

## Genes learn from stress

### How infantile trauma programs us for depression

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**E**arly-life stress induces persistent memory traces on our genes and programs the life-long risk for depression. Epigenetic marking of the arginine vasopressin (*AVP*) gene by early-life stress in mice underpins sustained expression and increased hypothalamic-pituitary-adrenal axis activity, triggering endocrine and behavioral alterations that are frequent features in depression. This epigenetic memory evolves in two steps coordinated by the epigenetic reader and writer MeCP2. While early derepression of *AVP* is driven by neuronal activity causing  $Ca^{2+}$ /calmodulin kinase-dependent phosphorylation and dissociation of MeCP2, subsequent hypomethylation at the *AVP* enhancer gradually develops to sustain derepression. In a vicious circle MeCP2 occupancy uncouples from the initial stimulus and leads to the hard-coding of early-life experience at the level of DNA methylation. The sequential order of these events demarcates the transition from a preliminary to a persistent, possibly irreversible, epigenetic memory and thus defines a critical time window for the timely therapy of severe trauma.

#### Farewell to the Biological Karma

While some humans will develop depression on exposure to severe trauma, others suffering from the same experience, will remain unaffected.<sup>1</sup> Such diversity has been ascribed so far to differences in our genetic inheritance. The genome has been widely viewed as immutable master plan that has been laid down with the inception of our lives. By oversimplification

the public debate has portrayed man as a marionette of his genes<sup>2</sup>—this fatalistic choreography has however to be urgently revised in the light of recent progress in the field of epigenetics.<sup>3-5</sup> In actual fact, our heritage undergoes a steady process of transitions. Not only do genes shape man but also does man mold the action of his genes.<sup>6</sup>

#### A Molecular Archive

How genes and environmental influences interact with each other is of utmost interest especially in Psychiatry. It has been more than 100 years since Sigmund Freud described the influence of traumatic experiences on the development of depression and major disorders. Many epidemiological studies have unequivocally confirmed this concept. However, the molecular mechanisms through which these experiences leave deep traces in our brain are only just beginning to become unraveled.

With the decoding of the human genome at the turn of the millennium, scientists pinned their hopes, above all, on the identification of gene variants coming along with an elevated risk of contracting a disease. However, it is becoming increasingly apparent that genetic factors and environmental influences are not independent of each other and that acquired pieces of information provide instructions how the genetic material is used.<sup>3,4</sup> Whether or not the risk manifests as an overt psychiatric disorder then seems to depend on how genes and environment conspire.<sup>7</sup>

Cells of a multicellular organism are genetically identical but structurally and functionally distinct owing to the

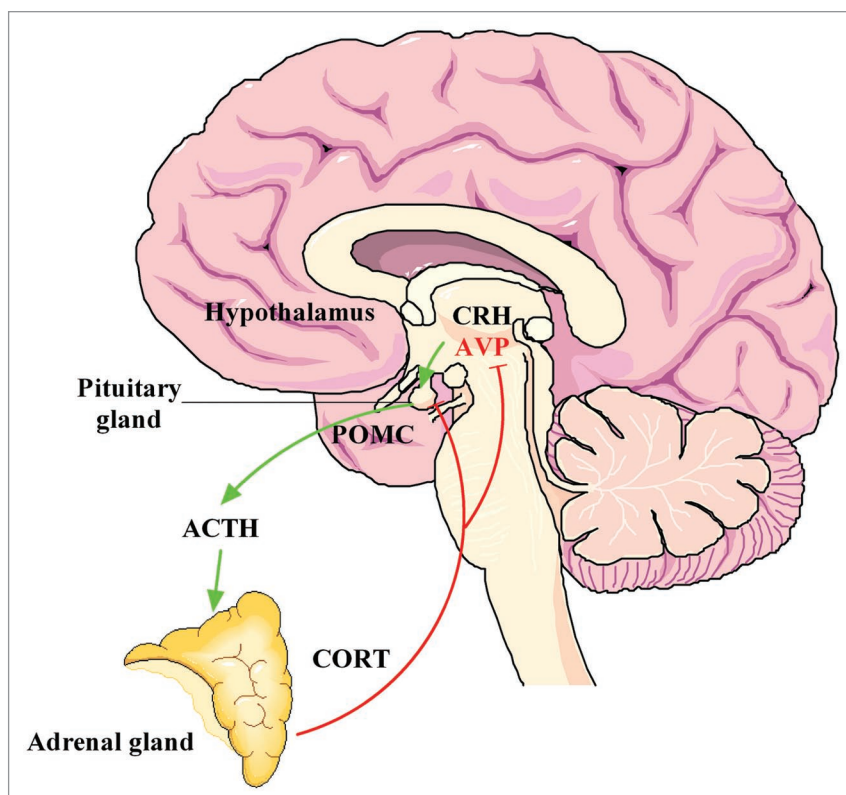
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**Figure 1.** The hypothalamo-pituitary-adrenal (HPA) stress axis. The neuropeptides corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) are expressed in the parvocellular neurons of the hypothalamic nucleus paraventricularis. The joint release of CRH and AVP into the portal blood vessels leads to potent stimulation of anterior pituitary ACTH secretion and POMC transcription. ACTH is derived from the POMC precursor mRNA and stimulates in turn secretion and synthesis of the stress hormone corticosterone by the adrenal glands. The activation effects of the HPA axis are counteracted by the inhibitory effects of corticosterone on the hypothalamus and pituitary and serve to attenuate and determinate the stress response.

## Echoes of the Past

Developmental plasticity defines the ability of an organism to develop in various ways depending on the particular environment or settings.<sup>10</sup> Many lines of evidence, including epidemiological data and data from extensive clinical and experimental studies, indicate that early-life events play a powerful role in influencing later susceptibility to diabetes, cardiovascular and psychiatric diseases.<sup>11,12</sup> During critical periods of prenatal and postnatal mammalian development, nutrition, stress and other stimuli can influence developmental pathways and thereby induce permanent structural and regulatory changes predisposing to disease susceptibility.

The biological mechanisms underlying this developmental origin of human disease are still poorly understood. Developmental plasticity requires stable modulation of gene expression, and this appears to be mediated, at least in part, by epigenetic processes such as histone modifications and DNA methylation. Thus, epigenetic mechanisms determine not only constitutive gene expression but also the potential to appropriately alter gene expression in response to extracellular signals. Early-life induced programming of gene activity can act as a pacemaker for disease susceptibility if the tailored epigenome does not match the environment to which an organism is exposed through lifetime.

## Stressed for Life

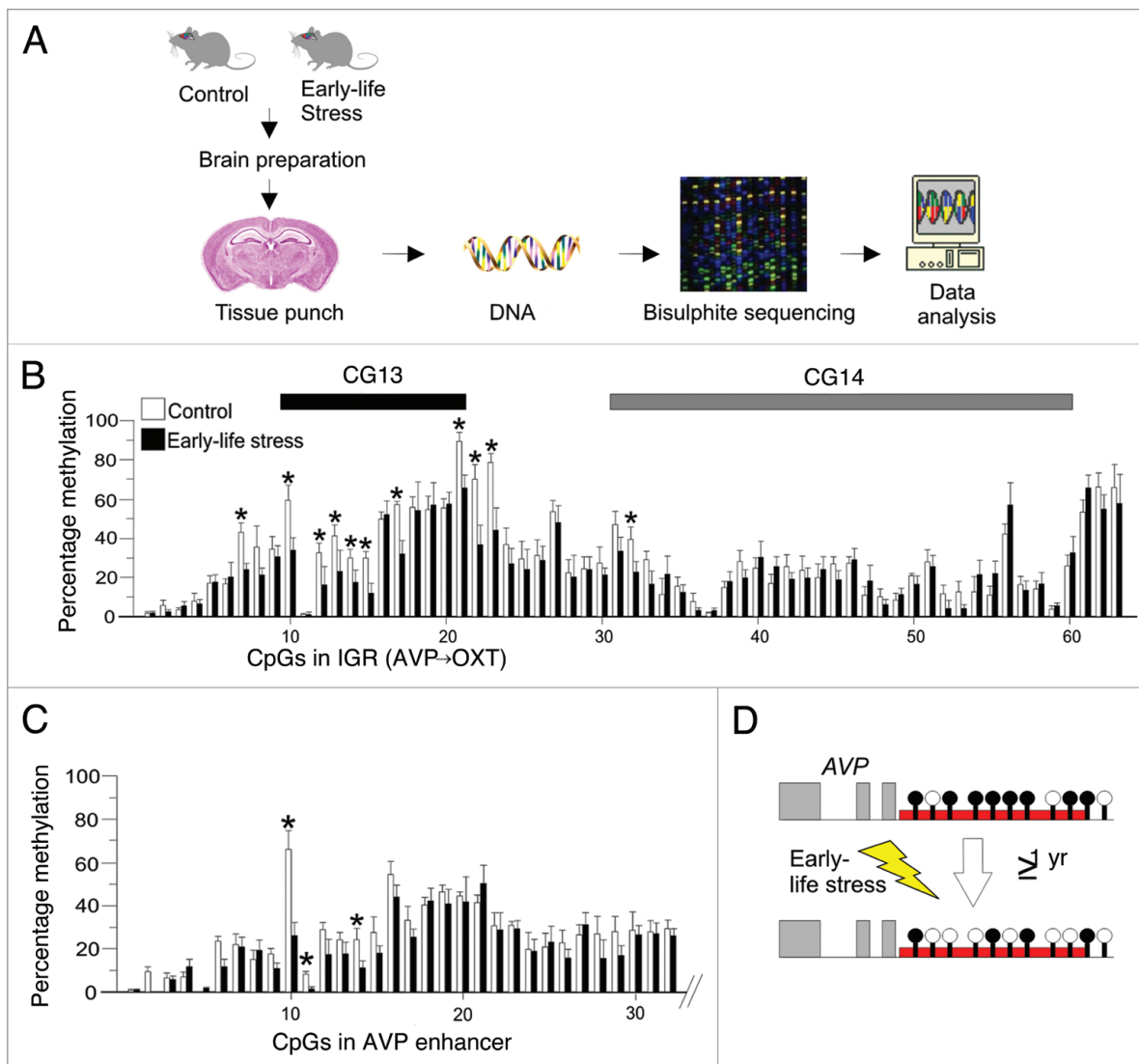
Early-life stress has long lasting effects on the brain and is thought to increase the vulnerability to stress-related disorders such as depression and anxiety.<sup>13,14</sup> Periodic infant-mother separation during early postnatal life is one of the most commonly used paradigms for inducing early-life stress in rodents. It is characterized by lifelong elevated glucocorticoid (GC) secretion (cortisol in man, corticosterone in mice), heightened endocrine responsiveness to subsequent stressors, and disruption of the homeostatic mechanisms that regulate the activity of the hypothalamo-pituitary-adrenal (HPA) axis (Fig. 1), all of which are considered to be pathogenic factors in disorders of mood

differential expression of genes. Many of these differences in gene expression arise during development and are subsequently retained through mitosis. The term epigenetics is now commonly used to describe the study of stable alterations in gene expression potential that arise during development, differentiation and the influence of the environment.<sup>8</sup>

The epigenome refers to the ensemble of coordinated epigenetic marks in the genome that govern the accessibility of the DNA to the machinery driving gene expression. Modification of histones that package the DNA and methylation of cytosines in cytosine-guanine (CpG) dinucleotides represent the best-understood epigenetic marks.<sup>3,4</sup> DNA methylation is heritable during cell division and typically underpins processes that demand sustained control of gene expression as exemplified by selective gene-silencing during cell

differentiation, parent-of-origin specific silencing (genomic imprinting) and suppression of transposable mobile elements.

Evidence that dietary or pharmacological interventions have the potential to modify epigenetic states has provided additional impetus for elucidating the epigenetic basis of disease, including disorders of the brain.<sup>9</sup> In this regard, the relevance of epigenetic mechanisms in facilitating adaptation of an organism to changing environments through alterations in gene expression has gained appreciation in the field of psychiatry. If cells are networked by their epigenome to the environment, DNA methylation may be an attractive mechanism to stably record past life experiences. What are then the specific settings and mechanisms that allow experience-dependent stimuli to couple to DNA methylation and leave a trace on our genes?

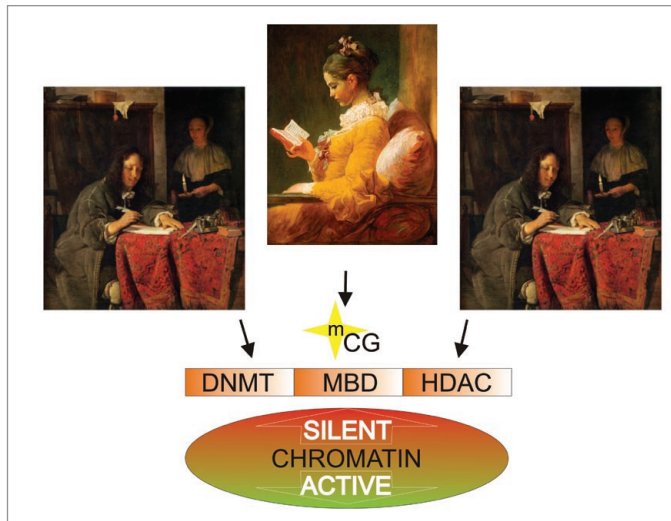


**Figure 2.** Early-life stress induced epigenetic programming of *AVP*. (A) Experimental flow chart. While control mice were grown up in the constant presence of the mother, early-life stress treated mice were separated from postnatal day 1–10 for 3 hr daily. Brain tissues from control and early-life stress treated mice were dissected to prepare punches of the nucleus paraventricularis containing parvocellular, *AVP* expressing neurons. DNA was isolated, purified and bisulfite treated. Methylation profiles were assembled from single clone sequence reads. (B) Early-life stress induced hypomethylation of the *AVP* gene. The downstream region of the *AVP* gene containing two adjacent CpG-islands (CGI3, CGI4) is shown. *AVP* expression is controlled by an enhancer mapping to CGI3. While early-life stress induced hypomethylation of multiple CpG residues at CGI3 in 6-week old mice, no effect was observed at CGI4. (C) Early-life stress induced *AVP* hypomethylation was maintained in 3-month (not shown) and 1 year old mice (C) and centered on the enhancer. (D) Scheme of early-life stressed induced programming of *AVP*. Persistent hypomethylation of the downstream enhancer region leads to sustained *AVP* expression.

and cognition.<sup>13–15</sup> The neural control of GC secretion involves two hypothalamic neuropeptides, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) which act synergistically to stimulate adrenocorticotropin (ACTH) secretion from the pituitary.<sup>16</sup> ACTH, in turn, stimulates synthesis and secretion of GC by the adrenals (Fig. 1). This top-down regulation of adrenocortical activity is normally counteracted through the negative feedback actions of GC at the

pituitary, hypothalamic and hippocampal levels.<sup>17</sup> Abundant evidence links AVP and CRH to mood and cognitive behaviors, making their receptors the targets of novel psychopharmacological agents.<sup>18</sup> In addition to playing an important role in the postnatal development and functional maturation of the pituitary-adrenal axis, AVP potentiates the actions of CRH under circumstances that demand sustained activation of the pituitary and adrenal glands.<sup>16,19</sup>

A recent study<sup>20</sup> showed that early-life stress in mice (daily 3-hour separation of mouse pups from their mother during postnatal day 1–10) caused persistent epigenetic marking (hypomethylation) of a key regulatory region of the *AVP* gene in the hypothalamic nucleus paraventricularis (PVN) (Fig. 2). DNA methylation is interpreted by a family of methyl CpG-binding domain (MBD) proteins comprising MeCP2, MBD1, MBD2, MBD3 and MBD4, the first



**Figure 3.** Epigenetic readers and writers. DNA methylation at CpG dinucleotides ( $mCG$ ) is interpreted by a family of methyl CpG-binding domain (MBD) proteins comprising MeCP2, MBD1, MBD2, MBD3 and MBD4. These epigenetic readers (except MBD4) can serve as an epigenetic platform upon which synergistic crosstalk between histone deacetylases (HDAC) and DNA methyltransferases (DNMT) is played out to confer transcriptional repression and gene silencing. Specific signals can trigger reductions in MBD occupancy and derepress gene activity in the short term. Additionally, MBD dissociation can initiate an erosion of DNA methylation at the respective recognition site, precluding MBD occupancy in the long term, and leading ultimately to sustained gene expression.

three of which couple DNA methylation to transcriptional repression (Fig. 3).<sup>3,21</sup> Early-life stress induced hypomethylation centered on high-affinity context-dependent DNA binding sites for MeCP2,<sup>22</sup> which mapped to the downstream *AVP* enhancer region. Early-life stress responsive CpG residues (dubbed methylation landmarks) were targeted specifically by MeCP2 and were spared from occupancy by other MBDs.<sup>20</sup>

These epigenetic events were accompanied by persistent upregulation of *AVP* mRNA expression in the parvocellular subdivision of the PVN and underpinned sustained hyperactivity of the HPA axis. The early-life stress induced endocrine phenotype lasted for at least 1 year following the initial adverse event. In addition, early-life stress treated mice showed reduced stress-coping ability and memory deficits; these neuroendocrine and behavioral alterations are frequent features in depression.<sup>13,14</sup> Importantly, treatment with an *AVP* V1b receptor antagonist reversed the mice's increased stress responses and impaired memory, indicating a central role for *AVP* in the early-life stress phenotype. Epigenetic marking of the methylation landmarks

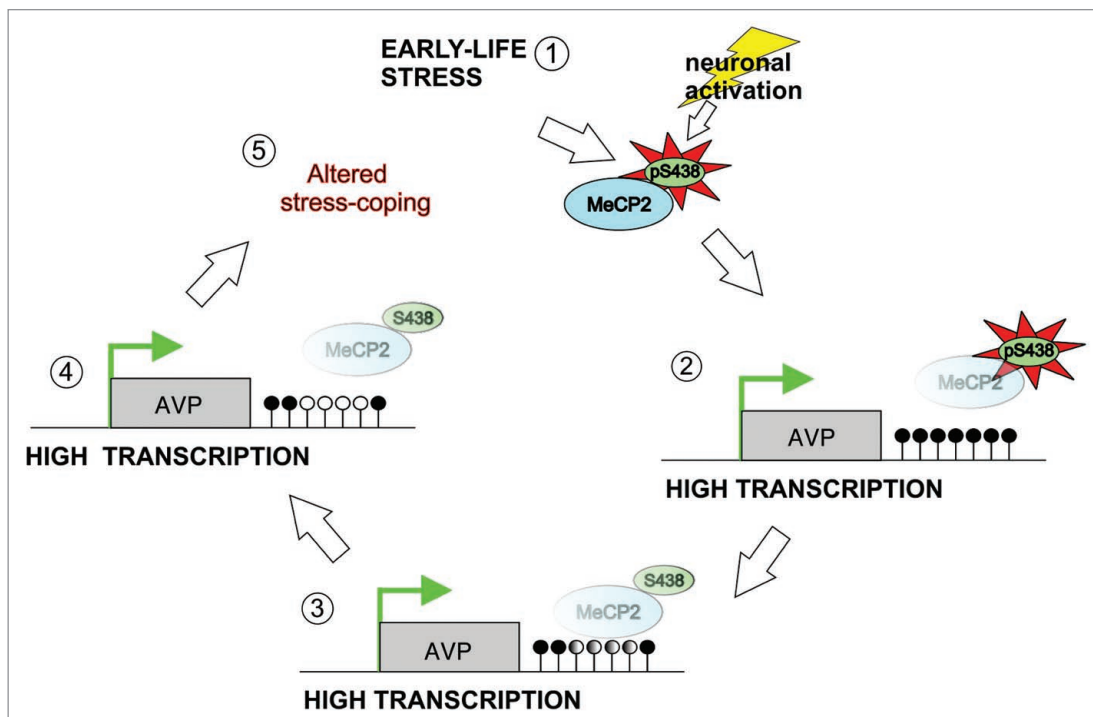
in the *AVP* enhancer, however, persisted under receptor blockade suggesting that early-life stress has engraved a permanent memory trace that conferred lifelong susceptibility to stress.<sup>20,23</sup>

### Initial Seeds

In contrast to the clear increase in *AVP* mRNA expression at postnatal day 10, DNA methylation did not differ between control and early-life stressed mice.<sup>20</sup> This result indicated that adverse early-life experiences were not straightforwardly translated into changes in DNA methylation but seemed to evolve gradually. Intriguingly, MeCP2 occupancy at the *AVP* enhancer was nevertheless strongly reduced during this early period in stressed mice.<sup>20</sup> MeCP2 serves as an epigenetic platform upon which synergistic crosstalk between histone deacetylation, H3K9 methylation and DNA methylation is played out to confer transcriptional repression and gene silencing (Fig. 3).<sup>21,24</sup> Thus, reduced MeCP2 occupancy conferred increases in active chromatin marks and relieve of *AVP* repression irrespective of changes in DNA methylation.<sup>20,23</sup> So what are then the signals

that control MeCP2 occupancy at this step?

Sensory experience and the resulting synaptic activity within the brain are critical for the development of neural circuits. Experience-driven synaptic activity causes membrane depolarization and calcium influx into select neurons within a neural circuit, which in turn induce a wide variety of cellular changes that alter the synaptic connectivity within the circuit.<sup>25</sup> Many of the signaling pathways that link neuronal activity to transcription and the regulation of complex programs of gene expression have been identified in the last years.<sup>26</sup> Among these, neuronal depolarization has also been shown to trigger  $Ca^{2+}$ -dependent phosphorylation of MeCP2, causing dissociation of MeCP2 from the *Bdnf* promoter and increased *Bdnf* transcription.<sup>27,28</sup> Recently, *de novo*  $Ca^{2+}$ /calmodulin-dependent protein kinase II (CaMKII) was shown to mediate phosphorylation of rat MeCP2 at serine 421 (S438 in mouse).<sup>29</sup> Compatible with these findings, MeCP2 dissociation from the *AVP* enhancer is regulated by depolarization through a  $Ca^{2+}$ /CaMKII dependent pathway and depends on phosphorylation of serine 438.<sup>20</sup> Importantly, MeCP2-S438 phosphorylation was robustly increased in parvocellular *AVP*-expressing neurons in the PVN of 10-day old early-life stressed mice. In addition, these neurons displayed increased phospho-CaMKII immunoreactivity in *AVP*-positive neurons, a finding that is in agreement with a role for this kinase in the mediation of activity-dependent MeCP2-S438 phosphorylation. In contrast, *AVP*-expressing neurons of the supraoptic nucleus, which do not participate in the regulation of the HPA-axis, showed neither differences in *AVP* expression nor in MeCP2-S438 phosphoimmunoreactivity in response to/following early-life stress.<sup>20</sup> Together, these findings support that early-life stress leads to activation of specific neuronal circuits controlling HPA-axis activity via depolarization of paraventricular neurons, which in turn increases activated  $Ca^{2+}$ /calmodulin-kinase II and MeCP2-S438 phosphorylation, and ultimately, relief of MeCP2 occupancy at the *AVP* enhancer.



**Figure 4.** The vicious circle of early-life stress. Maternal separation in mice leads to (1) phosphorylation of MeCP2 in response to neuronal activation in the parvocellular neurons of the hypothalamus. (2) Phosphorylated MeCP2 is no longer able to bind and repress *AVP* resulting in a higher transcription. Without the interaction of MeCP2 at the *AVP* enhancer—possibly in conjunctions with DNA methyltransferases—DNA methylation is insufficiently maintained. (3) Although MeCP2 is only transiently phosphorylated and regains its binding potential by early adulthood, binding is less due to impaired DNA methylation. (4) Consequently the ability of MeCP2 to bind to this region is further reduced resulting in higher transcription of *AVP*. (5) The result of altered *AVP* regulation manifests with impaired stress coping and HPA axis overactivity.

### One Way Ticket

Although early-life stress induced sustained *AVP* expression, differences in activated CaMKII and MeCP2-S438 phosphoimmunoreactivity faded away and were undistinguishable in adult (6-week old) early-life stressed and control mice. At the same time DNA hypomethylation gradually evolved in early-life stressed mice and underpinned reduced MeCP2 occupancy at the *AVP* enhancer.<sup>20</sup> This sequential course of events suggests that MeCP2 maintains *AVP* repression by the recruitment of DNA methyltransferases to protect DNA methylation in the long run. Experience-dependent phosphorylation and dissociation of MeCP2 thus initiates an insidious erosion of DNA methylation at the *AVP* enhancer that reinforces dissociation of MeCP2 and consequently primes further demethylation. At the end of the day, this vicious circle does not only uncouple MeCP2 occupancy from the initial stimulus but ultimately leads to the hard-coding of the early-life experience

at the level of DNA methylation (Fig. 4). The mice are permanently caught by their adverse early-life experience and their latent scars become easily unmasked by further stress exposure.

### Windows of Opportunities

A compelling insight from our work is that experience-dependent epigenetic marks are undergoing (at least in part) a transition from a preliminary, labile state to a hard-coded, stable state. The maturation of epigenetic marks may thus define a critical window for timely psychotherapeutic and pharmacological interventions following exposure to severe trauma. Conceivably, histone marks are more amenable to therapeutic interventions than DNA methylation. In fact, recent reports have implied the ability of certain antidepressants and mood stabilizers to modulate chromatin modification, while the post-mitotic nature of neurons still poses a major challenge to an easy remedy for methylation scars.<sup>30,31</sup> Dependent on the

quality and duration of a stressor, the neuronal circuits and the programmed genes involved, environmental manipulations may ameliorate early-life induced methylation stigmas.<sup>32,33</sup> Further work is needed to explore these possibilities.

In sum, the understanding of the initiation, maintenance and progression of enduring epigenetic marks in response to early and later life experiences has become an epicenter of modern psychiatry. This evolving field can deliver new guidelines for timely therapeutic interventions and the development of new pharmacological leads. More than 100 years after Sigmund Freud's seminal work, the discovery of an epigenetic record tracking severe life experiences demarcates a decisive turning point to pave the way for proactive interventions.

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