Mothering style and methylation

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Frequent licking and grooming by rat mothers increases the number of hippocampal glucocorticoid receptors in their pups, leading to tighter regulation of stress hormone levels. A study in this issue shows that this treatment alters DNA methylation of the glucocorticoid receptor gene and acetylation of histones early in life, providing a mechanism for these permanent changes in stress responses.

The style of parenting can influence the sort of adult a child becomes. This belief permeates everything from psychiatry (where parenting style has been shown to influence the risk of adult psychopathology in adopted children[1]), criminal defense strategies, and literature, to half the conversations on first dates. Though some have questioned the power of parental influences[2], and its importance can be erroneously, tragically overstated (for example, in debunked notions that particular parenting styles cause schizophrenia or determine sexual orientation), the influence of parenting is a subject of tremendous importance in developmental psychobiology. The key mechanistic question, of course, is how such influences become long lasting. On page 847 of this issue, Weaver *et al.* present an extraordinarily clear picture of one such route: specifically, they show how one facet of mothering style in the rat leads to a cascade of molecular and cellular changes, resulting in lifelong alterations in the nature of the stress response[3].

The phenomenon they explore has its roots in an early observation in psychobiology. If a neonatal rat is 'handled' daily—removed from its mother and placed in a new cage for 15 minutes—remarkable lifelong changes are triggered. As adults, such rats have tighter regulation of the secretion of a class of stress hormones, glucocorticoids (resulting in lower basal and post-stress levels)[4,5]. This tighter regulation is due to a lifelong increase in the number of glucocorticoid receptors in the hippocampus, a brain region that plays a crucial role in negative feedback regulation of the hormone's release. Collectively, handling results in a more exploratory, less fearful and stress-reactive adult that is more cognitively intact in old age. Such a profile is produced only if the handling occurs in the first few weeks of life; thus, the phenomenon represents a case of a critical period in development[6].

The group of researchers on this new report, led by Michael Meaney, had previously answered two questions raised about neonatal handling. First, the phenomenon obviously did not evolve in rats as an adaptive response to the experimental manipulations of graduate students—so what is its real-world relevance? Meaney and colleagues showed that rats differ stably in mothering style, and that those who lick, groom and do arched-back nursing at rates more than one standard deviation above the mean (high 'LG-ABN' females) produce the handling profile in their pups. This maturational outcome does not reflect an underlying genetic profile giving rise to both the mothering style and the handled profile; as evidence, cross-fostering neonates of low-LG-ABN mothers (one standard deviation below the mean) to high-LG-ABN ones (or the reverse) produces adults with the profile associated with the adoptive, rather than the biological, mothering style. Perhaps most remarkably, pups raised by a high-LG-ABN mother become high-LG-ABN mothers themselves as adults, thereby passing on the trait in a case of multigenerational, nonmendelian inheritance[7,8].

The second question was how being raised by a high-LG-ABN mother produced this profile. Meaney's group had previously demonstrated the acute consequences in pups of this mothering style. Specifically, high-LG-ABN rearing results in increased serotonergic tone in the hippocampus of pups, leading to activation of cAMP and cAMP-dependent protein kinase. Critically, this leads to the expression of the transcription factor nerve growth factor–inducible protein A (NGFI-A). The first exon of the glucocorticoid receptor gene contains a binding site for NGFI-A, and NGFI-A binding to it results in increased expression of the hormone receptor[9]; such increased receptor expression seems crucial to the suite of neuroendocrine changes seen in the offspring of high-LG-ABN mothers.

These findings help explain the immediate consequences of mothering style on pups. But these consequences are not necessarily self-perpetuating. How do these changes become lifelong? This is where the current study comes in.

The answer appears to involve two time-honored epigenomic mechanisms that cause prolonged differences in gene expression[10]. The first, methylation, is the process by which a gene can be stably silenced for substantial percentages of a lifetime. Specifically, this is accomplished by the attachment of a methyl group to nucleotides in a gene's promoter, with the methyl group then tightly binding a repressor protein. Thus, demethylation of a gene typically leads to marked expression of its protein product. The second mechanism concerns the fact that DNA in eukaryotic cells is wrapped around proteins called histones, with the DNA and histones collectively comprising chromatin. The compactness of histones in a particular region of chromatin determines how readily transcription factors access promoters in the DNA, and a variety of regulators can alter chromatin structure. In this new report, changes in both methylation state and chromatin structure are implicated in the handling phenomenon.

The authors first showed that the methylation state of the NGFI-A binding sequence of the first exon of the glucocorticoid receptor was quite plastic around the time of birth. Just before birth, this binding sequence was

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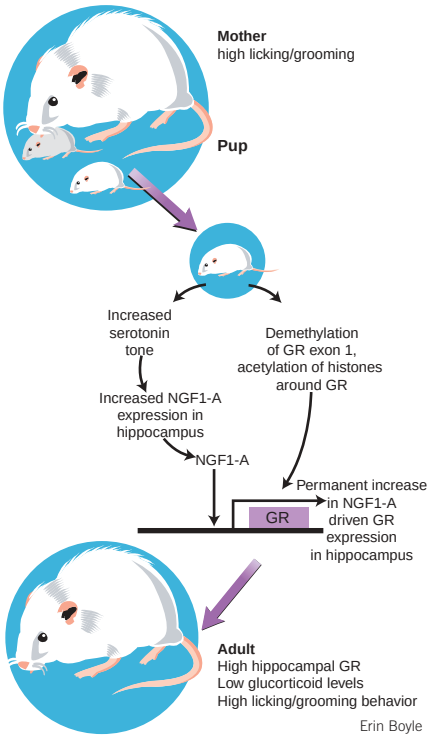

Figure 1 A cascade by which a constellation of traits in the adult rat is induced by the mothering style to which the rat was exposed as a pup. Mothering characterized by high levels of licking and grooming produces two interacting pathways of changes in the pup. In the first, increased serotonin tone in the hippocampus leads to increased expression of the transcription factor NGF1-A. In the second, the first exon of the glucocorticoid receptor gene in the hippocampus is demethylated, and the histones surrounding it are acetylated. The result is a glucocorticoid receptor gene that is permanently more open to transcriptional activation by NGF1-A. This produces higher numbers of glucocorticoid receptors in the hippocampus of the rat as an adult, which gives rise to other distinctive endocrine and behavioral features.

unmethylated, and it was then methylated by the first day after birth. Strikingly, over the course of the next week, exposure to high-LG-ABN mothering (by either the biological mother or a cross-fostering female) caused one key site in the binding sequence to be demethylated; in contrast, low-LG-ABN mothering did not reverse the methylation. Thus, this immediately suggests a long-term route by which NGFI-A can induce greater expression of glucocorticoid receptors in high-LG-ABN rats.

The authors then turned their attention to chromatin structure and histone interactions with DNA. Actylation of histones decreases their binding to DNA, thereby increasing access of transcription factors to promoters. The authors observed that histones surrounding the glucocorticoid receptor gene were more acetylated in high-LG-ABN animals, providing another long-term mechanism by which NGFI-A would have greater access to the gene in such animals, something the authors then demonstrated.

Then, in a key final step, the authors showed that methylation state and histone acetylation were not merely correlated with the adult consequences of mothering style, but caused those consequences. They made use of a pharmacological inhibitor of histone deacetylation that, prior work suggested, opened up the chromatin, thereby facilitating access of demethylating enzymes. Administering this inhibitor to adult rats who had been raised in a low-LG-ABN environment transformed these animals into ones with the high-LG-ABN adult profile: greater NGFI-A access to the now-demethylated promoter, more glucocorticoid receptors in the hippocampus and, finally, low circulating glucocorticoid levels. Inhibition of histone deactylation must surely have altered chromatin structure around, and methylation state of, genes other than just the glucocorticoid receptor. However, although the authors have not yet demonstrated that it is the subsequent demethylation of the glucocorticoid receptor gene, *per se*, which is critical, it is certainly the most plausible gene to be involved. Thus, remarkably, a pharmacological intervention replicated the adult consequences of a particular mothering style at the molecular, cellular and physiological levels.

Naturally, there is much more that needs to be known in the wake of this *tour-de-force* study. Some work must be done that would constitute a mopping-up operation: it must be shown whether the behavioral consequences of having been raised by a low-LG-ABN mother are also shifted by this pharmacological intervention.

Other work would represent something of a lateral shift of focus: the high- versus low-LG-ABN mothering style also produces lifelong changes in the physiology of anxiety (specifically, high-LG-ABN mothering produces lifelong increases in pups in numbers of receptors in the amygdala for benzodiazepines, which have antianxiety properties)[11]. The mechanisms underlying this consequence of mothering style remain to be identified, and could well be similar to those revealed by the present findings.

Another area of further work must go in a more reductive direction. The authors have answered the question "How does high-LG-ABN mothering cause a permanent increase in hippocampal glucocorticoid receptor number?" with "By changing the methylation state of the promoter for the receptor's gene." And thus, this redefines the question to "How does high mothering style regulate methylation in a pup?"

But the most interesting direction, arguably, is a more expansive one. Using a drug that inhibits histone deactylation is a fine experimental intervention to reverse the less-than-salutary consequences of the low-LG-ABN mothering style. Are there ever events in the natural world of an adult rat that might lead to inhibition of histone deactylation, thereby reversing the consequences of early mothering style? Obviously, some consequences of early experience are reversible. As but one example, prenatal stress can alter aspects of neuroendocrine function in adult rats, but this package of long-term consequences can be prevented by adoption by a different rat mother shortly after birth[12,13]. Thus, early experience can have lifelong consequences ranging from the molecular to the behavioral level, and Weaver *et al.* have revealed the scaffolding underlying one example of this process to an unprecedented extent. But, to the great relief of many of us, early experience is not necessarily destiny, and understanding the neurobiological mechanisms of intervention remains a vital challenge.

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