CHILD DEVELOPMENT

Child Development, January/February 2010, Volume 81, Number 1, Pages 41–79

Epigenetics and the Biological Definition of Gene × Environment Interactions

Michael J. Meaney
McGill University

Variations in phenotype reflect the influence of environmental conditions during development on cellular functions, including that of the genome. The recent integration of epigenetics into developmental psychobiology illustrates the processes by which environmental conditions in early life structurally alter DNA, providing a physical basis for the influence of the perinatal environmental signals on phenotype over the life of the individual. This review focuses on the enduring effects of naturally occurring variations in maternal care on gene expression and phenotype to provide an example of environmentally driven plasticity at the level of the DNA, revealing the interdependence of gene and environmental in the regulation of phenotype.

The nature–nurture debate is essentially a question of the determinants of individual differences in the expression of specific traits among members of the same species. The origin of the terms *nature* and *nurture* has been credited to Richard Mulcaster, a British teacher who imagined these influences as collaborative forces that shape child development (West & King, 1987). History has conspired to pervert Mulcaster's intent, casting genetic and environmental influences as independent agents in the field of development. The intent of this article is to examine the biological context in which genetic and environmental signals actually operate. The result of this analysis produces an impression that lies close to Mulcaster's formulation.

Any successful attempt to constructively leverage the remarkable advances of the genomic era will depend upon our ability to understand genetic influences and their interactions with the environmental context within which they operate. Hindering such efforts is the rather arcane notion that we can partition the causes of individual differences into distinct genetic *or* environmental spheres of influence. This issue is of particular importance in the area of child development where research that examines the origins of individual differences in complex traits often forms the basis for extensive intervention or prevention programs (Fisher, Gunnar, Chamberlain, & Reid, 2000; Olds et al., 1998). Our assumptions concerning the processes of child development influence the design of such programs. Thus, one of the great challenges at this time is that of integrating genomics into the design of prevention and intervention programs. In the social sciences, and particularly psychology, there has generally been an understandable bias in favor of explanations derived from the ''nurture'' perspective, which emphasizes the capacity for environmentally induced plasticity in brain structure and function. Nevertheless, over recent decades there has been a gradual, if at times reluctant acceptance that genomic variations contribute to individual differences in brain development and function. And thus the integration of genetics into psychology and psychiatry has become one of the more active research areas in the study of neural function. Now that we have come to accept the idea that genomic variation influences brain development and function in humans, it is time to consider what this should mean in the reformulation of intervention programs that target children and their families.

The idea that variation in the expression of neural functions would lie outside the dominion of genomic influences is biologically untenable. Like any tissue, the development of the mammalian brain and its function occurs as a result of coordinated influences that include heritable variation in genomic sequence. What remains as a major challenge for the psychological sciences is the development of a conceptual framework from which to meaningfully understand the results of studies that examine brain development and function at the level of the genotype. However, we remain mired in additive models of explanation within which phenotypic development rolls out as the cumulative by product of genetic + environmental

Correspondence concerning this article should be addressed to Michael J. Meaney, Douglas Mental Health University Institute, McGill University, 6875 LaSalle Blvd, Montreal, QC, Canada H4H 1R3. Electronic mail may be sent to michael.meaney@mcgill.ca.

influences, with each discipline adding independently to the equation. Science in the postgenomic era requires a more sophisticated, biologically informed understanding of the interplay between gene and environment in defining individual differences at the level of complex traits.

The fundamental question is that of the relation between genotype and phenotype: How do variations in the genotype influence selected traits at any point over the lifespan? This question is addressed very differently in epidemiology and the social sciences than it is in the biological sciences. Indeed, the actual meaning and interpretation of the term *gene* can differ across research domains (Griffiths & Tabery, 2008). For epidemiologists or those employing epidemiological approaches, genotype–phenotype relations are defined by statistical probabilities. A gene *for* aggression is thus defined as a genomic region within which a defined variation in sequence predicts a specific phenotypic outcome, in this case one associated with aggressive thoughts or actions. Here, genotype–phenotype relations are statistical. For the biological scientist, genotype–phenotype relations are defined by the actual physical operation of a genomic region in relation to the proximal cellular events that directly mediate the behavioral variation as well as the more distal influences that regulate the relevant cellular signals. Thus, a gene *for* aggression would be defined as a genomic region from which a transcribed product actively participates in a biological pathway that *directly* mediates the expression of aggressive behaviors. The studies of Young and colleagues (Donaldson & Young, 2008; Hammock & Young, 2005) on the sequence variation in the vasopressin 1A receptor gene provide an excellent example. Among voles, subspecies differences in social behaviors are associated with a sequence variation in the vasopressin 1A receptor gene and differential expression of the vasopressin 1A receptor. Reversing the pattern of vasopressin 1A receptor expression eliminates the differences in selected, vasopressin-mediated social behaviors (see Robinson, Fernald, & Clayton, 2008, for additional examples). These studies describe a causal genotype–phenotype relation. Within the biological sciences, the mere statistical relation between a genomic variation and phenotypic outcome would not be considered as sufficient basis for the establishment of a functional link between the gene (or its product) and the relevant variation in phenotype. Such differences in perspective have led to rather lively debates between, for example, researchers in quantitative behavioral genetics and researchers examining the biological basis for individual differences in neural function (Griffiths & Stotz, 2007; Griffiths & Tabery, 2008; Moss, 2005).

The issue becomes even more complicated when considering the interaction between gene and environment, since the statistical approaches to the study of Gene × Environment interactions are complicated and not yet fully developed. Indeed, since the time of Fisher and Haldane there has been debate concerning the adequacy of analysis of variance (ANOVA) models in detecting Gene × Environment interactions (Wahlsten, 1990; see also Griffiths & Stotz, 2007; Griffiths & Tabery, 2008, for historical reviews). The primary objective of this article is to examine the Gene × Environment interaction from the biological perspective. For whatever the difficulties in statistically defining Gene × Environment interactions, research in biology reveals that the genome cannot possibly operate independent of its environmental context. The biological perspective reveals the futility of the nature–nurture debate and of additive models of genetic and environmental influences in defining phenotype. Moreover, recent studies from the field of epigenetics provide insight into biological mechanisms whereby Gene × Environment interactions can biochemically alter the genome and thus stably influence individual differences in neural function. Developmentalists studying the enduring effects of early experience on brain development and function have long anticipated such processes (Bateson et al., 2004; Gottlieb, 1997, 1998; Schneirla, 1966). This approach envisions development as an active process of adaptation that occurs as a function of the continuous dialog between the genome and its environment. With the integration of epigenetics into developmental psychobiology, we can now begin to define the physical basis for this interactive process.

Statistical Approaches to the Study of the ''Instrumental'' Gene

Research examining the potential influence of heritable variations in DNA sequences on brain development and function in humans has relied largely upon two approaches. The first is a classical epidemiological approach that involves association or linkage analyses that essentially use correlational approaches to describe genotype–phenotype relations. Much as epidemiological research statistically associates specific exposures with health outcomes, such studies correlate variation in nucleotide sequence at specific sites in the genome with a

phenotypic outcome. This is certainly a reasonable approach in the initial phase of examining the *potential* role for a specific genomic variant (Plomin & Rutter, 1998). The advent of high-throughput technology permits genome-wide association studies that can potentially scan 1 million or more single nucleotide polymorphisms in individual subjects. While there are inherent statistical complications, such as controlling for multiple comparisons, the notion of being able to examine the relation between 10^6 sequence variants with a single trait of interest across a sufficiently large sample is incredibly seductive. Nevertheless, such studies do not inform at the level of causal mechanism. A correlation between a genomic variant and a developmental outcome is no less correlational and no more explanatory (or mechanistic) than is the correlation between, say, level of education or a dietary constituent and the same developmental outcome. The popular press often trumpets such findings (usually simply echoing the institute's own press release) as the identification of a gene *for* an outcome of interest. Studies that, for example, statistically identify a relation between a genomic polymorphism and the variation in the occurrence of conduct disorder are interpreted as successfully revealing the gene *for* conduct disorder. Such interpretations drive many scientists slightly mad, since the data are merely correlational and do not imply a cause–effect relation between the genomic variant and conduct disorder. The gene might indeed be expressed only in the liver and influence the development or activity of the relevant neural systems that regulate social behavior only through a very general metabolic pathway that fuels brain development in early life. There may be no direct effect on any neuronal population whatsoever, let alone the complex neural network that underlies the ability to regulate affect and behavior. Nevertheless, the genomic variant is indeed statistically associated with conduct disorder. The controversy surrounding the claims from association or linkage studies lie in the interpretation of the data and the implicit confusion of correlation with causation. The data are safely assumed to provide the identification of *potential* influence over the phenotype in question. However annoying the misuse of such correlational data in public or professional discourse, the findings serve as a starting point for research examining the possible functional relation between the genomic variant and the phenotype. In the case of our example, the nature of the relation between the genomic variant of interest and conduct disorder must then become a matter of more precise studies that directly examine the functional significance of that genomic variant, as well as the particular circumstances under which the genotype–phenotype relation is apparent (Plomin & Rutter, 1998).

A second statistical approach is one that has evoked greater controversy. Quantitative behavioral genetics does not examine genotype–phenotype relations at the level of specific genomic variants but rather attempts to partition the variance in the expression of a specific phenotypic traits across a population into that associated with ''genetic'' or ''environmental'' influences (Plomin, DeFries, & McClearn, 1990). Such studies actually examine so-called genetic effects without ever directly examining the genome let alone variation in the genome (Sokolowski & Wahlsten, 2001). Indeed within quantitative behavioral genetics the issue that is directly under study is that of heritability. Genetic effects are simply implied on the basis of data derived from studies of the heritability of traits. This statistical approach, based on the development of ANOVA models by Fisher and Haldane at the turn of the last century, has a long history in agriculture and animal sciences as the basis for selective breeding for specific traits (Griffiths & Tabery, 2008; Wahlsten, 2003). Such endeavors do not rely on the assumption that heritability necessarily reflects a genomic mechanism for the transmission of individual differences in the trait from parent to offspring. What is critical for selective breeding is the ability to reliably estimate the passage of traits from the parent to offspring. Traits with greater heritability are better candidates for selective breeding. The actual mechanism of inheritance is irrelevant. I think that the situation in the biological sciences is mixed. For some, heritability simply reflects familial transmission. For others, it has come to be equated with a true estimate of variation due to variation in genomic sequence. Estimates of heritability are commonly equated with ''genetic'' influences. It is this assumption that proves controversial.

Johannsen (1911) first introduced the terms *genotype* and *phenotype* and the relation between the two became perhaps the defining question in biology. In the study of genotype–phenotype relations, the term *phenotype* refers to all aspects of gene function and may thus be used to describe any observable characteristic of an organism, such as its morphology, biochemical or physiological properties, or behavior (Johannsen, 1911; Lewontin, 1974). The gene had actually been the subject of scientific analysis well before the early 20th century and long

before the identification of DNA as the physical basis for the ''genetic code.'' Over this period, the gene was considered as the hypothetical physical constituent of the cell, the transmission of which from parent to offspring served as the basis for Mendelian inheritance (Griffiths & Stotz, 2007; Griffiths & Tabery, 2008). The gene was defined as the ''unit of heredity.'' And thus it was understandable that during this period estimates of heredity were assumed to reflect a genetic influence. This was true by definition. Griffiths and Tabery (2008) thus refer to this entity as the ''traditional gene'' —the substance of inheritance. For the early pioneers in genetics such as Morgan, and many studying patterns of inheritance to this day, the actual physical identity and operation of the gene is irrelevant (Falk, 1986). This tradition continues within the study of quantitative behavioral genetics for which the gene is simply viewed as the unit of inheritance, with apparently little concern for the actual biology that might mediate the relation of the genomic variant, cellular function and the trait of interest.

A classical research approach in quantitative behavioral genetics has employed studies of twins to infer ''genetic'' influences. Twin studies rely on the level of similarity in specific traits between monozygotic and dizygotic twins to derive estimates of heritability (h^2). As noted earlier, such measures were developed in animal sciences using parent–offspring regressions to study intergenerational transmission of specific traits. The intent of such studies is not to identify the causal interplay between gene and environment in defining the development and expression of a particular trait in an individual but rather to estimate the relative contribution of genetic and environmental influences in defining the variation in the trait across a population (Plomin et al., 1990). The focus on ''relative contributions'' of gene and environment as *independent* forces is the hallmark of quantitative behavioral genetics and assumes that such forces can act independently of one another. In following the biometric tradition of Fisher and Haldane, the variance in the expression of a phenotypic trait across a population is examined using ANOVA models that partition variance in phenotype according to genetic and environmental factors. Note that within such analyses the ''genetic'' influences are defined *statistically* and not biologically, and of course the same is true for the definition of so-called environmental influences. Moreover, the resulting analyses are an attempt to understand the source of variation in phenotype across a population and not as an explanation for individual patterns of development (Griffiths & Tabery, 2008).

Studies of the heritability of selected traits thus yield *statistical* and not *biological* evidence for a genetic influence. The irritation derives from the interpretation of such findings, and in particular the notion of ''main effects,'' which implies that the genome and its environment can operate independently. And of course the angst should be no less for environmental main effects than for those associated with genotype. Again the issue is that of interpretation and an appreciation of what such findings actually do and do not represent.

Twin studies derive estimates of heritability. Such findings are of considerable importance for their own sake, particularly for those interested in issues surrounding familial transmission of vulnerability for illness. An indication of high heritability for a specific trait logically leads to the question of whether genomic variations at the level of sequence might serve as the mechanism for the passage of individual differences in phenotype from parent to offspring (prior to investing valuable research funds in genome-wide searchers, it is indeed useful to establish that the variation in the trait is actually subject to familial transmission). But the idea that estimates of heritability are the *equivalent* of a genetic effect is problematic. First, such estimates ignore Gene × Environment interactions. Indeed, heritability equates with a main effect ''genetic'' influences only if one assumes no Gene × Environment contribution. Second, the estimate of heritability reflects the importance of genomic mechanisms only if one assumes that there are no other biological mechanisms for inheritance. This is simply untrue. There are multiple potential mechanisms of inheritance, involving, for example, the passage of epigenetic marks (Chong & Whitelaw, 2004) through the germline, the passage of maternal RNA molecules into the embryo, the potential passage of prion proteins from parent to offspring, the biochemical state of the gametes at the time of conception, and the transmission of nutrients, bacteria, or antibodies from maternal circulation to that of the offspring, and so on (Bettegowda & Smith, 2007; Boulinier & Staszewski, 2008; Chong & Whitelaw, 2004; Grindstaff, Brodie, & Ketterson, 2003; Hasselquist & Nilsson, 2009; Rassoulzadegan et al., 2006; Shorter & Lindquist, 2005). Variations in genomic sequence are simply not the only mechanism of inheritance. All of these factors can and do influence the phenotype of the offspring. This is a serious problem for those assuming that heritability equates with main effects of genomic variation.

Apart from the methodological and biological pitfalls with such inferences (Lewontin, 1974; Sokolowski & Wahlsten, 2001; Wahlsten, 1990), the argument presented here is that the notion of a main effect of either ''gene'' or ''environment'' on the development of individual differences in complex traits is biologically fallacious. Instead, the development of the individual is best considered as the emergent property of a constant interplay between the genome and its environment (Gottlieb, 1991, 1997). This article briefly examines how the study of gene activity or expression reveals why main effects models are *biologically* implausible and then reviews studies on the effects of maternal care on glucocorticoid receptor expression in the rat as a model for the understanding one process by which Gene × Environment interactions might operate to influence the development of individual differences.

The Molecular Gene and Its Operation

The challenge within developmental psychology is that of understanding the operation of the genome and how the analysis of nucleotide-based variation can contribute to our understanding of individual differences in brain development and function at the level of cell biology, physiology, and emotional-cognitive states. One obvious way to start is to simply appreciate the role of DNA in cell biology and realize that DNA *directly* codes for molecules, more specifically for ribonucleic acids (RNAs), and not for higher levels of function. While there are indeed statistical relations between variation in nucleotide sequence and that in complex traits, at the level of biology there are no genes for intelligence, depression, athletic abilities, fashion sense, or any other such complex trait. Rather, there are certain variations in genomic sequences that can potentially alter either the DNA product (RNA) or the degree to which the DNA is transcribed (i.e., actively producing its molecular product). There are multiple and complex cellular processes that lie between the DNA sequence and the functional outcome associated with the gene product. The relation between genotype and phenotype, even at the level of cellular molecules, is anything but direct. Thus, the intent of this rather brief section is to simply illustrate the complexity of the processes that mediate genotype–phenotype relations even when considering the most fundamental aspects of gene function—the production of RNAs and protein products.

Throughout portions of this review I will largely focus on one gene, that encoding for the glucocorticoid receptor, both as an exemplar, for which it is rather well suited, and as a subject for our own research. The glucocorticoid receptor belongs to the nuclear receptor superfamily and is transcribed from a single mRNA. The glucocorticoid receptor protein is found both in the cytoplasm and in the nucleus of the cell. The receptor belongs to a class of intracellular proteins referred to as ligand-activated transcription factors. This simply refers to the fact that the binding of the hormone (the ligand) to the receptor alters the configuration of the glucocorticoid receptor, which then permits binding to specific sites on the DNA and the regulation of gene transcription. Glucocorticoids, a hallmark of the endocrine stress response, are hormones secreted by the adrenal cortex that bind to the glucocorticoid receptor to mediate various cellular processes including those involved in cardiovascular activity, appetite, metabolism, immune responses, electrolyte balance, as well as neuronal function and behavior (e.g., Dallman, Akana, Strack, Hanson, & Sebastian, 1995; Dallman, Pecoraro, & la Fleur, 2005; de Kloet, Karst, & Joels, 2008; Heitzer, Wolf, Sanchez, Witchel, & DeFranco, 2007; Kumar & Thompson, 2005; Munck, 2005; Munck, Guyre, & Holbrook, 1984; Sapolsky, Romero, & Munck, 2000; Seckl & Holmes, 2007; Walker, 2007). Thus, the gene encoding the glucocorticoid receptor has multiple, tissue-specific functions. The activation of the glucocorticoid receptor alters gene expression, the number and identity of which depends very much upon the target cell. When not bound by glucocorticoids, the glucocorticoid receptor resides in the cytoplasm of the cell associated with what is termed a chaperone protein (Kumar & Thompson, 2005; Lu & Cidlowski, 2006; McNally, Müller, Walker, Wolford, & Hager, 2000). Binding of glucocorticoids induces conformational changes in the receptor; dissociation from the chaperone proteins; association with another, activated glucocorticoid receptor protein, thus forming a dimer (association of two proteins); translocation into the nuclear compartment; and binding to specific sites on the DNA (Lu & Cidlowski, 2006 and references therein). The activated glucocorticoid receptor can also complex with other proteins, or cofactors, that then modify the effect of glucocorticoid receptor on the expression of target genes. The association of the cofactor may simply modify the ability of the glucocorticoid receptor to access DNA sites, and thus the magnitude of its transcriptional effect. However, the presence of the cofactor may determine the nature of the affected

genomic target. Thus, an activated glucocorticoid receptor associated with factor A may influence a different set of genes than the activated glucocorticoid receptor associated with factor B. Thus, the glucocorticoid receptor can affect two different cellular populations in very different ways depending upon the associated cofactor. The function of the glucocorticoid receptor gene product depends upon the context within which it operates. Indeed, the presence of other transcriptional factors can actually determine the direction of the effect of the glucocorticoid receptor on a common genomic target, increasing transcription in one context and decreasing it in another (e.g., Diamond, Miner, Yoshinaga, & Yamamoto, 1990). For example, activation of the glucocorticoid receptor promotes cell survival in liver cells but activates apoptosis (cell death) in thymocytes. Thus, a sequence-based variation in the glucocorticoid receptor could (and indeed does) have exactly the opposite effect on two different cellular populations. Of course, not all contextual effects are quite so dramatic. But the influence of such cofactors reveals a complexity that suggests that the consequences of even a highly functional sequence variation in the glucocorticoid receptor gene, as an example, will be determined by the cellular mileu in which the glucocorticoid receptor gene is operational. Some cells would inevitably be more affected than others. The presence and activity of such cofactors is regulated by a variety of extra- and intracellular signals and forms an important feature of the context within which glucocorticoid receptor activity is defined. This rather complicates the study of genotype–phenotype relations and the plausibility of simple main effects of either genome or environment at the level of cell biology. Rather, such processes suggest that the effect of genomic sequence variation is understood only in terms of its interaction with environmental signals represented in the cellular context. Such signals, of course, would also reflect sequence variation in other genomic regions. Since the transcriptional cofactors that influence glucocorticoid receptor activity are constantly regulated by the internal and external environment, the functional implications of a sequence-based variation in the glucocorticoid receptor gene are inexorably linked to context.

The glucocorticoid receptor can also alter gene expression and cellular function through processes that do not involve the interaction of the receptor with DNA. Intracellular proteins interact to form complexes. In certain cases, such interactions inhibit the action of one of the partners. One of the primary biological effects of the activated glucocorticoid receptor is to dampen inflammation (Munck, 2005; Munck et al., 1984). This effect involves, in part, the interaction of the glucocorticoid receptor with another protein, NFKß (e.g., Nissen & Yamamoto, 2000), which is a major participant in the activation of inflammatory responses. The binding of the glucocorticoid receptor renders NFKß unable to access its DNA targets, thus muting the inflammatory reaction. However, this glucocorticoid receptor effect is apparent only if there is an elevation in NFKß. Thus, the effects of either a genomic or environmental influence would depend upon the immunological status of the organism, further reflecting the importance of context. The function of the glucocorticoid receptor gene with such protein–protein interactions further illustrates why notions of main effects of gene or environment at the level of biological mechanism are untenable.

Figure 1 outlines the organization of the glucocorticoid receptor gene in the rat, which was cloned in large part by the group of Yamamoto (Miesfield et al., 1984) with a recent addition from our group in collaboration that of Jonathan Seckl (McCormick et al., 2000). The organization of the gene is similar in rat and human (Nobukuni, Smith, Hager, & Detera-Wadleigh, 1995; Turner & Muller, 2005). The glucocorticoid receptor gene comprised nine exons (an exon is a region of DNA that contributes directly to an mRNA molecule). Exons 2–9 are coding regions that produce mRNA for amino acids sequences included in the glucocorticoid receptor protein (as in DNA–RNA–protein). Exon 1 is a

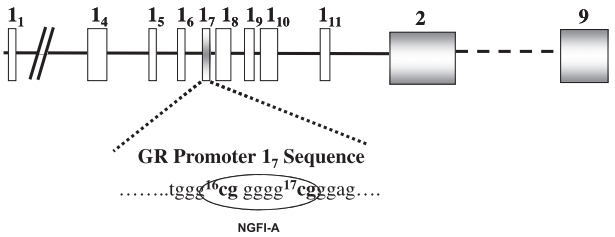

Figure 1. A schema describing the organization of the rat glucocorticoid receptor gene including nine exon regions.
Note. Exons 2–9 participate in the coding of the glucocorticoid receptor protein. Exon 1 comprised multiple regions, each of which is capable of activating gene transcription (i.e., promoter sequences). The various exon 1 promoters' actions are tissue-specific, with evidence suggesting that certain promoters are more active in areas such as liver or thymus, and others more active in brain (e.g., exon 17; based on McCormick et al., 2000; see Turner & Muller, 2005, for comparable data in humans). The consensus binding site for NGFI-A lying within the exon 1_7 promoter is highlighted. NGFI-A = nerve growth factor-inducible factor A.

large region containing a number of nucleotide sequences that are represented in the glucocorticoid receptor mRNA, but do not code for protein. This exon 1 region contains a number of regulatory elements, the activation of which alters the level of glucocorticoid receptor gene transcription. This exon 1 region is similarly organized in humans (Turner & Muller, 2005; see Zhang, Haws, & Wu, 2004, for examples of similar genomic organization). In the rat, each of the individual exon 1 segments displayed in Figure 1 contains a region that activates gene transcription (i.e., a transcriptional start site; McCormick et al., 2000) and is therefore referred to as a ''promoter.'' Thus, intracellular signals, referred to as transcription factors, bind to different regions on the exon 1 promoters to activate or repress the transcription of the glucocorticoid receptor gene. Figure 1 shows a small segment from the exon 1_7 promoter for the rat glucocorticoid receptor gene that binds the transcription factor nerve growth factor-inducible factor A (NGFI-A; Crosby, Puetz, Simburger, Fahrner, & Milbrandt, 1991).

The exon 1 region contains a number of promoters, and this complex level of regulation is essential for glucocorticoid receptor function. The glucocorticoid receptor gene is potentially active in virtually every cell in the body, with different functions across various cell types. In the fetal mammal, for example, the activation of the glucocorticoid receptor induces lung surfactant, a protein essential for the transition to a gaseous environment that marks birth. This effect is the basis for the glucocorticoid therapy of premature infants. Increasing glucocorticoid receptor function in pulmonary tissues in late fetal life is essential. Not so in the brain. Activation of glucocorticoid receptors in the mammalian forebrain is associated with a decrease in neurogenesis (Uno, Tarara, Else, Suleman, & Sapolsky, 1989) and synaptic plasticity (de Kloet et al., 2008; McEwen, 2007). Similar catabolic effects are apparent in several other tissues (Munck et al., 1984; Sapolsky et al., 2000). The ideal scenario is that of increasing glucocorticoid receptor expression in the lung while muting that in neurons and other active sites of growth (Bronnegard & Okret, 1991; Sapolsky & Meaney, 1986). Such tissue-specific expression of the glucocorticoid receptor gene is probably accomplished through the activation of different transcriptional promoters in various tissues. Indeed, different promoters are associated with the activation of the glucocorticoid receptor in lung, liver, thymus, and brain (McCormick et al., 2000). It is therefore possible to increase the expression of the glucocorticoid receptor in one tissue while leaving it unaltered or even decreased in another. Thus, a nucleotide-based variant in a glucocorticoid receptor promoter region might lead to altered transcription of the glucocorticoid receptor gene in one tissue, while the activation of the same gene in a different tissue is completely unaffected. The resulting genotype–phenotype interaction would then depend upon whether the trait under study is dependent upon a cellular population that employs the affected promoter sequence.

There is also variation of glucocorticoid receptor gene function at the level of the protein product (Lu & Cidlowski, 2006). Exons 2–9 encode multiple variants of the glucocorticoid receptor. Two of the variants (or isoforms), the α- and β-forms of the glucocorticoid receptor, are identical for the first 727 amino acids, beyond which they differ. Glucocorticoid receptor α has an additional 50 amino acids not contained in the ß-variant. The ß-variant has 15 distinct amino acids. The classic effects of glucocorticoids on gene expression are mediated through glucocorticoid receptor α. Glucocorticoid receptor ß seems to bind to glucocorticoid receptor α and to block its effects on gene transcription (Lu & Cidlowski, 2006). Indeed, increased expression of glucocorticoid receptor ß produces glucocorticoid resistance. What is remarkable is that two seemingly antagonistic proteins are actually encoded from the same gene! Further complicating the picture is the finding that the glucocorticoid receptor ß-variant is produced in only a subset of cells. The production of glucocorticoid receptor variants, and thus of glucocorticoid receptor gene, is entirely dependent upon the cellular context within which the gene operates.

The production of glucocorticoid receptor protein variants is not limited to the α- and ß-isoforms. More recent studies reveal at least eight different versions of the glucocorticoid receptor a protein, each produced from the same gene and even the same mRNA (Lu & Cidlowski, 2006). These glucocorticoid receptor α-isoforms are differentially distributed across various tissues and differ in their transcriptional activity on target genes. Certain genomic targets are regulated by all of the various glucocorticoid receptor α-isoforms, although transcription is regulated to a greater extent by some isoforms than by others. But some genes are regulated only by certain isoforms. In no case is this diversity in the genomic product associated with variation in nucleotide sequence. This complexity is an inherent feature of the glucocorticoid receptor system.

This brief review considers only the variation in the glucocorticoid receptor protein itself. If we factor into consideration the potential for such variation together with the multiple cofactors, each of which can alter glucocorticoid receptor function, the potential for diversity in signaling becomes substantially greater. If genotype–phenotype relations are so remarkably intricate at the level of protein, then imagine the complexity at the level of physiology and behavior! It is critical that we appreciate the degree to which the activity of the gene is dependent upon the cellular context within which it functions and to appreciate that this same context is also subject to the influences of sequence variations in other genes that operate within the same network. Both the form and the function of the protein product are defined by context. The function of the gene can only be fully understood in terms of the cellular environment in which it operates. And the cellular environment, of course, is dynamic, changing constantly as a result of signals from other cells, including those that derive from events occurring in the external environment. Ultimately, function can only be understood in terms of the interaction between environmental signals and the genome.

Gene Transcription

The most compelling evidence for the predominance of Gene × Environment interactions in cellular function emerges from the study of gene transcription. The transcription of a gene is a highly regulated event. At the heart of this process lies a class of proteins referred to as transcription factors (and see earlier). As the name implies, these proteins have the ability to bind to regulatory elements of the gene (e.g., see the exon 1 region of the glucocorticoid receptor gene) and to activate or repress gene transcription. Importantly, the expression and/or activation of the transcription factors themselves are dynamically regulated by environmental signals. Many of the earliest cellular responses to environmental stimuli involve either the activation of preexisting transcriptional signals through, for example, phosphorylation at specific sites, or the rapid synthesis of proteins (e.g., immediate early gene products) that then serve to regulate the activity of other genes. Thus, the binding of transcription factors to DNA sites is the biological machinery of the dynamic Gene × Environment interactions that result in altered rates of gene transcription.

Figure 1 portrays the organization of the glucocorticoid receptor gene. The schema is actually somewhat misleading. For reasons of graphic simplicity, we often describe the organization of a gene or the interactions between transcription factors and DNA as if the DNA were a linear molecule to which transcription factors gain unimpeded access. The reality of protein–DNA interactions is quite different. Figure 2 presents the classic crystallographic analysis of the organization of DNA (Luger, Mader, Richmond, Sargent, & Richmond, 1997). DNA is organized into units referred to as nucleosomes, each of which contains about 145–150 base pairs wrapped around a core region of histone proteins (Turner, 2001). The histones and DNA together are referred to as chromatin; the nucleosome is the organization of chromatin. Under normal conditions there is a tight physical relation between the histone proteins and its accompanying DNA, resulting in a rather closed nucleosome configuration. This restrictive configuration is maintained, in part, by electrostatic bonds between the positively charged histones and the negatively charged DNA. The closed configuration impedes transcription factor binding and is associated with a reduced level of gene expression. The activation of gene expression commonly requires chemical modifica-

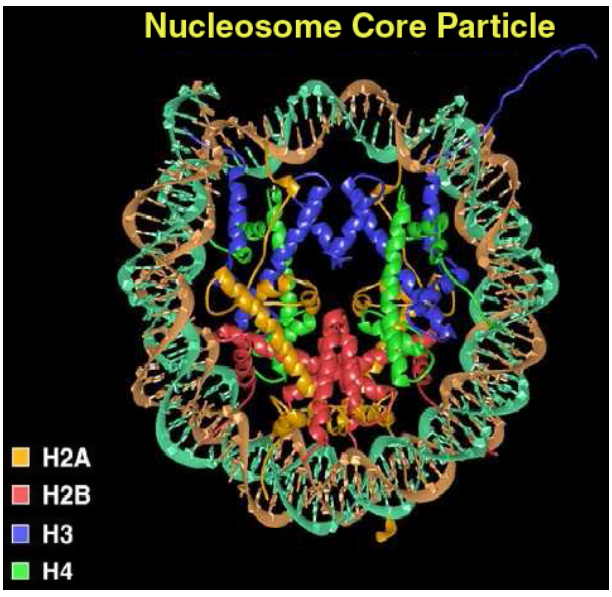

Figure 2. Crystallographic image of the nucleosome showing 146 bp wrapped around a histone complex that comprised histone 2A, 2B, 3, and 4 proteins (from Luger et al., 1997).
Note. The tight configuration is maintained, in part, by electrostatic bonds. Modifications, such as acetylation, to the histone regulate transcription factor binding and occur primarily at the histone tails protruding out of the nuclesome (pictured is the tail of histone 3).

tion of the chromatin that occurs on the histone proteins.

Histone Modifications

Chromatin remodeling is required for increased transcription factor binding to regulatory sites on the DNA and the activation of gene expression. The dynamic alteration of chromatin structure is achieved through modifications to the histone proteins at the ''tail'' regions that protrude outside of the nucleosome (Figure 2). This process is achieved through a series of enzymes that bind to the histone tails and modify the local chemical properties of specific amino acids along the tail (Grunstein, 1997; Hake & Allis, 2006; Jenuwein & Allis, 2001). The histone tails are chains of amino acids that include lysines and arginines that are primary targets for modification. For example, the enzyme histone acetyltransferase ''transfers'' an acetyl group onto specific lysines on the histone tails. The addition of the acetyl group diminishes the positive charge, loosening the relation between the histones and DNA, opening the chromatin, and improving the ability of transcription factors to access DNA sites. Thus, histone acetylation at specific lysine sites is commonly associated with active gene transcription. The functional antagonists of the histone acetyltransfereases are a class of enzymes known as histone deacetylases (HDACs). These enzymes remove acetyl groups and prevent further acetylation, thus serving to maintain a closed chromatin structure, decreasing transcription factor and gene expression. The reader should note that there are actually multiple modifications to histone tails including methylation (in this case on the histones), phosphorylation, ubiquitination, and so on. For the sake on simplicity, the discussion is limited to histone acetylation or deacetylation.

Regulation of Glucocorticoid Receptor Expression

Figure 3 summarizes the effect of the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]) on glucocorticoid receptor gene transcription in hippocampal neurons (Mitchell, Betito, Rowe, Boksa, & Meaney, 1992; Mitchell, Rowe, Boksa, & Meaney, 1990; Weaver et al., 2007). This effect is dependent upon the binding of the transcription factor NGFI-A to a specific binding site on the exon 1_7 GR promoter. We can precisely define the importance of this interaction. For example, mutating a single nucleotide, in this case simply exchanging a cytosine for an adenine, in the region of the promoter that normally

Summary of *In Vivo* and *In Vitro* Studies

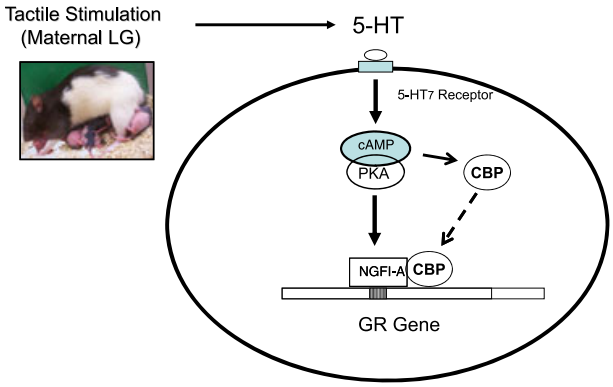

Figure 3. A summary of in vivo studies with hippocampal tissue samples from neonates and in vitro studies using primary hippocampal cell cultures.
Note. In vivo, an increased frequency of pup LG associates with hippocampal 5-HT turnover, activation of a 5-HT7 receptor positively coupled to cAMP and cyclic nucleotide-dependent kinases (PKA), and the induction of NGFI-A expression. In vivo, increased pup LG or artificial tactile stimulation induces NGFI-A expression as well as that of the CREB-binding protein, both of which show greater binding to the exon 17 promoter in the neonatal offspring High- compared with low-LG mothers. Results of in vitro studies show that blockade of cAMP, PKA, or NGFI-A abolish the effect of 5-HT of glucocorticoid receptor expression. cAMP = concentration of adenosine 3,5-monophosphate; CREB-binding protein = cAMP-response element-binding protein; 5-HT = 5-hydroxytryptamine; LG = licking/grooming; NGFI-A = nerve growth factor-inducible factor A; PKA = protein kinase A.

binds NGFI-A abolishes the ability of NGFI-A to associate with the exon 1_7 promoter and eliminates the effect of NGFI-A on gene transcription (Weaver et al., 2007). However, the ability of NGFI-A to bind to the exon 1_7 promoter is regulated by another protein, a cofactor, the concentration of adenosine 3,5-monophosphate (cAMP)-response element-binding (CREB) protein, that is, activated by the same 5-HT–cAMP/PKA (protein kinase A) signaling cascade (Figure 3). The CREB-binding protein is a histone acetyltransferase. The resulting increase in the association of the CREB-binding protein with the exon 1_7 promoter is accompanied by an increase in the acetylation of a specific lysine on the tail of histone 3 of the exon 1_7 promoter (Weaver et al., 2004; Weaver et al., 2007). Thus, 5-HT activates both NGFI-A and the CREB-binding protein, which appear to associate with one another prior to DNA binding. The CREB-binding protein acetylates histones associated with the exon 1_7 promoter, enhancing the ability of NGFI-A to bind and activate gene transcription.

Serotonin is a classic neurotransmitter that responds dynamically to environmental signals. This effect reflects the dependence of gene transcription on signals that derive from environmental events (note the relevant environmental event may be internal or external to the organism; e.g., a change in the availability of glucose, an electrical impulse, or a social interaction). Thus, the consequences of a variation in DNA sequence for phenotype will depend upon environmental signals. The genomic variant is relevant for phenotype only to the extent that the gene is actually expressed and participating in cellular function. If a particular tissue were to reveal a genomic variant in a region of the DNA that is transcriptionally inactive, then the implications for phenotype are minimal. The degree to which a genomic variant influences phenotype will depend upon the cellular context. Likewise, to the extent that an inherited genomic variant influences the capacity for transcription, then the influence of the environmental signal on phenotype will be affected by variation in nucleotide sequence. Such considerations showcase the interdependence of gene and environment.

Gene × Environment Interactions: The Case of the 5-HT Transporter

One of the best-studied genomic variations in biological psychiatry is that of the polymorphism in the promoter region of the *SLC6A4* gene that encodes for the 5-HT transporter (5-HTTP). The synaptic activity of 5-HT is terminated by reuptake into presynaptic terminals, which occurs via the 5-HTTP protein. Serotonin is a critical neurotransmitter in the regulation of emotional states (Lucki, 1998) and a major target for medications designed to treat a range of affective disorders (Blier & de Montigny, 1999; Gross & Hen, 2004). The gene for the 5-HTTP in humans shows a relatively common polymorphism characterized by a variable repeat sequence in the promoter region resulting in two common alleles: the short (*S*) variant comprising 14 copies of a 20–23 base-pair repeat unit, and the long (*L*) variant comprising 16 copies (Lesch et al., 1996).

The 5-HTTP promoter sequence polymorphism associates with differential transcription of the 5-HTTP gene, with more efficient expression from the *L* allele (Greenberg et al., 1999; Lesch et al., 1996). More recent studies suggest that further variation lying within the *L* allele also affects 5-HTTP gene function, resulting in subtypes of the *L* allele (Hu et al., 2005). The early studies of the functional correlates of the 5-HTTP promoter polymorphism as well as subsequent meta-analyses (Sen, Burmeister, & Ghosh, 2004; Schinka, Busch, & Robichaux-Keene, 2004; but also see Munafo, Clark, & Flint, 2005) reveal a statistical association with neuroticism, which is elevated among individuals that carry at least one copy of the *S* allele. Since neuroticism predicts an increased risk for affective disorders (Hettema, Neale, Myers, Prescott, & Kendler, 2006; Kendler, Kuhn, & Prescott, 2004), it is not surprising that the 5-HTTP promoter polymorphism is at least weakly associated with disorders of emotional function, namely, depression and anxiety (Canli & Lesch, 2007; Hariri, Emily, Drabant, & Weinberger, 2006).

Affective disorders associate with altered activity in the amygdala (Davis, 2006; Gorman, Kent, Sullivan, & Coplan, 2000; LeDoux, 2000; Meaney, LeDoux, & Leibowitz, 2008; Pérez-Edgar & Fox, 2005), a primary brain structure for emotional states of fear as well as a major target for 5-HT projections from the raphé nuclei. The expression of fear in rodents is inhibited by 5-HT activity in the hippocampus and amygdala (Stutzmann & LeDoux, 1999), and animals bearing mutations of 5-HT1A receptors show enhanced fearfulness (Gross & Hen, 2004). Aversive stimuli activate the amygdala in humans (LaBar et al., 1995; LaBar et al., 1998; Morris, Ohman, & Dolan, 1998) and individual differences in trait anxiety predict the response of the amygdala to threatening stimuli (Etkin, Egner, Peraza, Kande, & Hirsch, 2005; Etkin et al., 2004). Individuals carrying an *S* variant of the 5-HTTP promoter polymorphism show increased activation of the amygdala while processing fearful or angry faces (Hariri et al., 2002; Hariri et al., 2005; Pezawas et al., 2005). Similar effects of the 5-HTTP promoter polymorphism are obtained in subjects during processing of negative relative to neutral pictures (Heinz et al., 2005) or words (Canli et al., 2006). Moreover, the *S* allele of the 5-HTTP promoter is associated with increased activity at rest in both the amygdala and the hippocampus (Canli & Lesch, 2007).

An additional feature of anxiety is that of decreased inhibitory prefrontal regulation of evoked activity in the amygdala (Shin et al., 2004, 2005). Affective disorders associate with decreased volume in the subgenual region of the anterior cingulate cortex (Drevets et al., 1997), which projects to and regulates activity in the amygdala (Maren & Quirk, 2004). Gray matter volume in the subgenual anterior cingulate cortex is reduced in carriers of the *S* variant of the 5-HTTP promoter

polymorphism. In addition, individuals bearing the *S* promoter variant show decreased functional connectivity between the amygdala and anterior cingulate cortex (Pezawas et al., 2005), which could serve as the basis for the increased amygdala response to negative stimuli.

Since genomic variations are potentially operative at any time over the lifespan, it is interesting to consider the possibility that the 5-HTTP polymorphism could alter 5-HT activity during periods of neural development. Thus, Pezawas et al. (2005) proposed that the association between the 5-HTTP promoter polymorphism and vulnerability to depression reflects a developmental process that affects the structural connectivity and functional interactions within a neural circuit that regulates emotional reactivity and fear. They further proposed that these functional deficits could be exacerbated by environmental adverse experiences, possibly through an impaired capacity to regulate affective states during periods of adversity. Such an effect would be expected to result in an interaction between gene and environment that renders individuals vulnerable to affective disorders. There is considerable evidence in support of this developmental hypothesis. Studies with rodent models reveal a profound influence of 5-HT activity on neuronal development, including the sprouting of axonal processes. Hen and colleagues (Gross et al., 2002) showed that a null mutation of the 5-HT1A in mice associates with increased fearfulness. This knockout model, like that of human sequence polymorphisms, reveals effects from conception onward to the time of behavioral assessment. It is therefore unclear as to whether the associated change in phenotype reflects a developmental influence, an effect on neurotransmission at the time of assessment, or both. Hen's lab (Gross & Hen, 2004; Gross et al., 2002) resolved this issue by producing a conditional knockout model in which the gene that encodes for the 5-HT1A receptor is rendered inoperative only during selected periods over the lifespan of the mouse. The findings reveal that the condition in which the 5-HT1A receptor gene is muted for only during the first 3 weeks of life completely recapitulates the increased fearfulness observed in animals in which the 5-HT1A receptor was knocked out for the entire life of the animal. These findings suggest that genomic variants that alter 5-HT function can act during early development to regulate neural circuitry that mediates the expression of fear. Moreover, variants of the gene for the 5-HTTP are associated with negative temperament in infancy (Auerbach et al., 2002; Ebstein, 2003, 2006), further suggesting that the consequences of 5-HT alterations appear in early life. Here again, however, there is environmental modulation of the genotype–phenotype relation. Fox et al. (2005) reported that the quality of the maternal environment interacted with the 5-HTTP promoter genotype to determine behavioral inhibition in children.

Fox et al.'s (2005) report is representative of a growing body of studies revealing Gene × Environment interactions in emotional and cognitive development. Suomi, Bennett, and colleagues described a brilliant example of such gene–environment interdependence in the rhesus monkey (Bennett et al., 2002; Champoux et al., 2002; Suomi, 2006). The rhesus monkey shows a polymorphism in the 5-HTTP promoter that is comparable in form and function to that in humans such that the *S* 5-HTTP promoter variant in the monkey is also associated with decreased 5-HTTP levels in brain (Lesch et al., 1997). Likewise, the 5-HTTP promoter polymorphism affects both 5-HT metabolism and emotional function in the monkey. Monkeys bearing the *S* version of the 5-HTTP promoter polymorphism show reduced 5-HT activity, increased impulsivity, and are more aggressive than animals bearing the longer version of the promoter variant (Bennett et al., 2002; Champoux et al., 2002). However, the genotype–phenotype relation is strongly influenced by the rearing environment. The phenotypic differences described earlier are apparent among animals separated from their mothers and reared in peer–peer groups. In contrast, among animals reared by their mothers, the differences in phenotype are actually slightly reversed such that animals with the *S* variant of the 5-HTTP promoter show modestly greater 5-HT activity and are less impulsive and aggressive than those carrying the longer promoter variant. In these studies, the genotype–phenotype relations depended completely upon the rearing environment. Similar effects are apparent in the mouse where there is an interaction between a disruption of the 5-HTTP gene and the quality of maternal care on neural systems associated with fear behavior (Carola et al., 2008).

A series of landmark studies (e.g., Caspi & Moffit, 2006; Caspi et al., 2003) provide a parallel set of findings in human populations. In these studies, as shown in previous research, childhood maltreatment increases the probability of an episode of major depression. However, this effect is only apparent among individuals that bear at least one copy of the *S* 5-HTTP promoter allele. Individuals homozygous for the *L* promoter variant show no increase in the probability of depression despite the

experience of childhood maltreatment (see Caspi & Moffit, 2006, for a review of other, compelling examples of such Gene × Environment interactions). A more recent study shows evidence for the same Gene × Environment interaction in defining individual differences in neural functions that associate with depression (Canli et al., 2006). There is also evidence of a comparable Gene × Environment effect examining polymorphisms in other genes implicated in 5-HT neurotransmission (Jokela et al., 2007). In this case, the polymorphism in question is a SNP in the *5HTR2A* gene that encodes for the 5-HT2A receptor that is also linked to mood disorders. The authors found a significant interaction between the *5HTR2A* genotype and the quality of maternal care in defining the level of depressive symptoms.

The Caspi et al. (2003) finding of an interaction between the promoter polymorphism and either life stressors or childhood maltreatment has been replicated (Kaufman et al., 2004; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005), and other studies suggest further moderation by gender (Eley et al., 2004; Grabe et al., 2005) or social support (Kaufman et al., 2004). Two studies (Gillespie, Whitfield, Williams, Heath, & Martin, 2005; Surtees et al., 2006) failed to replicate this Gene × Environment interaction effect. However, we need to appreciate that an interaction between a *single* genomic variant and a broadly characterized environmental condition at one stage of life is still a rather narrow focus on the complex pathophysiology of mental disorders. Genes encode for RNAs and proteins that operate in complex networks within cell signaling pathways. Such networks form the basis for the interaction of gene products that are then revealed as gene–gene interactions in studies of genomic variations, including data sets from genome-wide association studies. A major advance in genetics is the emergence of computational approaches that apply a biologically informed, statistical analysis of the interactions between functionally related genes. Such bioinformatic approaches feature the description of gene networks, involving multiple genomic targets and their variations. We can thus anticipate studies of Gene Network × Environment interactions.

Arguments for the interdependence of gene and environment should be familiar to those in developmental psychology. Belsky (1997) and Rutter (2007) argue from the other point of view that environmental forces, such as familial influences, will not affect all children equally and that an obvious source of differential sensitivity lies in genomic variation. In the nonhuman primate studies cited earlier, animals bearing the *L* variant of the 5-HTTP promoter polymorphism are markedly less sensitive to peer versus maternal rearing than those carrying the *S* promoter variant (at least with respect to the measures used in these studies). The same argument could be made for the Caspi et al. (2003) findings that reveal an increased risk for depression associated with child maltreatment among those bearing *S/S* 5-HTTP promoter variants. Indeed, one might well imagine that certain genomic variants alter neuronal function of the child, affecting the response to environmental conditions. Studies examining the children with a polymorphism on the *DRD4* gene that encodes a receptor for the neurotransmitter dopamine bear on this point. This genomic variant is associated with differences in attachment (Gervai et al., 2007; Lakatos et al., 2002) as well as infant responses to novelty (Lakatos et al., 2003). The *DRD4* polymorphism interacts with the quality of parenting to determine the level of internalizing and externalizing behavior in children (Propper, Willoughby, Halpern, Carbone, & Cox, 2007). Moreover, the same polymorphism determines the effect of a parent-training intervention targeting maternal sensitivity on the level of externalizing behavior in children (Bakermans-Kranenburg, Van IJzendoorn, Pijlman, Mesman, & Juffer, 2008). In these studies, the genotype of the child determined the response to parenting. Likewise, considering the evidence for the effects of genomic variants on emotional functions in children, genomic variation might operate within specific familial settings (e.g., Fox et al., 2005) to influence the behavior of the child and thus the quality of the interactions with caregivers. The 5-HTTP polymorphism associates with differences in infant temperament and reactions to novelty (Auerbach et al., 1999; Ebstein, 2006; Lakatos et al., 2003), which in turn predicts sensitivity to parental influences (Belsky, 1997). Although the point is less emphasized in this article, the Gene × Environment perspective argues no less forcefully that environmental effects must be considered within the genomic context of the individual. The interdependence of gene and environment is bidirectional.

These findings underscore the importance of Gene × Environment interactions, and the studies with the rhesus monkey suggest that the developmental consequences of such interactions persist into adulthood. This consideration simply reflects the long-standing interest of psychologists on the potential influence of early experience (Gottlieb, 1991, 1997), particularly those involving the interaction of the parent and child, on phenotypic

development. In the preceding sections, we considered the molecular mechanisms that underlie Gene × Environment interactions with respect to immediate and transient changes in gene expression. But what are the mechanisms that might account for the enduring effects of environmental conditions on gene expression? How might parental influences stably alter phenotype? Could such effects involve the ''programming'' of gene expression? How might such stable influences occur? A response to such questions requires an understanding of the physical properties of Gene × Environment interactions that produce enduring effects on brain function.

Environmental Programming of Gene Expression

Studies in developmental psychobiology and physiology are replete with examples of the environmental programming of gene expression. Such studies commonly report that a variation in the early environment associates with changes in gene expression and function that persist into adulthood and thus well beyond the duration of the relevant environmental event. In the rat, for example, prenatal nutrient deprivation or enhanced exposure to hormonal signals associated with stress can stably alter, or program, the transcription of genes in the liver and other sites that are associated with glucose and fat metabolism, including the gene for the glucocorticoid receptor (Bateson et al., 2004; Gluckman & Hanson, 2004, 2007; Meaney, Szyf, & Seckl, 2007; Seckl & Holmes, 2007). These findings are assumed to represent instances in which the operation of a genomic region in adulthood varies as a function of early environmental influences. The results of recent studies suggest that such ''programming'' effects can derive from Gene × Environment interactions in early life that lead to a structural alteration of the DNA, which in turn mediates the effects on gene expression as well as more complex levels of phenotype (Meaney & Szyf, 2005; Meaney et al., 2007). These studies have been performed in rodents but were inspired by the vast literature reporting the pervasive effects of family environment on health outcomes in humans.

Epidemiological studies reveal the importance of family function and early life events as predictors of health in adulthood (Felitti et al., 1998; Leserman et al., 1996; Lissau & Sorensen, 1994; McCauley et al., 1997; Repetti, Taylor, & Seeman, 2002; Russak & Schwartz, 1997; Seckl, 2001). As adults, victims of childhood physical or sexual abuse, emotional neglect and harsh, inconsistent discipline are at considerably greater risk for mental illness, as well as for obesity, gastrointestinal illness, diabetes, and heart disease. ''Stress diathesis'' models are proposed as explanations for the relation between early experience and health (e.g., Gorman et al., 2000; Heim & Nemeroff, 2001; McEwen, 2003; Meaney, 2001; Meaney et al., 2007; Repetti et al., 2002; Seckl & Meaney, 1994). These models suggest that adversity in early life alters the development of neural and endocrine responses to stress in a manner that predisposes individuals to disease. Adversity or decreased quality of parental investment appears to directly increase the magnitude of emotional, autonomic, and endocrine responses to stress (collectively referred to here as *defensive responses*). Diathesis describes the interaction between development, including the potential influence of genetic variations at the level of sequence, and the prevailing level of stress in predicting health outcomes. Such models have considerable appeal and could identify both the origins of illness and the nature of the underlying vulnerability (as well as resilience). Moreover, such models have the virtue of providing a logical bridge between psychosocial (e.g., family function) and biological (endocrine responses to stressors) levels of analyses in understanding health outcomes.

A critical assumption in the stress diathesis models is that the increased expression of defensive responses endangers health. The idea has considerable support in clinical physiology (Chrousos & Gold, 1992; Dallman et al., 2005; McEwen, 2007). Defensive responses to stress are adaptive. Neural signals associated with the perception of the stressor increase the release of stress hormones into the bloodstream, including glucocorticoids from the adrenal gland and catecholamines, particularly norepinephrine, from the sympathetic nervous system. The combined actions of these hormones increase the availability of energy substrates, such as those derived from fat and glucose metabolism. Such effects maintain the normal cellular function and organ efficiency. These actions protect against catastrophes such as hypotensive shock. The release of catecholamines in the brain increases vigilance and provokes states of fear, and enhances avoidance learning and fear conditioning, which can serve to reduce the chances of further encounters with the same conditions.

Defensive responses are the logical outcome of the stress-induced changes in activity in the brain and endocrine organs, and are defined by increases in the synthesis and release of glucocorticoids and catecholamines (Dallman et al., 1995; Munck et al.,

1984; Rosen & Schulkin, 1998; Sapolsky et al., 2000). However, there is a cost associated with persistent activation of these same responses: chronically enhanced emotional arousal, sustained increases in blood sugars and fats, possible hyperinflammation, disruption of sleep and normal cognitive function, among others (Chrousos & Gold, 1992; Dallman et al., 2005; McEwen, 2007; Sapolsky et al., 2000; Walker, 2007). Thus, chronic activation of defensive responses can indeed predispose individuals to illnesses such as diabetes, heart disease, mood disorders, and so on. Not surprisingly, studies in behavioral medicine reveal that individuals with enhanced stress reactivity are at greater risk for such forms of chronic illness. However, it is also important to note that insufficient activation of defensive responses under conditions of threat also compromises health and is associated with chronic fatigue, chronic pain, posttraumatic stress disorder, and hyperinflammation (Munck et al., 1984; Raison & Miller, 2003; Yehuda & Bierer, 2008). We walk a fine line here. And this underscores the importance of an appropriate level of stress reactivity for the individual, one sufficient to ensure the maintenance of function during adversity, but not so excessive as to promote chronic illness. What is appropriate, of course, will vary depending upon the prevailing level of environmental demand. There is no single, ideal level of stress reactivity across all populations.

Individual Differences in Defensive Responses

In the late 1950s and early 1960s, psychologists Gig Levine and Victor Denenberg (Denenberg, 1964; Levine, 1970) reported that postnatal handling (or infantile stimulation) of infant rodents decreased the magnitude of both behavioral and hypothalamic–pituitary–adrenal (HPA) responses to stress in adulthood. These findings demonstrated the influence of the early environment over the development of rudimentary defensive responses to threat. The importance of such findings in this era should not be underestimated. This was a period when defensive responses were considered as ''innate,'' developing well outside the realm of experiential influence. Levine and others later suggested that the effects of handling were actually mediated by changes in maternal care. Thus, handling of the pups was thought to alter the behavior of the mother, which was then critical for the ''handling'' effects. The handling paradigm involves exposure of the neonate to a complex set of stimuli, including that of a novel physical environment (Tang, Akers, Reeb, Romeo, & McEwen, 2006). Nevertheless, postnatal handling of rat pups does indeed increase the licking/grooming (LG) of pups by the mother (e.g., Lee & Williams, 1977; Liu et al., 1997). Pup LG is a major source of tactile stimulation for the neonatal rat that regulates endocrine and cardiovascular function in the pup (Hofer, 2005; Levine, 1994; Schanberg, Evoniuk, & Kuhn, 1984). The question then was whether such variations in pup LG might directly alter the development of individual differences in defensive responses.

Subsequent findings revealed considerable evidence for the effect of maternal care on the behavioral and endocrine responses to stress in the offspring. One approach was to simply examine the consequences of naturally occurring variations in pup LG among lactating rats independent of any experimental manipulation. Among lactating rats there are considerable individual differences in the frequency of pup LG that are stable over the reproductive lifetime of the female (Champagne, Francis, Mar, & Meaney, 2003). The results of longitudinal studies are consistent with the Levine (1970) hypothesis. The male or female adult offspring of mothers that naturally exhibit increased levels of pup LG (i.e., high-LG mothers) show more modest behavioral and endocrine responses to stress compared to animals reared by low-LG mothers (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997; Menard, Champagne, & Meaney, 2004; Toki et al., 2007; Weaver et al., 2004; Zhang et al., 2006). Specifically, the offspring of high-LG mothers show reduced fearfulness and more modest HPA responses to stress. Cross-fostering studies, where pups born to high-LG mothers are fostered at birth to low-LG mothers (and vice versa), suggest a direct relation between maternal care and the postnatal development of individual differences in behavioral and HPA responses to stress (Caldji, Diorio, & Meaney, 2003; Caldji, Francis, Sharma, Plotsky, & Meaney, 2000; Francis et al., 1999; Weaver et al., 2004). In these studies, the rearing mother determined the phenotype of the offspring. Thus, variations within a normal range of parental care can dramatically alter phenotypic development in the rat.

The effects of maternal care on the development of defensive responses to stress in the rat involve alterations in the function of the corticotrophin-releasing factor (CRF) systems in selected brain regions (Figure 4). The CRF system furnishes the critical signal for the activation of behavioral, emotional, autonomic, and endocrine responses to stressors (Bale & Vale, 2004; Koob, Heinrichs, Menzaghi, Pich, & Britton, 1994; Plotsky, Cunning-

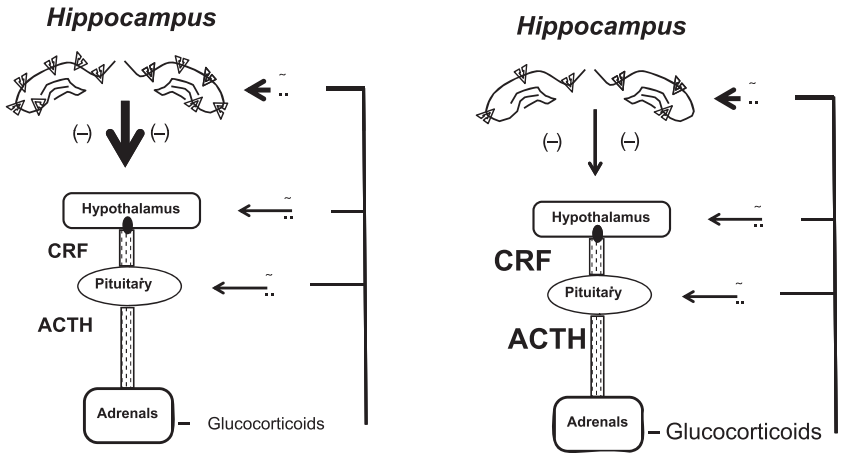

Figure 4. A schema outlining the function of the hypothalamic–pituitary–adrenal axis, the nexus of which are the corticotropin-releasing factor (CRF) neurons of the paraventricular nucleus of the hypothalamus.
Note. CRF is released into the portal system of the anterior pituitary stimulating the synthesis and release of adrenocorticotropin (ACTH), which then stimulates adrenal glucocorticoid release. Glucocorticoids act on glucocorticoid receptors in multiple brain regions, including the hippocampus, to inhibit the synthesis and release of CRF (i.e., glucocroticoid negative feedback). The adult offspring of high-LG mothers, by comparison to those of low-LG dams, show (a) increased glucocorticoid receptor expression, (b) enhanced negative-feedback sensitivity to glucocorticoids, (c) reduced CRF expression in the hypothalamus, and (d) more modest pituitary–adrenal responses to stress. LG = licking/grooming.

ham, & Widmaier, 1989). As adults, the offspring of high-LG mothers show decreased CRF expression in the hypothalamus, as well as reduced plasma adrenocorticotropin (ACTH) and glucocorticoid responses to acute stress by comparison to the adult offspring of low-LG mothers (Francis et al., 1999; Liu et al., 1997; Weaver et al., 2004; Weaver et al., 2005). Circulating glucocorticoids act at glucocorticoid receptor sites in corticolimbic structures, such as the hippocampus, to regulate HPA activity (Figure 4). Such feedback effects commonly inhibit hypothalamic CRF expression. The high-LG offspring showed significantly increased hippocampal glucocorticoid receptor expression, enhanced glucocorticoid negative-feedback sensitivity, and decreased hypothalamic CRF levels. Indeed, the magnitude of the glucocorticoid response to acute stress was significantly correlated with the frequency of pup LG during the 1st week of life, as was the level of both hippocampal glucocorticoid receptor and hypothalamic CRF expression (all *r*s > .70; Liu et al., 1997). Importantly, pharmacological manipulations that block the effect of the glucocorticoid receptor eliminate the maternal effect on the HPA response to stress, suggesting that the differences in hippocampal glucocorticoid receptor expression are directly related to those at the level of HPA function.

Pup LG is a major source of tactile stimulation for the neonate. Experimental models that directly apply tactile stimulation, through the stroking of the pup with a brush, provide direct evidence for the importance of tactile stimulation derived from pup LG. Thus, stroking pups over the 1st week of life increases hippocampal glucocorticoid receptor expression (Jutapakdeegul, Casalotti, Govitrapong, & Kotchabhakdi, 2003) and dampens behavioral and HPA responses to stress (Burton et al., 2007; Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001). Likewise, manipulations of lactating mothers that directly increase the frequency of pup LG also increase hippocampal glucocorticoid receptor expression and decrease HPA responses to stress (Francis et al., 1999; Toki et al., 2007).

The offspring of the high- and low-LG mothers also differed in behavioral responses to stress (Caldji et al., 1998; Caldji et al., 2003; Francis et al., 1999). As adults, the offspring of the high-LG mothers showed decreased startle responses, substantially less fearfulness in the presence of stressors, such as novel environments. Moreover, active defensive responses, such the burying of threatening stimuli, are more conspicuous among the adult offspring of low-LG mothers (Menard et al., 2004). While such pervasive effects are no doubt associated with alterations in multiple neural systems, there are robust effects of maternal care on the central CRF systems, including those that lie outside of the hypothalamus. The activation of fear behavior corresponds to an increase in CRF release from the amygdala and bed nucleus of the stria terminalis onto catecholaminergic cell bodies in the

locus coeruleus. CRF acts at CRF_1 receptors in the locus coeruleus to stimulate the release of norepinephrine in a variety of corticolimbic structures (Bale & Vale, 2004; Valentino, Curtis, Page, Pavcovich, & Florin-Lechner, 1998). Gamma-aminobutyric acid type A (GABA), which is the primary source of neural inhibition in the adult mammalian brain, dampens the activation of this CRF–catecholamine connection during stress. The offspring of the high-LG mothers show decreased CRF_1 receptor levels in the locus coeruleus and increased $GABA_A$/benzodiazepine receptor levels in the basolateral and central nucleus of the amygdala, as well as in the locus coeruleus and decreased CRF expression in the amygdala (Caldji et al., 1998; Caldji et al., 2003). The adult offspring of high-LG mothers are more sensitive to the inhibitory effects of benzodiazepines on fear behavior (Fries, Moragues, Caldji, Hellhammer, & Meaney, 2004). Receptors for the benzodiazepines (e.g., diazepam) are part of the $GABA_A$ receptor complex, and the anxiolytic effects of these compounds occur through an ability to enhance the effect of GABA at the $GABA_A$ receptor site. $GABA_A$, or benzodiazepine receptor agonists, suppress CRF expression in the amygdala, thus reducing activity within the amygdala–locus coeruleus and decreasing norepinephrine responses to stress. Predictably, stress-induced increases in norepinephrine that are normally stimulated by CRF are significantly higher in the offspring of the low-LG offspring. The increased release of norepinephrine is consistent with the enhanced fearfulness of these animals. Thus, increased pup LG is associated with the enhanced efficacy of systems that normally serve to inhibit the expression and actions of CRF. These systems include the hippocampal glucocorticoid receptor and the $GABA_A$ receptor in the amygdala. Both effects involve sustained alterations in gene expression as a function of maternal care.

The complexity of such maternal effects on gene expression is apparent in the alterations in $GABA_A$ receptor function. The $GABA_A$ receptor is a multiprotein complex that comprised five individual subunits, each of which is a product of a distinct genomic region. An interesting feature to this system is that there are at least 19 different subunits that can be employed in the formation of a $GABA_A$ receptor. The activity of the receptor is determined by its subunit composition. For example, the inclusion of an a_1 subunit confers an increased affinity of the receptor for GABA, enhancing GABAergic activity at the $GABA_A$ receptor. Others, such as the g_2 subunit, will define the presence of a benzodiazepine binding site that further increases the inhibitory function of the $GABA_A$ receptor. The adult offspring of high-LG mothers show significantly increased expression of both the a_1 and g_2 subunits. The effect is almost unique to the amygdala and is reversed with cross-fostering (Caldji et al., 2003).

The results of these studies suggest that the behavior of the mother toward her offspring can ''program'' stable changes in gene expression that then serve as the basis for individual differences in behavioral and neuroendocrine responses to stress in adulthood. The maternal effects on phenotype are associated with sustained changes in the expression of genes in brain regions that mediate responses to stress, and form the basis for stable individual differences in stress reactivity. These findings provide a potential mechanism for the influence of parental care on vulnerability/resistance to stress-induced illness over the lifespan. However appealing, this hypothesis has yet to be directly confirmed. But the critical issue for this article is simply that of *how* maternal care might stably affect gene expression. How are variations in the social interactions between the mother and her offspring ''biologically embedded'' so as to stably alter the activity of specific regions of the genome? The answers to these questions appear to involve the ability of social interactions in early development to actually modify the structure of the relevant genomic regions.

Epigenetic Regulation of the Genome

When we think of genomic influences we most commonly imagine effects associated with variation in nucleotide sequence—the genetic code. Yet, this is only one form of information contained on the DNA. Despite the reverence afforded DNA, a gene is basically like any other molecule in the cell; it is subject to physical modifications. These modifications alter the structure and chemical properties of the DNA, and thus gene expression. Collectively, the modifications to the DNA and its chromatin environment can be considered as an additional layer of information that is contained within the genome. This information is thus *epigenetic* in nature (the name derives from the Greek *epi* meaning ''upon'' and *genetics*). The acetylation of the histone proteins referred to earlier is one example of an epigenetic modification. Epigenetic modifications do not alter the sequence composition of the genome. Instead, epigenetic marks on the DNA and the other features of the chromatin regulate the operation of

the genome. Thus, *epigenetics* has been defined as a functional modification to the DNA that does not involve an alteration of sequence. While this definition has recently been subjected to revision (Bird, 2007; Hake & Allis, 2006), the essential features of epigenetic mechanisms are (a) structural modifications to chromatin either at the level of the histone proteins (Figure 2) or the DNA, (b) regulation of the structure and function of chromatin, (c) affects on gene expression, and (d) that these effects occur in the absence of any change in nucleotide sequence. The functional byproduct of the epigenetic modifications is that of a change in gene transcription.

The classic epigenetic alteration is that of DNA methylation, which involves the addition of a methyl group onto cytosines in the DNA (Bird, 1986; Holliday, 1989; Razin & Cedar, 1993; Razin & Riggs, 1980). The methylation of DNA is an active biochemical modification that in mammals selectively targets cytosines and is achieved through the actions of a class of enzymes, DNA methyltransferases, which transfer the methyl groups from methyl donors. There are two critical features to DNA methylation: First, it is a stable chemical modification, and second, it is associated with the silencing of gene transcription (Bestor, 1998; Bird, 2002; Bird & Wolffe, 1999; Razin, 1998).

Until recently, DNA methylation patterns on the genome were thought to be overlaid upon the genome only during early periods in embryonic development. This belief was derived in part from the experimental models commonly used to study DNA methylation. DNA methylation-induced gene silencing mediates two of the most commonly studied examples of epigenetic silencing, namely, X-chromosome inactivation and gene imprinting. Mammalian females bear two copies of the X-chromosome. The inactivation of one copy of the X-chromosome occurs in all mammalian females and is essential for normal function (i.e., maintaining a constant gene dosage in males and females). The silencing of the X-chromosome is associated with DNA methylation (Mohandas, Sparkes, & Shapiro, 1981; Riggs & Pfeifer, 1992; see Hellman & Chess, 2007, for a more current update). The second example of epigenetic-mediated gene silencing is that of gene imprinting (da Rocha & Ferguson-Smith, 2004; Reik, 2001), a remarkable subject in its own right, and one with considerable implications for growth and development (Charalambous, da Rocha, & Ferguson-Smith, 2007). For humans and other mammals, the expression-specific genes are determined by the parent of origin. For certain genes, the copy derived from the mother is active, while that emanating from the father is silenced—a ''maternally imprinted gene.'' In other cases, it is the reverse; the copy of the gene inherited from the father that is active, while that from the mother is silenced—a ''paternally imprinted gene.'' The silent copy is methylated in DNA regions that regulate gene expression and thus inactive. Again, the epigenetic marks associated with gene imprinting are established very early in life. These marks, as well as those associated with X-chromosome inactivation, are stable, leaving researchers in the field with the impression that under normal conditions DNA methylation occurs early in embryonic life and is largely irreversible. Indeed, it was commonly thought that DNA methylation was an actively dynamic process only during periods of cell division such that in mature, postmitotic cells further alteration of methylation patterns was improbable. Moreover, the loss of cytosine methylation in such models is associated with profound pathology. This perspective was further reinforced by findings showing that an alteration of DNA methylation at critical genomic targets (i.e., tumor suppressors) is associated with cancer (Eden, Gaudet, Waghmare, & Jaenisch, 2003; Feinberg, 2007; Laird, 2005).

While these assumptions concerning DNA methylation appear valid for the examples cited earlier, recent studies reveal that DNA methylation patterns are actively modified in mature (i.e., fully differentiated) cells, including, and perhaps especially, neurons and that such modifications can occur in animals in response to cellular signals driven by environmental events (Bird, 2007; Jirtle & Skinner, 2007; Meaney & Szyf, 2005). For example, variations in the diet of mice during gestation or later in development, such as the early postweaning period, can stably alter the methylation status of the DNA (Cooney, Dave, & Wolff, 2002; Waterland & Jirtle, 2003; Waterland, Lin, Smith, & Jirtle, 2006; Whitelaw & Whitelaw, 2006). Likewise, both mature lymphocytes (Bruniquel & Schwartz, 2003; Murayama et al., 2006) and neurons (e.g., Champagne, 2008; Champagne et al., 2006; Lubin, Roth, & Sweatt, 2008; Martinowich et al., 2003; Sweatt, 2009) show changes in the DNA methylation patterns at critical genomic regions in response to environmental stimuli that stably alter cellular function. The ability of environmental signals to actively remodel epigenetic marks that regulate gene expression is a rather radical change in our understanding of the environmental regulation of gene expression. Such epigenetic modifications are thus a candidate mechanism for the environmental ''programming'' of gene expression.

DNA Methylation and Gene Transcription

DNA methylation is associated with the silencing of gene transcription. This effect appears to be mediated in one of two ways (Bird, 2002). First, wide swaths of DNA can be methylated and the shear density of methylation precludes transcription factor binding to DNA sites, thus silencing gene expression. The second manner is subtler, and probably far more prevalent, in regions with more dynamic variations in gene transcription, such as the brain. In this case, selected cytosines are methylated and the presence of the methyl group attracts a class of proteins know as methylated-DNA binding proteins (Klose & Bird, 2006). These proteins, in turn, attract an entire cluster of proteins, known as the repressor complexes that are the active mediators of the gene silencing. The HDACs are a critical component of the repressor complex. HDACs prevent histone acetylation and favor a closed chromatin state that constrains transcription factor binding and gene expression (Figure 2 and see earlier). Compounds that inhibit HDACs can thus increase transcription from methylated DNA.

Epigenetics and the Social Environment

The following section describes studies of the molecular basis for the effects of maternal care on the development of individual differences in gene expression and stress responses. The mechanism for this interaction is epigenetic, involving alterations in DNA methylation at specific sites in the genome. In summary, variations in mother–infant interactions in the rat alter the extra- and intracellular environment of neurons in selected brain regions. Such alterations directly modify the epigenetic marks on regions of the DNA that regulate the transcription of the glucocorticoid receptor, which in turns regulates the HPA response to stress. These epigenetic marks are stable, enduring well beyond the period of maternal care, and thus provide a molecular basis for a stable maternal effect on the phenotype of the offspring. Thus, the behavior of the mother directly alters cellular signals that then actively sculpt the epigenetic landscape of the offspring, influencing the activity of specific regions of the genome and the phenotype of the offspring.

Epigenetic Effects of Variations in Maternal Care

The critical feature of the maternal effects described earlier is that of persistence. The differences in the frequency of pup LG between high- and low-LG mothers are limited to the 1st week of postnatal life. And yet the differences in gene expression and neural function are apparent in adulthood. How might the effects of an essentially social interaction stably alter the expression of the glucocorticoid receptor gene?

The focus of the epigenetic studies is the NGFI-A consensus sequence in the exon 1_7 promoter (Figure 1) that activates glucocorticoid receptor expression in hippocampal neurons. The tactile stimulation associated with pup LG increases 5-HT metabolism in the hippocampus. As described earlier (Figure 3), in vitro studies show that 5-HT acts on 5-HT_7 receptors to initiate a series of intracellular signals that culminate with an increase in the expression of NGFI-A as well as in the CREB-binding protein. Comparable effects occur in vivo. Manipulations that increase pup LG by lactating rats result in an increased level of cAMP as well as NGFI-A (Meaney et al., 2000). Pups reared by high-LG mothers show increased NGFI-A expression in hippocampal neurons as well as an increased binding of NGFI-A to the exon 1_7 promoter sequence (Hellstrom , Zhang, Diorio, & Meany, 2009; Weaver et al., 2007). Moreover, the binding of NGFI-A to the exon 1_7 promoter sequence is actively regulated by mother–pup interactions, such that there is increased NGFI-A bound to the exon 1_7 promoter immediately following a nursing bout but not at a period that follows 25 min without mother–pup contact (Hellstrom et al., 2009).

Nerve growth factor-inducible factor A and the CREB-binding protein form a complex that binds directly to the exon 1_7 promoter sequence and actively redesigns the methylation pattern at this region of the genome (Weaver et al., 2004; Weaver et al., 2007). Thus, as adults the offspring reared by high-LG mothers show very modest levels of methylation at the 5′-CpG of the NGFI-A consensus sequence (Figure 5). This effect on methylation is very precise. Lying only a few nucleotides removed from this site is 3′-CpG site (Figure 5), the methylation status of which is unaffected by maternal care.

A rather novel aspect to the effect of maternal care on DNA methylation was apparent in the results of a simple developmental study examining the methylation status of the 5′- and 3′-CpG sites from late in fetal life to adulthood (Weaver et al., 2004). Neither the 5′- nor the 3′-CpG site is methylated in hippocampal neurons from fetal rats, whereas both sites are heavily methylated on the day following birth, with no difference as a function of maternal care. These findings reflect what is

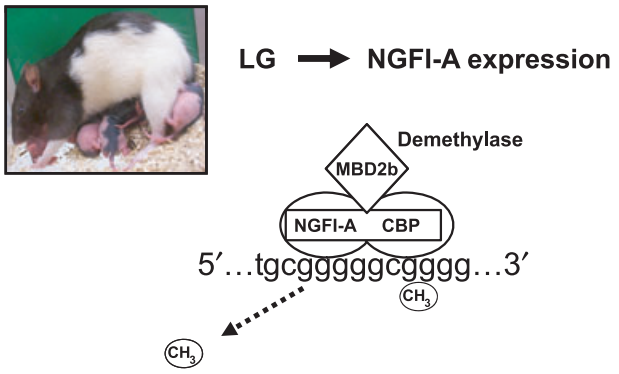

Figure 5. A working hypothesis for the experience (maternal care) driven remodeling of the epigenetic state of the NGFI-A consensus binding sequence over the 1st week of postnatal life in the offspring of high-LG mothers.
Note. The binding of a NGFI-A/CBP complex actively targets the association of a putative demethylase (MBD2) resulting in the removal of the methyl group from the 5′-CpG site of the NGFI-A binding site (Meaney & Szyf, 2005). CBP = CREB-binding protein; LG = licking/grooming; NGFI-A = nerve growth factor-inducible factor A.

referred to as *de novo methylation*, whereby a methyl group is applied to previously unmethylated sites. However, between the day following birth and the end of the 1st week of life, the 5′-CpG is ''demethylated'' in pups reared by high-, but not low-LG mothers. The difference then persists into adulthood. Importantly, the period over which the demethylation occurs is precisely that during which high- and low-LG mothers differ in the frequency of pup LG; the difference in pup LG between high- and low-LG mothers is not apparent in the 2nd week of postnatal life (Caldji et al., 1998; Champagne, Francis, et al., 2003).

The demethylation of the 5′-CpG site occurs as a function of the same 5-HT-activated signals that regulate glucocorticoid receptor gene expression in cultured hippocampal neurons (Weaver et al., 2007). Thus, when hippocampal neurons of embryonic origin are placed in culture and treated with 5-HT, which mimics the extracellular signal associated with maternal LG, the 5′-CpG site is demethylated; there is no effect at the 3′-CpG site. The binding of NGFI-A to the exon 1_7 site is critical. Hippocampal neurons that are rendered incapable of increasing NGFI-A expression through antisense or siRNA treatment show neither the demethylation of the 5′-CpG site nor the increase in glucocorticoid receptor expression (Weaver et al., 2007). Likewise, a mutation of the NGFI-A site that completely abolishes the binding of NGFI-A to the exon 1_7 promoter also prevents the demethylation of the 5′-CpG. Finally, the infection of hippocampal neurons with a virus containing a construct that was engineered to express high levels of NGFI-A produces demethylation of the 5′-CpG of the exon 1_7 promoter sequence and increased glucocorticoid receptor expression.

But there is a complication. If DNA methylation blocks transcription factor binding and the 5′-CpG site of the exon 1_7 promoter is heavily methylated in neonates, then how might maternally activated NGFI-A bind to and remodel the exon 1_7 region? And why is the effect apparent at the 5′- but not the 3′-CpG? The answer to these questions appears to involve other transcriptional signals that are affected by maternal care. Levels of the transcription factor specific protein-1 (SP-1) and the CREB-binding protein are also increased in the hippocampus of pups reared by high-LG mothers (Hellstrom et al., 2009; Weaver et al., 2007). The exon 1_7 promoter contains a DNA sequence that binds SP-1 and this region overlaps with that for NGFI-A. SP-1 can actively target both methylation and demethylation of CpG sites (Brandeis et al., 1994). The 5′-CpG site is the region of overlap in the binding sites. The CREB-binding protein, on the other hand, acts as a histone acetyltransferase, an enzyme capable of acetylating histone tails, including the exon 1_7 region, opening chromatin and permitting the binding of transcription factors such as NGFI-A and SP-1. Increasing histone acetylation can lead to transcription factor binding at previously methylated sites, and the subsequent demethylation of these regions (Szyf, Weaver, Champagne, Diorio, & Meaney, 2005).

The NGFI-A transcription factor binds to multiple sites across the genome. If NGFI-A-related complexes target demethylation, then one might assume that other NGFI-A-sensitive regions should show a maternal effect comparable to that observed with the glucocorticoid receptor. Zhang, Hellstrom, Wei, and Meaney (2009) showed that the hippocampal expression of the *GAD1* gene that encodes for glutamic acid decarboxylase, a rate limiting enzyme in the production of GABA, is increased in the adult offspring of high-LG mothers. This effect is associated with increased cytosine methylation of an NGFI-A response element. Moreover, as with the effect on the glucocorticoid receptor, an in vitro increase in NGFI-A expression mimics the effects of increased pup LG.

In summary, the maternally induced changes in specific intracellular signals in hippocampal neurons can physically remodel the genome. The increased binding of NGFI-A that derives from pup LG appears critical for the demethylation of the exon 1_7

promoter. We suggest that this process involves accompanying increases in SP-1 and the CREB-binding protein, and that the combination of these factors results in the active demethylation of the exon 1_7 promoter. It should be noted that there are important features of this model that remain to be clearly defined, including the identification of the enzyme that is directly responsible for the demethylation. Nevertheless, the events described to date represent a model by which the biological pathways activated by a social event may become imprinted onto the genome. This imprint is then physically apparent in the adult genome, resulting in stable alterations (or programming) of gene expression.

The Functional Importance of the Social Imprint

The presence of a methyl group on the 5′-CpG of the NGFI-A binding site is functionally related to glucocorticoid receptor gene expression in adult animals. In vitro studies reveal that the methylation of the 5′-CpG site reduces the ability of NGFI-A to bind to the exon 1_7 promoter and activate glucocorticoid receptor transcription (Weaver et al., 2007). These findings are consistent with the model described earlier, whereby DNA methylation impedes transcription factor binding and thus the activation of gene expression. The question concerns the in vivo situation. In contrast to the situation with neonates, there is no difference in NGFI-A expression as a function of maternal care among adult animals. However, the altered methylation of the exon 1_7 promoter would suggest differences in the access of NGFI-A to its binding site on the promoter. Chromatin-immunoprecipitation assays, which permit measurement of the direct interaction between a specific protein and a defined region of the DNA, reveal increased NGFI-A association with the exon 1_7 promoter in hippocampi from adult offspring of high- compared to low-LG mothers (Weaver et al., 2004; Weaver et al., 2005). These findings show that in the living animal, under normal conditions, there is more NGFI-A associated with the exon 1_7 promoter in hippocampal neurons of adult animals reared by high- compared with low-LG mothers.

There is also evidence that directly links the maternal effect on the epigenetic state of the exon 1_7 promoter to the changes in glucocorticoid receptor expression and thus HPA responses to stress. Recall that the methylation of specific CpG sites can diminish transcription factor binding through the recruitment of repressor complexes that include HDACs. The HDACs deactylate histone tails, thus favoring a closed chromatin configuration. Indeed, the exon 1_7 promoter is more prominently acetylated in hippocampi from adult offspring of high- compared with low-LG mothers (Weaver et al., 2004, 2005). This finding is consistent with the increased transcription of the glucocorticoid receptor gene in animals reared by high- versus low-LG mothers. A subsequent study (Weaver et al., 2004) examined the effects of directly blocking the actions of the HDACs in the adult offspring of high- and low-LG mothers. An HDAC inhibitor was infused directly into the hippocampus daily for 4 consecutive days. The treatment with the HDAC inhibitor produces a series of predictable results that reflect a cause–effect relation between DNA methylation and gene expression. First, HDAC blockade eliminates the differences in the acetylation of the histone tails of the exon 1_7 promoter in hippocampal samples from high- and low-LG mothers. Second, the increased histone acetylation of the exon 1_7 promoter in the offspring of low-LG mothers is associated with an increase in the binding of NGFI-A to the exon 1_7 promoter in the offspring of low-LG mothers, eliminating the maternal effect on NGFI-A binding to the exon 1_7 promoter. Comparable levels of NGFI-A binding to the exon 1_7 promoter then eliminate the maternal effect on hippocampal glucocorticoid receptor expression, such that glucocorticoid receptor levels in the adult offspring of low-LG mothers treated with the HDAC inhibitor are comparable to those in animals reared by high-LG mothers. And most importantly, the infusion of the HDAC inhibitor reversed the differences in the HPA response to stress.

Histone deacetylase inhibition increases NGFI-A binding to the exon 1_7 promoter in the offspring of low-LG mothers. The studies with neonates reveal that increased NGFI-A binding results in the demethylation of the 5′-CpG. In vitro, the introduction of a viral tool that leads to the increased expression of NGFI-A is sufficient to demethylate the exon 1_7 promoter. We (Weaver et al., 2007) argue that the binding of NGFI-A is critical for the demethylation of the 5′-CpG site. The same effect is apparent in vivo and even with the adult animals used in the studies described earlier. HDAC infusion into the hippocampus increases NGFI-A binding to the exon 1_7 promoter in the adult offspring of low-LG mothers and decreases the level of methylation of the 5′-CpG site on the exon 1_7 promoter. A subsequent study (Weaver et al., 2005) showed that the reverse pattern of results could be obtained in response to a dietary manipulation (methionine):

Greater methylation of the 5′-CpG in the offspring of high-LG mothers decreased NGFI-A binding and GR expression and increased HPA responses to stress (Weaver et al., 2005).

While these studies employ rather crude pharmacological manipulations, the results are critical as they suggest that fully mature neurons in an adult animal express the necessary enzymatic machinery to demethylate or remethylate DNA. Thus, it is possible that environmentally driven changes in neuronal transcriptional signals could potentially remodel the methylation state of specific regions of the DNA (Meaney & Szyf, 2005). The cytosine methylation state of a promoter for the brain-derived neurotrophic factor (*bdnf*) gene is also influenced by maternal care in early life (Roth, Lubin, Funk, & Sweatt, 2009). Indeed, Lubin et al. (2008) provided evidence for an alteration of the methylation state of the same *bdnf* promoter following contextual fear conditioning in adult rats (also see Bredy et al., 2007). These effects are consistent with previous reports of activity-dependent alterations in the methylation of the same *bdnf* promoter (Martinowich et al., 2003) and suggest that epigenetic states might be altered by a wide range of biologically relevant events that result in synaptic remodeling (Meaney & Szyf, 2005; Renthal & Nestler, 2008; Sweatt, 2009). Such epigenetic modifications might therefore underlie a wide range of stable changes in neural function following exposure to highly salient events (e.g., chronic stress, drugs of abuse, reproductive phases such as parenting, etc.), and are thus logical mechanisms for environmentally induced alterations in mental health (Akbarian & Huang, 2009; Jiang et al., 2008; Tsankova, Renthal, Kumar, & Nestler, 2007). While such effects have yet to be reported for DNA methylation, modifications of histone proteins are associated with exposure to drugs of abuse and stressors in rodent models (Renthal & Nestler, 2008; Renthal et al., 2007).

A set of recent studies (McGowan et al., 2009) suggests that comparable epigenetic modifications might occur in humans in response to variations in parent–offspring interactions. DNA was extracted from hippocampal samples obtained from victims of suicide or from individuals that had died suddenly from other causes (auto accidents, heart attacks, etc.). The samples were obtained from the Québec Suicide Brain Bank, which conducts forensic phenotyping that includes a validated assessment of psychiatric status and developmental history (e.g., McGirr, Renaud, Seguin, Alda, & Turecki, 2008). The studies examined the methylation status of the exon 1_F promoter of the glucocorticoid receptor, which corresponds to the exon 1_7 promoter in the rat (Turner & Muller, 2005). The results showed increased DNA methylation of the exon 1_F promoter in hippocampal samples from suicide victims compared with controls, but only if suicide was accompanied with a developmental history of child maltreatment. Child maltreatment, independent of psychiatric state, predicted the DNA methylation status of the exon 1_F promoter. As in the previous rodent studies, the methylation state of the exon 1_F promoter also determined the ability of NGFI-A to bind to the promoter and activate gene transcription. While such studies are obviously correlational, and limited by postmortem approaches, the results are nevertheless consistent with the hypothesis that variations in parental care can modify the epigenetic state of selected sites of the human genome. Moreover, the findings are also consistent with studies that link childhood abuse to individual differences in stress responses (Heim et al., 2000). Childhood abuse was associated with an increase in pituitary ACTH responses to stress among individuals with or without concurrent major depression. Heim et al.'s (2000) findings are particularly relevant since pituitary ACTH directly reflects central activation of the HPA stress response and hippocampal GR activation dampens HPA activity. These findings are consistent the rodent studies cited earlier investigating epigenetic regulation of the glucocorticoid receptor gene and with the hypothesis that early life events can alter the epigenetic state of relevant genomic regions, the expression of which may contribute to individual differences in the risk for psychopathology (Holsboer, 2000; Neigh & Nemeroff, 2006; Schatzberg, Rothschild, Langlais, Bird, & Cole, 1985).

Transgenerational Effects

Individual differences in stress responses in the adult rat are associated with naturally occurring variations in maternal care during infancy. Manipulations that alter mother–pup interactions in the rat alter patterns of gene expression and stress response in the offspring (e.g., Francis et al., 1999; Meaney, 2001; Roth et al., 2009; Toki et al., 2007). Such effects are certainly familiar to child psychologists working with parenting training programs. Moreover, these maternal effects might also serve as a possible nongenomic mechanism by which selected traits could be transmitted from one generation to another through variations in mother–pup

interactions. Interestingly, low-LG mothers are more fearful than are high-LG dams (Francis, Champagne, & Meaney, 2000). Individual differences in stress reactivity are apparently transmitted across generations: Fearful mothers beget more stress reactive offspring. The obvious question is whether the transmission of these traits occurs only as a function of genomic-based inheritance. If this is the case, then the differences in maternal behavior may be simply be an epiphenomenon and not causally related to the development of individual differences in stress responses. The issue is not one of inheritance but rather the mode of inheritance. However, the results of the previously cited cross-fostering studies indicate that individual differences at the level of gene expression or complex phenotype can be directly altered during the postnatal period by maternal behavior. This interpretation is buttressed from studies showing that tactile stimulation of pups by the experimenter with a brush, so-called stroking, increases hippocampal NGFI-A (Hellstrom et al., 2009) increases hippocampal GR expression (Jutapakdeegul et al., 2003), and dampens HPA responses to stress in adulthood (Burton et al., 2007; Gonzalez et al., 2001).

The cross-fostering studies also reveal that individual differences in maternal behavior are transmitted from mother to the female offspring. Hence, as adults, the female offspring of more fearful, low-LG mothers are also more fearful low-LG mothers (Francis et al., 1999; also see Fleming, O'Day, & Kraemer, 1999). Regardless of their biological origins, females that are reared by high-LG mothers are less fearful and show an increased frequency of pup LG. And the mechanism for the intergenerational transmission of such individual differences appears to be the difference in the frequency of pup LG during early postnatal life. Fleming and colleagues found that as adults, females rats deprived of maternal showed reduced frequencies of multiple forms of maternal behavior, and such effects were reversed if animals were provided with tactile stimulation by stroking the pups with a brush over the first weeks of life (Gonzalez et al., 2001; Lovic, Gonzalez, & Fleming, 2001; Melo et al., 2006). Moreover, the stroking in infancy also increased the expression of maternal behaviors in response to ovarian hormones in adulthood (Novakov & Fleming, 2005), an effect that is comparable to that associated with increased pup LG (Champagne, Diorio, Sharma, & Meaney, 2001; see also next).

The individual differences in pup LG involve differences in estrogen receptor α-gene expression in the medial preoptic area (MPOA) of the hypothalamus, a region that is critical for maternal behavior in the rat (Fleming et al., 1999; Numan & Insel, 2003). There is increased estrogen receptor a expression in female offspring of high-LG mothers (Champagne, Weaver, Diorio, Sharma, & Meaney, 2003). Estrogen acts during late gestation to increase oxytocin receptor levels in the MPOA (Fahrbach & Pfaff, 1986; Pedersen, 1997), and this effect is greater in the female offspring of high- compared to low-LG mothers (Champagne et al., 2001), reflecting the increased sensitivity to estrogen. Oxytocin appears to act at oxytocin receptor levels in the MPOA to facilitate the release of dopamine from neurons in the ventral tegmental nucleus, and the increased dopamine release then activates pup LG in lactating female rats (Champagne, Stevenson, Gratton, & Meaney, 2004; Shahrohk et al., in press). This effect is abolished with the infusion of an oxytocin receptor antagonist into the ventral tegmental area (Shahrohk et al., in press). Likewise, the same oxytocin receptor antagonist completely eliminates the differences in maternal behavior between high- and low-LG mothers (Champagne et al., 2001). Drugs that block the reuptake of synaptic dopamine, thus increasing the overall dopaminergic signal, increase pup LG in low-LG mothers and eliminate the difference in maternal behavior between high- and low-LG mothers. In summary, the increased estrogen receptor a expression in the MPOA of high-LG mothers leads to greater sensitivity to estrogen, an increased level of oxytocin levels in MPOA neurons that project directly to the dopamine neurons in the ventral tegemental regions, and greater activation of dopamine release during nursing bouts. And the differences in estrogen receptor a expression, like those in pup LG, are reversed with cross-fostering (Champagne, Francis, et al., 2003), suggesting that maternal care regulates the activity of the estrogen receptor in the MPOA, which then forms the basis for subsequent ''inherited'' differences in maternal behavior.

This finding reveals another example of a maternal effect on gene expression, and there is evidence for epigenetic mediation. The activation of the estrogen receptor α-gene in the rat brain occurs through the estrogen receptor 1B promoter. This promoter contains multiple cytosine sites that are potential targets for DNA methylation. Champagne et al. (2006) found increased cytosine methylation across the exon 1B promoter in the offspring of low-LG mothers. Activation of the exon 1B promoter occurs in response to the binding of the

transcription factor Stat5, and the adult offspring of low-LG mothers show decreased Stat5 association with the exon 1B promoter in the MPOA. These findings suggest that differences in DNA methylation may mediate the effect of maternal care on the expression of estrogen receptor α in the MPOA and thus serve as the molecular basis for the nongenomic transmission of individual differences in maternal behavior from the mother to her female offspring.

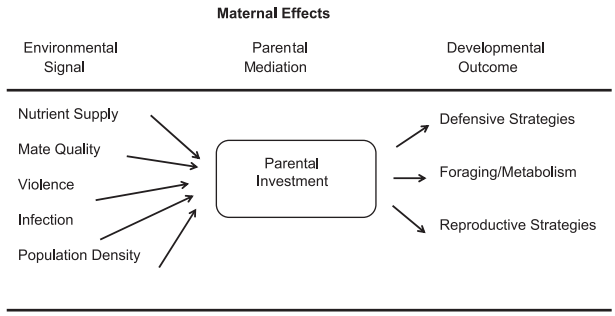

Figure 6. Summary of the literature from evolutionary biology on ''maternal effects.''
Note. The consistent theme reflected in these studies is that various environmental signals can alter multiple phenotypic outcomes through effects on parent–offspring interactions, broadly referred to as *parental investment*. The relevant form of the variation in parent–offspring interaction (investment) will vary depending upon the species. The principal idea is that of parental mediation and of coordinated effects on multiple phenotypic outcomes.

Adaptive Value of Epigenetic Maternal Effects

Variations in parental signals alter gene expression and thus the development of individual differences in complex phenotypes. But why bother? Why would nature configure such a process? Why transmit individual differences in stress reactivity or maternal behavior across generations through a process that is driven by parental care and mediated by the complex cellular machinery necessary to rearrange epigenetic markings on the DNA? Why not simply leave such issues of inheritance in the hands of classic genetic transmission? The answer may lie in the simple fact that unlike nucleotide sequence, epigenetic marks are dynamic and indeed reversible. The more flexible epigenetic mechanism would provide the basis for an adaptive parental effect: In the rat at least, parents can actively remodel epigenetic marks and thus affect patterns of gene expression in the offspring. Such effects do not occur at the level of nucleotide sequence.

Studies in the fields of evolutionary biology and ecology report maternal effects on phenotype in the offspring across a wide range of species (see Figure 6; Badyaev, 2008; Cameron et al., 2005; Meaney, 2007; Mousseau & Fox, 1998; Rossiter, 1998). Despite the fact that these studies have been performed largely with simpler organisms, such as plants, insects, and reptiles, the emerging theme is much the same as that described earlier in studies with human families: Environmental adversity decreases parental investment in the offspring and thus alters phenotypic development. And there is evidence that these phenotypic effects are adaptive within adverse settings (Mousseau & Fox, 1998).

Obviously, the form of ''parental investment'' varies across species. In plants, the variation may involve seed quality. Among certain insects ''investment'' would include the nutrient value of the ''propulgate,'' the food source left by the mother for the hatching offspring. While some insects do exercise postnatal behavioral care of the offspring, variations in this form of investment are most relevant for mammalian species. Nevertheless, the stability of the relation between the quality of the prevailing environment, parental investment, and phenotypic development in the offspring led Hinde (1986) to suggest that evolution may have actually shaped the offspring to ''use'' parental signals, such as variations in parental care behaviors, to forecast the quality of the environment in which they must function (also see Bateson, 1994; Bateson et al., 2004; Rossiter, 1998). Evolution should come to favor offspring that are able to accurately ''read'' variations in parental behavior as forecasts of environmental conditions and thus as useful signposts to guide developmental outcomes (Hinde, 1986). By definition, such responses should occur in reaction to variation within the normal range. Why evolve responses to forms of parental care that are unlikely to occur? Parents (or parent in some cases) are a logical source of such information since they are the one ''environmentally informed'' and constant experience for the offspring. Moreover, since parents are invested in the adaptive success of their biological offspring, one would expect that the fidelity of signals emanating from a parent would be greater than that of other adult conspecifics. Thus, to the extent that the parent and offspring share a common interest in the adaptive value of such phenotypic plasticity, selection may also act on the signaling capacity of the parent. Indeed, phenotypic plasticity in response to parental signals may also be thought of as a parental strategy, although it is clear that the benefits work in both directions as would be expected among organisms that share genes. In either case, the sensitivity of

the offspring to parental signals during critical phases of development may be thought of as a strategy that favors a highly predictable relation between environmental conditions, parental input, and phenotypic variation in defensive responses. For the offspring, parents should matter.

Hinde's (1986) formulation implies an adaptive function for parental effects in preparing the offspring for the environmental demands that are likely to prevail during development and into adulthood (also see Bateson et al., 2004; Gluckman & Hanson, 2004; Meaney, 2001). Indeed, it is probably true that the environmental conditions of the adult are a reasonable predictor of those facing the offspring (with modern, urbanized human populations as a rare exception; Gluckman & Hanson, 2004; West & King, 1987). However, environmental conditions can vary with the migration of populations. Moreover, even within a similar environment, the ability of individuals to access resources can vary from one individual to another as a function of, for example, social dominance hierarchies. Success requires the ability to adapt, to fine-tune phenotypic development in relation to the prevailing environmental demands. Learning, of course, would provide a mechanism for such phenotypic plasticity. But learning requires trial and, unfortunately, error. Nature does not always permit such indulgences. Epigenetic variation may provide a mechanism for the dynamic regulation of the genomic machinery in response to variation in prevailing environmental conditions during development (Jablonka & Lamb, 2005). Such epigenetic adaptations could then occur directly as a result of the relevant environment condition (e.g., nutrient availability) or indirectly through parental mediation. Such effects could, for example, explain the enduring effects of childhood socioeconomic status on health outcomes in humans.

We suggest that parental signals over the perinatal period serve as an important catalyst for epigenetic remodeling of the genome. If such effects support an adaptive function, then there should be a high degree of fidelity between the quality of the environment and parental care. This argument rests upon at least three assumptions. First, there is a consistent relation between the quality of the environment and that of parental care. Second, variations in specific developmental outcomes should be adaptive. This includes an adaptive value to traits that appear to increase vulnerability to illness, such as increased stress reactivity. And third, the ecology of the neonate reliably predicts the conditions of adulthood.

Stress and Parenting

Perhaps, the most compelling evidence for a direct effect of environmental adversity on parent–offspring interactions emerges from the studies of Rosenblum, Coplan, and colleagues with nonhuman primates (Coplan, Andrews, Rosenblum, & Nemeroff, 1996). Bonnet macaque mother–infant dyads were maintained under typical lab conditions, with free, unhindered access to food (a low-foraging-demand condition) or one in which the amount of available food varied and required long periods of searching (high-foraging-demand condition). The high-foraging-demand condition severely disrupted mother–infant interactions, producing significant conflict. Infants of mothers housed under these conditions were more timid and fearful, with some revealing signs of depression that are commonly observed in maternally separated macaque infants. Such reactions were even apparent among infants that were in contact with their mothers. As adolescents, the infants reared in the high-foraging-demand conditions were more fearful, submissive, and showed less social play behavior. As expected, these conditions also affected the development of neural systems that mediate behavioral and endocrine response to stress. Adult monkeys reared under variable foraging demand (VFD) conditions showed increased central levels of CRF and increased noradrenergic responses to stress. It would be fascinating to see if such traits would then be transmitted to the next generation. In some rather remarkable cross-fostering studies, Maestripieri (2005) has shown that individual differences in maternal behavior are transmitted across generations in the rhesus monkey.

The critical issue here is that of a direct effect of environmental adversity on maternal behavior. Stress during pregnancy decreases maternal responsivity in lactating rats (Fride, Dan, Gavish, & Weinstock, 1985; Kinsley, Mann, & Bridges, 1998; Moore & Power, 1986). Gestational stress also eliminates the differences in pup LG between high- and low-LG mothers (Champagne & Meaney, 2006). Gestational stress decreases the frequency of maternal LG in the high-, but not in low-LG mothers (also see Smith, Seckl, Evans, Costall, & Smythe, 2004), and the effect is apparent even with subsequent litters in the absence of further exposure to stress. As expected, the effects on maternal behavior are apparent in the development of the offspring. As adults, the offspring of high-LG/gestationally stressed mothers were comparable to those of low-LG dams on measures of maternal behavior, as well

as in fear behavior and hippocampal glucocorticoid receptor gene expression. These effects can be distinguished from those associated with prenatal stress in animals that were in utero during the imposition of the stressor by simply examining the offspring of subsequent litters in which the behavior of the mother remains affected, but in the absence of the stressor (Champagne & Meaney, 2006).

As mentioned earlier, there is considerable, albeit correlational, evidence for a relation between environmental adversity and parental care in humans. Environmental adversity influences emotional well-being in parents and these effects are reflected in alterations in parental care, commonly reflecting a decreased level of investment (Fleming, 1988; Repetti et al., 2002). Increased maternal stress is associated with less sensitive child care (Dix, 1991; Goldstein, Diener, & Mangelsdorf, 1996). The children of highly stressed primary caregivers tend to develop insecure parental attachment (Goldstein et al., 1996; Vaughn, Egeland, Sroufe, & Waters, 1979). Parents in conditions of poverty experience more negative emotions, irritability, depressed, and anxious moods, which lead to more punitive parenting (Belsky, 1993; Conger, McCarty, Yang, Lahey, & Kropp, 1984; Grolnick, Gurland, DeCourcey, & Jacob, 2002). The resulting patterns of parental behavior can affect the development of HPA responses to stress in the offspring (Heim et al., 2000; Pruessner, Champagne, Meaney, & Dagher, 2004), an effect that may associate with altered glucocorticoid receptor expression (McGowan et al., 2009). Hane and Fox (2006) found a direct relation between the quality of maternal care and behavioral inhibition in children. There is rather compelling evidence for parental mediation in the effects of impoverished environments on phenotypic development. Thus, the effects of poverty on emotional and cognitive development are, in part, mediated by variations in parent–offspring interactions. If parental care factors are statistically controlled, there no longer remains any discernible effect of poverty of child development (Conger, Ge, Elder, Lorenz, & Simons, 1994; McLloyd, 1998). Moreover, parents and parenting style are highly effective targets for intervention studies aimed at development outcomes in children living in adverse environmental conditions (Belsky, 1997; Fisher et al., 2000; Offord et al., 1992; Olds et al., 1998; Van den Boom, 1994).

Adaptive Value of Increased Stress Responses

Intergenerational transmission of individual differences in stress reactivity via parental behavior could represent an adaptive approach to development. Since the offspring usually inhabit a niche that is similar to their parents, the transmission of individual differences in traits from parent to offspring could serve to be adaptive with respect to survival. The conceptually challenging feature of this argument is that it requires that we identify an adaptive virtue to a phenotypic profile, such as increased HPA stress responses, that predict greater vulnerability to chronic illness.

Adverse environmental conditions such as those associated with poverty over the adult life of the parent have historically predicted more of the same for the offspring. The critical challenge over the course of development is to mold specific features of phenotype in a manner that is most appropriate to the level of environmental demand. This capacity for phenotypic plasticity (Agrawal, 2001; Mousseau & Fox, 1998) is a product of evolutionary forces. As such, it is critical to note that the ultimate measure of success for any phenotypic trait is the degree to which it enhances the probability for reproductive success. The health of the individual is a relevant consideration *only to the degree that it influences the ability to successfully reproduce.* Health will influence survival and the ability to attract mating opportunities, and may therefore be of relevance, at least during the period of active reproduction. However, the incidence of chronic illnesses that commonly occur in later life, once the prospects for reproductive activity have declined, is not a major consideration in evaluating the adaptive value of any specific trait in the evolutionary sense.

There is a very real divide between the way in which the success of development is evaluated by those working in the health sciences as compared to those in biology. Optimal development for those in the health sciences is judged by the quality of life and the absence of disease. For biologists, success is measured in the terms of the currency of natural selection—reproductive fitness. For biologists, there are no ideal phenotypes. Rather, the adaptive merits of phenotype are apparent only in relation to success within a particular set of environmental conditions: One single phenotype does not fit all. For example, an increase in the responsivity of an individual to threat (i.e., greater stress reactivity) is realistically considered as a risk factor for multiple forms of chronic illness. Thus, the catabolic effects associated with adrenal glucocorticoids or sympathetic catecholamines tend to be vilified, especially in psychology and psychiatry. Indeed, chronic elevations in the levels of these ''stress mediators'' can

directly promote illness (Chrousos & Gold, 1992; Dallman et al., 2005; McEwen, 2007; Sapolsky et al., 2000; Walker, 2007). However, the bitter truth of adaptation and survival in the face adversity is that such hormonal stress responses are essential for continued life. The survival interests of an individual during periods of increased environmental demand are well served by behavioral (e.g., vigilance, fearfulness) and endocrine (HPA and metabolic/cardiovascular) responses to stress. These responses promote detection of potential threat, fear conditioning to stimuli associated with threat and avoidance learning. Moreover, the hormonal effectors of sympathoadrenal and HPA stress responses mobilize energy reserves through effects of lipolysis, glycolysis, and gluconeogenesis. These effects are the hallmark of the shift to catabolism that occurs during periods of stress and are essential for animals exposed to chronic stress, particularly if the stressor is coupled to conditions of famine. Indeed, the ability to survive sustained periods of nutrient deprivation depends upon the capacity to increase circulating levels of glucocorticoids and catecholamines. Impoverished environments are also commonly associated with multiple sources of infection. Under such conditions adrenal glucocorticoids serve as a potent defense against septic shock (Munck et al., 1984; Sapolsky et al., 2000). Among rats, animals with increased HPA responses to agents such as bacterial endotoxins are at reduced risk for sepsis. Interestingly, adults exposed to a bacterial endotoxin during the 1st week of life exhibit increased HPA responses to stress as well increased resistance to sepsis upon subsequent exposure to bacterial infection (Shanks, Larocque, & Meaney, 1995; Shanks et al., 2000). Conversely, postnatal conditions that increase maternal LG and dampen HPA responses to stress *increase* vulnerability to endotoxin-induced sepsis. These findings underscore the potentially adaptive value of increased HPA and sympathetic responses to stress, especially for individuals living under conditions of impoverishment and infection. Thus, the decreased parental investment associated with more stressful conditions may in fact be of benefit to the offspring, if indeed such conditions remain stable over time. Decreased parental investment might enhance the stress responses of the offspring, which could serve to protect against repeated periods of nutrient deprivation and infection. This theme is apparent in the effects of maternal care on neurocognitive development in the rat.

Maternal Care and Neurocognitive Development

Pup LG in the rat dynamically alters the activity of endocrine systems that favor of somatic growth (Levine, 1994; Schanberg et al., 1984). Comparable effects are apparent in the hippocampus, a brain region intimately associated with learning and memory. The neonatal offspring of high-LG mothers show increased hippocampal expression of genes that encode for neurotrophic factors, which supports the sprouting and survival of synapses, this effect includes the expression glutamate receptor subunits. The activation of glutamate receptors in the hippocampus stimulates synaptogenesis (Kirkwood, Dudek, Gold, Aizenman, & Bear, 1993; Schatz, 1990). Thus, as adults, the offspring of high-LG mothers show increased hippocampal synaptic density (Bredy, Humpartzoomian, Cain, & Meaney, 2003; Champagne et al., 2008; Liu et al., 2000) associated with more extensive dendritic arborization of both pyramidal and granule cell populations (Bagot et al., 2009; Champagne et al., 2008). The effect of maternal care on synaptic development suggests an increased capacity for synaptic plasticity in the offspring of high-LG mothers. Long-term potentiation (LTP) is an electrophysiological model of synaptic plasticity at specific neural sites that is thought to mimic the structural remodeling of synaptic connections that underlie learning and memory. Hippocampal LTP formation is indeed stronger in the adult offspring of High compared to Low LG mothers (Bagot et al., 2009; Bredy et al., 2003; Champagne et al., 2008). And not surprisingly, the adult offspring of high-LG mothers show improved performance hippocampal-dependent tests of learning and memory, such as spatial learning (Bredy, Zhang, Grant, Diorio, & Meaney, 2004; Liu et al., 2000) and object recognition (Bredy et al., 2004).

These findings suggest a rather predictable effect of maternal care on hippocampal development, involving enhanced synaptic development in neonatal life and an increased capacity for synaptic plasticity in adulthood, revealed in electrophysiological as well as behavioral studies. Such findings would seem to reflect an advantage for the offspring of high-LG mothers. But these findings fail to consider the issue of context. Studies of in vitro LTP are performed with hippocampal slices obtained from animals in the resting state. Studies of in vivo LTP or behavioral tests of learning and memory are performed in animals habituated to the testing conditions. What if animals were tested under more demanding, stressful conditions? Champagne et al. (2008) and Bagot et al. (2009)

examined LTP in hippocampal slices obtained from the adult offspring of high- or low-LG mothers under basal conditions, or in the presence of stress-like levels of glucocorticoids. Glucocorticoids are an endocrine signature of the stress response and known to diminish hippocampal LTP (Bodnoff et al., 1995; Diamond & Rose, 1994; Joels, Karst, Krugers, & Lucassen, 2007; Kim & Yoon, 1998; Shors, Foy, Levine, & Thompson, 1990). This effect is readily apparent in sections obtained from high-LG mothers. LTP under basal conditions is markedly greater in hippocampal slices from high- compared to low-LG offspring, and the magnitude of LTP in slices from high-LG offspring is markedly reduced by glucocorticoids. The exact *opposite* is observed in hippocampal slices prepared from the offspring of low-LG mothers: Glucocorticoids significantly enhance LTP to the levels observed in hippocampal slices from high-LG mothers under basal conditions. This is true for LTP obtained from multiple regions within the hippocampus (i.e., for aficionados of hippocampal anatomy, this included neurons within both the Ammon's Horn and the dentate gyrus). And an analogous effect is apparent at the level of learning and memory. When the adult offspring of high- and low-LG mothers are tested not under the benign conditions described earlier but in tests on contextual fear conditioning, the performance of the offspring of the low-LG mothers is significantly improved (i.e., increased learning of the context–shock contingency) over that of high-LG dams (Bagot et al., 2009; Champagne et al., 2008). Contextual fear conditioning is also a hippocampal-dependent test of learning and memory (Fanselow, 2000; Maren & Quirk, 2004), in this case one that involves the association of a context with an aversive event (i.e., a mild shock). Thus, both hippocampal synaptic plasticity and hippocampal-dependent learning and memory are enhanced in the offspring of low-LG mothers *under stressful conditions*. The effects of maternal care on hippocampal function are context dependent.

Implications

All cellular processes derive from a constant dialogue between the genome and environmental signals. Thus, genotype–phenotype relations are defined by the context within which the genome operates. Likewise, the consequences for Gene × Environment interactions at the level of function are defined by the broader context, including the demands of the prevailing environment. The results of studies on the effects of maternal care on hippocampal development in the rat reflect an important point. Although it is tempting to assume that increased pup LG enhances synaptic plasticity and cognitive performance, such effects are apparent only under conditions that are minimally stressful. Under stressful conditions, such as those typified by contextual fear conditioning, it is the offspring of low-LG mothers that show improved synaptic plasticity and learning. We suggest that such findings represent a situation in which parental care shapes adaptive phenotypes (Hinde, 1986; Meaney, 2001; Bateson et al., 2004; Zhang et al., 2004; Gluckman & Hanson, 2007). In the rat, environmental stressors decrease parental investment and enhance behavioral and endocrine responses to stress, as well as learning and memory under stressful conditions. If indeed the decreased parental investment accurately reflects an increased level of environmental demand for the offspring, then such effects could be highly adaptive. Regardless of the merits of this proposal, these findings attest to the simple fact that the adaptive value of any phenotypic profile depends upon the environmental context: There is no universally ''ideal'' phenotype.

There is also evidence for the potentially adaptive effects of increased stress reactivity that more directly bears on the interests of child and adolescent psychology. The research of Farrington, Gallagher, Morley, St Ledger, and West (1988) and Tremblay (e.g., Haapasap & Tremblay, 1994) on young males growing up in impoverished, high-crime urban settings illustrates the potential advantages of increased emotional stress reactivity. Both studies show that shy and more timid males are most successful in avoiding the pitfalls associated with ''criminogenic'' environments. Under such conditions, behavioral inhibition emerges as a protective factor (Haapasap & Tremblay, 1994), despite the fact that this same profile is associated with an increased risk for mood disorders in later life (Pérez-Edgar & Fox, 2005). Moreover, under such adverse conditions a parental rearing style that favored the development of a greater level of stress reactivity to threat could be viewed as adaptive. If indeed there is no single ideal phenotype, then it should follow *that there is no single ideal form of parenting*. If this conclusion has worth, then it leads us to question the wisdom of establishing parenting programs that foster parental skills based on studies of families rearing children under more favorable conditions.

Caveats

The magnitude and scope of the maternal effects on rodent development should not be surprising. Hinde (1986), Bateson (1994), and others have described the logic for the importance of maternal signals for the developing mammal. Indeed, even the gametes include parental signals apart from genomic DNA, such as RNAs and proteins, which influence embryonic development. Nevertheless, it is important to understand that the rodent studies described earlier are designed to examine how variations in maternal *can* affect development; they do not address the issue of *how* and to *what degree* maternal care influences development under normal circumstances in the rat. Thus, the model described earlier examines maternal effects on neural development in animals that are then housed under highly standardized conditions following the weaning period, eliminating the possibility that maternal effects might be subsequently altered by variations in the postweaning environment. Indeed, there is evidence that postweaning environments can reverse the effects of preweaning variations in maternal care in the rat (Bredy et al., 2003; Champagne et al., 2006). These studies are perhaps best considered as a model for the mechanisms by which social experience in early life can influence the structure and operation of the genome with respect to specific phenotypic outcomes. Indeed, as suggested by Belsky (1997), Rutter (2007), and others, the effects of environmental influences, such as maternal care, will vary across individuals, and the studies of Fox et al. (2005) in humans and Suomi and colleagues (Bennett et al., 2002; Champoux et al., 2002; Suomi, 2006) in the nonhuman primate, suggest that variation in genomic sequence may be a critical factor in determining sensitivity to parental signals.

The study of epigenetics provides a molecular mechanism for environmental effects of gene expression. But the ultimate effect on gene transcription is best thought of as an emergent property of the interaction between the epigenetic state and the underlying genome. Thus, genomic sequence determines the effects of epigenetic states on gene transcription. While DNA methylation is linked to transcriptional silencing, such relations are not universal and the strength of the relation may be determined by the underlying genomic sequence (Weber et al., 2007). For example, among promoters with a lower percentage of CpG dinucleotides, there appears to be a weaker relation between the overall level of methylation and that of gene transcription. Likewise, the presence of histone modifications (H3K4me) that associate with transcriptionally active genes appears to be determined not only by DNA methylation but also by the underlying genomic sequence. Such findings should dissuade us from assuming that genomic sequence is merely passive player in the definition of epigenetic states. The underlying genomic sequence influences the nature of potential epigenetic states as well as their importance for gene transcription. Finally, many transcriptionally inactive genes show relatively unmethylated promoters. While this finding does not preclude the possibility that specific modifications in the methylation status at critical CpG sites may define changes in gene transcription, such as occurs for the *IL2* gene (Murayama et al., 2006), it is important to bear in mind that DNA methylation is only one level of influence in the very complex biochemical machinery that regulates gene transcription. These are still early days in the study of the molecular mechanisms for the environmental programming of gene expression. But the door has clearly been opened.

General Conclusions

The effects of maternal care on gene expression and neural function in the rat provide an understanding of how environmental events, including variations in parent–offspring interactions at the level of behavior, can become physically imprinted upon the genome. Maternal care can directly alter intracellular signals that, in turn, structurally alter the DNA and its operation. These structural modifications involve DNA methylation, a classic epigenetic mark that regulates gene transcription. More recent studies suggest that comparable epigenetic modifications associate with learning and memory (Lubin et al., 2008; Miller & Sweatt, 2007), chronic exposure to drugs of abuse (Renthal & Nestler, 2008), and psychiatric illness (Grayson et al., 2005; Ptak & Petronis, 2008; Tsankova et al., 2007). The dynamic genome is probably a slightly foreign concept to those who imagine the DNA as simply the repository of the sequence information that forms what is commonly referred to as the ''genetic code.'' But the research of the postgenomic era, with its focus on the operation rather than simply the composition of the genome, reveals that the DNA is an active target for remodeling by cellular signals that are activated by environmental events. The reality of the functional genome does not admit to main effects of either gene or environment, but rather to

a constant interaction between the DNA and its environment.

The study of epigenetic mechanisms reveals the importance of DNA remodeling for gene transcription, which is certainly the most fundamental operation of the genome. The transcription of the genome requires dynamic alterations to the chromatin structures within which the DNA operates. Such events involve transient modifications of the histone proteins that determine the accessibility of the DNA. Indeed, cellular signals that increase or decrease gene transcription often function within complexes that include enzymes that directly operate on chromatin. Potentially more stable signals, such as DNA methylation, are also regulated by environmental events and regulate chromatin activity over longer periods. Such epigenetic marks are thus a candidate mechanism for the environmental programming of gene expression. This level of complexity is an essential feature of biology. The ability of an organism to adapt to variations in environmental conditions over the course of its lifespan demands plasticity in genotype–phenotype relations.

The emergence of an integrative developmental perspective has been constrained by historical misunderstandings of the processes by which variations in genomic sequence and nongenomic factors contribute to the development of individual differences in any specific traits. While quantitative behavioral genetics is a valid approach in establishing patterns of familial transmission (i.e., heritability) that *might* imply a causal role for genomic variation, such studies are commonly subject to misinterpretation of genomic influences. The heritability of individual differences could result as a function of nongenomic biological signals such as RNA and protein emanating from the parent as well as from instances where epigenetic modifications enter the germline (Anway, Cupp, Uzumcu, & Skinner, 2005; Jablonka & Lamb, 2005; Vardhman et al., 2003; Whitelaw & Whitelaw, 2006). There are multiple processes by which individual differences in complex traits might be transmitted from parent to offspring. Moreover, estimates of the contribution of *either* genetic or environmental influences at the level of individual variation are complicated by the reality of Gene × Environment interactions (Lewontin, 1974). Most troublesome is the obvious disjunction between the definition of genomic influences that derives from statistical models and the actual biological reality of the genome. It is the operation of the genome that directly influences phenotype. The operation of the genome at any phase of the life cycle is an emergent property of the constant and very physical interaction of the genome with environmentally regulated, intracellular signals that directly alter chromatin structure. Thus, function at any level of biology emerges as a function of the continuous dialogue between the genome and its environment. Attempts to parse the influence of genomic and environmental influences on the expression of complex traits are inconsistent with even the most rudimentary understanding of gene function.

These issues are critical for the study of child development. Research in this field guides both the creation and the evaluation of intervention studies (e.g., Fisher et al., 2000; Olds et al., 1998). The science and technology of the genomic era offer remarkable opportunities to enhance the sophistication of our approach to these challenges. We are now positioned to study directly the origins of individual differences in sensitivity/resistance to treatment within the context of intervention studies (Belsky, 1997; Rutter, 2007). Indeed, the many years of remarkable research in child psychology and psychiatry forms the basis for hypothesis-driven studies of candidate Gene × Environment interactions of responses to treatment within the context of intervention studies. Thus, the response of a child to psychosocial interventions, such as those targeting mother–infant attachment, is influenced by the temperament or reactivity of the child (Belsky, 1997). More reactive children also seem to be more sensitive to the quality of the rearing environment (Boyce & Ellis, 2005). The temperament of the child is influenced by the presence of specific genomic variations in genes that encode for proteins involved in the function of the 5-HT and dopamine systems (Auerbach et al., 2002; Ebstein, 2003; Fox et al., 2005; Lakatos et al., 2003). Could such genomic variations mediate the observed effect of child temperament on the response to specific interventions? What might be a more appropriate intervention for ''treatment-resistant''populations? A more integrated Gene × Environment approach offers the opportunity to understand clearly the nature of individual differences in vulnerability or resistance for psychopathology and to more effectively target interventions.

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