Early Life Influences on the Ontogeny of the Neuroendocrine Stress Response in the Human Child

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Introduction

Living organisms are flexible; they can respond to changing conditions through a variety of morphological, physiological, and behavioral mechanisms. The processes that organisms use to change and respond to environmental challenges are posited to be evolved adaptations (West-Eberhard, 2003). Flexibility involves both immediate, temporary responses and longer-term developmental changes. The ability to generate a variety of phenotypes from a single genotype to adapt to environmental variations is called *phenotypic plasticity*.

Humans exhibit a most complex form of phenotypic plasticity. Our brain has unique information-processing capacities that we use to master the fast-paced dynamics of social networks and culture (Adolphs, 2003; Roth and Dicke, 2005). The stress axis appears to play an important role in modulating the aforementioned plasticity. Humans present an extraordinary sensitivity to stress that appears to arise at the earliest stages of development. Stress response may at first sight seem paradoxical because release of cortisol and other stress hormones may have attendant somatic costs and important consequences for human health (Flinn, 2007, 2008). Maternal depression and anxiety during pregnancy, for example, are associated with low birth weight, elevated stress reactivity, and subsequent health risks for the offspring (Barker, 1998; Weinstock, 2005; Gluckman and Hanson, 2006).

In this chapter, we discuss this apparent paradox. We also evaluate possible mechanisms and developmental trajectories that link early life events to physiological stress response, psychological development, and

health outcomes from the very moment of conception. We start by analyzing stress as a modulator of women's reproductive function and the conflicts that can arise between mother and fetus regarding the ultimate fate of the pregnancy during stressful times. Next, we discuss the effects that stress has on fetal development once pregnancy is firmly established (after the placentation process has taken place). The short- and long-term developmental consequences of stress appear to vary according to the time in which the stress challenge takes place during ontogeny. We review the role of the environment as a factor in "developmental programming" during ontogenetic processes. Then we focus on the specific effects that social challenges appear to have on the postnatal ontogeny of neuroendocrine stress response and subsequent health outcomes. We illustrate our ideas with brief overviews of our respective work in Guatemala and Dominica. We conclude by proposing the "depathologization" of the study of the developmental consequences of stress.

Stress and the Timing of Pregnancy

The quality of the mother's environment during pregnancy and soon after parturition can critically affect her offspring's chances of survival and overall quality. Consequently, a pregnancy conceived or maintained under stressful conditions may affect the mothers' lifetime reproductive success and that of her offspring (Penn and Smith, 2007). Being able to optimize the timing of new reproductive ventures would, therefore, be a valuable adaptation (Nepomnaschy et al., 2006). To test whether stress plays any role in regulating reproductive function in humans, Nepomnaschy and colleagues conducted research in a Mayan community in the highlands of southwest Guatemala. People in this community live under stringent conditions, enduring intervals of restricted food supply, associated threats of infectious diseases, and other seasonal, psychological, and environmental stressors. Longitudinal analyses of interview data and urine specimens collected continuously for a full year from this population uncovered several interesting relationships. First, these women's most important expressed concerns had to do with health issues affecting them or their immediate family and interpersonal problems (Nepomnaschy et al., in prep). Considering that in this population common health problems such as infant diarrhea, respiratory problems, and obstetric complications are not uncommon causes of death, and that in this society individuals are highly interdependent, the loss of a member of a woman's support network due to illness or conflict can carry with it serious practical, social, and economic consequences.

Importantly, women's self-reports of concerns were associated with elevated cortisol levels in their urine samples (Nepomnaschy et al., 2007). Cortisol is a key mediator in the body's response to a variety of psychosocial, energetic, and health challenges and is, therefore, frequently used as a marker of stress (Sapolsky, Romero, and Munck, 2000; Tilbrook, Tunner; and Clarke, 2000; Roberti, 2003). In turn, women's increases in cortisol levels were linked with changes in the profiles of the participants' reproductive hormones. Specifically, raised cortisol levels were associated with untimely increases in gonadotrophin and progestin levels during the follicular phase of the menstrual cycle. Increased cortisol levels were also associated with significantly lower progestin levels during the middle of the luteal phase (Nepomnaschy et al., 2004). These are important results because all of these hormonal changes have been previously found to negatively affect a female's chances to conceive (Baird et al., 1999; Ferin, 1999). Furthermore, during the first three weeks of gestation (the period in which the placenta begins to develop and becomes functional), pregnancy loss was almost three times higher in those women with increased cortisol levels (see Figure 16.1) (Nepomnaschy et al., 2006; compare Ellison et al., 2007).

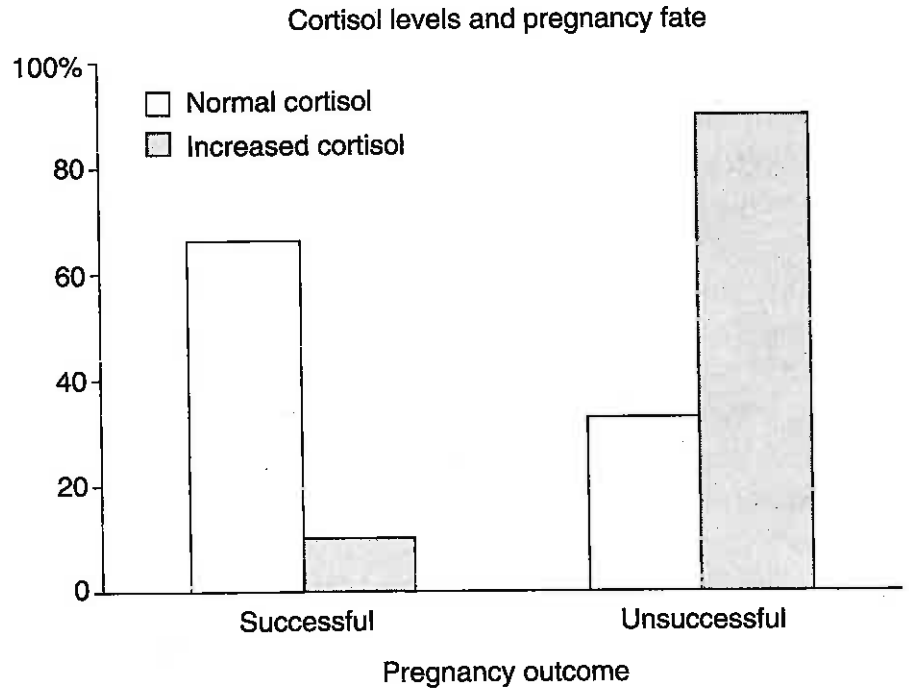

Figure 16.1 Cortisol levels and pregnancy outcome. Pregnancy outcomes stratified by average cortisol values between estimated time of ovulation and miscarriage or three weeks after ovulation, whichever came first. Pregnancies exposed to "high cortisol" were 2.7 times more likely to result in miscarriage *(unsuccessful)* than those exposed to "normal cortisol." Rao-Thomas $F(1,16)=3.4$; $p=0.03$.

In sum, what Nepomnaschy and colleagues uncovered was a connection between a woman expressing concerns, stress axis activation, and reproductive suppression. They argue that this connection could have an adaptive value: in unfavorable circumstances, avoiding or interrupting reproduction allows females to focus scarce resources on survival, improvement in overall condition, and investment in existing offspring (Nepomnaschy et al., 2004; Nepomnaschy et. al., 2006).

While the mechanisms described above may reduce a woman's chances to conceive or increase her chances of early spontaneous abortion, their effectiveness may decrease as her fetus begins to develop. After conception a fetus becomes an actor in determining the fate of its own gestation; yet mother and fetus may have different interests. Each fetus is a genetically unique entity, with only one opportunity to be born. Being alive is a prerequisite to achieving any other goals, and thus being born should be a fetus's first priority. Here is where conflicts of interest with the mother may arise. There will be conditions under which the mother would benefit from the interruption of gestation, and the fetus would benefit from promoting its continuation. These conflicts of interest might help explain why, within just hours after fertilization, embryos begin secreting a battery of metabolites that reduce the risk of miscarriage. In line with this argument Nepomnaschy and colleagues (2006) recently suggested that maternally derived abortive mechanisms may lose efficiency as the fetus progressively gains physiologic control of its own gestation. In other words, the "older" a fetus gets, the more capable it should be of surviving periods of maternal stress.

The Cost of Living: Consequences of Prenatal Stress

Whether the abortifacient effects of stress diminish as the fetus develops remains to be tested. A number of studies, however, suggest that pregnancies can continue despite acute stressors deriving from challenges experienced after placentation has taken place (for recent reviews, see de Kloet et al., 2005; Kaiser and Sachser, 2005; Murphy et al., 2006). Surviving stressful maternal conditions, though, can have serious physiologic and behavioral consequences for the fetus and lead to important variations in the resulting postnatal phenotype.

Alternative Phenotypes

Waddington (1959) termed the mysteries of the translation of information from genetic materials during development to the phenotype as the "great gap in biology." Ontogeny is an astonishingly complex process. All

living organisms have significant portions of their genomes and epigenomes that perform developmental "regulatory" functions, turning some genes off and others on during development. These regulatory "switches" are attuned to environmental conditions in ways that can result in phenotypic modifications.

The putative objective of developmental plasticity is to modify the phenotype so as to better meet present and future challenges. In terms of the future, the key problem is predictability. How reliable are the cues that are used to assess future contingencies? The difficulty of prediction increases with distances in time and space. Early preparation, however, allows for more specialization and economy of development. The sooner one can adjust development to fit future environments, the better; in this way resources need not be wasted covering other options. The balance between predictability and specialization during development influences the ontogenetic trajectories of phenotypic plasticity. "Critical periods" for environmental input involve this inherent temporal trade-off between the reliability of cues and the advantages of earlier specialization. In terms of development of the stress axis and all its associated phenotypic traits, gestation appears to be one of those critical periods.

Stress and Fetal Development

Experiments conducted in nonhuman mammals, mainly rodents and primates, suggest that prenatal stress can result in low birth weight and delayed physical growth postnatally and can affect motor and cognitive development. Significantly, one of the important consequences of prenatal stress appears to be a "reprogramming" of the hypothalamic-pituitary-adrenal (HPA) axis basal function and regulation (de Kloet et al., 2005). Animals experimentally exposed to prenatal stress show a shift in their circadian cortisol secretion profile (Koehl et al., 1999) and increased basal cortisol and adrenocorticotropic hormone (ACTH) levels (for example, Schneider, 1992; Weinstock et al., 1992; Clarke et al., 1994; Weinstock, 1997; Welberg, Seckl, and Holmes, 2000; Buitelaar et al., 2003). When prenataly stressed rats are postnatally challenged, they present a faster and stronger physiologic response to stressors than controls (Fride et al., 1986; McCormick, 1995). Furthermore, these rats also take longer to recover and to habituate to stressors (Takahashi, Turner, and Kalin, 1992). In line with these observations, prenatally stressed animals tend to have higher plasma glucose levels than controls (Vallée et al., 1997). Prenatally stressed animals also present fewer hippocampal glucocorticoid and mineralocorticoid receptors (Szuran et al., 2000; Weinstock, 2005).

Neurophysiologic changes observed in prenatally stressed animals have clear behavioral correlates. Animals experimentally subjected to stress during pregnancy display increased anxiety, shorter attention spans, and depressive-like symptoms such as learned helplessness and anhedonia. They also differ from controls in their behavioral strategies when facing novel situations such as the inclusion of new individuals in their social group, physical changes to their environment, or when exposed to an open field (for example, Frye and Wawrzycki, 2003; Morley-Fletcher et al., 2003).

Although human studies have been logically more limited in scope and design, available reports are consistent with what has been observed in animal experiments. Prenatal stress in humans has been linked with premature delivery; low birth weight for gestational age (Hedegaard et al., 1996; Wadhwa et al., 1998; Ruiz et al., 2002; Rondo et al., 2003); small head circumferences (Lou et al., 1994; Buitelaar et al., 2003); and variations in the rates of physical, mental, and motor development (Gluckman, Hanson, and Beedle, 2007). Importantly, in humans prenatal stress appears to also affect HPA axis regulation (example, Andrews and Matthews, 2004; Glover et al., 2004; Halligan et al., 2004). Gitau and colleagues (2001), for example, report that piercing of the fetal trunk around the time of pituitary maturation (eighteenth gestational week) leads to detectable increases of fetal beta-endorphin levels and that the same challenge two weeks later, when the adrenal glands mature, leads to increases in fetal cortisol. The effects of prenatal stress on the HPA axis can also be observed postnatally. Field and colleagues (2004) compared depressed and nondepressed pregnant women and their children as they develop postnatally. They report a strong association between the cortisol, catecholamines, and serotonin levels in their mother-children dyads. Similarly, Gutteling, de Weerth, and Buitelaar (2004) report that maternal cortisol during gestation and pregnancy-related fears were positively associated with their children's cortisol levels before and after being vaccinated at ages four to six and during the first days of the school year (Gutteling, de Weerth, and Buitelaar, 2005). In that study, prenataly stressed children also presented steeper circadian slopes.

Even while still in utero, stress exposure may affect the fetus's neurological development and its ability to habituate to challenges. Maternal stress levels during the third trimester have been associated with changes in fetal heart rate after vibroacoustic stimulation (Sandman et al., 1999). The behavioral effects of prenatal stress in the postnatal phenotype appear to be broad. Prenatally stressed children have been reported to present an increased tendency to depression, attention deficits (Buitelaar

et al., 2003), difficult temperament (Van den Bergh, 1992), emotional problems, hyperactivity (Linnet et al., 2003; O'Connor, Heron, et al., 2003), and difficulties in adapting to novel situations (Van den Bergh, 1992). Furthermore, prenatal stress has been linked to schizophrenia (Van Os and Selten, 1998; Hultman et al., 1999; Imamura et al., 1999; Watson et al., 1999; Koenig, Kirkpatrick, and Lee, 2002), criminal behavior, autism, and social withdrawal (Huttunen and Niskanen, 1978; Meijer, 1986; McIntosh, Mulkins, and Dean, 1995; Schneider, Moore, and Kraemer, 2004).

There are also studies that do not find the purported associations between prenatal stress and changes in HPA functioning, motor and cognitive development, and postnatal behavior. For example, while individuals prenatally exposed to the Dutch famine (1944–1945) show higher salivary cortisol baselines than controls who were not exposed, response to psychological stress appears not to differ between the two (de Rooij et al., 2006). Furthermore, there are studies that report positive effects of moderate gestational stress on development. After studying a group of healthy women, for example, DiPietro and colleagues (2006) found that mild maternal anxiety and depression were associated with enhanced motor maturation in two-year-old children.

These inconsistencies may be related to the broad range of differences in design between studies and to the multiple limitations affecting human research on this subject. Furthermore, these inconsistencies confirm that the relationship between prenatal stress and fetal programming is complex and highlight how little we still know about this phenomenon.

Mechanisms Mediating Maternal Effects on Fetal Development

The pathways through which prenatal stress may exert the effects described above are also poorly understood (Huizink, Mulder, and Buitelaar, 2004; de Weerth and Buitelaar, 2005). Glucocorticoids are logically suspected to be involved. Maternal cortisol levels are known to gradually increase during pregnancy. A placental physiologic barrier, however, keeps fetal cortisol levels 5 to 10 times lower than in the mother (Predline et al., 1979; Gitau et al., 1998). The placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) inactivates maternal cortisol by metabolizing it into cortisone before it crosses the placenta (Brown et al., 1993). Nonetheless, some maternal cortisol (about 10%) is believed to cross the placenta as cortisol (Gitau et al., 1998). Given the difference in basal levels between mother and fetus, even small amounts of maternal cortisol can cause significant changes in fetal physiology (de Weerth and Buitelaar, 2005).

Direct exposure to cortisol increases have been reported to affect fetal growth (Banks et al., 1999), birth weight (Bloom et al., 2001), head circumference (French et al., 1999), HPA axis functioning (Gitau et al., 1998), coronary function (Rotmensch et al., 1999; Subtil et al., 2003), and gastric function (Chin, Brodsky, and Bhandari, 2003). Stress metabolites may have additional effects on fetal development through less direct pathways. Placental production of corticotropin-releasing hormone (CRH) has been reported to increase with maternal stress (de Weerth and Buitelaar, 2005). CRH enters fetal circulation and can influence the development, distribution, and abundance of glucocorticoid receptors in the fetus's central nervous system (Meaney et al., 1996; Majzoub and Karalis, 1999). Increases in glucocorticoids and catecholamines can reduce uterine blood flow, which, in turn, may restrict fetal growth and contribute to fetal hypoxia. This mechanism has been proposed as a potential explanation for the link between prenatal stress, low birth weight, prematurity, and poor neurologic outcomes (Teixeira, Fisk, and Glover, 1999; Schneider, Moore, and Kraemer, 2004). Furthermore, prematurity and low birth weight have been linked with increased HPA reactivity in the adult (Phillips et al., 2000; Reynolds et al., 2001; Kajantie et al., 2002; Kajantie et al., 2003).

Another important factor to consider is interindividual variability in stress responses between mothers. There is evidence suggesting that pregnant women differ in their physiologic responses to stress, and that may differentially affect their fetuses' development (de Weerth and Buitelaar, 2005; Murphy et al., 2006). Furthermore, how much cortisol reaches the fetus is not only a function of the mothers' circulating levels of this glucocorticoid; it is also a function of the activity levels of placental 11β-HSD2. Placental 11β-HSD2 activity also varies between women and within each individual according to gestational stage, protein content of diet, oxygen levels, and hormonal levels related to placental function such as estradiol, progesterone, prostaglandins, and catecholamines (Welberg, Seckl, and Holmes, 2000; Bertram et al., 2001). Variations in placental 11β-HSD2 activity levels have been linked to changes in fetal HPA development; fetal growth; survivorship; birth weight; and adult coronary, renal, and hepatic functions (Murphy et al., 2006). Animal experiments suggest that alterations in placental function involving changes in the levels of prostaglandins, pro-opiomelanocortin (POMC), progesterone, and 11β-HSD2 activity may mediate some of the effects stress has on fetal development (Bloomfield et al., 2004).

Prenatal stress may exert its effects through a variety of other genetic, epigenetic, and ontogenetic pathways. Fetal development depends on the

synchronized occurrence of a large number of complex processes. Stress-related alterations of any of those processes or their tempos are likely to have an effect on the final outcome. At the neurological level, for example, disruptions of cell differentiation, migration, positioning, and connection appear to be linked to outcomes such as dyslexia, schizophrenia, and mental retardation (Watson et al., 1999). Finally, fetal factors are also likely to play an important role in any pathway explaining the relationship between gestational stress and fetal development (Murphy et al., 2006).

Understanding the multiple mechanisms through which prenatal stress affects fetal development and the resulting postnatal phenotype is a complex task that will require high-quality longitudinal research and a lot of patience. Future studies will need to be thorough in assessing the various factors that might be mediating the observed relationships. It would be important, for example, to (1) explore the genetic heritability of HPA function from mothers to children, (2) separate the influence of the maternal HPA functioning from stress-related behaviors (such as alcohol or caffeine consumption), and (3) assess the differential effects of prenatal stress on HPA functioning at the different stages of fetal development.

Postnatal Effects of Stress on Development

Adjustments to developmental trajectories begin with the fetus's adaptation to the maternal uterine environment and continue throughout infancy and childhood. There are, however, important differences in developmental responses to the pre- and postnatal environments. After birth, children have much more information available for phenotypic adjustments. They can see, hear, feel, taste, and smell the environment to a much greater extent. They become active participants in the social network, with new opportunities to influence the actions of others via communication. Compared with other social species, human infants are mentally precocial and experience a lengthy childhood and adolescence (Bogin, 1999; Bjorklund and Pellegrini, 2002). The evolutionary point of this costly extension of the juvenile period may be related to the development of a social brain that will have to master complex dynamic tasks such as learning the personalities and social biases of peers and adults and developing appropriate emotional responses to them (R. Alexander, 1990; Flinn, 2004, 2006a; Flinn and Coe, 2007).

Parents and other kin may be especially important for the child's mental development of social and cultural maps because they can be relied on

as landmarks that provide relatively honest information. Human mothers appear to have especially important roles in the development of their offsprings' sociocognitive development (Deater-Deckard, Atzaba-Poria, and Pike, 2004). Learning, practice, and experience are imperative for social success. The link between physiologic stress and responses to it may guide both the acute and long-term neurological plasticity necessary for adapting to the dynamic aspects of human sociality.

Psychosocial Stress and the Development of the Human Child

Natural selection appears to have favored the development of links between the neuropsychological mechanisms involved with assessment of the social environment and the neuroendocrine mechanisms that regulate stress response. Social challenges can trigger the activation of the HPA axis (Kirschbaum and Hellhammer, 1994; Gunnar, Bruce, and Donzella, 2000; Dickerson and Kemeny, 2004; Flinn, 2006b). The link between emotional domains and the stress axis may help manage the direction of mental processes to solving specific problems. For example, when dealing with the threat of an approaching bully, a child needs to allocate her cognitive efforts to the task at hand: prepare for immediate contingencies by recalling salient information, enhancing relevant sensory input, and activating circuits for appropriate actions.

ONTOGENY: EARLY POST NATAL TRAUMA → HPA DYSFUNCTION HYPOTHESIS

> [T]he development of individual differences in behavioral and neuroendocrine responses to stress can be influenced by events occurring at multiple stages in development. (Francis, Diorio, et al., 2002, 7843)

As discussed in previous sections, early experiences can have profound and permanent effects on HPA regulation and stress response (Suomi, 1997; Meaney, 2001; Maccari et al., 2003; Cameron et al., 2005; cf. Levine, 2005). Research on the developmental pathways has targeted the homeostatic mechanisms of the HPA system, which appear sensitive to exposure to high levels of glucocorticoids and CRH during ontogeny. Glucocorticoid receptors (GRs) and neurons in the hippocampus, which are part of the negative feedback loop regulating release of CRH and ACTH, can be damaged by the neurotoxic levels of cortisol or CRH associated with traumatic events (Sapolsky, 1990a, 2005). Hence early trauma is posited to result in permanent HPA dysregulation and hypercortisolemia, with consequent deleterious effects on the hippocampus, thymus, and other key neural, metabolic, and immune system components (Mirescu, Peters, and

Gould, 2004; Zhang et al., 2004). In primates these effects have additional consequences resulting from a high density of GRs in the prefrontal cortex (de Kloet, Oitzl, and Joels, 1999; Patel et al., 2000; Sanchez et al., 2000).

Finer-grained analysis of the epigenetic mechanisms involved with maternal effects on glucocorticoid negative feedback on CRH release indicates that DNA methylation affects hippocampal GR exon 17 promoter activity (Weaver, Diorio, et al., 2004). The permanence of DNA methylation, set during a sensitive period in the first week after birth in the rat, is a mechanism connecting diminished maternal care (licking, grooming, and arched-back nursing) with long-term elevations of HPA stress response. While the relationship between early trauma and variation in HPA development in humans has not been as well documented as in animal studies, similar effects and intervening mechanisms appear plausible (for example, Heim et al., 2000; Essex et al., 2002; Heim et al., 2002; O'Connor, Heron, et al., 2003; Teicher et al., 2003; Lupien et al., 2005).

Adaptive Phenotypic Plasticity: Programming the Limbic System and Neocortex

Neuroendocrine stress response may guide adaptive neural reorganization, such as enhancing predator detection and avoidance mechanisms (LeDoux, 2000; Meaney, 2001; Dal Zatto, Marti, and Armario, 2003; Wiedenmayer, 2004; Buwalda et al., 2005; Rodríguez Manzanares et al., 2005). Exposure to cats can have long-term effects on the central amygdala (right side) in mice, resulting in increased fear sensitization (Ademec, Blundell, and Burton, 2005). The potential evolutionary advantages of this neural phenotypic plasticity are apparent (Rodriguez Manzanares et al., 2005). Prey benefit from adjusting alertness to match the level of risk from predators in their environments. Post-traumatic stress disorder (PTSD) appears analogous to these fear-conditioning models and involves similar effects of noradrenergic (Pitman et al., 2002) and glucocorticoid systems (Roozendaal, Quirarte, and McGaugh, 2002) on associative long-term potentiation (LTP) of the amygdala and other neurological structures that underlie the emotional state of fear.

Social defeat also affects the amygdala and hippocampus, but in different locations (Koolhaas et al., 1997; Bartolomucci et al., 2005), suggesting that neural remodeling and LTP are targeted and domain specific (for example, Rumpel, et al., 2005). Glucocorticoids, perhaps in combination with peptide hormones and catecholamines, appear to facilitate the targeting of domain-specific remodeling and LTP. The potentiating effects of cortisol on emotional memories and other socially salient information

may be of special significance in humans (Pitman, 1989; Fenker et al., 2005; Lupien et al., 2005; Jackson et al., 2006; Roelofs, et al., 2007). The neurological effects of stress response may underlie adaptation to short-term contingencies and guide long-term ontogenetic adjustments of emotional regulation and associated behavioral strategies.

If physiological stress response promotes adaptive modification of neural circuits in the limbic and higher associative centers that function to solve psychosocial problems (Huether et al., 1999), then the apparent paradox of psychosocial stress would be partly resolved. Temporary elevations of cortisol in response to social challenges could have advantageous developmental effects involving synaptogenesis and neural reorganization (Huether, 1996, 1998; Buchanan and Lovallo, 2001). Such changes may be useful and necessary for coping with the demands of an unpredictable and dynamic social environment. Elevating stress hormones in response to social challenges makes evolutionary sense if it enhances specific acute mental functions and helps guide cortical remodeling, including the neurological structures involved with emotional regulation.

Chronic destabilization of neuronal networks in the hippocampus or cerebral cortex, combined with enhanced fear circuits in the amygdala (for example, Bauer, Ledoux, and Nader, 2001; Phan et al., 2006), however, could result in apparently pathological conditions such as PTSD (Yehuda, 2002) and some types of depression (Preussner et al., 2005). Even normal (but rather novel) everyday stressors in modern societies, such as social discordance between what we desire and what we have (Dressler and Bindon, 2000), might generate some maladaptive HPA responses. Individual differences in perception, emotional control, rumination, reappraisal, self-esteem, and social support networks seem likely cofactors (Chisholm et al., 2005; Ellis et al., 2006).

Hypotheses Involving Ontogenetic Processes

Testing ideas about relations among physiological stress response, neural remodeling, and adaptation to the social environment is not a simple or easy task (for example, Pine et al., 2001). Cortisol can affect cognitive functioning and emotional states; and cognitive processing and emotional regulation can affect cortisol response, all in an ongoing ontogenetic dance. Teasing out the causes and effects in ontogenetic sequence requires sequential data on physiological response profiles, environmental context, and perception. Extensive research on hormonal stress response has been conducted in clinical, experimental, school, and work settings (for reviews, see Weiner, 1992; Stansbury and Gunnar, 1994;

Panter-Brick and Pollard, 1999; Dickerson and Kemeny, 2004). We, however, know relatively little about stress neuroendocrinology among children in normal, everyday ("naturalistic") environments, particularly in nonindustrial societies. Investigation of childhood stress and its effects on development has been hampered in the past by the lack of noninvasive techniques to measure stress hormones.

The development of immunoassay techniques for saliva samples presents new opportunities to research stress response in everyday life. Saliva is relatively easy to collect and store, especially under the adverse field conditions typical of naturalistic research settings (Ellison, 1988). Longitudinal monitoring of a child's daily activities, stress hormones, and psychological conditions provides a powerful research design for investigating naturally occurring stressors. Analyzing hormone levels from saliva can be a useful tool for examining the child's imperfect world and its developmental consequences, especially when accompanied by detailed ethnographic, medical, and psychological information. Unfortunately, we do not yet have field techniques for assessment of corresponding ontogenetic changes in the relevant neurological mechanisms.

Assessment of relations among psychosocial stressors, emotional states, hormonal stress response, and health during child development is complex, requiring (1) longitudinal monitoring of social environment, emotional expressions, hormone levels, immune measures, and health; (2) control of extraneous effects from physical activity, circadian rhythms, and food consumption; (3) knowledge of individual differences in temperament, experience, and perception; and (4) awareness of specific social and cultural contexts. Multidisciplinary research that integrates human biology, psychology, and ethnography is necessary for meeting these demands. Physiological and medical assessment in concert with ethnography and co-residence with children and their families can provide intimate, prospective, longitudinal information that is not feasible to collect in clinical studies.

The Dominica Child Stress Project

For the past 20 years (1988–present), Flinn and colleagues have conducted research on childhood stress and health in the community of Bwa Mawego, located on the east coast of Dominica. In this study, Flinn and colleagues use sequential longitudinal monitoring to assess children's physiological stress response to everyday events, including social challenges. Their analyses indicate that social challenges are important stressors, with an emphasis on the family environment as both a primary

source and a mediator of stressful stimuli (Flinn and England, 1995, 2003).

High-stress events (cortisol increases from 100% to 2,000%) most commonly involved trauma from family conflict or change (Flinn et al., 1996). Punishment, quarreling, and residence change substantially increased cortisol levels, whereas calm, affectionate contact was associated with diminished (–10% to –50%) cortisol levels. Of all cortisol values that were more than two standard deviations above mean levels (that is, indicative of substantial stress), 19.2% were temporally associated with traumatic family events (residence change of child or parent/caretaker, punishment, "shame," serious quarreling, and/or fighting) within a 24-hour period. In addition, 42.1% of traumatic family events were temporally associated with substantially elevated cortisol (that is, at least one of the saliva samples collected within 24 hours was greater than two standard deviations above mean levels).

There was considerable variability among children in cortisol response to family disturbances. Not all individuals had detectable changes in cortisol levels associated with family trauma. Some children had significantly elevated cortisol levels during some episodes of family trauma but not during others. Cortisol response is not a simple or uniform phenomenon. Numerous factors, including preceding events, habituation, specific individual histories, context, and temperament, might affect how children respond to particular situations. Nonetheless, traumatic family events were associated with elevated cortisol levels for all ages of children more than any other factor that we examined. These results suggest that family interactions were a critical psychosocial stressor in most children's lives, although the sample collection during periods of relatively intense family interaction (early morning and late afternoon) may have exaggerated this association.

Chronic elevations of cortisol levels are also most often associated with family difficulties. Children usually became habituated to stressful events, but absence of a parent often resulted in abnormal patterns of elevated and/or subnormal cortisol levels. Children living in families with high levels of marital conflict (observed and reported serious quarreling, fighting, residence absence) were more likely to have abnormal cortisol profiles than children living in more amiable families (Flinn and England, 2003). Long-term stress, however, may result in diminished cortisol response. In some cases, chronically stressed children had blunted response to physical activities that normally evoked cortisol elevation. Comparison of cortisol levels during "non-stressful" periods (no reported or observed crying, punishment, anxiety, residence change, family conflict, or

health problem during 24-hour period before saliva collection) indicates a striking reduction and, in many cases, reversal of the family environment–stress association (Flinn and England, 2003). Chronically stressed children sometimes had subnormal cortisol levels when they were not in stressful situations. For example, cortisol levels immediately after school (walking home from school) and during noncompetitive play were lower among some chronically stressed children. Some chronically stressed children appeared socially "tough" or withdrawn and exhibited little or no arousal to the novelty of the first few days of the saliva collection procedure. These subnormal profiles may be similar in some respects to those of individuals with PTSD (for example, Yehuda et al., 2005). The relation between cortisol and level of arousal or interest is also apparent in the high reactivity of both shy (introverted) and surgent (extroverted) children to some types of social challenges.

Although elevated cortisol levels in children are usually associated with negative affect such as fear, anxiety, and anger, events that involve excitement and positive affect also stimulate stress response. For example, cortisol levels on the day before Christmas were more than one standard deviation above normal, with some of the children from two-parent households and those with the most positive expectations having the highest cortisol. Cortisol response appears sensitive to social challenges with different affective states. Other studies further suggest that the cognitive effects of cortisol may vary with affective states, such as perceived social support (Ahnert et al., 2004; Quas, Baver, and Boyce, 2004). There are, also, some age and sex differences in cortisol profiles, but it is difficult to assess the extent to which this is a consequence of neurological differences (for example, Butler et al., 2005), physical maturation processes, or the different social environments experienced, for example, during adolescence as compared with early childhood (Flinn et al., 1996).

The emerging picture of HPA stress response in the naturalistic context from the Dominica study is a combination of physical exertion and metabolic demands, on the one hand, and sensitivity to social challenges, on the other, consistent with clinical and experimental studies. The results further suggest that family environments and their developmental sequelae of affiliation, attachment, and security are an especially important source and mediator of stressful social challenges for children, consistent with other sources (for example, Garmezy, 1983; Gottman and Katz, 1989; Hetherington, 2003a, 2003b; Dunn, 2004).

Children in the Bwa Mawego study who were exposed to the stress of hurricanes and political upheavals during infancy or in utero do not have

any apparent differences in cortisol profiles in comparison with children who were not exposed to such stressors. Children exposed to the stress of parental divorce, death, or abuse (hereafter *early family trauma,* or EFT), however, have significantly higher cortisol levels at age 10 than other children (Figure 16.2). Based on analogy with the nonhuman research discussed previously, two key factors could be involved: (1) diminished hippocampal GR functioning, resulting in less effective negative feedback regulation of cortisol levels; and (2) enhanced sensitivity to perceived social threats, mediated in part by emotional regulation. Cortisol increases in response to common activities such as eating meals, active play, and hard work (for example, carrying loads of wood to bay oil stills) among healthy children but within an hour or two returns to normal levels. If EFT has affected the negative feedback loop, then recovery to normal cortisol levels would be slower. Resumption of normal cortisol levels after physical stressors, however, is similar regardless of early experience of family trauma. In contrast, cortisol profiles following social stressors indicate that EFT children sustain elevated cortisol levels longer than non-EFT children (Figure 16.3). Hence, the enhanced HPA

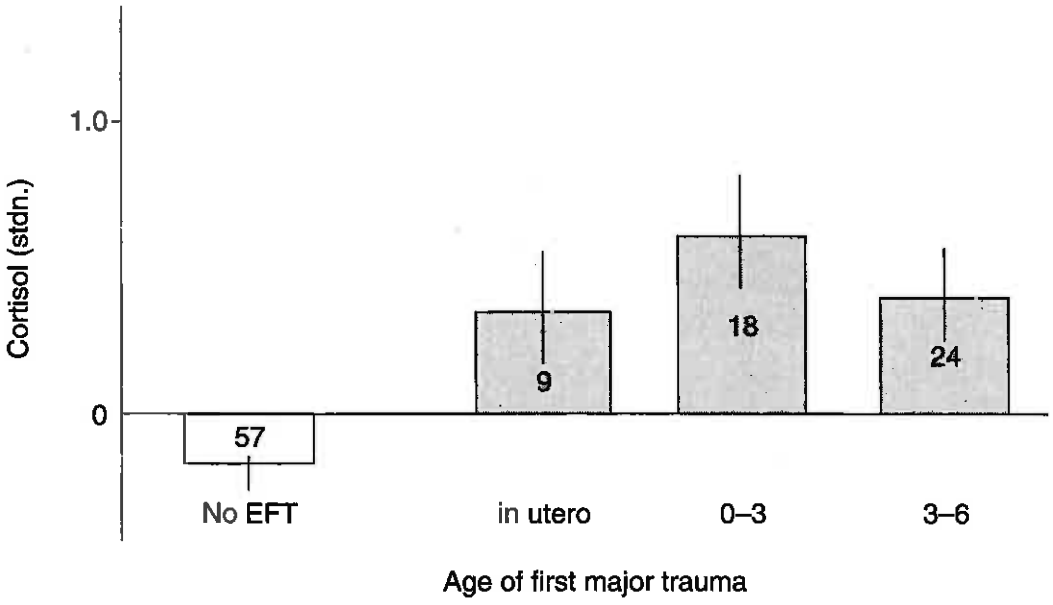

Figure 16.2 Early family trauma and cortisol at age 10 and older. Children exposed to early family trauma in utero or postnatally have higher average (means for each child) cortisol levels at ages 10 and above than children who were not exposed to early trauma (no EFT). Sample sizes (number of children) are in *bars*. *Vertical lines* represent 95% confidence intervals. Figure adapted from Flinn, 2006b.

stress response of children in this community who were exposed to EFT appears primarily focused on social challenges, suggesting that the ontogenetic effects of early trauma on stress response may be domain specific and even context specific. These results are consistent with studies of the effects of social defeat with nonhuman models (for example, Kaiser and Sachser, 2005).

Discussion and Concluding Remarks

Understanding the effects that early stress has on human ontogeny may have significant consequences for public health (Marmot, 2004; Flinn, 2007) because it could provide new insights into associations among stress response, social disparities, and perinatal programming, among other outcomes (Barker, 1998; Heim and Nemeroff, 2001; Maccari et al., 2003; Gluckman, Hanson, and Beedle, 2007). The analyses of the relationship between developmental stress and its postnatal consequences should, however, be guided by a more comprehensive theoretical framework than the one currently offered by the traditional medical sciences.

Many of the developmental consequences of stress are currently labeled *pathologies*. Pathologies, however, are usually understood as the result of malfunctions. In this chapter, we evaluated evidence suggesting that many of the consequences of stress may be the result of adaptive responses to developmental constraints rather than malfunctions. Several

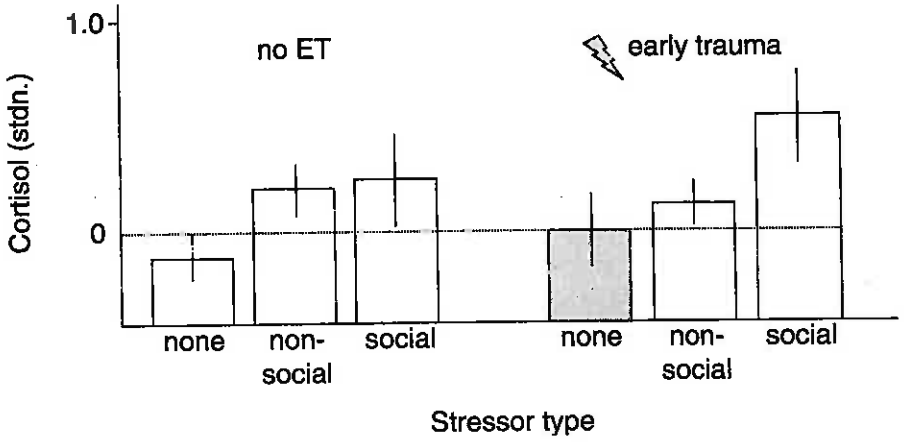

Figure 16.3 EFT and domain-specific stress response. Children exposed to EFT have higher cortisol levels in response to social stressors, but not nonsocial stressors, than no-EFT children. *Vertical lines* represent 95% confidence intervals. Figure adapted from Flinn, 2006b.

of these adaptations appear to have costly consequences later in life. True. But surviving is a prerequisite for everything else. Thus, while a depressive adult phenotype may not necessarily sound like a desirable outcome, from an evolutionary perspective a depressive adult is better than no adult at all. Furthermore, depression does not preclude reproduction and, within certain individual contexts, may actually enhance it.

The environmental constraints experienced by the developing fetus may contain important information regarding the world outside the uterus. Traits developed to face postnatal challenges may carry some costs but may be necessary for survival. The presence of these mechanisms in other species leads us to pose an obvious question: Do any of the so-called negative outcomes associated with prenatal stress in humans increase the carriers' chances of survival and reproduction? Appropriately answering this question in humans will require complex analyses of the true biologic costs and benefits of those "negative outcomes" across the entire life span of the individual, beginning from the moment of conception.

One of several challenges of such a project would be to control for both the postnatal environment of development and the environment in which individuals finally attempt reproduction. The postnatal environment of development continues to modify ontogeny as well as dictate some of the costs and benefits of the phenotypic changes triggered by prenatal stress. If individuals survive to reproductive age, the environments they face at that stage will dictate the final costs and benefits for their reproductive fitness of each particular trait affected by prenatal stress. Furthermore, as a species in which parental investment continues beyond the reproductive years (Hawkes et al., 1998), any stress-led phenotypic modifications that help the individual survive and reproduce but shorten postreproductive life may have a negative impact on inclusive fitness. One of several challenges of such a project would be to control for both the postnatal environment of development and the environment in which individuals finally attempt reproduction.

Nine months of gestation represents quite a short period compared to the length of the human life span, but information obtained during the prenatal period can still be critically relevant in the short and long terms. As discussed by Ellison (2005), certain aspects of the human environment and their related stressors are quite stable; others are not. Yet temporal domains of adaptive adjustment are not mutually exclusive. A mother's HPA axis could provide useful information to the fetus regarding both secular trends and abrupt changes. The mother's HPA baseline functioning, its reactivity, length of its refractory period, and other parameters may provide

"integrated" information about the stress landscape that the mother has faced over her lifetime. For example, despite the common perception to the contrary, socioeconomic level is highly inheritable (Duncan, Kalil, and Mayer, 2005). In turn, the socioeconomic status (SES) in which an individual is born is likely to affect the type, frequency, and length of many of the postnatal challenges an individual will face throughout the life span. Thus, in preparation for his or her future postnatal environment, it may be advantageous for the fetus to adjust the baseline functioning of its own HPA axis to that of its mother. Following a similar logic, sudden alterations to the mother's HPA baseline functioning (acute stress) could also indicate relevant changes in the conditions to be faced postnatally, and the fetus should benefit from adjusting its stress response to those changes as well. A simple example of this type of scenario could concern the loss of a contributing partner/father taking place during gestation. Such an event could trigger modifications in the mother's HPA functioning and also affect the prenatal and postnatal environments of development for the fetus. Again, neurophysiological changes that help the developing fetus to survive a fatherless gestation, first, and a fatherless childhood, second, should be positively selected. Whether the resulting adult phenotype enjoys his or her life or whether peers deem the individual a carrier of one pathology or another is irrelevant to the process of natural selection.

Some of the undesirable outcomes associated with stress may represent the unavoidable costs of adaptations that allowed the individual to survive exogenous challenges at some point during development or to be better adapted for the current environment. Labeling all stress outcomes as pathologies ignores the adaptive role of stress function, reduces our ability to achieve a complete understanding of the role stress plays in the unusual life history and ontogeny of the human fetus and child, and fails to help us curtail environments that lead to those undesirable outcomes.