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Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation

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Abstract

This study was conducted to examine the pathophysiologic mechanisms of long-term adverse effects by neonatal maternal separation on neurobehaviors of the offspring. Sprague–Dawley pups were separated from dam daily for 180 min during the first 2 weeks of life (MS) or undisturbed (NH), and subjected to behavioral sessions for ambulatory activity, forced swim, and elevated plus maze tests at 2 months of age. Serotonin reuptake transporter (5-HTT) mRNA levels in the raphe nucleus and the contents of serotonin (5-HT) and its metabolite 5-hydroxyindol acetic acid in the raphe and the hippocampus were examined as well. Ambulatory counts decreased and immobility duration in swim test increased in MS rats compared with NH rats. MS rats spent more time in the closed arms, less time in the open arms, of elevated plus maze, compared to NH rats. The hippocampal contents of 5-HT and the raphe expression of 5-HTT mRNA were decreased in MS rats compared with NH rats. These results suggest that neonatal maternal separation may result in the development of depression- and/or anxiety-like behaviors in later life, in which the long-term alterations in 5-HTergic neurotransmission may take a role.

Keywords: Early life stress; Gene expression; Hypothalamic-pituitary-adrenal axis; Maternal separation; Raphe nucleus; Serotonin transporter

1. Introduction

A number of studies have indicated a strong correlation between traumatic events during early life and the development of behavioral and neuroendocrine abnormalities later in life. For examples, loss of a parent during childhood, a stressful life event, increased the risk of developing major anxiety disorders (Kendler et al., 1992; Furukawa et al., 1999), and women with histories of childhood abuse displayed abnormal responses of hypothalamic-pituitary-adrenocortical (HPA) axis with signs of depression (Heim et al., 2000, 2001). Dysfunction in neurotransmission of serotonin (5-hydroxy-trypamine; 5-HT) is implicated in a variety of psychiatric disorders, including major depression (Mann et al., 1995; Drevets et al., 2000; Bhagwagar et al., 2002) and anxiety (Graeff, 1997; Nutt, 2001). Interestingly, many clinically effective antidepressants altering 5-HT neurotransmission improved both mood and hypercortisolemia (Ogren et al., 1979; Linkowski et al., 1987; Holsboer and Barden, 1996).

Neonatal maternal separation is considered as an animal model of stressful experience early in life. Many of studies have demonstrated its impact both on the activity of HPA axis; i.e., permanent alterations in the characteristics of the HPA response to stress (Ladd et al., 1996; Van Oers et al., 1998; Vazquez et al., 2000) and on the development of depression- (Ladd et al., 2000; Khoury et al., 2006) and anxiety-like behavior (Kalinichev et al., 2002; Daniels et al., 2004) later in life. 5-HT neurotransmission in the hippocampus is believed to be involved in the regulation of HPA axis activity throughout life. Indeed, it has been reported that periodic maternal separations during pre-weaning period result in persistent alterations in 5-HT concentrations (Matthews et al., 2001) and

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5-HTergic functions in selective brain regions including the hippocampus (Gartside et al., 2003; Arborelius et al., 2004; Van Riel et al., 2004). Furthermore, stress response of adrenocorticotrophic hormone (ACTH) was blunted concomitantly with increased 5-HT turnover in the hippocampus in rats with neonatal maternal separation (Daniels et al., 2004).

5-HT reuptake transporter (5-HTT) timely controls 5-HT neurotransmission by reuptake 5-HT from the synaptic cleft immediately after its release. Gene expression of 5-HTT in the dorsal raphe nucleus, where the largest population of 5-HTergic neurons is located, alters by brain 5-HT level (Linnet et al., 1995; Choi et al., 2003). Pharmacologic inhibition of 5-HTT with selective 5-HT reuptake inhibitors, such as fluoxetine, enhances 5-HT neurotransmission and decreases depression symptoms (Gorman and Kent, 1999). Reduced 5-HTT binding was observed in the raphe nuclei of depressed patients with single photon emission computed tomography (Malison et al., 1998). In rodents, decreased expression or lack of 5-HTT appears to correlate with the development of depression-like behavior. That is, 5-HTT mRNA expression was decreased in the dorsal raphe nucleus of *ob*/*ob* mouse showing symptoms of behavioral depression (Collin et al., 2000), and the knockout mice lacking 5-HTT expression exhibited depression-like behaviors with decreased neuronal activities of 5-HTergic neurons in the dorsal raphe nucleus (Lira et al., 2003). Furthermore, chronic treatment with selective 5-HT reuptake inhibitor improved depression-like behavior in an animal model of depression the Flinder Sensitive Line rats with/without neonatal maternal separation (Khoury et al., 2006). These reports together strongly support a functional significance of 5-HTT in the development of depression-like behaviors by maternal separation.

We previously reported that repeated maternal separation during the first 2 weeks of life in rats blunted mRNA expression of corticotropin-releasing hormone (CRH) responding to cold stress (Kim et al., 2005a) and the increase of plasma corticosterone responding to food deprivation, a stressful episode (Kim et al., 2005b). In this study, the hippocampal 5-HT contents and the raphe expression of 5-HTT mRNA were examined in our maternal separation model parallel with behavioral assessments for depression and anxiety.

2. Materials and Methods

2.1. Animals

Sprague–Dawley rats were purchased (Daehan biolink, Co., Korea), and cared in a specific-pathogen-free barrier area with constant control of temperature (22 ± 1 °C), humidity (55%), and a 12/12 h light/dark cycle (lights-on at 07:00 a.m.). Standard laboratory food (Purina Rodent Chow, Purina Co., Seoul, Korea) and membrane filtered purified water were available *ad libitum*. Animals were cared according to the Guideline for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guidelines for the Care and Use of Laboratory Animals, revised 1996. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University.

Nulliparous females and proven breeder males were used for breeding in the laboratory of the animal facility, and the pups were reared in a controlled manner to minimize and standardize unwanted environmental stimulation from *in utero* life. Twelve hours after confirming delivery (PND 1), pups were culled to five males and five females per litter. Each litter was assigned either for the maternal separation (MS) group or for the non-handled (NH) group. The MS group was removed from their dam and home cage and placed closely together in a new cage bedded with woodchips for 180 min, and then returned to their home cage and dam. Maternal separation was performed during 9:00–12:00 h daily from PND 1 through 14, and then the pups were left with their dam undisturbed until weaning on PND 22. The NH group remained undisturbed throughout the whole period except for cleaning the cages. Rats were single caged after weaning, and one male pup from each litter were hired for the present study and the rest were excluded. All rats had free access to rodent chow and water *ad libitum* until sacrificed. Body weight gain and 24 h food intake were recorded weekly after weaning.

2.2. Ambulatory activity

Rats were subjected to ambulatory activity test for three consecutive days from PND 61 (6 NH rats and 6 MS rats from 12 different litters). On each trial day, rat was placed in the center of the activity chamber (43.2 cm in length, 42.2 cm in width, and 30.5 cm in height, MED Associates, VT, USA), a transparent acryl chamber equipped with two horizontal planes of 16 infrared photocell-detector pairs placed in *x*, *y* dimension, spaced 2.5 cm apart, and its ambulatory activity for a 5 min session was monitored by computerized system. Ambulatory activity was measured as the total counts of beam interruptions in the horizontal sensor during the initial 3 min of the trial. The activity chamber was cleaned with 70% alcohol after each use to eliminate any olfactory cues of previously tested rat.

2.3. Forced swim test

Another group of rats from each group were subjected to forced swim test (8 NH rats and 8 MS rats from 16 different litters), according to the method previously described (Porsolt et al., 1977). Each rat was allowed to swim in a glass cylinder (54 cm in height and 24 cm in diameter) filled with water in 40 cm of depth (23–25 °C) for 15 min on PND 61, as pre-swim trials, and then removed from the cylinder, dried with paper towel and returned to their home cage. From the next day (PND 62), rats were subjected to 5 min of swim test daily for three consecutive days. All test sessions were recorded by a video camera from the side of the cylinder. Duration of rat's immobility in the water was scored from videotapes by a trained observer who was blinded to the experimental conditions. Immobility was defined as the state in which rats were judged to be making only the movements necessary to keep their head above the surface.

After the end session of swim test, rats were allowed to rest in their home cages for 7 days to minimize any effects of previous stress, and then subjected to elevated-plus maze test.

2.4. Elevated plus maze

Rats were subjected to the behavioral assessment in an elevated plus maze, a plus shaped acryl maze with two opposite open arms (50 cm in length and 10 cm in width) and two opposite closed arms (50 cm in length, 10 cm width, and 31 cm in height), extending out from a central platform (10 cm × 10 cm). The whole apparatus was elevated 50 cm above the floor. The test procedure was followed as previously described (Daniels et al., 2004). Each rat was placed in the center of the maze, and then allowed to explore the open or closed arms of the maze for 5 min. The time spent in the different arms was recorded, respectively. Two paws had to be inside the entrance line to each arm, which signaled the start of the time spent in the specific arm, and then the end time was recorded when all four paws were outside the line again. The maze was cleaned with 70% ethanol after each test to prevent influences of previously tested rat.

All behavioral assessments were performed between 9:00 a.m. and 12:00 p.m. of the day to avoid the influence of circadian variances.

2.5. 5-HT and 5-HIAA contents

Rats (8 NH rats and 6 MS rats from 14 different litters) were rapidly decapitated on PND 60 after brief anesthesia in a carbon dioxide chamber.

Tissue samples of the hippocampus and the midbrain raphe were rapidly dissected on ice immediately after decapitation, frozen in liquid nitrogen and stored at −80 °C until used. 5-HT or its metabolite 5-hydroxyindol acetic acid (5-HIAA) contents of the tissue samples were measured by high-performance liquid chromatography (Waters Instrument, Model 700, Milford, MA, USA) equipped with an ESA Coulochem II Elctrochemical Dector (ESA Inc., Chelmsford, MA, USA) packed by biophase ODS 5 μm (250 mm × 4.5 mm, Bioanalytical System Inc., West Lafayette, IN, USA) according to a slight modification of the method previously reported (Wagner et al., 1982). The mobile phase, comprising of acetonitrile 8% and 92% 0.15 M monochloroacetic acid buffer (0.55 mM sodium octyl sulfate, 2 mM disodium EDTA, pH 3.35) was pumped at a rate of 1 ml/min.

2.6. In situ Hybridization

Rats (8 NH rats and 9 MS rats from 17 different litters) were anesthetized with an overdose of sodium pentobarbital on PND 60. Once unresponsive, transcardiac perfusion was performed with heparinized isotonic saline (0.9% NaCl, 0.5% $NaNO_2$) followed by ice-cold fixative (4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2). Brains were rapidly dissected, blocked, and post-fixed in the same fixative for 3 h, and then transferred into 30% sucrose solution for 24 h for cryoprotection. Forty-micron coronal sections were cut on a freezing sliding microtome (MICROM Laborgeräte, Walldorf, Germany). Alternate sections of the raphe nucleus (between bregma −7.64 and −8.80 mm; Paxions and Watson, 1986) were collected into 20 ml glass scintillation vials containing ice-cold 2× SSC (0.3 M NaCl, 0.03 M sodium citrate). The SSC was pipetted off, and the sections were suspended in 1 ml of prehybridization buffer (50% formamide, 10% dextran sulfate, 2× SSC, 1× Denhardt's solution, 50 mM dithiothreitol, and 0.5 mg/ml denatured herring sperm DNA), incubated for 2 h at 48 °C. *In situ* hybridization was performed with radioactively labeled cDNA probes of 5-HTT (a 0.8 kb EcoR1 restriction fragment; Jahng et al., 1998) as we previously described (Choi et al., 2003). The tissue sections were then mounted on gelatin-subbed slides, air-dried, and apposed to Kodak BioMax film (Eastman Kodak Co., NY, USA) at 4 °C. Exposure times varied from 12 to 48 h to obtain autoradiographic images within a linear range of optical density after development in Kodak D-19 developer. Images on the autoradiographic films were analyzed as we previously described (Choi et al., 2003).

The data were analyzed by one way analysis of variance (ANOVA) and preplanned comparisons with the controls were performed by post hoc Fisher's PLSD test, post hoc Scheffe's test, or unpaired *t*-test using StatView software (Abacus, Berkeley, CA). All data were presented as the means ± S.E.

3. Results

Body weight and 24 h food intake were measured weekly after weaning. Body weight gain did not differ between the groups until PND 29 (NH; 97.25 ± 1.63 g versus MS; 101.00 ± 2.06 g), however, significantly increased in the MS group compared with the NH group from PND 36 [$F(1,14) = 15.935$, $P < 0.01$ on PND 36; $F(1,14) = 19.159$, $P < 0.001$ on PND 43; $F(1,14) = 23.113$, $P < 0.001$ on PND 50; $F(1,14) = 23.407$, $P < 0.001$ on PND 57] (Fig. 1A). Weight difference between the groups appeared to become bigger as they grew [$F(1,14) = 15.569$, $P < 0.01$, NH; 321.44 ± 8.43 g versus MS; 364.31 ± 6.86 g on PND 60]. Significant increases in daily chow intake were firstly found in MS rats on PND 43 [$F(1,14) = 7.696$, $P < 0.05$ versus NH], and persisted until sacrificed (Fig. 1B).

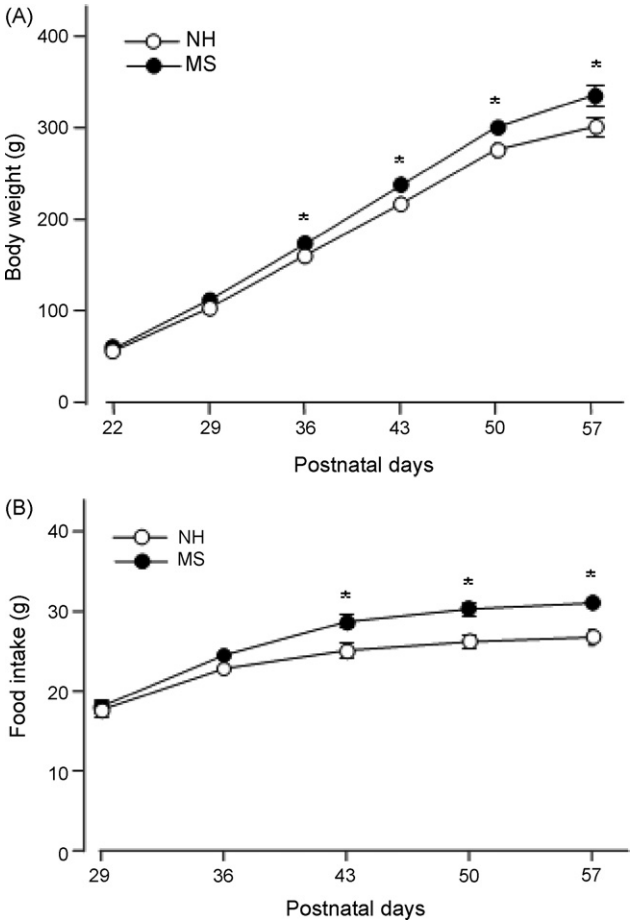

Fig. 1. Changes in body weight and daily food intake after weaning. (A) Body weight of maternal separation (MS) rats did not differ from non-handled (NH) rats until postnatal day (PND) 29. A significant increase in weight gain of MS rats was firstly found on PND 36 and persisted throughout the experimental period. $^{*}F(1,14) = 15.935$, $P < 0.01$ on PND 36; $^{*}F(1,14) = 19.159$, $P < 0.001$ on PND 43; $^{*}F(1,14) = 23.113$, $P < 0.001$ on PND 50; $^{*}F(1,14) = 23.407$, $P < 0.001$ on PND 57. (B) Daily chow intake was increased in the MS group from PND 43. $^{*}F(1,14) = 7.696$, $P < 0.05$ on PND 43; $^{*}F(1,14) = 12.216$, $P < 0.01$ on PND 50; $^{*}F(1,14) = 12.666$, $P < 0.01$ on PND 57; open circles refer NH rats and solid circles MS rats. $n = 8$, values are presented as means ± S.E.

3.1. Behavioral assessments

Ambulatory activities of NH or MS rats were assessed during PND 61–63, and measured as total beam breaking numbers for 5 min each day. Average counts of the first 3 min in each day were presented in Fig. 2. A gradual reduction in the activity counts was detected in NH rats over the three consecutive test days. Ambulatory counts was significantly reduced in MS rats compared with NH rats on test day 1 [$F(1,10) = 6.545$, $P < 0.05$], suggesting a behavioral depression in MS rats. A gradual reduction in the counts was not detected in the MS group following test days; i.e. MS rats were not habituated to the ambulation test.

In order to confirm whether or not the MS rats show depression-like behavior, another groups of rats in each group were subjected to forced swim test during PND 62–64, and immobility duration was measured (Fig. 3). Immobility duration of NH rats was gradually increased during three consecutive days of swim test, showing a typical pattern of learned helplessness. Immobility duration of MS rats during the first swim test were markedly increased [$F(1,14) = 25.450$,

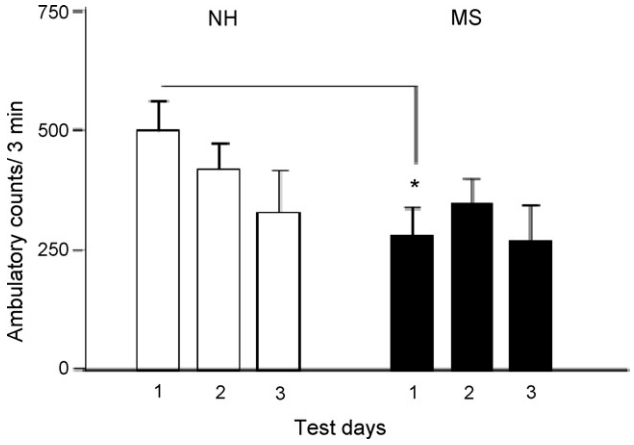

Fig. 2. Ambulatory activities of NH or MS rats assessed over three consecutive days (PND 61–63). Ambulatory activity was measured as the total counts of beam interruptions during the initial 3 min of the trial. Ambulatory activities of NH rats decreased gradually over the test days. Average activity counts of MS rats was significantly decreased on the first test day compared with NH rats [$^{*}F(1,10) = 6.545$, $P < 0.05$], and did not changed over the test days. Open bars refer NH rats and solid bars MS rats. $n = 6$, values are presented as means ± S.E.

$P < 0.001$ versus NH rats], showing depression-like behavior (Fig. 3). MS rats did not show a pattern of learned helplessness during repeated swim tests.

After the end session of swim test, rats were allowed to rest in their home cages for 7 days to minimize any effects of previous stress, and then subjected to elevated plus maze test for anxiety-like behavior. The time spent in the closed arms of the maze markedly increased in MS rats [$F(1,14) = 25.457$, $P < 0.001$] compared with NH rats (Fig. 4). Contrarily, MS rats spent less time in the open arms of the maze [$F(1,14) = 25.695$, $P < 0.001$ versus NH rats in the open arms].

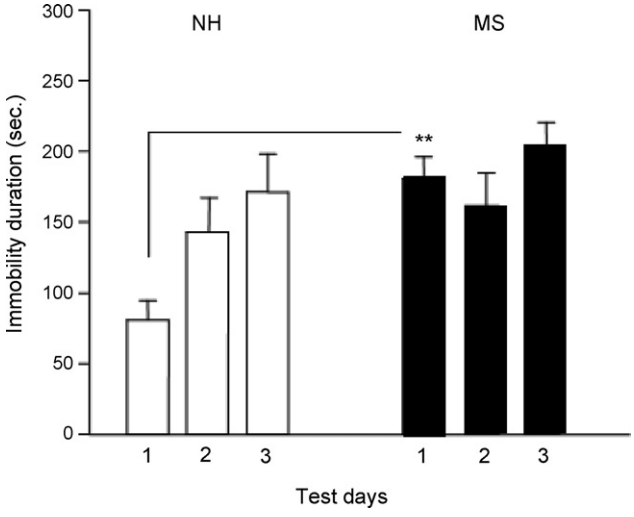

Fig. 3. Forced swim test assessed over three consecutive days (PND 62–64). Immobility duration during 5 min of forced swim on each test day was compared between the groups. Immobility duration of NH rats was gradually increased over the test days. MS rats showed a marked increase in immobility during the first swim test [$^{**}F(1,14) = 25.450$, $P < 0.001$ vs. NH rats]. Immobility duration of MS rats did not change over the test days. Open bars refer NH rats and solid bars MS rats. $n = 8$, values are presented as means ± S.E.

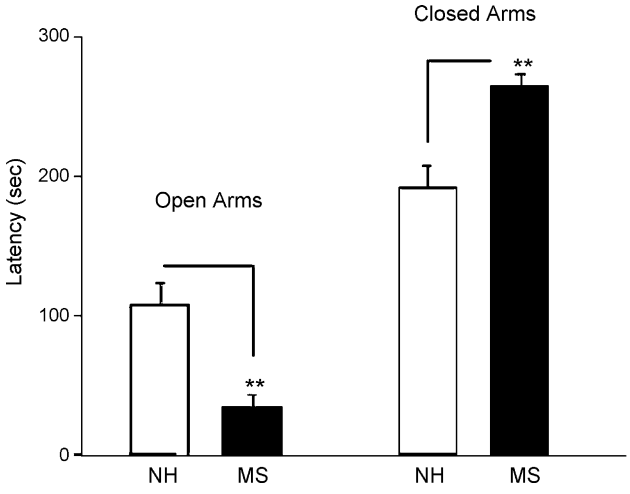

Fig. 4. Elevated plus maze tests of NH and MS rats. Seven days after the swim test, rats were subjected to elevated plus maze test. Each rat was placed in the center of the maze, and then allowed to explore the open or closed arms of the maze for 5 min. MS rats spent more time in the closed arms, less time in the open arms, compared with NH rats. $^{**}P < 0.001$ vs. NH group [open arms: $F(1,14) = 25.695$; closed arms: $F(1,14) = 25.457$]. Open bars refer NH rats and solid bars MS rats. $n = 8$, values are presented as means ± S.E.

3.2. 5-HT contents in the brain regions

Rats were sacrificed on PND 60 for HPLC analysis of 5-HT and 5-HIAA contents in the midbrain raphe and the hippocampus. Either 5-HT or 5-HIAA contents in the raphe nucleus of MS rats did not differ from NH rats (Fig. 5A). The hippocampal contents of 5-HT, but not of 5-HIAA, were decreased in MS rats [$F(1,12) = 6.663$, $P < 0.05$] compared with NH rats (Fig. 5B). Turnover rates of 5-HT (5-HIAA/5-HT) in the hippocampus tended to be increased in MS rats without statistical significance.

3.3. 5-HTT mRNA in situ hybridization

Rats were sacrificed on PND 60 to examine mRNA levels of serotonin reuptake transporter (5-HTT) in the dorsal raphe nucleus by *in situ* hybridization. 5-HTT mRNA expression in the dorsal raphe appeared to be decreased by experience of maternal separation (Fig. 6A). Quantificational analysis showed that 5-HTT mRNA levels in the dorsal raphe nucleus of MS rats were significantly decreased [$F(1,15) = 8.769$, $P < 0.01$] compared with NH rats (Fig. 6B).

4. Discussion

4.1. Depression- and anxiety-like behaviors in MS rats

Many human studies have reported that syndromal major depression and anxiety disorders are frequent in adults with a history of child hood abuse (Mullen et al., 1996; Stein et al., 1996; Felitti et al., 1998) and that early parental loss is related to unipolar and bipolar depression, as well as anxiety disorders, beyond familial or genetic factors (Kendler et al., 1992; Agid et al., 1999; Furukawa et al., 1999; Heim and Nemeroff, 2001).

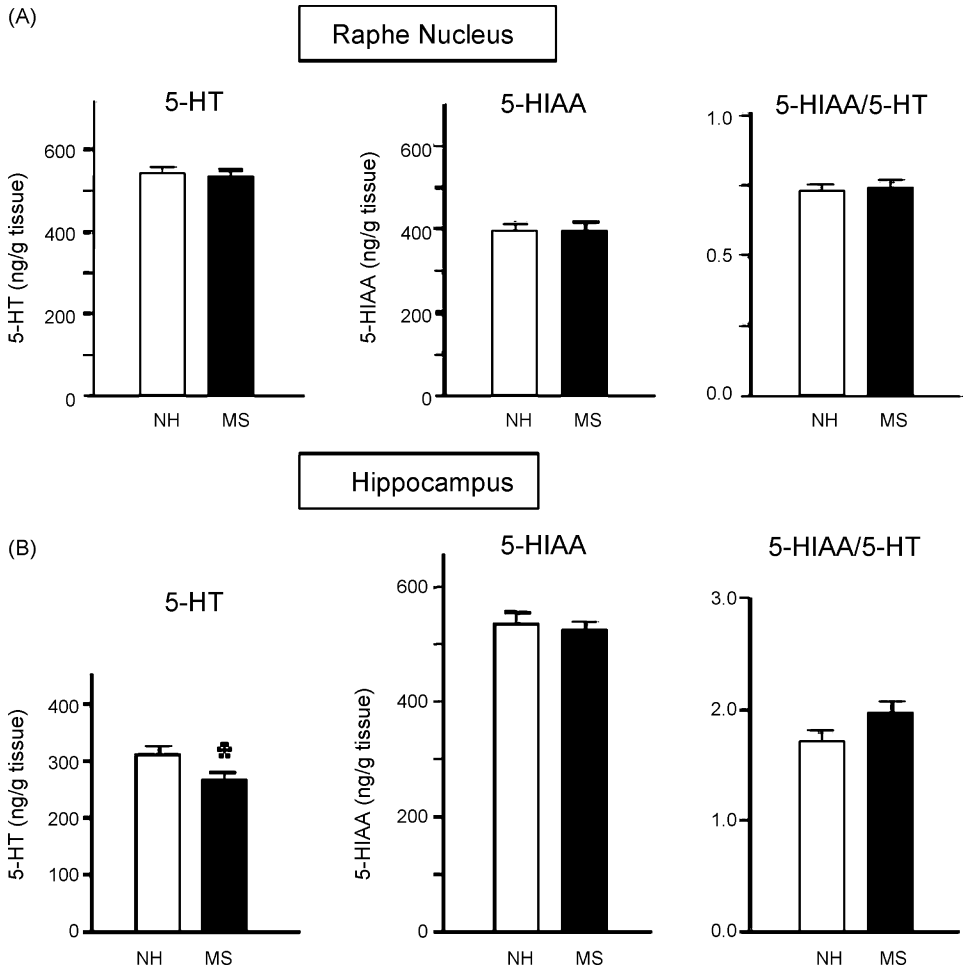

Fig. 5. HPLC analyses of 5-HT and 5-HIAA contents in the midbrain raphe and the hippocampus. Brain tissues were dissected immediately after decapitation on PND 60. (A) Either 5-HT or 5-HIAA contents in the raphe of MS rats did not differ from NH rats. (B) The hippocampal contents of 5-HT, but not of 5-HIAA, were decreased in MS rats compared with NH rats. Turnover rates of 5-HT (5-HIAA/5-HT) in the hippocampus tended to be increased in MS rats, but did not reach a statistical significance. $^{*}F(1,12) = 6.663$, $P < 0.05$ vs. NH, open bars refer NH rats and solid bars MS rats. 8 NH rats and 6 MS rats were analyzed. Values are presented as means ± S.E.

In this study, we demonstrated that neonatal maternal separation in rats results in depression- and anxiety-like behaviors at young adulthood. That is, MS rats exhibited decreased ambulatory activity and increased immobility in forced swim test, and spent significantly more time in the closed arms, less time in the open arms, of elevated plus maze, than NH rats. Our results are in accordance with reports by others following a similar separation paradigm (Ladd et al., 2000; Kalinichev et al., 2002; Newport et al., 2002; Daniels et al., 2004; Khoury et al., 2006), and further supports that neonatal maternal separation is an adequate rat model to study the molecular mechanism underlying the pathophysiology of affective disorders related with early life exposure to adverse events, including early parental neglect or loss, in human.

4.2. Decreased 5-HT contents in the hippocampus

Dysfunction in 5-HT neurotransmission is implicated in a variety of psychiatric disorders, including major depression (Mann et al., 1995; Drevets et al., 2000; Bhagwagar et al., 2002) and anxiety (Graeff, 1997; Nutt, 2001). In this study, 5-HT contents in the hippocampus of MS rats significantly decreased compared with NH rats. This result concurs with previous report by Matthews et al. (2001) that periodic maternal separation during neonatal period of rats decreases 5-HT contents of the hippocampus at adulthood. 5-HT neurotransmission in the hippocampus is known to be involved in the regulation of the HPA axis activity. That is, the hippocampus is known to regulate the HPA axis activity via mediation of glucocorticoid negative feedback, and *in vitro* study demonstrated that exposure to 5-HT significantly increased mRNA levels of glucocorticoid receptor in hippocampal neurons (Erdeljan et al., 2005). Thus, decreased 5-HT contents in the hippocampus of MS rats may implicate dysfunctions in their HPA axis activity. Indeed, we previously showed that CRH mRNA expression responding to cold stress (Kim et al., 2005a) and corticosterone increase responding to food deprivation (Kim et al., 2005b) were blunted in our animal model of maternal separation. It was also reported that rats experienced neonatal maternal separation showed anxious behavior with blunted ACTH response to stress, and their hippocampal 5-HTergic activity responding to stress was modulated (Daniels et al., 2004). Taken together with the

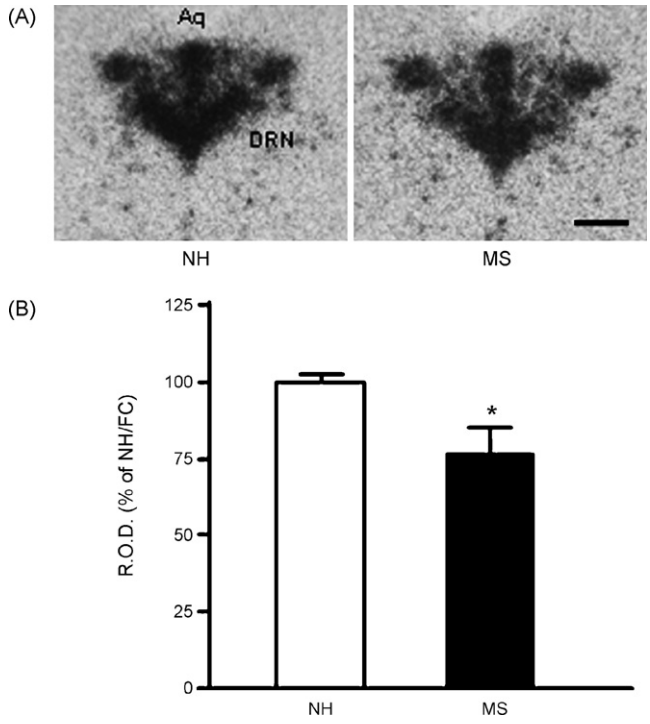

Fig. 6. Autoradiography and quantifications of 5-HTT mRNA *in situ* hybridization. Rats were sacrificed on PND 60, and the raphe sections were hybridized with 5-HTT cDNA probes labeled with ^{35}S-dATP. (A) 5-HTT *in situ* signals in the dorsal raphe nucleus appear to be decreased in MS rats. (B) Quantificational analysis shows a significant decrease in 5-HTT mRNA levels in the dorsal raphe nucleus of MS rats compared with NH rats. $^{*}F(1,15) = 8.769$, $P < 0.01$ vs. NH, aq: aqueduct; DRN: dorsal raphe nucleus; Scale bar: 100 μm; open bar refers NH rats and solid bar MS rats. 8 NH rats and 9 MS rats were analyzed. Values are presented as means ± S.E.

present results, it is concluded that decreased 5-HT neurotransmission in the hippocampus may take a role in the pathophysiology of behavioral depression and/or anxiety, possibly in relation with dysfunction of the HPA axis activity, in neonatal maternal separation model.

4.3. Decreased 5-HTT expression in the raphe nucleus

Most of 5-HT neurons innervated to the whole brain regions including the hippocampus are localized in the raphe nucleus. Previous reports suggested that neonatal maternal separation may decrease 5-HTergic activity of the raphe nucleus at adulthood (Gartside et al., 2003; Arborelius et al., 2004). In this study, we demonstrated that mRNA expression of serotonin reuptake transporter (5-HTT) in the raphe nucleus of adult rat is decreased by experience of neonatal maternal separation. 5-HTT reuptakes 5-HT from the synaptic cleft immediately after its release and ceases 5-HT neurotransmission, and 5-HTT mRNA expression in the raphe has been reported to be altered by brain 5-HT level (Linnet et al., 1995; Choi et al., 2003). Thus, it appears that the decreased mRNA levels of 5-HTT in the raphe nucleus of MS rats may, at least partly, be influenced by decreased 5-HT levels in the hippocampus. It has been suggested that decreased expression or lack of 5-HTT may correlate with the development of behavioral depression in rodents (Collin et al., 2000; Lira et al., 2003), and depressed patients showed decreased binding of 5-HTT in the raphe nuclei from single photon emission computed tomography (Malison et al., 1998). These reports support the idea that decreased expression of 5-HTT mRNA may take a role in the development of behavioral depression by experience of neonatal maternal separation. However, the underlying molecular mechanisms should be further studied.

4.4. Increases in weight gain and food intake

Previous studies have reported that repeated maternal separation during neonatal period transiently alters body weight gain in offspring. That is, MS males are slightly lighter than NH males shortly after the separation period, and thereafter MS males tended to be heavier than NH males without statistical significances (Iwasaki et al., 2000; Kalinichev et al., 2002). In our separation model, we did not measure body weights of the pups until weaning (PND 22) to minimize handling effects in the NH control group. Thus, we do not know whether the body weights of MS pups were lighter or not shortly after the separation period (PND 2–14). However, significant increases in body weight again were detected in MS rats from PND 36, compared to NH rats, and the difference persisted until sacrificed. Increases in daily chow intake appeared to contribute to the increased weight gain in MS rats. Since 5-HT plays a role in feeding behavior as an anorectic molecule (Curzon, 1990), it appears that increased food intake and weight gain in MS rats may, at least partly, be related with decreased 5-HTergic activities in their brain regions, such as decreased 5-HTT expression and 5-HT neurotransmission. Moreover, 5-HT turnover rate in the hypothalamus, where 5-HT exerts its anorectic effects (Leibowitz and Alexander, 1998), was decreased in MS rats compared with NH rats (unpublished our preliminary study). In addition, increased chow intake in our MS rats showing depression-like behavior could serve as an animal model system to study overeating behaviors accompanied with depression symptom (Riener et al., 2006).

In our previous study, MS rats were slightly heavier than NH rats at PND 22 and 29, and the weight differences between the NH and the MS groups became non-significant statistically from PND 36 (Kim et al., 2005b). Daily chow intake of MS rats used in our previous study did not differ from NH rats (unpublished observation). Our speculation is that the different housing methods after weaning might have led to the differences in weight gain and chow intake; i.e. pups were singly caged after weaning in this study as described, but five weanling pups were housed together in our previous study (Kim et al., 2005b). Lastly, our MS rat model that exhibits depressive and anxious behaviors with increased weight gain and food intake could serve as a good model system to study the pathophysiology of affective disorders related with early life adverse episodes, oftenly accompanied with eating disorders.

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