Early life adversity and the epigenetic programming of hypothalamic-pituitary-adrenal function

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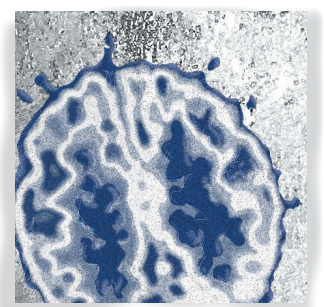

We review studies with human and nonhuman species that examine the hypothesis that epigenetic mechanisms, particularly those affecting the expression of genes implicated in stress responses, mediate the association between early childhood adversity and later risk of depression. The resulting studies provide evidence consistent with the idea that social adversity, particularly that involving parent–offspring interactions, alters the epigenetic state and expression of a wide range of genes, the products of which regulate hypothalamic-pituitary-adrenal function. We also address the challenges for future studies, including that of the translation of epigenetic studies towards improvements in treatments.

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Introduction

The quality of family life influences the development of individual differences in vulnerability for affective illnesses. Victims of childhood physical or sexual abuse or parental neglect are at considerably greater risk for affective disorders.[1-4] Epidemiological studies provide compelling support for the association between cumulative, adverse childhood experiences and the risk for depression.[4,5] This association remains significant when adjusting for living with a mentally ill parent, thus removing the effects of a gene–environment correlation. Moreover, there is commonly a dose–response relationship between the number of types of adversity associated and the magnitude of the risk for depression.[4-11] Statistical modeling demonstrates mediation by multiple intermediate variables, potentially acting with different degrees of effects at varying life stages. These include personality (neuroticism), low self-esteem, conduct disorder, increased risk of adverse life events, low social support, and difficulties in interpersonal relationships.[12,13] The results of prospective, longitudinal studies confirm the link between abuse/neglect and depression.[14] Moreover, childhood maltreatment also associates with an increased severity of illness, reduced treatment responsivity, and increased comorbidity.[14,15] Broader forms of familial dysfunction including persistent emotional and physical neglect, family conflict, cold, distant parent-child relationships, and conditions of harsh, inconsistent discipline compromise cognitive

Selected abbreviations and acronyms

ACTH	*adrenocorticotrophic hormone*
AVP	*vasopressin*
CRF	*corticotropin-releasing factor*
GR	*glucocorticoid receptor*
HDAC	*histone deacetylase*
HPA	*hypothalamic-pituitary-adrenal*
LG	*licking/grooming*
MDD	*major depressive disorder*
NGFI-A	*nerve-growth factor-inducible factor-A*

and emotional development and increase the risk for depression and anxiety disorders to a level comparable to that for abuse.[16-23] Family life also serves as a source of resilience in the face of chronic stress.[24] Thus, warm, nurturing families tend to promote resistance to stress and to diminish vulnerability to stress-induced illness.[18,25] The epidemiology of affective disorders reflects the profound influence of family life on neural development and mental health.

A major challenge is that of defining the biological pathways that link the quality of early life environments to sustained alterations in neural function and ultimately specific health outcomes. While earlier formulations of this issue emphasized the unique plasticity of the perinatal period for brain structure, it is now clear that neuroplasticity is a lifelong process, suggesting that alternative mechanisms must be invoked to explain the enduring influence of early experience. In this review we summarize evidence that links variations in parental care to the epigenetic mechanisms that regulate a specific phenotype, stress reactivity, that influences vulnerability for multiple forms of psychopathology. The review focuses on rodent models as well as translational research with human samples derived from individuals differing in familial history, with a particular emphasis on the development of individual differences in stress reactivity.

Parental influences on stress reactivity

The relationship between the quality of the early social environment and health in adulthood appears to be, in part, mediated by the development of individual differences in neural systems that underlie the expression of behavioral and endocrine responses to stress.[26-30] Thus, physical and sexual abuse in early life increases endocrine and autonomic responses to stress in adulthood.[31,32] Likewise, variations in parental care associate with individual differences in neuroendocrine and autonomic responses to stress in humans as well as emotional reactivity.[18,33-36] Finally, there is considerable evidence in favor of the hypothesis that individual differences in stress reactivity predict the risk for depression.[37] Thus, the influence of familial depressive illness is, in part at least, mediated by increased stress reactivity, enhancing the response of the individual to mild, regular stressors (ie, hassles). Individuals with early adverse experience appear to be sensitized to the depressive effects of acute stress in adulthood.[37,38]

Hypothalamic-pituitary-adrenal axis function and Major Depressive Disorder

Activity of the hypothalamic-pituitary-adrenal (HPA) axis activity is governed by the secretion of corticotropin-releasing factor (CRF) and vasopressin (AVP) from the paraventricular nucleus of the hypothalamus, which in turn activate the secretion of adrenocorticotrophic hormone (ACTH) from pituitary corticotropes, which then stimulates the synthesis and secretion of the glucocorticoids (cortisol in humans and other primates, and corticosterone in rodents) from the adrenal cortex. Cortisol or corticosterone bind to and activate both glucocorticoid (GR) and mineralocorticoid (MR) receptors in multiple target tissues including brain regions that influence hypothalamic synthesis of CRF and vasopressin, thus regulating the HPA activity. Activation of GR in particular leads to negative feedback inhibition of hypothalamic CRF and AVP from the hypothalamus and directly on secretion of ACTH from pituitary corticotropes.

Humans show a diurnal pattern of HPA activity. Cortisol levels rise in the later part of the night, with a further increase occurring about 30 min after waking that is followed by a gradual decline in cortisol levels over the day that reaches a nadir by evening.[39,40] Depression is associated with elevated levels of cortisol.[40,41] A recent meta-analysis confirmed the association of elevated basal levels of both ACTH and cortisol with depression and showed that the greatest difference in cortisol occurred during the afternoon, a time when cortisol levels are normally falling.[42] The increase in basal cortisol levels was particularly marked in melancholic and psychotic depression,[43] but not in atypical depression. Interestingly Cushing's disease or prolonged corticosteroid therapy

can precipitate Major Depressive Disorder (MDD), but also mania.[44] Since elevated levels of cortisol act to inhibit subsequent HPA activity, this finding suggests impaired glucocorticoid negative feedback in depressed patients. Administration of the GR antagonist, RU486, results in elevated levels of both ACTH and cortisol in humans, which implicates GR activation as a mediator of negative feedback inhibition.[45]

While increased HPA activity among depressed patients is not universal, it is of clinical significance. Elevations in evening cortisol levels predict a greater risk for persistent depressive episodes.[46] Successful antidepressant treatment is associated with resolution of the impairment in the negative feedback on the HPA axis by glucocorticoids.[47] Antidepressant treatment increases GR expression, GR function, and GR-mediated HPA axis feedback inhibition in rodents as well as in humans, thereby reducing resting and stimulated HPA axis activity.[40] Normalization of GR function by antidepressant treatment is a significant predictor of long-term clinical outcome.[47] In relation to the role of the GR in antidepressant action, polymorphisms in both the GR and the GR-associated heat-shock protein FKBP5 have been shown to predict clinical response.[48] FKBP5 binds to GR and prevents nuclear translocation and GR-mediated transcriptional activation.

Enhanced HPA activation is also apparent at the level of the brain. There are higher levels of both CRF and CRF-expressing neurons in postmortems samples from depressed compared with nondepressed individuals.[49-51] CRF1 receptors/binding sites are reduced in postmortem brains from suicide victims, many of whom showed a history of depression.[52,53] The downregulation of CRF1 receptors is consistent with increased levels of endogenous CRF. Cerebrospinal fluid (CSF) levels of CRF are reduced by successful treatment of depression by antidepressants.[54,55] Tricyclic antidepressants also reduce CSF levels of CRF in healthy individuals[55] suggesting a direct effect on CRF independent of clinical status. Further evidence for the clinical relevance of the increase in HPA activity is the finding that elevated CSF levels of CRF after antidepressant treatment is associated with greater risk of relapse.[56]

Developmental adversity and HPA function

Studies with adults reveal that childhood maltreatment is associated with an increased HPA response to stress. Women with a history of childhood abuse, either with or without current MDD, exhibit increased ACTH responses to a psychosocial stress compared with controls.[57,58] Subsequent statistical analyses revealed that childhood abuse was the strongest predictor of ACTH responsiveness, followed by the number of abuse events, adulthood traumas, and depression. An interaction term of childhood and adulthood trauma proved to be the most potent predictor of ACTH responses, suggesting that a history of childhood abuse per se is related to increased stress reactivity, which is further enhanced when additional trauma occurs in adulthood.[58] Among women with no history of MDD childhood trauma was similarly associated with and increased ACTH response to stress.[58]

Elevated CSF levels of CRF in adults associate with a history of childhood maltreatment,[58,59] a poor quality of parental care,[60] and childhood stressful experience.[61] Heim et al[58] showed that CSF CRF concentrations were correlated with the severity and duration of physical and sexual abuse. High CRF may arise due to GR downregulation and impaired negative feedback inhibition, as evidenced by the finding that childhood abuse[58,62] and parental loss[63] are associated with higher cortisol response to the dexamethasone (DEX)/corticotropin-releasing hormone (CRH) challenge test in adults with and without depression. However, DEX does not easily cross into the brain[64] suggesting that DEX-suppression tests largely target pituitary feedback mechanisms. Nevertheless, there is direct evidence for an association between childhood maltreatment and hippocampal GR expression.[65] This study examined human postmortem hippocampal samples from suicide victims or individuals who died accidentally, and for whom forensic information was available for both psychopathology and developmental history. Hippocampal samples from individuals with a history of abuse or extreme neglect showed decreased hippocampal GR mRNA levels.

Taken together, these findings suggest that childhood adversity stably influences HPA responses to stress. Moreover, childhood adversity moderates the relation between stressful life events in adulthood and depression, with increased risk for depression or anxiety in response to moderately stressful circumstances among individuals with a history of childhood adversity.[11,14,37] A critical question is that of how early life adversity might stably modify HPA activity under stressful conditions.

Developmental adversity and epigenetic regulation of HPA function

We explore the potential mechanisms for parental effects examining the influence of variations in maternal care in the rat on the development of individual differences in behavioral and endocrine responses to stress. Lactating female Long-Evans rats (an outbred strain of *rattus norvegicus*) exhibit considerable variation in the frequency of pup licking/grooming (LG). Individual differences in the frequency of pup LG among adult female rats are reliable across multiple litters, and thus a stable feature of the maternal phenotype. We use observational procedures to define mothers that consistently show high or low levels of pup LG (ie, High vs Low LG mothers). We and other labs find that variations in pup LG over the first week of postnatal life rat affect the development of behavioral and hypothalamic-pituitary-adrenal (HPA) responses to stress in adulthood.[66-75] Behavioral responses to environmental stressors include a cessation of exploration or appetitive behavior, as well as active attempts to escape from threat.[69,73] For example, in a novelty-induced suppression of feeding test in which food-deprived animals are provided food in a novel context, the adult offspring from High LG mothers show a shorter latency to begin eating and eat for a longer period of time.[67] The offspring of Low LG mothers also show increased vulnerability for stress-induced learned helplessness.[75]

Likewise, there are differences in hypothalamic-pituitary-adrenal responses to acute stress apparent in both circulating levels of pituitary adrenocorticotropin (ACTH) and adrenal corticosterone. As adults, the offspring of High LG mothers show more modest plasma ACTH and corticosterone responses to acute stress by comparison to animals reared by Low LG mothers.[66,70,71,74,75] Circulating glucocorticoids act at GR sites in corticolimbic structures, such as the hippocampus, to regulate HPA activity. Such feedback effects target CRF synthesis and release at the level of the paraventricular nucleus of the hypothalamus (PVNh). The offspring of High LG mothers show significantly increased hippocampal GR mRNA and protein expression, enhanced glucocorticoid negative feedback sensitivity and decreased hypothalamic CRF mRNA levels. Hippocampal infusion of a GR antagonist into the adult offspring of High and Low LG mothers completely eliminates the maternal effect on HPA responses to stress, suggesting a direct relation between hippocampal GR expression and the magnitude of the HPA response to stress.

The effects of maternal care on gene expression and stress responses of the adult offspring are reversed with cross-fostering (eg, refs 68,71): stress responses of adult animals born from Low LG mothers and reared by High LG dams are comparable to normal offspring of High LG mothers (and vice versa). Moreover, variations in the frequency of pup LG towards individual pups of the same mother are significantly correlated with hippocampal GR expression in adulthood.[76] Finally, studies directly examining the effects of the tactile stimulation associated with pup LG show that animals "brushed" for 15 min/day exhibit increased hippocampal GR expression.[77] These findings, as well as those from studies that directly manipulate the frequency of pup LG by the dam reveal a direct relation between maternal care and the phenotypic development of the offspring.

Tactile stimulation derived from maternal licking appears to be the critical environmental signal for the regulation of hippocampal GR expression in the neonate. In vivo studies with rat pups or in vitro studies using cultured hippocampal neurons suggest that maternal effects on hippocampal GR expression are mediated by increases in hippocampal serotonin (5-HT) turnover and the expression of the nerve-growth factor-inducible factor-A (NGFI-A) transcription factor.[78-82] Maternal licking or direct application of tactile stimulation that mimics the sensory effects of licking increases hippocampal expression of the transcription factor, nerve growth factor-inducible factor A (NGFI-A; AKA egr-1, zif268).[82] In vitro, 5-HT acts through a 5-HT_7 receptor to increase the activity of cyclic adenosine monophosphate (cAMP)-dependent signaling pathways in hippocampal neurons, resulting in elevated expression of NGFI-A. The effect of various 5-HT agonists on GR expression in hippocampal neurons is strongly correlated with the effect on cAMP formation. In cultured hippocampal neurons the effect of 5-HT on GR expression is: (i) blocked by 5-HT_7 receptor antagonists or inhibitors of protein kinase A; (ii) mimicked by 5-HT_7 receptor agonists or treatments with stable cAMP analogs; and (iii) eliminated by antisense or small interfering RNA (siRNA) knockdown of NGFI-A mRNA.[82,83] In vivo, the effect on GR is blocked with 5-HT receptor antagonists.[80,81] Moreover, the increase in hippocampal 5-HT activity is associated with a maternally-regulated increase in the conversion of thyroxine to triiodothyro-

nine (T3)[83]: T3 administration in neonatal period, which regulates the 5-HT systems activity, mimics the effects of increased pup LG on both NGFI-A expression and hippocampal GR programming.[82-84] Interestingly, the activation of ascending 5-HT systems during postnatal development also regulates the development of corticolimbic systems implicated in fear behavior.[85]

The 5' non-coding variable exon 1 region of the hippocampal GR gene contains multiple alternate promoter sequences including a neuron-specific, exon 1_7 sequence.[86] Increased pup LG enhances hippocampal expression of GR mRNA splice variants containing exon 1_7 sequence,[70,82,83,86] which contains an NGFI-A response element. Pup LG increases hippocampal NGFI-A expression and binding to the exon 1_7 promoter.[70,82,83] Co-transfection of an NGFI-A vector and an exon 1_7 - luciferase construct shows increased luciferase activity, reflecting NGFI-A-induced activation of transcription through the exon 1_7 promoter.[82,83] The effect of NGFI-A is eliminated by a site-directed mutation within the NGFI-A response element of the exon 1_7 promoter[83] revealing that it is the physical interaction of NGFI-A with its response element that triggers the increase in transcriptional activity. Moreover, infection of hippocampal neurons with an NGFI-A expression plasmid increases both total GR mRNA and exon 1_7-containing GR mRNA.[82] A series of in vivo studies show that the association of NGFI-A with the exon 1_7 promoter is actively regulated by pup LG and artificially generated tactile stimulation of the pups yields the same effect.[82] Thus, chromatin-immunoprecitipation (ChIP) assays reveal increased binding of NGFI-A to the exon 1_7 promoter in pups of High compared with Low LG mothers, but only in the period following a nursing bout with pup LG: hippocampal tissue samples obtained 20 min following a nursing bout, with no subsequent interaction between the mother and pup, do not reveal the difference in NGFI-A association. Perhaps most convincingly, artificial tactile stimulation of pups increases hippocampal NGFI-A expression and NGFI-A binding to the exon 1_7 promoter.

There is a similar effect on hippocampal *Gad1*,[87] an NGFI-A regulated gene that encodes for glutamic acid decarboxylase, the rate-limiting enzyme for GABA synthesis. The association of NGFI-A with the Gad1 promoter is increased in the offspring of High compared with Low LG mothers, but only following a nursing bout. Similarly, hippocampal neuronal cultures treated with 5-HT show an increase in *Gad1* expression and the effect is blocked by an siRNA targeting NGFI-A. These findings suggest that maternal care regulates the expression of a range of NGFI-A-sensitive genes.

The critical issue concerns the mechanism by which hippocampal GR expression remains elevated following weaning and separation from the mother? One possibility is that the increased NGFI-A - exon 1_7 interaction occurring within hippocampal neurons in the pups of High LG mothers might result in an epigenetic modification of the exon 1_7 sequence that alters NGFI-A binding and maintains the maternal effect into adulthood. We focused our initial studies on potential influences on DNA methylation with the assumption that this relatively stable covalent modification was a reasonable candidate mechanism for the enduring effects of maternal care on hippocampal gene expression in the rat.

Preliminary studies revealed greater methylation across the entire exon 1_7 GR promoter sequence in the hippocampus of adult offspring of Low LG mothers. These findings suggested a parental effect on DNA methylation patterns in the offspring. More focused approaches examined the methylation status of individual CpGs in the exon 1_7 sequence using sodium bisulfite mapping. The results reveal significant differences in methylation at the 5' CpG dinucleotide of the NGFI-A consensus sequence. This site is hypermethylated in the offspring Low LG mothers, and hypomethylated in those of High LG dams. Cross-fostering reverses the differences in the methylation of the 5' CpG site and suggests a direct relation between maternal care and DNA methylation of the exon 1_7 GR promoter.[70] The effect of maternal care involves significant alterations in the methylation status of the NGFI-A site. Nevertheless, although less striking, there are differences in the frequency of methylation at other CpG sites on the exon 1_7 promoter. Moreover, the difference in hippocampal GR expression associates with increased expression of promoters in addition to the exon 1_7 site.

An alternative form of DNA methylation, 5-hydroxymethocytosine, has been rediscovered, although its function is not fully understood.[88-91] The ten-eleven translocation (TET) family of enzymes can convert 5-methylcytosine to 5-hydroxymethylcytosine.[89-91] Bisulfite sequencing or PCR-based approaches to the study of DNA methylation cannot distinguish between

5-methylcytosine and 5-hydroxymethylcytosine. We analyzed levels of 5-hydroxymethylcytosine and 5-methylcytosine across the hippocampal GR exon 1_7 promoter in rats using antibody capture of hippocampal DNA and found the level of 5-hydroxymethylcytosine of the exon 1_7 GR promoter was three times higher in hippocampal samples from the offspring of Low compared with High-LG mothers.[92] In contrast, 5-methylcytosine-dependent immunoprecipitation revealed no differences across the exon 1_7 GR promoter. These findings suggest that the differences in DNA methylation at this site reflect, in part at least, differences in 5-hydroxymethylcytosine. This conclusion is consistent with the finding that 5-hydroxymethylcytosine is enriched in regions surrounding transcriptional start sites, which are commonly devoid of 5-methylcytosine.[93-95] The involvement of 5-hydroxymethylcytosine may also explain why our earlier studies with the exon 1_7 GR promoter had failed to reveal any increase in the binding of methylated-DNA binding proteins (eg, MeCP-2 or MBD-2) in hippocampus from the offspring of Low LG mothers, since 5-hydroxymethylcytosine does not attract these repressive mediators.[96] Nevertheless, in stem cells most 5-hydroxymethylcytosine-positive genes are not expressed (eg, ref 93) although this is less clear in neurons.[95]

The ability of DNA methylation to regulate the capacity for histone modifications, especially histone acetylation, forms a prominent link between methylation and transcription. The electrostatic bonds formed between the positively-charged histone proteins and their negatively-charged DNA partners demands an active chromatin remodeling process for transcriptional activation.[97,98] Chromatin remodeling is achieved through biochemical modifications to the histone proteins that control chromatin structure and thus genome function. The post-translational modifications to the histones occur through a series of enzymes that bind to the histone tails and modify the local chemical properties of specific amino acids.[98-100] For example, histone acetylation neutralizes the positive charge on the histone tail, opening chromatin and increasing the access of transcription factors to their DNA binding sites. Acetylation commonly occurs at lysine residues, such as the H3K9, and is catalyzed by histone acetyltransferases and reversed by histone deacetylases (HDACs). HDACs remove acetyl groups from histone tails and prevent subsequent acetylation.[99,101] Cytosine methylation attracts repressor complexes comprised of HDACs such that DNA methylation and histone acetylation are usually inversely related. H3K9ac associates with increased transcription and we found increased H3K9ac of the exon 1_7 GR promoter in hippocampus from the adult offspring of High compared with Low LG mothers.[70,83,92] This pattern is similar to maternal effects on hippocampal *Gad1* or *Grm1* expression; in each case decreased DNA methylation within promoter regions associates with increases in both H3K9ac and gene transcription.[87,92,102] Acetylation of lysine 9 on histone 3 (ie, H3K9ac) tends to associate with stably transcribed regions of the genome, which is consistent with the idea of a persistent increase in hippocampal GR transcription in the adult offspring of High LG mothers.

Additional histone modifications, notably histone methylation, influence transcription through indirect pathways that involve a complex array of transcriptional mediators.[98-101,103-106] Multiple lysine and arginine residues on the histone tails are subject to methylation, which is catalyzed by distinct histone methyltransferases and reversed by histone *de*methylases. This process provides a signaling pathway that begins with the activation of the intracellular signals that activate the individual methylating or demethylating enzymes producing a specific epigenetic profile on the histone tails. This process links specific intracellular signals to specific histone methylation marks. The methylation profile of the histone tails is highly variable. Methylation can occur at multiple sites along the histone tails and vary in the level of methylation (mono-, di-, or trimethylation). The resulting profile acts as a "code" for various protein complexes that remodel chromatin and alter transcriptional activity; thus the indirect influence of histone methylation on transcription.

Certain histone modifications covary. An example of relevance here is that of H3K9ac and trimethylation of lysine 4 on histone 3 (ie, H3K4me3). Both marks are generally present at actively transcribed regions of the genome.[105,106] Thus we find increased H3K9ac and H3K4me3 at both regions of the exon 1_7 GR promoter, and the levels of these individual marks are very highly correlated.[92] H3K4me, whether in the mono-, di- or trimethylated state, appears to protect CpG islands against methylation.[107,108] Thus, genome-wide analyses reveal a negative correlation between H3K4me and CpG methylation. The same relation was apparent across the exon 1_7 GR promoter, where the decreased level of DNA methylation was associated with an increased level of

H3K4me3.[92] H3K4me3 targets the chromatin remodeling factor (NURF) and the Yng1 protein in the NuA3 (nucleosomal acetyltransferase of histone H3) complex to genes, thus increasing the level of histone acetylation and transcriptional activation. This process explains the tight correlation between the levels of H3K4me3 and H3K9ac.

These findings suggest that variations in maternal care influence the methylation state of the exon 1_7 GR promoter in hippocampus, regulating NGFI-A binding, GR transcription and HPA stress responses. The effect of CpG methylation on gene expression is, in part, mediated by the recruitment of HDAC-containing repressor complexes. HDAC inhibitors permit chromatin remodeling and transcription factor binding, and may thus liberate the expression of genes from methylation-induced repression. HDAC inhibition also reverses the maternal effects on hippocampal GR expression.[70] Chronic, central infusion of adult offspring of Low LG mothers with the broad spectrum HDAC inhibitor, trichostatin A (TSA),[109] significantly increased H3K9ac, NGFI-A binding to the GR-1_7 promoter, and GR expression to levels comparable to those observed in the offspring of High LG mothers. TSA infusion also eliminated the effect of maternal care on HPA responses to acute stress. These results suggest a direct relation between maternal care, histone acetylation, DNA methylation of the GR-1_7 promoter, GR expression and HPA responses to stress.

These findings suggest that variations in parent–offspring interactions epigenetically "program" hippocampal GR expression and thus the nature of the HPA response to stress. However, subsequent studies reveal effects of early experience on multiple components of the HPA axis, and in each case there is evidence for stable epigenetic programming. In the mouse, prolonged periods of maternal separation alter the methylation state of the promoter for the arginine vasopressin gene (AVP), increasing hypothalamic AVP synthesis and HPA responses to stress.[110] This epigenetic programming of AVP expression in the parvocellular neurons of the paraventricular nucleus involves Ca(2+)/calmodulin kinase-mediated phosphorylation of the methyl-CpG binding domain protein MeCP2 leading to dissociation from its DNA binding site and de-repression of AVP gene transcription. The reduced occupancy of MeCP2 during this early stage of life facilitates the development of hypomethylation at the AVP enhancer, which sustains the de-repressed state of the AVP gene.

Environmental conditions that increased the frequency of pup LG in the rat are associated with decreased paraventricular CRF expression.[66,110-113] Baram and colleagues[114] showed that this maternally regulated decrease in CRF expression is accompanied by an increase in hippocampal GR expression. However, in these studies the decreased hypothalamic CRF expression occurs earlier in development than does the difference in hippocampal GR expression, suggesting that the difference in CRF expression develops independent of GR regulation. This is actually not surprising since hippocampal-mediated negative feedback emerges only about the time of puberty in the rat.[115] "Augmented" maternal care was associated with an increased hypothalamic expression of the transcriptional repressor NRSF and NRSF binding to a 21 bp sequence within the regulatory region (intron) of the *Crh* gene, which encodes for corticotrophin-releasing factor (CRF). In addition to active *Crh* repression, NRSF might also initiate chromatin modification. Korsoi et al[116] then showed that the number of excitatory synapses and the frequency of miniature excitatory synaptic currents onto CRF neurons were reduced as a function of augmented maternal care, as were the levels of the glutamate vesicular transporter vGlut2, A study using a procedure that disrupts the quality of maternal care in the mouse and enhances CRF expression[117] showed enhanced glutamatergic transmission to hypothalamic CRF neurons in the offspring.[118] Since neuronal activity can influence NRSF expression, the stable alteration in excitatory input together with later GR-mediated negative feedback inhibition from the hippocampus might sustain the effect of maternal care on hypothalamic CRF expression and HPA responses to stress.

Maternal regulation of HPA function extends to the level of the pituitary. Maternal separation of neonatal mice produces an enduring hypomethylation of the *Pomc* gene, which encodes for the ACTH pro-hormone, proopiomelanocortin,[119] increased *Pomc* mRNA expression and increased basal and CRF-induced levels of ACTH. Subsequent in vitro studies showed that methylation of this region of the promoter produced an MeCP2-mediated repression of *Pomc* expression. These findings reveal that the quality of postnatal maternal care epigenetically programs gene expression at multiple levels of the HPA axis to regulate both basal and stress-induced activity.

Epigenetic regulation of glucocorticoid receptor expression in humans

We established a translational program focusing on human hippocampus by virtue of the resources of the Québec Suicide Brain Bank (www.douglas.qc.ca/suicide). Approximately a third of individuals who die by suicide have histories of childhood adversity, including childhood sexual and physical abuse, as well as parental neglect. We[65,120] showed decreased hippocampal GR expression in samples from suicide completers with histories of childhood maltreatment compared with controls (sudden, involuntary fatalities). The program is strengthened by a validated forensic interview that establishes developmental history and mental health status. Regression analyses across the samples showed no significant correlations between psychopathology, notably depression and substance disorders, and hippocampal GR expression. Rather the decreased hippocampal GR expression associated with a history of childhood maltreatment. There were no differences in hippocampal GR expression in samples from suicides negative for a history of childhood maltreatment. Instead, the differences in hippocampal GR expression were unique to suicide completers with a history of childhood maltreatment.

Splice variant analysis revealed decreased expression of noncoding exons 1_B, 1_C, 1_F and 1_H in suicides with a history of childhood maltreatment compared with both controls and suicides without a history of maltreatment that correlated with differential DNA methylation patterns between groups in the corresponding exon 1 variant promoters. The exon 1_F sequence is of particular interest as it is the homolog of the rat exon 1_7, is highly expressed in brain and contains an NGFI-A response element.[65,121] Moreover, the exon 1_F sequence shows increased DNA methylation and decreased NGFI-A binding in samples from suicide victims with a history of maltreatment. These findings bear considerable similarity to the maternal effect in the rat and are suggestive of early-environment regulation of the neural epigenome in humans. Decreased expression levels of GR exon 1_B, 1_C, and 1_H transcripts were also associated with alterations in methylation of the respective sequences, with particular sites significantly correlated with expression levels. As expected on the basis of the expression data, the exon 1_B and 1_C regions showed increased methylation at predictive sites uniquely in samples from suicide/maltreatment subjects. However, analysis of the exon 1_H GR promoter yielded an interesting profile that contrasted starkly with that observed for the other exon 1 regions.[120] There was significantly increased DNA methylation of the exon 1_H promoter in hippocampal samples from both controls and suicide victims without a history of maltreatment by comparison to those positive for maltreatment. And the methylation of the exon 1_H promoter was *positively* correlated with hippocampal GR expression. Interestingly, most differentially methylated sites were found within putative transcription factor binding sites. These findings also point to the potential for bidirectional relation between transcription factor binding and transcriptional activity and that of DNA methylation.[104,122]

Forebrain GR activation inhibits HPA activity through tonic negative-feedback inhibition.[123,124] Thus, selective knockdown of GR expression in the corticolimbic system in rodents is associated with increased HPA activity under basal as well as stressful conditions.[125] Conversely, GR overexpression is associated with a dampened HPA response to acute stress.[126] These findings are thus consistent with a working hypothesis that links the early social environment to epigenetic modifications of the GR gene and GR expression, and HPA function.

Interindividual differences in DNA methylation can be tissue- and cell type-specific. Nevertheless, there are now a number of reports of associations between the quality of childhood experience and the methylation status of the exon 1F nuclear receptor (NR)3C1 gene promoter in peripheral cells. Perroud et al[127,128] used peripheral blood lymphocytes to show that childhood maltreatment associates with an increased level of exon 1_F methylation and, importantly, that the methylation status of the promoter was closely correlated with both the frequency and severity of maltreatment.[129] The offspring of mothers exposed to intimate partner violence also show an increased level of methylation of the exon 1_F NR3C1 gene promoter in blood cells.[130] Interestingly, Tyrka et al[131] reported increased methylation of the exon 1F NR3C1 gene promoter in leukocytes that associated with disruption of normal parent–offspring interactions or maltreatment. In this study, and others[132] childhood parental loss was associated with increased methylation of the exon 1F NR3C1 gene promoter (note ref 132 used salivary DNA, which is primarily of

leukocyte origin). Taken together, these studies provide support for the association between the quality of childhood experience and methylation of the NR3C1 gene.

Binder and colleagues[133] provided a comprehensive description of allele-specific demethylation of the GR co-regulator *FKBP5* in adults exposed to childhood trauma. This study demonstrated that polymorphisms in *FKBP5* influence the position of the nucleosome, which when combined with childhood trauma (postulated to increase circulating cortisol) resulted in an increased risk of post-traumatic stress disorder (PTSD). At the molecular level this interaction was associated with stable DNA demethylation, which in turn predicted increased *FKBP5* expression. Since *FKBP5* sequesters activated GR and prevents nuclear binding, the decreased *FKBP5* methylation and increased *FKBP5* expression associated with symptoms of glucocorticoid resistance.

Summary

The results of studies to date are consistent with a working hypothesis that positions epigenetic modifications of genes implicated in HPA function as a mediating process that links the quality of childhood experience to the risk for MDD. There are caveats that limit the degree to which this hypothesis might be applied across the population. Altered HPA activity is apparent only in a subset of MDD patients and it is not clear that this subset is defined by developmental history. It is clear that genotype moderates the impact of adverse childhood experience and of resulting epigenetic modifications (eg, ref 133). A genome-wide study of interindividual variation in DNA methylation showed that the vast majority of variably-methylated regions were best explained by a gene x environment interaction.[134] Unfortunately, with only a few exceptions, preclinical studies have yet to establish models of gene x environment interactions. Likewise, preclinical studies have yet to widely capitalize on the finding that adverse childhood experience alters the response to antidepressant medications.[15] This finding provides an obvious basis for studies of the mechanism by which developmental history might contribute to treatment resistance in MDD. Certainly one question of interest for the development of effective patient stratification is whether epigenetic marks associated with childhood adversity might identify individuals that are resistant to medications. We recently addressed this issue in PTSD patients and provide evidence suggesting that variation in the methylation of the exon 1_F GR promoter associated with PTSD symptoms and predicted treatment outcome.[135-137] One challenge for future research is that of integrating our knowledge of the importance of childhood experience into treatment models. ❑

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La adversidad en la niñez temprana y la programación epigenética de la función hipotálamo-hipófisis-adrenal

Se revisan estudios en humanos y en especies no humanas que examinan la hipótesis acerca de los mecanismos epigenéticos, especialmente los que afectan la expresión de genes involucrados en las respuestas al estrés, y que median en la asociación entre la adversidad en la niñez temprana y el riesgo posterior de depresión. Los resultados de los estudios aportan evidencia consistente con la idea que la adversidad social, especialmente aquella que involucra interacciones entre los padres y los hijos, altera el estado epigenético de una gran cantidad de genes, lo que se traduce en la regulación de la función hipotálamo-hipófisis-adrenal. También se abordan los desafíos de los estudios a futuro, incluyendo aquellos de traducción de las investigaciones epigenéticas en un progreso en la terapéutica.

Adversité précoce dans la vie et programmation épigénétique de la fonction hypothalamo-hypophyso-surrénalienne

Nous analysons les études pratiquées chez l'homme et chez d'autres espèces animales examinant l'hypothèse selon laquelle des mécanismes épigénétiques, en particulier ceux influant sur l'expression des gènes impliqués dans la réponse au stress, interviennent comme médiateurs dans l'association entre l'adversité survenant tôt dans l'enfance et le risque ultérieur de dépression. Les données de ces études concordent avec l'idée que l'adversité sociale, notamment celle qui concerne les interactions parents-enfants, modifie l'état épigénétique et l'expression d'un large éventail de gènes dont les produits régulent la fonction hypothalamo-hypophyso-surrénalienne. Nous abordons les difficultés des études à venir, dont celle de la traduction des études épigénétiques en améliorations thérapeutiques.

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