Behavioral Neuroscience
2013, Vol. 127, No. 5, 628–636

0735-7044/13/$12.00 DOI: 10.1037/a0034186

BEHAVIORAL NEUROSCIENCE AT 30

Early Environments, Glucocorticoid Receptors, and Behavioral Epigenetics

Frances A. Champagne
Columbia University

In 1985, a brief report published in *Behavioral Neuroscience* established the link between neonatal handling and concentrations of hippocampal glucocorticoid receptors (GR) in the adult rat, suggesting a neurobiological basis for the attenuated stress reactivity observed in handled versus nonhandled offspring. To celebrate the 30th anniversary of *Behavioral Neuroscience*, this article explores the research that preceded and followed from this brief but significant publication. Changes in hippocampal GR induced by handling were determined to be the outcome of a cascade of cellular and molecular events involving thyroid hormones, serotonin turnover, and transcription factor binding to the *Nr3c1* gene, leading to increased GR mRNA and protein. Though many hypotheses were proposed for the "handling effect," the role of handling-induced changes in maternal care, particularly pup licking/grooming (LG), generated a productive scientific framework for understanding the handling phenomenon. Indeed, LG has since been demonstrated to alter GR levels through the signaling pathways described for handling. Moreover, epigenetic mechanisms have been discovered to play a critical role in the effects of early life experience and particularly in the regulation of *Nr3c1*. Overall, the research avenues that have evolved from the initial finding of handling-induced changes in GR have broad applications to our understanding of plasticity, resilience, and the transmission of traits across generations.

Keywords: handling, maternal care, glucocorticoid receptor, hippocampus, epigenetic

In 1985, Michael J. Meaney and colleagues published a brief report in *Behavioral Neuroscience* illustrating that glucocorticoid receptor (GR) protein levels within the hippocampus and frontal cortex were increased in the brain of adult rats that had experienced postnatal handling (Meaney et al., 1985). This finding provided insight into the mechanism through which handling-induced reductions in hypothalamic-pituitary-adrenal (HPA) responsivity were achieved (Ader, 1970). In the 10 years following the publication of this initial report, several pathways were explored to determine the mechanism through which handling could alter GR levels, and evidence for the roles of thyroid hormone and serotonergic signaling emerged (Meaney et al., 2000; Smythe, Rowe, & Meaney, 1994). Moreover, handling was found to induce persistent increases in the transcription of the gene encoding GR (*Nr3c1*; O'Donnell, Larocque, Seckl, & Meaney, 1994). By the late 1990s, it had become evident that handling resulted in increased pup-directed maternal behavior by rat dams, suggesting that the "handling effect" may be due to variation in maternal–infant interactions (Liu et al., 1997). Similar to handling, high levels of maternal care, particularly pup licking/grooming (LG), were found to modify HPA response to stress and level of hippocampal GR (Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997). Advances in molecular approaches to the study of gene regulation paired with behavioral neuroscience research ultimately led to the discovery of the epigenetic pathways through which maternal care shaped GR (Weaver, Cervoni, et al., 2004). In the past 10 years, this finding has generated the ever-evolving field of behavioral epigenetics, in which the epigenetic basis of behavioral variation, and the environments that induce this variation, are explored across species—including humans. To celebrate the 30th anniversary of *Behavioral Neuroscience*, this article looks back and looks forward at the branches of research that unfolded prior to and following its publication of a brief but significant finding of handling-induced changes in GR.

A Little Separation Goes a Long Way

Epidemiological studies of childhood neglect (De Bellis, 2005; Horwitz, Widom, McLaughlin, & White, 2001; Norman et al., 2012) and Harry Harlow's primate studies of maternal deprivation (Harlow & Harlow, 1962; Harlow, Rowland, & Griffin, 1964) suggest a lasting impact of disruption in the degree of nurturing experienced in infancy. However, in studies of laboratory rats, it seemed to be the case that brief daily separations between mother and offspring could confer long-term benefits to health and a resistance to later life stressors. In 1957, Seymour Levine presented a report in *Science* illustrating that brief mother–infant separations of male rat pups—termed "handling"—during the postnatal period resulted in increased weaning weights and in adulthood was associated with decreased adrenal weights following exposure to a noxious stimuli (Levine, 1957). It should be noted that the "handling" procedure involved transferring pups out of the nest and into a holding compartment briefly before being

Correspondence concerning this article should be addressed to Frances A. Champagne, Columbia University, Department of Psychology, 406 Schermerhorn Hall, 1190 Amsterdam Avenue, New York, NY 10027. E-mail: fac2105@columbia.edu

returned to the nest, resulting in a 1- to 2-min duration of separation. Thus, it could be argued that the term "handling" is somewhat misleading, though, interestingly, these early reports of handling effects are consistent with the effects of being "gentled," in which pups are held and stroked (Weininger, 1954). Further investigation of the effects of handling on the HPA response to stress revealed that, compared with nonhandled rats, the experience of handling in infancy was associated with a more rapid decline in stress-induced HPA activity. Handled male and female rats returned to baseline levels of corticosterone within 1 hr following stress exposure, whereas nonhandled controls maintained elevated corticosterone levels at this time point (Ader, 1970). This finding was particularly relevant when considered in combination with the characterization of negative feedback effects within the HPA axis (Sapolsky, Meaney, & McEwen, 1985). Reduced levels of hippocampal GRs had been found to be associated with increased HPA output, with a particular effect on the ability of individuals to "recover" following stress and rapidly restore baseline stress hormone levels (Sapolsky, Krey, & McEwen, 1984; Sapolsky et al., 1985). Thus, the pattern of glucocorticoid release following a stressor in handled versus nonhandled rats was similar to those observed in individuals with elevated versus decreased density of hippocampal GRs. This raised the possibility that early life experiences could lead to variation in this critical negative feedback system with consequences for stress reactivity and health.

In the 1985 report in *Behavioral Neuroscience*, "Early Postnatal Handling Alters Glucocorticoid Receptor Concentrations in Selected Brain Regions," Michael J. Meaney and colleagues established the important link between the early life experience of handling and the development of the HPA negative feedback system (Meaney et al., 1985). They exposed litters of Long-Evans rats to handling (H), consisting of a 15-min period in which pups and mothers are separated and removed from the home cage, from the day of birth to weaning, and compared these offspring with nonhandled (NH) pups. In addition, following weaning, H and NH offspring were either housed in isolation (ISOL) or housed socially (SOC). At 120 to 150 days of age, pituitary and brain tissue were extracted and [^{3}H]-dexamethasone binding was implemented to determine the concentration of GRs in the pituitary, frontal cortex, hypothalamus, septum, amygdala, and hippocampus of male and female rats. The results, presented in Figure 1, illustrate the tissue-specific effects of environmental experience on GR protein levels. No effects of postnatal handling or postweaning housing conditions were observed within the pituitary, hypothalamus, septum, or

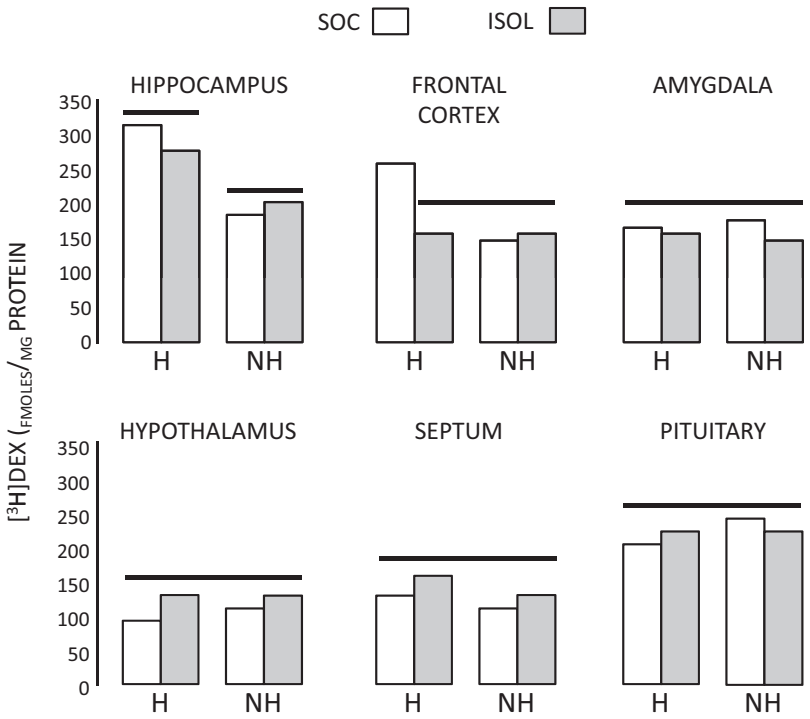

Figure 1. Effect of postnatal handling and postweaning housing on GR concentration. GR levels, as measured by [^{3}H]dexamethasone binding in the hippocampus, frontal cortex, amygdala, hypothalamus, septum, and pituitary were compared in adult rats that had experienced postnatal handling (H) or nonhandled (NH) rearing conditions, and then were either housed socially (SOC) or in isolation (ISOL) during the postweaning period. In the hippocampus, H rats had elevated GR compared to NH rats, regardless of postweaning housing conditions. In the frontal cortex, there was an interaction between postnatal and postweaning conditions in the prediction of GR. No group differences were observed in the other tissues examined. Adapted from "Early Postnatal Handling Alters Glucocorticoid Receptor Concentrations in Selected Brain Regions," by M. J. Meaney, D. H. Aitken, S. R. Bodnoff, L. J. Iny, J. E. Tatarewicz, & R. M. Sapolsky, 1985, *Behavioral Neuroscience*, *99*, p. 765–770. Copyright 1985 by the American Psychological Association.

amygdala. However, consistent with the hypothesis that handling was increasing HPA negative-feedback capacity, H rats had elevated GR levels in the hippocampus compared with NH rats regardless of the postweaning housing conditions. Interestingly, elevated GR was also observed in H versus NH rats within the frontal cortex; however, this effect was abolished if rats experienced postweaning social isolation, due to decreased GR in H-ISOL individuals. Thus, the link between early life experience and GR was clearly indicated, setting the stage for more in-depth studies of the mechanisms through which these effects are achieved.

At the time of the 1985 report on the modulation of GR levels in response to handling, two mechanisms were proposed that might account for these experiential effects—first, that the number of GR sites per cell was increased, and second, that handling was increasing postnatal neurogenesis in the hippocampus. Handled pups had been previously found to have cellular proliferation within the dentate gyrus of the hippocampus and neocortex compared with NH controls (Altman, Das, & Anderson, 1968), and thus there was certainly support for the second mechanistic hypothesis. However, as this field of research progressed, and as tools to study cellular and molecular processes in the brain became more advanced, significant insights into these mechanistic pathways was achieved. Handling was found to increase plasma thyroid hormone levels (thyroxine and triiodothyronine), and inhibition of thyroid synthesis blocked the effect of handling on hippocampal GR concentrations independent of plasma corticosterone levels (Meaney, Aitken, & Sapolsky, 1987). The inability to detect thyroid hormone effects on GR concentrations in vitro using hippocampal cell cultures (Meaney et al., 1987), suggested that there may be intermediary mechanistic pathways between thyroid signaling and levels of GR. Both handling and thyroid hormones increase serotonin (5-HT) turnover in hippocampal neurons, and lesions to 5-HT neurons results in reduced serotonergic input into the hippocampus, leading to reduced hippocampal GR density in adulthood (Mitchell, Iny, & Meaney, 1990). Unlike thyroid hormones, significant 5-HT effects on hippocampal GR concentrations have been illustrated in vitro, with dose-dependent increases in GR following 5-HT administration (Mitchell, Betito, Rowe, Boksa, & Meaney, 1992). Interestingly, the effects of 5-HT on GR concentrations persist for many weeks after the removal of 5-HT, consistent with the long-term effects of handling (Meaney et al., 1993). Further studies determined that 5-HT receptors play an important role in 5-HT effects on GR density; in vitro blockade of 5-HT_7 serotonin receptors inhibits 5-HT induced increases in hippocampal GR and blocks the effects of handling in vivo (Laplante, Diorio, & Meaney, 2002; Mitchell, Rowe, Boksa, & Meaney, 1990). The integration of serotonergic signaling into the mechanistic pathways underling the handling effects on GR observed in Meaney et al. (1985) may also account for the tissue specificity of GR increases. Handling was found to increase 5-HT turnover in the hippocampus and frontal cortex but not hypothalamus or amygdala and thus it may be through 5-HT signaling that hippocampal and frontal cortex-specific GR increases are achieved following postnatal handling (Mitchell, Iny, et al., 1990; Smythe et al., 1994).

The exploration of 5-HT signaling and its role in modulating hippocampal GR concentrations following handling suggests that handling may increase the number of GR sites per cell—the first of two hypotheses suggested in Meaney et al. (1985). This hypothesis implies handling-induced effects on the transcription of GR. The regulatory region of the GR gene (*Nr3c1*) contains transcription binding elements for activator protein-2 (AP-2) and nerve growth factor inducible factor A (NGFI-A; also *egr1*, *zif268*, *krox24*; Crosby, Puetz, Simburger, Fahrner, & Milbrandt, 1991; Nobukuni, Smith, Hager, & Detera-Wadleigh, 1995), both cAMP-protein kinase A (PKA) inducible transcription factors (Habener, 1990). Handling has been found to increase cAMP, and this effect is blocked by inhibition of thyroid hormone synthesis or 5-HT receptors (Meaney et al., 2000). Moreover, handling induces increases in hippocampal AP-2 and NGFI-A—an effect that is also blocked by inhibition of thyroid hormone synthesis or 5-HT receptors (Meaney et al., 2000). Both AP-2 and NGFI-A have been found to increase *Nr3c1* promoter activity through activation of their respective binding sequences, and handling has been found to increase not only the levels of these transcription factors but also the binding of the factors to the *Nr3c1* promoter (Meaney et al., 2000). Though there is a single GR protein, within the rat, there are multiple *Nr3c1* exon 1 sequences, many of which are expressed in the hippocampus (McCormick et al., 2000). Interestingly, it is the exon 1_7 transcript that is specifically altered by the experience of neonatal handling (McCormick et al., 2000). Increased exon 1_7-containing GR mRNA is detected in the dentate gyrus, CA1, and CA3 hippocampal regions following handling and within the genomic region immediately upstream of exon 1_7, there is a consensus binding site for NGFI-A (Crosby et al., 1991; McCormick et al., 2000). Taken together, these mechanistic studies support a pathway whereby handling-induced changes in thyroid and 5-HT signaling within the hippocampus leads to increased transcription factor binding to the *Nr3c1* gene, leading to increased GR mRNA and protein in the hippocampus and enhanced HPA negative-feedback following stress (Figure 2). Though expression of exon 1_7-containing GR mRNA was not found to be elevated in the frontal cortex following handling, these pathways likely account for the long-term effects of this early life experience on GR and stress reactivity (Meaney et al., 1993). A question raised by these findings regards "how" handling comes to trigger these cellular/molecular events upstream of the changes to thyroid/5-HT signaling.

Licking: More or Less

The experience of brief maternal separation during postnatal development clearly has long-term consequences for neurobiology and behavior. The question of "how"—what it is about handling that is meaningful in shaping these outcomes—has stimulated considerable speculation (Russell, 1971). Several theories have emerged in light of the effects of handling on HPA reactivity and emotionality. Seymour Levine proposed the "direct action" hypothesis, suggesting that the sensory experience of the handling procedure was a critical mediating variable (Levine, 1962). The particular modality of the sensory experience of importance was suggested to be the tactile/kinesthetic stimulation received, and it was further suggested that the total amount of tactile stimulation experienced prior to weaning shaped these developmental outcomes (Denenberg, 1964). However, it was argued that, in light of the intense tactile stimulation provided by mothers during the postnatal period, it was unlikely that handling would provide a

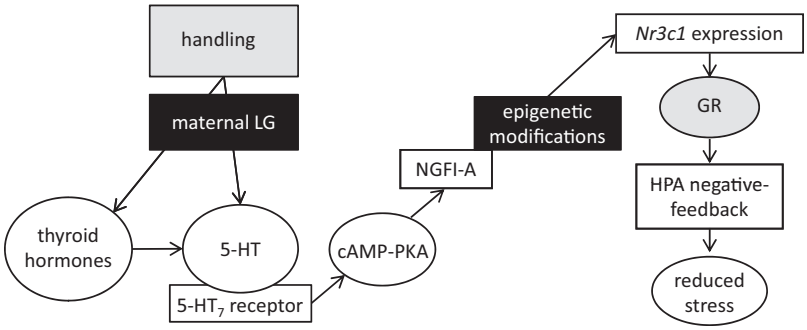

Figure 2. Cellular and molecular pathways linking handling and maternal LG to changes in hippocampal GR and reduced stress reactivity. Adapted from "Molecular Basis for the Development of Individual Differences in the Hypothalamic-Pituitary-Adrenal Stress Response," by M. J. Meaney, S. Bhatnagar, J. Diorio, S. Larocque, D. Francis, D. O'Donnell, . . . V. Viau, 1993, *Cellular and Molecular Neurobiology*, *13*, p. 321–347. Copyright 1993 by Kluwer Academic Publishers-Plenum Publishers. Also adapted from "Early environmental regulation of hippocampal glucocorticoid receptor gene expression: Characterization of intracellular mediators and potential genomic target sites" by I. C. Weaver, J. Diorio, J. R. Seckl, M. Szyf, & M. J. Meaney, 2004, Annals of the New York Academy of Sciences, 1024, p. 182–212. Copyright 2004 by New York Academy of Sciences.

sufficiently salient increase in stimulation capable of shifting development so profoundly. A second hypothesis, the "cooling hypothesis," focused on the hypothermia induced by the separation from the mother (Russell, 1971; Schaefer, Weingarten, & Towne, 1962). It was observed that preventing a drop in body temperature during the handling procedure could, in some instances, prevent the handling effect (Hutchings, 1963). However, it was deemed unlikely that this "cooling" effect could account entirely for the effects of handling. A third suggestion, the "stress hypothesis," conceptualized handling as a stressor and suggested that exposure to neonatal stress could "immunize" the individual from the experience of adverse effects deriving from later life stressors (Russell, 1971). It has been demonstrated that, following handling, pups experience an increase in corticosterone (Denenberg, Brumaghim, Haltmeyer, & Zarrow, 1967), and, in recent years, the "stress-inoculation" phenomenon, whereby mild stressors experienced in infancy are protective in later life, has gained momentum, and the role of the prefrontal cortex in this process has been described in primates and humans (Katz et al., 2009; Lyons & Macri, 2011; Lyons & Parker, 2007). Finally, a fourth hypothesis posits that the effects of handling are attributable to changes in maternal behavior experienced by handled pups (Russell, 1971). The "maternal-mediation hypothesis" was based on the premise that handling increased the incentive value of pups (i.e., increased ultrasonic vocalizations), which stimulates increased maternal behavior on her reunion with the pups (Bell, 1974; Smotherman, Brown, & Levine, 1977). The increase in mother–infant interactions induced by handling was proposed to attenuate the HPA response to stress. Though each of these hypotheses have merit and likely contribute to the developmental outcomes observed following handling, it is the "maternal mediation hypothesis" that is most relevant to the current research progress on the mechanisms through which early life experience shape the levels of hippocampal GR.

Maternal behavior during the postnatal period serves to promote growth and survival of offspring. However, this critical feature of the early environment also exhibits a high degree of plasticity. The handling procedure has been found to increase pup-directed maternal behavior in rats, particularly LG (Lee & Williams, 1974; Liu et al., 1997). This finding suggests the possibility that the effects of handling on the HPA response to stress are induced by increased maternal LG—a hypothesis that has generated significant support and that has expanded our understanding of the origins of individual differences in neurobiological and behavioral phenotypes. In rats, LG has been found to be a critical experience in shaping sexual dimorphism and in the behavioral responses of male and female offspring (Moore, 1992; Moore & Power, 1992). Though variation in LG can be induced by handling and other environmental experiences (Champagne & Meaney, 2006, 2007; Moore & Power, 1986), variation in LG can also be observed within unmanipulated laboratory rats (Champagne, Francis, Mar, & Meaney, 2003). Thus, natural variations in maternal behavior (i.e., low LG and high LG) can be characterized and the effects of the experience of differential LG can be explored. Comparison of the offspring of low-LG versus high-LG mothers reveals striking parallels with nonhandled versus handled offspring on HPA-related measures. Male offspring that experience low LG during the postnatal period have increased ACTH and corticosterone in response to stress and exhibit a slower recovery of glucocorticoid levels toward baseline following the cessation of the stressor (Liu et al., 1997). Consistent with this apparent effect on HPA negative feedback, male offspring of low-LG mothers have reduced hippocampal GR mRNA in the DG, CA1, and CA3 (Liu et al., 1997). Interestingly, if the male offspring of low-LG mothers are handled, they become indistinguishable from the offspring of high-LG mothers on measures of hippocampal GR expression (Francis et al., 1999)—a finding that provides strong support for the maternal mediation hypothesis of handling. These neurobiological and physiological effects of LG are also apparent in the increased anxiety-like behavior of low-LG male offspring (Caldji et al., 1998; Francis et al., 1999). Moreover, the signaling pathways whereby handling has been found to induce increased hippocampal GR expression seem to be activated by maternal LG: male offspring of high-LG mothers have increased thyroid hormone levels, increased NGFI-A levels, and increased association between NGFI-A and the *Nr3c1* 1_7 promoter (Hellstrom, Dhir, Diorio, &

Meaney, 2012). Licking-like tactile stimulation provided using a paintbrush has also been found to increase thyroid hormone and increase association between NGFI-A and the *Nr3c1* 1_7 promoter (Hellstrom et al., 2012). This effect of tactile stimulation is, like handling, 5-HT dependent. Overall, research in the past 15 years has provided a wealth of data on the impact of maternal LG on cellular, molecular, neurobiological, and behavioral outcomes that creates clear parallels to the early studies of handling, and is also continuing to extend the exploration of the effects of early life experience to variation in learning/memory (Liu, Diorio, Day, Francis, & Meaney, 2000), social behavior (Parent, Del Corpo, Cameron, & Meaney, 2012; Parent & Meaney, 2008), and reproduction (Cameron, Fish, & Meaney, 2008; Champagne et al., 2003). A future challenge for these studies will be to determine the mechanisms of species- specific and/or sex-specific responses to maternal LG (that is, whether these mechanistic pathways are involved in the effects of LG on female offspring; see Pedersen, Vadlamudi, Boccia, & Moy, 2011), particularly in light of differences in maternal behavior received by male and female pups (Moore & Morelli, 1979).

Eureka: Maternal Care and the Epigenetic Light Switch

The regulation of gene expression is a complex and dynamic process. In studies of handling and maternal LG, it is apparent that the effects of these postnatal experiences on GR protein and gene expression are long term, persisting into adulthood—well beyond the period of mother–infant interactions. In the case of both handling and maternal LG, it is evident that the critical period for inducing these effects on GR in rats is within the first week of life (Caldji et al., 1998; Meaney & Aitken, 1985). This observation raises questions as to the mechanism through which gene expression can be stably maintained. The question of mechanism led this research to the phenomenon of DNA methylation, whereby cytosine nucleotide bases in DNA become modified through addition of a methyl group, generating 5-methylcytosine (Ehrlich & Wang, 1981). Though this chemical modification is not a mutation, it can impact the accessibility of DNA to transcription factor binding and reduce gene expression—particularly when the 5-methylcytosine is located within the promoter region of the gene (Razin, 1998; Razin & Riggs, 1980). DNA methylation is one of many "epigenetic" modifications that can alter gene expression without altering the underling DNA sequence. However, among the various epigenetic mechanisms (i.e., posttranslational histone modifications, microRNAs), DNA methylation is notable for its stability and mitotic heritability (Razin, 1998). In fact, the process of cellular differentiation whereby cells maintain a cell-type specific gene expression/phenotypic profile is due, in large part, to the gene silencing role of DNA methylation. Given the stability of DNA methylation, it seemed plausible that this epigenetic mechanism would play a role in the differential expression of GR induced by early life experience. Indeed, in the past 10 years, it has become increasingly apparent that epigenetic modifications can be dynamically altered by early life experience and stably maintained throughout the life span (Champagne, 2010; Champagne & Curley, 2009). This is particularly evident in studies of environmental modulation of GR. Male offspring of low-LG mothers have elevated hippocampal cytosine methylation levels within the *Nr3c1* promoter region, particularly within the NGFI-A binding sequence (Weaver, Cervoni, et al., 2004). These molecular changes emerge during the first postnatal week, coinciding with the timing of the most profound differences in maternal LG and the window during which handling effects have been observed to increase hippocampal GR concentrations (Meaney & Aitken, 1985; Weaver, Cervoni, et al., 2004). Through subsequent in vitro and in vivo studies, it has become apparent that NGFI-A binding to the *Nr3c1* gene is a critical mediator of the differential DNA methylation induced by LG or serotonergic signaling (Hellstrom et al., 2012). Thus, the experience of high levels of maternal LG during infancy has been proposed to increase thyroid/serotonin signaling, increase NGFI-A levels and the association between this transcription factor and the *Nr3c1* gene, and promote a molecular environment that increases *Nr3c1* expression and protein levels through reduced levels of epigenetic silencing of the *Nr3c1* gene (Weaver et al., 2007; Weaver, Diorio, Seckl, Szyf, & Meaney, 2004; see Figure 2). Within the hippocampus, LG-associated effects on DNA methyltransferases (enzymes that add methyl groups to DNA) may also be important regulatory factors within these pathways and certainly account to the genome-wide transcriptional effects observed as a consequence of variation in maternal care (Weaver, Meaney, & Szyf, 2006; Zhang et al., 2010).

The discovery of the role of DNA methylation in mediating the effects of maternal care on offspring development continues to challenge our conception of the stability versus plasticity of this epigenetic mark. At present, it is unclear how this plasticity is achieved and what target genes may be most susceptible to long-term programming versus dynamic epigenetic variation. However, it is becoming increasingly evident that variation in *Nr3c1* promoter methylation can serve as a marker of early life experience—particularly adversity. In humans, postmortem analyses of hippocampal tissue have indicated that a history of childhood abuse is associated with increased *Nr3c1* methylation at the NGFI-A sequence and reduced GR mRNA levels (McGowan et al., 2009). In blood samples, increased *Nr3c1* methylation profile has also been observed as a consequence of childhood maltreatment and reduced nurturing (Tyrka, Price, Marsit, Walters, & Carpenter, 2012). Maternal depression during pregnancy is associated with increased *Nr3c1* methylation in fetal cord blood, the levels of which predict stress reactivity of infants at 3 months of age (Oberlander et al., 2008). Similar epigenetic effects in cord blood have been found consequent to maternal anxiety during pregnancy (Hompes et al., 2013). These characterizations of the epigenetic effect of adversity are an important first step in (a) determining the biological basis of adversity induced disease risk, and (b) developing novel strategies for intervention. Though DNA methylation can be a persistent epigenetic mark, studies of low- versus high-LG offspring suggest that pharmacological targeting of these modifications using drugs that alter DNA methylation/histone modifications can reverse the effects of early life experience in adulthood (Weaver, Cervoni, et al., 2004; Weaver et al., 2005, 2006). Further, the application of this research to humans reflects the across-species relevance of epigenetics to the understanding of variation in neurobiological and behavioral phenotypes. The field of behavioral epigenetics has emerged from this perspective and continues to illustrate the dynamic ways in which epigenetic modifications can generate phenotypic plasticity.

Lifelong Plasticity

In the initial report by Meaney et al. (1985), the effects of handling observed in the frontal cortex add an intriguing level of complexity to the study of early life experiences. When rats were housed socially, the effects of handling on GR were apparent. However, under conditions of postweaning social isolation, the handling effects were abolished due to the decrease in GR among handled rats that experienced subsequent social isolation (see Figure 1). This finding suggests that GR maintains a heightened degree of plasticity within the frontal cortex beyond the postnatal period. Though studies of postweaning modulation of GR have generally produced inconsistent findings, the notion of lifelong plasticity is an important consideration within studies of individual differences in emotionality and HPA reactivity (Champagne, 2010; Curley, Jensen, Mashoodh, & Champagne, 2011). The effects of neonatal exposure to prolonged periods of maternal separation (typically 2 to 3 hr in duration), which, unlike handling, typically produce an increased HPA response to stress, can be attenuated by subsequent exposure to environmental enrichment (consisting of group housing in a complex environment with toys; Francis, Diorio, Plotsky, & Meaney, 2002). Postweaning environmental enrichment reduces stress-induced corticosterone and increases exploration of a novel environment by adult rats that had experienced maternal separation. However, consistent with the finding that postweaning environment was not capable of shifting levels of hippocampal GR, maternal-separation-induced decreases in GR mRNA in the DG, CA1, and CA3 were not altered by environmental enrichment (Francis et al., 2002). Thus, the shift in the physiological and behavioral response to stress observed following environmental enrichment may be attributable to cellular/molecular changes outside the hippocampus, such as the frontal cortex. However, targets other than GR within the hippocampus may retain plasticity during the postweaning period. The effects of low LG on hippocampal alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor binding, N-methyl-D-aspartate (NMDA) subunit expression, and deficits in learning and memory can be reversed through postnatal environmental enrichment (Bredy, Humpartzoomian, Cain, & Meaney, 2003; Bredy, Zhang, Grant, Diorio, & Meaney, 2004). This plasticity extends to the epigenetic mechanisms that may underlie the effects of environmental experience, as there is increasing evidence that experiences spanning from fetal development through to adulthood can induce changes in DNA methylation and histone modifications, with implications for neurobiological functioning and behavior (Champagne, 2010).

Transgenerational Plasticity

The plasticity observed in response to environmental exposures may also have implications for subsequent generations. In the 1960s, Victor Denenberg reported that if a female rat pup was exposed to handling during infancy, and then mated in adulthood, her offspring had higher weaning weights (Denenberg & Whimby, 1963). Cross-fostering manipulations revealed that this outcome was predicted by whether the offspring were reared by a handled female or not—offspring born to a nonhandled mother and then reared by a handled mother were found to have elevated weaning weights. Analyses of open-field behavior revealed a complex influence of both prenatal and postnatal maternal factors in predicting the effect of handling on offspring (Denenberg & Whimby, 1963). These handling effects may even persist across multiple generations, as the handling experience of grandmothers, interacting with the housing conditions of mothers, was a predictor of grand-offspring weaning weight and open-field activity (Denenberg & Rosenberg, 1967). One possible mechanism for these across-generation effects are changes in the subsequent maternal behavior of handled females—a possibility supported by findings that handling increases the frequency of maternal LG among low-LG females (Francis et al., 1999). The transgenerational continuity of maternal LG is now well established and cross-fostering studies support the hypothesis that this transmission is determined by the experience of LG rather than genetic or prenatal factors (Champagne et al., 2003, 2006; Champagne & Meaney, 2001). Mechanistically, this transmission involves maternal LG-induced epigenetic changes within the hypothalamic estrogen receptor alpha (*Esr1*) gene, whereby low levels of LG lead to epigenetic silencing of *Esr1* and reduced estrogen sensitivity and maternal behavior (Champagne et al., 2006). Due to the transmission of maternal LG across generations, there is also transgenerational continuity in outcomes associated with the experience of LG, such as hippocampal GR and the HPA response to stress.

The maternal transmission of traits represents a critical "non-genomic" pathway through which the experience of one generation can alter the next. However, in light of advances in our understanding of epigenetics and the plasticity of molecular modifications such as DNA methylation, there has been increasing speculation that environmentally induced epigenetic variation may be heritable across generations (Daxinger & Whitelaw, 2012; Guerrero-Bosagna & Skinner, 2012). Though the mechanisms through which this transmission occurs have yet to be elucidated, it has been proposed that epigenetic changes in the germline that are not erased at the time of postfertilization epigenetic programming may become heritable, leading to altered developmental outcomes. For example, prenatal exposure to the pesticide vinclozolin has been found to produce disease risk and impaired fertility that persist to the F3 generation of offspring in the patriline, and these outcomes are associated with altered DNA methylation levels in sperm and brain (Anway, Cupp, Uzumcu, & Skinner, 2005; Skinner, Anway, Savenkova, Gore, & Crews, 2008). The experience of maternal separation by male offspring during postnatal development has also been demonstrated to alter DNA methylation levels in the sperm and brain of offspring and grand-offspring, with implications for social behavior (Franklin, Linder, Russig, Thony, & Mansuy, 2011; Franklin et al., 2010). It is notable that these proposed germline epigenetic effects have been observed in fathers rather than mothers, and it is perhaps the case that although mothers can shape offspring through direct mother–infant interactions, fathers, particularly in species that do not engage in parental care, have evolved a strategy for transmitting their environmentally induced phenotypes *via* the germline instead. However, it should also be noted that dissociating the influence of maternal care from those of inherited germline effects is challenging (Curley, Mashoodh, & Champagne, 2011; Dietz et al., 2011; Mashoodh, Franks, Curley, & Champagne, 2012), and it is likely the case that we must incorporate multiple genomic and "non-genomic" inheritance mechanisms into our thinking in order to fully understand the impact of early life experience.

Concluding Remarks

Scientific progress is made through the careful research steps that build a foundation that is larger than the sum of its parts. In the case of the establishment of the link between handling and tissue-specific GR levels in the brain reported in *Behavioral Neuroscience* in the early days of this journal (Meaney et al., 1985), this finding was part of an extensive scientific history of studies on the effects of early life experience and formed the basis for furthering our understanding of the behavioral and molecular pathways that are shaped by the environment. Perhaps most importantly, the research avenues generated from this early work continue to evolve and grow, addressing questions regarding the impact of the social environment on brain development, the importance of the tactile experience of infants, resilience to early life adversity, epigenetic pathways through which the environment acts to turn genes "on" or "off," and the transmission of phenotypic variation across generations. Moreover, the research being generated from these investigations has had a significant impact on our conceptualization of nature versus nurture (Meaney, 2001; Pigliucci, 2001), with growing acceptance of the molecular interplay between our genes and the environment that makes discussions of the relative importance of one or the other a part of history rather than the present or future.

References

Ader, R. (1970). The effects of early experience on the adrenocortical response to different magnitudes of stimulation. *Physiology & Behavior, 5,* 837–839. doi:10.1016/0031-9384(70)90168-X

Altman, J., Das, G. D., & Anderson, W. J. (1968). Effects of infantile handling on morphological development of the rat brain: An exploratory study. *Developmental Psychobiology, 1,* 10–20. doi:10.1002/dev.420010104

Anway, M. D., Cupp, A. S., Uzumcu, M., & Skinner, M. K. (2005, June 3). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science, 308,* 1466–1469. doi:10.1126/science.1108190

Bell, R. W. (1974). Ultrasounds in small rodents: Arousal-produced and arousal-producing. *Developmental Psychobiology, 7,* 39–42. doi:10.1002/dev.420070107

Bredy, T. W., Humpartzoomian, R. A., Cain, D. P., & Meaney, M. J. (2003). Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. *Neuroscience, 118,* 571–576. doi:10.1016/S0306-4522(02)00918-1

Bredy, T. W., Zhang, T. Y., Grant, R. J., Diorio, J., & Meaney, M. J. (2004). Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. *European Journal of Neuroscience, 20,* 1355–1362. doi:10.1111/j.1460-9568.2004.03599.x

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *PNAS Proceedings of the National Academy of Sciences of the United States of America, 95,* 5335–5340. doi:10.1073/pnas.95.9.5335

Cameron, N. M., Fish, E. W., & Meaney, M. J. (2008). Maternal influences on the sexual behavior and reproductive success of the female rat. *Hormones and Behavior, 54,* 178–184. doi:10.1016/j.yhbeh.2008.02.013

Champagne, F. A. (2010). Epigenetic influence of social experiences across the lifespan. *Developmental Psychobiology, 52,* 299–311. doi:10.1002/dev.20436

Champagne, F. A., & Curley, J. P. (2009). Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neuroscience and Biobehavioral Reviews, 33,* 593–600. doi:10.1016/j.neubiorev.2007.10.009

Champagne, F. A., Francis, D. D., Mar, A., & Meaney, M. J. (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiology & Behavior, 79,* 359–371. doi:10.1016/S0031-9384(03)00149-5

Champagne, F., & Meaney, M. J. (2001). Like mother, like daughter: Evidence for non-genomic transmission of parental behavior and stress responsivity. *Progress in Brain Research, 133,* 287–302. doi:10.1016/S0079-6123(01)33022-4

Champagne, F. A., & Meaney, M. J. (2006). Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. *Biological Psychiatry, 59,* 1227–1235. doi:10.1016/j.biopsych.2005.10.016

Champagne, F. A., & Meaney, M. J. (2007). Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. *Behavioral Neuroscience, 121,* 1353–1363. doi:10.1037/0735-7044.121.6.1353

Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology, 147,* 2909–2915. doi:10.1210/en.2005-1119

Crosby, S. D., Puetz, J. J., Simburger, K. S., Fahrner, T. J., & Milbrandt, J. (1991). The early response gene NGFI-C encodes a zinc finger transcriptional activator and is a member of the GCGGGGGCG (GSG) element-binding protein family. *Molecular and Cellular Biology, 11,* 3835–3841.

Curley, J. P., Jensen, C. L., Mashoodh, R., & Champagne, F. A. (2011). Social influences on neurobiology and behavior: Epigenetic effects during development. *Psychoneuroendocrinology, 36,* 352–371. doi:10.1016/j.psyneuen.2010.06.005

Curley, J. P., Mashoodh, R., & Champagne, F. A. (2011). Epigenetics and the origins of paternal effects. *Hormones and Behavior, 59,* 306–314. doi:10.1016/j.yhbeh.2010.06.018

Daxinger, L., & Whitelaw, E. (2012). Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nature Reviews Genetics, 13,* 153–162. doi:10.1038/nrg3188

De Bellis, M. D. (2005). The psychobiology of neglect. *Child Maltreatment, 10,* 150–172. doi:10.1177/1077559505275116

Denenberg, V. H. (1964). Critical periods, stimulus input, and emotional reactivity. *Psychological Review, 71,* 335–351. doi:10.1037/h0042567

Denenberg, V. H., Brumaghim, J. T., Haltmeyer, G. C., & Zarrow, M. X. (1967). Increased adrenocortical activity in the neonatal rat following handling. *Endocrinology, 81,* 1047–1052. doi:10.1210/endo-81-5-1047

Denenberg, V. H., & Rosenberg, K. M. (1967, November 11). Nongenetic transmission of information. *Nature, 216,* 549–550. doi:10.1038/216549a0

Denenberg, V. H., & Whimby, A. E. (1963, November 29). Behavior of adult rats is modified by the experiences their mothers had as infants. *Science, 142,* 1192–1193. doi:10.1126/science.142.3596.1192

Dietz, D. M., Laplant, Q., Watts, E. L., Hodes, G. E., Russo, S. J., Feng, J., . . . Nestler, E. J. (2011). Paternal transmission of stress-induced pathologies. *Biological Psychiatry, 70,* 408–414. doi:10.1016/j.biopsych.2011.05.005

Ehrlich, M., & Wang, R. Y. (1981, June 19). 5-Methylcytosine in eukaryotic DNA. *Science, 212,* 1350–1357. doi:10.1126/science.6262918

Francis, D., Diorio, J., Liu, D., & Meaney, M. J. (1999, November 5). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286,* 1155–1158. doi:10.1126/science.286.5442.1155

Francis, D. D., Diorio, J., Plotsky, P. M., & Meaney, M. J. (2002). Environmental enrichment reverses the effects of maternal separation on stress reactivity. *The Journal of Neuroscience, 22,* 7840–7843.

Franklin, T. B., Linder, N., Russig, H., Thony, B., & Mansuy, I. M. (2011). Influence of early stress on social abilities and serotonergic functions across generations in mice. *PLoS One, 6,* e21842. doi:10.1371/journal.pone.0021842

Franklin, T. B., Russig, H., Weiss, I. C., Graff, J., Linder, N., Michalon, A., . . . Mansuy, I. M. (2010). Epigenetic transmission of the impact of early stress across generations. *Biological Psychiatry, 68,* 408–415. doi:10.1016/j.biopsych.2010.05.036

Guerrero-Bosagna, C., & Skinner, M. K. (2012). Environmentally induced epigenetic transgenerational inheritance of phenotype and disease. *Molecular and Cellular Endocrinology, 354,* 3–8. doi:10.1016/j.mce.2011.10.004

Habener, J. F. (1990). Cyclic AMP response element binding proteins: A cornucopia of transcription factors. *Molecular Endocrinology, 4,* 1087–1094. doi:10.1210/mend-4-8-1087

Harlow, H. F., & Harlow, M. (1962). Social deprivation in monkeys. *Scientific American, 207,* 136–147. doi:10.1038/scientificamerican1162-136

Harlow, H. F., Rowland, G. L., & Griffin, G. A. (1964). The effect of total social deprivation on the development of monkey behavior. *Psychiatric Research Reports, 19,* 116–135.

Hellstrom, I. C., Dhir, S. K., Diorio, J. C., & Meaney, M. J. (2012). Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. *Philosophical Transactions of the Royal Society of London*. Series B: Biological Sciences, *367,* 2495–2510. doi:10.1098/rstb.2012.0223

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., Pexsters, A., . . . Claes, S. (2013). Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. *Journal of Psychiatric Research, 47,* 880–891. doi:10.1016/j.jpsychires.2013.03.009

Horwitz, A. V., Widom, C. S., McLaughlin, J., & White, H. R. (2001). The impact of childhood abuse and neglect on adult mental health: A prospective study. *Journal of Health and Social Behavior, 42,* 184–201. doi:10.2307/3090177

Hutchings, D. E. (1963). Early "experience" and its effects on later behavioral processes in rats. III. Effects of infantile handling and body temperature reduction on later emotionality. *Transactions of the New York Academy of Sciences, 25,* 890–901. doi:10.1111/j.2164-0947.1963.tb01926.x

Katz, M., Liu, C., Schaer, M., Parker, K. J., Ottet, M. C., Epps, A., . . . Lyons, D. M. (2009). Prefrontal plasticity and stress inoculation-induced resilience. *Developmental Neuroscience, 31,* 293–299. doi:10.1159/000216540

Laplante, P., Diorio, J., & Meaney, M. J. (2002). Serotonin regulates hippocampal glucocorticoid receptor expression via a 5-HT7 receptor. *Brain Research*. Developmental Brain Research, *139,* 199–203. doi:10.1016/S0165-3806(02)00550-3

Lee, M. H. S., & Williams, D. I. (1974). Changes in licking behaviour of rat mother following handling of young. *Animal Behaviour, 22,* 679–681. doi:10.1016/S0003-3472(74)80016-3

Levine, S. (1957, August 30). Infantile experience and resistance to physiological stress. *Science, 126,* 405. doi:10.1126/science.126.3270.405

Levine, S. (1962). The effects of infantile experience on adult behaviour. In A. J. Bachrach (Ed.), *Experimental foundations of clinical psychology* (pp. 139–169). New York, NY: Basic Books.

Liu, D., Diorio, J., Day, J. C., Francis, D. D., & Meaney, M. J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience, 3,* 799–806. doi:10.1038/77702

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., . . . Meaney, M. J. (1997, September 12). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277,* 1659–1662. doi:10.1126/science.277.5332.1659

Lyons, D. M., & Macri, S. (2011). Resilience and adaptive aspects of stress in neurobehavioral development. *Neuroscience and Biobehavioral Reviews, 35,* 1451. doi:10.1016/j.neubiorev.2010.09.004

Lyons, D. M., & Parker, K. J. (2007). Stress inoculation-induced indications of resilience in monkeys. *Journal of Trauma Stress, 20,* 423–433. doi:10.1002/jts.20265

Mashoodh, R., Franks, B., Curley, J. P., & Champagne, F. A. (2012). Paternal social enrichment effects on maternal behavior and offspring growth. *PNAS Proceedings of the National Academy of Sciences of the United States of America, 109,* 17232–17238. doi:10.1073/pnas.1121083109

McCormick, J. A., Lyons, V., Jacobson, M. D., Noble, J., Diorio, J., Nyirenda, M., . . . Chapman, K. E. (2000). 5′-heterogeneity of glucocorticoid receptor messenger RNA is tissue specific: Differential regulation of variant transcripts by early-life events. *Molecular Endocrinology, 14,* 506–517. doi:10.1210/me.14.4.506

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonte, B., Szyf, M., . . . Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience, 12,* 342–348. doi:10.1038/nn.2270

Meaney, M. J. (2001). Nature, nurture, and the disunity of knowledge. *Annals of the New York Academy of Sciences, 935,* 50–61. doi:10.1111/j.1749-6632.2001.tb03470.x

Meaney, M. J., & Aitken, D. H. (1985). The effects of early postnatal handling on hippocampal glucocorticoid receptor concentrations: Temporal parameters. *Brain Research, 354,* 301–304.

Meaney, M. J., Aitken, D. H., Bodnoff, S. R., Iny, L. J., Tatarewicz, J. E., & Sapolsky, R. M. (1985). Early postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. *Behavioral Neuroscience, 99,* 765–770. doi:10.1037/0735-7044.99.4.765

Meaney, M. J., Aitken, D. H., & Sapolsky, R. M. (1987). Thyroid hormones influence the development of hippocampal glucocorticoid receptors in the rat: A mechanism for the effects of postnatal handling on the development of the adrenocortical stress response. *Neuroendocrinology, 45,* 278–283. doi:10.1159/000124741

Meaney, M. J., Bhatnagar, S., Diorio, J., Larocque, S., Francis, D., O'Donnell, D., . . . Viau, V. (1993). Molecular basis for the development of individual differences in the hypothalamic-pituitary-adrenal stress response. *Cellular and Molecular Neurobiology, 13,* 321–347. doi:10.1007/BF00711576

Meaney, M. J., Diorio, J., Francis, D., Weaver, S., Yau, J., Chapman, K., & Seckl, J. R. (2000). Postnatal handling increases the expression of cAMP-inducible transcription factors in the rat hippocampus: The effects of thyroid hormones and serotonin. *The Journal of Neuroscience, 20,* 3926–3935.

Mitchell, J. B., Betito, K., Rowe, W., Boksa, P., & Meaney, M. J. (1992). Serotonergic regulation of type II corticosteroid receptor binding in hippocampal cell cultures: Evidence for the importance of serotonin-induced changes in cAMP levels. *Neuroscience, 48,* 631–639. doi:10.1016/0306-4522(92)90407-S

Mitchell, J. B., Iny, L. J., & Meaney, M. J. (1990). The role of serotonin in the development and environmental regulation of type II corticosteroid receptor binding in rat hippocampus. *Brain Research*. Developmental Brain Research, *55,* 231–235. doi:10.1016/0165-3806(90)90204-C

Mitchell, J. B., Rowe, W., Boksa, P., & Meaney, M. J. (1990). Serotonin regulates type II corticosteroid receptor binding in hippocampal cell cultures. *Journal of Neuroscience, 10,* 1745–1752.

Moore, C. L. (1992). The role of maternal stimulation in the development of sexual behavior and its neural basis. *Annals of the New York Academy of Sciences, 662,* 160–177. doi:10.1111/j.1749-6632.1992.tb22859.x

Moore, C. L., & Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. *Journal of Comparative and Physiological Psychology, 93,* 677–684. doi:10.1037/h0077599

Moore, C. L., & Power, K. L. (1986). Prenatal stress affects mother–infant interaction in Norway rats. *Developmental Psychobiology, 19,* 235–245. doi:10.1002/dev.420190309

Moore, C. L., & Power, K. L. (1992). Variation in maternal care and individual differences in play, exploration, and grooming of juvenile Norway rat offspring. *Developmental Psychobiology, 25,* 165–182. doi:10.1002/dev.420250303

Nobukuni, Y., Smith, C. L., Hager, G. L., & Detera-Wadleigh, S. D. (1995). Characterization of the human glucocorticoid receptor promoter. *Biochemistry, 34,* 8207–8214. doi:10.1021/bi00025a028

Norman, R. E., Byambaa, M., De, R., Butchart, A., Scott, J., & Vos, T. (2012). The long-term health consequences of child physical abuse, emotional abuse, and neglect: A systematic review and meta-analysis. *PLoS Med, 9,* e1001349. doi:10.1371/journal.pmed.1001349

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics, 3,* 97–106. doi:10.4161/epi.3.2.6034

O'Donnell, D., Larocque, S., Seckl, J. R., & Meaney, M. J. (1994). Postnatal handling alters glucocorticoid, but not mineralocorticoid messenger RNA expression in the hippocampus of adult rats. *Brain Research*. Molecular Brain Research, *26,* 242–248. doi:10.1016/0169-328X(94)90096-5

Parent, C. I., Del Corpo, A., Cameron, N. M., & Meaney, M. J. (2012). Maternal care associates with play dominance rank among adult female rats. *Developmental Psychobiology*. Advance online publication. doi:10.1002/dev.21070

Parent, C. I., & Meaney, M. J. (2008). The influence of natural variations in maternal care on play fighting in the rat. *Developmental Psychobiology, 50,* 767–776. doi:10.1002/dev.20342

Pedersen, C. A., Vadlamudi, S., Boccia, M. L., & Moy, S. S. (2011). Variations in maternal behavior in C57BL/6J mice: Behavioral comparisons between adult offspring of high and low pup-licking mothers. *Frontiers in Psychiatry, 2,* 42. doi:10.3389/fpsyt.2011.00042

Pigliucci, M. (2001). *Phenotypic plasticity: Beyond nature and nurture*. Baltimore, MD: Johns Hopkins University Press.

Razin, A. (1998). CpG methylation, chromatin structure and gene silencing-a three-way connection. *Embo Journal, 17,* 4905–4908. doi:10.1093/emboj/17.17.4905

Razin, A., & Riggs, A. D. (1980, November 7). DNA methylation and gene function. *Science, 210,* 604–610. doi:10.1126/science.6254144

Russell, P. A. (1971). "Infantile stimulation" in rodents: A consideration of possible mechanisms. *Psychological Bulletin, 75,* 192–202. doi:10.1037/h0030426

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1984). Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *PNAS Proceedings of the National Academy of Sciences of the United States of America, 81,* 6174–6177. doi:10.1073/pnas.81.19.6174

Sapolsky, R. M., Meaney, M. J., & McEwen, B. S. (1985). The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. *Brain Research, 350,* 169–173.

Schaefer, T., Jr., Weingarten, F. S., & Towne, J. C. (1962, January 5). Temperature change: The basic variable in the early handling phenomenon? *Science, 135,* 41–42. doi:10.1126/science.135.3497.41

Skinner, M. K., Anway, M. D., Savenkova, M. I., Gore, A. C., & Crews, D. (2008). Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. *PLoS One, 3,* e3745. doi:10.1371/journal.pone.0003745

Smotherman, W. P., Brown, C. P., & Levine, S. (1977). Maternal responsiveness following differential pup treatment and mother-pup interactions. *Hormones and Behavior, 8,* 242–253. doi:10.1016/0018-506X(77)90041-1

Smythe, J. W., Rowe, W. B., & Meaney, M. J. (1994). Neonatal handling alters serotonin (5-HT) turnover and 5-HT2 receptor binding in selected brain regions: Relationship to the handling effect on glucocorticoid receptor expression. *Developmental Brain Research, 80,* 183–189. doi:10.1016/0165-3806(94)90103-1

Tyrka, A. R., Price, L. H., Marsit, C., Walters, O. C., & Carpenter, L. L. (2012). Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: Preliminary findings in healthy adults. *PLoS One, 7,* e30148. doi:10.1371/journal.pone.0030148

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience, 7,* 847–854. doi:10.1038/nn1276

Weaver, I. C., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J., & Szyf, M. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: Altering epigenetic marking later in life. *The Journal of Neuroscience, 25,* 11045–11054. doi:10.1523/JNEUROSCI.3652-05.2005

Weaver, I. C., Diorio, J., Seckl, J. R., Szyf, M., & Meaney, M. J. (2004). Early environmental regulation of hippocampal glucocorticoid receptor gene expression: Characterization of intracellular mediators and potential genomic target sites. *Annals of the New York Academy of Sciences, 1024,* 182–212. doi:10.1196/annals.1321.099

Weaver, I. C., D'Alessio, A. C., Brown, S. E., Hellstrom, I. C., Dymov, S., Sharma, S., . . . Meaney, M. J. (2007). The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming: Altering epigenetic marks by immediate-early genes. *Journal of Neuroscience, 27,* 1756–1768. doi:10.1523/JNEUROSCI.4164-06.2007

Weaver, I. C., Meaney, M. J., & Szyf, M. (2006). Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *PNAS Proceedings of the National Academy of Sciences of the United States of America, 103,* 3480–3485. doi:10.1073/pnas.0507526103

Weininger, O. (1954, February 26). Physiological damage under emotional stress as a function of early experience. *Science, 119,* 285–286. doi:10.1126/science.119.3087.285

Zhang, T. Y., Hellstrom, I. C., Bagot, R. C., Wen, X., Diorio, J., & Meaney, M. J. (2010). Maternal care and DNA methylation of a glutamic acid decarboxylase 1 promoter in rat hippocampus. *The Journal of Neuroscience, 30,* 13130–13137. doi:10.1523/JNEUROSCI.1039-10.2010

Received June 20, 2013
Revision received July 28, 2013
Accepted July 28, 2013 ■