The Journal of Neuroscience, September 15, 2002, 22(18):7840–7843

Brief Communication

Environmental Enrichment Reverses the Effects of Maternal Separation on Stress Reactivity

Darlene D. Francis,[1] Josie Diorio,[1] Paul M. Plotsky,[2] and Michael J. Meaney[1]

[1]*Developmental Neuroendocrinology Laboratory, Douglas Hospital Research Centre, Departments of Psychiatry, and Neurology and Neurosurgery, McGill University, Montréal, Québec H4H 1R3, Canada, and* [2]*Department of Psychiatry and Behavioral Science, Emory University, Atlanta, Georgia 30322*

Postnatal maternal separation increases hypothalamic corticotropin-releasing factor (CRF) gene expression and hypothalamic-pituitary-adrenal (HPA) and behavioral responses to stress. We report here that environmental enrichment during the peripubertal period completely reverses the effects of maternal separation on both HPA and behavioral responses to stress, with no effect on CRF mRNA expression. We conclude that environmental enrichment leads to a functional reversal of the effects of maternal separation through compensation for, rather than reversal of, the neural effects of early life adversity.

Key words: early experience; environmental enrichment; maternal separation; stress; corticotropin-releasing factor; glucocorticoid receptors

In rodents or nonhuman primates, prolonged periods of maternal separation (MS) in early life increase the magnitude of neuroendocrine and fear responses to stress and thus vulnerability for stress-related illness (Higley et al., 1991; Plotsky and Meaney, 1993; Suomi, 1997; Hall et al., 1999; Caldji et al., 2000; Ladd et al., 2000; Liu et al., 2000; Meaney, 2001). Under stable living conditions, these effects endure over the lifespan, suggesting a certain degree of permanence. However, the question of reversibility has never been addressed directly in the maternal separation model. In light of the effects of peripubertal environmental enrichment on emotional development in more stress-reactive strains of mice or rats (Fernandez-Teruel et al., 1997; Chapillon et al., 1999), we wondered whether enrichment could reverse effects of MS on hypothalamic-pituitary-adrenal (HPA) responses to stress. Two questions are critical: (1) are the effects of MS on stress reactivity reversible at later stages in development? and (2) might such "functional reversibility" be reflected by the reversibility at the level of the underlying neural mechanisms thought to mediate the effects of MS?

Postnatal MS (3 hr/d; days 2–14 of life) enhances HPA responses to stressors (Plotsky and Meaney, 1993; Ladd et al., 2000; Liu et al., 2000). In contrast, postnatal handling, which involves only a brief (15 min) period of mother–pup separation, dampens both HPA and behavioral responses to stress (Levine, 1957; Meaney et al., 1989; Meaney, 2001). Corticotropin-releasing factor (CRF) from the paraventricular nucleus of the hypothalamus (PVNh) and the central nucleus of the amygdala is a primary regulator of HPA and behavioral responses to stress (Nemeroff, 1996; Valentino et al., 1998; Bakshi et al., 2000). CRF mRNA expression in both regions is increased by MS and decreased by postnatal handling (Plotsky and Meaney, 1993; Francis et al., 1999; Ladd et al., 2000). MS also affects the development of neural systems that regulate CRF gene expression. Hypothalamic CRF synthesis and release is inhibited via glucocorticoid negative feedback (Dallman et al., 1993; Jacobson and Sapolsky, 1991; De Kloet et al., 1998), an effect that is, in part, mediated by hippocampal glucocorticoid receptors (GRs) (Jacobson and Sapolsky 1991; De Kloet et al., 1998). MS decreases hippocampal glucocorticoid receptor expression and glucocorticoid feedback sensitivity (Ladd et al., 2000). Postnatal handling has precisely the opposite effect, increasing hippocampal glucocorticoid receptor expression, enhancing feedback inhibition and decreasing CRF mRNA levels in the PVNh (Meaney, 2001). Such alterations in glucocorticoid receptor expression are critical; reversing the differences in hippocampal glucocorticoid receptor levels eliminates the differences in HPA responses to stress between handled and nonhandled animals (Meaney et al., 1989). Moreover, manipulations that increase hippocampal glucocorticoid receptor expression and decrease hypothalamic CRF levels eliminate the maternal separation effect on HPA function (Ladd et al., 2000).

To examine the question of reversibility, animals were exposed to either handling or MS daily for the first 2 weeks of life. At the time of weaning, animals were then transferred into conditions of either environmental enrichment or standard social housing until day 70. In adulthood, the animals were tested for the reversal of early life conditions at the level of both function (behavioral and HPA responses to stress) and mechanism (CRF and GR mRNA expression).

MATERIALS AND METHODS

Animals. Litters from pregnant Long–Evans rats from an in-house breeding colony of Charles River Laboratories (St. Constant, Québec, Canada) stock were standardized to 8–13 pups per dam and exposed to one of the following rearing conditions from days 1–14 inclusive: (1) handled (H) animals were exposed to a daily 15 min period in which the dam was removed to an adjacent cage and the litter removed and placed in an incubator or (2) MS in which dams and pups were treated in the same manner for a 180 min period of separation. Litters were removed as a group, weighed, and placed as a group into a plastic cage (15 × 15 cm) in an adjacent room lined with bedding material and contained within an incubator maintained at 32 ± 0.5°C (days 1–5) or 30 ± 0.5°C (days 6–14). After the separation period, pups were returned to the nest and rolled in

Received Jan. 23, 2002; revised June 11, 2002; accepted June 27, 2002.

This work was supported by grants from the National Institute of Mental Health (P.M.P., M.J.M) and the Canadian Institutes for Health Research (M.J.M.). M.J.M. holds a Senior Scientist award, and D.D.F. holds a postdoctoral fellowship from the Canadian Institutes for Health Research.

Correspondence should be addressed to Michael J. Meaney, Developmental Neuroendocrinology Laboratory, Douglas Hospital Research Centre, 6875 Boulevard LaSalle, Montréal, Québec H4H 1R3, Canada. E-mail: michael.meaney@mcgill.ca.

 0270-6474/02/227840-04$15.00/0

home cage bedding material, and the dam was returned. In the rat, the mother is routinely off the litter for periods of 20–25 min (Jans and Woodside, 1990). Thus, MS, but not handling, results in a deprivation of maternal care. Animals were otherwise left undisturbed from days 0–15.

Offspring were weaned on day 22 and housed in same-rearing groups under either standard or enriched conditions with food and water available *ad libitum*. Animals in the enrichment condition were housed in groups of eight animals within a series of large 60 × 30 × 60 cm cages interconnected with a burrow system and filled with toys that were replaced regularly. Standard laboratory conditions were defined as two animals housed in a 20 × 40 × 30 cm clear plastic cage. At 70 d of age, all animals were then housed in same-treatment groups, two per cage until testing began on day 110. All procedures were approved by the McGill University Animal Care Committee.

Behavioral testing. For the measures of novelty-induced suppression of appetitive behavior (Britton and Thatcher-Britton, 1981), separate groups of animals were food deprived for 24 hr before testing and then provided with lab chow in either a novel environment (180 × 180 × 30 cm arena) or the home cage with food provided in a cylindrical wire-mesh hopper located in the center of the testing environment. During the 10 min test session, the experimenter scored the latency (in seconds) to begin feeding and the total amount of time spent feeding.

Another set of animals were examined in an open-field test of exploration. Animals were placed, one at a time, in a novel, circular open field, 1.6 m in diameter for 5 min. The critical measure was the time (in seconds) the animal spent exploring the inner area of the novel arena. Exploration was defined as the entire body of the animal being away from the immediate vicinity of the wall (>10 cm) enclosing the open field. The open field was cleaned between each subject to prevent olfactory cues from affecting the behavior of subsequently tested rats.

Stress testing and blood sampling. Restraint stress was performed between 12:00 P.M. and 3:00 P.M. Prestress blood samples were taken from rats within 30 sec of removal from the cage, and restraint stress was performed between 12:00 P.M. and 3:00 P.M. with blood sampling (300 μl) from the tail vein at 0, 20, 60, and 120 min after the onset of restraint (Meaney et al., 1989). Plasma (10 μl) corticosterone was measured by RIA with a highly specific B antiserum (B3–163; Endocrine Sciences, Tarzana, CA) and [^{3}H]corticosterone (101 Ci/mmol; NEN, Boston, MA) as tracer. The antiserum cross-reacts slightly with desoxycorticosterone (~4%) but not with aldosterone, cortisol, and progesterone (<1%). The intra-assay and interassay coefficients of variation were 8.8 and 10.4%, respectively.

In situ *hybridization.* Brains were rapidly removed and frozen in isopentane (−70°C). Coronal sections (15 μm) were thaw mounted onto poly-D-lysine-coated slides and stored at −80°C under RNase-free conditions. Before hybridization, slides were warmed to room temperature, postfixed in 4% paraformaldehyde in 0.1 phosphate buffer, pH 7.0, for 10 min, and washed in three changes of 2× SSC (0.3 M NaCl and 0.03 M sodium citrate) in sterile water containing 0.2% diethylpyrocarbonate.

CRF mRNA *in situ* hybridization was performed using an 48 bp oligonucleotide sequence (CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC). Slides were warmed to room temperature, postfixed in 4% paraformaldehyde in 0.1 phosphate buffer, pH 7.4 for 10 min, washed in 2× SSC in sterile water containing 0.2% diethylpyrocarbonate, rinsed once in TEA (triethanolamine and HCl)–2× SSC–0.25% acetic anhydride for 10 min and once in 2× SSC, dehydrated in increasing concentrations of ethanol, and placed in chloroform for 10 min and then 100 and 95% EtOH.

Hybridization was performed at 37°C for 18 hr in buffer containing 50% deionized formamide, 1.2 M NaCl, 20 mM Tris, pH 7.5, 100× Denhardt's solution, 20 mM EDTA, pH 8.0, 200 μg/ml denatured salmon sperm DNA, 200 μg/ml yeast tRNA, 10% dextran sulfate, 10 mM dithiothreitol, and 1×10^7 cpm/ml [^{35}S]ATP-labeled CRF oligoprobe. Slides were washed four times for 30 min in 1× SSC at 55°C, rinsed briefly in water, dried, and apposed to Hyperfilm for 21 d along with sections of ^{35}S-labeled standards prepared with known amounts of ^{35}S in a brain paste.

Preparation of glucocorticoid receptor riboprobes as well as the *in situ* hybridization procedure and sense controls have been described previously (Liu et al., 1997). Briefly, sections were treated as above and hybridized using [^{35}S]UTP-labeled cRNA antisense probes transcribed *in vitro* from plasmid vectors containing the appropriate cDNA insert. GR cRNA was transcribed from a 674 bp *Pst*I-*Eco*R1 fragment of the rat GR cDNA (steroid binding domain obtained from Dr. R. Meisfield, University of Arizona, Tucson, AZ), linearized with *Ava*I, and transcribed with T7 RNA polymerase. Hybridization was performed as described above. Slides were rinsed in 2× SSC and treated with RNase A (20 μg/ml) for 45 min at 37°C. Slides were then washed in decreasing salt concentrations to a final stringency of 0.1× SSC (containing 14 mM β-mercaptoethanol) at 60°C. Sections were dehydrated in increasing concentrations of ethanol (in 0.3 M sodium acetate), dried, and apposed to Hyperfilm for 21 d along with sections of ^{35}S-labeled standards.

The hybridization signal within the parvocellular subregion of the paraventricular nucleus of the hypothalamus (CRF mRNA) or the dorsal hippocampus (GR mRNA) was quantified using densitometry with an image analysis system (microcomputer imaging device; Imaging Research, St. Catherines, Ontario, Canada). The data are presented as arbitrary optical density (absorbance) units after correction for background. The anatomical level of analysis was verified using the rat brain atlas of Paxinos and Watson (1986) with Nissl staining of sections after autoradiography. The hippocampal GR mRNA data were analyzed using a two-way ANOVA (group × region), whereas the CRF mRNA data were analyzed with a one-way ANOVA (group).

Statistical analysis. Statistical comparisons were performed using the appropriate ANOVA model with Tukey's *post hoc* tests.

RESULTS

Enrichment reverses effects of MS on HPA function

Plasma corticosterone responses to stress were significantly greater in MS compared with H rats (Fig. 1*a*,*b*), and the group differences were completely eliminated among animals reared under conditions of environmental enrichment. Statistical analysis revealed a significant postnatal rearing × peripubertal rearing × time interaction ($F = 5.5$; df = 3,84; $p < 0.05$). *Post hoc* analysis revealed that plasma corticosterone levels during and after acute stress were significantly ($p < 0.01$) lower in H animals compared with control MS rats but not compared with MS rats exposed to environmental enrichment during the peripubertal period. There was no effect of peripubertal rearing condition on the HPA responses to stress in the H animals. For the area-under-the-curve analysis (Fig. 1*b*), the statistical analysis revealed a significant interaction between the postnatal and peripubertal rearing conditions ($F = 20.8$; df = 1,28; $p < 0.0001$), reflecting the significantly increased corticosterone levels in the MS control animals.

A similar pattern of results emerged from behavioral tests of fearfulness. Statistical analysis revealed a significant postnatal rearing × peripubertal rearing interaction ($F = 5.0$; df = 1,31; $p < 0.05$). *Post hoc* analysis revealed a significantly ($p < 0.01$) decreased exploration in the MS control animals. In an open-field test of exploration, H animals as well as MS–environmental enrichment animals spent significantly more time exploring the inner area of the novel environment than did control MS rats (Fig. 1*c*). Likewise, there was a significant postnatal rearing × peripubertal rearing interaction ($F = 5.0$; df = 1,28; $p < 0.05$) in the test of novelty-induced suppression of appetitive behavior. There was a significantly ($p < 0.01$) increased novelty-induced suppression of feeding in the MS control animals when animals were food deprived for 24 hr and provided lab chow in a novel environment; H as well as MS–environmental enrichment animals ate more readily than did control MS rats (Fig. 1*d*). In each case, the control MS rats, unlike those reared in environmental enrichment, were more behaviorally inhibited under conditions of novelty. Thus, peripubertal environmental enrichment completely reversed the effects of postnatal MS on both HPA and behavioral responses to stress.

Enrichment does not reverse effects of MS on CRF gene expression

Environmental enrichment did not reverse the effects of MS on CRF gene expression We found significant effects only for the postnatal rearing condition on levels of either CRF mRNA in the PVNh or glucocorticoid receptor mRNA in the hippocampus.

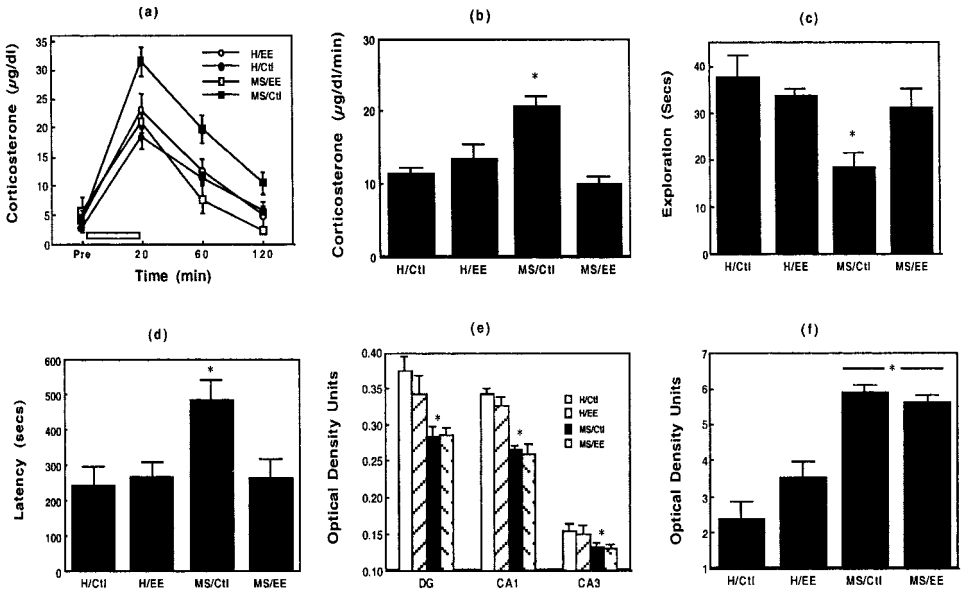

Figure 1. *a*, Mean ± SEM plasma level of corticosterone (in micrograms per deciliters; $n = 8$ per group) in response to a 20 min period of restraint stress (*open bar*) in H and MS animals reared under standard lab procedures (*Ctl*) or environmental enrichment (*EE*) during postnatal days 22–70 and tested on days 110–120. *b*, Mean ± SEM area-under-the-curve analysis for the corticosterone data displayed in *a* calculated using the trapezoidal rule. *c*, Mean ± SEM time spent exploring the inner area of a 2 × 2 m novel open-field environment ($n = 8$–10 per group). *d*, Mean ± SEM latency to begin eating food presented to food-deprived animals in a novel environment ($n = 8$–9 per group). *e*, Mean ± SEM levels of glucocorticoid receptor mRNA in the dentate gyrus (*DG*) and CA1 and CA3 cell fields of the hippocampus, expressed as optical density units, on autoradiograms from *in situ* hybridization studies with a ^{35}S-labeled riboprobe for the rat glucocorticoid receptor ($n = 3$–5 per group). *f*, Mean ± SEM levels of CRF mRNA in the paraventricular nucleus of the hypothalamus, expressed as optical density units, on autoradiograms from *in situ* hybridization studies with a ^{35}S-labeled oligonucleotide probe for the rat CRF ($n = 3$–5 per group).

Environmental enrichment had no effect on either measure in the MS rats. For glucocorticoid receptor mRNA expression, there was a significant effect of postnatal rearing for each region (dentate gyrus, $F = 16.5$, df = 1,12, $p < 0.005$; CA1, $F = 51.2$, df = 1,12, $p < 0.0001$; CA3, $F = 5.5$, df = 1,12, $p < 0.05$) but no significant interaction effect. Likewise, the statistical analysis revealed a significant effect of postnatal rearing ($F = 6.5$; df = 1,12; $p < 0.01$) on CRF mRNA levels in the paraventricular region of the hypothalamus, with no significant interaction effect.

DISCUSSION

Child intervention programs in humans can serve to offset the risk associated with family stress for intellectual and emotional development, and such effects are most apparent in individuals whose development was compromised as a function of early life adversity (Ramey and Ramey, 1998). Interestingly, MS but not H animals were affected by environmental enrichment. It is not clear from human studies whether the outcomes associated with adversity have actually been established and then reversed or whether enrichment has served to negate the influence of adversity such that the more severe scenarios simply never emerge.

Animals studies reveal an apparent reversal of the effects of early life events. For example, postnatal handling reverses the effects of prenatal stress on the development of the HPA axis as well as on behavioral reactivity to stress (Maccari et al., 1995; Vallée et al., 1997; Weinstock, 1997), and postweaning housing conditions can reverse the effects of treatments imposed over the first week of life (Whimbey and Denenberg, 1967). These studies demonstrate reversibility at the level of function and raise the question of whether this functional reversibility is reflected in reversibility of underlying neural mechanisms. Functional reversibility could represent a process whereby cellular and molecular effects occurring at one stage of early life, which would otherwise persist into adulthood, are actually reversed by events at a later stage in development. Alternatively, such functional reversal could also represent a process whereby events at later stages in development serve to promote the development of "compensatory" effects, which might then effectively offset the effects of early trauma. These are not mutually exclusive possibilities. Development might include examples of both reversal as well as compensation. Our data provide little evidence for reversibility at the level of cellular mechanism and suggest that some measure of compensation occurs in MS rats as a result of environmental enrichment. Hippocampal glucocorticoid receptor gene expression, which mediates the effects of early experience on CRF expression, was unaffected by environmental enrichment. Indeed, even at the level of CRF mRNA expression in the paraventricular nucleus of the hypothalamus, there was no evidence for any reversal-like effect as a function of environmental enrichment.

These findings suggest that alterations in gene expression associated with MS are rather resistant to subsequent environmental influences. This finding may be related to the mechanisms underlying the changes in gene expression. In recent studies, we (Weaver et al., 2001) provided evidence for differences in DNA methylation of the exon 17 region of the glucocorticoid receptor promoter. The exon 17 promoter appears to be particularly active in neurons and is regulated by early experience, including postnatal handling as well as maternal care (McCormick et al., 2000). The effects of handling or maternal care on both glucocorticoid receptor mRNA expression and methylation of exon 17 are apparent by day 8 of life (Meaney and Aitken, 1985; Weaver et al., 2001). DNA methylation is an excellent candidate as a mediator for early environmental "programming effects" because it is a covalent modification of DNA that is extremely stable and affects gene expression. DNA methylation is a very active process in early development that is commonly associated with the inactivation of genes (Robertson and Wolffe, 2000). CpG islands represent a potent target for methylation and are common in the promoters such as the exon 17 sequence. It may be that such structural changes in DNA are beyond reversal and that subsequent changes in function are derived from compensatory effects at other levels within the system.

The nature of the proposed compensatory effect remains a matter of speculation, but the hippocampus and prefrontal cortex emerge as potentially interesting sites for consideration. Environmental enrichment alters frontal cortex function, and the medial prefrontal cortex provides inhibitory regulation over HPA re-

sponses to stress (Diorio et al., 1993). Environmental enrichment increases 5-HT_{1A} mRNA and receptor binding in the hippocampus (Rasmuson et al., 1998). Interestingly, enhanced hippocampal 5-HT_{1A} receptor binding is a crucial feature for the behavioral effects of antidepressant medications (Blier and de Montigny, 1999; Pineyro and Blier, 1999), and these medications are known to dampen HPA as well as fear responses to stress. Antidepressant drugs attenuate the effect of MS on HPA responses to stress (Ladd et al., 2000). We found previously that neither postnatal handling nor MS altered 5-HT_{1A} receptor mRNA or binding, suggesting that plasticity within this system may emerge at a later date and thus serve as a substrate for the effects of postweaning environments. Thus, it may be that both antidepressant drugs and environmental enrichment serve to dampen HPA activity through effects on the 5-HT_{1A} receptor system in the hippocampus and, perhaps, the frontal cortex and that these actions represent a form of compensation for the effects of early adversity. Interestingly, antidepressant drugs, like enrichment, have little effect on HPA responses to stress in H animals (Ladd et al., 2000).

Although the nature of compensatory mechanisms is currently a matter of speculation, these findings clearly suggest that the development of individual differences in behavioral and neuroendocrine responses to stress can be influenced by events occurring at multiple stages in development, including the peripubertal period, and that these effects occur as a result of alterations at different levels with the relevant neural systems. Effects at later stages in development might then serve to effectively compensate for the influence of adversity in earlier stages of development. Moreover, the environmental effects occurring at one stage of development appear to depend on previous forms of experience. The current data are clear in suggesting that possible compensatory effects derived from enrichment can serve to mask the effects of previous adversity. The question now lies in the identification of these processes and the mechanisms by which they serve to mask effects from earlier events. The data presented here are consistent with a hierarchical organization within neural systems that regulate behavioral and endocrine stress responses, suggesting that later developing systems, or at least systems with a more prolonged period of plasticity, can serve to override effects at other levels within the system.

REFERENCES

Bakshi VP, Shelton SE, Kalin NH (2000) Neurobiological correlates of defensive behaviors. Prog Brain Res 122:105–115.

Blier P, de Montigny C (1999) Serotonin and drug-induced therapeutic responses in major depression, obsessive-compulsive and panic disorders. Neuropsychopharmacology 21:91S–98S.

Britton DR, Thatcher-Britton R (1981) A sensitive-open-field measure of anxiolytic drug action. Pharmacol Biochem Behav 15:577–580.

Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ (2000) The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22:219–229.

Chapillon P, Manneche C, Belzung C, Caston J (1999) Rearing environmental enrichment in two inbred strains of mice. I. Effects on emotional reactivity. Behav Genet 29:41–46.

Dallman MF, Akana SF, Scribner KA, Bradbury MJ, Walker C-D, Strack AM, Cascio CS (1993) Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. J Neuroendocrinol 4:517–526.

De Kloet ER, Vregdenhil E, Oitzl MS, Joels M (1998) Brain corticosteroid receptor balance in health and disease. Endocr Rev 19:269–301.

Diorio D, Viau V, Meaney MJ (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J Neurosci 13:3839–3849.

Fernandez-Teruel A, Ecorihuela RM, Castellano B, Gonzalez B, Tobena A (1997) Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking and age-related cognitive and hippocampal impairments: focus on the Roman rat lines. Behav Genet 27:513–526.

Francis DD, Diorio J, Liu D, Meaney MJ (1999) Nongenomic transmission across generations in maternal behavior and stress responses in the rat. Science 286:1155–1158.

Hall FS, Wilkinson LS, Humby T, Robbins TW (1999) Maternal deprivation of neonatal rats produces enduring changes in dopamine function. Synapse 32:37–43.

Higley JD, Haser MF, Suomi SJ, Linnoila M (1991) Nonhuman primate model of alcohol abuse: effects of early experience, personality and stress on alcohol consumption. Proc Natl Acad Sci USA 88:7261–7265.

Jacobson L, Sapolsky RM (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenal axis. Endocr Rev 12:118–134.

Jans J, Woodside BC (1990) Nest temperature: effects on maternal behavior, pup development, and interactions with handling. Dev Psychobiol 23:519–534.

Ladd CO, Huot RL, Thrivikraman T, Nemeroff CB, Meaney MJ, Plotsky PM (2000) Long-term behavioral and neuroendocrine adaptations to adverse early experience. Prog Brain Res 122:79–101.

Levine S (1957) Infantile experience and resistance to physiological stress. Science 126:405–406.

Liu D, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ (1997) Maternal care, hippocampal glucocorticoid receptor gene expression and hypothalamic-pituitary-adrenal responses to stress. Science 277:1659–1662.

Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ (2000) The effects of early life events on in vivo release of norepinepherine in the paraventricular nucleus of the hypothalamus and hypothalamic-pituitary-adrenal responses during stress. J Neuroendocrinol 12:5–12.

Maccari S, Piazza PV, Kabbaj M, Barbazanges A, Simon H, Le Moal M (1995) Adoption reverses the long-term impairments in glucocorticoid feedback induced by prenatal stress. J Neurosci 15:110–116.

McCormick JA, Lyons V, Jacobson MD, Noble J, Diorio J, Nyirenda M, Weaver S, Ester W, Yau JLW, Meaney MJ, Seckl JR, Chapman KE (2000) 5′Heterogeneity of glucocorticoid receptor messenger ribonucleic acid is tissue-specific: differential regulation of variant transcripts by early life events. Mol Endocrinol 14:506–517.

Meaney MJ (2001) The development of individual differences in behavioral and endocrine responses to stress. Annu Rev Neurosci 24:1161–1192.

Meaney MJ, Aitken DH (1985) The effects of early postnatal handling on the development of hippocampal glucocorticoid receptors: temporal parameters. Dev Brain Res 22:301–304.

Meaney MJ, Aitken DH, Sharma S, Viau V, Sarrieau A (1989) Postnatal handling increases hippocampal type II, glucocorticoid receptors and enhances adrencocortical negative-feedback efficacy in the rat. Neuroendocrinology 51:597–604.

Nemeroff CB (1996) The corticotropin-releasing factor (CRF) hypothesis of depression: new Findings and new directions. Mol Psychiatry 1:336–342.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. San Diego: Academic.

Pineyro G, Blier P (1999) Autoregulation of serotonin neurons: role of antidepressant drug action. Pharmcol Rev 51:533–591.

Plotsky PM, Meaney MJ (1993) 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.

Robertson KD, Wolffe AP (2000) DNA methylation and chromatiin structure. Nat Rev Gen 1:11–19.

Ramey CT, Ramey SL (1998) Early intervention and early experience. Am Psychol 53:109–120.

Rasmuson S, Olsson T, Henriksson BG, Kelly PA, Holmes MC, Seckl JR, Mohammed AH (1998) Environmental enrichment selectively increases 5-HT1A receptor mRNA expression and binding in the rat hippocampus. Brain Res Mol Brain Res 53:285–290.

Suomi (1997) Early determinants of behaviour: evidence from primate studies. Br Med Bull 53:170–184.

Valentino RJ, Curtis AL, Page ME, Pavcovich LA, Florin-Lechner SM (1998) Activation of the locus ceruleus brain noradrenergic system during stress: circuitry, consequences, and regulation. Adv Pharmacol 42:781–784.

Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S (1997) Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J Neurosci 17:2626–2636.

Weaver ICG, Cervoni N, Diorio J, Szyf M, Meaney MJ (2001) Maternal behavior in infancy regulates methylation of the hippocampal glucocorticoid receptor promoter. Soc Neurosci Abstr 27:697.15

Weinstock M (1997) Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? Neurosci Biobehav Rev 21:1–10.

Whimbey AE, Denenberg VH (1967) Experimental programming of life histories: the factor structure underlying experimentally created individual differences. Behaviour 29:296–314.