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Research report

Environmental enrichment and chronic restraint stress in ICR mice: Effects on prepulse inhibition of startle and Y-maze spatial recognition memory

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ABSTRACT

In most studies regarding the improving or therapeutical effects induced by enriched environment (EE), EE was performed after the stress treatment or in patients with certain diseases. In the current study, the effects of chronic restraint stress (6 h/day) in mice living in an enriched environment or standard environment (SE) were tested. Mice were randomly divided into 4 groups: non-stressed or stressed mice housed in SE or EE conditions (SE, stress + SE, EE, stress + EE). Prepulse inhibition (PPI) of startle was tested after the 2 weeks or 4 weeks stress and/or EE treatment and 1 or 2 weeks withdrawal from the 4 weeks treatment. After the 4 weeks treatment, spatial recognition memory in Y-maze was also tested. The results showed that EE increased PPI in stressed and non-stressed mice after 2 weeks treatment. No effect of EE on PPI was found after the 4 weeks treatment. 4 weeks chronic restraint stress increased PPI in mice housed in standard but not EE conditions. Stressed mice showed deficits on the 1 h delay version of the Y-maze which could be prevented by living in an enriched environment. Our results indicated that living in an enriched environment reversed the impairing effects of chronic restraint stress on spatial recognition memory. However, EE did not change the effects of stress on PPI.

1. Introduction

Repeated stress is an important risk factor for the development of mental disorders. Chronic stress produces depressive-like symptoms, such as anhedonia, anxiety and neophobia (for review, see [1]). Chronic stress caused by isolation rearing has received considerable attention as an animal model of sensorimotor gating deficits in schizophrenia [2,3]. Chronic stress altered hippocampal structure and resulted in cognitive deficiency. Enhancement of corticosterone levels by restraint stress resulted in impaired performance of spatial learning [4]. Three weeks of daily 6 h restraint impaired spatial memory in male rats tested on the 8-arm radial maze [5] and the Y-maze [6].

Lots of studies showed that environmental enrichment (EE) had positive effects on the brain and brain function (for review, see [7]). EE improves memory, early stimulation by EE leads to improved learning abilities in various learning tasks. EE has beneficial effects in psychiatric and neurodegenerative disorders, such as schizophrenia, depression, Huntington's, and Parkinson's diseases (for review, see [8]). EE ameliorates the impairing effects of stress. Living in an enriched environment during adolescence reversed the effects of prenatal stress on social play behavior and HPA axis reactivity in rats [9]. EE treatment after early life stress (limited nesting/bedding materials) prevented learning and memory impairments in rats [10]. A recent research showed that exposure to enriched environment (EE) following restraint ameliorated the depressive symptoms caused by stress and restored the survival and differentiation of the progenitor cells in the dentate gyrus (DG) [11]. These findings indicated that EE might be used to prevent the impairing effects of postnatal or acute stress on mnemonic processes. However, in all these researches, EE treatment was done posterior to the termination of stress treatment. As to the humans, many people live a both stressful and environmental enriched life. Thus, the effects of stress in animals living in enriched environment need further research.

Prepulse inhibition (PPI) of the startle response is a measure of inhibitory function and time-linked information processing by which a weak sensory stimulus (the prepulse) inhibits the startle response caused by a sudden intense stimulus [12–14]. PPI is commonly viewed as an operational measure of a process

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called "sensorimotor gating", by which excess or trivial stimuli are screened or "gated out" of awareness, so that an individual can focus its attention on the most salient aspects of the stimulus-laden environment [15]. Several psychiatric disorders, such as schizophrenia, show impaired sensorimotor gating, expressed as reduced PPI [15–17].

Y-maze is a simple 2-trial recognition test for measuring spatial recognition memory. It is based on the innate tendency of rodents to explore novel environments [18]. This paradigm avoids the effects of punishment (such as electric shock) or reward (such as food) that is commonly used in avoidance and other memory paradigms and may have non-specific effects on the results. In addition, it does not require learning of a rule. Thus, it is useful for studying memory in rodents [18–20].

The purpose of the current study was to investigate if chronic restraint stress affects behaviors differently in EE housed and standard housed mice. Male ICR mice were restrained daily, 6 h a day, housed in EE or standard environments. After 2 or 4 weeks, mice were tested for PPI. PPI were also tested 1 and 2 weeks withdrawal from the 4 weeks treatment to study if there were long lasting effects of stress and/or EE treatment on PPI after the treatment stopped. A spatial recognition memory version of the Y-maze was tested after the 4 weeks treatment. We hypothesized that chronic stress-induced sensory gating and spatial learning memory changes might be less in EE housed than standard housed mice.

2. Methods and materials

2.1. Animals

144 weaned male ICR (Imprinting Control Region) mice (11–15 g body weight, at age of 3 weeks) from Experimental Animal Institute Sichuan Academy of Medical Science were used. Animals were housed on a 12-h light/dark cycle with light on from 07:00 to 19:00 h. They had food and water available ad lib and were allowed to familiarize with the experimenter and adapt to the laboratory conditions for 1 week before the experiment started. The experiments were carried out during the light phase of the cycle. The experiments were conducted in accordance with the guidelines for the National Care and Use of Animals approved by the National Animal Research Authority.

2.2. Housing conditions

Mice were housed in either standard conditions (6 mice per Plexiglas cage, 30 cm × 20 cm × 16 cm, length × width × high) or EE. EE consisted of 6 mice per Plexiglas cage (45 cm × 35 cm × 20 cm), which contained movable (small PVC fittings and nesting material) and immovable objects (tunnels, running wheels and pots). The materials used in EE experiments were cleaned weekly and the moveable and some of the immoveable objects such as pots were put at different places each week.

2.3. Chronic stress

Restraint stress was accomplished by placing the mouse in a 50 ml centrifuge tube (3 cm in diameter and 10 cm in length) that prevented forward and backward movement and limited side-to-side mobility but did not discomfort the animal in any other way. About 50 holes (2 mm in diameter) were made on the side of the centrifuge tube to ensure that the mouse get enough air to breath. Mice were restrained in the tubes for 6 h per day from 09:00 to 15:00 for 14 days (2 weeks treatment, 2-W-T) or 28 days (4 weeks treatment, 4-W-T) and were weighed weekly. Both standard and EE housed mice were returned to their home cages during restraint.

2.4. Prepulse inhibition of the acoustic startle response

Startle measures were performed with a 4-unit automated acoustic startle response testing instrument. Each unit contained a small plexiglas cylinder (4.5 cm in diameter and 8 cm in length) fixed on a platform under which a sensitive sensor was attached. Stimuli were delivered and the startle response signals were sampled by software running on a PC in an adjacent room. During experiments, mice were remained in the cylinders within a sound-attenuating cabinet with 65 dB SPL white background noise. Acoustic stimuli were delivered through a speaker above the cylinders.

In the test session, mice were first placed in the testing cylinder of the instrument for a 5-min acclimation period, during which rats received 10 trials of randomly delivered 115 dB SPL pulse-alone stimuli or prepulsed startle stimuli. After the acclimation period, mice were exposed to 50 trials of randomly delivered stimuli which consisted of 10 trials of 115 dB SPL pulse-alone stimuli, 10 trials during which no stimuli were delivered (NOSTIM), and 30 trials of prepulsed startle stimuli. Prepulsed startle included a single 20 ms 115 dB SPL pulse preceded by 100 ms of a 20 ms white noised non-startling stimulus of 5, 10 and 15 dB SPL over 65 dB SPL background noise (PPI 5, PPI 10 and PPI 15, respectively). Inter-trial intervals (ITI) were randomly assigned to 27–32 s. Percentage of PPI was calculated as [(startle response to the 115 dB SPL startle stimuli–response to pulses with the prepulse)/startle response to the 115 dB SPL startle stimuli × 100].

2.5. The recognition Y-maze test

The behavioral apparatus used in the recognition Y-maze test was the same as we used before [21–23]. Three arms were randomly designated: start arm, in which the mouse started to explore (always open), novel arm, which was blocked at the 1st trial, but opened at the 2nd trial, and other arm (always open). The start arm and other arm were designed randomly to avoid the spatial memory error. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Visual cues were placed on the walls of the maze to differentiate different arms.

The Y-maze test consisted of 2 trials separated by an inter-trial interval (ITI). The 1st trial (training) was 10 min duration and allowed the mouse to explore 2 arms (start arm and other arm) of the maze, with the 3rd arm (novel arm) being blocked. After a 1 h or 4 h ITI, the 2nd trial (retention) was conducted. For the 2nd trial, the mouse was placed back in the maze in the same starting arm, with free access to all 3 arms for 5 min. Using a ceiling-mounted CCD camera, all trials were recorded on a VCR. Video recordings were later analyzed and the number of entries and the time spent in each arm were analyzed. The percentage of time spent in and entries into the novel arms score the spatial recognition memory.

2.6. Experimental procedure

Four separate groups ($n = 12$ for each group) were used in all behavioral tasks: non-stressed mice housed in standard conditions (SE); non-stressed mice housed in EE conditions (EE); chronically stressed mice housed in standard conditions (Stress + SE) and chronically stressed rats housed in enriched conditions (Stress + EE). Behavioral assessment began on the day after restraint ended.

2.6.1. Experiment 1

Effects of 2 weeks EE and/or restrain stress on prepulse inhibition. Mice were started restraint stress and/or EE treatment when 4 weeks old, after 2 weeks EE and/or restrain treatment, PPI were tested.

2.6.2. Experiment 2

Effects of 4 weeks EE and/or restrain stress on prepulse inhibition. Mice were started restraint stress and/or EE treatment when 4 weeks old, after 4 weeks, PPI were tested. PPI were also tested 1 and 2 week's withdrawal from the treatment.

2.6.3. Experiment 3

Effects of 4 weeks EE and/or restrain stress on Y-maze spatial recognition memory. We started restraint stress and/or EE treatment when the mice were 4 weeks old, after 4 weeks, the treatment were stopped and Y-maze spatial recognition memory were tested weekly for 2 weeks. The inter-trial interval (ITI) of the training and testing trials in the 2 tests were 1 h and 4 h, respectively.

2.7. Statistical analysis

Data were presented as mean ± standard error of the mean. All data were analyzed with analysis of variance (ANOVA) with repeated measures when necessary, using SPSS 13.0. Between-group factors were the EE treatment (2 levels: EE housed or SE housed) and the chronic restraint treatment (2 levels: restraint or non-restraint). Within-group factors were prepulse intensity, arm visits or body weights, when analyzing different data forms.

3. Results

3.1. Effects of 2 weeks EE and/or restrain stress on prepulse inhibition

One-way ANOVA with *post hoc* LSD showed that after 2 weeks stress and/or EE treatment, the mean startle amplitudes did not differ between each treatment group (2-W-T: $F(3,44) = 0.06$, $P = 0.98$). Thus, 2 weeks EE and/or stress treatment did not alter the startle responses in mice.

Repeated measure showed that EE increased PPI after 2 weeks treatment (effects of EE: $F(1,44) = 4.06$, $P = 0.005$). *Post hoc* LSD showed a significant higher PPI in non-stressed EE mice or stressed EE mice compared with the standard housed mice (SE vs. EE: $P = 0.039$; SE vs. Stress + EE: $P = 0.023$) (Fig. 1). Stress did not show

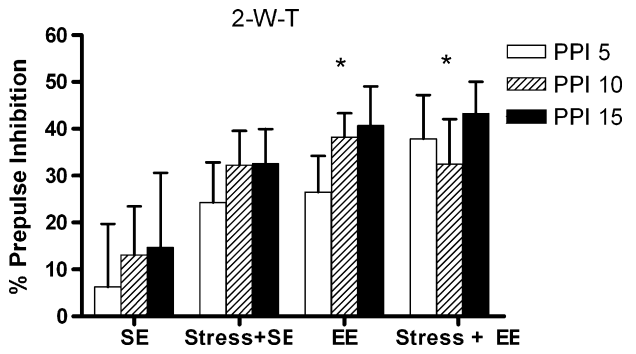

Fig. 1. The effects of 2 weeks restrain stress and/or EE treatment on prepulse inhibition of startle (PPI). 4 weeks old mice were kept free or restraint 6 h per day in standard or EE conditions for 2 weeks. PPI were tested the day after the treatment. Both non-stressed and stressed mice housed in EE conditions increased PPI compared with the controls. $n = 12$ for each group. Data were expressed as mean ± S.E.M. $^{*}P < 0.05$ for difference in PPI compared with control mice.

any effects on PPI after 2 weeks treatment ($F(1,44) = 1.76$, $P = 0.19$). No EE × stress interaction was found ($F(1,44) = 0.98$, $P = 0.33$).

3.2. Effects of 4 weeks EE and/or restrain stress treatment and 1 or 2 weeks withdrawal from the treatment on prepulse inhibition

The mean startle amplitudes of mice did not differ between each treatment group after 4 weeks stress and/or EE treatment ($F(3,44) = 1.25$, $P = 0.30$, One-way ANOVA). 1 or 2 weeks withdrawal from the treatment also did not change the startle amplitudes in mice (1-W-W: $F(3,44) = 0.92$, $P = 0.44$; 2-W-W: $F(3,44) = 0.05$, $P = 0.99$).

After 4 weeks EE and/or stress treatment, combined ANOVA showed no effects of stress or EE on PPI, no interactions between factors were found. However, when compared PPI between stressed and non-stressed mice housed in standard conditions, stressed mice showed increased PPI than their non-stressed counterparts ($F(1,21) = 6.40$, $P = 0.019$) (Fig. 2a).

One-week withdrawal from the 4 weeks treatment, repeated measure showed no difference in PPI between each treatment group (Fig. 2b).

Two weeks withdrawal from the 4 weeks treatment, combined analysis showed a main effect of stress ($F(1,43) = 4.44$, $P = 0.04$) on PPI. The effect of restraint stress was different between EE housed and standard housed mice (interaction of EE × stress $F(1,43) = 3.01$, P =0.01). Restraint stress had no effects on PPI in standard housed mice ($F(1,21) = 0.07$, $P = 0.80$). However, restraint stress increased PPI in EE housed mice ($F(1,22) = 7.40$, $P = 0.01$) (Fig. 2c).

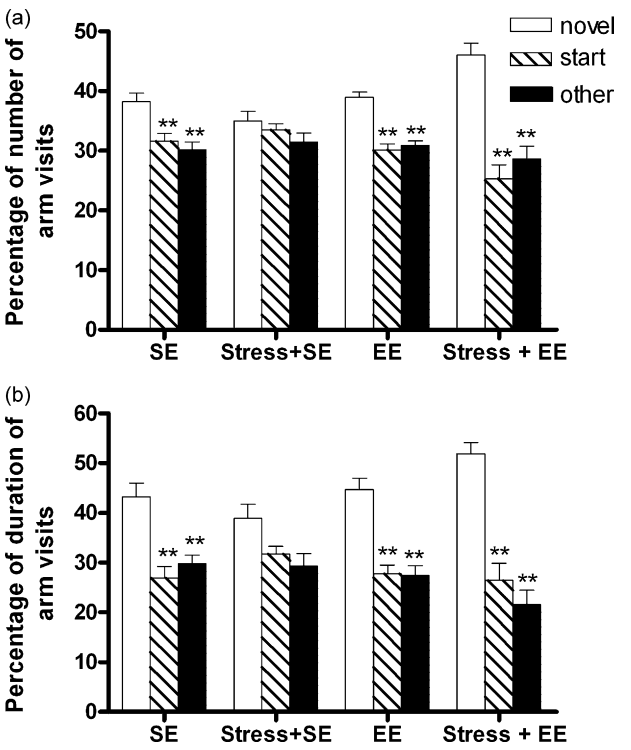

Fig. 3. Effects of 4 weeks restraint stress and/or EE treatment on the spatial recognition memory in Y-maze after 1 h ITI. Panels a and b show the percentage of number and duration of arm visits for mice visiting the novel, start and other arms. Stressed mice housed in standard conditions (Stress + SE, $n = 11$) showed impaired spatial recognition memory while stressed mice housed in EE conditions (Stress + EE, $n = 8$) showed intact spatial recognition memory by exploring the novel arms more often and spent more time in it as the non-stressed mice housed in standard conditions (SE, $n = 12$) and EE conditions (EE, $n = 12$). Data were expressed as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ for difference in performance of mice in the novel arm vs. the start and other arms. Open bar: the novel arm, hatched bar: the start arm, solid bar: the other arm.

3.3. Effects of 4 weeks restraint stress and/or EE treatment on the spatial recognition memory in Y-maze after 1 h ITI

3.3.1. Percentage of number of arm visits in the 5 min retention test

As shown in Fig. 3a, percentage of number of arm visits in the novel arm was significantly increased compared with the start and other arms after 1 h ITI in Y-maze (main effect of arm: $F(2,78) = 38.24$, $P < 0.001$). Combined ANOVA showed a significant stress × arm interaction ($F(2,78) = 9.64$, $P < 0.001$) and

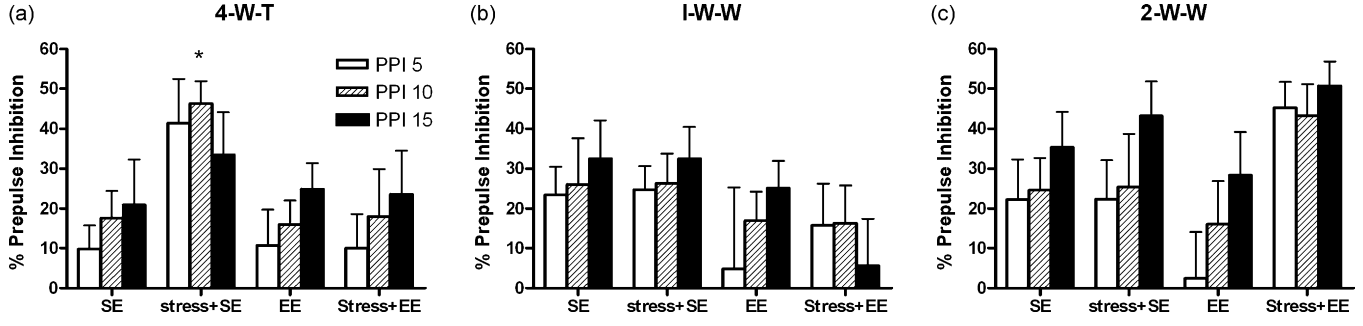

Fig. 2. The effects of 4 weeks EE and/or restrain stress treatment and 1 or 2 weeks withdrawal from the treatment on prepulse inhibition. 4 weeks old mice were kept free or restraint 6 h per day in standard or EE conditions for 4 weeks. PPI were tested the day after the treatment and 1 and 2 weeks withdrawal from the treatment. Panel a shows that after the 4 weeks EE and/or stress treatment, stressed mice housed in standard conditions showed increased PPI than other groups. Panel b shows that when PPI was tested after 1-week withdrawal, no differences were found between treatment groups. Panel c shows that the PPI after 2 weeks withdrawal, stressed mice housed in EE conditions showed increased PPI compared with stressed mice housed in standard conditions. $^{*}P < 0.05$ for difference in PPI compared with EE and Stress + EE mice.

stress × EE × arm interaction ($F(2,78) = 9.64$, $P < 0.001$). No EE × arm interaction was found ($F(2,78) = 1.0$, $P = 0.375$).

When the arm differences in each group were analyzed, percentage of number of arm visits in the novel arm was significantly increased than in the start and other arm in non-stressed mice housed in standard conditions and stressed and non-stressed mice housed in EE conditions (SE: $F(2,20) = 6.67$, $P = 0.006$, novel vs. start: $P = 0.021$, novel vs. other: $P = 0.007$; EE: $F(2,22) = 19.46$, $P < 0.001$, novel vs. start: $P < 0.001$, novel vs. other: $P < 0.001$; Stress + EE: ($F(2,14) = 17.44$, $P = 0.001$, novel vs. start: $P = 0.003$, novel vs. other: $P = 0.004$).

3.3.2. Percentage of duration of arm visits in the 5 min retention test

As shown in Fig. 3b, the percentage of duration of arm visits in the novel arm was significantly increased compared with the start and other arms after 1 h ITI in Y-maze (main effect of arm: $F(2,78) = 45.43$, $P < 0.001$). Combined ANOVA showed a significant stress × arm interaction ($F(2,78) = 4.84$, $P = 0.01$). The interaction of stress × EE × arm was proximal but not significant ($F(2,78) = 2.93$, $P = 0.059$). No EE × arm interaction was found ($F(2,78) = 1.0$, $P = 0.375$).

When the arm differences in each group were analyzed, percentage of duration of arm visits in the novel arm was significantly increased than in the start and other arm in non-stressed mice housed in standard conditions and stressed and non-stressed mice housed in EE conditions (SE: $F(2,20) = 9.47$, $P = 0.001$, novel vs. start: $P = 0.007$, novel vs. other: $P = 0.007$; EE: $F(2,22) = 16.20$, $P < 0.001$, novel vs. start: $P = 0.001$, novel vs. other: $P = 0.001$, Stress + EE: ($F(2,14) = 21.49$, $P < 0.001$, novel vs. start: $P = 0.001$, novel vs. other: $P < 0.001$).

3.4. Effects of 4 weeks restraint stress and/or EE treatment on the spatial recognition memory in Y-maze after 4 h ITI

3.4.1. Percentage of number of arm visits in the 5 min retention test

Percentage of number of arm visits in the novel arm was significantly increased compared with the start and other arms after 1 h ITI in Y-maze (main effect of arm: $F(2,80) = 10.17$, $P < 0.001$). No significant stress × arm, EE × arm and stress × EE × arm interactions were found (Fig. 4a).

3.4.2. Percentage of duration of arm visits in the 5 min retention test

Percentage of duration of arm visits in the novel arm was significantly increased compared with the start and other arms after 1 h ITI in Y-maze (main effect of arm: $F(2,80) = 6.72$, $P = 0.002$). No significant stress × arm interaction, EE × arm interaction and stress × EE × arm interaction were found (Fig. 4b).

3.5. Body weights during the 4-week-treatment and withdrawal

The mean body weight of stressed or non-stressed mice housed in standard or EE conditions during the experiment was shown in Fig. 5a. Mice were weighted weekly. Body weights increased with age (overall effect of age: $F(6,258) = 340.33$, $P < 0.001$). Stressed mice gained weight significantly slower than non-stressed mice (interaction of stress × age: $F(6,258) = 12.79$, $P < 0.001$). Stressed mice living in both SE and EE conditions were lighter than their non-stressed counterparts (overall effect of stress: $F(1,43) = 19.77$, $P < 0.001$). EE housed mice gained weight significantly quicker than mice housed in standard conditions (interaction of EE × age $F(6,258) = 3.09$, $P = 0.006$). However, there was no stress × EE × age interaction.

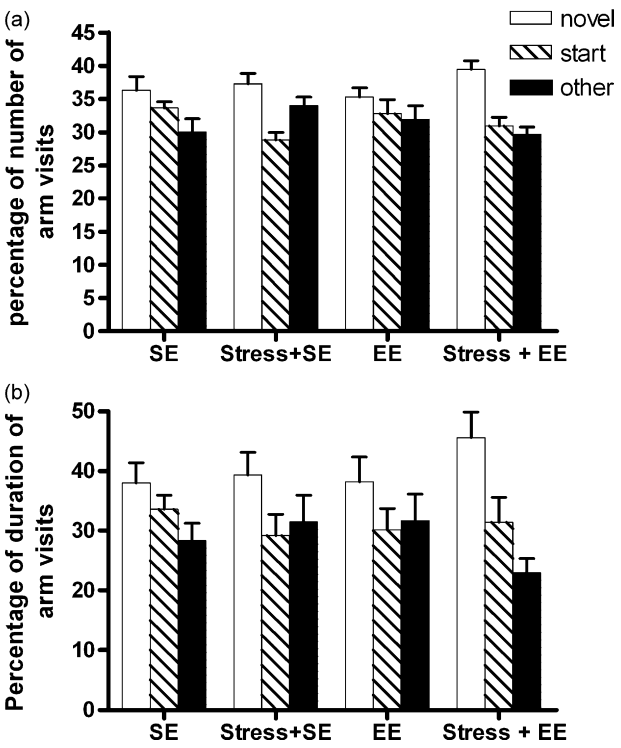

Fig. 4. Effects of 4 weeks restraint stress and/or EE treatment on the spatial recognition memory in Y-maze after 4 h ITI. Y-maze was tested a week after the 4 weeks treatment. Panels a and b show the percentage of number and duration of arm visits for mice visiting the novel, start and other arms. No effects of EE or stress on arm visits were found. Data were expressed as mean ± S.E.M. Open bar: the novel arm, hatched bar: the start arm, and solid bar: the other arm.

One-way ANOVA with *post hoc* LSD showed that when the experiment started, no difference was found in the body weight between each group (Fig. 5b). In mice raised in standard conditions, after 4 weeks restraint stress, stressed mice housed in standard conditions were lighter than their non-stressed counterparts ($P = 0.01$). However, the difference disappeared after 1 week and 2 weeks withdrawal from the stress. In EE conditions, stressed mice showed lower body weights than non-stressed mice ($P = 0.000$) and this difference lasted until 1 week ($P = 0.026$) and 2 weeks ($P = 0.006$) withdrawal from the stress. Mice housed in EE conditions did not show any difference in body weight from mice housed in standard conditions during the treatment and 1-week withdrawal from the treatment, however, EE mice were heavier than any other groups when the experiment finished ($F(3,43) = 4.94$, $P = 0.005$, EE vs. SE: $P = 0.012$; EE vs. Stress: $P = 0.001$; EE vs. Stress + EE: $P = 0.006$).

4. Discussion

In the current study, post weaning mice reared either in EE conditions or standard conditions were chronically treated with restraint stress (6 h per day) for 2 or 4 weeks, prepulse inhibition of startle were tested. PPI were also tested 1-week and 2 weeks withdrawal from the 4 weeks stress and/or EE treatment. We also tested spatial recognition memory in a 2-trial recognition Y-maze after mice received 4 weeks EE and/or stress treatment. The aim of the present study was to investigate if living in an enriched environment would alleviate or reverse the effects of daily stress on sensory gating and spatial recognition memory. Our results showed that environmental enrichment increased PPI both in stressed and non-stressed mice after 2 weeks treatment. However, this effect diminished after 4 weeks treatment and during the withdrawal. 4 weeks restraint stress resulted in increased PPI in standard housed

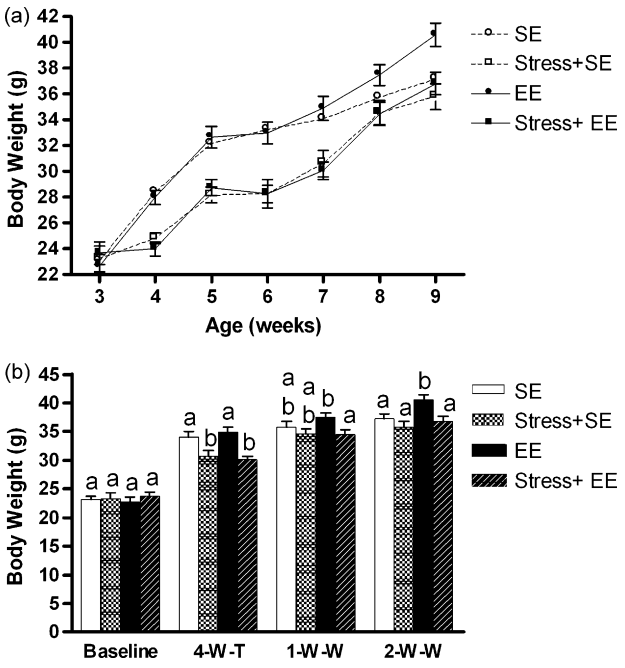

Fig. 5. Effects of restraint stress and/or EE treatment on body weight in mice. Panel a shows the body weight of mice during the experiment. Panel b shows the body weight when the experiment started (baseline), after 4 weeks treatment (4-W-T), a week withdrawal from the treatment (1-W-W) and 2 weeks withdrawal from the treatment (2-W-W). No difference was found in the baseline body weight of each group, stressed mice housed in standard conditions (Stress + SE, $n = 11$) showed lower body weight than their non-stressed counterpart (SE, $n = 12$) after 4 weeks treatment. After 1-week withdrawal from the treatment, stressed EE mice (Stress + EE, $n = 12$) showed lower body weight than non-stressed EE mice (EE, $n = 12$). After 2 weeks withdrawal from the treatment, EE mice were heavier than other mice. Data were expressed as mean ± S.E.M. Body weights indicated by the same letter were significantly different from those with different letters.

but not EE housed mice. In the Y-maze, our results demonstrated that EE prevented the impairing effects of chronic restraint stress on spatial recognition memory.

In the current study, stressed mice housed in standard conditions showed increased PPI after 4 weeks treatment. This was consistent with studies which showed increased PPI after stress treatment. It was reported that prenatal stress (repeated restraint of pregnant mothers) increased PPI in their offsprings [24]. In a threat-of-shock experiment, PPI was increased by shock anticipation which might result from the increase in the general level of alertness that facilitated the processing of the prepulse [25]. Repeated mild foot shock treatment has also been found to increase PPI in rats, which was thought to be due to a decreased sensitivity of the mesolimbic dopamine system [26]. However, some researches reported a disrupted PPI caused by stress [3,27]. Acute stress might increase the release of dopamine in the caudate putamen and nucleus accumbens, brain regions implicated in the regulation of PPI. Overactivity of the dopaminergic system has been hypothesized to contribute to aspects of schizophrenia (for review, see [28]). Thus, the effect of stress on PPI was inconsistent and needed more researches. The procedural differences between experiments, such as different durations and types of restraint, the ages at which stress started and the use of different strains of mice may cause different results. In our study, we used repeated restraint stress which was conducted to adolescent mice and found an increased PPI after 4 weeks restraint stress.

In many studies, enriched environment was found to have no effect on PPI. No significant difference in PPI between EE and control mice was found after being housed in EE for 5 weeks [29]. In rats housed in enriched environment for 8 weeks, they showed normal PPI compared with controls [30]. In the current study, we also found that 4 weeks treatment of EE did not affect PPI. However, increased PPI were found after 2 weeks of EE treatment. We hypothesized that the transient increase of PPI might result from some unknown changes induced by EE which differed between short-term and long-term treatment. EE has been suggested to represent a mechanism of stress inoculation. The repeated introduction of novel objects and the opportunity to explore them is comparable to repeated mild stress exposures [31]. EE is known to activate the hypothalamic–pituitary–adrenal (HPA) axis and usually stimulates glucocorticoids release [32,33]. This slight hyperactivation of HPA axis was probably related to repeated exposure to novel objects and the complex environment [32,34]. In the current study, the activation of HPA axis might less in mice housed in EE for 2 weeks than 4 weeks. Furthermore, studies have found that EE treatment increased novelty seeking behavior in rat [35]. The novelty seeking behavior in mice housed in EE conditions for 2 weeks might be more than mice housed in EE for 4 weeks because of the habituation to the enriched environment. Thus, the mild stress condition and HPA activation in 2 weeks EE housed mice, and the increased novelty seeking behavior and behavioral arousal after 2 weeks when compared with 4 weeks in the EE condition might explain the increased PPI in 2 weeks EE treated mice.

In a recent study, no significant effect of EE on PPI was observed for wild-type mice, however, decreased PPI in a knockout mice line modeling schizophrenia was rescued by enriched rearing [36]. In the present study, we did not find any interactions between chronic restraint stress and enriched environment as the independent variables of PPI, which indicated that stress and EE might affect sensory gating in a different way in the current study.

One week after the EE and/or stress treatment stopped, no differences in PPI were found between each treatment group, which indicated that the PPI changes induced by chronic restraint stress and/or EE treatment would not last for several days. However, the main effect of stress on PPI was found 2 weeks after the treatment stopped. Stressed EE mice showed a significant increased PPI compared with non-stressed EE mice. These results demonstrated that the long-term effects of stress and/or EE treatment after the termination were complicated and how long these effects would exist after the treatment stopped might need further researches.

Environmental enrichment during the peripubertal period has been found to completely reverse the effects of maternal separation on both HPA and behavioral responses to stress [37]. In the current study, EE completely prevented the effects of chronic restraint stress on spatial recognition memory. However, EE did not take any effects in modulating the effects of stress on PPI or body weight. The present data indicated that EE might have some beneficial effects on neural systems involved in spatial memory rather than PPI and body weight. Hippocampus is a well-known brain area associated with spatial memory (for review, see [38]). Hippocampus is always found to be involved in the memory deficits caused by chronic stress because chronic stress produces morphological changes in the hippocampus. Chronic restraint causes retraction of apical dendrites in the CA3 region of the hippocampus [39]. By contract, EE increased the survival of newborn cells in dentate gyrus of hippocampus [40,41]. Decreases of synaptic density in cerebrum in senescence could be prevented by rearing rats under enriched environment [42]. Our results were consistent with the hypothesis that EE could reverse spatial memory deficits induced by chronic restraint stress.

EE has been proved to improve memory. Enriched rats learned the Morris water maze faster than their impoverished counterparts [43,44]. In the current study, EE mice did not show any difference in performing the spatial recognition memory Y-maze task compared with the standard housed mice. This might be due to the learning paradigm we used. Memory deficient could be detected

easily with Y-maze but it might not be sensitive enough to test the improvement of a memory because of a ceiling effects.

During the restraint/EE treatment, the body weights of EE mice did not differ from standard housed mice. However, stressed mice housed in both conditions gained dramatically less weight over the 28-day stress phase than their non-stressed counterparts. This was consistent with studies showing that stress resulted in lower body weights [45]. EE did not interact with stress to affect the body weights of mice. However, after 2 weeks withdrawal from the treatment, EE mice were the weightiest of the all. This indicated that the transition from an enriched environment which involves access to running wheels into standard conditions might result in weight increment in mice.

In most studies regarding the improving or therapeutical effects induced by enriched environment, EE was performed after stress treatment or in patients with certain diseases. For instance, enriched environment treatment in early postnatal periods can cause a recovery from the prenatal stress-induced hippocampal synaptic changes [46]. EE delayed the onset of Huntington's disease symptoms in a mouse genetic model [47]. EE may alter behavioral, cellular and molecular aspects of pathogenesis in a range of transgenic AD mouse models, PD patients have shown significant improvement in motor function as well as increased life span following physical therapy or EE (for review, see [8]). In the current study, we studied the effects of stress in mice housed in EE conditions. Stress and EE took place on the same day which resembled the stressful life of some people living in modern cities. Our results indicated that EE increased PPI in stressed and non-stressed mice after 2 weeks treatment. Living in an enriched environment prevented the impairing effects of chronic restraint stress on spatial recognition memory.

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