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PERSPECTIVE

Epigenetic programming by stress and glucocorticoids along the human lifespan

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Psychosocial stress triggers a set of behavioral, neural, hormonal, and molecular responses that can be a driving force for survival when adaptive and time-limited, but may also contribute to a host of disease states if dysregulated or chronic. The beneficial or detrimental effects of stress are largely mediated by the hypothalamic-pituitary axis, a highly conserved neurohormonal cascade that culminates in systemic secretion of glucocorticoids. Glucocorticoids activate the glucocorticoid receptor, a ubiquitous nuclear receptor that not only causes widespread changes in transcriptional programs, but also induces lasting epigenetic modifications in many target tissues. While the epigenome remains sensitive to stressors throughout life, we propose two key principles that may govern the epigenetics of stress and glucocorticoids along the lifespan: first, the presence of distinct life periods, during which the epigenome shows heightened plasticity to stress exposure, such as in early development and at advanced age; and, second, the potential of stress-induced epigenetic changes to accumulate throughout life both in select chromatin regions and at the genome-wide level. These principles have important clinical and translational implications, and they show striking parallels with the existence of sensitive developmental periods and the cumulative impact of stressful experiences on the development of stress-related phenotypes. We hope that this conceptual mechanistic framework will stimulate fruitful research that aims at unraveling the molecular pathways through which our life stories sculpt genomic function to contribute to complex behavioral and somatic phenotypes.

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INTRODUCTION

Psychosocial stress triggers a set of behavioral, neural, hormonal, and molecular responses that can be a driving force for survival when adaptive and time-limited, but may also contribute to a host of disease states if dysregulated or chronic. A primary effector of the stress response is the hypothalamic-pituitary-adrenal axis, which is set into motion by hypothalamic secretion of corticotropin-releasing hormone and arginine vasopressin in the brain and culminates in adrenal secretion of glucocorticoids in the systemic circulation. At the molecular level, the effects of hypothalamic-pituitary-adrenal axis activation and glucocorticoid signaling are largely mediated by the glucocorticoid receptor (GR), a ubiquitous ligand-dependent transcription factor that drives the genomic actions of glucocorticoids in essentially every body tissue.

While the genomic effects of time-limited stress and glucocorticoid exposure can be largely ascribed to the transcriptional activity of the GR, the lasting consequences of acute, repetitive, or chronic stress are obscure and subject to scientific enquiries. Can exposure to an acute stressor influence genomic regulation long after cessation of the stimulus? Are there distinct periods in life during which genomic regulation may be particularly sensitive to the impact of stress exposure? And, may the effects of concurrently multiple or repetitive stressors over time accumulate and sculpt genomic function, contributing to the development of distinct phenotypes? This perspective addresses these questions and highlights evidence supporting epigenetic regulation as a crucial mechanism that underlies the lasting consequences of stress exposure on genomic function and stress-related phenotypes along the lifespan.

A BRIEF INTRODUCTION TO 'EPIGENESIS'

Epigenetics—an Aristotelian composite term derived from the Greek prefix 'epi-', meaning 'over', and the word 'genetics'—encompasses, in its modern use, an ever-growing repertoire of biological processes that regulate gene expression without changing the underlying DNA sequence. These processes include covalent DNA modifications, such as DNA cytosine methylation and hydroxymethylation, posttranslational histone modifications, noncoding RNAs, and changes in higher-order chromatin conformation.[1] Epigenetic mechanisms are well known for their instrumental role in cell differentiation, but they also act as a molecular interface between the genome and the environment. The latter function stems from the epigenome's ability to dynamically respond to environmental exposures, including life stress, and to adapt genomic function to distinct environments.[2] Despite this dynamic responsivity, certain epigenetic signatures can be stabilized during life and even across generations,[3–5] having lasting consequences on genomic function and contributing to the development of complex phenotypes. Consequently, epigenetic regulation represents a prime mechanism for examining the impact of stressful experiences on behavioral and somatic phenotypes.

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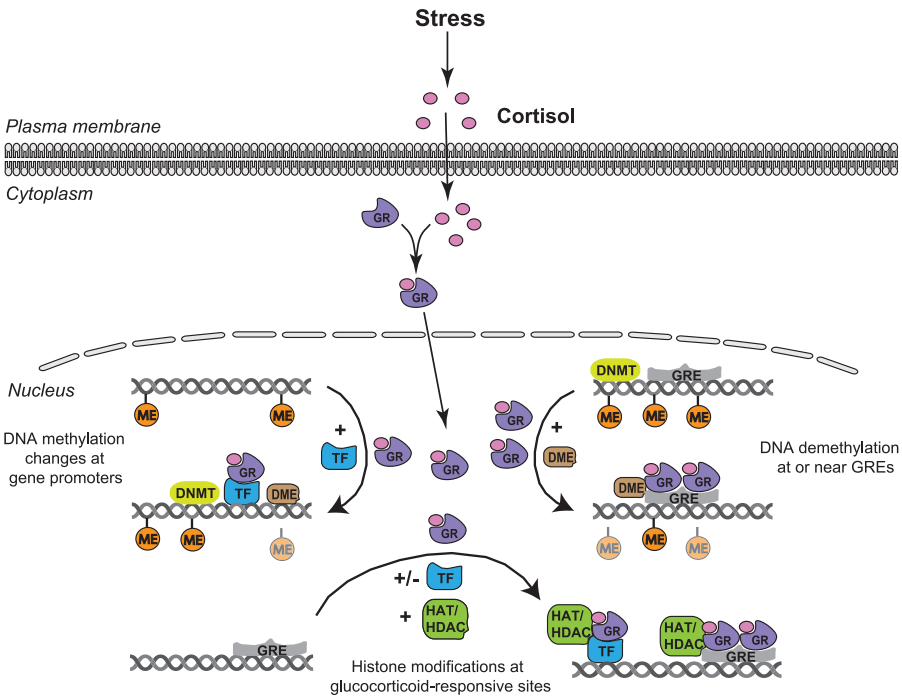

Figure 1. Simplified scheme depicting the mechanisms that underlie the effects of stress and glucocorticoids on the epigenome. Stress exposure leads to peripheral secretion of glucocorticoids (cortisol in humans), which enters the cell and binds to the glucocorticoid receptor (GR). The GR acts as a transcription factor that translocates to the nucleus and induces a host of epigenetic modifications. DME, demethylating enzymes; DNMT, DNA methyltransferases; GRE, glucocorticoid response element; HAT, histone acetyltransferases; HDAC, histone deacetylase; ME, CpG methylation; TF, transcription factors other than the GR.

GLUCOCORTICOID SIGNALING AS DRIVER OF EPIGENETIC MODIFICATIONS

The GR primarily acts as a ligand-activated transcription factor, which regulates gene transcription in a tissue-specific manner either by directly binding to glucocorticoid response elements (GRE)-conserved DNA sequences in glucocorticoid-responsive genes- or by transactivating or transrepressing other transcription factors via GRE-dependent or -independent mechanisms.[6] Beyond these rapid effects on gene transcription, however, accumulating evidence shows that glucocorticoid signaling can also cause epigenetic modifications via a number of distinct mechanisms (Figure 1). Glucocorticoids can rapidly induce methylation changes in cytosine-guanine dinucleotides (CpG) of the DNA (DNA methylation), most notably global decreases in methylation, as well as local demethylation of CpGs located at or near GREs.[7–13] Studies have also documented glucocorticoid-induced DNA hypermethylation in some sites, such as in gene promoter CpGs,[14] though this methylation change may be less common than demethylation.[10] Although the mechanisms underlying these effects have not been adequately explored, glucocorticoids increase the expression of enzymes involved in active demethylation, such as the Tet family of 5-methylcytosine dioxygenases (TET),[12,15] and decrease the expression and activity of the maintenance methyltransferase DNMT1[16,17] and the levels of the *de novo* methyltransferase DNMT3a.[12] Notably, the effects on DNA methylation may be contingent upon the specific developmental and genomic context. For example, prenatal stress induces both TET1 and DNMT1 in a tissue-specific manner,[18,19] suggesting that methylation and demethylation processes are in some cases activated concurrently, promoting dynamic changes in the methylome. Despite this dynamic landscape, some methylation changes are stabilized in select genomic regions through as yet undetermined mechanisms. Glucocorticoid signaling can also induce histone modifications, such as changes in histone methylation and acetylation.[6] This can occur at sites of direct GR binding but also by recruitment of other transcription factors; for example, the GR interacts with the RelB/p52 transcription factor and recruits the histone acetyltransferase CREB-binding protein and histone deacetylase-1 at target promoter sites, promoting dynamic acetylation and deacetylation of histone 3 lysine 9.[20,21] Emerging evidence also supports the role of glucocorticoid signaling in the regulation of several microRNAs (miRNAs), such as miR-29a, miR-124 and miR-218.[22,23] Finally, glucocorticoids can promote dynamic chromatin remodeling that may alter accessibility of GR-binding sites to the transcriptional machinery.[6]

Importantly, the effects of GR activation on the epigenome are characterized by tissue specificity,[8,24] which may be directed by the cell-type-specific binding of the GR to target genomic sites[6,8] and may last long after removal of the stimulus. The enduring effects of GR activation on the epigenome have been supported by studies in mice showing that glucocorticoid-induced changes in DNA methylation persist several weeks after cessation of glucocorticoid action.[14,25] In line with this observation, and, as discussed below, stress exposure, which may influence glucocorticoid signaling, can also result in lasting epigenetic modifications that persist throughout life and even across generations. Such long-lasting changes have been observed for DNA methylation,[3,10,26] but also for other components of the epigenetic machinery, including histone modifications and the regulation of specific miRNAs.[27–29]

In the following sections, we propose and separately examine two key principles that may govern the epigenetic effects of stress and glucocorticoids along the lifespan: first, the presence of distinct life periods during which the epigenome shows heightened plasticity in its response to stress and to aberrant glucocorticoid signaling; and, second, the potential of stress-induced epigenetic changes to accumulate throughout life both in

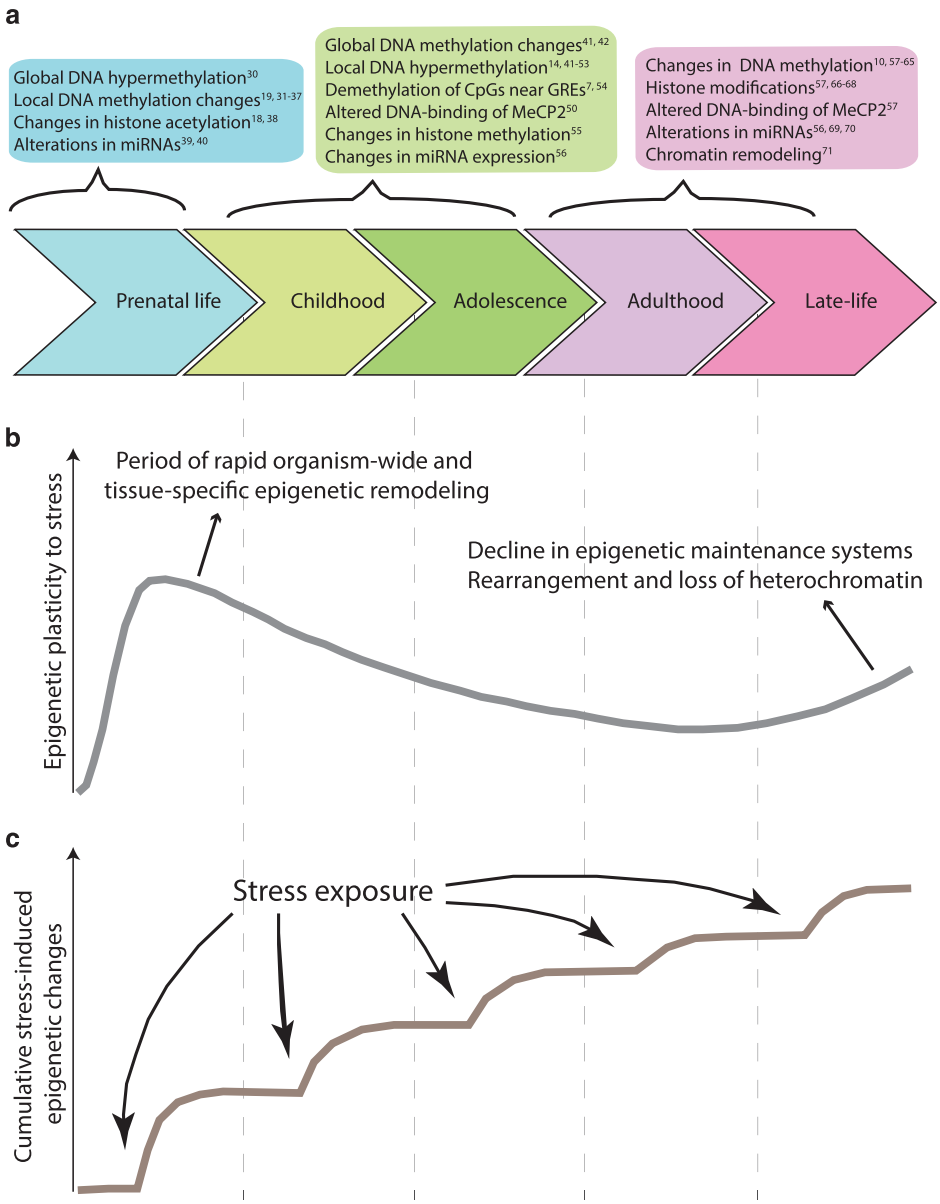

Figure 2. Conceptual model depicting the epigenetic effects of psychosocial stress along the lifespan. (**a**) Arrow of mammalian development with the distinct stages and the stress-induced epigenetic modifications that have been described for each stage. Relevant references are cited in the text inserts. Dotted lines demarcate developmental stages across figure panels. (**b**) Proposed model for the epigenetic plasticity to stress across developmental stages. While the epigenome remains sensitive to stress exposure throughout life, increased plasticity may be observed in periods of rapid epigenetic remodeling, such as prenatally and in the first years of life, and when there is a decline in the epigenetic maintenance systems, such as in late-life. The curve depicts the global epigenetic plasticity, though there may be select genomic sites that remain sensitive during periods of low global plasticity. (**c**) The potential accumulation of stress-induced epigenetic changes through repetitive exposure to stressors along the lifespan. The impact on the epigenome may be stronger and lasting when stressors occur during periods of high plasticity. For illustration purposes, exposure to a single stressor is shown for each developmental stage. GRE, glucocorticoid response element.

select chromatin regions and at the genome-wide level via the effects of the GR at susceptible genomic sites.

PLASTICITY PERIODS FOR STRESS-INDUCED EPIGENETIC CHANGES

Although a detailed account of all studies examining the epigenetics of psychosocial and/or physical stress is beyond the scope of this article, we highlight work in both humans and rodents showing that stress exposure in various phases of the mammalian life, occurring as early as prenatally and during childhood, but also later in adolescence and adulthood, can result in lasting epigenetic modifications (Figure 2a). *In utero* exposure to stress can induce profound changes in DNA methylation, including global hypermethylation[30] and region-specific hypermethylation or demethylation,[19,31–37] as well as alterations in histone acetylation and in the expression of several specific miRNAs.[18,38–40] Stress exposure during childhood and adolescence can similarly lead to lasting changes in DNA methylation, including tissue-specific global hypo- or hypermethylation,[41,42] hypermethylation of select CpGs in promoter and other regulatory DNA regions,[14,41–53] and demethylation of CpGs located near

GREs,[7,54] as well as alterations in DNA-binding of the methyl CpG binding protein 2 (MeCP2),[50] histone methylation marks,[55] and miRNA expression.[56] Finally, stressors occurring during adulthood, most notably chronic stress exposure, can induce a wide range of epigenetic modifications, including hypo- or hypermethylation of promoter and age-related CpGs,[10,57–65] changes in histone acetylation and methylation,[57,66–68] altered DNA-binding of MeCP2,[57] alterations in miRNAs,[56,69,70] and chromatin remodeling.[71]

While these studies show that stress exposure in essentially every stage of the mammalian life can set into motion the epigenetic machinery, there is a paucity of studies comparing multiple stressors that occur at distinct life stages and their potential to induce, modulate, and/or stabilize such modifications along the lifespan. In a study examining both childhood and adulthood stress in humans, lasting demethylation of select CpGs located within or in the vicinity of GREs of the stress-responsive *FKBP5* gene were shown to result only from childhood trauma,[7] indicating that stress exposure early in development may be essential for stabilization of this epigenetic signature. Supporting this finding mechanistically, the same stress-susceptible *FKBP5* CpGs were demethylated by exposure to the glucocorticoid receptor agonist dexamethasone (DEX) during the proliferation and differentiation stages of hippocampal progenitor cells, whereas demethylation was not observed if DEX exposure occurred after cell differentiation.[7] In a separate study examining the effects of lifetime stress exposure on epigenetic aging measured by a composite CpG methylation-based age predictor,[72] life stressors more strongly influenced epigenetic aging in older as compared to younger individuals,[10] suggesting enhanced sensitivity of the epigenome to the 'wear and tear' effects of stress with advancing age. Thus, similarly to observations that diet and other environmental exposures can induce certain epigenetic changes only when occurring in critical stages of an organism's development,[73–75] we propose the presence of distinct 'sensitive' or 'high-plasticity periods,' most strikingly early in development, but also in older ages, during which stressors of certain type and duration can have pronounced and lasting effects on the epigenome (Figure 2b). Despite this global fluctuation in epigenetic plasticity, there may be select regions that remain sensitive to stressors throughout life or during periods of low global plasticity. For example, stressor timing at different developmental stages can determine which CpG sites will be affected,[49,51,76] with some sites being more susceptible to adulthood than childhood stressors.[76] Thus, the plasticity of the epigenome to stressors should be examined both at the global and region-specific levels.

Several mechanisms could explain the life course changes in the epigenetic plasticity to stress. Global increases in the sensitivity of the epigenome to environmental insults may coincide with periods of rapid epigenetic remodeling, such as during the global reprogramming of DNA methylation in the very early prenatal life.[74] Beyond such organism-wide epigenetic changes, tissue-specific fine-tuning of epigenetic marks occurs during early development, but also throughout life, and guides tissue differentiation and adaptations through the communication of cells to the internal and external milieu. Rapid epigenetic rearrangements can promote susceptibility of certain genomic regions to GR binding,[77] rendering the genome more sensitive to aberrations in glucocorticoid signaling. Aberrant glucocorticoid levels in early life can occur, for example, in response to chronic stress that deregulates the circadian and ultradian rhythmicity of glucocorticoid secretion,[78] as well as *in utero* by maternal exposure to psychosocial stress and deregulated secretion of maternal cortisol, which strongly correlates with cortisol levels in the fetal circulation.[79] At the other end of the life spectrum, pronounced chromatin remodeling is observed with aging, which is associated with rearrangement and loss of heterochromatin coupled with extensive changes in histone marks.[80] Furthermore, aging results in telomere attrition, which further contributes to genomic instability. A body of evidence has linked telomere shortening with life stress exposure,[81,82] an effect that in interaction with other stress-induced changes might increase susceptibility of the epigenome to stress. Finally, aging could increase the epigenetic plasticity to stress through the synergistic effects of stressors with other environmental factors and with the age-related decline in DNA repair and maintenance mechanisms. The protective role of epigenetic maintenance systems has been demonstrated for other environmental exposures; for instance, aging downregulates DNMT1,[83] increasing susceptibility of the methylome to dietary deficits.[84]

In conclusion, the epigenome remains sensitive to stressors across all stages of mammalian life, but it may be particularly susceptible during periods of rapid epigenetic remodeling or periods of inadequate functioning of epigenetic maintenance systems, such as in early development or at advanced age. As we discuss below, this epigenetic plasticity facilitates the accumulation of tissue-specific epigenetic modifications induced by repetitive exposure to stressors along the lifespan, eventually contributing to the development of complex behavioral and somatic phenotypes.

CUMULATIVE EPIGENETIC EFFECTS OF STRESS ALONG THE LIFESPAN

The potential of epigenetic modifications to last long after environmental encounters implies that stress-induced epigenetic signatures, embedded in susceptible genomic sites during distinct windows of epigenetic plasticity, may accumulate as life progresses and stressful experiences accrue. This hypothesis is supported by a limited number of studies in humans showing that increasing levels of cumulative life stress or higher numbers of stressors across multiple life stages induce stronger effects on the methylome. For example, increasing numbers of life stressors until the age of 15 years are associated with allele-specific increases in CpG methylation of the promoter of the serotonin transporter gene,[51] while higher numbers of stressors until early adulthood predict lower methylation of the promoter of the catechol-O-methyltransferase gene in a dose-response manner.[65] Such cumulative effects of stress on specific CpG sites could be mediated by chronic exposure to aberrant glucocorticoid levels. This has been demonstrated in mice for glucocorticoid-sensitive CpGs within the *FKBP5* gene, where the extent of demethylation correlates with the total burden of glucocorticoid exposure.[85] Beyond the cumulative effects at specific CpGs, epigenetic modifications may accumulate at a systems level via the impact of stress on multiple sites. Supporting this hypothesis, cumulative lifetime stress, but not recent stress alone, was shown to accelerate epigenetic aging.[10] The acceleration of epigenetic aging may be driven by GR binding at or near age-related CpGs, which colocalize with GREs and dynamically respond to glucocorticoid exposure. Notably, the effect of lifetime stress was blunted in individuals exposed to higher levels of childhood maltreatment,[10] suggesting that repetitive stressors may interact in complex ways, which either promote or attenuate the susceptibility of the epigenome to subsequent life stressors. We propose that stress-induced epigenetic effects accumulate along the lifespan -both in distinct genomic regions and at a systems level- at a rate contingent upon the stage-and context-specific epigenetic plasticity (Figure 2c). Eventually, these cumulative effects may contribute to epigenetic remodeling and sculpt genomic function along the lifespan.

The mechanisms underlying the cumulative effects of stress and determining which epigenetic signatures will be stabilized, reversed, or propagated in response to lifetime stressful experiences are unknown. These mechanisms could involve synergistic actions of several components of the epigenetic machinery that

are differentially regulated by stressors of different type, timing, and duration. For example, opposite changes in histone 3 methylation and histone 4 acetylation marks can be induced by chronic vs acute stressors.[86,87] Initial exposure to a stressor could also prime certain components of the epigenetic machinery that are then activated by subsequent stressors. Stress-induced priming has been demonstrated for changes in histone acetylation and in the regulation of several critical pathways, such as the ERK/MAPK pathway.[87,88] Such priming could involve the cooperative induction of different epigenetic changes that show extensive crosstalk; for example, histone modifications may direct DNA methylation changes and *vice versa*.[89] Future studies may uncover these intriguing mechanisms.

CLINICAL AND TRANSLATIONAL IMPLICATIONS

The principles outlined above show striking parallels with key processes that may govern the development of stress-related disorders. Similarly to the existence of 'plasticity periods' for the effects of stress on the epigenome, stressful experiences strongly contribute to behavioral and psychiatric phenotypes when they occur during 'susceptibility windows,' such as in early life.[2,90,91] Repetitive or cumulative stress exposure along the lifespan, through the potential accumulation of stress-induced epigenetic changes and their impact on genomic function, may contribute to the development of diverse behavioral or nosologic phenotypes, including general mental health, major depression, and psychosis.[92–94] The long-term establishment of stress-induced epigenetic changes and the development of stress-related phenotypes may be contingent upon the timing, duration, intensity, and burden of lifetime stress exposure, as well as the genetic predisposition of the individual.

CONCLUDING REMARK

In this article, we present a conceptual mechanistic framework for the epigenetic effects of stress along the lifespan, focusing on the GR as a prime example mediator of these effects. We hope that this framework will stimulate fruitful research that aims at unraveling the molecular mechanisms via which our life stories, through stressful events that happen at sensitive time periods and accrue throughout life, can sculpt our epigenome and shape genomic function. Because psychosocial stress is an independent risk factor for several diseases that are currently the leading causes of morbidity and mortality worldwide,[95–101] elucidating the molecular mechanisms that shape this risk could open new opportunities for the prevention and treatment of stress-related pathologies. Intriguingly, pharmaceutical modulation of several stress-induced epigenetic modifications has been achieved in preclinical studies. Examples include the rescue of maltreatment-induced DNA hypermethylation of genes encoding neurotrophic factors by the use of methyltransferase inhibitors,[43] the prevention of isolation stress-induced CpG hypermethylation by antagonizing GR activity,[14] and the reversal of maternal separation-induced decreases in methylation of histone 3 lysine 9 with the use of sodium valproate.[55] Nevertheless, the exact circumstances and tissues in which the modulation of epigenetic signatures will be feasible or desirable remain unclear. While certain stress-induced epigenetic changes may contribute to the pathogenesis of aberrant phenotypes, others may be essential for successful adaptation to stress and survival in ever-changing environments.[2] Dissecting the functionally divergent epigenetic changes induced by stressors of different type, timing, and duration will be a formidable task for future studies, but it will provide invaluable insights into the pathogenesis of stress-related disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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