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REVIEW ARTICLE

Long-Term Impact of Early Life Events on Physiology and Behaviour

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This review discusses the effects of stress and nutrition throughout development and summarises studies investigating how exposure to stress or alterations in nutrition during the pre-conception, prenatal and early postnatal periods can affect the long-term health of an individual. In general, the data presented here suggest that that anything signalling potential adverse conditions later in life, such as high levels of stress or low levels of food availability, will lead to alterations in the offspring, possibly of an epigenetic nature, preparing the offspring for these conditions later in life. However, when similar environmental conditions are not met in adulthood, these alterations may have maladaptive consequences, resulting in obesity and heightened stress sensitivity. The data also suggest that the mechanism underlying these adult phenotypes might be dependent on the type and the timing of exposure.

Key words: stress, nutrition, peri-natal, animal model

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The importance of the prenatal and early postnatal environment for the health of the individual has become clear from studies investigating the offspring of mothers exposed to natural and other disasters. It became clear from epidemiological studies after the nuclear reactor accidents in Chernobyl, Ukraine and Three Mile Island, USA, as well as the release of toxic gas in Bhopal, India, that direct exposure to toxins during pregnancy can lead to birth defects, as well as cognitive and emotional disorders, although the effects are variable (1–3). Interestingly, not only direct exposure to toxins, but also stress and anxiety induced by the disaster may lead to defects in offspring. A Swedish study showed that the amount and severity of emotional and cognitive disorders in a group of children whose mothers were pregnant during the Chernobyl disaster did not correlate with the radiation dose that their mothers were exposed to. However, the severity of symptoms in the offspring was correlated with the anxiety and stress levels of their mothers (1). More recent evidence for the idea that stress exposure during pregnancy may have severe consequences for the offspring comes from the Project Ice Storm study showing that offspring of mothers exposed to the ice storm had lower cognitive and language abilities at 5.5 years of age (4).

The nutritional environment during pregnancy also plays a significant role in the disease risk of the offspring. Children born during or right after the Dutch Hunger Winter have been shown to have an increased risk to psychiatric and metabolic disorders (5–7). Observations such as these have lead to the concept of the 'Developmental Origins of Health and Disease (DOHaD)', which proposes that adverse influences early in development, and particularly during intrauterine life, can result in persistent changes in physiology and metabolism, which results in an increased disease risk in adulthood. This concept was originally proposed Dr David Barker and has also been referred to as the 'Barker Hypothesis' (8–10). Although epidemiological studies in humans clearly indicate that the prenatal and early postnatal environments affect the health of the individual in later life, ethical considerations prevent controlled causal studies in humans, leading to the development of several animal models that aim to study mechanistic processes and potential interventions. In this review, we present several studies using rodent models for early life perturbations that aim to investigate the effects of stress and nutrition during the pre-gestation, prenatal and early postnatal periods on the long-term health of the individual.

Table 1. Summary of Studies Investigating Pre-Conception Stress Discussed in the Present Review (i.e. Not Intended to be an Exhaustive Review of the Topic).

Article		Description of stress			Effects on the offspring		
Author	Reference	Sex	Type	Timing	Brain	Endocrine	Behaviour
Ryzhavskii *et al.*	12	F	3-week RS	PM10-31	↓ brain weight ↑ layer V neuronal nuclei ↓ parietal cortex	–	↓ exploration EPM
Ryzhavskii *et al.*	13	F	3-week RS	PM10-31	↓ cerebellum mass ↑% purkinje cells ↓ lipids concentration white matter	–	–
Li *et al.*	14	F	3-week CVS	NS	↓ 5-HT HYPO ↓ NE HPC ↓ P-CREB HPC	–	↓ sucrose consumption ↓ MWM performance
Huang *et al.*	15	F	3-week CVS	NS	↓ DAT mPFC ↓ COMT mPFC	↑ serum corticosterone	↓ activity OF ↑ immobility FST
Dietz *et al.*	17	M	10-day social defeat	PM28-38	–	↑ serum corticosterone ↑ VEGF	↓ exploration EPM ↓ social interaction ↓ sucrose consumption ↑ immobility FST
Mychasiuk *et al.*	18	M	27-day elevated platform	NS	↑ global DNA methylation	–	↑ exploration OF ↑ novelty seeking ↓ geotaxis (d9)
Rodgers *et al.*	19	M	42-day CVS	PM28-70, PM56-98	≈ CRFr1 ≈ POMC ≈ adrenal Mc2r ≈ 11βHSD	↓ corticosterone RST	≈ TST ≈ barnes maze ≈ light–dark box

↑, increased in treatment group; ↓, decreased in treatment group; ≈, no difference; 5-HT, 5-hydroxytryptamine; 11β-HSD, 11β-hydroxysteroid dehydrogenase; COMT, cathechol-O-methyl transferase; CVS, chronic variable stress; CRF, corticotrophin-releasing factor; DAT, dopamine active transporter; EPM, elevated plus maze; F, female FST, forced swim test; HYPO, hypothalamus; HPC, hippocampus; M, Male; Mc2r, melanocortin receptor 2; MWM, Morris water maze; mPFC, medial prefrontal cortex; NE, norepinephrine; NOR, novel object recognition; NS, not specified; OF, open field; POMC, pro-opiomelanocortin; PM, pre-mating day; RS, restraint stress; RST, restraint stress test; TST, tail suspension test.

Stress

It has long been known that chronic stress exposure in adulthood has negative health consequences, with reports of an increased risk for metabolic, cardiovascular and psychiatric disorders (11). Stress responses, however, should be seen as normal adaptations to deal with the ongoing environmental conditions that will ultimately benefit the survival chances of the individual. Stress-induced pathologies may develop when the stress exposure becomes chronic and the adaptations do not lead to an improvement of the environmental conditions. From an evolutionary stand point, preparing the offspring for the potential adverse environmental conditions following birth and weaning may be crucial to ensure survival. This environmental pressure may have resulted in the alterations in offspring physiology that are observed after prenatal stress exposure. Below, we review the effects of perinatal stress exposure, either pre-conception or during pregnancy, as well as stress exposure during the early postnatal period on the development of the rats.

Pre-conception stress

Starting from the hypothesis that adaptations in the development and physiology of the offspring occur to optimally prepare the offspring for the environmental conditions that they might encounter later in life, it is reasonable to argue that exposing the parents to stressors prior to gestation may signal potential adverse conditions expected later in life. This may, in turn lead to alterations in gene expression in the progeny that influence the development of the offspring (Table 1).

Maternal pre-conception stress

Neurodevelopmental effects of maternal pre-gestation stress were reported by Ryzhavskii *et al.* (12,13), showing that the progeny of dams exposed to 3 weeks of restraint stress 10 days prior to pregnancy had reduced brain mass (12), smaller cerebella (13) and decreased parietal cortex thickness (12). Behaviourally, their progeny were characterised by lower exploratory activity during an elevated plus maze test, suggestive of increased anxiety levels (12). Behavioural deficits as a result of maternal pre-conception stress were confirmed by Li *et al.* (14). Progeny of pre-conception stressed mothers showed decreased sucrose consumption, suggestive of a depression-like phenotype (14). Additionally, during a Morris water maze test, as used to evaluate spatial memory, the progeny of pre-conception stressed mothers displayed impaired retention, although their acquisition was not affected (14). Finally, a recent study using

pre-conception chronic variable stress showed decreased exploration in an open field test, increased immobility in a forced swim test and decreased sucrose consumption in the offspring, together suggesting greater anxiety- and depressive-like behaviour (15). The mechanism underlying this behavioral phenotype might include alterations in the hypothalamic-pituitary-adrenal (HPA)-axis because serum corticosterone and corticotrophin-releasing hormone levels were higher in pre-conceptionally stressed offspring (15). Additionally, the dopaminergic system may play a role as well because alterations in dopamine synthesis and turnover were observed in this model (15). A caveat in studies investigating pre-conception stress is that the stress paradigm used typically leads to considerable alterations in the stress levels in the dam that last throughout gestation, which leaves the potential of effects of pre-conception stress being partially mediated by persistent stress or depression-like phenotypes in the dam during pregnancy. Nevertheless, this approach does model the situation in pregnant humans and, regardless of whether or not the effects of pre-gestation stress exposure linger through the pregnancy, the impact of stress exposure during this period has significant consequences for the offspring. The effects of pre-conception parental stress on the offspring likely result from epigenetic changes. Epigenetic refers to the stably heritable phenotype resulting from changes in chromatin without alterations in the DNA sequence (16), such as alterations in DNA methylation (DNAm), micro (mi)RNAs and histone configuration. To our knowledge, there are no studies showing alterations in epigenetic marks in the offspring of mothers stressed pre-conception.

Paternal stress

Not only the offspring of stressed mothers are possibly affected, but also the offspring of fathers that were stressed prior to breeding may show altered phenotypes. Offspring of male mice exposed to social defeat were shown to spend less time on the open arm of an elevated plus maze, decreased time interacting with a conspecific in a social interaction task, and had decreased sucrose intake, all suggestive of anxiety-like or depression-like phenotypes in these mice (17). By contrast, Mychasiuk *et al.* (18) found that paternally stressed rat offspring spent more time in the centre of an open field test, suggesting decreased anxiety levels. Furthermore, paternal stress exposure leads to decreases in HPA axis reactivity, with lower corticosterone levels during a restraint stress test in both male and female mouse offspring (19). Paternal stress may also affect maturation of the brain because 9-day old offspring of stressed fathers were shown to have delayed negative geotaxis (automatic, reliable, stimulus-bound, orientations and movements directed against gravitational cues) development, although these effects appear to be transient because no differences in negative geotaxis or learning and memory were observed by postnatal day (PND)10 (18). It may be hypothesised that the phenotype of the paternally stress offspring may be mediated through alterations in the expression of genes involved in HPA axis functioning; however, a study investigating the offspring of chronically stressed male mice showed that genes directly affecting HPA axis output in the pituitary and adrenals, such as those for corticotrophin releasing factor-receptor 1, pro-opiomelanocortin, melanocortin-2 receptor and 11β-hydroxy steroid dehydrogenase (HSD)-1, were not altered, which may suggest, at least in this model, that the HPA axis might not play an important role (19). Some recent studies suggest that paternal stress induced epigenetic alterations. In both male and female offspring, global DNA methylation levels were increased in the hippocampus of the paternal stress group at PND21 (18). How increases in global DNA methylation may affect the expression of specific genes is currently unclear. More evidence for a role of epigenetics in the phenotype of paternally stressed offspring comes from a gene set enrichment study. In the pareventricular nucleus (PVN) and bed nucleus of the stria terminalis (BNST), C3 genes sets were shown to have been enriched in paternally stressed rats (2.1% in PVN and 14.7% in BNST). Gene sets in the C3 collection consist of genes sharing a cis-regulatory motif, and share motifs for transcription factors or miRNAs (20). More specifically, enrichment was found in gene sets involved in the glucocorticoid receptor (PVN), chromatin modification, neuronal survival, the MiR-154 family, the MiR-8 family and several other miRNAs (19). Finally, the effects might already occur at the level of the sperm cell because several miRNAs were increased in sperm cells of the stressed dads and these miRNAs appear to target genes that have a predicted function in epigenetic regulation, such as *Dnmt3a*, *Tnrc6b* and *Hdac9* (19). Overall, these studies suggest that paternal stress may induce epigenetic alterations at different levels, which, in turn, may mediate the phenotype of the offspring. Future research is, however, needed to determine further causal pathways.

Prenatal stress

The effects of stress during pregnancy have been studied extensively over recent decades. Epidemiological studies in humans suggest that prenatal stress exposure might be linked to schizophrenia (21) and autism (22). Additionally, children of mothers stressed during pregnancy are more anxious and have a higher risk for developing attention deficit hyperactivity disorder and conduct disorders (23,24). A recurring problem in human studies is that it is almost impossible to dissociate prenatal from early postnatal effects. However, pregnancy-specific anxiety was also associated with heightened anxiety in the offspring, suggesting that there are prenatal specific effects on the health of the offspring in humans (25). The effects of prenatal stress exposure can more definitively be studied using animal models. In nonhuman primates, prenatal stress exposure increased anxiety associated behaviours and decreased attention span (26). Several studies in rodent models have shown depression-like behaviour, such as increases in immobility in a forced swim test and decreases in social interaction in rats exposed to prenatal stress (27–30). Additionally, increases in anxiety-like behaviour, such as reduced exploration in an open field test and decreased time spent on the open arm of an elevated plus maze, have been reported (31). With respect to energy metabolism, prenatally stressed (PNS) rats were shown to gain more weight when weaned on a high-fat diet and show signs of impaired glucose

homeostasis (32). Furthermore, deficits in spatial memory and extinction of fear memory have been reported, at least in male offspring (33). Finally, male offspring have been shown to have a more female-like phenotype (34). Although, in general, prenatal stress appears to predispose the offspring to the development of psychiatric and metabolic disorders, the phenotype of these animals is not always consistent and appears to be highly dependent on the type of stressor, the timing of the stress exposure and the sex of the offspring. Below, we discuss some of the potential mechanisms underlying prenatal stress induced alterations in rodents (Table 2).

Effects on neuronal development

The behavioural phenotype of PNS rats may originate in changes in neuronal development. Prenatally stressed offspring are characterised by decreased neuronal proliferation in the hippocampus, nucleus accumbens and stem cells in the subependymal zone of the lateral ventricle in the foetus (35,36). At weaning, these rats show less myelination and greater microstructural impairment in hippocampal neurones (37), as well as apical dendritic atrophy (38). In adolescence and adulthood, exposure to prenatal stress has been shown to alter neurogenesis, neuronal arborisation, neuronal density, dendritic architecture and synaptic connectivity, particularly in the hippocampus and amygdala (39).

Role of glucocorticoids

Recent data show that, according to the type of stress, many organs could be involved in the effects of the prenatal stress. Chronic cold stress during gestation produced modifications in ovarian development and function leading to deficiencies in reproductive function of the female progeny. The mechanism underlying this behavioural phenotype might be alterations in the HPA axis or in the autonomic nerves–brain axis because norepinephrine was lower in the progeny of stressed rats (40). Potentially the most frequently proposed hypothesis on the origin of prenatal stress effects is that increases in maternal glucocorticoid levels during pregnancy lead to changes in foetal development and, consequently, alterations in the phenotype of the offspring. This hypothesis is supported by the observation that the administration of exogenous glucocorticoids induces a phenotype in the offspring similar to that resulting from prenatal stress (41). Further support comes from a study in which the effects of prenatal stress on stress reactivity in the offspring were reversed by adrenalectomising the pregnant dams and clamping corticosterone at baseline levels throughout pregnancy (42). Higher levels of circulating glucocorticoids could affect the development of the offspring through several pathways. Glucocorticoids may directly affect gene expression in the foetus through glucocorticoid receptor binding sites that are present on numerous genes involved in neuronal development (43).

Role of the placenta

Because the placenta is the main barrier between the mother and foetus, alterations in placental function may play a critical role in the effects of maternal stress upon the offspring. For example, the developing foetus is normally protected from high levels of maternal circulating glucocorticoids by the enzyme 11β-HSD-2 in the placenta. This enzyme converts the active cortisol (corticosterone in rodents) to its inactive form, cortisone (44). As a result of this enzyme, the foetal cortisol concentration is approximately 13-fold lower than the maternal cortisol concentrations (45). The effects of prenatal stress on the expression and activity of 11β-HSD-2 in the placenta are somewhat inconsistent; some studies have reported unchanged 11β-HSD-2 expression in PNS rats (46), whereas others report decreased 11β-HSD-2 mRNA expression and activity (47,48). A recent study by Jensen Peña *et al.* (49) suggests that the difference in 11β-HSD-2 expression in the placenta might be mediated by alterations of DNA methylation of the 11β-HSD-2 promoter. Increased DNA methylation was observed at specific CpG sites within the 11β-HSD-2 gene promoter, as well as increased mRNA levels of the DNA methyltransferase 3a (DNMT3a) (49). Additionally, placental expression of glucose transporters is altered in PNS rats and the expression of glucose transporter type 1 (GLUT1) is decreased, whereas GLUT3 and GLUT4 are slightly increased, which suggests that glucose transport over the placenta may be altered in PNS rats (47). Finally, studies in humans suggest that stress during pregnancy may affect blood flow in umbilical and uterine arteries, which would affect the blood circulation and nutrient supply to the foetus (50,51).

The placenta might also provide biomarkers that can be used to predict prenatal stress exposure. Using a genomics and proteomics approach, Howerton *et al.* (52) identified a placental biomarker O-GlcNAc transferase that was significantly down-regulated in PNS male offspring predicted to have a risk phenotype (52).

Early postnatal stress

Stress exposure during the early postnatal period has also been shown to have developmental and health consequences (Table 3). In humans, exposure to childhood abuse or neglect, for example, has been associated with an increased risk of anxiety and mood disorders, obesity, cardiovascular disease and addiction (53). To model early life adversity such as neglect, the maternal separation model has been developed. Although several different experimental paradigms are employed, in general, pups are separated from their mother for 2–8 h for multiple days. Long-term maternally separated rodents consistently show anxiety- and depression-like phenotypes, such as reduced negative-feedback of the HPA axis and neophobia (54,55). However, it appears as though the rat strain plays an important role in the effects of maternal separation because, in Wistar rats, the reports are variable (56–59). Furthermore, maternally separated male and female rats displayed more rapid eye movement sleep (60,61) but no other sleep changes, consistent with those observed in depressed humans, suggesting that these animals exhibit normal sleep regulation. Immune function appears to be affected by maternal separation because studies show that maternally separated mice are more vulnerable to dextran sulphate sodium induced colitis (62), and rats exposed to maternal separation are more susceptible to primary parasitic

Table 2. Summary of Studies Investigating Gestational Stress Discussed in the Present Review (i.e. Not Intended to be an Exhaustive Review of the Topic).

Article		Description stress			Effects on the offspring		
Authors	Reference	Species	Type	Timing	Brain	Endocrine	Behaviour
Khasan *et al.*	21	Human	Adverse life events	–	–	–	↑ schizophrenia
Beversdorf *et al.*	22	Human	Life events	G147–G224	–	–	↑ autism
O'Connor *et al.*	23	Human	Anxiety	G168–G252	–	–	↑ emotional problems
Van den Bergh and Marcoen	24	Human	Anxiety	–	–	–	↑ ADHD ↑ anxiety ↑ externalising problems
Huizink *et al.*	25	Human	Anxiety	–	↓ mental development ↓ motor development	–	–
Schneider *et al.*	26	Rhesus monkey	Noise and hormone	–	↓ motor development	↓ birth weight ↑ cortisol during RST	↓ activity ↓ exploration
Lee *et al.*	27	Rat	CVS	G14–G21	–	–	↓ social interaction
Rayen *et al.*	28	Rat	RS	G15–G20	≈ HPC volume ↓ neuronal proliferation ↓ neurogenesis	–	↑ immobility FST ↓ exploration OF
De Souza *et al.*	29	Rat	RS	G14–G21	↓ oxytocin neurones ↓ vasopressin in PVN	–	↑ anxiety ↑ aggression
Mueller and Bale	30	Mice	CVS	G1–G7	↑ response to citalopram (M) SERT HPC (M) ↑ DNAm GR exon 1(7) (M) ≈ DNAm BDNF, CRF (M)	↑ Corticosterone RST (M) ↑ placental PPARα, IGFBP-1, GLUT4, Hif3α (M) ↑ placental DNMT1	↑ immobility FST, TST ↓ sucrose intake basal ↑ sucrose intake RS
Vallee *et al.*	31	Rat	RS	G14–G21	–	↑ corticosterone during RST	↑ anxiety EMP ↓ exploration OF ≈ MWM ≈ Y-maze
Tamashiro *et al.*	32	Rat	CVS	G14–G21	–	↑ glucose ↑ insulin ↑ body weight	–
Markam *et al.*	33	Rat	CVS	G14–G21	–	–	↑ exploration OF ↓ performance NOR ↓ performance MWM ↓ fear extinction ≈ distinctive memory ↓ spatial memory ↓ visual memory
Morgan and Bale	34	Mice	CVS	G1–G7	↓ masculine gene profile (M) (F2)	↓ Anogenital length (M) (F2) ↓ testis weight (M) (F2) ≈ corticosterone during RST (M,F) (F2)	–
Kippin *et al.*	35	Hamster	RS	G9–G16	↓ neural stem cells	–	–
Kawamura *et al.*	36	Rat	RS	G14–G21	↓ neurogenesis ↓ neuronal proliferation	–	–
Xu *et al.*	37	Rat	RS	G7—G14 or G14–G21	↓ myelination HPC neuronal microstructure impairments HPC	–	–
Jia *et al.*	38	Rat	RS	G14–G20	↑ glutamate HPC ↑ dendritic atrophy pyramidal neurones HPC	–	–
Barra *et al.*	40	Rat	Cold stress	G1–G20	–	↓ sensitivity FSH ↓ follicular recruitment ↓ follicular development	–

(continued)

Table 2 (continued)

Article Authors	Reference	Description stress Species	Type	Timing	Effects on the offspring Brain	Endocrine	Behaviour
Barbazanges *et al.*	42	Rat	RS	G14–G21	↑ type I hippocampal corticosteroid receptors	↑ corticosterone during RST	–
Welberg *et al.*	46	Rat	RS	G14–G21	–	≈ basal 11β-HSD ↓ stress 11β-HSD	–
Mairesse *et al.*	47	Rat	RS	G14–G21	–	↓ placental 11β-HSD ↓ placental GLUT1 ↑ placental GLUT3 ↑ placental GLUT4	–
Lucassen *et al.*	48	Rat	RS + social	G5–G20	↓ neurogenesis HPC	↓ placental 11β-HSD	–
Jensen-Pena *et al.*	49	Rat	CVS	G14–G21	–	↓ placental 11β-HSD ↑ DNAm 11β-HSD promoter ↑ DNMT3a	–
Sjostrom *et al.*	50	Human	Anxiety	–	–	↑ PI values umbilical artery ↓ PI values fetal middle cerebral artery	–
Teixera *et al.*	51	Human	Anxiety	–	–	↑ uterine resistance	–
Howerton *et al.*	52	Mice	CVS	G1–G7	–	↓ placental OGT	–

↑, increased in treatment group; ↓, decreased in treatment group; ≈, no difference; 11β-HSD, 11β-hydroxysteroid dehydrogenase; ADHD, attention deficit disorder; CVS, chronic variable stress; DNMT, DNA methyltransferase; EPM, elevated plus maze; F, female; F2, second filial generation; FSH, follicle-stimulating hormone; FST, forced swim test; G, gestational day; GLUT, glucose transporter; GR, glucocorticoid receptor; HPC, hippocampus; IGFBP, insulin-like growth factor-binding protein; M, Male; MWM, Morris water maze; NOR, novel object recognition; OF, open field; OGT, O-GlcNAc transferase; PPAR, peroxisome proliferator-activated receptors; PVN, paraventricular nucleus; RS, restraint stress; RST, restraint stress test; SERT, serotonin transporter; TST, tail suspension test.

infection and parasite-induced infections (63). Additionally, male maternally separated rats acquire self-administration of cocaine faster and have an increased preference for morphine and ethanol, suggestive of a more addiction-prone phenotype. These changes might be mediated by sex-specific alterations in monamine responses (64). There is more controversy in respect to some of the other reported phenotypes. Maternally separated rodents are reported to have both increased and decreased performance in memory tasks such as the Morris water maze and active avoidance tasks (65,66). In mice, long-term maternal separation leads to chronically increased corticosterone levels and altered negative-feedback within the HPA axis. In addition to parameters related to the HPA axis, alterations in arginine vasopressin (AVP) expression have been reported. Again, changes in DNA methylation may be at the basis of these effects because maternal separation in mice led to hypomethylation of the AVP gene (67).

Short-term (< 24 h) maternal separation has also been shown to have consequences for the adult animal. One important aspect to emphasise when assessing the effects of short-term maternal separation is the age at which the separation takes place. Separation at PND3, for example, resulted in higher HPA axis activity and no changes in anxiety related behaviours, whereas separation on PND11 resulted in lower HPA axis activation and increased anxiety-like behaviour (68). Based on these data, it appears that, depending on the timing of the separation, maternal separation may have both adverse and protective effects on stress responsivity.

Decreased maternal care

Another model for stress exposure during the early postnatal period might be that of offspring raised by dams characterised by low licking and grooming behaviour. Within a natural population, dams differ in the level of maternal care they provide, which can be quantified by the amount of licking and grooming behaviour displayed by the dams. The offspring of low maternal care dams display several neurodevelopmental alterations. Below, we summarise some of these characteristics; however, a full review of the phenotype is provided by Fish *et al.* (69). In general, offspring of low maternal care dams are characterised by impairments in learning and memory tasks such as the Morris water maze and novel object recognition. Furthermore, these offspring have increased levels of novelty-induced anxiety, indicated by increased startle responses, decreased exploration in novel environment and increased latencies to enter a novel environment. Finally, low maternal care exposed offspring display increased corticosterone levels in response to acute stress, a phenomenon that appears to be induced by decreased negative-feedback through the HPA axis. From a neurobiological view, low maternal care offspring display, amongst other factors, decreased expression of the glucocorticoid receptor, brain-derived neurotrophic factor and corticotrophin-releasing hormone (69). Decreased hippocampal expression of the glucocorticoid receptor in low maternal care offspring has been associated with greater DNA methylation of the 1_7 promoter region of the glucocorticoid receptor gene in that

Table 3. Summary of Studies Investigating Early Post-Natal Stress Discussed in the Present Review (i.e. Not Intended to be an Exhaustive Review of the Topic).

Article		Description stress			Effects on the offspring		
Authors	Reference	Species	Type	Timing	Brain	Endocrine	Behaviour
Felitti *et al.*	53	Humans	NS	P0–P18	–	↑ obesity ↑ ischaemic heart disease ↑ cancer ↑ chronic lung disease ↑ skeletal fractures ↑ liver disease	↑ alcoholism ↑ drug abuse ↑ depression ↑ suicide attempt
Ladd *et al.*	54	Rats	MS	P2–P14	↑ HPC MR ↓ cortical and HPC GR	–	–
Caldji *et al.*	55	Rats	MS	P2–P14	↓ GABAA receptor LC, NTS ↓ CBZ AMG, PFC, LC, NTS ↓ gamma 2 subunit GABAA	–	↑ startle ↓ exploration OF ↑ NIS of feeding
Guijarro *et al.*	57	Rats	MS	P2–P14		≈ ACTH and corticosterone	↓ freezing behavior in emotional memory tasks
Leon Roderiguez and Duenas	59	Rats	MS	P2–P14	↓ $GABA_A$ receptor-α-subunit immunoreactivity		↑exploration OF ↑exploration EPM
Tiba *et al.*	60	Rats	MS	P2–P14	–	↑ paradoxical sleep (M)	–
Tiba *et al.*	61	Rats	MS	P2–P14	–	↑ total sleep time (F) ↑% REM sleep (F) ↓ corticosterone to CS	
Milde *et al.*	62	Rats	MS	P1–P14	–	↑ DSS-induced colitis ↑ corticosterone to FS	↑ startle to FS
Barreau *et al.*	63	Rats	MS	P2–P14	–	↑ parasitic infection	–
Kawakam *et al.*i	64	Mice	MS	P1–P14	↑ 5-HT levels in the hippocampus in males ↑ DA and 5-HT levels in PFC in females	↑ corticosterone response to chronic ethanol	
Aisa *et al.*	65	Rats	MS	P2–P14	↓ NCAM ↓ BDNF ↓ synaptophysin ↓ Cell proliferation	–	↓ MWM performance
Pryce *et al.*	66	Rats	MS	P2–P14	nm	≈ corticosterone to CS	≈ MWM performance ≈ ASC ≈ 2wAA
Murgatroyd *et al.*	67	Mice	MS	P1–P10	↑ AVP in PVN ↓ DNA methylation AVP	↑corticosterone	↑ passive stress coping ↓ memory
Weaver *et al.*	70	Rats	MC	P1–P21	↓ GR expression ↑ histone acetylation ↑ DNA methylation GR ↑ NGFI-A binding	–	↑ corticosterone to RST
Roth *et al.*	71	Rats	MS	P1–P7	↓ BDNF PFC ≈ BDNF HPC ↑ DNAm BDNF exon IV ↑ DNAm BDNF exon IX	–	–
Gomes *et al.*	73	Rats	NH	P1–P10	↑LHRH content MPOA	↓ E levels ↓ Pr levels ↓ LH ↓ FSH ↓ PRL anovulatory oestrous cycle	↓ sexual receptiveness

(continued)

Table 3 (continued)

Article Authors	Reference	Description stress Species	Type	Timing	Effects on the offspring Brain	Endocrine	Behaviour
Mazaro and Lamano-Carvalho	74	Rats	NH	P1–P14	–	↓ testicular weight ↓ seminiferous tubule diameter ↓ germinal epithelium thickness ↓ daily sperm production ↓ number of mature spermatids ↓ Sertoli cells ↓ weight and # spermatozoa	–
Raineki *et al.*	75	Rats	NH	P2–P14	–	–	↓ maternal odor preference ↓ adult partner preference
Camazzato *et al.*	76	Rats	NH	P1–P10	↓ cell proliferation MPOA ↓ cell number MPOA	–	–
Raineki *et al.*	77	Rats	NH	P2–P14	↓ NAergic activity ↓ NO levels	–	–

↑, increased in treatment group; ↓, decreased in treatment group; ≈, no difference; 2wAA, two-way active avoidance; AMG, amygdala; AVP, arginine vasopressin; Bdnf, brain derived neurotrophic factor; CS, cold stress; DDS, dextran sodium sulphate; E, oestradiol; EPM, elevated plus maze; F, female offspring; FS, foot shock; FSH, follicle-stimulating hormone; GABA, gamma-aminobutyric acid; GR, glucocorticoid receptor; HYPO, hypothalamus; HPC, hippocampus; LC, locus coeruleus; LH, luteinising hormone; LHRH, luteinising hormone releasing hormone; M, male offspring; MWM, Morris water maze; MHPG, 3-methoxy-4-hydroxyphenylglycol; MPOA, medial preoptic area; MR, mineralocorticoid receptor; MS, maternal separation; NCAM, neural cell adhesion molecule; NH, neonatal handling; NIS, novelty induced suppression; NS, not specified; NTS, nucleus tractus solitarii; OF, open field; Pr, progesterone; P, postnatal day; PFC, prefrontal cortex; PVN, paraventricular nucleus; PRL, prolactin; REM, rapid eye movement; RST, restraint stress test.

brain region (70). Additionally, exons IV and VI of brain-derived neurotrophic factor in the hippocampus were found to be hypermethylated in rats exposed to low maternal care (71).

Neonatal handling

By contrast to the effects of maternal separation or decreased maternal care, neonatal handling, a procedure during which rodent offspring are briefly separated from the dam and handled during the first week of life (72), appears to have clear protective effects against adverse behavioural phenotypes. Neonatally handled rats have reduced stress responses and increased exploratory behaviour in novel environments. However, neonatal handing reduces reproductive behaviour in both males and females (73–75). In males, lower testicular weight and reduced seminiferous tubule diameter and germinal epithelium thickness have been reported. This results in a reduction in sperm production, leading to decreased reproductive success in male rats (74). Whether the neonatal handling procedure leads to alterations in brain areas important for reproduction in males has, to our knowledge, not been studied. In females, the effects of neonatal handling on reproductive function may be mediated by decreased hormone activity because plasma levels of luteinising hormone (LH), follicle-stimulating hormone (FSH) and prolactin were decreased during the pro-oestrous in handled rats. Furthermore, the number of cells in the medial preoptic area (mPOA) was decreased in neonatally handled female rats (76). Additionally, neonatal handling decreased activity of the noradrenergic and nitric oxidergic systems in the mPOA during pro-oestrus, which is involved in the control of LH and FSH secretion (77). Although considered as a mild environmental intervention, the neonatal handling procedure may induce stable changes in relevant neuroendocrine systems, such as the HPA and hypothalamic pituitary gonadal axis.

Diet

In addition to stress exposure, nutrition during the perinatal period may also influence the susceptibility to metabolic, immunological and cognitive disorders. Both over-nutrition and under-nutrition during the perinatal period have been associated with adverse health consequences later in life. Below, we discuss the effects of diet throughout the perinatal period (Table 4).

Pre-conception diet

It is well documented that both maternal obesity and being underweight have clear consequences for reproductive success, and several studies suggest that maternal weight may influence the health of the offspring. The use of animal models gives us the opportunity to tease apart the effects of maternal diet, maternal weight and maternal metabolic status, which could help with respect to understanding the underlying mechanisms for adverse outcomes in offspring.

Maternal pre-conception diet

Most studies investigating maternal nutrition have focused on maternal high-fat diet intake. By contrast, there is relatively less known about the effects of pre-conception high or low protein or carbohydrate diets on the phenotype of the offspring. With regard

Table 4. Summary of Studies Investigating Peri-Natal Diet Discussed in the Present Review (i.e. Not Intended to be an Exhaustive Review of the Topic).

Article		Description diet			Effects on the offspring	
Authors	Reference	In	Type	Timing	Brain	Endocrine
White *et al.*	78	Rats	Maternal HF	Prior to mating	–	≈ body weight ≈ glucose tolerance
Carone *et al.*	79	Mice	Paternal HF	Prior to mating	–	↑ hepatic expression lipid genes ↑ cholesterol biosynthesis genes ↓ cholesterol esters
Ng *et al.*	80	Rats	Paternal HF	Prior to mating	–	↑ body weight, ↑ adiposity, ↓ glucose tolerance, ≠ gene expression pancreas
Dunn and Bale	81	Mice	Maternal HF	4 weeks prior to mating + G1–G21 + L1–L21	↓ GHSR transcriptional repression	↑ body length ↑ body weight ↑ plasma leptin ↓ insulin sensitivity
Sun *et al.*	82	Rats	Maternal HF	G1–G21 + L1–L21	↓ leptin sensitivity	↑ body weight, ↑ adiposity ↓ glucose tolerance
Zheng *et al.*	85	Rats	Maternal HF	G1–G21 + L1–L21	–	↓ p16 expression mammary gland
Dudley *et al.*	86	Rats	Maternal HF	G1–G21 + L1–L21	–	↓ liver weight ≠ gene expression liver
Jimenez-Chillaron *et al.*	87	Mice	Maternal FR	G12.5–G18.5	–	↑ basal secretion insulin ↓ glucose responsivity ↑ hexokinase activity
Wilson and Hughes	88	Rats	Maternal PR	G1–G21 + L1–L21	–	↓ glucose-stimulated insulin release ↓ glucose tolerance
Hyatt *et al.*	91	Sheep	Maternal PR	G30–G80	–	↑ hepatic triglyceride, ↑ Ppargamma, ↑ PGC1α
Vieau *et al.*	92	Rats	Maternal FR	G14–G21 +L1–L21	–	↓ HPA axis activity in pups ↑ HPA axis activity adult
Burdge *et al.*	93	Rats	Maternal PR	G1–G21	–	↓ hepatic PPARα promoter methylation ↓ hepatic GR promoter methylation
Jing-Bo *et al.*	94	Pigs	Maternal PR	G1–G114	–	↑ hepatic GR ↑ PPARα, ↑ PPARgamma ↑ fatty acid synthase ↑ PEPCK
Plagemann *et al.*	99	Rats	Small size litters	L1–L21	–	↑ body weight ↑ adiposity ↓ glucose tolerance
Velkoska *et al.*	100	Rats	Small size litters	L1–L21	–	↑ body weight ↑ 11β-HSD in white adipose tissue ↓ UCP-1 in brown adipose tissue
Xiao *et al.*	101	Rats	Small size litters	L1–L21	–	↑ body weight, ↓ UCP-1 in brown adipose tissue, ↓ sympathetic β 3-adrenergic receptor ↓ response to isoproterenol
Rodrigues *et al.*	102	Rats	Small size litters	L1–L21	≈ leptin signalling in HYPO ↑ leptin receptors (Ob-R) in PIT ↑ JAK2 and ↑ p-STAT3 in PIT	↑ visceral fat ↑ TSH, ↑ T3 and ↑ T4 in pups ↓ T3 and ↓ T4 in adults
Leonhardt *et al.*	103	Rats	Maternal FR	L1–L21	–	↓ body weight ↓ adiposity ↓ leptin ↓ FSH ↓ onset puberty

(continued)

Table 4 (continued)

Article Authors	Reference	Description diet In	Type	Timing	Effects on the offspring Brain	Endocrine
Moura *et al.*	104	Rats	Maternal PR	L1–L10	–	↓ insulin secretion ↓ glucose tolerance
Laborie *et al.*	105	Rats	Maternal FR	G7–G21 + L1–L21	–	↑ responsiveness sympathoadrenal system ≠ adrenomedullary gene expression
Sebaai *et al.*	106	Rats	Maternal FR	G1–G21 + L1–L21	↓POMC	↑ HPA axis activity adult
Reyes-Castro *et al.*	107	Rats	Maternal FR	L1–L21		↑ HPA axis activity adult

↑, increased in treatment group; ↓, decreased in treatment group; ≈, no difference; ≠, altered in treatment group; 11β-HSD, 11β-hydroxysteroid dehydrogenase; FR, food restriction; FSH, follicle stimulating hormone; G, gestational day; GHSR, growth hormone secretagogue receptor; HF, high-fat diet; HPA, hypothalamic-pituitary-adrenal; HYPO, hypothalamus; L, lactation day; P16, cyclin-dependent kinase inhibitor 2A; PEPCK, phosphoenolpyruvate *carboxykinase*; PIT, pituitary; POMC, proopomelanocortin; PPAR, peroxisome proliferator-activated receptor; PR, protein restriction; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; UCP, uncoupling protein; VEGF, vascular endothelial growth factor.

to pre-conception high-fat diets, most of these studies have not separated maternal obesity effects from maternal diet effects. An exception is a study by White *et al.* (78) comparing female rats fed a high-fat diet with those that were pair-fed to a low-fat diet so that they would ingest a high-fat diet in the absence of the associated weight gain. Interestingly, the results obtained suggested that the metabolic impairments in the offspring were a result of maternal obesity rather than maternal diet because no impairments were observed in the offspring of non-obese high-fat diet fed dams.

Paternal pre-conception diet

The paternal diet may also affect the offspring's phenotype. Offspring of males fed a low protein diet prior to mating were shown to have decreased levels of cholesterol esters. Additionally, these rats had increase expression of genes involved in the lipid and cholesterol biosynthesis in the liver (79). A paternal high-fat diet led to increased body weight, greater adiposity and impaired glucose homeostasis in female offspring. The alterations in glucose homeostasis in these female offspring were attributed, at least in part, to changes in the expression of genes in pancreatic islet cells (80). It is very likely that there is an epigenetic mechanism underlying the offspring's phenotype. This hypothesis is supported by the observation that the offspring of fathers on a low protein diet showed an increase in methylation of a CpG island upstream of the Ppar-α gene, a transcription factor involved in the lipid metabolism in the liver. (79). In female offspring of high-fat diet fed fathers, hypomethylation of the interleukin-13-α-receptor-2 promoter has been reported (80). These studies suggest a role of DNA methylation in paternal diet-induced alterations in the offspring.

Prenatal diet

Prenatal over-nutrition

Several different models of maternal over-nutrition have been employed to investigate the effects of maternal diet on the offspring. Although there were differences in severity of symptoms, regardless of whether the dams were fed a high-fat diet, a high-fat/high sucrose diet or received a high-fat diet gastric infusion, the adult offspring of over-fed dams typically display elevations in body adiposity and abnormalities in glucose homeostasis (81,82); for a systematic review, see Ainge *et al.* (83). A maternal high-fat diet is suggested to alter hypothalamic signalling in the offspring, which may lead to the observed metabolic abnormalities. Prenatal high-fat diet offspring were, for example, shown to be less sensitive to leptin (82). However, the results also suggest that the timing of the diet exposure plays an important role because cross-fostering of high-fat diet pups, on PND1, to control diet fed dams and vice versa implicated the importance of the diet during the lactation period. Furthermore, exercise during the early postnatal period can reverse the prenatal high-fat diet-induced changes in leptin signalling and the consequent metabolic phenotype (84). It is suggested that epigenetic alterations may play a role in the effects of a maternal high-fat diet. The effects of a prenatal high-fat diet on the epigenome have only recently been studied. A study examining in the effects of a prenatal high-fat diet on breast cancer risk reports that a prenatal high-fat diet induced down-regulation of p16 (INK4a) transcription in the mammary gland and that this was associated with reduced acetylation of histone H4 and increased recruitment of histone deacetylase 3 within the p16 (INK4a) promoter region (85). The hepatic cell cycle inhibitor Cdkn1a was shown to be hypomethylated at specific CpG dinucleotides and its first exon, which may indicate that the prenatal diet may modulate hepatic metabolism through alterations in DNAm (86).

Prenatal under-nutrition

Studies investigating maternal under-nutrition can generally be divided into two groups: those that restrict total diet intake and those that restrict the intake of a specific nutrient, most commonly proteins. Although there are differences in the effects of both paradigms, both have been linked to enhanced metabolic risk. Specifically, maternal under-nutrition has been associated with impaired

pancreas development, resulting in a decrease of insulin production in β-cells of up to 25% (87,88), impaired glucose tolerance and greater insulin resistance (88,89). Furthermore, offspring of under-nourished dams were shown to have an increased risk to hypertension (90) and decreases in lipid metabolism in the liver have been reported (91). In addition to metabolic derangements, maternal under-nutrition has been associated with abnormalities in brain development and HPA axis function. Interestingly, maternal under-nutrition lead to reduced HPA axis activity in the foetus, which is associated with decreased placental 11β-HSD-2 activity. However, in the adult offspring, maternal under-nutrition leads to hyperactivity of the HPA axis, resulting in higher circulating glucocorticoid levels (92). These alterations in HPA axis development and function may play an important role in risk with respect to developing depression and anxiety disorders. There are several epigenetic pathways that may underlie the phenotype: first, the offspring of a low protein diet were shown to have decreased DNA methylation of promoter regions of the Ppar-α and GR genes (93). Similar alterations in DNA methylation of these genes were observed in intrauterine growth restricted piglets (94), suggesting common pathways.

Deficits in placental function resulting from alterations in maternal diet could also influence an offspring's phenotype. Maternal under-nutrition results in decreases in the expression of glucose transporter 3 and cationic amino acid transporter, two nutrient transporters that are critical in transferring nutrients from maternal circulation to the foetus (95). Additionally, maternal under-nutrition in rats led to vascular dysfunction in the placenta, a process that is likely mediated by vascular endothelial growth factor, which stimulates the release of nitric oxide (NO) which in turn was shown to be less synthesised and active in the placenta's of protein-restricted pigs (96). Interestingly, placentas derived from intrauterine growth-restricted (IUGR) foetuses were shown to have decreased vascular endothelial NO synthase (eNOS) activity, whereas arterial eNOS activity was increased (97). IUGR is one of the major consequences of maternal under-nutrition that could affect or interact with some of the effects of maternal under-nutrition. IUGR is defined in humans as having a weight below the 10th percentile for gestational age. The behavioural phenotype of IUGR rats is remarkably similar to that of maternally under-nutrition offspring, even when IUGR is not induced by changes is nutrient content, which suggests that common underlying pathways may play a role. The changes in eNOS activity may again be controlled by epigenetic modifications because alteration in DNA methylation of the eNOS3 promoter was observed in the umbilical artery and vein endothelial cells of IUGR compared to normal foetuses (97,98). These alterations in turn may be mediated by DNA methylatransferase 1 (DNMT1) because DNMT1 knockdown restored eNOS levels in IUGR umbilical artery and vein endothelial cells to control levels (97,98).

Early postnatal diet

The diet during the early postnatal period, during lactation, may have important consequences for the development of metabolic and other disorders. Although there is a vast body of literature on the influences of several micronutrients during lactation on the development of the child, such as folic acid and vitamin D, we focus on caloric over-nutrition and under-nutrition during this time period.

Early postnatal over-nutrition

Cross-fostering studies, in which rats from control dams were cross-fostered to dams fed a high-fat diet, can be used assess the effects of early postnatal over-nutrition, independent of the prenatal maternal diet. This work suggests that the exposure to a high-fat diet during lactation leads to impaired metabolic parameters in adulthood (99). However, in these studies, the possibility remains that changes in the offspring induced through the diet during gestation still linger on during lactation. A well established model for early postnatal over-nutrition in the rodent is litter size reduction, in which litters of healthy control dams are reduced in size to increase milk availability for the remaining pups. Adult rats raised in small litters are characterised by hyperphagia, increased body adiposity, hypertension, insulin resistance and leptin resistance (100,101). Furthermore, energy expenditure may also be affected by over-nutrition during lactation. In brown adipose tissue, expression levels of uncoupling protein 1 were decreased in rats raised in small litters and sympathetic outflow was decreased in these rats (102). Additionally, the activity of the thyroid is altered in rats raised in small litters; at weaning, higher levels of triiodothyronine, thyroxine and thyroid-stimulating hormone were reported, whereas these same hormones were decreased in adulthood compared to rats raised in control litters (103). Overall, the increased energy intake and decreased energy expenditure observed in the small-litter rats may contribute equally to the obesity prone phenotype of these rats. To date, alterations in leptin signalling have been implicated to play a crucial role in both the altered energy intake and the energy expenditure pathways induced by early postnatal over-nutrition (101,103) and studies are ongoing to unravel the contribution of epigenetic processes in these leptin dependent pathways.

Early postnatal under-nutrition

By contrast to maternal over-nutrition or maternal under-nutrition during gestation, maternal under-nutrition during lactation has not been associated with obesity. Rather maternal under-nutrition during lactation led to decreased body weight, decreased adipose weight and decreased leptin levels in adulthood (104). Although normal glycaemia is maintained in general, there are alterations in glucose homeostasis in these rats. The vagal tone to the pancreas is decreased, leading to lower insulin secretion in rats under-nourished during lactation (105). Maternal under-nutrition during lactation may, however, have large consequences for the stress responsivity because increased HPA axis activity and decreased sympathetic activity have been reported (106,107). It may be argued that these alterations in the stress system may prime these under-nourished rats for stress, depression or anxiety-like phenotypes in adulthood, although a recent study reports that under-nutrition during lactation did not influence anxiety or stress-related behaviours

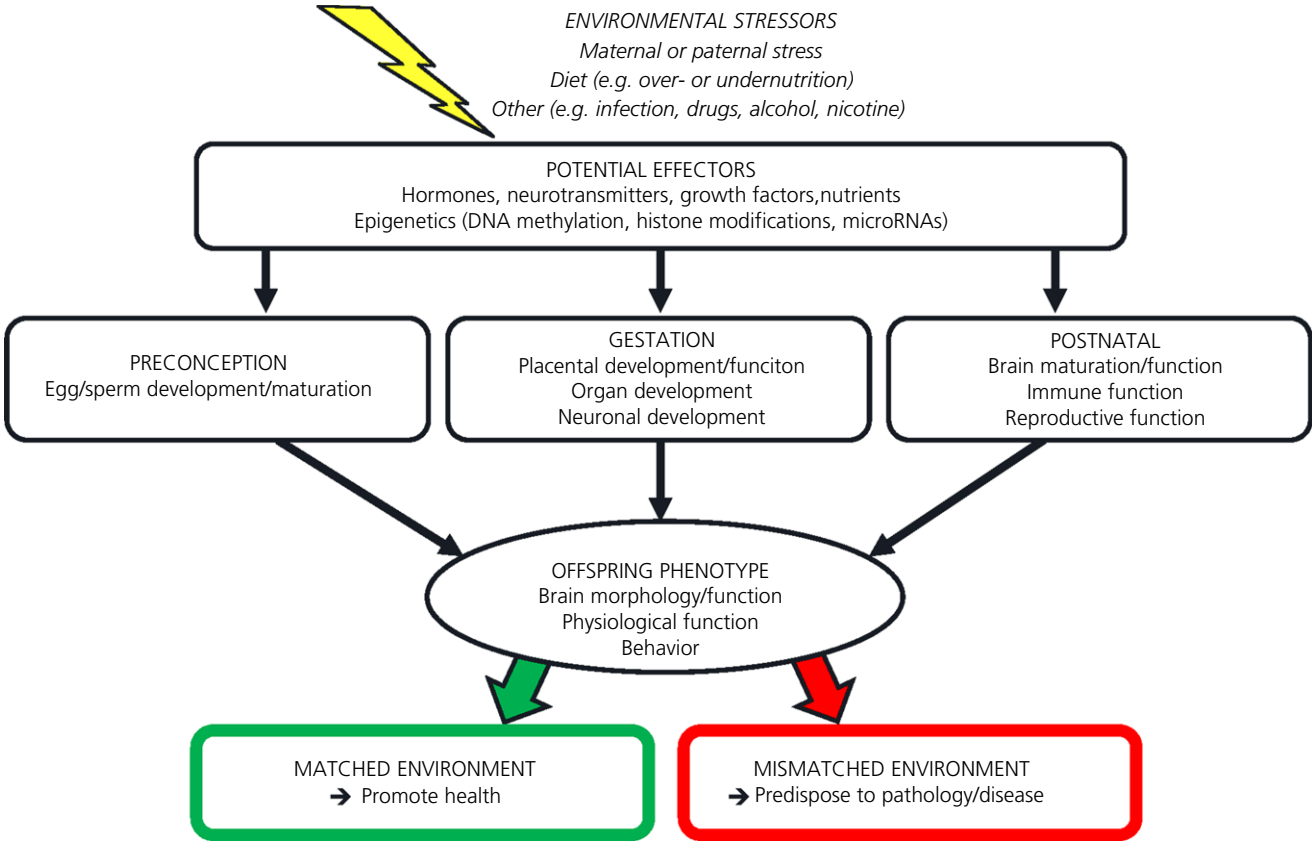

Fig. 1. Environmental stressors may be communicated to developing offspring via different effectors including, but not limited to, circulating factors and epigenetic modifications. Critical periods during development include the pre-conception, gestation and early postnatal environments. Exposure to stressors during these critical periods can influence the development of specific tissues and organ systems, resulting in alterations in structure and function. Ultimately, the effects of the perinatal environment on the phenotype of the offspring depends on whether the perinatal adaptations of the offspring match (promoting health) or are mis-matched with (leading to pathology and disease) the later postnatal environment.

in elevated plus maze or open field tests (98). By contrast, malnutrition during lactation does impair learning and memory processes and motivation to work for a food reward (108,109).

Future perspectives

It is clear that dietary and other environmental perturbations during the peri-natal period have major consequences for the development and health of the offspring. In general, it may be argued that anything signalling potential adverse conditions later in life, such as high levels of stress or low levels of food availability, will lead to alterations in the offspring that will prepare the offspring for these conditions later in life. However, when similar environmental conditions are not met in adulthood, these alterations will have maladaptive consequences, such as obesity and heightened stress sensitivity. Based on this hypothesis, all alterations induced by peri-natal stress should have an adaptive origin. This case is easy to make when focusing on energy balance and stress phenotypes. It may be argued that the anxiety prone phenotype, for example, is a more conservative and safe phenotype under 'dangerous' highly stressful situations. Furthermore, the differences in offspring phenotypes observed among varying stress paradigms may represent adaptations that are specific to the environmental stressors expected. However, the deficits in memory and learning observed in several of the peri-natal stress paradigms are more difficult to explain from this evolutionary adaptation hypothesis. Several studies show decreased memory formation, as well as decreases in neuronal plasticity, in peri-natally stressed individuals. It is not clear how decreased memory formation might have adaptive properties under stressful conditions. An additional question that remains is how the timing of a stressor may lead to differential phenotypes. One may expect that the phenotype might be dependent on the neuronal development ongoing at the time of the stressor. However, the data presented suggest that, although the mechanism underlying these adult phenotypes might be dependent on the timing of the exposure, the final phenotype is remarkably similar (Fig. 1).

By contrast to this adaptation hypothesis are studies looking at peri-natal over-nutrition. In this situation, the logical argument is that the peri-natal environment would signal an abundant environmental condition, which would suggest that, in the offspring, either a control phenotype would be observed or even a very lean phenotype. However, peri-natally overfed rats display an obesity prone phenotype. One possible explanation for this is that the peri-natal exposure to a high-fat diet might be signalling fluctuating

environmental conditions, which could merit an energy-conserving response. Alternatively, the effects of peri-natal over-nutrition might be a maladaptive response to begin with, meaning that the diet leads to a pathological condition early in life, which is in contrast to the potentially adaptive responses observed in the peri-natal over-nutrition and stress studies, where a mismatch with the later life environment is causal to the pathological phenotype. This last hypothesis might be strengthened by the observation that early exposure to voluntary running, leading to normalisation of circulating nutrient and hormone levels, will counteract the effects of peri-natal over-nutrition (110). According to the fluctuating environmental conditions hypothesis, alterations in activity levels should again signal fluctuating conditions and thus amplify the obesity prone phenotype. Additional studies looking at common underlying pathways and different pathways within the different environmental perturbation models rather than within one model and a control should be carried out aiming to determine the evolutionary background of these maladaptive phenotypes resulting from peri-natal perturbations.

In the present review, peri-natal stress and peri-natal diet have been discussed separately; however, it is likely that these peri-natal perturbations have overlapping mechanisms. Alterations in peri-natal diet may induce physiological and potentially even psychosocial stress in the parents, which in turn may affect the offsprings' phenotype. In a similar fashion, peri-natal stress may affect parental nutrition because stress has been shown to lead to both hyper- and hypo-phagia in different models, and thereby the effects of peri-natal stress exposure may be partly mediated by effects on the peri-natal diet. To date, few studies have investigated these potential interactions and future studies might focus on this.

Although the role of gene–environment interactions has not specifically been discussed in the present review, they could play a significant role in some of the observed phenotypes, particularly in human literature. Studies in individuals with a polymorphism in the serotonin transporter gene, for example, show that these individual have an increased risk for psychiatric disorders after exposure to early life stress (111), which suggests that individuals may respond differently to early life perturbations depending on their genetic phenotype. Further research is necessary to understand how the early life environment, the epigenome and the genotype of the individual interact to determine their susceptibility for psychiatric, metabolic and other pathologies later in life. Finally, research needs to be designed aiming to understand how we can manipulate the environmental and epigenetic factors to prevent or treat these disorders in individuals who are at risk.

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