Korean J Physiol Pharmacol
Vol 2: 677−685, December, 1998

Effects of Neonatal Footshock Stress on Glucocorticoid and 5- $HT_{2A/2C}$ Receptor Bindings and Exploratory Behavior

Dong Goo Kim, Seoul Lee, Dong Won Kang[1], and Jong Su Lim

Department of Pharmacology and Yonsei Brain Research Institute, Yonsei University College of Medicine, Seoul 120−752, Korea; [1]Department of Pharmacology, Kwandong University College of Medicine, Kangnung 210−701, Korea

To investigate the effects of neonatal stress on behavior and neurochemistry, rats were exposed to the footshock stress on postnatal day (PND) 14 or PNDs 14 and 21. Rats were exposed to uncontrollable electric shocks delivered to the floor with a constant current (0.8 mA) for 5 sec period. Daily sessions consisted of 60 trials on a random time schedule with an average of 55 sec. The first exposure to footshocks on PND 14 decreased body weight gain for 1 day. However, the second exposure to footshocks on PND 21 did not affect body weight gain. Exploratory activity was measured by exposing a rat to a novel environment 24 h after experience of footshocks. Similar to the body weight changes, a decreased activity was noted after the first exposure to footshocks, while no changed activity was noted after the second exposure to footshocks. However, the Bmax value of 5-$HT_{2A/2C}$ receptors in the cortex decreased by the second exposure to footshocks, but not by the first exposure to footshocks. Moreover, an autoradiographic study revealed that the density of [^{3}H]dexamethasone binding in hippocampus decreased in rats exposed to footshocks 4 times during PND 14∼20. These results suggest that the uncontrollable footshock stress changes 5-hydroxytryptamine and glucocorticoid receptor systems acutely and that the repeated exposure to the same stress may not elicit behavioral alterations by the compensatory activity of young brain although changes in some neurochemistry exist.

Key Words: Footshock, Neonatal stress, 5-Hydroxytryptamine, Glucocorticoid, Exploratory behavior

INTRODUCTION

It has long been known that exposure to a stressor elicits stress response mediated by the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. Activated sympathetic nervous system stimulates its target organs and adrenal medulla from which epinephrine and norepinephrine are released into the blood stream. When the HPA axis is activated, glucocorticoid is eventually released from the adrenal cortex. Corticosterone, the principal glucocorticoid in rats, binds at least two different types of corticosteroid receptors, the type I (mineralocorticoid) and the type II (glucocorticoid) receptors (see De Kloet, 1991; De Kloet et al, 1994). Mineralocorticoid receptors (MRs) display a 10-fold higher affinity for corticosterone relatively to glucocorticoid receptors (GRs). Therefore, MRs are extensively occupied at low levels of circulating corticosterone, whereas GRs are effectively occupied at higher levels of circulating corticosterone following exposure to the stressor.

The stress response results in various consequences according to the degree of controllability to the stressor. Controllable stressor elicits active coping response, and uncontrollable stressor results in passive helplessness which developed into anxiety or depression (see huether, 1996). The controllability is believed to be determined by the psychoemotional status established by the previous experiences of the org-

Corresponding to: Dong Goo Kim, Department of Pharmacology, Yonsei University College of Medicine Seoul 120-752, Korea. (Tel) 02-361-5227 (Fax) 02-313-1894, E-mail: dgkimpharm@yumc.yonsei.ac.kr

anism. We hypothesized that central 5-HT played an important role for determining controllability to the stressor because 5-HT might have a permissive role in the activation and propagation of the central stress response rather than act as a direct trigger (Spoont, 1992). The permissive role of 5-HT has been reported in dopamine release (Korsgaard et al, 1985; Yoshimoto & McBride, 1992), hippocampal and sympathetic activities (Anderson et al, 1992; Calogero et al, 1990).

Most reports concerning neonatal stress used a model of postnatal handling. This procedure usually consisted of separating the mother and pups once per day for a period of 15 min/day, for the first 3 weeks of life. This early life manipulation resulted permanently in altered coping response to stress where enhanced glucocorticoid negative-feedback sensitivity exists (Ader & Grota, 1969; Hess et al, 1969; Levine, 1957, 1961; Levine et al, 1967). Meaney and colleagues (Meaney et al, 1985, 1988, 1989, 1991, 1992; Viau et al, 1993; Weaver et al, 1997) revealed that handled rats respond to stressors with smaller ACTH and corticosterone elevation. They also found increased GR density in hippocampus which is one of the responsible brain areas for negative feedback effect of glucocorticoid.

In wildlife, it is expected that mother-pup separation occurs more than once per day for a period of 15 min/day. Therefore, we regard the handling as a normal physiologic environmental stimulation (controllable stress), rather than a stressful event (uncontrollable stress). It has been also found that the learning ability of handled rats was higher than that of nonhandled rats when tested at older ages (Meaney et al, 1988).

We were interested in the effect of neonatal exposure to a severe stress, rather than a mild repeated stress like the handling experiment. Although the long-term effect of neonatal handling has been well established, the effect of severe stress early in life has not been established yet. We report here the acute effect of neonatal footshock stress on behavior, and 5-HT and glucocorticoid receptor systems. Rat pups were subjected to uncontrollable and unpredictable footshocks on postnatal day (PND) 14 or PNDs 14 and 21. One day after exposure to the footshocks, the exploratory activity was measured, and 5-hydroxytryptamine (5-HT) $_{2A/2C}$ receptor binding assay was done in the cortex. In addition, in vivo autoradiographic study for GRs in hippocampus was performed.

METHODS

Animal breeding and grouping

Animals were maintained at a constant temperature with a 12 h light-dark cycle. Lights were on at 0700 hr. Food and tap water were available *ad libitum*. To minimize and standardize unwanted environmental stimulation from *in utero* life (Sparber, 1991; Spear & File, 1996), Sprague-Dawley rats were mated and bred in a controlled manner. Nulliparous female and proven breeder male Sprague-Dawley rats were used for breeding. Providing each rat from different litters ensured genetic heterogeneity. Four females and one male were placed into a cage at 1900 hr and removed at 0700 hr on the next day. Pregnancy was confirmed by vaginal smear. From gestation day 15 until parturition, rats were housed individually. Beginning on gestation day 20 until delivery, nesting cages were examined at 0700 hr and 1900 hr for the presence of pups. Twelve hours after confirming delivery (average 18 h after birth), pups were divided by sex, weighed and culled. We used litters which had 5 males and 5 females or more, and pups were culled to 5 males and 5 females in a litter.

For the present study, we used male pups from 10 litters. Five male pups in a litter were assigned to 5 different groups; the non-handled control (NH), exposure to footshocks only on PND 14 (S1), exposure to footshocks on both PNDs 14 and 21 (S2), and non-shocked controls for the S1 group (NS1) and for the S2 group (NS2). The mean body weights of the 5 groups were not different since the groups were assigned by the Latin square method according to the order of body weights within a litter on PND 14. Pups of the NH group had not been separated from their mother except occasional weighing. Those of non-shocked group were subjected to the same procedure for the footshock delivery on PND 14 (NS1) or PNDs 14 and 21 (NS2), but they did not receive footshocks.

Delivery of footshocks

A rat was placed into the non-escapable chamber ($20.5 \times 17 \times 20$ cm) equipped with a grid floor of stainless steel bars (2.0 mm diameter, spaced 8 mm apart) within an opaque sound attenuating cubicle. A constant current (0.8 mA) shock was delivered to the floor for 5 sec period through the shock generator via

a grid scrambler (ENV-410, ENV-412, Med Associates, IN, USA). When the shocked group was exposed to footshocks, the non-shocked control group was also exposed to another chamber within an opaque sound-attenuating cubicle for the same period of the experiment. The opaque sound-attenuating cubicle prevented the non-shocked group from being stressed by the procedure for the footshock delivery. Daily sessions consisted of 60 trials on a random time schedule, ranging from 10 to 100 sec, with an average of 55 sec.

Measurement of exploratory activity

Twenty four h after the experience of footshocks, a rat was placed into a novel environment, and its explorative activity was measured. The experiment measuring the exploratory activity after the second exposure to the footshocks did not use the rats which had been exposed to a novel environment once on PND 15. Activity meters (Opto-Verimex Mini, Columbus Instruments, USA) were used. A rat was placed into the activity chamber (45×37×25 cm), a novel environment, and its ambulatory activity was measured for 30 min at intervals of 3 min. The transparent acryl chamber was equipped with 15 infrared photocells spaced 2.54 cm apart. The number of consecutive beam interruptions was counted as the ambulatory activity. Chambers were cleaned with alcohol (70%) after each use to prevent the influence of a tested rat previously.

[^{3}H]Ketanserin binding assay

Rats were killed by guillotine immediately after measuring exploratory behavior. After decapitation, the whole brain was removed, placed immediately on ice, and cerebral cortex was dissected out and stored at −80°C until the time of assay. On the day of assay, the tissue was homozenized briefly in 10 volumes of cold 0.25 M sucrose solution by using cell disruptor (Ultra-Turrax T25, Janke & Kunkel GMBH & Co., Germany). The homogenate was centrifuged at 1,000 xg for 10 min. The supernatant was centrifuged at 40,000 xg for 15 min. The pellet was suspended in ice-cold 40 volumes of 50 mM Tris-HCl buffer (pH 7.6) and centrifuged at 40,000 xg for 15 min. The resulting pellet was suspended in 40 volumes of 50 mM Tris-HCl buffer (pH 7.6) and incubated at 37°C for 20 min for the elimination of endogenous 5-HT. Then it was centrifuged at 40,000 xg for 15 min. The pellet was finally resuspended in 50 mM Tris-HCl buffer (pH 7.6, 40 volumes of original weight) and used for the [^{3}H]ketanserin binding assay.

We used [^{3}H]ketanserin as a ligand for the 5-$HT_{2A/2C}$ receptor. For the binding assay, the method described by Leysen et al (1982) was used with slight modification. Aliquots (400 μl) of resuspended tissue described above were placed into polypropylene tubes, and after the addition of [^{3}H]ketansein (50 μl , 0.0625-5 nM, specific activity 83.10 Ci/mmol) and methysergide (50 μl, 1 μM), the tubes were incubated at 37°C for 15 min. The samples were then rapidly filtered through Whatman GF/B filters under vaccum using the Cell Harvester (Brandel, MD, USA). The filters were rapidly rinsed 3 times with a 5 ml ice-cold buffer. The filters were then taken and placed into the polyethylene counting vial with 4 ml of liguid scintillation cocktail (Ready Safe™, Beckman Instruments, Inc., USA). Radioactivity was determined by liquid scintillation spectrometry using a beta-counter (Hewlett Packard, USA). Nonspecific binding was defined as the radioactivity which remained on the filter with membrane samples incubated in the presence of 1 μM methysergide, and specific binding as the difference between the radioactivity of a given sample (total binding) and the nonspecific binding. The data were analyzed by the Ligand program, and pKd (-log Kd) and Bmax values of [^{3}H]ketanserin binding were calculated.

In vivo autoradiography

For this experiment, we performed another breeding with the same method described above. We had 13 litters and 3 out of 5 male rats in a litter was assigned to 3 different groups; the non-handled control (NH), exposure to footshocks on PNDs 14, 16, 18 and 20 (S4), and non-shocked controls for the S4 group (NS4). Pups of the NH group had not been separated from their mother except occasional weighing. Those of non-shocked group were subjected to the same procedure for the footshock delivery on PNDs 14, 16, 18 ad 20, but they did not receive footshocks. In the S4 group, 4 rats died during or after the 4th exposure to footshocks. These 4 litters were discarded for the next procedure.

Twenty four h after the last experience of footshocks, both adrenal gland were removed under ether

anesthesia to eliminate endogenous corticosteroids. Adrenalectomized rats were given 0.9% NaCl solution instead of drinking water. We used [^{3}H]dexamethasone as a ligand for the type II glucocorticoid receptor. Sixteen h after adrenalectomy, [^{3}H]dexamethasone was injected intraperitoneally at a dose of 1.25 μCi/g body weight. Rats were sacrificed by guillotine 150 min after injection with [^{3}H]dexamethasone, and their brains were rapidly removed and frozen with Spot Freeze (Nabakem, Nambang Chemical Co., Japan), and then stored at -70°C. Brains were cut coronally into slices (20 μm), and the slices at bregma-5.3 mm (plate 41 In: The Rat Brain in Stereotaxic Coordinates, Paxinos G & Watson C, 4th ed., Academic Press, 1998) were mounted on the slide glass and placed into the Image Plate (Fuji Co., Japan). Then they were developed for 10 days at -70°C. After developing the Image Plate, the image of the autoradiogram was analyzed, by the Image Analyzer (Fugi Co., Japan). The image was digitized into 100 μm^2 pixels with 256 grayscales. Data were expressed as photo-stimulated luminescence (PSL). We chose the hippocampal area to be analyzed and the PSL of the hippocampal area was subtracted by the PSL of the cortical area of the same slice, a back ground activity. The area term was included in the final quantitative data, and then they were expressed as (hippocampal PSL cortical BG)/mm^2.

Statistical analysis

Data were expressed as means$\pm$standard errors (S.E.). Statistical comparisons were made by overall analysis of variance (ANOVA), and preplanned multiple comparisons were done by appropriate statistical tests. The analyses of the exploratory behavior were done by ANOVA on the activity of the given time period and by repeated measures ANOVA on the cumulative activity during the total period.

RESULTS

Effects of footshock stress on body weight gain

No differences in body weights were noted between groups just before the exposure to the footshocks on PND 14 or 21. However, the difference was evident in weight gain for 24 h after the experience of footshock stress on PND 14 by overall ANOVA test ($F_{(2,43)}$=20.53, $p<0.01$) (Fig. 1A). Weight gain of the NH group after the experience of footshocks was 2.2$\pm$0.2 g. Weight gain of the rats subjected to the stress manipulation but not received footshocks (the NS1 group) was 1.1$\pm$0.2 g ($p<0.05$ vs. the NS group, Scheffe's F test). Body weight of the shocked group (the S1 group) showed no increase during 24 h period after the experience of footshocks. In fact, their weight gain was 0.3$\pm$0.3 g ($p<0.01$ vs. the NH and the NS groups, Scheffe's F test).

When these rats were exposed to the identical shock parameters again on PND 21, exposure to the stress manipulation with/without footshocks did not resulted in a significant decrease in weight gain (Fig. 1B). The weight gain of the NH, NS2 or S2 groups was 4.6$\pm$1.9 g, 1.0$\pm$1.9 g or 0.6$\pm$2.0 g, respec-

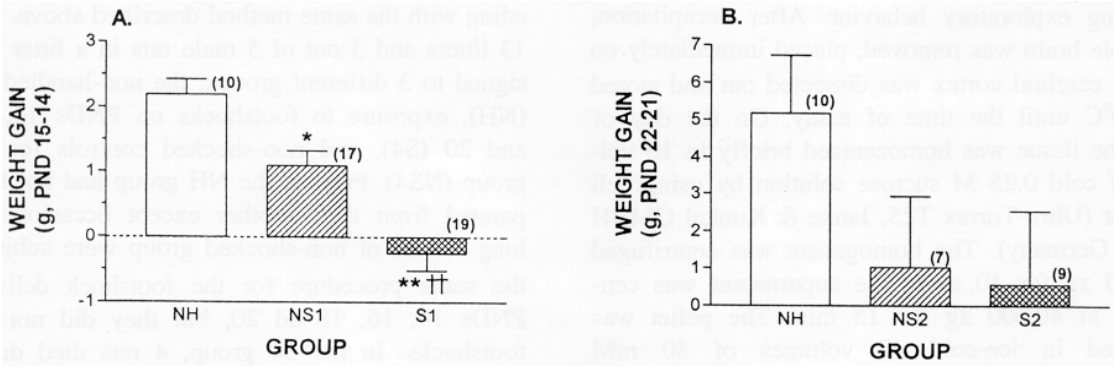

Fig. 1. Weight gain after exposure to footshocks on PND 14 (A) or on PNDs 14 and 21 (B). Weight gain was measured 24 hours after exposure to 60 uncontrollable and unpredictable footshocks in a session. Other legends are the same as Table 1. *$p<0.05$, **$p<0.01$ vs. the NH group (Scheffe's F test). $^{\dagger\dagger}p<0.01$ vs. the NSl group (Scheffe's F test).

tively.

Effects of footshock stress on exploratory activity

Twenty four h after the experience of footshock stress on PND 14 or 21, ambulatory activities of rats were measured for 30 min during the exposure to a novel environment. The ambulatory activities of all 3 different groups decreased gradually on the exposure to a novel environment after experiencing the footshock on PND 14 or 21 as expected since rats adapt themselves to the new environment rapidly. When the data were analysed more carafully, there was a tendency that the degree of decreasing activity in the course of time was greater in the S1 group than in the NH or the NS1 group on PND 15 (24 h after PND 14). This effect was marginal when data were analyzed by the unit time of measurement (Fig. 2A). However, the effect became evident by the repeated measures ANOVA of cumulative ambulatory activity (Fig. 2B). There was a significant effect in the group by the exposure time interaction ($F_{(18,243)}$=1.75, $p<0.05$). Subsequent analyses revealed that the time-based cumulative exploratory activity of the S1 group was less than that of the NH group ($F_{(9,162)}$=2.16, $p<0.05$) or the NS1 group ($F_{(9,162)}$=3.14, $p<0.01$). However, similar to the weight gain data, no group differences in the activity were found on PND 22 (24 h after PND 21) (Fig. 2C,D).

[^{3}H]Ketanserin binding

Immediately after measuring the exploratory behavior, that is PND 15 or 22, rats were decapitated, and [^{3}H]ketanserin binding was done in cerebral cortex for the 5-$HT_{2A/2C}$ receptor. After the first exposure to the footshocks, the density (Bmax) and the affinity (pKd) of [^{3}H]ketanserin bindings in the cerebral

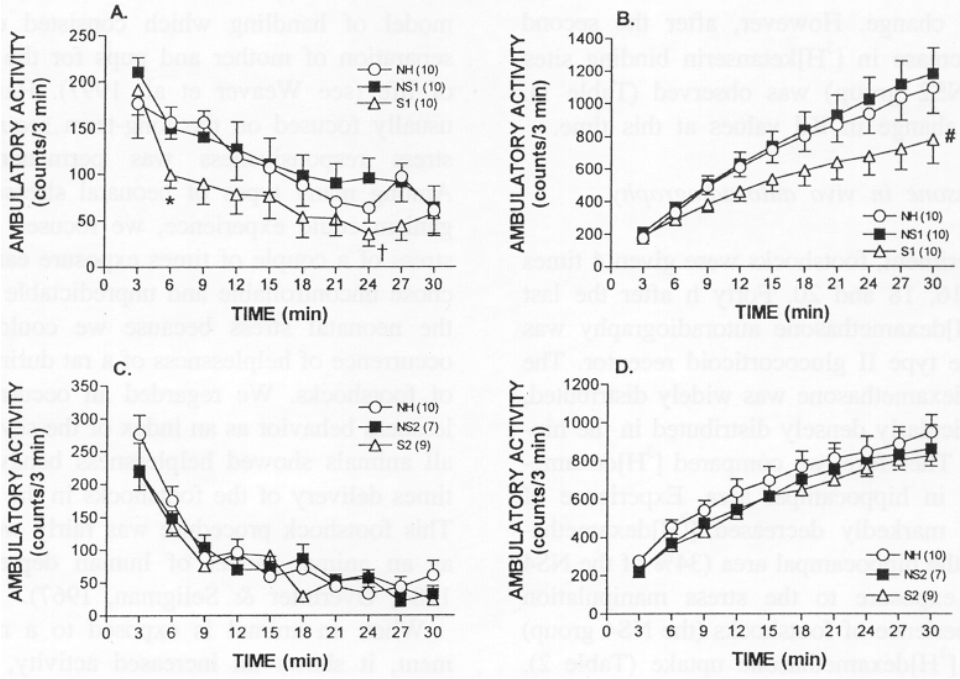

Fig. 2. Exploratory activity after exposure to footshocks on PND 14 (A, B) or PNDs 14 and 21 (C, D). Ambulatory activity was measured for 24 hours after exposure to 60 uncontrollable and unpredictable footshocks in a session. Data were collected for 30 min exposure to a novel environment at intervals of 3 min. Other legends are the same as Table 1. Data were analyzed by the unit time interval of measurement (A, C) or by the cumulative activity during the total period of measurement (B, D). *$p<0.05$ vs. the NH group (Fisher's PLSD test). $^{\dagger}p<0.05$ vs. the NS1 group (Fisher's PLSD test). $^{\#}$ Analyses by repeated measures ANOVA revealed differences vs. the NH group ($p<0.05$) and vs. the NS1 group ($p<0.01$).

Table 1. [^{3}H]Ketanserin binding in the cerebral cortex 24 h after the exposure to footshock stress on PND 14 or PNDs 14 and 21 in rats.

Group	Bmax (fmol/mg tissue)	pKd (M, -log Kd)
NH (5)	4.6±1.13	9.2±0.20
NS1 (7)	6.6±1.23	8.7±0.13
S1 (8)	8.3±0.92	8.7±0.11
NS2 (7)	7.9±1.52	8.7±0.15
S2 (6)	3.0±1.24*	9.2±0.16

Values are means±S.E.. Numbers in parentheses represent the number of animals. NH, the non-handled control; NS1, the non-shocked control for the S1 group; S1, the shocked group exposed to footshocks on PND 14; NS2, the non-shocked control for the S2 group; S2, the shocked group exposed to footshocks on PNDs 14 and 21. *$p<0.05$ vs. the NS2 group (Scheffe's F test).

Table 2. Photostimulated luminescence value of [^{3}H]dexamethasone autoradiography in hippocampal area after the exposure to footshock stress 4 times during PND 14~20 in rats.

Group	(PSL-BG)/mm^2
NH (7)	5.01±0.91
NS4 (6)	5.57±1.10
S4 (9)	1.92±0.86*

Values are means±S.E.. Numbers in parentheses represent the number of animals. Data are the back ground (BG)-subtracted hippocampal photostimulated luminescence (PSL) in the unit area (mm^2). NH, the non-handled control; NS4, the non-shocked control for the S4 group; S4, the shocked group exposed to footshocks on PNDs 14, 16, 18 and 24. *$p<0.05$ vs. the NH and the NS2 groups (Scheffe's F test).

cortex did not change. However, after the second exposure, a decrease in [^{3}H]ketanserin binding sites (38% of the NS2 group) was observed (Table 1). There was no change in Kd values at this time.

[^{3}H]Dexamethasone in vivo autoradiography

For this experiment, footshocks were given 4 times on PNDs 14, 16, 18 and 20. Forty h after the last footshocks, [^{3}H]dexamethasone autoradiography was assessed for the type II glucocorticoid receptor. The uptake of [^{3}H]dexamethasone was widely distributed, and it was particularly densely distributed in the hippocampal area. Therefore, we compared [^{3}H]dexamethasone uptake in hippocampal area. Experience of the footshocks markedly decreased [^{3}H]dexamethasone uptake in the hippocampal area (34% of the NS4 group), while exposure to the stress manipulation without the experience of footshocks (the NS4 group) did not affect [^{3}H]dexamethasone uptake (Table 2).

DISCUSSION

This study clearly showed that the footshock stress early in life decreased the exploratory activity and cortical 5-HT$_{2A/2C}$ receptor density. This effect was observed 24 h after the experience of footshocks on PND 14. Most studies on neonatal stress used a model of handling which consisted of daily brief separation of mother and pups for the first 3 weeks of life (see Weaver et al, 1997). Handling studies usually focused on the long-term sequelae in which stress responsiveness was permanently changed. Among many types of neonatal stresses that an organism could experience, we focused on the severe stress of a couple of times exposure early in life. We chose uncontrollable and unpredictable footshocks as the neonatal stress because we could confirm the occurrence of helplessness of a rat during the delivery of footshocks. We regarded an occurrence of helplessness behavior as an index of the severe stress, and all animals showed helplessness behavior within 60 times delivery of the footshocks in the present study. This footshock procedure was fairly well established as an animal model of human depression (Maier, 1984; Overmier & Seligman, 1967).

When an animal is exposed to a novel environment, it shows an increased activity, called exploratory activity. The balance between the curiosity-induced exploratory trait and fear-induced freezing determines this activity. It is well known that a delivery of footshock decreases the responding of the schedule-maintained behavior. This phenomenon is called the conditioned emotional response, and this suppression of responding is believed to be elicited by fear (Estes & Skinner, 1941, Blackman, 1977). The footshock is delivered on the ongoing operant

responding in a classical conditioned emotional response procedure. Unlike this procedure, we measured exploratory activity 24 h after the experience of footshock. This delayed effect of footshocks has been reported to decrease the exploratory activity (Heinsbroek et al, 1991; Mendella & Zacharko, 1996) or have no effect (Harris et al, 1997) or variable effect (Sandi et al, 1992a,b) on the exploratory behavior. Ferretti et al (1995) reported that five 10 sec 1mA footshocks decreased exploratory activity 15 min after the experience of footshock, whereas no significant changes in exploratory activity after acute stress was observed in rats which had been subjected to chronic stress. This observation is similar to our results in which exposure to the footshocks for the first time decreased exploratory activity, whereas no changed exploratory activity was found on the second exposure.

Acute stress increases 5-HT synthesis by enhancing tryptophan availability and stimulating tryptophan hydroxylase activity, and thereby a depletion of 5-HT from serotonergic neurons is avoided (Curzon et al, 1972; Dunn, 1988). Handling stress also increased 5-HT turnover in the hippocampus and frontal cortex (Mitchell & Meaney, 1990; Smythe et al, 1994). However, stress-induced changes in 5-HT receptors were not consistent. Twenty four h after immobilization, increased (Torda et al, 1990) and not changed (Mendelson & McEwen, 1991) 5-HT_2 receptor binding was reported in the cortex and in the hippocampus, respectively. In case of acute footshock stress, the stress decreased density of the 5-HT_2 receptors in cortex, whereas the density of the 5-HT_{1A} receptors did not change (Ferretti et al, 1995). This data is consistent with our results although it is not yet clear why acute footshock stress downregulates cortical 5-HT_2 receptors.

In cultured hippocampal cells, 5-HT increased GR levels dose-dependently, but it had no effect on MR levels. Moreover, 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, prevented 5-HT-induced increase in GR levels (Mitchell et al, 1990). These findings suggest that hippocampal GR expression is mediated by the activity of hippocampal 5-HT_2 receptors. On the other hand, 5-HT increased cAMP in the cultured hippocampal cells (Mitchell et al, 1992). Since 5-HT_2 receptors is known to be coupled to inositol phosphate hydrolysis system, it is unlikely that hippocampal 5-HT_2 receptors play an important role in the GR regulation. Therefore, it is possible that cortical 5-HT_2 receptors, but not those in hippocampus, modify cortical function and in turn modify the hippocampal GR expression. We found decreased cortical 5-HT_2 receptor density with decreased GR density in hippocampus. This result implies a close relationship between the cortical activity of 5-HT_2 receptors and the hippocampal GR expression.

In the present study, the footshocks of the same parameter were delivered twice early in life. We chose the neonatal period as the time of exposure to stress because the young brain exhibits higher plasticity. We hoped that we could detect the stress response easily, perhaps permanently. In fact, we were able to observe decreased exploratory activity 24 h after the exposure to footshocks on PND 14. This stress response, however, disappeared after the second exposure to the footshocks on PND 21. At this point, we can only speculate as to the mechanism of different stress responses, according to the frequency of exposure to the same environmental manipulation. An adaptation (learning) from the previous exposure may exert a critical role in this phenomenon. This belief is supported by the fact that the decrease in weight gain, a physical response, after the first exposure also disappeared after the second exposure. This observation suggested that a physical adaptation also occurred. It is also possible that the different age when the rat was exposed to the footshocks caused differential age-dependent stress reponses. We can't speculate about the relative contribution of the age factor from the results of the present study. It is, however, certain that the experimental conditions of the present study is quite similar to the real life in which the age factor should be included in case of repeated exposure to stress. In case of neurochemical stress responses, unlike weight gain or behavioral changes, the density of 5-$HT_{2A/2C}$ receptors decreased after the second exposure, but not after the first exposure. It seems that stress-induced neurochemical damages are additive upon repetitive exposure, although the mechanism remains to be clarified. The age may not be a factor for this phenomenon because increased 5-HT turnover was found after handling stress even in 7-day-old rat pups (Smythe et al, 1994). Considering no changed behavior upon altered neurochemistry on PND 21, it is quite possible that active compensatory mechanisms of the young brain exert a role in shaping an unimpaired bahavior (see Reuhl, 1991), a final intergrated output of the brain function.

In summary, the present study showed that a re-

peated exposure to stress attenuates physical and behavioral stress responses even under the existence of neurochemical alterations. The results suggest that the young brain has active compensatory mechanisms which express an unimpaired behavior by overcoming minor neurochemical damages. The present study also demonstrated simultaneous decrease in 5-$HT_{2A/2C}$ receptors in the cortex and in GRs in hippocampus, suggesting a close relationship between the 5-HT and the glucocorticoid systems. Further studies are needed to clarify the relationship between the behavioral and the neurochemical stress responses.

ACKNOWLEDGEMENT

This work was supported by the Basic Medical Research Fund from the Ministry of Education, 1995~1996, Republic of Korea.

REFERENCES

Ader R, Grota L. Effects of early experience on adrenocortical activity. *Physiol Behav* 4: 303−305, 1969

Anderson IK, Martin GR, Ramage AG. Central administration of 5-HT activates 5-HT_{1A} receptors to cause sympathoexitation and 5-HT_2/5-HT_{1C} receptors to release vasopressin in anesthetized rats. *Br J Pharmacol* 107: 1020−1028, 1992

Blackman D. Conditioned suppression and the effects of classical conditioning on operant behavior. In: Honig WK, Staddon JER ed, *Handbook of operant behavior*. Prentice-Hall Inc., Englewood Cliffs, NJ, p340−363, 1977

Calogero AE, Bagdy G, Szemeredi K, Taartaglia ME, Gold PW, Chrousos GP. Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic pituitary-adrenal axis in the rat. *Endocrinology* 126: 1888−1894, 1990

Chaouloff F. Physiopharmacological interactions between stress hormones and central serotonergic systems. *Brain Res Rev* 18: 1−32, 1993

Curzon G, Joseph MH, Knott PJ. Effects of immobilization and food deprivation on rat brain tryptophan metabolism. *J Neurochem* 19: 1967−1974, 1972

De Kloet ER, Rots NY, Van Den Verg DTWM, Oitzl MS. Brain mineralocorticoid receptor function. *Ann NY Acad Sci* 746: 8−21, 1994

De Kloet ER. Brain corticosteroid receptor balance and homeostatic control. *Front Neuroendocrinol* 12: 95−164, 1991

Dunn AJ. Changes in plasma and brain tryptophan and brain serotonin and 5-hydroxyindoleacetic acid after footshock stress. *Life Sci* 42: 1847−1853, 1988

Estes WK, Skinner BF. Some quantitative properties of anxiety. *J Exp Anal Behav* 29: 390−340, 1941

Ferretti C, Blengio M, Gamalero SR, Ghi P. Biochemical and behavior changes induced by acute stress in a chroic bariates stress model of depression: the effect of amitriptyline. *Eur J Pharmacol* 280: 19−26, 1995

Harris RB, Zhou J, Youngblood BD, Smagin GN, Rya DH: failure to change exploration or saccharin preference in rats exposed to chronic mild stress. *Physiol Behav* 63: 91−100, 1997

Heinsbroek RPW, Van Haaren F, Feenstra MGP, Boon Pien, Van de Poll NE. Controllable and uncontrollable footshock and monoaminergic activity in the frontal cortex of male and female rats. *Brain Res* 551: 247−255, 1991

Hess J, Denenberg V, Zarrow M, Pfeifer W. Modification of the corticosterone response curve as a function of handling in infancy. *Physiol Behav* 4: 109−111, 1969

Huether G. The central adaptation syndrome: psychosocial stress as a trigger for adaptive modifications of brain structure and brain function. *Prog in Neurobiol* 48: 569−612, 1996

Korsgaard S, Gerlack J, Christenson E. Behavioral aspects of serotonin-dopamime interaction in the monkey. *Eur J Pharmacol* 118: 245−252, 1985

Levine S, Haltmeyer G, Karas G, Denenberg V. Physiological and behavioral effects of infantile stimulation. *Physiol Behav* 2: 55−59, 1967

Levine S. Infantile experience and resistance to physiological stress. *Science* 126: 405, 1957

Levine S. Plasma-free corticosteroid response to electric shock in rats stimulated in infancy. *Science* 135: 795−796, 1961

Leysen JE, Niemegeers CJE, Van Nueten JM, Laduron PM. {^{3}H}ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin2 receptor binding sites. *Mol Pharmacol* 21: 301−314, 1982

Maier SF. Learned helplessness and animal models of depression. *Prog NeuroPsychopharmacol & Biol Psychiat* 8: 435−446, 1984

Meaney MJ, Aitken DH, Bodnoff S, Iny L, Sapolsky RM. The effects of postnatal handling on the development of the glucocorticoid receptor systems and stress recovery in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* 9: 731−734, 1985

Meaney MJ, Aitken DH, Sharma S, Viau V. Basal ACTH, corticosterone and corticosterone-binding globulin levels over the diurnal cycle, and age-related changes in hippocampal type I and type II corticosteroid receptor binding capacity in young and aged, handled and nonhandled rats. *Neuroendocrinology* 55: 204−213,

1992

Meaney MJ, Aitken DH, Van Berkel C, Bhatnagar S, Sapolsky RM. Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science* 239: 766−768, 1988

Meaney MJ, Aitken DH, Viau V, Sharma S, Sarrieau A. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology* 50: 597−604, 1989

Meaney MJ, Viau V, Aitken DH, Bhatnagar S. Stress-induced occupancy and translocation of hippocampal glucocorticoid receptors. *Brain Res* 445: 198−203, 1988

Meaney MJ, Viau V, Bhatnagar S, Betito K, Iny L, O'Donnell D, Mitchell J. Cellular mechanisms underlying the development and expression of individual differences in the hypothalamic-pituitary-adrenal stress response. *J Steroid Biochem Mol Biol* 39: 265−274, 1991

Mendella PD, Zacharko RM. Variation of motor activity following intraventricular (D-Pen 2, D-Pen 5)-enkephalin administration: interaction with acute uncontrollable foot-shock in mice. *Pharmacol Biochem & Behav* 53: 877−888, 1996

Mendelson SD, McEwen BS. Autoradiographic analyses of the effects of restraint-induced stress on 5-HT_{1A}, 5-HT_{1C} and 5-HT_2 receptors in the dorsal hippocampus of male and female rats. *Neuroendocrinology* 54: 454−461, 1991

Mitchell J, Betito K, Rowe W, Boksa P, Meaney MJ. Serotonergic regulation of type II corticosteroid receptor binding in hippocampal cell cultures: evidence for the importance of serotonin-induced changes in cAMP levels. *Neuroscience* 48: 631−639, 1992

Mitchell J, Iny L, Meaney MJ. The role of serotonin in the development and environmental regulation of type II corticosteroid receptor binding in rat hippocampus. *Dev Brain Res* 55: 231−235, 1990

Mitchell MJ, Rowe W, Boksa P, Meaney MJ. Serotonin regulates type II corticosteroid receptor binding in hippocampal cell cultures. *J Neurosci* 10: 1745−1752, 1990

Overmier JB, Seligman MEP. Effects of inescapable shock upon subsequent escape and avoidance learning. *J Comp Physiol Psychol* 63: 23−33, 1967

Reuhl KR. Delayed expression on neuropathy: The problem of silent damage. *Neurotoxicol* 12: 341−346, 1991

Sandi C, Borrell J, Guaza C. Behavioral factors in stress-induced immunomodulation. *Behav Brain Res* 48: 95−98, 1992

Sandi C, Borrell J, Guaza C. Behavioral, neuroendocrine and immunological outcomes of escapable or inescapable shocks. *Physiol Behav* 51: 651−656, 1992

Smythe JW, Rowe WB, Meaney MJ. Neonatal handling alters serotonin (5-HT) turnover and 5-HT_2 receptor binding in selected brain regions: relationship to the handling effect on glucocorticoid receptor expression. *Dev Brain Res* 80: 183−189, 1994

Sparber SB. Measurement of drug-induced physiological and behavioral delays and abnormalities in animal studies: A general framework. *NIDA Research Monograph Series* 114: 148−164 , 1991

Spear LP, File SE. Methodological considerations in neurobehavioral teratology. *Pharm Biochem Behav* 55: 455−457, 1996

Spoont MR. Modulatory role of serotonin in neural information processing: Implications for human psychopathology. *Psychobiol Bull* 112: 330−350, 1992

Torda T, Murgas K, Cechova E, Kiss A, Saavedra JM. Adrenergic regulation of [^{3}H]ketanserin binding sites during immobilization stress in the rat frontal cortex. *Brain Res* 527: 198−203, 1990

Viau V, Shama S, Plotsky PM, Meaney MJ. Increased plasma ACTH responses to stress in nonhandled compared with handled rats require basal corticosterone levels and are associated with increased levels of ACTH secretagogues in the median eminence. *J Neurosci* 13: 1097−1105, 1993

Weaver SA, Meaney MJ. Regulation of forebrain glucocorticoid receptor systems and the development of individual differences in stress responses in the rat. *Current Opinion in Endocrinology and Diabetes* 4: 148−157, 1997

Yoshimoto K, McBride WJ. Regulation of nucleus accumbens dopamine release by the dorsal rephe nucleus in the rat. *Neurochem Res* 17: 401−407, 1992