1 The Nature of Nurture: Maternal Effects and Chromatin Remodeling

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My Life in Science as a John Mellencamp Song

Considering the developmental focus of the work undertaken by my colleagues and me, it seems appropriate to begin with some reference to my own academic, early experience. I began my life in science under the tutelage of Professor Jane Stewart, a preeminent behavioral neuroscientist. One can do no better. I have never really understood what was meant by the word *instinct*. An action repeated effortlessly, immutable in form, unerring in consequence? Jane's ability to mentor research fellows was such. I believe she is without peer. Jane was married to Dalbir Bindra, perhaps the leading theorist in psychology of his day. D.B., as he was called, was a titan of dignity, charm, and intellectual generosity. To my great fortune, D.B. had a bad back that precluded shoveling snow. And it snows considerably in Montreal. Thus it came to pass, that following any significant snowfall, we would, appropriately enough, ascend the hill to where Jane and D.B. lived, and clear the driveway. Our reward was to then breakfast with Jane and D.B. on eggs, juice, and a discussion of the most recent version of a chapter of D.B.'s book. I love snowfalls in ways that no one else can ever appreciate.

Following my doctoral studies, I moved to the the Rockefeller University and the laboratory of Professor Bruce McEwen. I suspect there are few who will read this without having some impression of Bruce's remarkable reputation for excellence in science and mentorship. It is all well deserved. There is little good in my career that has not in some way been derived from my sojourn in Bruce's lab.

The parenting was thus sublime. And in the midst of all this was I, focused from my earliest days as an undergraduate on precisely the same scientific interest, the biology of individual differences. For an offspring of Montreal, the home of Hans Selye and Donald Hebb, my studies of the development of individual differences in endocrine responses to stress seem as if destiny. I fell in love with this topic as a sophomore. My affection has never wavered. Scientifically, I married my childhood sweetheart. I still live in my hometown. And I yearn to transmit to mine what was bestowed upon me. Actually, it has all been rather simple.

You Can't Get There from Here

Following a public lecture, a journalist approached the renowned psychologist Donald Hebb and asked for his opinion on which contributed more to personality, nature or nurture. Hebb responded that this was akin to asking what contributed more to the area of a rectangle, the length or the width. Like all good urban myths, there are multiple versions of this story. The context changes somewhat, but Hebb's reply remains intact in its piercing brilliance. Forty some years later, we pace about in the same state of confusion, pondering the same foolish question, armed with the impressive tools of a new millennium, but without the wisdom of Hebb.

We have ample reason to celebrate the advances associated with the human genome project, yet the same technology bears the risk of expanding the divide between the biological and social sciences. One group of scientists is blindly infatuated with the explanations derived from gene sequencing consortiums; the other huddles in fear at the thought of a biological maze in which it is lost. Such divisions, by definition, only further confuse the study of development as scientists from different disciplines retreat further into their respect comfort zones. Can you imagine the study of "rectangularity" composed of those who study "lengths" and those who study "widths"? Ultimately, one would hope, individuals would emerge demanding an integrative approach that recognizes only the study of rectangles, dismissing the notion that anything meaningful can come from the study of "lengths" or "widths" alone. Such an advance would require no new tools, but rather a change in the way we think about rectangles.

Life does not emerge as a function of either nature or nurture. And it is equally wrong to assume that phenotype derives from *both* nature *and* nurture. For this is only to repeat the misunderstanding in kinder, gentler terms. Both conclusions are derived from additive models of determinism where gene + environment = phenotype. Such models make no biological sense whatsoever. To paraphrase Lewontin (1980), life emerges only from the interaction between the two: there are no genetic factors that can be studied independent of the environment, and there are no environmental factors that function independent of the genome. Phenotype emerges only from the interaction of gene and environment. Nature and nurture do not exist in a manner that can ever be considered as independently quantifiable. At no level can the function of a gene be separated

from its cellular environment; it is biologically absurd to assume otherwise. Every trait is a function of the interaction between gene X and the environment. And, lest you think I am simply some environmental wolf in sheep's clothing, it is equally absurd to believe that the environmental factors can be studied independent of the genome and the constraints it places on the neural systems that serve as the inevitable bridge between environment and effect.

Dearest Mommy, Do Parents Really Matter?

My laboratory examines gene—environment interactions through studies of the effects of maternal care on gene expression and phenotypic development in mammals. The developmental outcomes involve measures of endocrine and behavioral responses to adversity, or stress. Indeed, our studies follow from a well-established theme in biology: maternal effects on the development of defensive responses to threat. Such effects are apparent in virtually all forms of life. In a remarkable paper, Agarwal, Laforsch, and Tollrian (1999) provided evidence for transgenerational, maternal effects in two models: one a plant, and the other an insect. Herbivory results in the expression of inducible defenses (defensive reactions occurring in response to specific forms of provocation) in plants. In the radish, damage from a caterpillar induces an increased production of mustard oil glycosides and a greater density of setose trichomes on newly formed leaves. These defenses protect against subsequent attacks. Plants expressing such defenses have a significantly greater lifetime seed production. And there are consequences for the next generation. The seedlings derived from the caterpillar-damaged radishes showed significant changes in glycosinolate

profiles and altered trichome expression: the number of tri-chomes per leaf was increased in seedlings as a function of maternal herbivory. Only the mothers, and not the seedlings themselves, had ever been exposed to herbivory in any form. Such changes were adaptive. Caterpillars gained significantly less weight, presumably from reduced consumption, when exposed to seedlings from damaged versus undamaged mothers.

This is but one example of maternal effects. The capacity for flight in grasshoppers, the tail length of lizards, and the helmet size of water fleas are all determined by maternal effects acting through unknown mechanisms. The fundamental principle here is that of maternal regulation of the development of rudi-mentary defensive responses to threat. These are classic exam-ples of epigenetic, or nongenomic, inheritance, where traits of the parents are transmitted to offspring in a manner not dependent on information encoded in the nuclear genes. Mater-nal effects in plants and insects alter the form and intensity of defensive responses to threat. The environmental experience of the mother is thus translated through an epigenetic mechanism of inheritance into phenotypic variation in the offspring. Indeed, maternal effects could result in the transmission of adaptive responses across generations. My colleagues and I argue that similar effects occur in mammals and are derived, in part, from variations in maternal care during postnatal life. As in nonmammalian species, these effects also target rudimentary defensive responses.

Le Rat de Ville (and the Other One)

Amazingly, a female rat commonly gives birth to a litter of 10–15 pups that, before weaning, will weigh more than she

does. Over this period, the dam remains the sole source of nutrients and fluids. In a laboratory setting, lactating mother rats vary little in the amount of time spent in physical contact with their pups, alternating between nursing bouts with their offspring and time alone to attend to their deeply challenged metabolic equilibrium. A nursing bout commences when the mother approaches the nest, gathers the pups underneath her ventral surface, and licks and grooms her offspring. The licking and grooming arouses the pups, which then vigorously attach to a nipple and suckle. In the next minutes, there ensues a milk letdown and a relaxation of the pups. The dam again licks the pups, which despite being engorged with milk, scurry underneath her and compete enthusiastically for nipples in the misguided assumption that a Wisconsin dairy farm lurks only a few millimeters down the road. This is nonnutritive suckling as competitive sport. Over the first week of life, about 30 percent of the maternal licking is directed toward the pups' anogenital region. This is essential. Pups will not otherwise urinate. The bounty for the mother lies in the ingestion of the sodium-enriched urine.

There are highly stable individual differences in licking and grooming (LG) such that over the first week of life some (i.e., high-LG) mothers consistently lick and groom their pups about three times as frequently as do other (i.e., low-LG) mothers. This information is gleaned from hours of observations per day of individual mothers with their litters under perfectly undisturbed conditions. It is an ideal activity for long Canadian winters.

These naturally occurring variations in maternal care are associated with individual differences in hypothalamic-pituitary-adrenal (HPA) axis responses to stress (there are also differences

in behavioral responses to stress, but for the sake of this essay I will limit my comments to the HPA axis). As adults, the offspring of mothers that undertake frequent licking and grooming and arched-back nursing (high-LG-ABN mothers) are behaviorally less fearful and show more modest HPA responses to stress than the offspring of mothers that do not (low-LG-ABN mothers; Liu et al., 1997). Cross-fostering studies show that the biological offspring of low-LG-ABN mothers reared by high-LG-ABN dams resemble the normal offspring of high-LG-ABN (and vice versa; Francis, Dioro, Liu, & Meaney, 1999). These findings suggest that variations in maternal behavior can directly influence the development of HPA responses to stress (which is, of course, an inducible defense).

Maternal behavior in the rat permanently alters the development of hypothalamic-pituitary-adrenal responses to stress through tissue-specific effects on gene expression. The magnitude of the HPA response to stress is a function of the neuronal stimulation of hypothalamic corticotropin-releasing factor (CRF) release that then activates the pituitary-adrenal system, as well as modulatory influences, such as glucocorticoid negative feedback that inhibits CRF synthesis and release and thus dampens the HPA response to stress. The adult offspring of high-LG compared with low-LG mothers show increased hippocampal glucocorticoid receptor expression and enhanced glucocorticoid feedback sensitivity. Predictably, the offspring of high-LG mothers also show decreased hypothalamic CRF expression and more modest HPA responses to stress. Eliminating the difference in hippocampal glucocorticoid receptor levels abolishes the effects of early experience on HPA responses to stress in adulthood, suggesting that the difference in hippocampal glucocorticoid receptor expression serves as a

mechanism for the effect of early experience on the develop-
ment of individual differences in HPA responses to stress.

In vivo and in vitro studies suggest that maternal licking
and grooming increases glucocorticoid receptor gene expression
through increased hippocampal serotonin (5-HT) activity at 5-
HT_7 receptors, and the subsequent intracellular enzyme activity.
Both the in vitro effect of 5-HT, defined using primary hip-
pocampal cell cultures, and the in vivo effect of maternal behav-
ior on glucocorticoid receptor gene expression are accompanied
by an increased hippocampal expression of the transcription
factor, nerve growth factor–inducible factor A (NGFI-A). The
noncoding (a segment of the DNA that does not code for a func-
tional protein) exon 1 region of the hippocampal glucocorticoid
receptor includes a promoter region, exon 1_7, containing a
binding site for NGFI-A. Noncoding regions of the DNA do
not code for functional proteins and are commonly contain
sequences that regulate the expression of the "downstream"
coding segment. The exon 1 region contains several promoter
sequences that can alter gene expression. The exon 1_7 sequence
functions as such a promoter, is apparently unique to neurons,
and is more active in the offspring of high-LG mothers or
following manipulations that increase maternal licking and
grooming, which suggests that use of this promoter is enhanced
as a function of maternal care. Moreover, maternal LG increases
the binding of NGFI-A to the exon 1_7 sequence. Although these
findings might explain the increased glucocorticoid receptor
expression in the neonate, left unanswered is the question of
how the effect of maternal care might persist into adulthood.

Transcription factors such as nerve growth factor–inducible
factor A regulate gene expression, and thus provide a cellular
interface between environment and gene. But the relationship

is constrained. DNA operates within a chromatin context, which forms the DNA-packaging system inside the cell nucleus. Wrapped around the histone proteins, the DNA sequences that form our genetic code is only variably accessible to transcription factors. The positively charged histones and negatively charged DNA form bonds that preclude transcription factor binding to DNA sites. Enter the histone acetyl transferases (HATs) that acetylate (what else?) histone tails, neutralizing the charge, and relaxing the histone-DNA relationship to a state where transcription factors can enter the fray and bind to DNA sites. For those who considered DNA sites as mundane, passive recipients of intracellular influences, the science of chromatin remodeling through acetylation or other modifications renders the world a wonderfully interesting place.

Likewise, there are structural changes to DNA that result in far more stable silencing of DNA transcription. DNA methylation is a stable, epigenomic mark that occurs at cytosine nucleotides commonly found within promoter sequences. DNA methylation attracts a class of enzymes known as "histone deacetylases," which prevent histone acetylation, and preserve the tight histone-DNA relationship. DNA methylation is therefore associated with a stable suppression in gene transcription and is the pathway by which genes are turned off during early embryonic development. It may also be the mechanism by which maternal care during postnatal life can program the expression of specific genes in the brain and elsewhere.

In our studies, my colleagues and I focus on the methylation of the exon 1_7 glucocorticoid receptor promoter. The results reveal significant differences in the methylation of the exon 1_7 glucocorticoid receptor promoter sequence as a function of maternal care. Of greatest interest is the significant difference in

a single cytosine within the NGFI-A consensus sequence (the DNA sequence to which NGFI-A binds), which is always methylated in the offspring of low-LG mothers, but rarely so in those of high-LG mothers. This difference in DNA methylation occurs at a single nucleotide and emerges over the first week of life, which corresponds perfectly to the time during which high-LG and low-LG mothers differ in maternal care. Moreover, an adoption study in which the biological offspring of high- or low-LG mothers were cross-fostered to either high- or low-LG mothers within 12 hours of birth produced a pattern of exon 1_7 glucocorticoid receptor promoter methylation that was associated with the rearing mother thus reversing the difference in methylation at the cytosine within the NGFI-A consensus sequence in animals born to low-LG, but reared by high-LG, mothers.

Such differences in cytosine methylation are functionally relevant. Both in vivo and in vitro studies have shown that the methylation of the critical cytosine within the NGFI-A consensus sequence eliminates binding of the transcription factor to the exon 1_7 sequence of the glucocorticoid receptor. Presumably, the offspring of the low-LG-ABN mothers thus lose the ability to increase hippocampal glucocorticoid receptor expression through NGFI-A activation.

The offspring of high-LG mothers exhibit increased hippocampal glucocorticoid receptor expression from the exon 1_7 promoter and dampened hypothalamic-pituitary-adrenal axis response to stress. The differential pattern of methylation of the exon 1_7 glucocorticoid receptor promoter is proposed as a critical mechanism. DNA methylation attracts histone deacetylases that stabilize the tight histone-DNA configuration and prevent transcription factors, such as NGFI-A, from binding to promoter sites. In support of this idea, we found that trichostatin A, a

compound that inhibits histone deacetylases, reverses the differences in NGFI-A binding to the exon 1_7 promoter of the hippocampal glucocorticoid receptor gene in the offspring of low-LG-ABN mothers, increasing receptor expression and reversing the differences in HPA responses to stress. Thus DNA methylation does appear to be one mechanism for the enduring maternal effects on the development of defensive responses to threat in mammals.

Parenting as a Competitive Sport

Maternal effects on the expression of defensive responses, such as increased hypothalamic-pituitary-adrenal activity, are a common theme in biology. Alteration of the methylation status of targeted DNA sites in response to variations in environmental stimulation might ultimately be a process mediating such maternal effects. DNA methylation could serve as an intermediate process that imprints dynamic environmental experiences on the fixed genome resulting in stable alterations in phenotype.

But what are the origins of the individual differences in maternal behavior? Perhaps the pivotal feature of this model is the increased estrogen receptor expression in the medial preoptic area (mPOA) of the hypothalamus of high-LG mothers; the mPOA is heavily implicated in the expression of maternal behavior in the rat. The increased estrogen sensitivity enhances oxytocin activity in the mPOA that, in turn, increases dopamine release from the nucleus accumbens. The increased dopamine release in the nucleus accumbens then drives pup licking and grooming. Now all this is great fun if you enjoy the details of neuroendocrinology, which I do immensely. But I could easily

forgive readers for wondering if we have not missed the essential question. Why are not all mothers equally investing in their offspring?

Anxious human mothers are generally less sensitive to their offspring. Lactating Bonnet monkeys that are chronically stressed postpartum develop far more contentious relationships with their infants (Coplan et al., 1996). Likewise, when my colleagues and I stressed normally high-LG-ABN dams postpartum, they became low-LG-ABN dams. For reasons that are very poorly understood at the level of mechanism, stress seems to consistently decrease parental investment in the young, resulting in patterns of parental care that increase stress reactivity in the offspring.

Environmental adversity is translated into a pattern of maternal care that enhances the defensive responses of the offspring and likely reflects a very adaptive pattern of development. Children inherit not only genes from their parents, but also an environment: Englishmen inherit England, as Francis Galton remarked. Under conditions of increased environmental demand, it is commonly in the animal's interest to enhance its behavioral responsivity (e.g., vigilance, fearfulness) as well as its endocrine (HPA and metabolic or cardiovascular) responsivity to stress. These responses promote detection of potential threat, avoidance learning, and metabolic or cardiovascular responses that are essential under the increased demands of the stressor. Because the offspring usually inhabit a niche that is similar to their parents, the transmission of these traits from parent to offspring could serve to be adaptive. The key issue here is that of the potential adaptive advantage of the increased level of stress reactivity apparent in the offspring of low-LG mothers. In the present context, the research of Farrington and colleagues

(1988) and Tremblay (e.g., Haapasalo & Tremblay, 1994) on young males growing up in impoverished, high-crime environments in urban environments provides an excellent illustration of the potential advantages of increased stress reactivity. In this environment, the shier and more timid males were most successful in avoiding the pitfalls associated with such "criminogenic" environments. Under such conditions a parental rearing style that favored the development of increased stress reactivity to threat would be adaptive. Thus it is understandable that parents occupying a highly demanding environment might transmit to their young an enhanced level of stress reactivity in "anticipation" of a high level of environmental adversity. Such a pessimistic developmental profile would be characterized by an increased corticotropin-releasing factor gene expression, and by patterns of gene expression that dampen the capacity of inhibitory systems, such as the hippocampal glucocorticoid receptor system. The quality of the environment influences the behavior of the parent, which in turn is the critical factor in determining whether development proceeds along an optimistic versus a pessimistic pattern of development. In mammals, as in the radish or water flea, parental signals serve as a "forecast" of the level of adversity that lies ahead. The obvious conclusion is that there is no single ideal form of parenting: various levels of environmental demand require different traits in the offspring. This is a simple, even obvious message, with significant social implications.

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