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Beneficial Effects of Highly Palatable Food on the Behavioral and Neural Adversities induced by Early Life Stress Experience in Female Rats

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Abstract

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This study examined the effects of highly palatable food during adolescence on the psycho-emotional and neural disturbances caused by early life stress experience in female rats. Female Sprague-Dawley pups were separated from dam for 3 h daily during the first two weeks of birth (MS) or left undisturbed (NH). Half of MS females received free access to chocolate cookies in addition to *ad libitum* chow from postnatal day 28. Pups were subjected to the behavioral tests during young adulthood. The plasma corticosterone response to acute stress, ΔFosB and brain-derived neurotrophic factor (BDNF) levels in the brain regions were analyzed. Total caloric intake and body weight gain during the whole experimental period did not differ among the experimental groups. Cookie access during adolescence and youth improved anxiety-/depression-like behaviors by MS experience. ΔFosB expression was decreased, but BDNF was increased in the nucleus accumbens of MS females, and ΔFosB expression was normalized and BDNF was further increased following cookie access. Corticosterone response to acute stress was blunted by MS experience and cookie access did not improve it. Results suggest that cookie access during adolescence improves the psycho-emotional disturbances of MS females, and ΔFosB and/or BDNF expression in the nucleus accumbens may play a role in its underlying nneural mechanism.

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Keyword: early life stress, reward system, preferred diet, depression, anxiety
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Contents

Ⅰ. Introduction

There is a growing body of evidence that identical dietary manipulations can have divergent responses between the sexes. At the molecular level, it has been demonstrated that there are sexually dimorphic responses of the hippocampal transcriptome between male and female rats exposed to the same diet.[1]At the metabolic/neuroendocrine level, female rats exhibit different hypothalamic neuropeptide responses to a prolonged high fat diet[2] and higher capacity than males to compensate a high lipid influx[3]. Short term high fat-fed adult females have decreased glucocorticoid receptor mRNA levels in the hippocampus and their hypothalamic- pituitary-adrenal (HPA) axis responds differently from males to a subsequent stress.[4, 5] At the behavioral level, short term exposure of adult rats to fat diet reduces anxiety and increases exploration in males, while it has the opposite effect in females.[6]Puberty is a crucial developmental period characterized by increased endocrine plasticity and changes in stress responsiveness.[7] Studies have suggested that post-weaning high fat diet can modify the basal HPA axis activity and the endocrine responses to an acute stress by affecting both stress and metabolic media tors in a sexually dimorphic manner.[8,9] We have previously found that prolonged consumption of highly palatable food during adolescence increases anxiety- and depression-like behaviors in male rats, but not in female rats.[10]Prolonged consumption of cafeteria diet high in fat (32% fat content) improved behavioral adversities both in male and female rats that subjected to a similar maternal separation (MS) protocol used in this study, with greater beneficial effects in males.[11] The behavioral and neuroendocrine adversities observed in our female MS rats [12] appeared to differ from ones in

male MS rats.[13,14] In our previous study, prolonged access to highly palatable food, a moderate fat diet (~21% fat),[6,15] during adolescence and youth improved some anxiety-related symptoms and the HPA axis dysfunction in male MS rats.[14] Studies have suggested that modulation of the stress axis function is implicated in the positive emotional behaviors by highly palatable diet. That is; exposure to a highly preferred diet high in fat was suggested to reduce stress sensitivity.[16] individuals offered with highly palatable food had more pleasant emotions such as satisfaction, enjoyment, and desire[17] and consumption of palatable food decreased sympathetic responses following psychological and immunological stresses,[18] stress hormone levels following restraint[19] and anxiety-like behaviors during the elevated plus maze test in rats.[20] However, in this study, the moderate fat diet(~21% fat) during adolescence and youth did not improve the HPA axis dysfunction of MS females, although it improved not only anxiety- but also depression-like behaviors. In order to investigate the neural mechanisms underlying the psycho-emotional effect of highly palatable diet access in our MS females, we have examined brain-derived neurotrophic factor (BDNF) and ΔFosB levels in the nucleus accumbens (Nac). The Nac, a basal forebrain structure constituting a mesolimbic dopaminergic pathway, has a role in reward, motivation, and reinforcement.[21] Development of anhedonia, a core symptom of major depressive disorder, has been ascribed to dysfunction of the reward pathway, in which the Nac plays a pivotal role.[22,23]The Nac neurons are activated responding to behavioral stress paradigm,[24, 25] and have been implicated in anxiety disorders.[26,27] The mesolimbic dopaminergic activity and the stress-induced activation of the Nac neurons were blunted in our male MS rats that showed anxiety and depression-like behaviors.[13,28] BDNF was sugg-

-ested to be involved in hedonic feeding via modulation of the mesolimbic dopamine system,[29, 30] and exposure to a palatable diet increased BDNF and ΔfosB levels and dopamine receptor D1 binding in the Nac.[16, 31, 32]

Ⅱ. Materials and Methods

Animal

Sprague-Dawley rats were purchased (Samatako Bio, Osan, Korea), and cared in a specific-pathogen-free barrier area with constant control of temperature (22±1℃), humidity(55%), and a 12/12hr light/dark cycle (lights-on at 7:00 AM). Standard laboratory food (Pruina 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 Seoul National University.

Experimental protocol

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 [postnatal day (PND)1], pups were manipulated as we previously described.[13, 14, 33 - 35] Each litter was assigned either for the maternal separation (MS) group or for the non-handled (NH) group. MS pups were removed from their dam and home cage and placed closely together in a new cage bedded with woodchips (Aspen shaving, Animal JS Bedding, Cheongyang, Korea) during 9:00 h - 12:00 h, and then returned to their home cage and dam. No additional treat-

-ment to keep the pups warm during the separation period was offered. MS was performed 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 until weaning except for routine cage cleaning performed twice a week. On the weaning day, 2 NH and 4 MS female pups were randomly selected from each NH or MS litter, respectively, and placed 2 NH or 2 MS pups together in each cage. Two female MS pups housed together received free access to highly palatable food (HPF) (Oreo cookie, Kraft Foods Global, Inc., East Hanover, NJ, USA), in addition to ad libitum chow from PND 28 (MS+HPF group), and the rest 2 female MS pups in each litter (MS group) and NH pups (NH group) received standard chow only. The nutrient composition formulae of standard chow and Oreo cookie is shown in Table 1. Daily food intake and weight gain were recorded from PND 29. For the evaluation of 24 h food intake, premeasured amount of chow and cookies was provided, and on the next day, left amount of chow and cookies was weighed and subtracted from the value provided on the previous day. Special care was taken to include spillage. Caloric intake was calculated according to the nutrient composition formulae of chow and cookies. Total amounts of food consumed by the pups in each cage were divided by the number of pups in each cage and the each calculated value was considered as n=1. Water was freely available to all experimental groups, and the food conditions were continued throughout the whole experimental period. The schematic diagram of experimental protocol is provided in Figure 1.

Ambulatory Activity

NH, MS and MS+HPF females (n=8 from 4 different litters in each group;

total 24 pups from 8 different litters) were subjected to the ambulatory test on PND 54. On each trial, the 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 was monitored by the computerized system for 30 min. Light condition of the test room was maintained at the same intensity with animal rooms under day-light condition. Ambulatory activity was measured as the total counts of beam interruptions in the horizontal sensor during each consecutive 5 min session. Defecation activity, weight of fecal boli, during the ambulation test of each rat was scored as well. Grooming activity was further analyzed; i.e., forepaw and head grooming was considered as rostral grooming, and body, legs, and tail/genital grooming as caudal grooming.[36] The activity chamber was cleaned with 70% ethanol after each use to eliminate any olfactory cues of the previously tested rat.

Elevated Plus Maze

Two days after the ambulatory activity test (PND 56), 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 in width, and 31 cm in height), extending out from a central platform (10 cm x 10 cm). The whole apparatus was elevated 50 cm above the floor. The test procedure was followed as previously described.[37] Each rat was placed in the center of the maze facing one of the open arms, 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. Four 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 the previously tested rat.

Forced Swim Test

Three days after the elevated plus maze test (PND 59), rats were subjected to the forced swim test, according to the method previously described.[38] Each rat was allowed to swim in a glass cylinder(54cm in height and 24cm in diameter) filled with water in 40cm of depth (23~25℃) for 5 min, and the test sessions were recorded by a video camera from the side of cylinder. Duration of rat's immobility in the water was scored from videotapes using a stopwatch. 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 of water.

Plasma corticosterone assay

A week after the end of behavioral sessions, rats were placed in a restraint box for 2 h, in which rats were able to move their four limbs but not to change their body orientation. Tail blood was collected at 0, 30, 60 and 120 min time points during the restraint period, and centrifuged at 2,000 rpm for 20 min. The plasma samples were frozen in liquid nitrogen, and stored at - 80 °C until used for the assay. Plasma levels of corticosterone were determined by radioimmunoassay using 125I-labelled Coat-A-Count kit (Siemens, CA, USA). The sensitivity of the assay was 5.7ng/ml. The intra-assay coefficient of varia-

-tion was 4-12.2 %.

Western blot analysis

Rats that are naïve from the behavioral tests (n=6 from 3 different litters in each group; total 18 pups from 6 different litters) were sacrificed on PND 62 for the western blot analysis of ΔFosB and BDNF levels in the brain regions. Retroperitoneal fat pads were collected at the time of sacrifice and the brains were removed immediately after decapitation. Tissue samples of nucleus accumbens (NAc) and hippocampus were rapidly dissected on ice, frozen in liquid nitrogen and stored at - 80 °C until used. The NAc tissue dissection was performed using a fine blade according to the method used in our previous studies;[28, 39] however, a possible inclusion of nearby ventro-medial striatum cannot be avoided. The tissues were homogenized in a single detergent lysis buffer (50 mM Tris, pH 8.0; 150 mM NaCl; 1% Triton X-100; protease and phosphatase inhibitor cocktail 0.5%) and then centrifuged at 13,000 g for 20 min at 4°C. The supernatants transferred into new tubes were measured for protein content using a protein assay kit (Biorad DC, Biorad, Inc., Hercules, CA), aliquoted at an 80 µg/20 µl concentration in lysis buffer, and stored at – 80 °C, otherwise used in the same day. The samples were mixed with loading buffer (100 mM Tris, pH 6.8; 200 mM dithiothreitol; 4% SDS; 20% glycerol; 0.2% bromophenol blue) at 1:1 dilution, boiled for 5 min, quickly chilled on ice, and then electrophorazed on 12% SDS-polyacrylamide Tris-glycine gels. The proteins transferred onto nitrocellulose membranes (Hybond-C, Amersham, Bucks, UK) were treated with 5% nonfat dry milk in 1X Tris-buffered saline-Tween (10 mM C4H11NO3 0.145 M NaCl; 0.2 % SDS; 0.1 % Tween 20) overnight at 40C. The membranes were reacted with polyclonal rabbit anti-

ΔFosB (1:1000 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) or anti-BDNF (1:500 dilution; Millipore, Temecula, CA, USA), and the bound antibodies were detected with chemiluminescence according to the manufacturer's instructions (Lumi-light western blotting substrate; Roche, Indianapolis, IN, USA), and quantified using a digital image analysis system (LAS-1000, Fuji film, Tokyo, Japan). Digitized values of each sample were normalized to the loading control β-actin, and then all values were converted to relative values to the averaged value of NH group.

Statistical analysis

Data were analyzed by one- or two-way [corticosterone data; treatment (handling or food condition, 2 levels each) X time (4 levels)] analysis of variance (ANOVA), and preplanned comparisons between groups was performed by post hoc Fisher' PLSD test when necessary, using StatView software (Abacus, Berkeley, CA, USA). Body weight and food intake data were further analyzed by repeated measures ANOVA, followed by Bonferroni correction for P value adjustment. The level of significance was set at $P < 0.05$, and all values were presented as means ± S.E.M.

Ⅲ. Results

1. Food intake and body weight gain

MS females appeared to be lighter than the age-matching NH females until PND 39 and the weight difference was not observed thereafter (Figure 2). Statistically significant differences ($P<0.05$) between NH and MS females were observed during PND 32 - 39, except on PND 36 and 37. Palatable food access diminished the weight difference by MS experience, and the statistical significance between NH and MS+HPF disappeared after PND 36. Repeated measures ANOVA revealed that body weight gain over time is different between MS and MS+HPF [$F(1,780)=2.146$; $P=0.0008$], but not between NH and MS. Daily chow intake of MS females did not differ from the age-matching NH females (Figure 3). Cookie access suppressed daily chow intake of MS females, but daily caloric intake tended to be increased by cookie access without statistical significances. Analysis of caloric intake with repeated measures ANOVA revealed no effects of maternal separation and food condition. Total caloric intake during the whole experimental period (PND 28 - 62) did not differ among the experimental groups (Figure 4A). About 40 % of the total calorie consumed by MS+HPF females originated from the cookies (3259.921 ± 211.657 kcal from chow, 2184.641 ± 186.077 kcal from cookies). Retroperitoneal fat pad of MS females on PND 62 did not differ from the age-matching NH females, and tended to be increased with cookie access without statistical significance ($P=0.0833$, MS vs. MS+HPF) (Figure 4B).

2. Behavioral assessments

Ambulatory activities of NH, MS and MS+HPF females were measured in a computerized activity chamber on PND 54. Ambulatory counts of MS females during the first (0 - 15 min) and the later session (15 - 30 min) were decreased significantly compared with NH females; however, a significant decrease (P<0.05) relative to NH was observed only during the later session in MS+HPF group (Figure 5A). Total distance traveled during the first 15 min session was significantly decreased in MS (P<0.05), but not in MS+HPF, compared to NH (Figure 5B). Grooming behavior and defecation activity were scored during the ambulatory activity test (Figure 3C & D). MS experience significantly increased rostral grooming (P<0.05, NH vs. MS) while cookie access reduced it (P<0.05, MS vs. MS+HPF) (Figure 5C). Defecation activity of MS females tended to be increased relative to NH without statistical significance, and cookie access significantly reduced it (P<0.05, MS vs. MS+HPF) (Figure 5D). In order to further assess the anxiety-like behaviors, rats were subjected to an elevated plus maze test 2 days after the ambulatory activity test (PND 56). Time spent in the open arms was significantly decreased in MS females (P < 0.05), but not in MS+HPF, compared with NH (Figure 6A). Percent open arm entry did not differ among the experimental groups (Figure 6B). To assess depression-like behaviors, rats were subjected to forced swimming test 3 days after the elevated plus maze test (PND 59). Immobility duration during the 5 min of forced swimming test session was significantly increased in MS females (P < 0.05) compared with NH, and the immobility score of MS+HPF females did not differ from NH (Figure 7).

3. Plasma corticosterone levels

A week after the swim test, rats received restraint stress and the tail bloods

were collected at 0, 30, 60 and 120 min time points during 2 h of restraint session, and was used for the plasma corticosterone assay (Figure 8). The basal corticosterone levels (0 time point) did not differ among the groups; however, the stress-induced increases of corticosterone level were lower in MS females than in NH at 30 and 60 min time points after the onset of stress (P<0.05, NH vs. MS at each time point). The plasma corticosterone levels of MS+HPF did not differ from NH at 30 min after the onset of stress, but was lower than NH at 60 min time point (P < 0.05; 394.29 ± 38.35 ng/ml in NH vs. 247.48 ± 24.57 ng/ml in MS+HPF). Analysis of the stress-induced corticosterone levels with 2-way ANOVA revealed main effects of maternal separation [F(1,56)=8.814, P=0.0045] and time [F(3,56)=9.335, P<0.0001], and no effect of food condition. Significant interactions between maternal separation and time or between food condition and time were not found.

4. ΔFosB and BDNF western blots

ΔFosB and BDNF levels in the NAc were examined with western blot analysis (Figure 6). ΔFosB was significantly reduced, but BDNF was increased in the NAc of MS females (P < 0.05) compared with NH (Figure 9A & B). ΔFosB level in the NAc of MS females was normalized by cookie access; i.e. no difference between NH and MS+HPF, and BDNF level was further increased (P < 0.05, MS vs. MS+HPF). BDNF levels in the hippocampus of MS females were markedly decreased relative to NH (P < 0.05) and it was not recovered by cookie access (Figure 9C).

Ⅳ. Discussion

Palatable food access improved the psycho- emotional behaviors of MS females

In this study, the behavioral scores representing anxiety and depression, such as ambulatory activity, rostral grooming and defecation activity during activity test; open arms stay during elevated plus maze test; immobility during forced swim test, were improved in MS females with free access to Oreo cookies during adolescence and youth. The corticosterone response to acute stress was blunted in MS females, as reported in MS males.[14] The blunted corticosterone response seems to be a consequence of MS experience; i.e. experience of repeated stress, since the HPA axis responses to acute stress challenge appeared to be blunted following experiences of chronic repeated stress.[40,41] Studies suggested that exposure to highly preferred diet high in fat can modify the basal HPA axis activity and the endocrine responses to an acute stress, [9] improve stress responses[17,19] and decrease anxiety-like behaviors. [18,20] Also, free access to Oreo cookies (~21% fat content; a moderate fat diet) during adolescence and youth normalized the blunted HPA axis function and improved anxiety-like behaviors in male MS rats.[14] That is, it is likely that adolescent cookie access may improve the blunted HPA axis function by MS experience, repeated stress in early life, and ameliorate the behavioral adversities. However, in this study, the cookie access during adolescence and youth did not improve the HPA axis activity responding to acute stress in female MS rats. It is suggested that the anxiolytic and/or antidepressant efficacies of adolescence cookie access in female MS rats may not be related with the HPA axis function, though it was in male MS rats.

Male and female rats differ in numerous neuroendocrine and behavioral parameters, and vulnerability to stress is gender dependent.[42,43] Previous study reported that a 7-day moderate fat diet protocol leads to a male-selective exaggerated corticosterone release following an acute stress.[6]

Palatable food access and neuronal function in the NAc of female rats

The present study demonstrated that ΔFosB expression is decreased and BDNF increased in the NAc of female rats by MS experience. It has been reported that either psychologic or metabolic stress increases ΔFosB expression in the NAc.[44-46] Studies have suggested that transcription factor ΔFosB is related with BDNF expression in the NAc neurons.[32,47-49] Taken together, it is suggested that decreased ΔFosB and increased BDNF expression in the NAc may be a long-term consequence of MS stress in early life, possibly modulating the NAc neuronal function. The NAc neuronal function was suggested to be modulated by behavioral stress paradigm[24,25] and its dysfunction has been implicated in depression and anxiety disorders.[22,23,26,27] Indeed, increased BDNF signaling in the NAc has been reported in stress-induced models of depression,[50-52] and stress-induced depressive effects was blunted in mice over-expressing ΔFosB in striatum.[53] Thus, it is likely that depression- and/or anxiety-like behaviors of female MS rats may be related with decreased Δ FosB and increased BDNF expression in the NAc. In this study, cookie access during adolescent period increased ΔFosB and BDNF expressions in female MS rats. This result concurs with previous reports showing that exposure to a palatable diet results in increased levels of ΔFosB in the NAc[31], and that high fat diet increased BDNF levels in

the NAc of ΔFosB over-expressing mice.[32] Considering previous report revealing that increased ΔFosB expression in striatum exerts resilience to stress-induced depressive effects,[53] it is concluded that increased ΔFosB in the NAc of our MS females with cookie access; i.e. normalized to its basal level, might have contributed to the antidepressant and/or anxiolytic efficacy of adolescence cookie access. However, it is not clear if increased BDNF level in the NAc of MS females with cookie access is implicated in its antidepressant and anxiolytic effects, since increased BDNF signaling in the NAc was reported mostly in depressive models,[50-52] but rarely in antidepressant models. Further studies are warranted.

Effects of MS and HPF on the hippocampal BDNF levels

Decreased BDNF level in the hippocampus has been reported both in male and female rats that were subjected to a similar MS protocol used in this study.[54, 55]Concurrently, BDNF level was decreased in the hippocampus of our female MS rats relative to NH controls in this study. Hippocampal neurogenesis has been implicated in symptoms of anxiety and depression,[56, 57] and the hippocampus is well known to be involved in the feedback regulation of the HPA axis activity. Recalling that the HPA activity was blunted in our MS females, it is likely that decreased BDNF levels in the hippocampus may be implicated in anxiety and/or depression disorders by MS experience, possibly in relation with the blunted HPA axis activity. The relation between the hippocampal BDNF level and the HPA axis activity in our MS females was further supported by the fact that cookie access did not improve both of them in this study. Previous study showed that prolonged consumption of high fat diet (32% fat) increases BDNF expression in the hippocampus of male MS

rats subjected to a similar MS protocol that was used in this study.[58] The effect of cookie access during adolescence (~21% fat) on the hippocampal BDNF levels of male MS rats is currently under investigation.

Behavioral effects of fat/sugar contents in Oreo cookie

In this study, free access to Oreo cookies improved the psycho-emotional adversities in female rats with early life stressful experience. Oreo cookie is a chocolate cookie, not only high in fat but also high in sugar as shown in Table 1. In human study, eating chocolate reduced negative mood compared to drinking water, whereas no effects were found on neutral and positive moods.[59] And the mood improving effect of chocolate was dependent on the palatability of chocolate (milk chocolate vs. plain chocolate), suggesting that eating sweet palatable food improves an experimentally induced negative mood state. It was reported that sucrose craving is increased in depressed animals with chronic mild stress and palatable milk chocolate craving is increased specifically in subjects with depressed mood.[60] Free choice of sucrose and/or lard in addition to chow all modulated the stress axis responses to acute stress. [61] Also, short-term exposure to a moderate fat diet (20 % corn oil; similar fat content with Oreo cookies) induced neuroendocrine and behavioral alterations in a sexually dimorphic manner.[4-6] Taken together, it is concluded that sugar and fat contents of Oreo cookies might have contributed to improve the neural and behavioral adversities in MS females. Further studies are warranted to examine if the same amount of fat or sugar provision as Oreo cookie access would produce similar improvements in MS females observed in this study. Lastly, retroperitoneal fat depot tended to be increased in MS females by cookie access in this study. In addition to its mod-

-ulation effect on the stress axis function, palatable food access markedly increased circulating leptin and insulin levels with increased fat depot.[15, 61] Both leptin and insulin have been suggested to exert a regulatory function in the mesolimbic reward system, and especially insulin increase expression of dopamine transporters in the ventral tegmental area.[62, 63] As described above, the mesolimbic reward system is highly implicated in the psycho-emotional disorders in relation with the stress axis function. [22-27] Thus, a tentative modulation, if any, in the mesolimbic reward system by increased leptin and/or insulin with increased fat depot is suggested to play a role in the mood elevation by palatable food access. Indeed, prolonged consumption of a high fat diet (32% fat content) reduced anxiety- like behaviors and increased plasma leptin and insulin levels with markedly increased fat depot in female rats that subjected to a similar MS protocol used in this study.[11] However, it is not yet clear whether or not the behavioral improvements observed in our MS females with cookie access (a moderate fat diet with ~21% fat content) is related with increased fat depot, because the increase in retroperitoneal fat depot by cookie access did not reach a statistical significance and further, neither circulating leptin nor insulin was measured in the current study.

Ⅴ. Conclusion

Dysfunctions in the HPA axis, the NAc neurons, and the hippocampus appeared to be implicated in the psycho-emotional adversities of young female MS rats by experience of maternal separation during the first two weeks of birth. Free access to highly palatable food, a moderate fat diet, during adolescence and youth improved anxiety- and depression- like beha-

-viors in MS females without affecting body weight gain, and functional modulation in the NAc neurons may play a role in its underlying neural mechanisms.

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Table

Nutrient	Chow	Cookie
Protein	28.507	4.25
Fat	13.496	21.26
Carbohydrate	57.996	36.15
Sugar	0	38.27
Vitamine	0.001	0
Sodium	0	0.05

Table 1. Nutrient contents (%) in standard chow and Oreo cookie

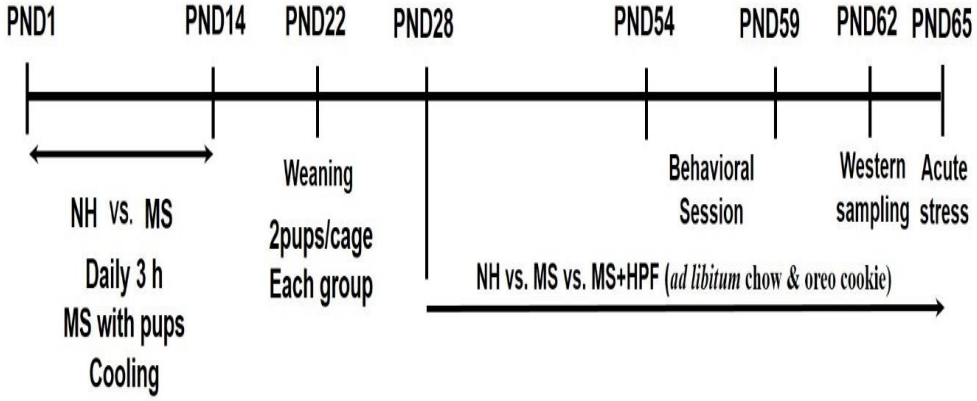

Figure 1. Experimental protocol.

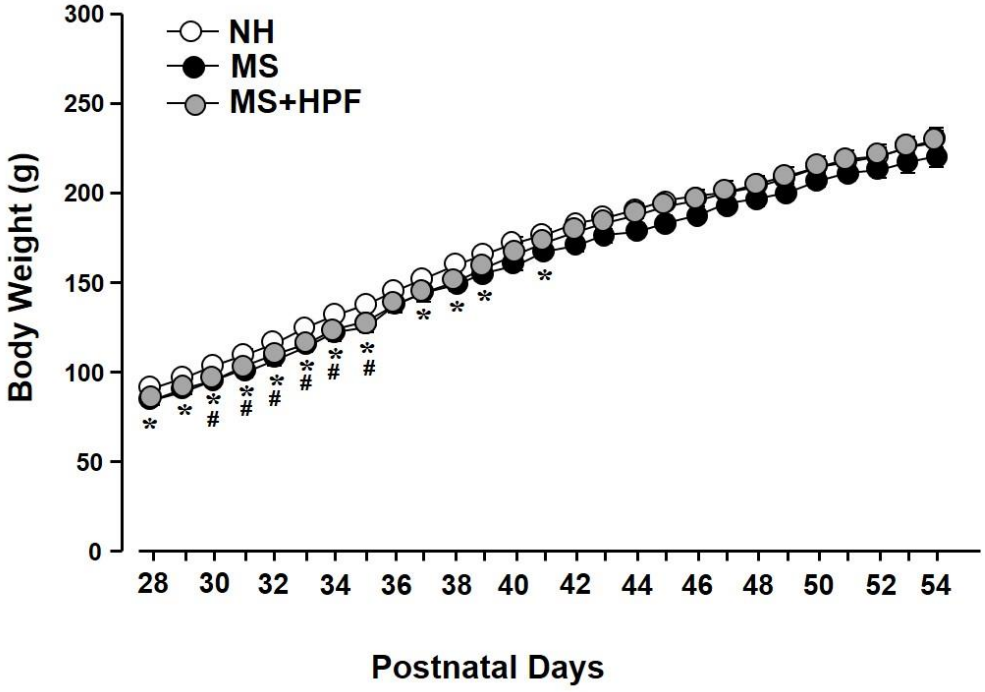

Figure 2. Body weight gain NH; Non-Handled fed with chow only, MS; Maternal separation fed with chow only, MS+HPF; Maternal separation fed with chow and cookie, HPF: Highly palatable food, $^{*}P < 0.05$ NH vs. MS $^{\#}P < 0.05$ NH vs. MS+HPF, n=14 in each group, Data are presented by mean ± S.E.M.

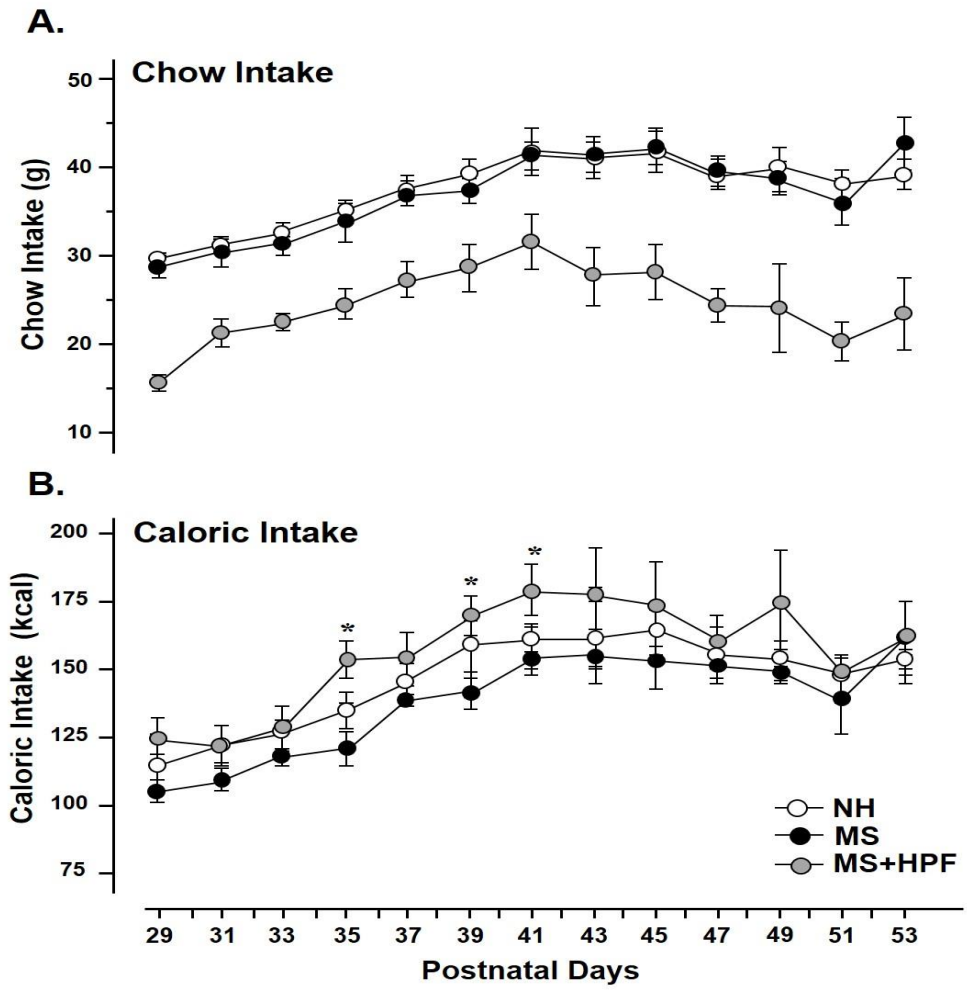

Figure 3. Daily intake of chow (A) and caloric (B). NH; Non-Handled fed with chow only, MS; Maternal separation fed with chow only, MS+HPF; Maternal separation fed with chow and cookie, HPF: Highly palatable food, $^{*}P < 0.05$ NH vs. MS, $^{\#}P < 0.05$ NH vs. MS+HPF, n=14 in each group, Data are presented by mean ± S.E.M.

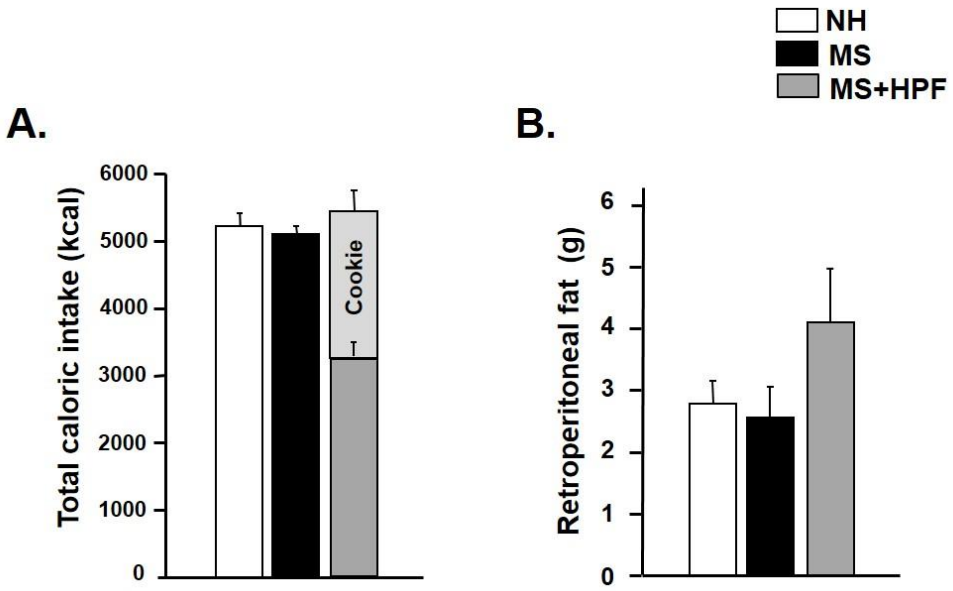

Figure 4. Total caloric intake (A) and weight of retroperitoneal fat pad (B). NH; Non-Handled fed with chow only, MS; Maternal separation fed with chow only, MS+HPF; Maternal separation fed with chow and cookie, HPF: Highly palatable food, $^{*}P < 0.05$ NH vs. MS, $^{\#}P < 0.05$ NH vs. MS+HPF, n=14 in each group, Data are presented by mean ± S.E.M.

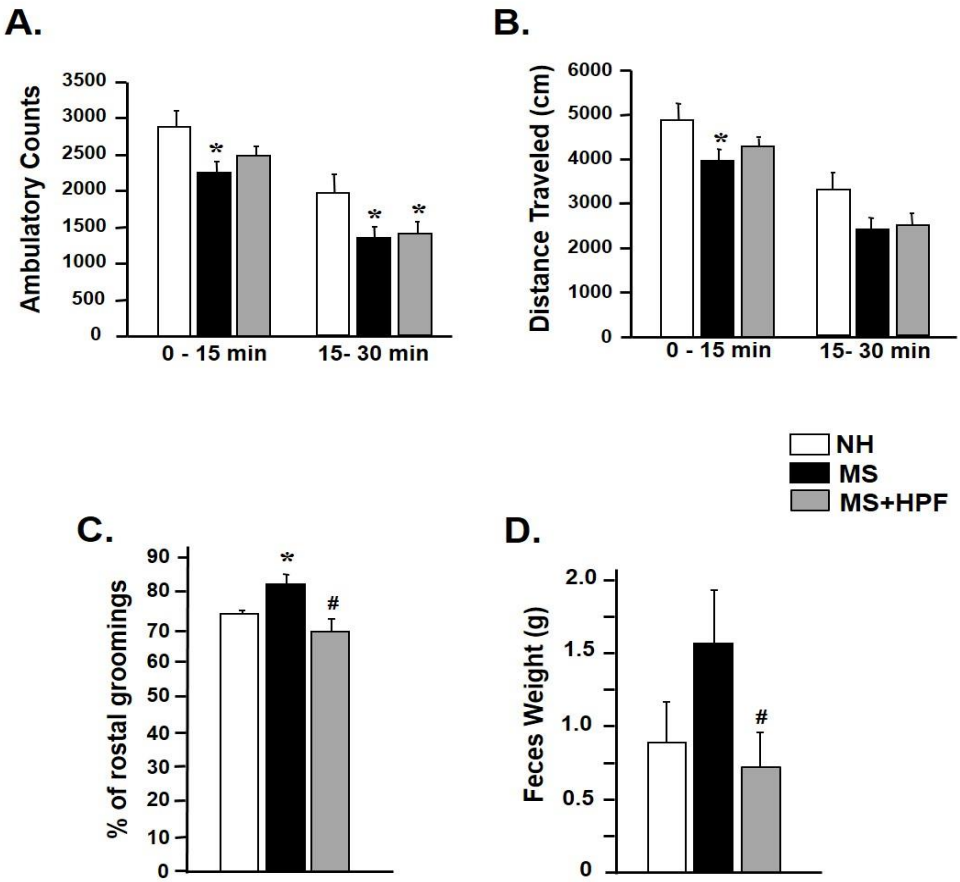

Figure 5. Ambulatory activity test performed on PND 54. Ambulatory counts scored consecutively at every 15 min session. Grooming behaviors and defecation activity during 30 min of the ambulatory activity test were scored. (A). Total ambulatory counts (B). Traveled distance (C). Rostral grooming (D). Feces weight NH; Non-Handled fed with chow only, MS; Maternal separation fed with chow only, MS+HPF; Maternal separation fed with chow and cookie, HPF: Highly palatable food, $^{*}P < 0.05$ NH vs. MS, $^{\#}P < 0.05$ NH vs. MS+HPF, n=8 in each group, Data are presented by mean ± S.E.M.

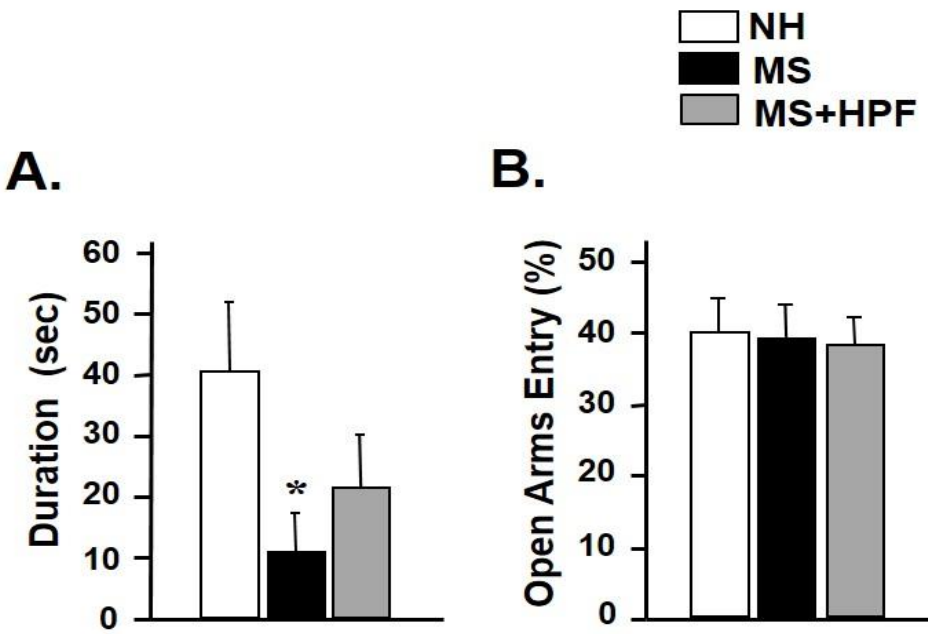

Figure 6. Elevated plus maze test. (A). Time spent in open arms (B). Entry to open arms NH; Non-Handled fed with chow only, MS; Maternal separation fed with chow only, MS+HPF; Maternal separation fed with chow and cookie, HPF: Highly palatable food, $^{*}P < 0.05$ NH vs. MS, $^{\#}P < 0.05$ NH vs. MS+HPF, n=8 in each group, Data are presented by mean ± S.E.M.

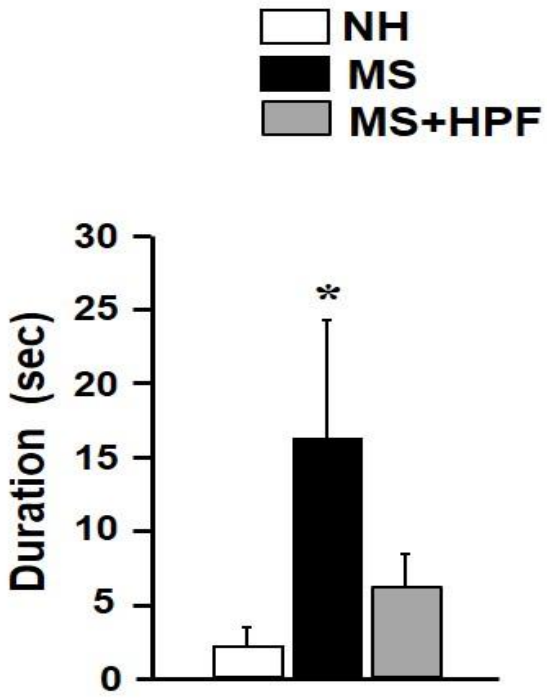

Figure 7. Immobility during forced swim test. NH; Non-Handled fed with chow only, MS; Maternal separation fed with chow only, MS+HPF; Maternal separation fed with chow and cookie, HPF: Highly palatable food, $^{*}P < 0.05$ NH vs. MS, $^{\#}P < 0.05$ NH vs. MS+HPF, n=8 in each group, Data are presented by mean ± S.E.M.

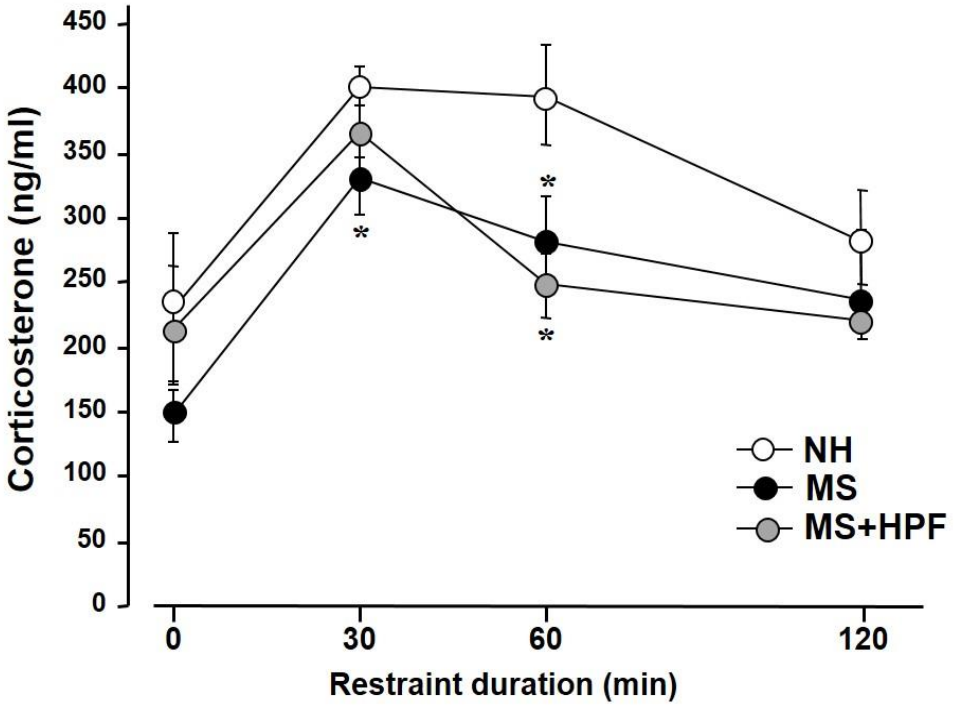

Figure 8. Plasma corticosterone levels during 2 h of restraint session. Rats were subjected to restraint stress following a week of recovery from the forced swim test. Feeding condition continued during the recovery period. Rats were placed in the restraint box and tail blood was collected at each time point. NH; non-handled fed with chow only, MS; maternal separation fed with chow only, MS+HPF; maternal separation fed with chow and cookie, HPF; highly palatable food, *P<0.05 vs. NH at each time point, n=8 in each group, Data are presented by means ± S.E.M.

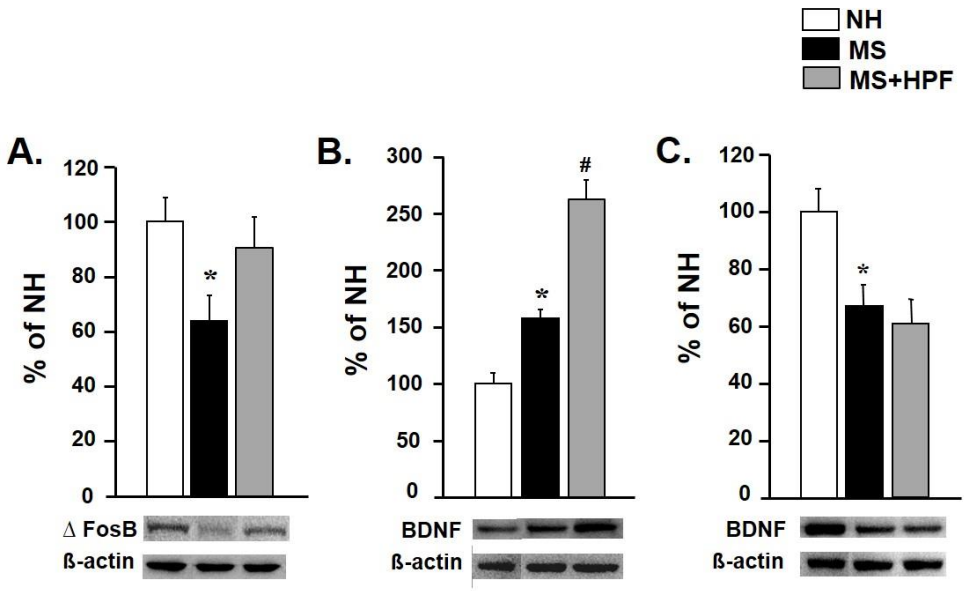

Figure 9. Western blot analyses. (A). ΔFosB, and (B).BDNF levels in the NAc. (C). BDNF level in the hippocampus. Rats that are naïve from the behavioral tests were sacrificed on PND 62 to collect the tissue samples for western blot analysis. NH; non-handled fed with chow only, MS; maternal separation fed with chow only, MS+HPF; maternal separation fed with chow and cookie, HPF; highly palatable food, *P<0.05 vs. NH, #P<0.05 vs. MS, n=6 in each group, Data are presented by means ± S.E.M.

국문초록

백서의 생애초기 스트레스 경험에 의한 신경행동장애에서 청소년기 고열량 선호식 섭취에 의한 개선효과

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김 진 영

1. 목적

동물과 인간에 있어 청소년기는 아동기에서 성인기에 이르는 과도기로서 생리적, 정서적, 사회적 발달이 활발히 이루어지는 중요한 시기이다. 이 시기에 영양분의 섭취 및 외부적인 스트레스요소는 뇌기능과 정서심리행동발달에 지대한 영향을 미치게 된다. 뇌보상회로는 약물중독회로와 유사한 작용기전을 가지며, 선호식 (highly palatable food)의 섭취는 쾌감적인 자극을 유발하여 보상회로를 활성화함으로서 심리행동에 영향을 끼치게 된다. 변연계 도파민 회로는 동기부여, 보상, 행동을 담당하는 보상회로로서 중요한 역할을 하며 선호식 섭취 및 스트레스는 뇌보상센터 중격의지핵에서 도파민의 분비를 유도한다.

뇌보상센터에서 도파민의 분비는 생리 기전뿐 만 아니라 심리행동에도 영향을 미친다. 본 연구실의 이전 연구에서 생애초기 모자분리 스트레스 경험은 성장 후 정서심리행동장애를 유발하는데, 이러한 행동장애가 청소년기 동안 선호식의 섭취에 의해 개선됨을 수컷 백서를 사용하여 밝힌 바 있다. 또한 이는 생애초기 모자분리 스트레스 경험에 의한 스트레스 대응 축 활성의 이상이 청소년기 동안 고열량 선호식 섭취에 의해 정상화 된 사실과 연관 있을 것임을 보고하였다. 스트레스반응을 포함한 여러 가지 생리반응에서 성별의 차이가 뚜렷한 사실이 보고된 바, 본 연구에서는 생애초기 모자분리 스트레스 경험과 청소년기 고열량 선호식 섭취가 암컷 백서의 정서심리행동에 미치는 영향과 그 신경화학적 기전을 연구하였다.

2. 재료 및 방법

Sprague-Dawley 종의 수컷과 암컷을 실험실에서 교배하여 출산을 시작한지 12시간 이후부터 PND(postnadal day) 1으로 하고, 출산 후 어미 한 마리 당 새끼의 성비를 5:5 로 조정하여 유지하였다. PND 1일차부터 모자분리군(maternal separation, MS)은 PND 14일까지 어미로부터 매일 3시간씩 분리를 하였고, PND 15부터 PND 21일까지는 아무런 처치를 하지 않고 어미와 함께 그대로 두었다. 대조군(non-handle, NH)은 전 기간 동안 아무런 처치를 하지 않았다. 모자분리군 및 대조군 모두 PND 22일에 젖떼기(weaning)를 하고 암컷 새끼만 선별하여 사용하였다. 모자분리&쿠키군 (maternal separation, MS+HPF)군에게는 PND 28일부터 일반사료와 함께 쿠키를 자유롭게 섭취하도록 공급하였다.

PND 54일에 Ambulatory activity test 를 하였다. 매 테스트를 시행할 때마다 암컷 백서를 활성 챔버(43.2cm x 42.2cm x 30.5cm) 가운데에 두고 30분동안 5분 간격으로 컴퓨터 자동화 시스템으로 활동량 및 횟수를 측정하였고 테스트 중에 불안관련행동패턴으로 나타나는 grooming, 배변활동을 분석하였다. PND 56일에 행동 검색을 위해 Elevated plus maze 행동실험을 진행하였다. 매 실험방식은 5분동안 50cm 높이에 있는 십자가 형태로 되어 있는 두 개의 open arms과 두 개의 close arms 가운데에 두고 각각의 arms 에 머무르는 시간, arms를 지나는 횟수를 측정하였다. PND 59일에 우울증 관련 행동변화를 알아보기 위해 Forced swim test를 진행 하였다. 물이 40cm채워진 원통형 수조에 암컷 백서를 넣고 5분 동안 우울관련행동패턴인 immobility, struggling, swimming 세가지 패턴으로 분석하였다. 모든 행동검색이 완료된 후, 스트레스 축의 활성화 정도를 알아보기 위해 구속 스트레스에 노출된 2시간 동안 4개의 시간구역 (0, 30, 60, 120 분)에서 백서의 꼬리 혈액을 채취하여 corticosterone을 조사하였다. 보상회로의 분자기전을 알아보기 위해 western blot analysis으로 중격의지핵 (nucleus accumbens)의 ΔFosB, Brain Derived Neurotrophic Factor (BDNF) 와 단백질 발현 정도를 확인하였고, 해마 부위의 Brain Derived Neurotrophic Factor (BDNF) 발현을 관찰하였다.

3. 결과

대조군(NH), 모자분리군(MS), 모자분리&쿠키군(MS+HPF) 세 군으로 나누어 분석한 결과, 전 실험기간 동안 몸무게, 칼로리 섭취양의

변화에서 실험군 간의 차이는 없었다. 청소년기 고열량 선호식의 무제한적 섭취는 모자분리를 경험한 암컷 백서에서 불안, 우울증 관련 행동들을 개선시켰다. 모자분리경험군에서 스트레스에 대한 Corticosterone 반응이 둔화되었고, 이는 청소년기 쿠키섭취에 의해 개선되지 않았다. 중격의지핵에서 모자분리군의 ΔFosB의 발현정도는 대조군에 비해 감소하였으나, BDNF의 발현은 증가하였다. 중격의지핵에서 ΔFosB의 발현은 쿠키섭취로 인해 정상화되었으나, BDNF의 발현은 더 증가한 것으로 나타났다. 모자분리 스트레스 경험은 해마에서 BDNF 발현을 감소시켰으며, 이는 청소년기 고열량 선호식 섭취에 의해 회복되지 않았다.

4. 결론

암컷 백서에서 생애초기 모자분리 스트레스 경험에 의한 정서심리행동장애에는 HPA axis 축, 중격의지핵 및 해마의 기능 이상이 관련되었을 것으로 사료된다. 청소년기—동안에 적절한 지방이 첨가된 고열량 선호식의 무제한적 섭취는 체중에는 영향을 주지 않았지만 모자분리를 경험한 암컷 백서의 불안장애 및 우울증 관련을 개선시킨 것으로 나타났다. 그 기전에는 고열량 선호식 섭취에 의한 중격의지핵 뉴런의 활성변조 효과가 관련되었을 것으로 사료되었다.

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주요어: 생애초기 스트레스 경험, 뇌보상회로, 선호식, 우울증, 불안장애
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