Perinatal programming of adult hippocampal structure and function: emerging roles of stress, nutrition and epigenetics

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Early-life stress lastingly affects adult cognition and increases vulnerability to psychopathology, but the underlying mechanisms remain elusive. In this Opinion article, we propose that early nutritional input together with stress hormones and sensory stimuli from the mother during the perinatal period act synergistically to program the adult brain, possibly via epigenetic mechanisms. We hypothesize that stress during gestation or lactation affects the intake of macro- and micronutrients, including dietary methyl donors, and/or impairs the dam’s metabolism, thereby altering nutrient composition and intake by the offspring. In turn, this may persistently modulate gene expression via epigenetic programming, thus altering hippocampal structure and cognition. Understanding how the combination of stress, nutrition, and epigenetics shapes the adult brain is essential for effective therapies.

Early-life environment programs the brain structure and function

Early-life (EL) is a period of unique sensitivity. It is well known that perinatal environmental conditions exert lasting effects on adult brain structure and function, and on the susceptibility to developing psychopathology [1,2]. Most EL experiences are embedded in the parent–offspring relationship [3], and alterations in maternal care [4], including sensory stimulation, warmth, and nutrition [5], can affect the development and function of the offspring’s brain (Figure 1). Furthermore, clinical data suggest a direct association between early-life stress (ELS) (e.g., maternal depression [2], the 9/11 attacks [6] and abuse [7–9]), and the incidence of psychiatric disorders and cognitive impairments.

Interestingly, similar impairments to those observed following ELS are found in children exposed to perinatal...
malnutrition [5,10–12] or famine [13] (but see [14]). The quality of early nutrition has major effects on adult cognitive function [15], suggesting that dietary elements are possibly instrumental in mediating the ELS and EL malnutrition induced impairments. To develop appropriate interventions, it is important to understand the mechanisms by which ELS and EL malnutrition exert their long-lasting effects on the brain and disease susceptibility. As evident from the above-mentioned examples, stress and malnutrition often occur simultaneously, and are interrelated. Feeding behavior and metabolism are closely regulated by neuroendocrine mechanisms that are influenced by stressful events, and malnutrition affects the stress system as well (Figure 1). Up to now, most research directed at understanding the processes underlying programming (see Glossary) by the EL environment viewed stress hormones and nutritional elements as independent factors [16–18]. Here, we propose that to fully understand the processes underlying the programming of the brain by ELS, it is key to study the interplay of these elements and how they mediate the programming of the brain: for example, possibly via epigenetic mechanisms.

In the following sections we review some of the evidence that ELS as well as EL nutrition affect hippocampal structure, plasticity, and function (Box 1). After addressing the role of maternal sensory stimuli, circulating stress hormones/neuropeptides, and nutrient availability in mediating these effects, we introduce the importance of examining the coordinated interaction of these elements and discuss how these effects could be mediated by epigenetic mechanisms.

The hippocampus, highly susceptible to early-life experiences
To understand how EL experiences affect mental health and cognition, numerous studies have focused on the hippocampus, as this brain region is implicated in both cognition [19] and regulation of the stress response [20]. In fact, the hippocampus is particularly sensitive to the EL environment because it mostly develops postnatally, is highly plastic, and is rich in stress-hormone receptors.

The human hippocampus develops between the last trimester of gestation and 16 years of age [21], whereas the rodent hippocampus develops between embryonic
day 18 and postnatal weeks 2–3 [22]. The hippocampus exhibits a high degree of structural and synaptic plasticity and undergoes dynamic changes in neuronal connectivity that can be assessed morphologically or functionally (Box 1).

Furthermore, the dentate gyrus of the hippocampus is one of the few brain regions that exhibits the ability to generate new neurons during adulthood [23]. This fundamental form of structural plasticity is termed adult neurogenesis and is regulated by various factors (e.g., it is inhibited by stress and stimulated by exercise or enrichment [24]). Dysregulation of neurogenesis [25] and impairments in long-term potentiation (LTP) [26] or dendritic complexity [27] have been implicated in reduced hippocampus-dependent cognition (e.g., spatial memory, declarative memory, and pattern separation [19]).

In the following sections, we discuss the evidence that alterations in hippocampal structure and plasticity might underlie the lasting effects of EL stress and malnutrition.
Importantly, postnatal strain often suggests vulnerabilities that are associated with affected hippocampal structure. For instance, in rats, exposure to stress during gestation impaired spatial learning in adult offspring, suppressed LTP [31,32], altered spine density and dendritic length [33], and reduced levels of proliferation and newborn cell survival [29,34]. These effects were already evident at postnatal day 1 (P1) [35,36] and lasted up to 22 months of age [29]. Similarly, postnatal stressors in rodents, such as maternal deprivation [30], repeated maternal separation [37,38], or chronic ELS [26,39], impair the acquisition of spatial information and are associated with impaired LTP, aberrant mossy fiber growth, dendritic atrophy [26,40,41], and changes in levels of adult neurogenesis [30,42–46]. Importantly, whereas stress effects during adulthood are often reversible [47,48], ELS-induced hippocampal structural changes and cognitive deficits persist throughout life [49]. Interestingly, whereas adult rat offspring from maternally deprived or from low-caring mothers show impaired learning and reduced synaptic plasticity under basal conditions, they exhibit improved contextual learning and enhanced LTP under stressful conditions [30,41]. This suggests that ELS, rather than exerting ‘deleterious effects’ in general, prepares the organism to respond optimally under comparable situations encountered later in life, a concept known as the match–mismatch theory [50].

In conclusion, perinatal stress alters cognition into and throughout adulthood. Although alterations in hippocampal plasticity and synaptic integrity are likely to be instrumental, the specific elements in the early environment (e.g., sensory stimuli and nutrition), and the molecules and molecular mechanisms mediating these long-term effects are only partly resolved.

Programming effects of perinatal nutrition
Given the high metabolic activity and energy demand of the brain, its functioning requires adequate supply of micro- and macronutrients. Even minor dietary insufficiencies can have adverse effects, especially during critical stages of development, when they can permanently change brain structure and cognitive functioning. For instance, children exposed to perinatal malnutrition exhibit cognitive deficits and increased risks for psychopathology in adulthood [5,10–12,14]. Preclinical studies also demonstrate that offspring of malnourished dams exhibit cognitive deficits [51–53] (but see [54]). Many nutrients are essential for neuronal growth and brain development, but during the perinatal period, the intake of iron, zinc, selenium, iodine, folate, vitamin A, vitamin B6, vitamin B12, choline, long-chain polyunsaturated fatty acids, and proteins overall is of particular importance. For example, fetal and neonatal iron and protein deficiency results in long-term deficits in memory functions [12].

Although it is unclear whether alterations in hippocampal structure and synaptic plasticity are instrumental in mediating these cognitive deficits, there is evidence that perinatal manipulations in nutritional status induce alterations in hippocampal neurogenesis [55,56], as well as reduce granular cell size, dendritic complexity, and synaptic spine density [57]. These structural changes are associated with enhanced interneuron-mediated inhibition [58] and deficits in LTP in malnourished animals [59]. Furthermore, vitamin B6 and B12 deficiencies during gestation and lactation persistently impair hippocampal structure and function [12,60]. Finally, protein malnutrition results in reduced neuronal DNA and RNA content and an altered fatty acid profile that, in turn, could change neuronal function, synapse number, and/or dendritic arborization [61,62].

These data indicate that synapses in the malnourished hippocampus might be less capable of supporting plasticity and that alterations in the hippocampal circuitry during development could account for cognitive deficits induced by perinatal malnutrition. However, further research is needed to understand which nutrients are most relevant and how exactly nutritional deficiencies affect hippocampal structure and function.

The role of sensory stimulation from the mother and stress hormones
Alterations in tactile stimulation from the mother (potentially induced by maternal stress exposure as well as malnutrition; see next section) are instrumental in mediating the consequences of EL experiences. The key role of these sensory stimuli has been established by studies demonstrating that both artificial manipulation of maternal care (via maternal separation or deprivation, chronic ELS, and handling; Box 2) [40,63,64] and the natural variation in maternal care between [41,65] and within litters [66] programs the brain and behavior of the adult offspring. In line with this, stroking (simulating maternal tactile stimuli) reversed the effects of maternal separation in rats [67]; and in (pre)term human neonates, moderate touch (e.g., massage) reduces reactivity to stress at adult ages [68]. In the next section we examine how these sensory stimuli can be affected by other elements of the EL environment (stress and nutrition).

Next to maternal sensory stimulation, EL experience-induced alterations in circulating levels of stress hormones and stress-related peptides are considered to be instrumental in mediating the lasting effects of EL experience on the brain and behavior. This includes lasting changes in the hippocampus and cognitive functions. EL experience programs the neuroendocrine system (the hypothalamic–pituitary–adrenal (HPA) axis), which is activated on stress exposure. When the HPA axis is activated, corticotrophin-releasing hormone (CRH; also known as corticotrophin-releasing factor (CRF)) is released from the hypothalamic paraventricular nucleus. In turn, CRH stimulates the pituitary to release adrenocorticotropic hormone (ACTH), resulting in the synthesis and release of glucocorticoids (corticosterone (CORT)) from the adrenal glands. There is ample evidence that EL experience affects CRH [69], glucocorticoid receptors (GRs), and mineralocorticoid
Box 2. Animal models to study the programming effects of early-life stress and nutrition

The ‘developmental origins of health and disease’ hypothesis proposes that the early-life (EL) environment, from gestation till puberty, can set the stage for adult pathology. During this developmental period, quality of the EL environment critically depends on the mother providing the prenatal environment and forming the primary source of nutrition, warmth, and tactile stimulation during postnatal EL. These critical components are often manipulated in animal models used to study the long-term effects of EL experiences.

Commonly used manipulations to induce prenatal stress in rodents include exposure of the pregnant dam to single or repeated stress (Figure IA). Postnatal ELS can be induced by single prolonged separation of dam and pups for 24 hours, for example, at postnatal day 3 (P3) (maternal deprivation; Figure IB), or by repeated daily separations for 2–5 hours (maternal separation; Figure IC). Furthermore, a powerful method to induce chronic EL stress consists of reducing the amount of nesting and bedding material during the first postnatal week (Figure ID). This induces fragmented maternal care, thereby mimicking aspects of a human chronic ELS situation in which the mother is present but unable to provide appropriate care. Besides experimentally induced alterations in maternal care, selection based on natural variation is used to compare offspring that received low versus high levels of maternal care (Figure IE). For the above-described models, the effects on the level of maternal sensory stimuli are well characterized. Indeed, the lasting effects of these manipulations on brain structure and function have been mostly attributed to altered maternal sensory input, although other key components of the dam–pup interaction (e.g., nutrition and warmth) also have a role.

EL malnutrition is usually induced by altering maternal diet during pregnancy and/or lactation (e.g., overnutrition with a high-fat diet (Figure IA), protein intake restriction (Figure IB), or global dietary restriction (Figure IC)). Again, in most of these studies, only the manipulated element is considered as the main player, ignoring the fact that nutritional manipulation itself might affect maternal care and stress hormones. However, when addressing the mechanisms underlying EL programming of the brain, it is key to consider which environmental elements are involved and how these components interact.

Figure I. Frequently used experimental manipulations to induce early-life stress in rodents.

Figure II. Frequently used experimental manipulations to alter early-life nutrition in rodents.
receptors (MRs) [70], as well as arginine vasopressin (AVP) [71] and brain-derived neurotrophic factor (BDNF) [72]. If these persistently altered factors are responsible for the functional consequences, then modulating these changes pharmacologically should prevent or reverse the functional consequences. Because CORT–GR/MR and CRH–CRF receptor type 1 (CRF1) have received the most attention so far, we will discuss these in detail.

EL experience has lasting consequences on CORT levels [39,73,74] and affects GR and MR expression [70,75]. An elegant series of experiments highlighted the relevance of GR exon I for later life consequences and its potential as a target for reversal of EL effects [70]. However, it is not clear whether these alterations are directly responsible for mediating the lasting effects of ELs. In fact, even though CORT is a logical candidate, there is some controversy in the literature because CORT alteration following ELs is not consistent across animal models [40,45,76]. Neonatal treatment with the synthetic glucocorticoid dexamethasone lastingly impairs spatial learning and reduces hippocampal synaptic plasticity [77,78], and reducing CORT levels by adrenalectomy of adult mice exposed to ELs restores neurogenesis to control levels [45]. However, permanently reducing CORT levels by adrenalectomy at P10 does not alter subsequent levels of adult neurogenesis [79], and suppressing the rise of CORT induced by maternal deprivation does not prevent the HPA-axis alterations discussed above [80]. Thus, the complexity of corticosteroid regulation by ELs points to the need for further research in this area.

CRH expression is persistently altered by EL experience in the hypothalamic paraventricular nucleus (PVN) [39,40,63,69,81] and in the hippocampus [26]. Several lines of evidence indicate a critical role for CRH in mediating lasting EL effects. For example, chronic exposure to CRH and chronic ELs have similar effects on hippocampal structure [40]. Moreover, CRH repression is the first alteration in the HPA axis that occurs after handling [82]. Blocking CRF1 in control rats (P10–P17) improves cognitive functions [75], and blocking CRF1 after chronic ELs prevents ELs-induced LTP impairment and dentritic atrophy, and preserves hippocampal cognition [26]. Finally, in conditional CRF1-knockout mice subjected to chronic ELs, cognition, LTP, and spine density are restored [83].

In line with these preclinical data, single-nucleotide polymorphisms in the Crhr1 gene protect against depression in childhood-maltreated individuals [84]. Therefore, pharmacological targeting of GR and CRF signaling may enhance resilience to ELs-related cognitive impairment and affective disorders [40].

**Interaction among maternal care, stress system, and nutrient availability**

Based on the studies discussed so far, it is clear that maternal tactile stimulation and nutrient availability are key factors in the early environment, and that stress hormones and neuropeptides are instrumental in mediating the lasting effects of these EL experiences. Most studies consider these elements individually, but for optimal intervention it is fundamental to understand how these environmental elements and molecules interact and influence each other. We therefore next examine the available evidence supporting a coordinated interaction of these elements in the lasting effects of EL experience.

Food intake and HPA-axis activity are closely interrelated with overlaying neuronal pathways that respond to and integrate both nutritional and stressful stimuli. Indeed, basal HPA-axis activity and stress responsiveness are altered in genetically obese rats [85] and in rodents fed a high-fat diet [86] or subjected to perinatal food restriction [87]. Conversely, chronic stress conditions have been correlated with changes in food intake [16]. The HPA axis is sensitive to modulation by metabolic signals, including leptin, insulin, glucose, and ghrelin [88,89]. This is also true early in life [90,91], when food intake and nutrition of the progeny depend on maternal care and diet. Indeed, next to quality and quantity of maternal care and circulating stress hormones, metabolic signals are crucial in programming the HPA axis [90]. For instance, only combined food administration and sensory stimulation of pups during maternal deprivation prevents the deleterious effects of this EL stressor on the HPA axis and on hippocampal GR expression, whereas stroking alone is not sufficient to achieve this recovery [80]. In addition to changes in HPA-axis tone, maternal separation reduces plasma glucose and leptin levels, and increases ghrelin levels in the offspring [90]. Pharmacologically blocking this reduction in glucose, or the increase in ghrelin, attenuates the HPA-axis response to maternal separation [90]. This suggests that metabolic signals play an important part in triggering the HPA-axis response of the neonate to maternal separation.

Furthermore, plasma leptin levels in the offspring are modified by the availability and composition of the maternal milk. Maternal milk is rich in fat and is required for growth and brain development [91–93]. Feeding mothers a high-fat diet from gestational day 14 and throughout lactation increased maternal milk fatty acid and leptin content and persistently increased the offspring’s plasma fat and leptin levels [92]. These metabolic changes are associated with a blunted hormonal stress response [94], and increased GR expression and anxiety [95]. Although this effect on the HPA axis could be directly due to the increases in leptin levels [91], differences in maternal diet might have affected maternal behavior as well [17], thereby contributing indirectly to the observed effects.

In a similar way to fat content, protein restriction [96] and general undernutrition during gestation and/or lactation [97] affect the HPA axis of the progeny. In addition to the direct effects of protein restriction on the quality of maternal milk, these nutritional restrictions increased expression of the genes encoding CORT and decreased expression of the gene encoding placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). Protein restriction also possibly led to fetal overexposure to maternal CORT, which could be partly responsible for some of the alterations in the HPA axis of the progeny. This exemplifies again the tight relationship among stress, nutrition, and metabolic signals.

Interestingly, the effects of perinatal malnutrition on adult stress responsiveness and cognitive function resemble several of the long-term deficits induced by perinatal...
stress. Both insults result in cognitive deficits and an increased susceptibility to psychiatric disorders [98,99]. One possibility is that impairments in adult cognition and stress responsiveness observed following both perinatal stress and malnutrition depend on the combined effects of lack of key nutrients, affected maternal behavior, and altered HPA-axis activity (Figure 1). In line with this hypothesis, maternal undernutrition results in altered maternal behavior [17] and in high plasma CORT levels in the adult offspring along with reduced GR expression [100]. This suggests that the deleterious effects of maternal nutrient restriction could also result from differential sensory input from the mother and enhanced HPA-axis activity.

Certainly, the opposite possibility should be considered as well. During (EL) stress, regulation of appetite and metabolism are altered, thereby affecting the intake of essential macro- and micronutrients [101]. For instance, exposure to perinatal stress increased the risk of developing obesity later in life and affected feeding regulation in both clinical [102,103] and preclinical studies [104–106]. These effects of stress on appetite regulation and metabolism are in large part mediated by glucocorticoids and stress-related peptides such as CRH and urocortins [107], which affect the neural circuits and hormones involved in the regulation of feeding behavior. A detailed analysis of how these systems interact has been conducted [18].

In conclusion, there is some evidence that, in addition to maternal sensory stimulation and stress hormones, the lack of key nutrients affects the brain directly in the case of both restricted nutrition and perinatal stress. The next question then is which molecular mechanisms mediate the programming effects of EL experience. Thus, below we explore the role of epigenetic modifications.

### Early-life stress and epigenetic mechanisms

Various reports have implicated epigenetic mechanisms in mediating persistent effects of EL experience [69–72] (Table 1). Epigenetic modifications determine whether a gene is transcribed or repressed without changing the DNA sequence. In contrast to the genome, the epigenome is dynamic, thereby allowing the organism to adapt to the environment, and therefore it is an excellent candidate for mediating the effects of EL experiences on the brain. For example, increased hippocampal GR expression induced by high levels of maternal care is associated with decreased DNA methylation and increased histone acetylation binding to the GR promoter [70] (but see [108]). Similarly, translational research has found lower hippocampal GR expression and increased GR promoter DNA methylation in suicide victims with a history of childhood abuse or neglect [109]. Furthermore, ELS-induced increases in AVP levels are associated with DNA hypomethylation [71], whereas ELS-induced reduced BDNF expression in the prefrontal cortex is accompanied by increased DNA methylation [72], and maternal deprivation-induced increases in CRH expression are accompanied by decreased DNA methylation [69]. Finally, the handling-induced reduction in CRH expression is associated with persistently increased levels of neuron restrictive silencing factor [81], further indicating that environmental factors during EL can trigger the epigenetic machinery and persistently change transcription of important regulatory genes.

There is evidence that the epigenome is also affected more globally, as the epigenetic response to maternal care is coordinated in clusters across broad genomic areas [110], and maternal separation in mice changed global levels of histone deacetylases [111]. Accordingly, whole-genome DNA methylation is significantly different

### Table 1. Examples of epigenetic modifications induced by early-life experiences

<table>
<thead>
<tr>
<th>Early-life intervention</th>
<th>Altered gene expression</th>
<th>Tissue</th>
<th>Epigenetically regulated</th>
<th>Species</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low maternal care</td>
<td>↑ Glucocorticoid receptor</td>
<td>Brain (hippocampus)</td>
<td>↑ DNA methylation of exon 1 of GR promoter</td>
<td>Rats</td>
<td>[70]</td>
</tr>
<tr>
<td>Maternal deprivation (P1–10 for 3 hours)</td>
<td>↑ AVP</td>
<td>Brain (hypothalamus)</td>
<td>↓ DNA methylation</td>
<td>Mice</td>
<td>[71]</td>
</tr>
<tr>
<td>Exposure to stress-abusive dam (P1–7)</td>
<td>↑ BDNF</td>
<td>Brain (prefrontal cortex)</td>
<td>↑ DNA methylation IV exon promoter</td>
<td>Rats</td>
<td>[72]</td>
</tr>
<tr>
<td>Maternal deprivation</td>
<td>↑ CRH</td>
<td>Brain (hypothalamus)</td>
<td>↓ DNA methylation of CRH promoter CRE</td>
<td>Rats</td>
<td>[69]</td>
</tr>
<tr>
<td>Handling</td>
<td>↓ CRH</td>
<td>Brain (hypothalamus)</td>
<td>↑ NRSF</td>
<td>Rats</td>
<td>[81]</td>
</tr>
<tr>
<td>Maternal separation</td>
<td>Genome wide</td>
<td>Brain (forebrain)</td>
<td>↑ HDAC expression</td>
<td>Mice</td>
<td>[111]</td>
</tr>
<tr>
<td>Childhood abuse/neglect</td>
<td>↑ Glucocorticoid receptor</td>
<td>Brain (hippocampus)</td>
<td>↑ DNA methylation</td>
<td>Human</td>
<td>[109]</td>
</tr>
<tr>
<td>Institutionized children</td>
<td>Genome wide</td>
<td>Periphery (blood)</td>
<td>Differential patterns of DNA methylation</td>
<td>Human</td>
<td>[112]</td>
</tr>
<tr>
<td>Postnatal overfeeding</td>
<td>↑ Glucocorticoid receptor</td>
<td>Periphery (adipose tissue)</td>
<td>?</td>
<td>Rats</td>
<td>[134]</td>
</tr>
<tr>
<td>Protein restriction during gestation</td>
<td>↑ Glucocorticoid receptor</td>
<td>Periphery (liver)</td>
<td>↓ DNA methylation of exon 10 of GR promoter</td>
<td>Rats</td>
<td>[127]</td>
</tr>
<tr>
<td>Periconception maternal vitamin B/methionine restriction</td>
<td>Genome wide</td>
<td>Periphery (fetal liver)</td>
<td>Differential patterns of DNA methylation</td>
<td>Sheep</td>
<td>[113]</td>
</tr>
<tr>
<td>High maternal choline intake (third trimester)</td>
<td>↓ CRH</td>
<td>Periphery (placenta)</td>
<td>↓ DNA methylation of CRH promoter</td>
<td>Human</td>
<td>[115]</td>
</tr>
</tbody>
</table>

*Abbreviations: AVP, arginine vasopressin; BDNF, brain-derived neurotrophic factor; CRH, corticotrophin-releasing hormone; GR, glucocorticoid receptor; H3K9me2, histone H3 lysine 9 dimethylation; H4K12ac, histone H4 lysine 12 acetylation; HDAC, histone deacetylase; MeCP2, methyl CpG-binding protein 2; NRSF, neuron restrictive silencing factor.
between institutionalized children and children raised by their biological parents [112].

Thus, epigenetic mechanisms both targeted at specific genes and genome wide seem to be good candidates for mediating the programming effects of EL.

**Effects of nutrition on the epigenome**

Interestingly, early nutrition modulates the epigenome in several peripheral tissues. In both humans and animals, diet is a potent modulator of epigenetic marks during the perinatal period [113–115]. EL nutrition can modulate the epigenome by alterations in: (i) the supply of methyl donors; (ii) the activities of DNA methyltransferases (DNMTs); or (iii) activities of specific transcription factors [116]. Here, we review the evidence that such mechanisms could be at work in the brain.

The dietary methyl donors folate, vitamins B6 and B12, methionine, choline, and betaine all affect DNA and histone methylation [117]. Fetal choline availability is essential for normal brain development, and maternal choline deficiency alters development and neurogenesis in the fetal mouse hippocampus [118]. Alterations in choline availability during fetal brain development induce epigenetic modifications of genes directly involved in epigenetic machinery [119], signal transduction [120], HPA-axis reactivity [115], and neuronal differentiation [121,122]. In fact, pregnant and lactating women possess a system assuring the necessary choline intake by the fetus and infant, respectively [123]. In rodents, maternal diets supplemented with choline improved the memory of the offspring and were associated with increases in neurogenesis in the embryonic brain [124,125].

Finally, perturbations in maternal diet can alter DNMT expression: for example, pregnant rats fed a protein-restricted diet showed increased blood homocysteine concentration [126], which was associated with a reduction in DNMT1 expression and inhibited binding of DNMT1 to the liver GR promoter [127]. Because DNMT1 expression is regulated by homocysteine and folic acid [127], modulation of DNMT1 expression by differences in one-carbon metabolism could provide a link between maternal diet and epigenetic regulation of the offspring’s gene expression. However, whether a lack of specific nutrients during the critical developmental period affects brain structure and function, and whether this involves epigenetic mechanisms remains to be determined (Box 3).

**Can dietary intervention reverse the effects of perinatal stress and/or malnutrition?**

Despite the apparent stability of methylation marks, alterations in DNA methylation induced by maternal diet or differential nurturing behavior can be prevented and reversed by interventions in postnatal life [128]. Both folate and glycine supplementation to maternal diet reversed the effects of protein restriction during pregnancy on blood pressure and vascular function of the offspring [129], and prevented the aforementioned epigenetic changes [130]. In addition, the phenotype and gene expression of the offspring from protein-restricted dams were altered by folate supplementation during the juvenile–pubertal period [131]. Finally, central infusion of l-methionine at P90 reversed the maternal care-induced effects on DNA methylation [132]. Accordingly, formula fortified with protein and high energy improved neural development of children who suffered brain damage [133]. Taken together, early nutrition appears to be a promising candidate for modulating (some of) the lasting consequences of EL experience on adult brain structure and function.

**Concluding remarks**

In summary, adverse experiences during critical developmental periods persistently affect gene expression, which ultimately might determine cognitive outcomes and disease susceptibility in adulthood. Hippocampal development, various forms of neuronal plasticity, and synapse formation are tightly regulated by maternal sensory stimuli, exposure to hormones and neuropeptides (e.g., CORT and CRH), the availability of macro- and micronutrients, and epigenetic mechanisms. Therefore, EL events that alter any of these components will interfere with outcome measures later in life.

It remains difficult to dissect the contributions of the EL environment and nutrition to (epigenetic) programming of hippocampal structure and function, as they influence each other and often occur simultaneously. Yet understanding the mechanisms by which nutrition and other environmental cues influence epigenetic regulation and identifying the periods of susceptibility and stability of the induced changes is critical for the identification of individuals at risk and for the development of novel intervention strategies.

Much evidence points towards a crucial role for epigenetic mechanisms in mediating the lasting effects of adverse EL experiences on hippocampal structure and function. Next to maternal care, stress hormones, and neuropeptides, early nutrition seems to play an important part in modulating these epigenetic influences on hippocampal anatomy and physiology. Therefore, non-invasive interventions targeted at maternal nutrition, which are relatively easy to implement, could have a significant effect on the brain and on the behavior of the offspring in the long term.
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