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Chronic mild stress impairs cognition in mice: From brain homeostasis to behavior

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ABSTRACT

Exposure to chronic stress in rodents and psychosocial stress in humans has been shown to alter cognitive functions and has been linked to the pathophysiology of mood disorders. The purpose of the present study was to investigate effects and possible mechanisms of a chronic mild stress (CMS) procedure on cognitive behaviors in Swiss albino mice using the object recognition test (ORT) and object location test (OLT). Results showed that CMS exposure impaired cognitive performance and produced amnesia of acquired information in both ORT and OLT. Furthermore, the cognitive impairment was coexistent with increased plasma levels of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), as well as with enhanced plasma levels of corticosterone (CORT), corticotrophin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH). In addition, severe neuronal cell damage was found, as bromodeoxyuridine (BrdU) positive cells and the expression of brain derived neurotrophic factor (BDNF) in dentate gyrus (DG) of hippocampus were decreased after 5 weeks CMS procedure. Taken together, these findings indicated that CMS exposure-induced impairment of cognitive behaviors might be attributed to the stress-related alterations in brain homeostasis that were reflected in changes in the neuroimmune and neuroendocrine systems as well as in neurogenesis.

Introduction

Stress is defined as any environmental change, whether endogenous or exogenous, that disturbs the maintenance of brain homeostasis. For humans, psychosocial stress plays an important role in the development and maintenance of both mood disorders and cognitive impairments (Sapolsky, 1996; Simoens et al., 2007; Oei et al., 2006). However, the pathophysiological mechanisms underlying these disease states still remain unclear. Many animal models have been established and applied to experimental examination of possible mediators in stress-induced cognitive or emotional alterations to better understand the complicated interactions between stress, emotion and cognition. In recent studies (Song et al., 2006; Stemmelin et al., 2007), our laboratory and others have examined the association between stress and cognitive function or mood disorder by employing a well-established animal model, the chronic mild stress (CMS) (Willner et al., 1992; Willner, 1997). The results of those studies indicated that CMS produced several changes similar to those found in human depression, including elevated plasma corticosterone concentration and reduced levels of cAMP responsive element binding protein and brain derived neurotrophic factor (BDNF). Furthermore, in those studies, CMS was also found to be associated with spatial cognition impairment in the Morris water maze task. These results suggested that, along with relevant depression-like behaviors, CMS could also induce significant changes in cognitive function. The specific underlying mechanisms for these changes are not well understood, but there are several possible neurohumoral mediators that may participate in the cognitive alterations associated with exposure to CMS.

The recent achievements in neuroscience and neuropsychological research demonstrate a clear new understanding that neurons do not work in isolation, that they need constant interactions with other brain components and systems to process information. Brain homeostasis is now known to involve complex interactions among the neuroimmune and the neuroendocrine systems and neurons themselves, and plays important roles in regulation of brain functions, such as emotion and cognition. For instance, depression is associated with excessive secretion of cytokines and hormones, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, interferon, corticosterone, corticotrophin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) (Smith 1991; Jozuka et al., 2003; Aihara et al., 2007). These cytokines and hormones induce depressive symptomatology and "sickness behavior" (Connor and Leonard, 1998; Dantzer et al., 1999; Pollak and Yirmiya, 2002; Wichers and Maes, 2002) and are involved in cognitive functions (Solerte et al., 2000; Reichenberg et al., 2001; Marsland et al., 2006; Medeiros et al., 2007; Gemma and Bickford, 2007; Schram et al., 2007). That evidence suggests that there might be a physiologically-mediated relationship among stress, mood disorders and cognitive impairment, perhaps involving endocrine and immune mechanisms. To further investigate

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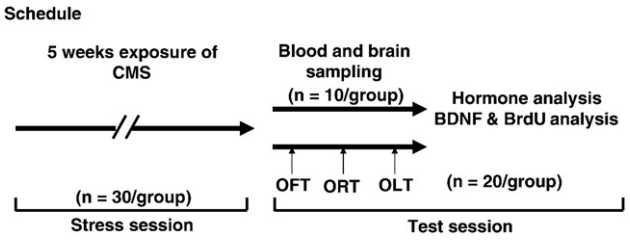

Fig. 1. Experimental schedule. Animals were exposed to 5 weeks chronic mild stress (CMS). After the stress session, one third of the animals were sacrificed for blood and brain sampling and analysis. Two thirds of the animals were submitted to a test session composed of 3 different behavioral tests: open field test (OFT), object recognition test (ORT) and object location test (OLT).

the association of cognitive and psychological disruptions that were related to stress, the present study was undertaken to examine the impact of CMS on brain homeostasis.

Materials and methods

Animals

Adult male Swiss albino mice (18–22 g) were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University. Before the CMS procedure, animals were housed in groups under standard conditions (12 h light/dark cycle; lights on from 0730 to 1930; 22±2 °C ambient temperature; 55±10% relative humidity). They were fed with standard diet and water ad libitum and were allowed to acclimatize 7 days before they were used. The present study was performed according to the experimental schedule shown in Fig. 1. After 1 week of sucrose consumption training phase, animals were randomized into 2 groups with 30 mice per group, and the mice of the stressed group were subjected to CMS. At the end of the 5-week stress session, 10 animals of each group were administered BrdU and then sacrificed for blood and brain sampling and analysis. The remaining 2/3 of the animals were submitted to a test session composed of 3 different behavioral tests. The experimental procedures involving animals and their care were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Chronic mild stress procedure

The CMS procedure was performed as described (Song et al., 2006; Li et al., 2007). Initially, the animals were given 1% sucrose solution for a 48-h period in their home cages with no food or water following food and water deprivation for 18 h. Subsequently, they were housed individually and given sucrose for 1 h per day on five consecutive days. The sucrose intake was measured at the end of the training phase in order to group the mice. The mice in the experimental group were then subjected to CMS for 5 weeks. The CMS procedure consisted of a variety of unpredictable mild stressors including one period (2 h) of paired caging, one period (3 h) of tilted cage (45°), one period of food and water deprivation (18 h), one period (1 h) of restricted access to food, one period (1 h) of exposure to an empty bottle, one 21 h period with wet cage (200 ml water in 100 g sawdust bedding) and one period with 36 h of continuous light. These stressors were randomly scheduled over a one-week period and repeated throughout the 5-week

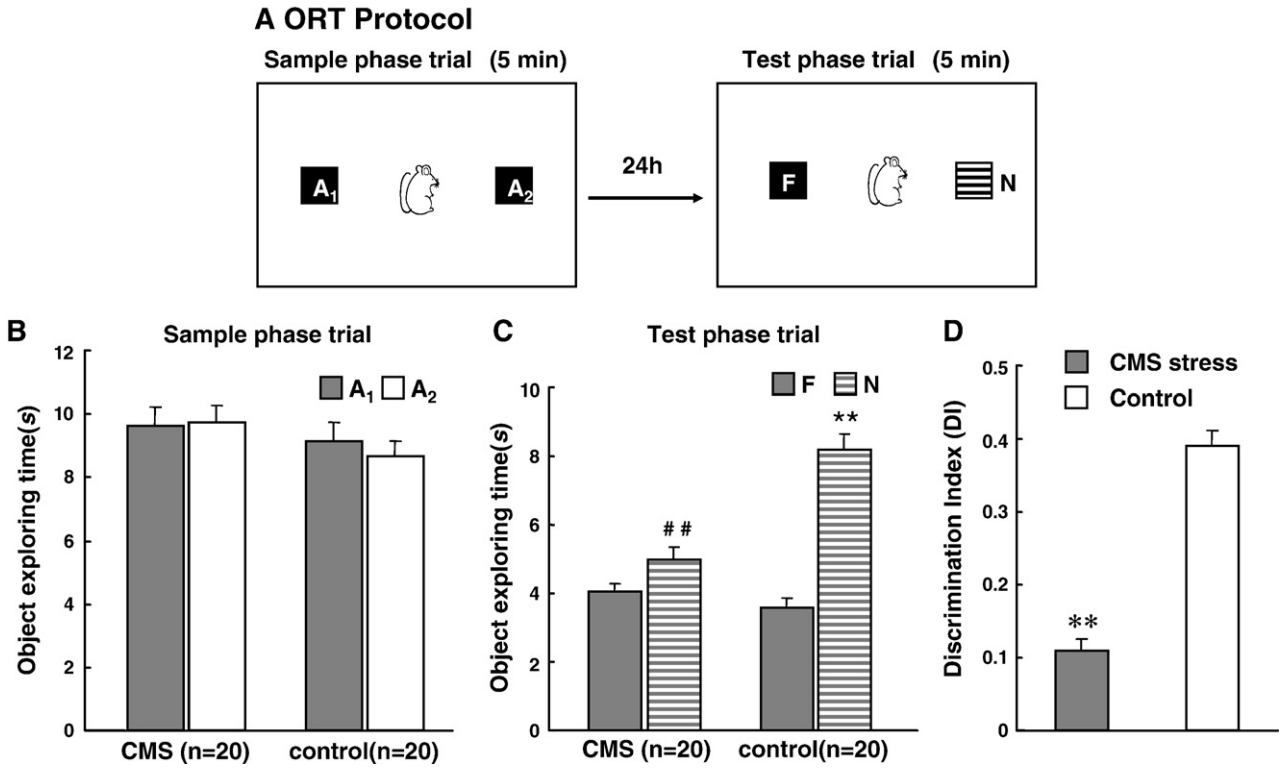

Fig. 2. A: Representation of sample and test phase trial conditions in the object recognition test (ORT). The animals received three sessions of 10-min duration in the empty arena to acclimatize to the apparatus and test room. B: Object recognition performance of CMS stressed mice in the sample phase trials. Animal was first placed in the box for a 5-min and exposed to two identical sample objects, A1 and A2. The total time the mouse spent exploring each object was measured. C: Object recognition performance in the test phase trial. Test phase trials in which both objects were replaced with the two identical copies (objects F and N), one of which contained a black-and-white pattern (object N), were performed 24 h after the sample phase trials. Each data point represents the mean±S.E.M. of control (n=20) or CMS mice (n=20). ** P<0.01 compared with respective time spent for the familiar object. $^{\#\#}$ P<0.01 compared with control group. D: Discrimination index (DI): The index was calculated on the basis of the data obtained in the test trials using the equation indicated in the text. ** P<0.01 compared with control group.

experiment. In contrast to other previous procedures in rats, nociceptive stressors were excluded, and only environmental and social disturbances were applied (Pardon et al., 2000). The non-stressed control animals were housed in normal conditions without any manipulations. Sucrose intake (1% sucrose solution) and body weight of all animals were measured once a week, on separate days, during a 1-h window after 18 h of food and water deprivation in both CMS and control group. Consumption was measured by weighing the pre-weighed bottle at the end of the test. The intake was expressed in relation to the animals' body weight (g/kg). All animals were housed in groups (5 per cage) during the stress session but housed individually for measurement of sucrose consumption.

Open field test (OFT)

Twenty-four hours after exposure to the last stressor, the open field test was performed. The open field consisted of a base (100×100 cm) and black walls (20 cm) divided into 25 (5×5) identical sectors (20×20 cm) by white stripes. The squares were subdivided into peripheral and central sector, where the central sector included the 9 central squares (3×3) and the peripheral sector contained the squares close to the wall. The animals were placed in the central sector and their activity recorded for 5 min by a video camera and taped for further analysis. The open field arena was thoroughly cleaned with 75% ethanol between each test. The room was lit by a dim red light. No stressor was applied to the animals for at least 24 h before the test. Open field activity was scored manually. Motility was scored when an animal crossed a sector border with both its hind-limbs. The following activities were scored: Peripheral activity: the number of peripheral sectors crossed; Central activity: number of central squares crossed; Total activity: the overall activity, in both peripheral and central areas during the 5 min test; Climbing: number of exploring with forepaws on the wall of arena; Rearing: number of standing on hind legs with nose in the air for exploration; Grooming: number of times cleaning the fur and skin.

Object recognition test

The object recognition test ORT was performed by slightly modifying the method described by De Rosa et al. (2005; Fig. 2A). The observation arena, with the size of 50×35×20 cm, was made of white polyethylene, located in a testing room dimly lit by a constant illumination of about 40 lux. The objects chosen are triple copies of cuboid plastic blocks (A; 5×5×5 cm). These objects were heavy enough that the mice were unable to move them. Performance of the animals in this test was video-recorded for later analysis. The ORT consisted of a sample phase trial and a test phase trial, and lasted for 3 days. On day 1, the animals received three sessions of 10-min duration in the empty box to acclimatize them to the apparatus and test room. On day 2 of sample phase trial, two identical objects (A1, A2) for each arena were placed on opposite sides and leaving 8 cm from the walls, with the distance of 34 cm between each other. The mice were placed between two objects to start a 5-min object exploring training. The total time the mouse spent exploring each of two objects was measured and then the mouse was returned to its cage. On day 3, test phase trials were performed 24 h after the sample phase trials. In this trial, one of the two objects was replaced by its identical copy (familiar object, F) and the other by a new one with a black-and-white pattern (new object, N). Mice were left to explore the objects for 5 min. The total time spent exploring each of the two objects was measured.

Object location test

The sample phase trials of the object location test (OLT) were performed in the same way as those of the ORT (Fig. 3A). The objects used in the sample phase trials were two white cylinder plastic bottles

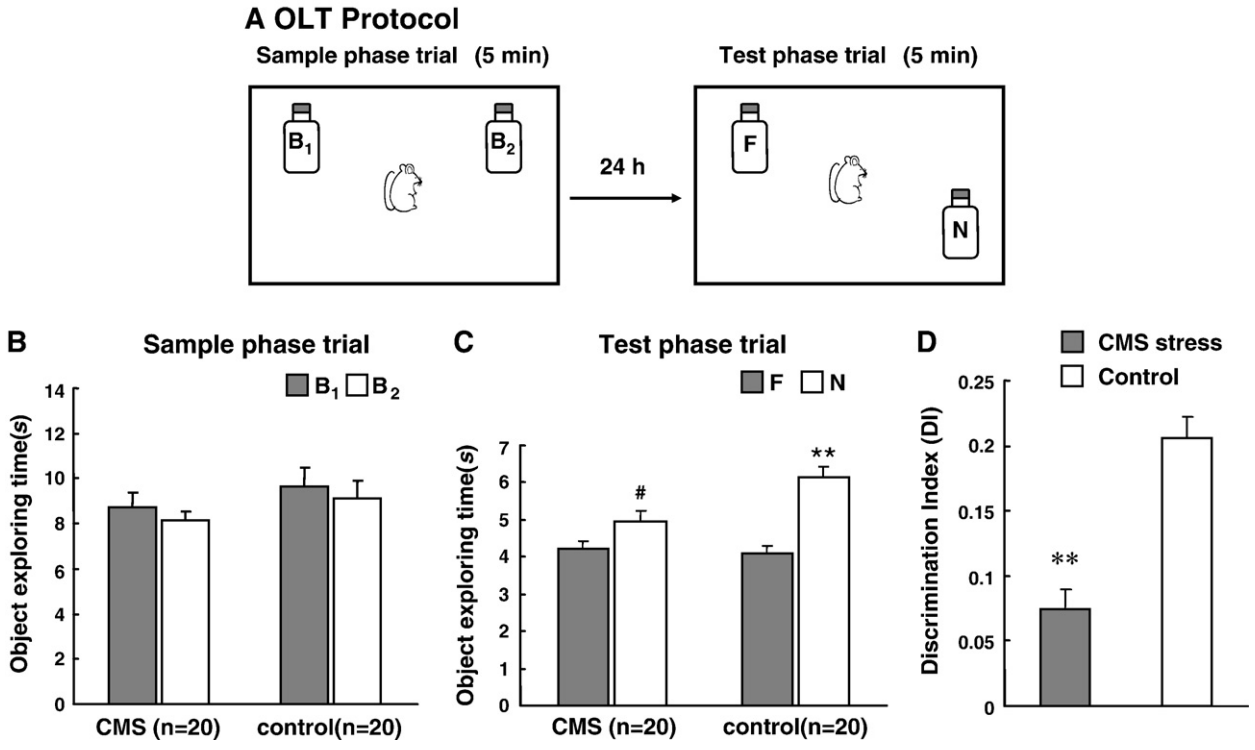

Fig. 3. A: Representation of sample and test phase trial conditions in the object location test (OLT). B: Object recognition performance of CMS stressed mice in the sample phase trials. Each mouse was first placed in the box for 5 min and exposed to two identical sample objects, B1 and B2. The total time the mouse spent exploring each object was measured. C: Object recognition performance in the test phase trial. Test phase trials in which both objects were replaced with the two identical copies (objects F and N), one of which was moved to the opposite corner (object N), were performed 24 h after the sample phase trials. Each data point represents the mean ± S.E.M. of control (n=20) or CMS mice (n=20). ** $P<0.01$ compared with respective time spent for the familiar location. $^{\#}$ $P<0.05$ compared with control group. D: Discrimination index (DI): The index was calculated on the basis of the data obtained in the test trials using the equation indicated in the text. ** $P<0.01$ compared with control group.

Table 1
Effects of 5-week CMS exposure on the motor activity of mice in the open field test

Group	Locomotor activity			Rearing	Climbing	Grooming
	Central activity	Peripheral activity	Total activity			
Control	29.9±10.3	57.9±13.8	87.8±19.4	4.0±2.6	11.9±2.5	2.9±1.1
CMS stress	30.2±8.2	60.7±9.9	90.9±16.1	3.9±2.4	11.7±3.6	3.0±1.4

Each value represent the mean±S.E.M (n=20) of counts in 5 min open field test.

(B1, B2; height 8 cm, diameter 5 cm) filled with water. After a delay of 24 h, the test phase trials were conducted. In this trial, the objects were replaced by their identical copies, one of which was placed in the same position as it was in the sample phase trial (familiar location, F), whereas the other one was moved to the adjacent corner (new location, N), so that the two objects were now in diagonally opposite corners. In the test phase trials, both objects were equally familiar to the animals, but one had changed location. The mice were exposed to the objects for 5 min and the total time spent exploring each of the two objects was measured.

In both ORT and OLT, object exploration was defined as directing the nose to the object at a distance of <2 cm and touching it with the nose, in accordance with a previous report (De Rosa et al., 2005). Turning around, climbing over, or sitting on the object were not recorded as exploration. If the exploration time in the sample phase trials was <3 s, the mice were discarded from the sample. Moreover, if the mice spent <1 s exploring both new and familiar objects in the test phase trials, they were also excluded from the sample (De Rosa et al., 2005). Objects were thoroughly cleaned with 75% ethanol after each individual trial to prevent a build-up of olfactory cues. A discrimination index (DI) was calculated according to the following equation (Blalock et al., 2003; De Rosa et al., 2005):

$$DI = (Tn - Tf)/(Tf + Tn)$$

Here, Tn and Tf represent the time spent to explore new and familiar objects (locations) during a 5-min observation period, respectively.

Hippocampal section preparation

For bromodeoxyuridine (BrdU; Sigma, St. Louis, Missouri) labeling, 10 mice in each group were administered BrdU (4×75 mg/kg, ip, every 2 h) after exposure to the last stressor. Twenty-four hours after the last BrdU injection, mice were sacrificed. Blood was collected for plasma hormone and cytokine analysis and all brains were post-fixed overnight in paraformaldehyde (with shaking) at 4 °C and stored at 4 °C in 30% sucrose. Serial sections of the brains were prepared (30 μm sections) through the entire hippocampus on a freezing microtome, and sections were stored at −20 °C. Part of sections was stained with hematoxylin-eosin (HE) for morphological analysis.

Immunohistochemistry determination and morphological analysis

Free-floating sections were used in the determination of BrdU labeling or BDNF expression. After DNA denaturation and several PBS rinses, sections were incubated for 30 min in 2 N HCl and then for 10 min in boric acid. After washing in PBS, sections were incubated for 30 min in 3% H_2O_2 to eliminate endogenous peroxidases. After blocking with 3% normal goat serum in 0.01% Triton X-100, sections were incubated with anti-mouse BrdU or BDNF (1:400; Boehringer-Mannheim, Indianapolis, IN) overnight at 4 °C. Sections were then incubated for 1 h with secondary antibody (biotinylated goat anti-mouse; Vector Laboratories, Burlingame, CA) followed by amplification with an avidin–biotin complex, and were visualized with diaminobenzidine (DAB). Finally, BrdU-positive cells or BDNF level was observed and pictures were taken with microscope (Olympus BX60, Japan). The expression of BDNF proteins in the hippocampus was analyzed using the image analysis system (MetaMorph/CoolSNAP fx/ AX70 image analysis system, Germany) and was measured by inspecting the average gray value and average optical density. Coronal sections (30 μm) were prepared through the rostral hippocampus. For the assessment of neural damage, sections were stained with HE. The number of surviving and apoptotic neurons in the dentate gyrus regions from each animal was counted by an investigator unaware of the treatment that the animal had received. Neuronal survival cell and apoptotic cell were quantified within a 0.09 mm^2 area selected in each hippocampal subfield.

Plasma corticosterone, ACTH and CRH assay

Ten of 30 animals administered BrdU were also used for the measurement of plasma corticosterone, ACTH and CRH levels. The animals were sacrificed after 5 weeks of CMS procedure. The blood samples of mice were collected between 0800 and 1000 A.M. and kept on ice and then centrifuged immediately at 2000 ×g at 4 °C for 15 min. The obtained plasma was kept at −80 °C until analysis. Corticosterone, CRH and ACTH levels were measured using commercially available radioimmunoassay (RIA) kits (ICN Biomedicals, Costa Mesa, CA).

IL-1β, IL-6 and TNF-α determination

Plasma TNF-α, IL-1β, and IL-6 levels were measured, with same samples for plasma hormone testing, using enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo, CA), according to manufacturer instructions. Details of these assays have been provided in previous studies (Francis et al., 2003; Woods et al.,

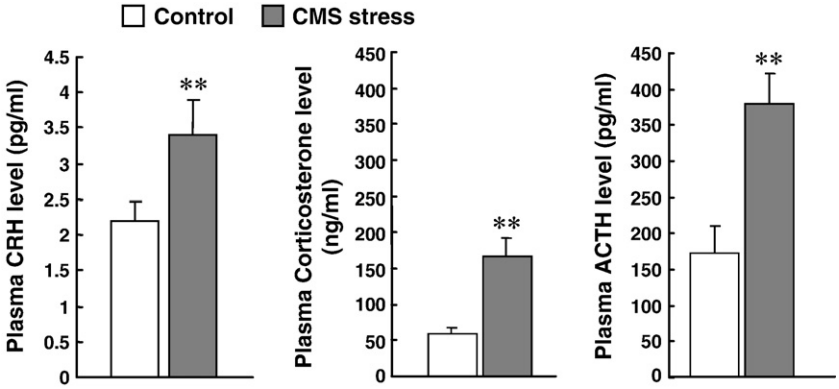

Fig. 4. Effects of chronic mild stress (CMS) on plasma CRH, corticosterone and ACTH levels of mice. Data are presented as mean±S.E.M. of control or CMS stressed mice; ** P<0.01 compared with control group (n=10).

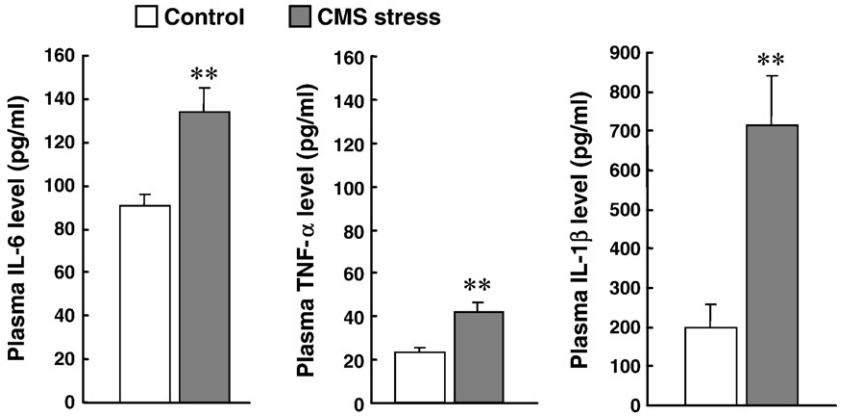

Fig. 5. Effects of CMS on plasma IL-1β, IL-6 and TNF-α levels in mice. Values are presented as mean±S.E.M. of control or CMS stressed mice; ** $P<0.01$ compared with the control group ($n=10$).

2001; Urakubo et al., 2001). The minimum detectable concentrations were <0.1 pg/ml for TNF-α, <3 pg/ml for IL-1β, and 20 pg/ml for IL-6.

Statistical analyses

All data were expressed as the mean±S.E.M.. Statistical analyses were performed with paired Student's *t*-tests. Differences of $P<0.05$ were considered significant.

Results

Sucrose consumption

CMS gradually reduced the consumption of the sucrose solution. As compared to the 14.7 g/kg intake in the baseline test, 5 weeks later the sucrose intake was reduced to 7.9 g/kg in the CMS stressed animals.

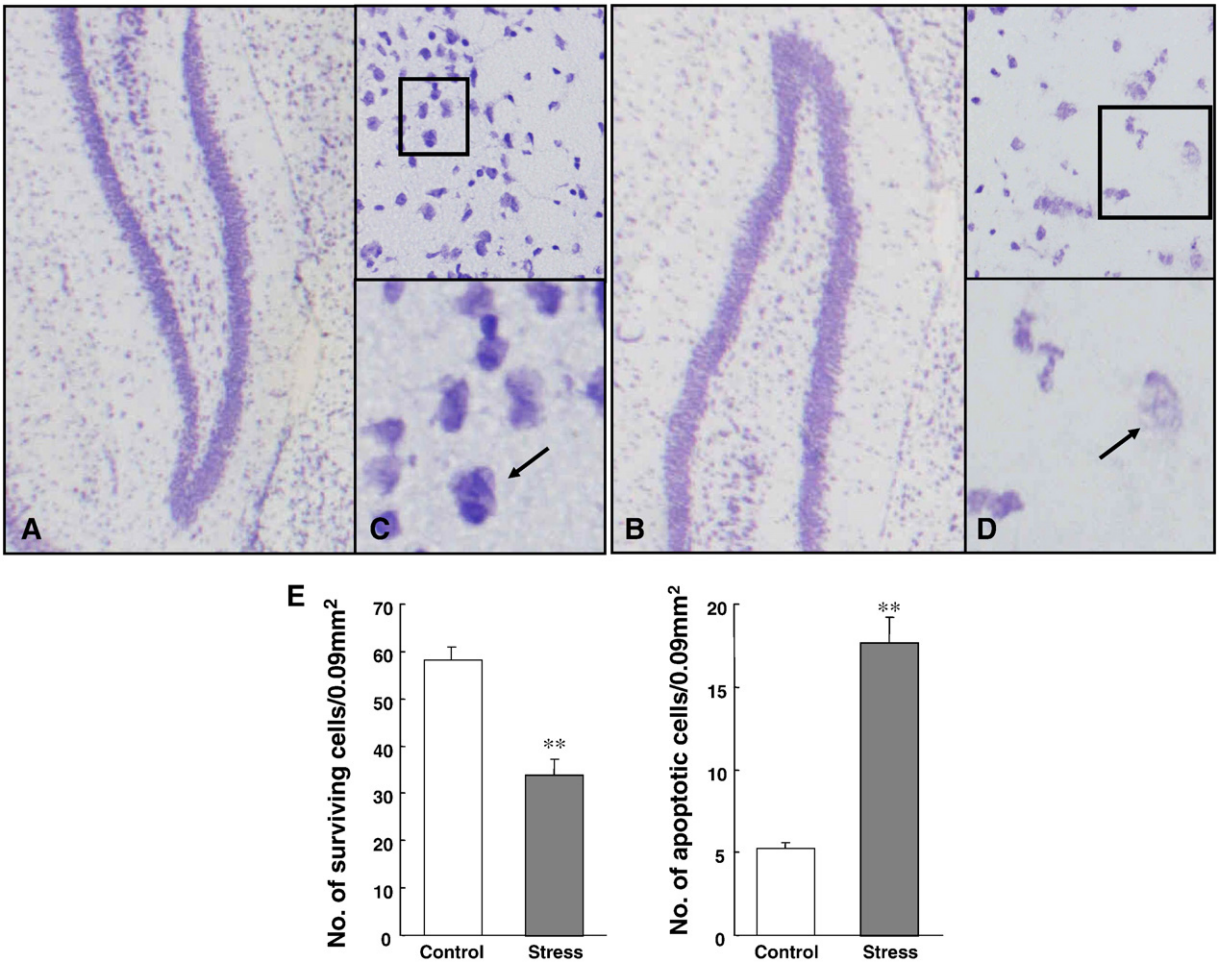

Fig. 6. Morphologic changes in dentate gyrus of mice induced by chronic mild stress (HE staining). A: control group; B: CMS stressed group. Chronic stress resulted in significant neuronal cell damage, reflected in increased occurrence of apoptotic cells and decreased number of survival cells, in the dentate gyrus. Black arrows showed examples of normal survival (C) and apoptotic cell (D). E: Effect of CMS on number of survival and apoptotic cells in the dentate gyrus of hippocampus. Data are expressed as mean±S.E.M. ($n=10$). ** $P<0.01$ compared with un-stressed control group.

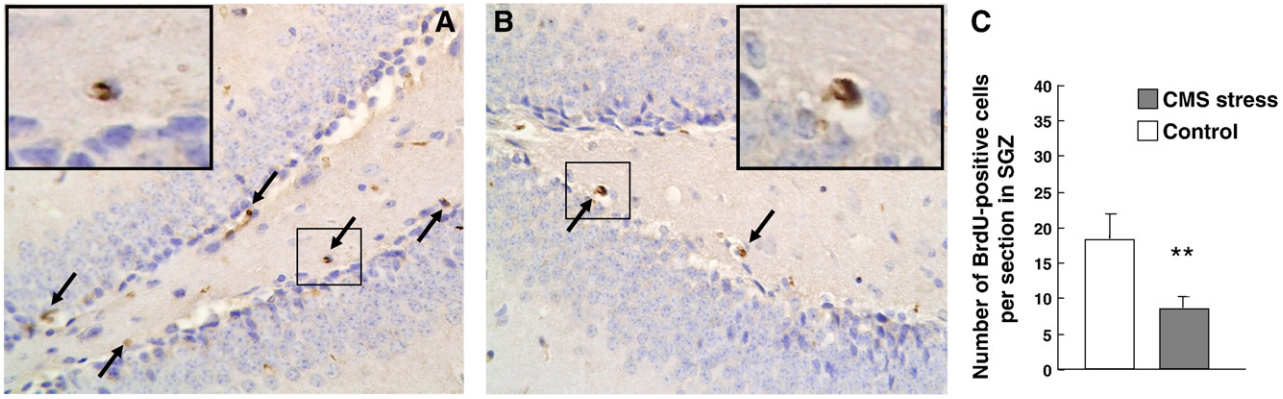

Fig. 7. Influence of CMS on number of BrdU positive cells in the subgranular zone (SGZ) of hippocampus of mice. Chronic mild stressed mice received injections of BrdU after the last stressor and were killed 24 h after the last BrdU injection. A: BrdU positive cells (brown) in SGZ of hippocampus in mice of control group; B: BrdU positive cells in SGZ of hippocampus in CMS stressed mice; C: Number of BrdU positive cells in SGZ of hippocampus. Data are expressed as mean ± S.E.M. (n = 10). ** P<0.01 compared with un-stressed control group.

Open field test

The CMS treated animals showed no differences in total activity compared with non-stressed control animals in 5 min open field test (Table 1).

Object recognition test

The effect of CMS on cognitive performance that is related to episodic memory in humans was elucidated using the object recognition test in animals. Analyses of performance in the sample phase trials of the ORT revealed no differences in total exploring time between un-stressed control and CMS stressed animals (P>0.05, Fig. 2B). These results eliminate the possibility that chronic stress causes deficits of motivation and sensory motor function. However, in the test phase trials conducted 24 h after the sample phase trial, the control mice spent a significantly longer time exploring a new object than exploring a familiar one and exhibited a clear preference for the novel object (P<0.01, Fig. 2C). In contrast, CMS stressed mice showed no difference in the exploring time for both familiar and novel object (P>0.05, Fig. 2C). When the data were analyzed as the discrimination index (DI), CMS mice exhibited a significant impairment of ability to discriminate between familiar and new objects compared to control group (P<0.01, Fig. 2D).

Object location test

Analysis of the sample phase trial revealed that there were no significant differences in the exploring time between unstressed control group and CMS stressed group (P>0.05, Fig. 3B). In the test phase trials, exploration times for the control group clearly showed a preference for the object placed in a new location compared to the object placed in a familiar location (P<0.01, Fig. 3C). In contrast, the animals of the CMS group showed that they were unable to discriminate a novel location from a familiar location (P>0.05, Fig. 3C). DI revealed a significant impairment of ability to discriminate between familiar and new location compared to the control group (P<0.01, Fig. 3D).

Plasma corticosterone, CRH and ACTH level

As shown in Fig. 4, the plasma CRH, corticosterone and ACTH level of the CMS treated mice were significantly higher than those of the un-stressed control animals. These results indicated that the stressed animals might show an impaired feedback regulation in the hypothalamic-pituitary-adrenal (HPA) axis after exposure to CMS procedure.

Plasma IL-1β, IL-6 and TNF-α level

As shown in Fig. 5, a significant increase in plasma IL-1β, IL-6 and TNF-α levels in mice exposed to the CMS procedure was observed (P<0.01).

Morphological analysis

As shown in Fig. 6, CMS procedure resulted in marked neuronal cell damage, as reflected in the increased occurrence of apoptotic cells and decreased number of surviving cells in the DG (Fig. 6E. P<0.01).

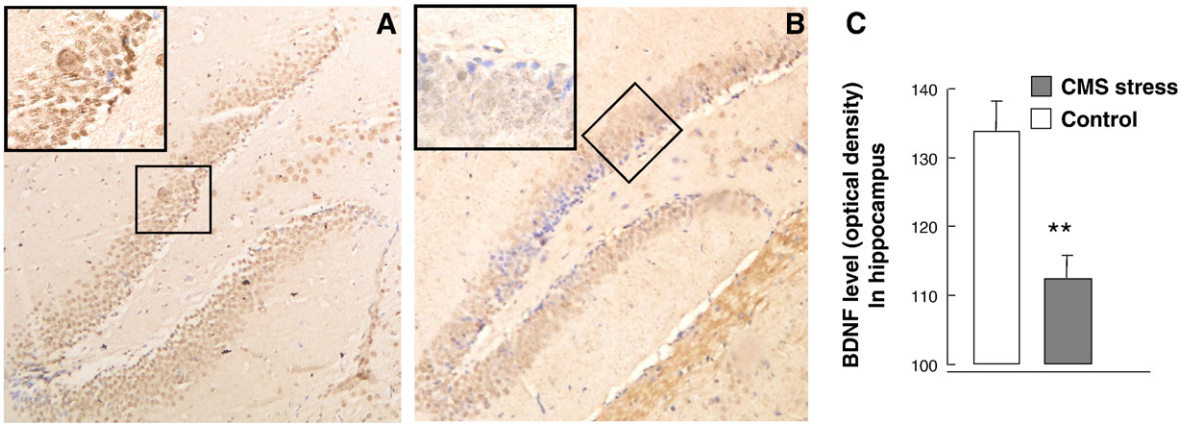

Fig. 8. Effect of CMS on the BDNF level in hippocampus of mice. Shown are representative photomicrographs. A: control group; B: CMS group; BDNF levels were determined by immunohistochemical assay and the cells in brown were BDNF positive. C: Effect of CMS on BDNF level of hippocampus. Data are expressed as mean ± S.E.M. (n = 10). ** P<0.01 compared with un-stressed control group.

BrdU assay of hippocampal neurogenesis

Bromodeoxyuridine (BrdU) was administered to both control and CMS stressed animals, and 24 h later, mice were sacrificed for immunohistochemical analysis. BrdU-positive cells are seen along the subgranular zone (SGZ) of dentate gyrus (Fig. 7A, B). After 5 weeks of CMS procedure, the number of BrdU-positive cells was decreased in CMS stressed animals as compared with the un-stressed control group (Fig. 7C. $P<0.05$).

BDNF level in hippocampus

BDNF has been reported to be important for neuronal survival, depression and cognition. Moreover, the expression of BDNF in the hippocampus is known to be down-regulated in response to acute or repeated stress. In the present study, the average BDNF level decreased in the hippocampus of chronic mild stressed mice ($P<0.05$, Fig. 8C) compared with un-stressed control animals, which was shown as the decreased gray value.

Discussion

Behaviors related to stress-induced mood disorders and cognitive impairments are closely linked via physiological mechanisms. The present study investigated specific neuroimmune, neuroendocrine and neurogenesis changes resulting from the presentation of a reliable animal model for depression, chronic mild stress. The CMS procedure has been employed to investigate specific aspects of mood disorders, including both behavioral and physiological changes (Willner et al., 1994; Anisman et al., 1996; Solberg et al., 1999; Gittos and Papp, 2001; Kubera et al., 2001; Duncko et al., 2001). Results obtained from the present study demonstrate that CMS induces a profile of neuroendocrine and neuroimmune systems activation that has the potential to promote neuronal cell damage, decrease neurogenesis and impair cognitive function in mice.

Neuroendocrine and cognitive function

The present results showed that circulating hormones were significantly elevated in the CMS mice accompanied with cognitive impairment. Evidence had suggested a close association between depressive disorders and neuroendocrine alterations (Jozuka et al., 2003). Circulating corticosterone has been shown to be elevated in approximately half of adult depressed patients (Asnis et al., 1987). Also, levels of corticosterone were elevated in the Flinders Sensitive Line of rats exposed to CMS (Ayensu et al., 1995). Endocrine changes in the HPA axis were observed following CMS, mirroring those changes seen in human depression. Furthermore, an extensive body of studies has found that enhanced HPA axis activities also impair cognitive functions both in rodents and in humans (Song et al., 2006; Csernansky et al., 2006; Maccari and Morley-Fletcher, 2007; Aisa et al., 2007) through glutamate and NMDA pathways that are involved in neuron apoptosis and neurotoxicity.

Neuroimmune and cognitive function

Cognitive impairment and the coexistent neuroendocrine changes in CMS are also related to neuroimmune system activation. The present results showed that circulating cytokines TNF-α, IL-1β and IL-6 were significantly elevated in the CMS group relative to the control group, following exposure to 5 weeks CMS. These findings are consistent with data from Suarez et al. (2003), who showed that low to moderate levels of depressive symptomatology in men were associated with an overexpression of proinflammatory cytokines, including IL-1α, IL-1β, and TNF-α. Cytokines serve as growth factors in peripheral immune cells. These signaling molecules and their receptors have also been identified within the brain; they increase in response to traumatic brain injury, neurological disturbances (Roth et al., 2004; Rothwell, 1999; Vezzani et al., 2002; Wang and Shuaib, 2002), and affective disorders (Leonard, 2001; Anisman and Merali, 2002; Dubas-Slemp et al., 2003). Perhaps most interesting is the finding that CMS, which leads to depression-like behaviors (anhedonia) and cognitive impairments (amnesia), acts on the central and peripheral nervous systems to influence immune and endocrine function in the absence of any peripheral injury, infection, or inflammation. Cytokines produced in the periphery as a result of stress can gain access to the central nervous system by way of the circumventricular organs, through the blood-brain-barrier by selective transporters, via neurally-mediated mechanisms involving sensory nerves, or by stimulating de novo production of central cytokines (Connor and Leonard, 1998; Nguyen et al., 2000). Cytokines could influence the cognitive functions (Schram et al., 2007; Marsland et al., 2006; Reichenberg et al., 2001), and the interactions of cytokines with central processes, such as activation of the HPA axis (Dunn et al., 1999; Pollak and Yirmiya, 2002), might also be important mechanisms that underlie stress-related affective disorders and cognitive deficits. Cytokines acting in key stress-related regions such as the hypothalamus might contribute to the progression of depression. Corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus are key central nervous system targets of TNF-α and IL-1β (Chrousos, 2000). Activation of corticotropin-releasing hormone neurons stimulating the HPA axis is an established feature of major depression (Nemeroff, 1996). Furthermore, since TNF-α and IL-1β stimulate corticotropin-releasing hormone production (Lozovaya and Miller, 2003; Felder et al., 2003), their higher concentration presented in CMS may imply an intense stimulation of the HPA axis. This interaction influences downstream functions such as the release of corticosterone, and further regulation of immune factors (Wichers and Maes, 2002; Francis et al., 2003) also leading to alteration in neurogenesis and the subsequent cognitive impairments.

Neurotrophin, neurogenesis and cognitive function

Ultimately, neurotrophic factors such as BDNF also play an important role in both cognitive functions (such as learning and memory) and mood disorders (depression). In our present study, CMS could reduce hippocampal BDNF expression and induce significant neuronal cell death. These effects might be due to the elevated HPA axis activity and enhanced plasma cytokines. A large number of studies have demonstrated that over-activated HPA axis impaired expression of neurotrophic factors (Ridder et al., 2005; Uys et al., 2006; Mitsukawa et al., 2006; Burton et al., 2007; Faure et al., 2007), while results of previous studies have also demonstrated that neurotrophic factors could be affected by cytokines (Rage et al., 2006; Mattson et al., 1997, 2004; del Porto et al., 2006; Benoit et al., 2001; Maher et al., 2004; Miklic et al., 2004; Nagatsu et al., 2000). Thus, stressor-induced variations of cytokines may ultimately come to influence neurotrophic factors and neurogenesis, and conversely, certain brain cytokines may be influenced by neurotrophic factors (Bayas et al., 2003). Moreover, intra-hippocampal infusion of an IL-1 antagonist prevented the reduction of BDNF mRNA within the DG and CA3 region of mice exposed to social isolation (Barrientos et al., 2004). In light of such findings it should be considered that cytokines and HPA axis might influence mood states and cognition as a result of processes related to neurogenesis, just as interplay with neurotrophins (Nagatsu et al., 2000).

Conclusion

The important observation in the present study is that cognitive impairment induced by CMS in male Swiss albino mice was related to specific alterations in brain homeostasis comprising the following aspects: neuroimmune and neuroendocrine systems as well as

neurogenesis. These findings provide a basis for examining more directly the neural and humoral pathways and interactions that underlie the link between stress and related cognitive impairments.

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