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Symposium Report

Epigenetic programming of neuroendocrine systems during early life

Chris Murgatroyd

School of Healthcare Science, Manchester Metropolitan University, Manchester M1 5GD, UK

New findings

- **What is the topic of this review?**
  Behavioural epigenetics and its role in early-life programming and adaptation is allowing us to understand how psychiatric diseases can develop through interactions of genes and environments.
- **What advances does it highlight?**
  The ability of methyl of Methyl-CpG binding protein 2 to regulate Avp gene expression, in response to early-life stress, and induce DNA methylation, occurs through the recruitment of components of the epigenetic machinery.

Arginine vasopressin plays a pivotal role in the control of long-lasting effects of early-life stress on the brain. We previously reported that maternal separation in mice persistently upregulates *Avp* gene expression associated with reduced DNA methylation of a region in the *Avp* enhancer. This early-life stress-responsive region serves as a binding site for the methyl-CpG binding protein 2, which in turn is controlled through neuronal activity. We also found that the ability of methyl-CpG binding protein 2 to regulate transcription of the *Avp* gene and induce DNA methylation occured through the recruitment of components of the epigenetic machinery. Understanding the sequential events involved in the epigenetic regulation of a gene should allow for targeted approaches aimed at reprogramming expression during development and possibly later life.

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Corresponding author C. Murgatroyd: School of Healthcare Science, Manchester Metropolitan University, Chester Street, Manchester, M1 5GD, UK. Email: c.murgatroyd@mmu.ac.uk

Early-life adversity can have long-lasting consequences for mental health by shaping individual differences in vulnerability to stress-related disorders throughout life (Heim & Nemeroff, 2002). These epidemiological findings raise the intriguing question of how adverse early experiences become integrated at the cellular and molecular level in the developing brain architecture. Accruing evidence suggests that the genetic blueprint is strongly shaped by environmental factors (Fig. 1). Animal models in which the early environment can be manipulated in a controlled fashion can help to improve understanding of gene–environment interactions and elucidate the pathways through which programming in response to early-life experiences occurs.

Epigenetic mechanisms, comprising covalent DNA and histone modifications, are prime candidates for the regulation of gene expression and allow integration of intrinsic and environmental signals in the genome (Jaenisch & Bird, 2003). In this respect, epigenetic mechanisms have been suggested to underpin brain plasticity through different life stages, a process requiring stable modulation of gene expression (Hunter & McEwan, 2013; Patchev *et al.* 2013). Although DNA methylation is one of the most intensely studied epigenetic mechanisms, its role in the translation of life experiences into lasting changes in postmitotic gene expression still remains poorly understood.

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Adverse conditions during early life can impact on experience-dependent maturation of structures underlying emotional functions and endocrine responses to stress, such as the hypothalamic–pituitary–adrenal (HPA) axis (a major part of the body's stress system), leading to increased stress responsivity in adulthood (Seckl & Meaney, 2004). Consistent with this concept, depressed patients with a history of childhood abuse or neglect are often characterized by hyperactivity of the HPA axis (Heim & Nemeroff, 2002).

In order to translate findings from humans to mice, we used periodic infant–mother separation [known as maternal separation (MS); 3 h per day from postnatal day 1 to 10] during early postnatal life (Murgatroyd *et al.* 2009). This is one of the most commonly used models for inducing early-life stress in rodents (Nishi *et al.* 2013), characterized by lifelong elevated glucocorticoid secretion, heightened endocrine responsiveness to subsequent stressors, and disruption of the homeostatic mechanisms that regulate the activity of the HPA axis. All of these signs are considered to be pathogenic factors in disorders of mood and cognition (Holsboer, 2000; Pariante & Lightman, 2008).

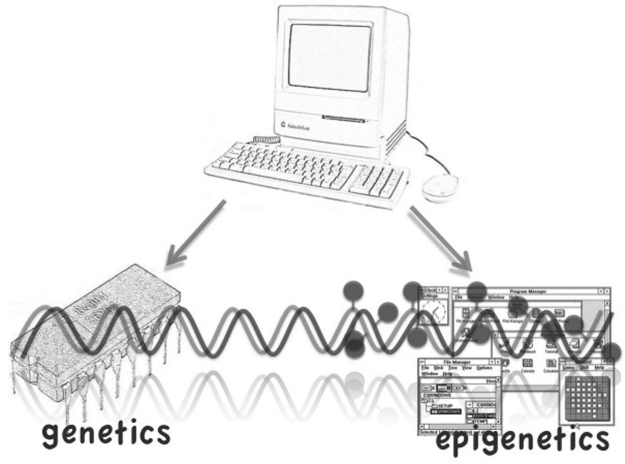

Figure 1. Epigenetics allows the genome to dynamically respond to the environment
DNA can be compared to the hardware of a computer, while the operating system of the DNA, the epigenetic programming, enables the processing of decisions regarding which functions the DNA hardware does and does not perform. Environmental conditions can be thought of as inputted data that allow the epigenetic software to meet nature's goals of adaptation.

Using this rodent model of early-life adversity, we examined the coupling of experience-driven neuronal activity with DNA methylation and gene expression. We focused on the expression of the two hypothalamic neuropeptides that regulate HPA axis activity by increasing the synthesis and release of pituitary adrenocorticotrophin, namely, arginine vasopressin (AVP) and corticotrophin-releasing hormone. Abundant evidence links AVP and corticotrophin-releasing hormone to mood and cognitive behaviours, making their receptors targets for potential psychopharmacological interventions (Bao & Swaab, 2010).

Specifically, we showed that MS leads to reduced DNA methylation at a downstream enhancer of the *Avp* gene within the parvocellular subdivision of the paraventricular nucleus of the hypothalamus. These epigenetic events are accompanied by persistent upregulation of *Avp* mRNA expression and consequently, sustained hyperactivity of the HPA axis. Importantly, the MS-induced endocrine phenotype lasted for at least 1 year following the initial adverse event and could be normalized through administration of an AVP V1b receptor antagonist. Moreover, we identified specific cytosine residues, within cytosine–guanine (CpG) dinucleotide residues, at the *Avp* enhancer whose sustained hypomethylation after MS is critical for the regulation of *Avp* expression. These residues correspond to high-affinity, context-specific binding sites for the methyl-CpG residue-binding protein Mecp2. Furthermore, depolarization of hypothalamic cells induced site-specific phosphorylation of Mecp2 via calmodulin kinase II and thus controlled the function of Mecp2 as reader and interpreter of the DNA methylation signal at the *Avp* enhancer. This result is consistent with previous findings that neuronal depolarization-dependent Ca^{2+} influx and activation of calmodulin kinases causes phosphorylation of Mecp2 (Zhou *et al.* 2006). Such modification is considered to impair the ability of Mecp2 to bind methylated DNA

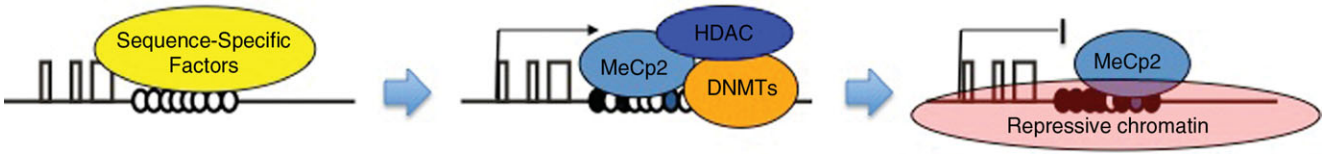

Figure 2. A stepwise pathway in the epigenetic programming of *Avp* during hypothalamic development in a time-dependent manner
Methyl-CpG binding protein 2 (MeCP2) binds to *Avp* enhancer, following prior binding of sequence-specific factors, and recruits histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) to target DNA methylation and repressive chromatin.

and relieve repression of target genes. The concept that experience-dependent stimuli dynamically control the methylation of the *Avp* enhancer is further supported by the observation that MS induces contemporaneous increases in calmodulin kinase II activation and Mecp2 phosphorylation, indicating that the sequential order of these events plays a major role in the establishment of epigenetic marks. Once established, the observed differences in *Avp* enhancer methylation centred on Mecp2 binding sites, which appeared to be actively maintained in MS mice.

Collectively, we propose that MS-induced depolarization of neurons in the paraventricular nucleus drives Mecp2 phosphorylation and enhanced *Avp* expression, thereby serving as a key mediator of the effects of MS. Moreover, MS tilts the balance towards persistent hypomethylation and *Avp* overexpression by lasting reductions in Mecp2 binding (Murgatroyd & Spengler, 2011).

Following on, we investigated how Mecp2 regulates and establishes epigenetic marking at the *Avp* enhancer. We now show that Mecp2 co-associates with repressive histone marks, histone deacetylases 1 and 2 and DNA methyltransferases 3a and 3b to establish repressive epigenetic marks. In addition, we show that Mecp2 is recruited during neurodevelopment to the *Avp* enhancer following prior binding of early developmental factors known to recruit histone- and DNA methylation-modifying enzymes. We propose that this lays the ground for further Mecp2 occupancy at the AVP enhancer. Given that early social experiences influence specific brain circuits during specific developmental stages (Andersen & Teicher, 2008; Loman & Gunnar, 2010), elucidation of the pathway establishing epigenetic regulation of *Avp* during hypothalamic development could enable the targeting of part of the epigenetic machinery in a time-dependent manner to regulate long-term programming of *Avp* expression in response to early experience (Fig. 2).

Another rodent model uses chronic social stress in rats to model postpartum depression and anxiety and allows testing of adult maternal behaviour and programming of neuroendocrine genes (Nephew & Bridges, 2011). Dams exposed to early-life chronic social stress as infants displayed long-term effects on the neuroendocrinology of maternal care, consisting of reduced oxytocin, prolactin and *Avp* gene activity in brain nuclei involved in the control of maternal behaviour, with the overall result being a decreased nursing efficiency in the adult dams (Murgatroyd & Nephew, 2013). Further studies are aiming to elucidate the epigenetic mechanisms linking exposure to chronic social stress during early development to the long-term effects on adult maternal behaviour and oxytocin and prolactin activity.

Perspectives

Behavioural epigenetics and its role in early-life programming and adaptation is a relatively new research field crucial in aiding our understanding of psychiatric diseases, through interactions of genes and environments. It is important to comprehend the mechanisms of how such processes encode DNA memories so that we might be able to take advantage of these early opportunities in neurodevelopmental processes (Nagy & Turecki, 2012). The identification of potential windows for timely therapeutic interventions in DNA memory-mediated disease states following early social stress is likely to be more effective and less costly than addressing problems at a later age (Hoffmann & Spengler, 2012). Refinements to translational animal models are an important way to address the impact of environmental interactions, such as maternal care and early-life stress. This will advance our understanding of how nature and nurture conspire in early life and ultimately allow us to foster appropriate preventive measures in the management of complex diseases and behaviours.

Call for comments

Readers are invited to give their opinion on this article. To submit a comment, go to: http://ep.physoc.org/letters/submit/expphysiol;99/1/62.

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Additional Information

Competing interests

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