Importance of early environment in the development of post-traumatic stress disorder-like behaviors

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Abstract

A number of clinical studies in which early adversities were defined retrospectively, demonstrated that early adverse experiences increased the morbidity rate of post-traumatic stress disorder (PTSD) in later life. However, no prospective studies have yet been conducted to elucidate whether early adversity affects the risk or severity of PTSD. Thus, we examined whether early adversity would strengthen the severity of PTSD symptoms in later life by using neonatal isolation (NI) and single prolonged stress (SPS) as an animal model of PTSD. We measured anxiety-like behavior in the elevated plus maze (EPM), contextual freezing in the contextual fear (CF) test, and analgesia in the flinch-jump and hot-plate tests in four groups of adult rats (sham, NI, SPS, and NI + SPS). NI significantly enhanced the SPS-induced decrease in the percentage of open arm time and open arm entries in the EPM, enhanced the SPS-induced increase in contextual freezing, and strengthened SPS-induced analgesia, without any changes in locomotor activity in the open field locomotor test. In addition, we examined the effect of environmental enrichment (EE). Repeated exposure to EE ameliorated the NI-induced enhancement of contextual freezing, but not anxiety-like behavior or analgesia, in response to SPS. The results of the present study demonstrated that while early adversity strengthened PTSD-like symptoms, EE alleviated the enhanced contextual freezing by NI and SPS. These findings suggest that early adversity may worsen dysfunction of the amygdala and hippocampus in PTSD, and an early intervention may alleviate the early adversity-mediated enhancement of hippocampal dysfunction in PTSD.

Keywords: Neonatal isolation (NI); Post-traumatic stress disorder (PTSD); Environmental enrichment (EE); Elevated plus maze test; Contextual fear conditioning; Analgesia

1. Introduction

Epidemiological studies on post-traumatic stress disorder (PTSD) suggest that early adversity, such as physical abuse or psychological maltreatment, is a major risk factor for PTSD. For example, Widom [39] reported that childhood abuse as well as neglect increased an individual’s risk for subsequent PTSD. In addition, psychoanalysts and child psychiatrists have reported that the mother–child relationship and fostering environment in early childhood strongly affect later mental development and influence the prevalence rate of various psychiatric disorders including PTSD [4,24,40]. However, since these clinical studies were conducted retrospectively and the evaluations of early trauma were based on patient self-reports, it remains uncertain whether early adversity precipitates the onset of PTSD.

Recent behavioral and molecular studies in rodents have demonstrated that early adversity such as neonatal isolation (NI), maternal separation, or low maternal care, is closely associated with the development of stress vulnerability in adolescence and adulthood. A series of studies conducted by Meaney and co-workers demonstrated that low maternal care resulted in decreased negative feedback on the hyperfunctioning of the hypothalamic–pituitary–adrenal (HPA) axis in response to stress through the increased methylation of the promoter of hippocampal glucocorticoid receptors (GRs) [38]. It has been also reported that maternal separation or lack of maternal care results in

Abbreviations: NI, neonatal isolation; PTSD, post-traumatic stress disorder; SPS, single prolonged stress; EE, environmental enrichment; GRs, glucocorticoid receptors; CRF, corticotropin releasing factor; MRs, mineralocorticoid receptors; CREB, cyclic AMP response element-binding protein

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increased corticotropin releasing factor (CRF) mRNA expression in hypothalamic and limbic regions, decreased glucocorticoid receptor density in the hippocampus, decreased neurogenesis in the hippocampus, and decreased GABA$_A$ and central benzodiazepine receptor binding [5,6,22,23,25,30]. Based on these findings, it is postulated that early adverse experiences increase stress vulnerability and the subsequent prevalence of stress-related psychiatric disorders, such as major depression and anxiety disorders.

As the development of appropriate animal models of depression promoted our understanding of the molecular pathophysiology of the disease [27,37], the pathogenesis of PTSD was revealed when an animal model of PTSD involving single prolonged stress (SPS) was developed. Rats subjected to SPS exhibit enhanced HPA negative feedback in response to glucocorticoid administration, which resembles the enhanced suppression of plasma cortisol levels by the dexamethasone suppression test in patients with PTSD [20,21]. In addition, rats exposed to SPS show an exaggerated acoustic startle response, which is similar to the increased arousal observed in PTSD patients [17]. Based on these findings, it has been suggested that SPS could be an appropriate animal model of PTSD. To elucidate whether early adversity increases the severity of PTSD symptoms, we examined whether NI would affect several PTSD-like behaviors in adult rats subjected to SPS.

It has been recently shown that environmental enrichment (EE) has protective effects against stress vulnerability induced by early adversity. It has been reported that EE after weaning alleviates the adverse effects of maternal separation on both HPA axis function and the behavioral response to stress [11]. In addition, EE has protective effects against cognitive deficits and some associated physiological and neural processes [3,31]. Therefore, we also examined whether EE after weaning ameliorates the enhanced severity of PTSD-like behaviors in adult rats subjected to NI.

2. Materials and methods

2.1. Animals

Pregnant female Sprague–Dawley rats were purchased from Charles River (Yokohama, Japan). The rats were housed individually at constant room temperatures (23 ± 2 °C) and humidity (60%) with a 12 h/12 h light–dark cycle (12-h light-12 h dark cycle, lights on at 08:00 h). Food (Rodent Lab Diet EQ 5L37, Japan SLC Inc.) and water, conforming to the Water Quality Standard required by the Japanese Waterworks Law, were provided ad libitum. Male rats in the litters were weaned on postnatal (PN) day 22. After weaning, the rats in all groups except for the NI group were housed in groups of three per cage (38 cm × 23 cm × 20 cm stainless steel cage) and were maintained under normal conditions. The rats in the EE group were housed in groups of two per cage (as described in Section 2.4).

The present study consisted of two experiments. In the first experiment, we examined the influence of NI and SPS on adulthood behaviors (Experiment 1). Rats were randomly assigned to four groups (Sham, NI, Sham + SPS, NI + SPS) to investigate the influences of NI and SPS on adulthood behaviors. The Sham group was left undisturbed. The NI group received NI only on PN days 2–9 without SPS. The Sham + SPS group received only SPS on PN day 56 without NI. The NI + SPS group received NI followed by SPS on PN day 56. Behavioral experiments including the open field locomotor test (Sham: n = 8, NI: n = 8, Sham + SPS: n = 8, NI + SPS: n = 8), elevated plus maze test (Sham + EE: n = 12, NI + EE: n = 14, Sham + EE + SPS: n = 12, NI + EE + SPS: n = 12), contextual fear conditioning test (Sham + EE: n = 10, NI + EE: n = 13, Sham + EE + SPS: n = 12, NI + EE + SPS: n = 13), and flinch-jump test (Sham + EE: n = 12, NI + EE: n = 11, Sham + EE + SPS: n = 12, NI + EE + SPS: n = 11)]. A different set of rats was used for each of the experiments.

In the second experiment, we examined the effects of EE on adulthood behaviors (Experiment 2). In this experiment, eight groups were included: Sham + EE, NI + EE, Sham + EE + SPS, NI + EE + SPS, as well as the four groups described in Experiment 1. The Sham + EE group received only EE, the NI + EE group received both NI and EE without SPS, the Sham + EE + SPS group received both EE and SPS without NI, and the NI + EE + SPS received all treatments. These groups received EE on PN days 22–49. To control for the effect of handling, rats were housed under normal condition (38 cm × 23 cm × 20 cm stainless steel cage) from PN 49 to 56 days. SPS and behavioral experiments (open field locomotor test, elevated plus maze test, contextual fear conditioning test, and flinch-jump test) were performed on PN day 63 (Fig. 2). Different sets of rats were used in Experiments 1 and 2 for each behavioral assessment [open field locomotor test (Sham + EE: n = 8, NI + EE: n = 8, Sham + EE + SPS: n = 8, NI + EE + SPS: n = 8), elevated plus maze test (Sham + EE: n = 12, NI + EE: n = 14, Sham + EE + SPS: n = 12, NI + EE + SPS: n = 12), contextual fear conditioning test (Sham + EE: n = 10, NI + EE: n = 13, Sham + EE + SPS: n = 12, NI + EE + SPS: n = 13), and flinch-jump test (Sham + EE: n = 12, NI + EE: n = 11, Sham + EE + SPS: n = 12, NI + EE + SPS: n = 11)]. A different set of rats was used for each of the experiments.

All animal procedures were approved by the Hiroshima University Medical Science Animal Care Committee.

2.2. Neonatal isolation (NI) treatment

After birth, the pups and mothers were housed together in their home cages (38 cm × 23 cm × 20 cm clear plastic cages) until weaning. Kehoe and Bronzino’s method [16] was used for NI treatment. The first 24-h period after birth was designated postnatal day 1 (PN1). In the NI group, pups were isolated from the dam, nest, and siblings, and placed in individual opaque round containers (7 cm diameter and 8 cm depth) without bedding in a temperature and humidity-controlled chamber for 1 h per day on postnatal days 2–9. Containers were placed 20 cm apart. Isolation was carried out between 9:00 AM and 12:00 noon each day. The other group of rats (Sham group) was housed under normal conditions and left undisturbed, except for cage cleaning, until weaning.
2.5.2. Elevated plus maze test

The test room was dimly illuminated with indirect white lighting. For 20 min before the start of the session, and the testing sessions lasted for 5 min. The test box (50 cm × 32.5 cm) was made of transparent acrylic resin on three sides and aluminum on the other two. One of the metal sides had a speaker and three 24 V lights. A clear plexiglass window allowed the rat to be continually observed. The chamber was equipped with an 18-bar insulated shock grid floor. The floor was removable, and the temperature of the chamber was maintained at 25 ± 2°C. Each testing session was 5 min long. Twenty-four hours later, rats were placed again in the same conditioning chamber and contextual freezing was assessed. Conditioning was assessed based on measurements of freezing, defined as the total absence of body and head movement except for that associated with breathing. Freezing behavior of the rat was recorded using a video recorder, and later scored blindly by the experimenter. Fear was quantified as the amount of time (in seconds) spent freezing.

2.5.3. Contextual fear conditioning test

Fear conditioning tests were performed as follows. The conditioning chamber was located in a windowless room and housed in a soundproof box (70 cm × 60 cm × 60 cm). The conditioning chamber was an open field test apparatus (24 cm diameter, 50 cm height), filled two-thirds with water. After recuperating for 15 min, rats were exposed to diethyl ether until the loss of consciousness. All rats were restrained for 2 h, followed immediately by a 20 min forced swim in 24 °C water. The forced swim was conducted in a clear acrylic cylinder (24 cm diameter, 50 cm height), filled to two-thirds with water. After recuperating for 15 min, rats were exposed to diethyl ether until the loss of consciousness and then left undisturbed in their home cage for 7 days (PN 56–63 days).

2.4. Environmental enrichment (EE) treatment

EE was performed on PN days 22–49 immediately after weaning. The enrichment condition in this study consisted of two different housing conditions. The rats in the EE condition were housed in groups of two rats per cage in the home cage (38 cm × 23 cm × 20 cm stainless steel cage) with a few toys replaced regularly for 22 h every day. In addition, these rats were housed in groups of six rats within a series of large 60 cm × 40 cm × 60 cm cages interconnected with a burrow system and filled with toys, which were replaced regularly, for 2 h every day.

2.5. Behavioral studies

All behavioral experiments were undertaken on PN day 63. Rats were tested between 08:00 and 14:00 h. All behavioral data were collected by a blind observer who was seated inside the testing room.

2.5.1. Open field locomotor test

In the open field locomotor test, rats were placed at the centre of a cubic chamber (48 cm × 48 cm × 48 cm). The animal’s horizontal movements, measured by automatic actography (SCANET MV-10; Melquest, Toyama, Japan), were estimated as the number of interruptions of the near infrared rays (photobeams in the animal chamber). All animals were habituated to the testing room for 20 min before the start of the session, and the testing sessions lasted for 5 min. The test room was dimly illuminated with indirect white lighting.

2.5.2. Elevated plus maze test

The plus maze consisted of two open (50 cm × 10 cm) and two closed (50 cm × 10 cm × 38 cm) arms, arranged perpendicularly, and was elevated 73 cm above the floor. Each rat was placed in the center of the apparatus and the number of entries and time spent per open and closed arms was recorded via a video camera mounted above center of the apparatus. Each rat was habituated to the testing room for at least 20 min before being placed in the center of the maze. The apparatus was cleaned with alcohol after each rat was tested. The trial lasted for 5 min, after which the rat was removed from the maze and returned to its home cage. The test room was dimly illuminated with indirect white lighting. The percentage of open arm entries (number of entries into the open arm divided by total number of entries in both arms), and the percentage of time in the open arms (time in the open arms divided by the time in both arms) were calculated.

Fig. 2. Animal treatment paradigms in Experiment 2 (effect of EE on adulthood behaviors). To investigate the effects of EE on adulthood behaviors, four additional groups were included: (E) Sham + EE, (F) NI + EE, (G) Sham + EE + SPS, and (H) NI + EE + SPS. The Sham + EE group received only EE. The NI + EE group received both NI and EE without SPS. The Sham + EE + SPS group received both EE and SPS without NI. The NI + EE + SPS group received all treatments. All groups received EE on PN days 22–49. To minimize handling effects, rats were undisturbed from PN days 49 to 56. SPS and behavioral experiments were performed likewise.

2.3. Single prolonged stress (SPS) paradigm

Rats were restrained for 2 h, followed immediately by a 20 min forced swim in 24 °C water. The forced swim was conducted in a clear acrylic cylinder (24 cm diameter, 50 cm height), filled two-thirds with water. After recuperating for 15 min, rats were exposed to diethyl ether until the loss of consciousness, and then left undisturbed in their home cage for 7 days (PN 56–63 days).

2.5.5. Hot-plate test

The hot-plate test is one of the most commonly used methods for determining analgesic efficacy in rodents. Each rat was placed in a glass beaker on a hot plate (HPT-1; Melquest, Toyama, Japan). A hot-plate algasia meter, maintained at 52.5°C, was used for this experiment. Latency to flinch or raise hind paws was recorded. To prevent tissue damage, the rat was removed from the hot plate if it did not respond within 30 s [35].

2.5.6. Statistical analysis

Behavioral parameters were expressed as the mean ± S.E.M. Two-way analysis of variance (ANOVA) was performed in the neonatal treatment and stress exposure study (Experiment 1). One factor was neonatal treatment (sham treatment or NI), and another factor was stress (non-SPS or SPS). Post hoc comparisons were performed using Tukey’s test. Three-way ANOVA was used in the EE study (Experiment 2). In the EE study, the influence of neonatal treatment, condition (non-EE or EE), and stress was investigated. Post hoc comparisons
were performed using Tukey’s test. Significant differences between groups were defined by a p value less than 0.05.

3. Results

3.1. Influence of NI on behavior in adult rats subjected to SPS

3.1.1. Open field locomotor test

In the open field locomotor test, the horizontal movement in the Sham, NI, Sham + SPS, and NI + SPS groups was 2110.12 ± 357.01, 2286.88 ± 127.62, 2112.63 ± 328.04, and 2188.88 ± 212.71 counts/5 min, respectively. Two-way ANOVA revealed no significant interaction between neonatal treatment and stress on locomotor activity, and no significant main effect of either neonatal treatment or stress.

3.1.2. Elevated plus maze test

In the elevated plus maze test, two-way ANOVA revealed a significant interaction between neonatal treatment and stress [F(1, 46) = 4.398, p < 0.05], and significant effects of neonatal treatment [F(1, 46) = 7.964, p < 0.05], and stress [F(1, 46) = 32.793, p < 0.01] on the percentage of time spent in the open arms (% open arm time) (Fig. 3a). Post hoc analysis revealed that SPS treatment significantly decreased the percentage open arm time in both the Sham and NI groups (p = 0.03, 0.0001, respectively) (Fig. 3a). The percentage open arm time in the NI + SPS group was significantly lower than that in the Sham + SPS group (p = 0.008) (Fig. 3a).

Similarly, two-way ANOVA revealed a significant interaction between neonatal treatment and stress [F(1, 46) = 4.159, p < 0.05], and significant effects of neonatal treatment [F(1, 46) = 4.733, p < 0.05] and stress [F(1, 46) = 40.337, p < 0.05] on the percentage of entries in the open arms (% open arm entries) (Fig. 3b). Post hoc analysis revealed that SPS significantly decreased the percentage of open arm entries in both the Sham and NI groups (p = 0.014, 0.0001, respectively) (Fig. 3b). The percentage of open arm entries in the NI + SPS group was significantly lower than that in the Sham + SPS group (p = 0.03) (Fig. 3b).

3.1.3. Contextual fear conditioning test

In the contextual fear conditioning test, two-way ANOVA revealed no significant effect of neonatal treatment [F(1, 43) = 2.328, p = 0.134], a significant effect of stress [F(1, 43) = 45.852, p < 0.0001], and a significant interaction between neonatal treatment and stress [F(1, 43) = 5.985, p < 0.05] on contextual freezing time (Fig. 4). Post hoc analysis revealed that the enhanced contextual freezing time in the Sham + SPS group was significantly greater than that in the Sham group (p = 0.02), and the NI + SPS group exhibited a significantly enhanced freezing time compared with the Sham + SPS group (p = 0.03) (Fig. 4).

3.1.4. Flinch-jump test

In the flinch-jump test, no significant interaction between neonatal treatment and stress was found on the flinch reaction to painful stimulation. In addition, no significant effect of neonatal treatment or stress was found. On the vocalization reaction, two-way ANOVA revealed no significant interaction between neonatal treatment and stress. In contrast, significant effects of neonatal treatment [F(1, 35) = 5.384, p < 0.05], and stress [F(1, 35) = 27.970, p < 0.0001] were found (Fig. 5a). Post hoc analysis revealed that the shock thresholds in the Sham + SPS and NI + SPS groups were significantly higher than that of the Sham group.
Fig. 5. Comparison of shock threshold (mA) on vocalization (a) and jump reactions (b) in the flinch-jump test. Data were expressed as mean ± S.E.M. The number of rats in each group varied from 8 to 11 (Sham: n = 8, NI: n = 9, Sham + SPS: n = 11, NI + SPS: n = 11), **p < 0.01.

3.1.5. Hot-plate test

In the hot-plate test, two-way ANOVA revealed no significant interaction between neonatal treatment and stress [F(1, 35) = 0.529, p = 0.472] and no significant effect of neonatal treatment [F(1, 36) = 0.222, p = 0.64], but a significant effect of stress [F(1, 36) = 34.336, p < 0.0001], on latency to flinch or raise hind paws (Fig. 6). Post hoc analysis revealed that the latencies in the Sham + SPS and NI + SPS groups were significantly longer than that in Sham group (p = 0.005, 0.003, respectively) (Fig. 6). No significant difference in the latency between the NI + SPS and Sham + SPS group was found (Fig. 6).

3.2. Effects of EE on the behaviors in adult rats subjected to NI and SPS

3.2.1. Effect of EE on the open field locomotor test

In the open field locomotor test, the horizontal movement in the Sham, NI, Sham + SPS, NI + SPS, Sham + EE, NI + EE, Sham + EE + SPS, and NI + EE + SPS group was 2110.12 ± 357.01, 2286.88 ± 127.62, 2112.63 ± 328.04, 2188.88 ± 212.71, 2372.50 ± 54.49, 2316.50 ± 148.66, 2259.63 ± 104.45, and 2350.25 ± 100.97 counts/5 min, respectively. Three-way ANOVA revealed no significant effects of neonatal treatment, condition, or stress on locomotor activity. There were also no significant interactions among these groups.

3.2.2. Effect of EE on the elevated plus maze test

In the elevated plus maze test, three-way ANOVA revealed significant effects of neonatal treatment [F(1, 93) = 12.877, p < 0.05], and stress [F(1, 93) = 59.506, p < 0.01], but not condition [F(1, 93) = 3.350, p = 0.07] on the percentage of time spent in the open arms (% open arm time). There were no significant interactions among these groups.

Three-way ANOVA revealed significant effects of neonatal treatment [F(1, 93) = 51.819, p < 0.05], condition [F(1, 93) = 36.999, p < 0.01], and stress [F(1, 93) = 25.134, p < 0.001] on the percentage of entries in the open arms (% open arm entries) (Fig. 7). There were also significant interactions between neonatal treatment and condition [F(1, 93) = 10.585, p < 0.01], neonatal treatment and stress [F(1, 93) = 4.392, p < 0.01], and condition and stress [F(1, 93) = 2.044, p < 0.05] (Fig. 7). Post hoc analysis revealed that while the percentage of open arm entries in the Sham + EE + SPS group was significantly higher than that in the Sham + SPS group (p = 0.012), the percentage of open arm entries in the NI + EE + SPS group was similar to that in NI + SPS group (Fig. 7).

3.2.3. Effect of EE on the contextual fear conditioning test

In the contextual fear conditioning test, three-way ANOVA revealed significant effects of neonatal treatment [F(1, 88) = 20.183, p < 0.0001], stress [F(1, 88) = 35.079, p < 0.0001]
and condition \(F(1, 88) = 15.626, p < 0.0001\) on contextual freezing time (Fig. 8). There were also significant interactions between neonatal treatment and condition \(F(1, 88) = 7.077, p < 0.01\), stress and condition \(F(1, 88) = 6.868, p < 0.01\), and neonatal treatment and stress \(F(1, 88) = 4.392, p < 0.05\) (Fig. 8).

Post hoc analysis revealed that the exposure to EE significantly downregulated the SPS-induced enhancement in contextual freezing time in both the Sham + SPS (\(p = 0.002\)) and NI + SPS group (\(p = 0.03\)) (Fig. 8).

### 3.2.4. Effect of EE on the flinch-jump test

In the flinch-jump test, three-way ANOVA revealed a significant effect of stress \(F(1, 78) = 92.720, p < 0.0001\), but not neonatal treatment \(F(1, 78) = 14.172, p = 0.01\), or condition \(F(1, 78) = 0.224, p = 0.637\) on the jump reaction to the painful stimulation (Fig. 9). There were no significant interactions among these groups. The exposure to EE downregulated the SPS-induced increase in the jump threshold of the Sham + SPS and the NI + SPS groups (Fig. 9).

### 4. Discussion

Since rats subjected to SPS exhibited enhanced glucocorticoid negative feedback through the upregulation of GRs and downregulation of mineralocorticoid receptors (MRs) in the hippocampus 7 days after SPS [21], we postulated that subjecting rats to SPS is useful for modeling human PTSD. In addition, Khan and Liberzon demonstrated that SPS significantly exaggerated the acoustic startle response in rats [17]. Similarly, the results of the present study demonstrated that SPS markedly enhanced contextual freezing in sham-treated rats. Furthermore, the results from the flinch-jump and hot-plate tests indicated that, in sham-treated rats, SPS produced stress-induced analgesia, which is often seen in patients with PTSD [29]. Based on these findings, we conclude that SPS is an appropriate animal model of PTSD.

The aim of Experiment 1 in this study was to examine whether an early adversity could enhance PTSD-like behaviors in adult rats. The results of the elevated plus maze test demonstrated that both the percentage of open arm time and entry in the NI + SPS group was significantly lower than in the NI alone or Sham + SPS.
groups, suggesting that NI markedly exaggerated anxiety-like behaviors in rats subjected to SPS. In addition, the assessment of contextual fear conditioning revealed that the contextual freezing time in the NI + SPS group was significantly longer than that in the NI alone or Sham + SPS group, suggesting that NI significantly increased contextual freezing in rats subjected to SPS. Furthermore, the flinch-jump test showed that the jump threshold, but not the vocalization threshold, in the NI + SPS group was significantly higher than that in the NI or Sham + SPS groups, and no differences existed in latency in the hot-plate test between the Sham + SPS and NI + SPS groups. These findings suggest that the degree of stress-induced analgesia in response to SPS was, at least in part, enhanced by NI. Overall, the results of the present study indicate that early adversity worsens anxiety-like behaviors and fearful memory depending on the spatial context, and, to a lesser extent, stress-induced analgesia, in PTSD model rats.

During the juvenile stage, as well as the neonatal stage, an exposure to stress has been reported to induce long-lasting changes in stress reactivity in adulthood. Avital and Richter-Levin demonstrated that rats exposed to a combination of juvenile and adulthood stress had increased anxiety levels and acoustic startle responses, as compared with juvenile stress alone or adulthood stress [2]. Consistent with this study, it is known that various types of juvenile stress, such as social stress and elevated-platform stress, induce long-lasting impairment of the stress-coping response [1,2,14]. Therefore, the possibility that juvenile adversity, such as isolation, worsens PTSD-like behaviors cannot be ruled out, although we did not examine whether juvenile isolation would affect these behaviors in SPS rats.

It has been reported that the patients with PTSD exposed to repeated early adverse events are likely to have severe psychiatric symptoms, and that their symptoms do not respond well to standard treatments for PTSD [7–9]. In this context, it is supposed that the pathophysiology and symptoms of PTSD in patients with early adverse experiences may be more evident than in typical PTSD patients. The results of the present study demonstrated that NI significantly worsened PTSD-like symptoms in rats subjected to SPS. Although it is difficult to compare results from rodent studies to those in human studies, our findings in Experiment 1 are consistent with clinical observations in PTSD. Considering the findings in both animal and human studies, it is conceivable that early adverse experiences may increase the severity of PTSD symptoms in later life.

Experiment 2 was undertaken to investigate whether EE after weaning ameliorates NI-induced enhancement of PTSD-like behaviors in adult rats subjected to SPS. The results of the elevated plus maze test demonstrated that although EE prevented the marked reduction in the percentage of open arm entries in the Sham + SPS group, EE had no effect on the NI-induced enhancement of the percentage of open arm entries in rats subjected to SPS. Likewise, EE treatment did not ameliorate the NI-induced increase in the jump threshold of rats subjected to SPS. These findings suggest that treatment with EE failed to ameliorate the NI-induced enhancement of anxiety-like behavior and stress-induced analgesia in PTSD model rats. In contrast, the contextual fear test showed that EE markedly downregulated the NI-induced enhancement of contextual freezing in rats subjected to SPS, and that the contextual freezing time in the NI + EE + SPS group returned to a level comparable to that of the Sham + SPS group, suggesting that EE ameliorated the NI-induced enhancement of fearful memory in PTSD model rats.

Since it has been revealed that the hippocampus, amygdala, and medial prefrontal cortex are intimately involved in the neuronal network of contextual fear conditioning [28,32,33], the results of the contextual fear conditioning test suggest that repeated EE treatment immediately after weaning in rats subjected to SPS may ameliorate the NI-induced enhancement of dysfunction in these brain regions. In contrast, the finding that EE did not ameliorate the NI-induced enhancement of anxiety-like behavior in the elevated plus maze, is inconsistent with the possible effect of EE derived from the contextual fear conditioning test. However, in addition to the amygdala and medial prefrontal cortex, several other brain regions such as the medial hypothalamus, dorsomedial hypothalamus, and rostral perirhinal cortex, mediate anxiety-like behavior in the elevated plus maze [13,19,34]. Thus, these findings raise the possibility that EE may ameliorate the NI-induced enhancement of dysfunction in hippocampus, amygdala, and medial prefrontal cortex, but not in these other brain regions. Together, these results suggest that early great care in abused or maltreated children may prevent the enhancement of fearful memory depending on the spatial context, such as flashlight, in patients with PTSD.

It is well known that early adverse experiences induce various changes in the central nervous system [15]. For example, maternal separation was reported to decrease central GABAergic signal transduction and enhance CRF-mediated signal transduction [5,10]. Based on these findings, it is conceivable that the significant enhancement of anxiety-like behavior in rats subjected to NI and SPS may be due to downregulation of GABA-mediated signals and/or the upregulation of CRF-mediated signals, although the precise effect of SPS on these systems remains unknown. A number of studies have demonstrated that the phosphorylation of cyclic AMP response element-binding protein (CREB) plays an important role in the development of contextual fear in rodents [12,18]. In addition, Morinobu et al. reported that NI significantly upregulated the phosphorylation of CREB in the hippocampus in response to a single restraint stress in adult rats [26]. Taken together, it is plausible that the enhanced phosphorylation of CREB in the hippocampus in response to re-exposure to the context, may be, at least in part, involved in the exaggerated contextual freezing in rats subjected to NI and SPS.

In summary, repeated NI significantly enhanced anxiety-like behavior in the elevated plus maze, as well as contextual freezing and stress-induced analgesia, in adult rats subjected to SPS, an animal model of PTSD. In rats subjected to SPS, repeated EE ameliorated the NI-induced enhancement of contextual freezing but did not affect anxiety-like behavior or stress-induced analgesia. Since these findings indicate the possibility that early adversities worsen the symptoms of PTSD, further studies examining the molecular mechanisms of the effects of NI and EE on PTSD-like behaviors may contribute to more effective treatment and prevention of PTSD in early traumatized patients.
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References


