotional information to large and dispersed areas of the brain. Many studies from different research fields support the belief. The most important monoamine neurotransmitters are serotonin, noradrenaline and dopamine, which share many properties. that all three of the monoamines, serotonin (5-HT), dopamine (DA) and norepinephrine (NE) are essential in several psychiatric disorders such as depression, psychosis, attention-deficit hyperactivity disorder, anxiety, and behavioral disturbances. It is reported by Hugo Lövheim that as long as none of the monoamines transmit exactly the same information as any other (which seems unlikely) [51], there will still be a three-dimensional space, although each monoamine neurotransmitter represents a different aspect of emotion should not, however, be interpreted to mean that the monoamines are independent. The study provides two important findings: 1. Significant reduction in levels of monoamine neurotransmitters (DA, NE and 5-HT) in hippocampus of maternal chronic stress rat during pregnancy' offspring; 2. Enriched environment treatment increased the level of NE, DA and 5-HT in OPS group. Overall, the results of present study indicated that exposure to maternal stress during pregnancy induces alterations in hippocampal monoamine neurotransmitters (play role in emotion functions), which might be the possible cause of emotional dysfunction. Anxious/depressive-like behaviors as well as altered hippocampal monoamine neurotransmitters (DA, NE and 5-HT) in adult mice [52]. Another factor be worth considering was that EE decreased corticosterone of offspring resulting from maternal stress during pregnancy, known to be involved in the etiopathology of depression [53].

In conclusion, the results obtained in the present study provide strong evidence for extreme sensitivity of enriched environment treatment repaired emotion deficits induced by maternal chronic stress rat during pregnancy. Thus, it is suggested that alterations caused in increasing of hippocampal monoamine neurotransmitters and decreasing of corticosterone of offspring after enriched environment. In view of these findings and to investigate other factors involved in emotional dysfunction of offspring induced by maternal chronic stress rat during pregnancy, further study is required.

Acknowledgements

This work was supported by Grants from Youth Science Fund of Xinjiang Uighur Autonomous Region (2013211B50). The funding agency did not play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

Address correspondence to: Ji-Wen Liu, Department of Occupational Health and Environmental Health, College of Public Health, Xinjiang Medical University, Urumqi 830011, P. R. China. Tel: 86-9914365004; Fax: 86-9914365004; E-mail: liujiwendr@163.com

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