

REVIEW

The future is now: early life events preset adult behaviour*

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Abstract

To consider the evidence that human and animal behaviours are epigenetically programmed by lifetime experiences. Extensive PubMed searches were carried out to gain a broad view of the topic, in particular from the perspective of human psychopathologies such as mood and anxiety disorders. The selected literature cited is complemented by previously unpublished data from the authors' laboratories. Evidence that physiological and behavioural functions are particularly sensitive to the programming effects of environmental factors such as stress and nutrition during early life, and perhaps at later stages of life, is reviewed and extended. Definition of stimulus- and function-specific critical periods of programmability together with deeper understanding of the molecular basis of epigenetic regulation will deliver greater appreciation of the full potential of the brain's plasticity while providing evidence-based social, psychological and pharmacological interventions to promote lifetime well-being.

Keywords adolescent obesity, behaviour, early life stress, epigenetics, psychopathology.

The future is now. From conception onwards, our physical and social environments trigger a series of biochemical, morphological and physiological responses that act on the genetic blueprint to adjust development and lifelong programs of somatic and mental function. This continuous gene–environment dialogue can lead to persistent ‘anticipatory’ changes in the phenotype. This essential process is frequently referred to as ‘programming’ and allows the organism to make homeostatic adaptations (including resetting of physiological sensors and thresholds) that ensure compatibility with changing environments and demands and therefore survival. In some cases, however, programming may result in maladaptation due to inherent (genetic) constraints, asynchronous or exaggerated adjustments in the chain of responses leading to elicitation of an overt physiological or behavioural output, or inflexibility induced by a preceding (programming) event. Epigenetic mechanisms – involving methylation or hydroxymethylation of DNA

[at cytosine–phosphate–guanine dinucleotide sites (CpG)] or post-translational chromatin modification (histone acetylation, methylation, ubiquitination, phosphorylation, sumoylation) – are emerging as a powerful way to explain *long-term* (or even *lifetime*) programming and reprogramming of phenotype. Other authors have provided comprehensive reviews of the mechanisms underlying the genome–environment dialogue and determination of phenotype (Meaney 2010, Hoffmann & Spengler 2012, Baker-Andresen *et al.* 2013, Hunter & McEwen 2013).

The term ‘epigenome’ refers to the ensemble of coordinated epigenetic marks that govern accessibility of the machinery driving gene expression to DNA. Specific histone modifications and DNA methylation reciprocally control each other's deposition whereby inaccessible genes are silenced and accessible ones can be actively transcribed. Importantly, epigenetic modifications such as DNA methylation do not alter the genetic sequence itself but control the accessibility of

regulatory factors to the control regions of genes. Chromatin remodelling by histone modifications is generally considered to be reversible, allowing transient adaptations in physiology and behaviour. On the other hand, DNA methylation results in more stable, if not irreversible, changes that manifest themselves in long-term altered patterns of gene expression, often interpreted as the encoding of 'memories' into genes. It is important to emphasize at the outset, however, that epigenetic alterations in gene activity occur in a dynamic (sometimes transient) fashion (see Ptashne 2013). Not every stimulus necessarily evokes an epigenetic adjustment, even though the overall response may be influenced by previous (epigenetic trace leaving) experience. Interestingly, DNA methylation does not occur in all organisms (e.g. flies, worms); yet, all organisms survive by making *immediate (acute)* biochemical and physiological responses to multitudinal challenges received on a time scale ranging from milliseconds to hours or days, by primarily exploiting their genetically determined cellular hardware.

The epigenetic dimension of neuroplasticity

Epigenetic programming plays an important role in cancer but also in the regulation of the physiology and pathology of other proliferating cells. Although the view that memory could be encoded in DNA more than 40 years ago (Griffith & Mahler 1969), the idea that postmitotic neurones have the potential to be epigenetically programmed by experience (learning) gained broad currency only in the last few years.

The developing brain is highly plastic during gestation and early postnatal life (Toga *et al.* 2006). It is estimated that the embryonic human brain grows by some 250 000 new cells per minute (Cowan 1979) that eventually organize themselves into functional units. During the last trimester of pregnancy, synapses are formed at the rate of 40 000 min⁻¹ (Bourgeois 1997), and efficient transmission is ensured through myelination of axons. However, suggestions that the existence of windows ('sensitive' or 'critical' periods), during puberty and perhaps even beyond, when the architecture of the brain is reorganized in response to changing environments, is supported by neuroimaging studies that have revealed the dynamic growth and functional connectivity (during the first 2 years); also to be noted is that neurons in the human brain undergo extensive pruning and reorganization from early postnatal life right through early adulthood, but especially during puberty (Huttenlocher & Dabholkar 1997, Levitt 2003, Knickmeyer *et al.* 2008, Gao *et al.* 2011, Jernigan *et al.* 2011). Moreover, it is now known that certain brain regions can produce new neurones even in adulthood (Eriksson *et al.* 1998,

Kempermann 2012), expanding the plastic potential of the brain in terms of connections and incorporation of new epigenetic information (Ma *et al.* 2010, Castrén & Hen 2013, Mateus-Pinheiro *et al.* 2013). Thus, experiential memories can be laid down through epigenetic marking (DNA and histone memories) and/or histomorphological adaptations (structural memories).

There is accumulating evidence that programming of physiological and behavioural functions is not limited to the intra-uterine or neonatal period. For example, recent studies show that adolescence and puberty, as well as adulthood, represent permissive windows for programming by stress (Lupien *et al.* 2009, Beardslee *et al.* 2012, Hunter & McEwen 2013) and nutritional status (Pervanidou & Chrousos 2012) in rodents and non-human primates. Conditions such as stress and obesity, on the one hand, and obesity and mental well-being, on the other, appear to be interdependent (Dauncey 2013, Sinha & Jastreboff 2013); accordingly, it will be interesting to tease apart the contribution of the individual stimuli to the programming of physiology and behaviour. Other open questions concern how social history and the social context in which exposure occurs factor into the complex equation that defines phenotype, in particular psychopathological phenotypes (see Rutter 2012), and the mechanisms through which epigenetic programs are apparently transmitted across generations (Bohacek *et al.* 2012). Recently, Keverne (2013) suggested that the mechanisms that predispose an individual to epigenetic changes, rather than parental adaptations, can be transferred to subsequent generations (cf. Niwa *et al.* 2013).

Adverse lifetime events and mental health

Developmental psychobiologists have long been interested in the 'Nature vs. Nurture' question (for a comprehensive review, see Sameroff 2010). This area has recently generated much interest in psychiatry and neurology with growing recognition that historical (lifetime) events can have an impact on the etiopathogenesis of mood, affective and cognitive disorders (Tsankova *et al.* 2007, Lupien *et al.* 2009). For instance, trauma during childhood correlates with risk of developing anxiety and major depression in adulthood (Heim & Binder 2012), and parent–infant relationships are reflected in executive functioning (Bernier *et al.* 2010, Hedges & Woon 2011); similarly, early life adversity is associated with significant cognitive and emotional impairments in mice (Murgatroyd *et al.* 2009, Meaney 2010).

The above observations illustrate that brain health, like somatic health (Gluckman & Hanson 2004, Wells

2007, Hanson & Gluckman 2011), is subject to adaptive programming induced by environmental cues. Notably, an authoritative policy paper observed that the 'mental wealth of nations' can be potentially improved by considering improvements in the social, economic health and educational environment that individuals are exposed to over the whole lifespan (Beddington *et al.* 2008), findings echoed in other studies (Duncan *et al.* 2010, Santos *et al.* 2012). Further, it is important to note that lifetime events do not necessarily leave an irreversible imprint on the brain. This is particularly well exemplified by research from the Bucharest Early Intervention Project (www.bucharestearlyinterventionproject.org/), which shows that foster care can, at least partially, reverse impairments in brain activity and cognition suffered by orphaned children (Smyke *et al.* 2010, Vanderwert *et al.* 2010, Fox *et al.* 2011, Sheridan *et al.* 2012, also see Rutter & O'Connor 2004). Moreover, our own studies have shown spontaneous recovery from stress-induced neuromorphological and behavioural changes in the rat brain (Sousa & Almeida 2012) and pharmacological correction of early life stress (ELS)-induced alterations in neuroendocrine, emotional and cognitive impairments in mice (Murgatroyd *et al.* 2009). The ability to effectively alter environment-triggered alterations in brain structure and function most likely depends on the quality, magnitude and duration of the initial 'insult' as well as on the exploitation of critical 'windows of interventional opportunity'.

The present review focuses on the epigenetic regulation of gene expression by ELS and its role in establishing neuroendocrine and behavioural phenotypes relevant to neuropsychiatric conditions. There are now strong links between stress (acute and accumulated) and disorders of cognition, mood and affect (Holsboer & Ising 2010, Sousa & Almeida 2012), and drug and alcohol abuse (Rodrigues *et al.* 2011); further, stress (and depression) is thought to predispose individuals to Alzheimer's disease (Sotiropoulos *et al.* 2011). In addition, stress is causally linked to metabolic disorders, such as overweight, obesity and diabetes (Noll *et al.* 2007, Scott *et al.* 2008, Kivimäki *et al.* 2011); on the one hand, these conditions are considered to be important risk factors for disturbances in mood, affect and cognition (Noll *et al.* 2007, Scott *et al.* 2008, Xu *et al.* 2011), and on the other hand, they may influence the efficacy of pharmacotherapies for certain brain disorders (Kloiber *et al.* 2007). Some of the best illustrations of how lifelong energy homeostasis and susceptibility to metabolic and cardiac disease are programmed by the intra-uterine environment and comes from studies on foetal nutrition (Kyle & Pichard 2006, Wells 2007, Gluckman 2011, Lillycrop & Burdge 2011).

Increased glucocorticoid (GC) secretion is a key physiological response to stress. This response normally serves to restore physiological and behavioural homeostasis through actions in a variety of cells, tissues and organs, including the brain. As their name implies, a major role of GC is to regulate energy (glucose) mobilization with concomitant increases in insulin secretion during stressful periods; their contribution to the adaptive response to stress includes suppression of immune and reproductive functions and increased cardiac output and blood supply to essential central and peripheral tissues (Selye 1998), as well as a diverse set of behavioural changes (e.g. hyperarousal and recall of contextual memories). However, the adaptation-promoting actions of GC can become deleterious for the organism when the regulatory mechanisms that operate to eventually restrain GC secretion go awry, especially when the type, strength or duration of the stressor exceeds certain thresholds.

Persistent GC hypersecretion may be considered as 'malprogramming' insofar that the rheostatic mechanisms that regulate the quality and magnitude of neuroendocrine responses (and subsequently, physiological, metabolic and behavioural responses) become dysregulated and compromise health and future adaptation. Excess GC levels in adults have been linked to major depression and anxiety (Holsboer & Ising 2010) and a variety of other psychiatric, metabolic and immune disorders (Chrousos & Kino 2007, Aguilera 2011); in addition, high levels of GC are associated with impairments in certain cognitive domains (Marin *et al.* 2011). Interestingly, both the mood- and cognition-impairing effects of GC in humans have been shown to be (at least transiently) reversible after correction of the underlying endocrine anomalies (Hook *et al.* 2007, Schatzberg & Lindley 2008). While the ability of GC to alter adult brain architecture and connectivity in humans and experimental animals (Marin *et al.* 2011, Sousa & Almeida 2012) is established, the influence of adverse early life experiences (and early life exposure to GC) on lifelong dysregulation of behavioural and GC secretion has only relatively recently become a major area of research.

Prenatal stress and GC exposure

Being small, lipophilic molecules, GC can readily traverse the placental barrier and influence brain development *in utero*, placing the foetuses of stressed mothers at special risk. Also at risk, are the offspring of women at risk of premature delivery because these subjects often receive GC therapy to accelerate maturation of the foetal lung (Hallman *et al.* 2010). The

placenta has its own protective mechanism to reduce GC load on the foetus; it expresses high levels of the enzyme 11 β -hydroxysteroid dehydrogenase 2 (11 β -HSD2), which converts active endogenous GC (cortisol and corticosterone) into inactive metabolites (cortisone and 11 β -dehydrocorticosterone) that cannot activate glucocorticoid receptors (GR; Wyrwoll *et al.* 2011) (synthetic GCs, such as dexamethasone, that are used for therapeutic purposes are, however, not 11 β -HSD2 substrates). Interestingly, 11 β -HSD2 is also expressed in the brain, including areas involved in the control of emotion and cognition (Wyrwoll *et al.* 2011), thus serving as an additional protective gate against elevated GC levels.

The brain programming potential of prenatal GC exposure was recently reviewed by us (Mesquita *et al.* 2009, Rodrigues *et al.* 2011). We previously showed that treatment of rat dams with dexamethasone during the last trimester of pregnancy results in offspring that have a marked anxious phenotype (Oliveira *et al.* 2006). We also found that intra-uterine exposure to dexamethasone reduces the number of dopamine-producing cells and dopaminergic tone in the mesolimbic (ventral tegmentum–nucleus accumbens, VTA-NAc) reward pathway, with a significant reduction in the volume of NAc dendrites (Leão *et al.* 2007). Behavioural correlates of these neuromorphochemical changes were subsequently found: antenatal dexamethasone-treated animals displayed greater sensitivity to the psychostimulatory actions of the opiate morphine and increased preference for alcohol and morphine (drug-seeking phenotype) when tested as adults (Rodrigues *et al.* 2012). Molecular analyses carried out in the same study identified an epigenetic basis for these observations. Specifically, they revealed that prenatal dexamethasone leads to abnormal methylation of regulatory regions of the gene encoding the dopamine receptor (*Drd2*) and increased mRNA levels of *Drd2* in the NAc. The latter was down-regulated after repeated administration of morphine, with parallel increases in methylation of the *Drd2* promoter. Further, administration of L-DOPA, a substrate for dopamine synthesis, normalized the hypodopaminergic state, methylation of the *Drd2* promoter, restored dendritic arborization in the NAc and reversed the drug-seeking behaviour shown by these animals. These findings show how GC pharmacotherapy in early life programs a particular behaviour (drug-seeking), a condition that frequently accompanies mood and affective disorders in humans.

The question of how prenatal stress or GC accesses the brain was recently addressed in an important paper, which showed that restraint stress during pregnancy in rats leads to concomitant decreases in placental expression of 11 β -Hsd2 (associated with

increased DNA methylation at specific CpG sites within the *HSD11b2* promoter) and increases in DNA methyltransferase 3a mRNA (Jensen Peña *et al.* 2012). In humans, maternal GC levels correlate with those found in the foetus (Gitau *et al.* 1998), indicating that the placental barrier can be breached with implications for the development of psychopathology in the offspring. A recent study reported heightened endocrine responses to stress and depressive-like behaviour in the male offspring of mouse dams that experienced stress during early gestation (before the presence of a foetal brain); this phenotype was characterized by increased expression of the gene encoding corticotrophin-releasing hormone (*Crh*, a neuropeptide responsible for driving GC secretion), correlating with reduced methylation of the *Crh* promoter (Mueller & Bale 2008). Jensen Peña *et al.* (2012) noted that maladaptive behaviours in the offspring of mothers experiencing high GC levels during pregnancy may be viewed as the cost of survival. At this juncture, it deserves mention that, as Seymour Levine, a pioneer of this aspect of developmental psychobiology, observed, certain stress paradigms may actually evoke 'positive' effects (Levine 1957). For example, mild GC elevations during prenatal and neonatal life in rodents improve cognition and emotional responses (Catalani *et al.* 2011) and may facilitate adaptive adjustments to future challenges (Macrì *et al.* 2011). These ideas may be fundamental to understanding the origins of individual differences in resisting stress-related psychological and somatic disease (see Levine 2005).

Early postnatal programming

Primates and rodents are altricial species, so survival of their young depends on parental (usually, maternal) provision of nutrients and water, hygiene/grooming, warmth and protection from other inclement environmental factors, including predators. Neglect during childhood is known to have detrimental effects on overall neurodevelopment and brain structure (Kaufman & Charney 2001, Hedges & Woon 2011, Cisler *et al.* 2013) and is strongly associated with psychiatric disorders such as depression, post-traumatic disorder and schizophrenia (Morgan & Fisher 2007, Heim & Binder 2012). A series of pioneering studies in rats by Michael Meaney's group reported that the offspring of dams showing higher levels of maternal care (licking and grooming) develop into adults with lower endocrine (GC) responses to stress, as compared to the offspring of mothers that display less intense maternal behaviour (for review, see Zhang *et al.* 2013). The former offspring also showed higher levels of expression of hippocampal GR and, subsequently, faster 'shut off' of the endocrine response to stress and reduced hypothalamic

Crb mRNA expression, both reflecting superior GC negative feedback regulation. Cross-fostering studies suggested that epigenetic mechanisms might be responsible for the observed phenotype, a view upheld by the discovery of differential histone acetylation and DNA methylation of a specific exon in the *GR* promoter in the hippocampi of offspring of high vs. low maternal caregivers. The potential translational importance of these findings is reflected in another finding from the Meaney laboratory, which reported that suicide victims who had experienced childhood abuse express low hippocampal levels of *GR* mRNA in association with increased methylation of the *GR* promoter (McGowan *et al.* 2009).

Our laboratory employs a different ELS paradigm in mice (C57/Bl6n strain) in an attempt to understand the relationship between stress and psychopathology from an epigenetic perspective (Murgatroyd *et al.* 2009). Our ELS protocol involves daily separation (3 h, variable times of the day) of mouse litters from their dams during the first ten postnatal days, leading to characteristic endocrine and behavioural phenotypes in adulthood. Specifically, male ELS-exposed mice display learned helplessness (immobility) in the forced swim test and impaired spatial and fear-conditioned learning. At the same time, ELS-treated mice have elevated GC levels under baseline conditions and show stress-induced GC secretory responses that are exaggerated in terms of magnitude and duration (indicating impaired GC negative feedback in the brain and pituitary and/or hyperactivation of the hypothalamic neurones responsible for positive regulation of GC secretion). Following a candidate gene approach, we searched for epigenetic modifications in a number of genes in the limbic-hypothalamic-pituitary circuitry that are known to be important for regulation of GC secretion. Among others, this analysis revealed hypomethylation of the so-called enhancer region of *Avp*, the gene that encodes arginine vasopressin (*Avp*), a hypothalamic neuropeptide that, under sustained demand (e.g. chronic stress or adrenalectomy), plays a critical role in ensuring higher corticotrope-GC output (Tilders *et al.* 1985, Davis *et al.* 1986, Hellbach *et al.* 1998). Consistent with our demonstration that this hypomethylation resulted in attenuated binding of methyl CpG-binding protein 2 (*Mecp2*) to the *Avp* enhancer (*Mecp2* serves as a scaffold for the assembly of repressor complexes including histone deacetylases and DNA methyltransferases), we observed an up-regulation of *Avp* mRNA expression (Murgatroyd *et al.* 2009). Further analysis revealed that ELS-induced DNA hypomethylation is preceded by loss of *Mecp2* occupancy during early life; the latter event depends on activity-driven phosphorylation of *Mecp2* through the mediation of

Ca²⁺-calmodulin-dependent protein kinase II (CaMKII). The observation that loss of *Mecp2* occupancy requires neural activity is important: it dismisses a role for GC in the mechanisms leading to hypomethylation of the *Avp* enhancer. In fact, the rodent adrenal first produces detectable levels of GC from about postnatal day 14; until this age, rodents are considered to be 'stress-hyporesponsive' (Sapolsky & Meaney 1986) even though they can emit ultrasonic calls (Hofer 1996) and release catecholamines (Sullivan 2003) from birth.

The above-reported molecular and endocrine responses induced by ELS were first seen at day 10 of life (at the end of the ELS paradigm) and were detectable for at least 1 year (Murgatroyd *et al.* 2009). Without necessarily excluding other mechanisms, these sustained changes in endocrine and behavioural function can be largely attributed to ELS-induced modifications in *Avp* expression because the phenotype was reversible with an AVP receptor antagonist (Murgatroyd *et al.* 2009). Another interesting aspect of this study is that the epigenetic changes observed occurred in (presumably) postmitotic neurones; although neurogenesis has been described in certain hypothalamic nuclei, the parvocellular paraventricular nucleus, in which the AVP neurones of interest to this work are located, is not known to be a neuroproliferative zone.

The peripubertal window – adolescent obesity and adult behaviour

Body mass and adiposity in childhood and adolescence appear to be good predictors of risk of obesity in adulthood. Comorbidity of adult overweight and depression and anxiety disorders occurs frequently (especially among women), with clinical evidence pointing to bilateral interactions between weight and mood/emotional states during adolescence and adulthood (Heo *et al.* 2006, Noll *et al.* 2007, Strine *et al.* 2008, Wouters *et al.* 2011). A number of interacting intra- and interdomain factors are likely to contribute to the final behavioural and metabolic phenotype, including psychological (e.g. family/social stability, self-esteem, dissatisfaction with body image, stigmatization, role models, motivation, behavioural control), physiological (e.g. multiple endocrine factors, including insulin, leptin, GC, sex steroids; energy absorption and utilization) and environmental (e.g. peer pressure, promotional media, physical activity, socio-economic circumstances) (Bornstein *et al.* 2006, Hyde *et al.* 2008, Cole *et al.* 2012). While body mass and adiposity are determined by the amount of energy consumed and the amount of energy expended, it is interesting that depression becomes more prevalent in girls than

in boys around the onset of puberty (Cyranowski *et al.* 2000, Angold & Costello 2006).

In a recent (unpublished) study, we examined how transient diet-induced obesity (DIO) in peripubertal rats influences adult behaviour in male and female rats (from between days 21 and days 80 of life, with testing when rats were 180 days old; see Fig. 1). Our experimental design was chosen against the background that reward (here delivered in the form of a palatable cafeteria-type diet) and mood are closely linked (Scott *et al.* 2008, Kivimäki *et al.* 2011, Dauncey 2013, Sinha & Jastreboff 2013), with both being subject to modulation by peripheral endocrine and metabolic signals. The paradigm used to achieve DIO involved giving animals a choice between standard chow and cafeteria diet (CD) that changed on a daily basis. While the use of CD in rodent experiments has certain advantages over HFD (Sampey *et al.* 2011), the CD approach is limited by the fact that the experimenter does not have complete control of the composition (individual ingredients and energy) of the food. To increase palatability of the CD, investigators commonly use foods that contain animal fat products or chocolate; we however avoided such foods for ethical reasons as well as hygiene and reproducibility and instead increased the incentive salience of the CD by providing chocolate-free diets containing nuts, marzipan, fruit-flavoured cereal bars and shortbread. Control animals only received standard chow.

Animals consumed high amounts of the CD, but they nevertheless continued to consume small amounts of the standard chow and showed rapid body weight gain, when compared to age-matched controls (Fig. 2a). Interestingly, when the CD was withdrawn at the age of 80 days, female rats showed reduced BW gain, whereas male rats continued to show body weight increases above those seen in age-matched control animals (Fig. 2b). Consistent with these feeding and growth patterns, CD-exposed males were found to have larger fat pads at necropsy (Fig. 2c).

Behavioural phenotyping (see Sousa *et al.* 2006 for methodological background) performed when animals were approx. 180 days old (100 days after last exposure to CD) revealed that the peripubertal DIO paradigm led to alterations in anxiety- and depression-like behaviour. Both males and females that had been exposed to the CD during peripubertal life displayed stronger signs of depression-like behaviour when tested in the forced swim test (Fig. 3a). Depression and anxiety frequently co-exist in humans, with greater comorbidity occurring in women (Alexander *et al.* 2007). We here observed, however, that whereas CD-exposed males were significantly more anxious than their age-matched controls in two different behavioural assays (open arena and an elevated plus maze), CD-exposed females were, if anything, less anxious than their corresponding controls (Fig. 3b).

As mentioned earlier, there are strong correlations between stress, GC levels and mood and emotional behaviour (Holsboer & Ising 2010). In view of this, we also examined GC levels in control and CD-exposed rats at the age of 180 days. Surprisingly, no differences were observed in daytime (nadir) and night-time (peak) GC levels when control and CD-treated male rats were compared (Fig. 4a). As expected, control female rats displayed higher levels of GC secretion than males (both time points) (Fig. 4a). Daytime and night-time GC levels were significantly higher in CD-exposed females as compared to their corresponding standard diet-fed controls (Fig. 4a). The latter results were supported by measures of GR mRNA expression in the hippocampus, a key brain area involved in the mediation of GC negative feedback: as shown in Figure 4b, the CD-exposed females were the only group to exhibit any treatment-related changes (decreases) in hippocampal GR expression. Lastly, the female CD-treated rats stood out from among the other experimental groups in that they showed the largest GC secretory response to an acute stressor (at 30 min). The above-reported data suggest that CD exposure during peripubertal

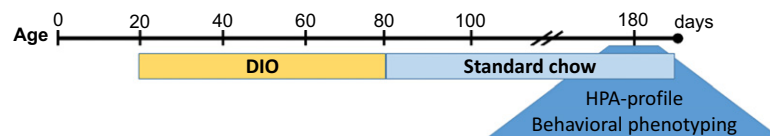


Figure 1 Experimental protocol used to examine the influence of diet-induced obesity (DIO) on activity of the hypothalamo-pituitary-adrenal (HPA) axis and depression-like and anxiety-related behaviour in rats. Male and female Wistar rats were weaned on postnatal day 21 and maintained under standard laboratory conditions. Animals were provided *ad libitum* with either standard laboratory chow (controls) or a cafeteria diet (CD) from days 21 to 80 of life to induce peripubertal DIO (ppDIO). From day 80 onwards, all animals were kept on standard laboratory chow. On postnatal day 180 (100 days after withdrawal of the CD), animals were successively tested for anxiety-related and depression-like behaviours (using the elevated plus maze test and forced swim test, respectively) and HPA axis activity under basal and acute stress (2-min air puff) conditions. There was an interval of 1 week between each of the tests.

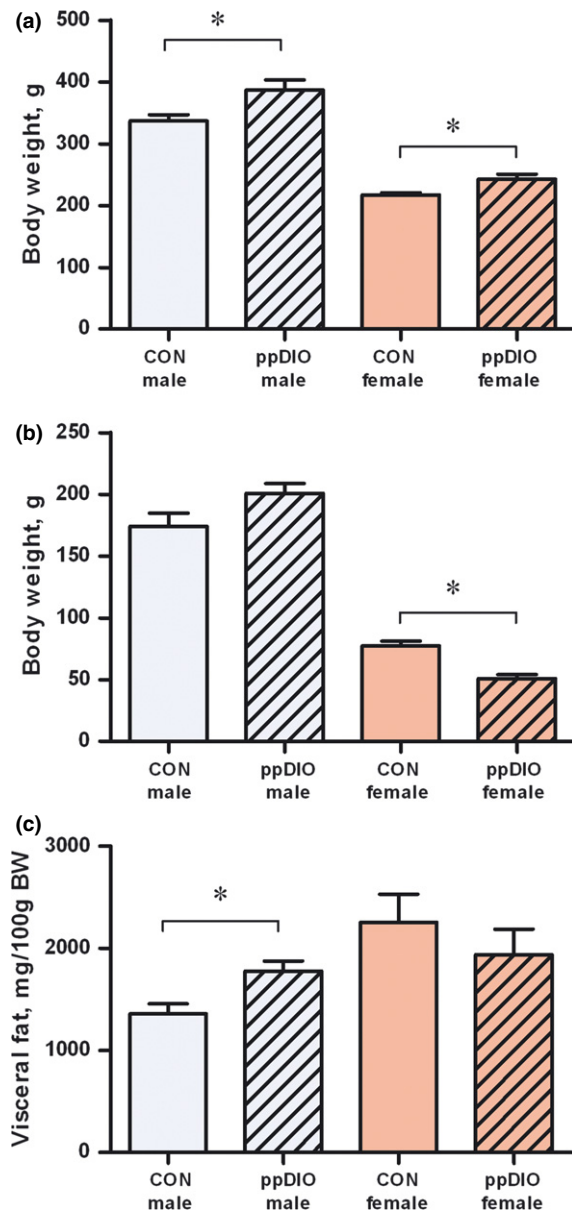


Figure 2 Body weight changes and visceral fat mass. (a) Male and female rats with access to cafeteria diet (ppDIO) from days 21 to 80 of life displayed gained body weight gains that were significantly greater than chow-fed controls ($P \leq 0.05$). (b) A small (non-significant) increase in body weight was observed in ppDIO males that were returned to standard chow between the ages of 80 and 180 days; in contrast, similarly treated females showed a small but significant slowdown of weight gain ($P \leq 0.05$). (c) Although ppDIO males that were returned to standard chow between the ages of 80 and 180 did not show weight gain, they displayed a significantly higher mass of visceral fat ($P \leq 0.05$). All values shown are mean \pm SEM ($n = 5-7$ per group). Asterisks indicate $P < 0.05$ (t -test comparisons of same-sex groups). ppDIO, peripubertal diet-induced obesity.

development leads to sex-specific disruption of the normal processes regulating GC secretion under basal and stressful conditions and a dissociation of GC

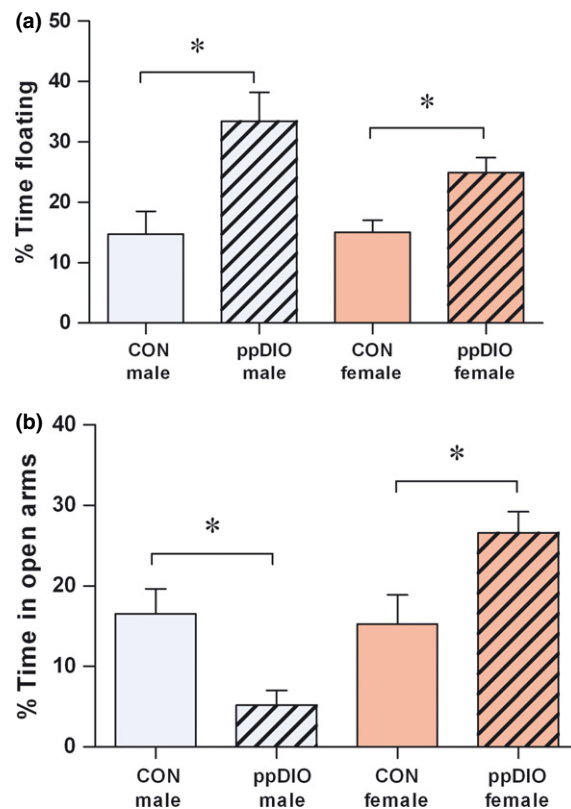


Figure 3 Behavioural phenotype of rats with a history of peripubertal diet-induced obesity (ppDIO). (a) Both male and female rats with a history of ppDIO showed significantly increased immobility in the forced swim test, a measure of depression-like behaviour ($P \leq 0.05$). The forced swim test was performed during the dark phase of the daily light–dark cycle, on two consecutive days. On the first day, the test lasted 15 min; on the second day, the test was 5 min long, and only data from this second session were video-recorded and subsequently quantified; time spent immobile (floating) is shown as a percentage of total time (5 min) in the test apparatus. (b) As compared to ppDIO females, ppDIO males displayed significantly higher ($P \leq 0.05$) levels of anxiety-related behaviour in the elevated plus maze (EPM), as measured by the percentage of time they spent exploring the open arms of the maze. Data shown are mean \pm SEM ($n = 5-7$ per group). Asterisks indicate $P < 0.05$ (t -test comparisons of same-sex groups).

secretory levels from the expression of depression- and anxiety-like behaviours.

Back to the future

Life events clearly influence physiological and behavioural trajectories. As reviewed here, early life experiences exert their effects, at least in part, through epigenetic mechanisms that lead to long-term changes in gene expression potential and, as has been recognized for decades, the brain is accessible to environment-induced

plasticity from very early gestation through to puberty. Obviously, much still remains to be done in order to identify those external stimuli that have the greatest impact on any given function of interest, as well as to assess to what extent that stimulus alters quality of life.

Survival is ensured by dynamic adaptations at the multilayered levels of organization and regulation of physiological processes; it will be therefore important to know the extent of ‘permanency’ of the snapshot phenotypes that we and others have recorded to date. Growing acceptance of the view that even DNA methylation may not result in indelible epigenetic marks

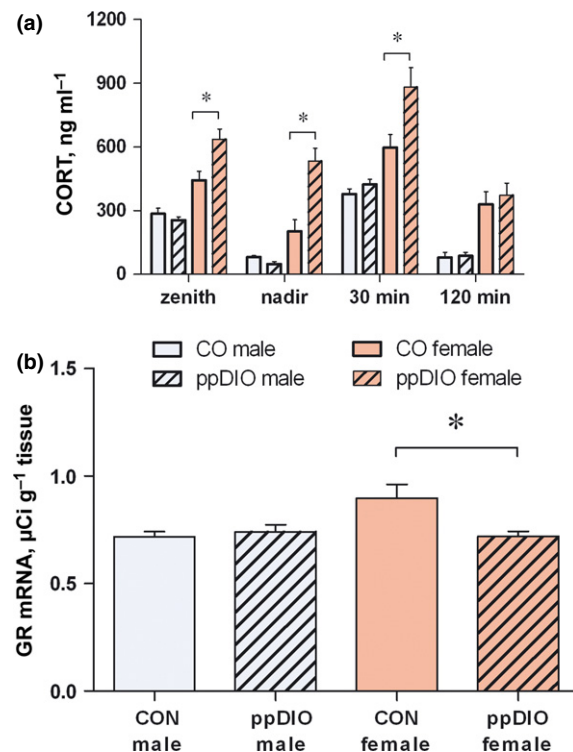


Figure 4 Peripubertal diet-induced obesity (ppDIO) effects on regulation of hypothalamo–pituitary–adrenal (HPA) axis activity. (a) Activity of the HPA axis was assessed under basal (resting) conditions during the daily zenith and nadir of corticosterone (CORT) secretion, as well as 30 or 120 min after exposure to an acute (air puff) stressor. Serum CORT was measured using a commercial radioimmunoassay kit (MP Biochemicals, Costa Mesa, CA, USA). Data show that ppDIO blunts the circadian fluctuations in HPA axis activity in female, but not in male, rats ($P \leq 0.05$). Further, ppDIO rats showed an exaggerated response to acute stress. (b) Shows results of semiquantitative *in situ* hybridization analysis of glucocorticoid receptor (GR) mRNA expression in the dentate gyrus of the hippocampal formation. There was a significant down-regulation of GR mRNA in ppDIO females ($P \leq 0.05$). All data are shown mean \pm SEM ($n = 5$ –7 per group). Asterisks indicate $P < 0.05$ (*t*-test comparisons of same-sex groups).

(Métivier *et al.* 2008, Ma *et al.* 2009, Lee *et al.* 2010, Guo *et al.* 2011a,b, Unternaehrer *et al.* 2012) prompts the question of how different signals arriving at intervals over lifetime act on the pre-existing epigenome to (re)shape the next phase of life. This necessarily includes definition of the spectrum of windows that may be accessible to epigenetic modification; it may be too early to exclude that these can open, narrow, close and even re-open in cell-, network- and environment-specific ways (Fig. 5). Keeping an open mind to these possibilities will not only help physiologists to appreciate the full potential of the brain’s plasticity but may also provide biological explanations for how otherwise fixed programs become reversible through social, psychological and pharmacological interventions, some examples of which were given earlier in this article.

Epigenetic mechanisms bring a promising and exciting new perspective to the physiologist’s quest to comprehend the complex systems as well as their integration that result in coordinated responses to internal and external demands. Remembering Leonardo da

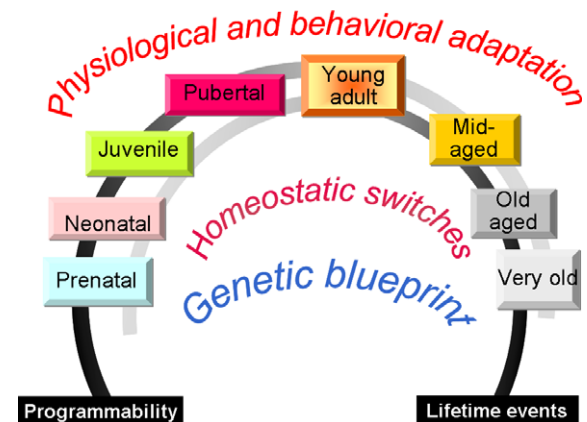


Figure 5 Maintenance of physiological and behavioural homeostasis during different phases of the lifespan. Lifetime events (e.g. stress, altered nutrition) can act directly (and rapidly) on genetically determined physiological switches, but may also induce epigenetic changes in varying degrees of permanency (not shown). As indicated by the decreasing intensity of shading of the ‘programmability’ curve, from prenatal to very old ages, it is thought that the brain of young individuals is more amenable to programming (epigenetic or otherwise) than that of older individuals. The schema shows that older subjects will have experienced more life events (positive and negative; note the increasing shading intensity of the ‘lifetime events’ curve, from prenatal to very old ages) that will serve as a ‘reference library’ for responding to future demands. This cumulative experience, ‘archived’ in previously adjusted physiological switches or epigenetic modifications, will play a decisive role in whether a subject ages healthily or unhealthily.

Vinci's adage '*Simplicity is the ultimate sophistication*' may be useful in designing future experiments to examine *gene × environment* interactions in the context of behavioural or psychiatric disorders; by definition, these disorders are complex as they involve interplay between different neural networks that are themselves subject to local regulation and modulation. Standardizing animal models and molecular and phenotypic analyses and being aware that a behaviour is unlikely to depend on a single gene or brain region may help us reach our goal more effectively. Moreover, biomedical experimentalists need to exert more care in extrapolating findings in model organisms to humans. As recently demonstrated in the context of modelling human psychiatric disease in animals, the impact of a given environmental stimulus may depend heavily upon predisposing genetic risks (Niwa *et al.* 2013). Interpretation of many of the animal studies discussed in this article is based on anthropomorphism; thus, extrapolations from experiments in animal models of disease to psychopathological conditions in humans require a certain degree of caution (see Seok *et al.* 2013). On the other hand, given that many molecular pathways and mechanisms are conserved across species, animal research undoubtedly serves to advance our understanding of complex behaviours, with further advances expected as investigators refine their models. Moreover, although developments in computational neuroscience and sophisticated minimally invasive neurotechnologies will facilitate exploration of the intricate circuitries that regulate human behaviour, the molecular dissection of normal and pathological human behaviour remains a long shot, not least because of safety and ethical considerations.

Conflict of interest

None of the authors have any conflict of interests to declare. The funding agency did not have any influence over the conception, design, interpretation or writing of this article or over the authors' unpublished data described in this article.

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