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Periodic Maternal Deprivation Alters Stress Response in Adult Offspring: Potentiates the Negative Feedback Regulation of Restraint Stress-Induced Adrenocortical Response and Reduces the Frequencies of Open Field-Induced Behaviors

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OGAWA, T., M. MIKUNI, Y. KURODA, K. MUNEOKA, K. J. MORI AND K. TAKAHASHI. *Periodic maternal deprivation alters stress response in adult offspring: Potentiates the negative feedback regulation of restraint stress-induced adrenocortical response and reduces the frequencies of open field-induced behaviors.* PHARMACOL BIOCHEM BEHAV **49**(4) 961–967, 1994.—The effects of periodic maternal deprivation (PMD) treatment on the adrenocortical stress response and on open-field behavior in adult offspring were investigated. Sprague–Dawley rat pups were deprived of mothers daily for 4.5 h during the first 3 weeks of life. PMD treatment resulted in lower corticosterone levels during restraint stress later in life. The result of dexamethasone suppression test indicated that PMD treatment caused a potentiation of the negative feedback function of adrenocortical response. These effects of PMD were not accompanied by an increased density of the hippocampal glucocorticoid receptor which has been reported to be induced in neonatal handling treatment (brief 15-min maternal deprivation). Serotonin (5-HT)-2 and β-adrenergic binding sites were also examined in cerebral cortex and no change of binding capacities were induced by PMD treatment. In the open-field test, PMD treatment decreased the number of ambulations and rearings but did not affect a frequency of defecation. From these results, it is suggested that PMD treatment leads rats to be insensitive to environmental stimuli in adulthood.

Maternal deprivation Glucocorticoid receptor Monoaminergic receptor Adrenocortical stress response Open-field test Dexamethasone suppression test

NORMAL mother-infant interactions are critical for growth and development in many mammalian species, and maternal deprivation is a profound stress that affects physiological and behavioral functions in the offspring (1,5,8,15,31). However, it is well known that beginning on about postnatal day 2 and continuing into the second week of life, rat pups fail to respond or respond only weakly to a variety of stressful stimuli, including maternal deprivation, ether, and surgery stresses (28,30,32,33). Moreover, basal corticosterone levels in plasma decline dramatically during this period (17,28). Thus, this period of adrenocortical quiescence has been termed the stress hyporesponsive period. On the other hand, it is also well

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known that in the immature brain, many neurotransmitters act as developmental signals or regulators, resulting in permanent changes in the densities of several neurotransmitter receptors once that the brain has matured (26,34,35). Thus, the recent concept of a programmable receptor depending on levels of neurotransmitters during development could explain many findings of altered receptor density seen in the matured brains from human disease states or predisposition (34). Several lines of evidence suggest that further studies are needed to determine whether or not stressful stimuli during the stress hyporesponsive period can alter the adrenocortical stress response and several neurotransmitter receptors in the adult brain.

Brief 15-min maternal deprivation periods for the first 3 weeks of life, termed postnatal handling, is well documented to reduce hypothalamo-pituitary-adrenal (HPA) responses to a stressful stimulus in adulthood (16). This difference is reflected in smaller increases in plasma adrenocorticotropin (ACTH) and corticosterone levels during stress and a faster return to basal levels following the termination of stress (16). In addition, it has been demonstrated that the brief 15-min handling-induced hypothermia resulted in an increase in the thyroid hormone secretion and 5-hydroxytryptamine (5-HT) turnover producing the increased binding capacity of glucocorticoid receptors in the hippocampus (14,18,19). On the contrary, Plotsky and Meaney have reported that rat pups exposed to 3 h of periodic maternal deprivation for 2 weeks in early life showed increased hypothalamic corticotropin-releasing hormone (CRH) mRNA levels and CRH content in median eminence compared with the brief 15-min handled animals in adulthood, and showed a significant difference in corticosterone secretion during and after restraint stress, compared with the brief 15-min handled animals (24). However, prolonged maternal deprivation for 16 h is known to result in stress-induced hyporesponsiveness of emotionality in adulthood (31). Little is known about the mechanisms that underlie the differences in HPA activity between postnatal handled and nonhandled animals and about the specificity of the 15-minute maternal deprivation. Thus, we have investigated the effect of 4.5 h maternal deprivation for the first 3 weeks of life on the negative feedback activity against adrenocortical stress response and stress-induced behaviors in adulthood. We also investigated the densities of several receptors including neurotransmitter receptors and glucocorticoid receptors in the adult brain.

METHOD

Animals

We used a total of 60 male pups from 14 litters obtained from pregnant Sprague-Dawley rats, which were purchased on gestation day 7 from Japan Clea Laboratories. On the day of birth (day 0) litters were crossfostered. Crossfostering consisted of removing half number of a litter from their biological mother and placing them with another lactating female (nonbiological mother). Seven litters were designed as periodic maternal deprivation groups (PMD). PMD consisted of removing the mother and then 10 pups (six to seven male and three to four female) from their home cage, and placing the pups into another cage without bedding material for 4.5 h. The mothers were left in their home cages during this period. PMD occurred once per day (1000–1430 h) for the first 3 weeks of life. The other seven litters as control groups, were left completely undisturbed throughout this period except for cleaning the bedding material once a week. On day 22, the animals were weaned and housed in same-treatment groups of two or three. Only male rats were used in the following experiments. In addition, we used 32 rats from 16 litters, which were treated neonatally with 5,7-dihydroxytryptamine, antidepressant, or vehicle, to investigate the correlation between body weight and stress-induced corticosterone release at 7 weeks of life.

Blood Sampling Under Basal Conditions and During Restraint Stress

Restraint stress was performed using tubular stainless steel restrainers. At 7 weeks of life, the rats of PMD groups and control groups were restrained for 2 h and blood samples (25–50 μl) were taken from the tail vein at various times during and following stress, to compare the stress-induced corticosterone response. A blood sample for basal corticosterone level was taken from the animal in the home cage before the animal was placed into the restrainer. In the suppression experiments, animals at 8 weeks of life were injected subcutaneously with dexamethasone (DEX, 10 or 100 μg/kg) 3 h prior to a 20-min restraint stress and a blood sample was taken 20 min following the onset of restraint. Two-hour restraint stress-induced corticosterone response and dexamethasone suppression tests were repeated at 20 and 21 weeks of life, respectively. At 14 weeks of life, the rats were treated with 1 μg/kg of corticotropin-releasing hormone (CRH, ovine CRH, Bachem, CA) subcutaneously and a blood sample was taken 20 min following the CRH administration to measure corticosterone levels. To estimate the pituitary response to CRH, we handled animals for a few minutes and treated with saline for 5 consecutive days to minimize the injection stress before CRH administration.

The corticosterone level was measured by radioimmunoassay as previously described (9). Blood sample (20 or 50 μl) was extracted with 1 ml of ethanol and centrifuged at 2300 $\times$ g for 30 min. The solvent (50–500 μl) was decanted into a glass tube and dried by exposure to nitrogen gas. The dried extract was assayed in duplicate by adding phosphate assay buffer, 100 μl of antiserum (UCB-Bioproducts S.A. Belgium, dilution factor 1 : 40000) and 100 μl of [1,2,6,7,-^{3}H (N)]-corticosterone (New England Nuclear, Boston, MA, approximately 10000 cpm) to a final volume of 700 μl. The tubes were incubated at 37°C for 30 min followed by overnight incubation at 4°C. The separation of bound and free hormone fractions was achieved by adding 500 μl of dextran/charcoal (0.25%/0.025% in phosphate buffer). The bound radioactivity was determined using a liquid scintillation counter. The standard curve ranged from 4 to 2000 pg/tube of corticosterone. The method has a sensitivity of 4 pg/tube and intraassay variation between 5.7 and 7.3%.

Glucocorticoid Receptor Binding Assay

At 10 weeks of life, the density of glucocorticoid receptor binding sites was compared in the hippocampus from rats of the PMD groups and control groups.

The hippocampi were dissected from rats that were adrenalectomized under sodium pentobarbital anesthesia (Nembutal, Abbott, 40 mg/kg, IP) 16 h before decapitation. The tissue was homogenized in an ice-cold 30 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM sodium molybdate, 10% (v/v) glycerol, and 1 mM dithiothreitol buffer (pH 7.4; TEDGM buffer) and centrifuged at 105,000 $\times$ g for 60 min (16,18). A resulting cytosol extract was then incubated with [^{3}H]dexamethasone (New England Nuclear, spec. act. 44.7 Ci/mmol; 9 nM). Nonspecific binding was determined

with parallel incubation containing a 500-fold excess of nonradioactive corticosterone. After 20 h incubation at 4°C, dextran/charcoal (2.5%/0.25% in TEDGM buffer) was added to the incubate and then the solution was centrifuged at 3000 rpm. Radioactivity of the supernatant was determined by liquid scintillation spectrometry. The protein content in the cytosol extract was determined by the method of Lowry (11).

Adrenergic and Serotonergic Receptor Binding Assay

At 10 weeks of life, the densities of serotonin (5-HT)-2 receptor and β-adrenergic receptor binding sites in the cerebral cortex from the two groups of rats were also compared.

Cerebrocortical tissues were homogenized in 25 vol (w/v) of ice-cold 50 mM Tris-HCl buffer containing 1 mM EDTA (pH 7.7 at 25°C) and then centrifuged at 49000 × *g* for 15 min. The resultant pellets were resuspended in the same buffer without EDTA and centrifuged. This procedure was repeated once more and the final membrane pellets were suspended in 50 vol of the same buffer. The membrane suspensions were incubated with 0.5 nM [ethylene-^{3}H]ketanserin hydrochloride (New England Nuclear, Boston, MA) at 25°C for 60 min or ^{3}H-CGP12177 [4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazol-2-one hydrochloride] at six different concentrations of 0.06–2.0 nM at 37°C for 30 min. Nonspecific binding was defined with 10 μM of methysergide for ketanserin binding and with 10 μM of (−)propranolol for CGP12177 binding. The incubation was terminated by rapid filtration through GF/B filter (Whatman) under reduced pressure. The filter was washed three times with 5 ml of ice-cold buffer. Radioactivity of the filter was determined by liquid scintillation spectrometry.

Open-Field Test

The open-field test was performed at 6 weeks of life. The open-field apparatus was a circular arena (60 cm in diameter). Its floor was divided into 19 squares. Animals were placed at a starting point in the center of the field and the number of squares entered with all four paws, rearings, and fecal pellets were scored for 5 min. This test was carried out between 0930 h and 1300 h under vivarium lights.

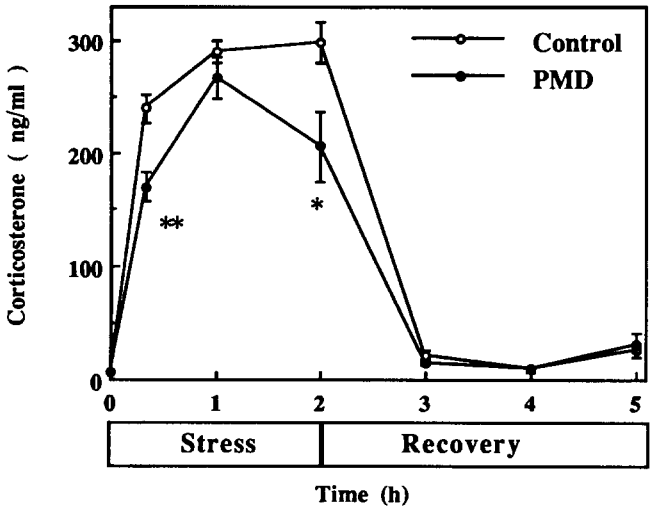

FIG. 1. Corticosterone levels prior to, during, and following the termination of a 2 h restraint stress at 7 weeks of life. The values are mean ± SEM of eight rats per group. A significant effect of PMD treatment, $F(1, 14) = 8.61$, $p < 0.01$, and of time, $F(2, 28) = 15.9$, $p < 0.0001$, and no PMD × time interaction, $F(2, 28) = 3.3$, during stress were indicated. *,**Significantly different from control group at $p < 0.05$, 0.01 by Student's *t*-test.

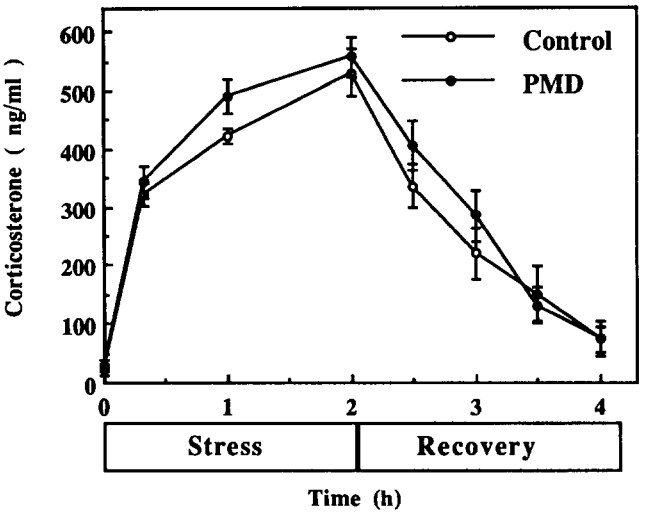

FIG. 2. Corticosterone levels prior to, during, and following the termination of a 2 h restraint stress at 20 weeks of life. The values are mean ± SEM of eight rats. No significant effect of PMD treatment was indicated, $F(1, 14) = 1.28$.

Statistics

The resultant values were analysed for significance using a two-way repeated measures ANOVA followed by a Student's *t*-test for corticosterone levels and a Mann–Whitney *U*-test for behavioral measures.

RESULTS

Corticosterone Levels Under Basal Condition and Stressful Condition

The corticosterone secretion during 2-h restraint stress treatment at 7 weeks of life of rats treated with periodic maternal deprivation (PMD) was less than that of the control groups (Fig. 1). This difference in the corticosterone secretion during stress treatment is statistically significant, although the basal corticosterone levels and the corticosterone secretion after the termination of stress treatment were not different between the two groups. However, this lower response in corticosterone secretion to restraint stress in the PMD groups was no longer apparent at 20 weeks of life (Fig. 2). In addition, the corticosterone levels 20 min after corticotropin-releasing hormone (CRH) administration to the control and PMD groups at 14 weeks of life were 85.8 ± 13.8 and 105.7 ± 12.1 ng/ml, respectively. CRH-induced corticosterone secretion was not significantly different between the two groups at 14 weeks of life. The PMD groups were conspicuously lighter than control groups at weaning (control, 50.3 ± 1.2 g; PMD, 44.6 ± 1.0 g, $n = 21$/group, $p < 0.01$), and this tendency continued until 8 weeks of life, but not at 20 weeks of life. In addition, there is no correlation between body weight and restraint stress-induced corticosterone levels in same age animals and with same range of body weight ($r = 0.1$).

Dexamethasone Suppression Test

Dexamethasone pretreatment potently inhibited the restraint stress-induced corticosterone secretion with a tendency of dose-dependent manner (Fig. 3). In the PMD groups, pretreatment with dexamethasone inhibited stress-induced corticosterone secretion to a greater extent than in the control group at 8 weeks of life by 68% for PMD and by 42% for controls (Fig. 3). This result showed that dexamethasone tended to be more potently inhibited in the PMD group than in the control group, and this potentiated dexamethasone suppression in the PMD group was confirmed at 21 weeks of life (Fig. 4). PMD-produced potentiation of negative feedback regulation of stress-induced corticosterone secretion in adulthood was unlikely due to weight loss produced by malnutrition during the nursing period, because the mother-deprived animals had similar weights as the control animals at 21 weeks of life.

Glucocorticoid Receptor and Monoaminergic Receptor Binding in the Brain

As shown in Fig. 5, there was no difference in dexamethasone binding density in the hippocampus between the PMD and control groups. More than 80% of the specific binding of dexamethasone defined by 5 μM corticosterone in the hippocampus was displaced by 1 μM of specific glucocorticoid receptor ligand, Ru 28362 or Ru 38486. We conclude that these dexamethasone binding densities mainly express the density of the glucocorticoid receptors in the hippocampus.

As shown in Table 1, there were also no differences in the densities of 5-HT-2 and β-adrenergic receptors in the cerebral cortex labeled by ketanserin and CGP12177, respectively, between the two groups.

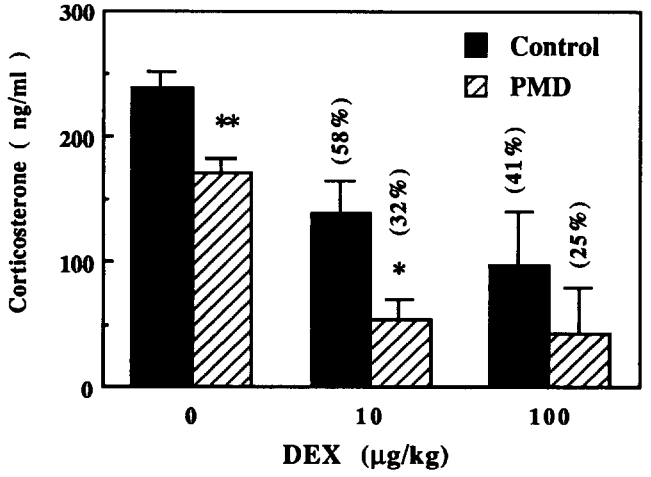

FIG. 3. Effect of pretreatment with dexamethasone (DEX) on corticosterone levels 20 min following an onset of restraint stress. Three hours before restraint stress, rats were injected with 10 or 100 μg/kg of DEX at 8 weeks of life. Data at a dose of 0 μg/kg were taken from Fig. 1. The values are mean ± SEM of eight rats per group. Significant effects of PMD treatment, $F(1, 14) = 6.45$, $p < 0.05$, and of DEX, $F(2, 28) = 15.8$, $p < 0.0001$, were indicated. *,**Significantly different from control group at $p < 0.05$, 0.01 by Student's t-test. The values in parenthesis indicate the percentage of corticosterone levels to levels at 0 μg/kg of each group. These values show a tendency that 10 μg/kg of DEX caused more potent inhibitory effect in PMD group than in control group ($p < 0.06$ by Student's t-test).

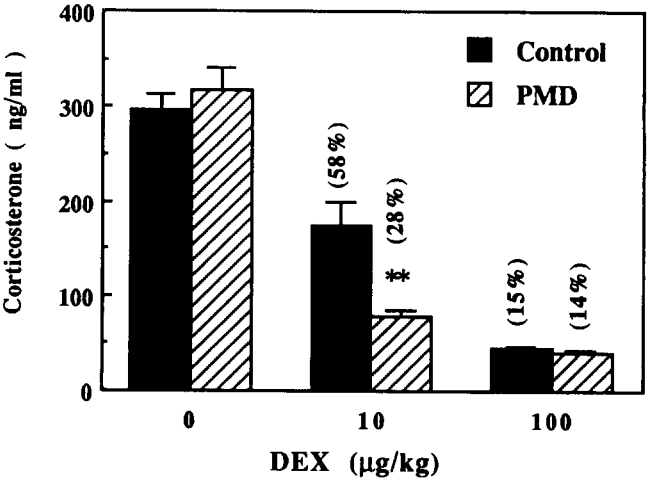

FIG. 4. Effect of pretreatment with dexamethasone (DEX) on corticosterone levels 20 min following an onset of restraint stress. Three hours before restraint stress, rats at 21 weeks of life were injected with 10 or 100 μg/kg of DEX. Data at a dose of 0 μg/kg were taken from Fig. 2. No significant effect of PMD treatment, $F(1, 14) = 3.87$, and a significant effect of DEX, $F(2, 28) = 135.9$, $p < 0.0001$, and a significant interaction PMD × DEX, $F(2, 28) = 5.95$, $p < 0.01$, were indicated. **Significantly different from control at $p < 0.01$ by Student's t-test. The values in parenthesis show the percentage of corticosterone levels to levels at 0 μg/kg of each group and indicate that 10 μg/kg of DEX caused more potent inhibitory effect in PMD group than in control group ($p < 0.01$, by Student's t-test).

Open-Field Test

Significant reductions in the numbers of squares crossed and rearings were observed in the PMD group at 6 weeks of life, while defecation scores were not different between the two groups (Fig. 6).

DISCUSSION

In the present study, it was clearly demonstrated that periodic maternal deprivation (PMD) for 4.5 h during the first 3

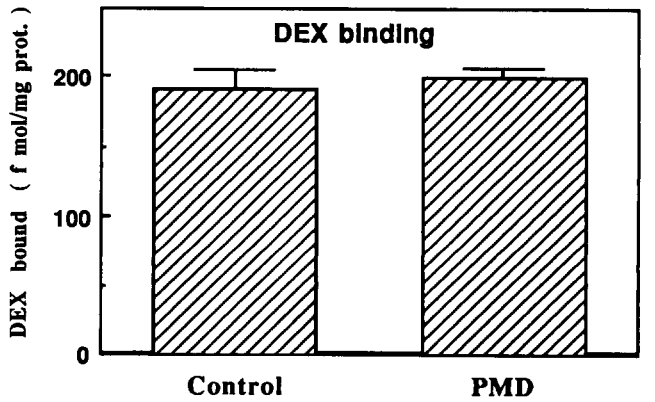

FIG. 5. [^{3}H]Dexamethasone binding capacities of hippocampal tissues from rats at 10 weeks of life that were subjected to stress tests at 7–8 weeks of life. The values are mean ± SEM of eight rats per group. [^{3}H]Dexamethasone binding capacities in the hippocampus were estimated using a single point assay.

TABLE 1

[^{3}H]KETANSERIN AND [^{3}H]CGP12177 BINDING IN CEREBROCORTICAL TISSUES FROM RATS AT 10 WEEKS OF LIFE

Group	[^{3}H]Ketanserin Bound (fmol/mg prot.)	[^{3}H]CGP12177 Bound B_{max}(fmol/mg prot.)	K_d(nM)
Control	125.6 ± 2.98	102.4 ± 2.35	0.23 ± 0.003
PMD	131.0 ± 2.19	98.4 ± 0.79	0.23 ± 0.009

The values are mean ± SEM of six rats per group. ^{3}H-ketanserin binding capacities in the cerebral cortex were estimated using a single point assay.

weeks of life resulted in the potentiation of dexamethasone-induced inhibitory effect on restraint stress-induced corticosterone secretion and in the reduction in the numbers of squares crossed and rearings in adulthood. It was also demonstrated that these alterations in the neuroendocrine function and behavior, manifested under stressful conditions, were not correlated with the alterations in the densities of the glucocorticoid receptors in the hippocampus, or of 5-HT-2 and β-adrenergic receptors in the cerebral cortex.

One may question if difference in body weight induced by PMD affects the stress-induced corticosterone response and the inhibitory potency of dexamethasone. It should be noted, however, that PMD treatment potentiated the inhibitory potency of dexamethasone to suppress restraint stress-induced corticosterone response at 21 weeks of life as well as 8 weeks of life, although the PMD group was no longer lighter than control group at 21 weeks of life. In addition, there is no correlation between body weight and restraint stress-induced corticosterone levels.

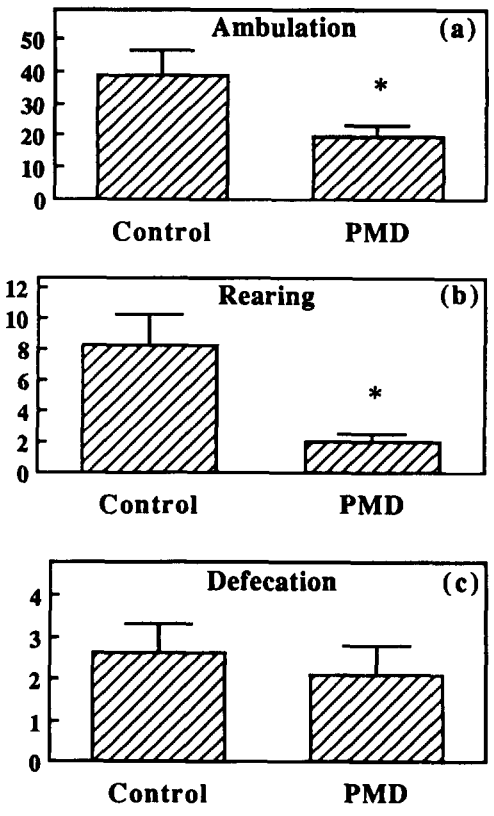

FIG. 6. Open-field behavior (ambulation (a), rearing (b), and defecation (c)) during 5 min at 6 weeks of life. The values are mean ± SEM of 14 rats per group. *Significantly different from control group at $p < 0.05$ by Mann-Whitney U-test.

Brief 15-min maternal deprivation for the first 3 weeks of life, termed postnatal handling is well documented to reduce hypothalamo-pituitary-adrenal (HPA) responses to a stressful stimulus in adulthood (16). This difference has been most commonly reported as a smaller increase in plasma corticosterone levels during stress and a faster return to basal corticosterone levels following the termination of the stress, and an increase in hippocampal glucocorticoid receptor binding capacity, which appears to enhance the efficacy of negative feedback inhibition on HPA activity (16,27). It is clear that some of the effects of 4.5-h periodic maternal deprivation on the regulating mechanisms of HPA response to stressful stimuli are similar to those of the brief 15-min handling, suggesting that stressful stimuli during early 3 weeks of life may alter the stress response in adulthood, and the brief 15-min handling is not specific regarding this effect. The most reliable and pronounced difference in the HPA response to stress between the PMD groups and control groups is the potentiation of exogenous steroid, dexamethasone-induced inhibitory effect on stress-induced corticosterone secretion. However, regarding the effect of the brief 15-min handling, the lower response in corticosterone secretion to restraint stress in the PMD groups seems to be transient. This may be due to some alterations in the regulatory activities occurring at various sites of the limbic-hypothalamo-pituitary-adrenal axis during the development until the young adult stage. This lower response was no longer apparent at 20 weeks of age and CRH-induced corticosterone secretion was not significantly different between the PMD groups and controls at 14 weeks of life. In addition, a faster return to basal levels following the termination of the stress was not observed in the PMD groups, although the most pronounced difference in the HPA response to stress between handled and nonhandled animals is considered to be the elevated poststress levels of corticosterone in the nonhandled animals (16). Recently, Plotsky and Meaney have reported that rat pups exposed to 3 h of periodic maternal deprivation for 2 weeks in early life showed increased hypothalamic CRH mRNA levels and CRH contents in median eminence compared with control animals in adulthood, but showed no difference in corticosterone secretion during and after restraint stress, compared with control animals (24). Thus, these authors have emphasized that the effects of brief 15-min handling and periodic maternal deprivation for 2 weeks on corticosterone secretion during and after stressful stimuli were in opposition. It is well-known that in the course of normal mother-pup interactions the dam routinely leaves her pups for periods of 15–25 min, whereas prolonged maternal deprivation is unusual and more stressful for pups. However, it is difficult to conclude that the effects of the brief 15-min handling and prolonged maternal deprivation on adult HPA function were in complete opposition. This is because both brief 15-min handling and PMD, as tested here, potently enhanced the negative feedback sensitivity to glucocorticoid against stress-induced corticosterone response, although brief 15-min handled and PMD-treated animals differ in some neuroendocrine response to stress in adulthood. Several studies have demonstrated that neonatal rats exhibit an enhanced pituitary and hypothalamic sensitivity to glucocorticoid during the stress hyporesponsive period (SHRP) and that the expres-

sion of hypothalamic CRH gene and pituitary POMC gene is reduced during the first week of life within the SHRP, suggesting that an impaired regulation of the CRH-synthesizing neurons may account for the SHRP. Thus, the repeated stress such as PMD or exogenous CRH administration during SHRP may confer effects on the development of the neuronal circuit, which regulates stress-induced adrenocortical response, and may alter the activity of the glucocorticoid-responsive elements in the promoter region of the CRH gene and/or POMC gene. Indeed, Insel et al. have reported that the repeated administration of CRH during neonatal period resulted in blunted corticosterone response to stress in adulthood (6). The present study has also demonstrated that the PMD treatment produced an enhanced negative feedback sensitivity to dexamethasone inhibiting stress-induced corticosterone secretion in adulthood.

Glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in the hippocampus appear to mediate the negative feedback effects of steroids on HPA axis (3,4,7,25). GR has a low affinity for corticosterone and appears to regulate the secretion of CRH associated with stress, while MR has a high affinity for corticosterone and appears to mediate the negative feedback effects of the low levels of corticosterone on the basal circadian rhythm of the pituitary-adrenal secretion (2–4,25). Sarrieau et al. have reported that the brief 15-min handling treatment resulted in an increase in GR binding capacity in the hippocampus but not in MR binding capacity (29). In the present study, however, PMD treatment did not alter the binding density of [^{3}H]dexamethasone in the hippocampus, even though PMD treatment as well as brief 15 min handling produced the potentiation of inhibitory effect of dexamethasone on stress-induced corticosterone secretion. This discrepancy may suggest the possibility that dexamethasone binding sites in other brain areas including the hypothalamus and the pituitary are also responsible for PMD-induced or brief handling-induced potentiation of the inhibitory effect of dexamethasone on stress-induced corticosterone response. Another possible explanation of the discrepancy is the different strains of rats used. Mitchell et al. have clearly demonstrated that brief 15-min handling-induced hypothermia resulted in an increase in the thyroid hormone secretion and 5-HT turnover, which produced the increased binding capacity of GR, and in addition, the blockade of 5-HT transmission during early life produced a decreased binding capacity of GR in the hippocampus of Long-Evans rats (18,19). In contrast, we found that neonatal treatment with a 5-HT neurotoxin, 5,7-dihydroxytryptamine failed to alter the adrenocortical response to stressful stimuli and the binding capacity of [^{3}H]dexamethasone in the hippocampus of Sprague-Dawley rats (20). Further investigations are needed to clarify the responsible sites, using methods such as in situ hybridization histochemistry of GR mRNA, because adrenalectomy treatment, which is needed to avoid the endogenous corticosterone competing with [^{3}H]dexamethasone binding, may, consequently, alter the state of the HPA axis.

Several investigators have demonstrated that chronic treatment with restraint stress, foot shock stress, or running wheel stress produced a decrease in β-adrenergic receptor density and an increase in serotonin (5-HT)-2 receptor density in the cerebral cortex of adult rats, but we and other investigators did not (12,13,21). Recent progress in developmental neurobiology clearly demonstrates that an altered monoamine metabolism in an immature brain permanently determines the density of monoaminergic receptors in a mature brain (22,23,34). Thus, it had been expected that some stressful condition in the early life might alter the densities of 5-HT-2 and β-adrenergic receptors in the cerebral cortex in adulthood, if the metabolism of monoaminergic transmitters, which may act as developmental signals, are altered in the immature brain under stressful conditions such as prolonged maternal deprivation. Unexpectedly, PMD treatment did not alter the densities of 5-HT-2 and β-adrenergic receptors in the cerebral cortex in adulthood.

In the open-field test, the rats treated with the PMD showed a reduction in the frequencies of squares crossed and rearings; this may suggest that these rats were more anxious than controls. This speculation, however, is not supported, because no difference was found on defecation scores between the PMD and control groups. The works reported by Levine et al. are well known to have demonstrated that brief 3-min handled rats during first 3 weeks were more active in the open field in adulthood (10). They had examined open-field behavior for 4 consecutive days, and found that handled rats showed more activity on the last 3 days, but were less active on the first day (10). Thus, it is noteworthy that the effects of PMD, demonstrated in the present study, and brief handling on behavioral response to stressful situation in adulthood may not be a contradiction, even though we did not examine open-field behavior for consecutive days.

Considering the present results of endocrinological and behavioral responses to stressful situation, it can be concluded that the PMD treatment leads rats to be insensitive to environmental stimuli in adulthood.

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REFERENCES

1. Ackerman, S. H.; Hofer, M. A.; Weiner, H. Age at maternal separation and gastric erosion susceptibility in the rat. Psychosom. Med. 37:180–184; 1975.
2. Akana, S. F.; Cascio, C. S.; Du, J.-Z.; Levin, N.; Dallman, M. F. Reset of feedback in the adrenocortical system: An apparent shift in sensitivity of adrenocorticotropin to inhibition by corticosterone between morning and evening. Endocrinology 119:2325–2332; 1986.
3. Dallman, M. F.; Akana, S. F. Negative feedback of glucocorticoids on the brain and pituitary: Role of feedback and its interactions with circadian and stress signals. In: Costa, E.; Paul, S. M., eds. FIDIA research foundation symposium series, vol. 8. Neurosteroids and brain function. New York: Thieme Medical Publishers; 1991:25–29.
4. Dallman, M. F.; Levin, N.; Cascio, C. S.; Akana, S. F.; Jacobson, L.; Kuhn, R. W. Pharmacological evidence that the inhibition of diurnal adrenocorticotropin secretion by corticosteroids is mediated via type I corticosterone-preferring receptors. Endocrinology 124:2844–2850; 1989.
5. Dörner, G.; Tonjes, R.; Hecht, K.; Hinz, G.; Poppe, I.; Poppei, M.; Tsamaloukas, A. Pyridostigmine administration in newborn rats prevents permanent mental III-effects produced by maternal deprivation. Endokrinologie 77:101–104; 1981.
6. Insel, T. R.; Battaglia, G.; De Souza, E. B. Brain corticotropin

releasing factor and development. In: Breznitz, S.; Zinder, D., eds. Molecular biology of stress. New York: Alan R. Liss Inc.; 1989:19-30.
7. Jacobson, L.; Sapolsky, R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr. Rev. 12:118-134; 1991.
8. Janus, K. Early separation of young rats from the mother and the development of play fighting. Physiol. Behav. 39:471-476; 1987.
9. Kuroda, Y.; Mikuni, M.; Ogawa, T.; Takahashi, K. Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT_2 receptor binding sites in neocortex of rat forebrain and 5-HT_2 receptor-mediated wet-dog shake behaviors. Psychopharmacology (Berlin) 108:27-32; 1992.
10. Levine, S.; Haltmeyer, G. C.; Karas, G. G.; Denenberg, V. H. Physiological and behavioral efects of infantile stimulation. Physiol. Behav. 2:55-59; 1967.
11. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275; 1951.
12. Martin, J. V.; Edwards, E.; Johnson, J. O.; Henn, F. A. Monoamine receptors in an animal model of affective disorder. J. Neurochem. 55:1142-1148; 1990.
13. Mayeda, A. R.; Simon, J. R.; Hingtgen, J. N.; Hofstetter, J. R.; Aprison, M. H. Activity-wheel stress and serotonergic hypersensitivity in rats. Pharmacol. Biochem. Behav. 33:349-353; 1989.
14. Meaney, M. J.; Aitken, D. H.; Sapolsky, R. M. Thyroid hormones influence the development of hippocampal glucocorticoid receptors in the rat: A mechanism for the effects of postnatal handling on the development of the adrenocortical stress response. Neuroendocrinology 45:278-283; 1987.
15. Meaney, M. J.; Aitken, D. H.; van Berkel, C.; Bhatnagar, S.; Sapolsky, R. M. Effect of neonatal handling on age-related impairments associated with the hippocampus. Science 239:766-768; 1987.
16. Meaney, M. J.; Aitken, D. H.; 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.
17. Meaney, M. J.; Sapolsky, R. M.; McEwen, B. S. The development of the glucocorticoid receptor system in the rat limbic brain. I. Ontogeny and autoregulation. Dev. Brain Res. 18:159-164; 1985.
18. Mitchell, J. B.; Iny, L. J.; Meaney, M. J. 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.
19. Mitchell, J. B.; Rowe, W.; Boksa, P.; Meaney, M. J. Serotonin regulates type II corticosteroid receptor binding in hippocampal cell cultures. J. Neurosci. 10:1745-1752; 1990.
20. Ogawa, T.; Mikuni, M.; Kuroda, Y.; Muneoka, K.; Mori, K. J.; Takahashi, K. Effects of the altered serotonergic signalling by neonatal treatment with 5,7-dihydroxytryptamine, ritanserin and clomipramine on the adrenocortical stress response and the glucocorticoid receptor binding in the hippocampus in adult rats. J. Neural Transm. 96:113-123; 1994.
21. Ohi, K.; Mikuni, M.; Takahashi, K. Stress adaptation and hypersensitivity in 5-HT neuronal systems after repeated foot shock. Pharmacol. Biochem. Behav. 34:603-608; 1989.
22. Peters, D. A. V. Effects of maternal stress during different gestational periods on the serotonergic system in adult rat offspring. Pharmacol. Biochem. Behav. 31:839-843; 1989.
23. Peters, D. A. V. Maternal stress increases fetal brain and neonatal cerebral cortex 5-hydroxytryptamine synthesis in rats: A possible mechanism by which stress influences brain development. Pharmacol. Biochem. Behav. 35:943-947; 1990.
24. Plotsky, P. M.; Meaney, M. J. Early postnatal experience alters hypothalamic corticotropin releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Mol. Brain Res. 18:195-200; 1993.
25. Reul, J. M. H. M.; de Kloet, E.R. Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology 117:2505-2511; 1985.
26. Rosengarten, H.; Friedhoff, A. J. Enduring changes in dopamine receptor cells of pups from drug administration to pregnant and nursing rats. Science 203:1133-1135; 1979.
27. Sapolsky, R. M.; Krey, L. C.; McEwen, B. S. Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proc. Natl. Acad. Sci. USA 81: 6174-6177; 1984.
28. Sapolsky, R.; Meaney, M. J. Maturation of the adrenocortical stress response: Neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. Rev. 11:65-76; 1986.
29. Sarrieau, A.; Sharma, S.; Meaney, M. J. Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors. Dev. Brain Res. 43:158-162; 1988.
30. Stanton, M. E.; Gutierrez, Y. R.; Levine, S. Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. Behav. Neurosci. 102:692-700; 1988.
31. Tönjes, R.; Hecht, K.; Brautzsch, M.; Lucius, R.; Duörner, G. Behavioural changes in adult rats produced by early postnatal maternal deprivation and treatment with choline chloride. Exp. Clin. Endocrinol. 88:151-157; 1986.
32. Walker, C.-D.; Scribner, K. A.; Cascio, C. S.; Dallman, M. F. The pituitary-adrenocortical system of neonatal rats is responsive to stress throughout development in a time-dependent and stressor-specific fashion. Endocrinology 128:1385-1395; 1991.
33. Walker, C.-D.; Perrin, M.; Vale, W.; Rivier, C. Ontogeny of the stress response in the rat: Role of the pituitary and the hypothalamus. Endocrinology 118:1445-1451; 1986.
34. Whitaker-Azmitia, P. M. Role of serotonin and other neurotransmitter receptors in brain development: Basis for developmental pharmacology. Pharmacol. Rev. 43:553-561; 1991.
35. Whitaker-Azmitia, P. M.; Lauder, J. M.; Shemmer, A.; Azmitia, E. C. Postnatal changes in serotonin1 receptors. Dev. Brain Res. 33:285-289; 1987.