

Early Life Experiences: Enduring Behavioral, Neurological and Endocrinological Consequences

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Glossary:

Adrenocorticotrophic hormone: (ACTH) a peptide hormone released from the anterior pituitary gland that mediates the production and secretion of hormones of the adrenal cortex.

Arginine Vasopressin: (AVP) a peptide hormone that participates in the release of stress-related hormones, such as ACTH.

Corticosterone: (CORT) a steroid hormone released by the adrenal cortex in response to stress.

Corticotropin-Releasing Hormone: (CRH) a peptide hormone that participates in the release of stress-related hormones, such as ACTH. This hormone is also referred to as corticotropin-releasing factor (CRF).

Glucocorticoid Receptor: (GR) a low affinity steroid hormone receptor for the corticoid steroids, such as CORT.

Hypothalamic-Pituitary-Adrenal Axis: (HPA Axis) the major neuroendocrine axis that mediates the hormonal stress response.

Mineralocorticoid Receptor: (MR) a high affinity steroid hormone receptor for the corticoid steroids, such as CORT.

Paraventricular Nucleus of the Hypothalamus: (PVN) a nucleus in the hypothalamus that contains CRH and AVP neurosecretory cells that regulate the release of stress-related hormones.

Synopsis:

Though individuals function in the present, we carry with us previous experiences that can fundamentally change how we respond physiologically and behaviorally to internal and external challenges. In this chapter, we highlight some classic and recent studies regarding how experiences neonatally and/or pubertally can influence later adult functioning. Specifically, we emphasize the role of early experience such as neonatal handling, novelty exposure, maternal deprivation and odor-shock conditioning on immediate and long-term emotionality and cognitive abilities. As the neonatal period is not the only developmental stage when individuals are susceptible to both positive and negative influences, we also discuss how exposure to stressors during adolescence modify later stress responsiveness and emotional behavior. Although it is clear that early life experiences can have many enduring neurobehavioral consequences, it is important to keep in mind that much work needs to be done. Given the importance of past experiences on the future health and development of an individual, a greater appreciation and understanding of early life experiences on enduring behavioral, neurological and endocrinological consequences remain vital.

1 INTRODUCTION

Though individuals function in the present, we carry with us previous experiences that can fundamentally change how we respond physiologically and behaviorally to internal and external challenges. Since the seminal work of Weininger (Weininger, 1954) on “gentling” and Levine (Levine, 1957) on infantile experiences in rats, an ever-growing body of literature has indicated that experiences early in development can have long-lasting effects on an individual’s physiological and behavioral potentials. In fact, some of these effects of early experience are so enduring that they can be trans-generational (Denenberg and Rosenberg, 1967). The purpose of the present chapter is to highlight some recent studies regarding how experiences neonatally and/or pubertally can modulate later adult functioning. Specifically, we emphasize the role of early experience such as neonatal handling, novelty exposure, maternal deprivation and odor-shock conditioning on immediate and long-term emotionality and cognitive abilities. As the neonatal period is not the only developmental stage when individuals are susceptible to both positive and negative influences, we also discuss how exposure to stressors during adolescence modify later stress responsiveness and emotional behavior. We conclude by briefly describing some provocative experiments that indicate experiences during puberty can offset or mitigate developmental insults that occur perinatally. These data indicate that at least some enduring consequences of early life experience remain malleable, even well into adolescence and adulthood.

The hormones released during stressful events appear to be a common thread in how early experiences during the neonatal or pubertal stage of development affect an individual’s immediate and long-term physiological and behavioral function. Thus, we

begin this chapter by briefly examining the hypothalamic-pituitary-adrenal (HPA) axis, the major neuroendocrine axis mediating the hormonal stress response (Herman and Cullinan, 1997; Herman *et al.*, 2003). Below, we discuss the components that comprise this axis and its neonatal and pubertal maturation.

2 HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

The release of stress-related hormones by the HPA axis is driven by a cascade of signals beginning with the release of the neuropeptides corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus (PVN). CRH and AVP are released into the hypophyseal portal system, which bring about the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH then stimulates the production and secretion of glucocorticoids (i.e., cortisol in primates and corticosterone in many rodent species) from the cortex of the adrenal gland. The hormones secreted by the HPA axis control their own secretion through a neuroendocrine negative feedback loop. That is, glucocorticoids feedback on the PVN and extrahypothalamic sites (e.g., pituitary, hippocampus and prefrontal cortex) to inhibit the further release of hypothalamic CRH and AVP (Herman *et al.*, 2003) (Figure 1) **<Figure 1 near here>**.

Two receptors mediate the actions of glucocorticoids in the central nervous system: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). These steroid receptors are found in relatively high concentrations throughout the neural-pituitary network that controls both negative feedback and activation of the HPA axis (Sapolsky *et al.*, 2000). The high affinity MR is typically saturated at basal

glucocorticoid levels, while the low affinity GR is primarily occupied only when elevated concentrations of glucocorticoids are present (de Kloet *et al.*, 1998). Thus, when glucocorticoid levels rise in response to stressors, the negative feedback on the HPA axis is primarily mediated by the GR (de Kloet *et al.*, 1998). However, the MR also appears to play a role in glucocorticoid-mediated negative feedback under mildly stressful conditions (Pace and Spencer, 2005).

The hormonal stress response is essential to survival as it allows an organism to cope with the internal and external demands imposed by a challenging event. This response attempts to restore the organism to homeostasis, a process termed allostasis (McEwen and Stellar, 1993). However, prolonged or more chronic exposures to stress and stress-induced hormones can lead to allostatic overload, resulting in a number of negative effects, particularly in regards to neurobiological and emotional function (Herbert *et al.*, 2006; McEwen, 2003; McEwen, 2004; McEwen and Stellar, 1993; Sapolsky, 1999; van Praag, 2004).

Both the magnitude and duration of the hormonal stress response change dramatically throughout an organism's lifespan. For instance, neonatal animals show reduced stress reactivity in response to stressors that typically elicit robust stress responses in adults (Sapolsky and Meaney, 1986). The reduced stress reactivity experienced by neonates has been posited to protect the developing organism from the negative influences of stress hormones (Sapolsky and Meaney, 1986). Conversely, aged animals show heightened and more prolonged stress responses compared to younger adults (Sapolsky, 1999). This has been proposed to contribute to the decline in neurophysiological and cognitive function observed during aging (Sapolsky, 1999;

Sapolsky *et al.*, 1985). Thus, parameters that change the responsiveness of the HPA axis, such as development, may have profound consequences on whether stressors lead to adaptive or maladaptive responses. The next section will briefly describe some of the major changes that occur in HPA function during neonatal and pubertal maturation.

2.1 Development of the HPA Axis

2.1.1 Neonatal development of the HPA axis

For the first two weeks of life, basal plasma corticosterone (CORT) concentrations are relatively low (Henning, 1978). In rats, these basal levels of CORT begin to increase around postnatal day (PND) 15 in both males and females, and peak around PND 24 (Henning, 1978; Meaney *et al.*, 1985c). As alluded to above, the neonatal stage of development is marked by a particularly striking change in HPA reactivity. That is, the ability of stressors to evoke CORT secretion in neonatal rats (PND 1-14) is greatly reduced compared to that in adults (Butte *et al.*, 1973; Cote and Yasumura, 1975; Guillet and Michaelson, 1978; Guillet *et al.*, 1980; Levine *et al.*, 1967). This period of reduced HPA responsiveness during neonatal development has been termed the “stress hypo-responsive period” (SHRP).

It appears that all levels of the HPA axis are involved in mediating the SHRP (Sapolsky and Meaney, 1986). At the level of the hypothalamus, CRH levels in the neonatal PVN are lower compared to those found in adults (Walker *et al.*, 1986a). At the level of the pituitary, CRH-induced ACTH release is reduced (Walker *et al.*, 1986a), in part due to increases in CORT-induced negative feedback on the neonatal pituitary

gland (Walker *et al.*, 1986b). At the level of the adrenal gland, the neonatal adrenal cortex shows reduced glucocorticoid output compared to adults (Martin *et al.*, 1977).

In addition to these changes in the HPA axis, extrahypothalamic areas involved in negative feedback, such as the hippocampus, also change substantially during neonatal development. Hippocampal GR levels increase during the first two weeks of life and stabilize at adult-like levels during puberty (Meaney *et al.*, 1985c; Meaney *et al.*, 1985d). In adulthood, hippocampal GR levels are regulated by the glucocorticoids such that higher levels of glucocorticoids down-regulate GR expression (Sapolsky *et al.*, 1984b). However, this auto-regulation does not occur neonatally (Meaney *et al.*, 1985c), presumably allowing GR levels to increase during later neonatal maturation independently of simultaneously rising glucocorticoid levels during this stage of development. Therefore, changes in GR levels in key modulatory sites of the HPA axis, such as the hippocampus, and possibly other extrahypothalamic sites, contribute to the neonatal maturation of the HPA axis and the emergence of stress responsiveness in the pubertal and adult animal.

The adaptive significance of the SHRP is presently unclear. It may protect the developing neonate from the negative influences of stress-related hormones. Indeed, neonates administered high levels of CORT show reduced brain volume and have fewer neurons than control animals (Balazs and Cotterrell, 1972; Cotterrell *et al.*, 1972). However, moderate exposure to CORT during this developmental stage may be beneficial in that neonates exposed to CORT via the dam's milk show superior performance on the Morris water maze task, a test of spatial memory (McCormick *et al.*,

2001). Future research will be needed to more fully understand and appreciate the significance of this hypo-responsive period in the neonate.

2.1.2 Pubertal development of the HPA axis

Though changes in the hypothalamic-pituitary-gonadal (HPG) axis have been well studied, the pubertal maturation of the HPA axis has recently begun to receive more experimental attention (reviewed in (McCormick and Mathews, 2007; Romeo, 2005)). It has been consistently observed that although basal and stress-induced ACTH and CORT secretion are similar in prepubertal (25-28 days of age) and adult (>65 days of age) animals, prepubertal animals have a significantly prolonged hormonal response compared to adults. Specifically, the ACTH and CORT (free and total) levels of either prepubertal male or female rats exposed to a single, acute physical and/or psychological stressor take at least 45 to 60 min longer to return to baseline compared to adults (Goldman *et al.*, 1973; Romeo *et al.*, 2006a; Romeo *et al.*, 2006b; Romeo *et al.*, 2004a; Romeo *et al.*, 2004b; Vazquez and Akil, 1993; Figure 2) **<Figure 2 near here>**.

Gonadal hormones are known to influence stress responsiveness in adult animals (Viau, 2002). However, the protracted stress responses observed in prepubertal males and females are not modulated by the presence or absence of gonadal hormones (Romeo *et al.*, 2004a; Romeo *et al.*, 2004b). Thus, this differential responsiveness before and after pubertal development appears to be independent of the influence of gonadal hormones on the HPA axis. It should be noted, however, that androgens can modulate HPA reactivity in mid-pubertal animals (40 days of age)

(Gomez *et al.*, 2004), suggesting that pubertal development does not have to be complete before gonadal hormones can modulate HPA responsiveness.

In addition to development, experience with stressors can also affect stress reactivity. For example, in adults, repeated exposure to a homotypic stressor leads to habituation of the stress response, such that peak stress hormone levels are blunted (Girotti *et al.*, 2006; Harris *et al.*, 2004; Helmreich *et al.*, 1997; Magarinos and McEwen, 1995; Marti and Armario, 1997). Interestingly, experience and pubertal maturation interact to affect HPA axis plasticity (Romeo *et al.*, 2006a). Specifically, in contrast to the extended response observed after acute stress, repeated stress (30min/day for 7 days) results in prepubertal males exhibiting a higher peak ACTH and CORT response immediately following the stressor, but a faster return to baseline, compared to adults (Romeo *et al.*, 2006a; Figure 3) **<Figure 3 near here>**. Mid-pubertal rats (40 days of age) exposed to a repeated homotypic stressor begin to show a diminished HPA response similar to adults (Gomez *et al.*, 2002). Therefore, it appears that within the adolescent window of development the HPA axis begins to react to homotypic stressors in a similar manner as adults. The physiological and neurobehavioral implications of this differential stress reactivity in adolescent and adult animals remain largely unknown.

It is unclear what mechanisms contribute to the pubertal change in stress responsiveness. Volumetric analyses of the PVN have not revealed any gross anatomical differences between the pubertal and adult PVN. Specifically, estimated volume, somal area and cell number in both the magnocellular and parvocellular aspects of the PVN are similar in prepubertal and adult animals (Romeo *et al.*, 2007).

Furthermore, peripheral injection of the retrograde tracer Fluoro-Gold reveals similar numbers of anterior pituitary projecting neurosecretory neurons in the parvocellular region of the PVN in both prepubertal and adult males (Romeo *et al.*, 2007).

Despite these similarities, it is interesting to note that CRH cells in the PVN show greater activation, as indexed by Fos immunohistochemistry, in prepubertal compared to adult animals in response to acute or repeated restraint stress (Romeo *et al.*, 2006a). There are also developmental changes in CRH mRNA expression in the PVN such that prepubertal males have greater basal CRH expression than adults (Romeo *et al.*, 2007). Though baseline levels of CRH mRNA are higher in juvenile animals, social stressors or restraint lead to increases in CRH expression in both the pubertal and adult PVN (McCormick *et al.*, 2006; Romeo *et al.*, 2007; Viau *et al.*, 2005). Together, it appears the basal and stress-induced regulation of CRH cells in the PVN may contribute to the differential HPA reactivity exhibited by adolescent and adult animals. However, it will be important to continue to investigate the role that possible differential release of CRH, sensitivity of the anterior pituitary to CRH (and other secretagogues such as AVP) and/or the sensitivity of the adrenal cortex to ACTH may play in these age-dependent differential responses.

Given the importance of the hippocampus on glucocorticoid-mediated negative feedback on the HPA axis (Herman *et al.*, 2003; Sapolsky *et al.*, 1984a), a few studies have assessed both baseline and stress-induced changes in hippocampal GR expression in adolescent animals. Meaney and colleagues (Meaney *et al.*, 1985c) have shown a slight decrease in GR concentrations in hippocampal homogenates in mid-pubertal (i.e., 35d) compared to adult (90-120d) males, while mRNA studies have found

no difference in hippocampal GR levels between prepubertal, mid-pubertal or adult animals (Romeo *et al.*, in press; Vazquez, 1998). Moreover, acute stress (30 min of restraint) decreases GR mRNA in the hippocampal formation in both prepubertal and adult males (Romeo *et al.*, in press). In general these data indicate that there are more similarities than differences in GR expression in the pubertal and adult hippocampus, and suggest hippocampal GR-mediated negative feedback contributes little to this robust change in HPA responsiveness exhibited by adolescent animals. However, GR-mediated negative feedback on the HPA axis can be mediated at other neural loci such as the medial prefrontal cortex and also at the pituitary gland (Herman and Cullinan, 1997; Herman *et al.*, 2003). It is also becoming apparent that, at least in adulthood, MRs play a role in glucocorticoid-mediated negative feedback under mildly stressful conditions (Pace and Spencer, 2005). Clearly, future studies will need to explore pubertal changes in GR and MR content and their regulation and function in the entire neural-pituitary network that mediates HPA reactivity.

3 NEONATAL EXPERIENCES AND ENDURING BEHAVIORAL, NEUROLOGICAL AND ENDOCRINOLOGICAL CONSEQUENCES

3.1 Individual Differences in the Development of the HPA Axis and Neonatal Experience

One of the most enduring findings concerning the development of the HPA axis is its modifiability or plasticity by a diverse range of environmental variables across different stages of development. This plasticity provides a source of individual differences in the regulation of the HPA stress response, which in turn, modulates a

wide range of psychological and physiological functions. Much progress has been made in terms of characterizing and documenting the specific experimental conditions or experiences that create specific behavioral, neural and endocrinological changes. However, utilizing recent methodological developments in neuroscience and behavioral sciences, the relationship among different experiential manipulations remain to be understood, inconsistent findings remain to be explained, and an unifying framework for integrating these diverse findings remain to be developed.

Two historically significant ideas appeared to be responsible for the development of the two most popular early experience manipulations: neonatal handling and maternal separation/deprivation paradigms. The first is the construct of stress introduced by Hans Selye. With the publication of his seminal work in *Nature* (Selye, 1936), Selye introduced what might have been the antecedent to a class of experimental paradigms in which the effects of stressful events were characterized in rodents. In an attempt to study effects of stress inflicted by electrical shock, Levine accidentally discovered something rather surprising (Levine, 1960), in that the bodily and behavioral responses of the rats to shocks, which also entails a certain amount of experimenter handling, was no different from that of the rats who were only handled but received no shock (handled group). Furthermore, in comparison to the control rats that just stayed in their home environment (referred to as non-handled rats), both groups showed reduce emotionality and a less sluggish HPA response to stressful events. This accidental discovery has led to the creation of a large body of literature built upon the manipulation referred to as neonatal handling (Daly, 1973; Denenberg, 1964; Denenberg, 1978; Levine, 1957; Meaney *et al.*, 1988).

The second is the idea that early mother-infant experiences influence adult outcomes. Although initially considered radical, convergence of data from both clinical observations and basic research gradually led to its acceptance. Specifically, astute clinical observations identified a link between disturbed maternal care or maternal separation and disturbed emotional and cognitive functioning that began in infancy and lasted into adolescence (Bowlby, 1951). Concurrent work by Harlow on infant monkeys separated from their mother appeared to mirror the strong emotional and physical stunting of orphaned infants (Harlow and Harlow, 1965). These data provided the foundation for early life experience related research with the unifying clinical and basic research theme that maternal care was critical for the normal development of infants. Because infant separation from the mother could be clearly defined and measured, it became an important variable for manipulating early life experience (Bowlby, 1951; Bowlby, 1969; Bowlby, 1982).

Over the past five decades, these two lines of research each independently revealed information that advanced our understanding of their very often opposite effects on HPA development and their functional consequences. Each research paradigm has its distinct advantages and faces its unique theoretical and practical challenges. The experimental procedure in neonatal handling involves assigning an entire litter, or rat family, to the handled and non-handled condition, and is relatively easy to carry out. It produces a set of effects on behavior, brain, and the HPA axis that are generally considered desirable (Levine, 1960; but see recent discussions of effect on reproductive behavior (Greisen *et al.*, 2005). The handling literature also faces major conceptual and interpretational difficulties that were initially pointed out over three

decades ago by Daly (1973). Using this procedure, it was impossible to tease apart several distinct, yet confounding factors, which differed between the handled and non-handled pups. With the exception of the effects on HPA function and measures of emotionality, handling effects on a variety of brain and behavioral functions could not be replicated.

Since then, research in neonatal handling appeared to have passed its golden days, but was revived by the elegant work of Meaney and colleagues showing parallel changes in hippocampal-dependent spatial learning, hippocampal cell counts, and a variety of HPA related measures (Meaney *et al.*, 1988). This work has led to new approaches in the understanding of how maternal care contributes to emotional and neuroendocrine development (Liu *et al.*, 1997). This work also motivated research efforts to directly address the difficult methodological issues raised in Daly's critical review (Daly, 1973). Specifically, an experimental paradigm was developed using a split-litter (within-family) design to better isolate the known confounding factors in neonatal handling (see Section 3.3 *Neonatal Novelty Exposure*; (Tang, 2001).

Another major line of research of maternal separation/deprivation involves exposing entire litters of pups to relatively prolonged periods of pup-dam separation (≥ 3 hrs). Different from the neonatal handling manipulation, maternal separation produces a set of effects that are generally considered negative, with a relatively clear correspondence to real world scenarios in child development. Consequently, practical implications of findings associated with this paradigm can be easily discussed. Today, while the rodent early experience research continues to rely on the maternal separation/deprivation paradigm, understanding the relatively subtler caregiver

behaviors and contrasting quantity and quality of maternal care have become increasingly important. This direction of research differs from the earlier maternal separation/deprivation work in their potential implications. While the maternal deprivation/separation literature provided mainly characterization of functional deficits, the maternal care literature offered insights into potential beneficial, or at least opposing, effects from those induced by maternal deprivation or separation.

The interest in the role of maternal care in early experience effects on development has been at least as old as the neonatal handling literature. The most notable hypothesis is the maternal mediation hypothesis (for review see; Macri and Wurbel, 2006). In its most extreme form, this hypothesis states that the effects of neonatal handling have nothing to do with handling-induced activation of the pup's HPA axis, but solely mediated by a handling effect on maternal behavior, which in turn resulted in changes in offspring development. Since then, many have categorized and quantified a variety of maternal behaviors and conducted carefully controlled studies in which the influence of maternal care on behavior, brain, and neuroendocrine function were determined (Meaney, 2001)

In the past decade, while some continue to focus on identifying more molecular markers within the brain that correlate with natural variations in the amount of specific maternal behaviors, such as licking and grooming (Weaver *et al.*, 2004), others have shown that early stimulation via handling can affect the offspring's brain and HPA axis in the absence of maternal behaviors (Denenberg, 1999). Additional situations have also been noted where effects of neonatal handling treatment, and other early stimulation manipulations, could not be explained solely by increased maternal care (Macri *et al.*,

2004; Macri and Wurbel, 2006; Tang *et al.*, 2006), and in some situations where an increase in maternal care was negatively associated with offspring development (Macri *et al.*, 2004). These recent observations suggest that a new theory of early experience other than maternal mediation is needed to provide a unifying explanation for how early experience and maternal influence jointly shape offspring behavioral, neural and endocrine development.

Following the two historical traditions of neonatal handling and maternal deprivation, we will discuss recent findings from studies using the neonatal novelty exposure and the odor-shocking conditioning paradigms to support an alternative theory of maternal modulation. Instead of affecting offspring development through maternal behavior, early stimulation exerts direct, independent effects on the offspring. Maternal variables modulate these direct stimulation effects, thereby jointly shaping offspring development.

3.2 Neonatal Handling

Behavioral, brain, and HPA changes as a result of neonatal handling have been reviewed throughout its history of the past half century. For comprehensive reviews, we would like to refer the readers to several manuscripts from various periods (Daly, 1973; Denenberg, 1964; Macri and Wurbel, 2006; Meaney *et al.*, 1996). Here, instead of reiterating previously documented findings, we will present the neonatal handling paradigm from a methodological point of view in order to present recent progress and to arrive at a modern interpretation of the neonatal handling literature.

3.2.1 Neonatal handling and behavior

Neonatal handling, also known as postnatal handling, is an early life behavioral manipulation with several defining features. First, it is a procedure carried out on all pups from an entire litter from a single birth. Specifically, an litter of pups are assigned to either the experimental group (handled) or the control group (non-handled). This feature determines that the litter is the unit of experimental manipulation and the results of such a manipulation provide information on between-litter differences in the offspring, but not within-litter differences (i.e., between-sibling differences). This feature further implies that pups within each litter might be highly correlated due to the fact that they share the same dam and home housing environment prior to weaning. Thus, to draw statistically sound conclusions, one needs to use litter as the unit of analysis, an exception to this rule, however, can be made if one fails to find sufficient strong within-litter correlation (equivalent to a non-significant litter effect). Many handling studies do not use appropriate statistical treatment of the data, and this might explain why some handling effects cannot be reliably reproduced.

Second, neonatal handling is a procedure involving multiple distinct procedural differences, including differences in experimenter handling, absence of the dam, absence of litter siblings, and experience of a relatively novel non-home environment. One consequence of having these multiple factors is that it is difficult, if not impossible, to pin point the precise cause of any observed difference between the experimental and control groups. In the case of a handling effect on spatial learning, improved learning is equally likely a result of maternal separation, touching by experimenters, or experiencing a relatively novel or unfamiliar environment, thus making it difficult to

determine how to use these research findings to optimize the early life environment to facilitate cognitive development. It would be a non-trivial mistake if one implements an intervention program by separating the infants from their mothers, or by having physical contact with strangers, when the true cause of improved learning is the early experience of novelty. This state of confusion is reflected by the fact that some researchers still use the phrase “maternal separation” to refer to the same handling manipulation. This implies a causal relation between maternal absence and the observed differences between the so-called handled and non-handled rats, even though carefully controlled experiments have demonstrated that exposure to a novel environment (Benetti *et al.*, 2007) and tactile stimulation (Jutapakdeegul *et al.*, 2003) can both contribute to developmental differences.

In terms of outcome assessment from this early environmental manipulation, the most reliable behavioral finding is a change in the activity of the offspring in an open field test (Denenberg, 1969). The specific parameters of the open field tests vary a great deal, including the number of days, number of trials, and the duration of trials. Regardless of these variations, the handling procedure appears to reliably produce some differences in open field activity. On the other hand, the interpretation of open field activity is less clear. Initially, an open field was used as a fear or anxiety inducing stimulus and it was reasoned that animals that were highly emotionally reactive to this novelty would show different levels of activity compared to animals showing relatively lower emotional reactivity. Multivariate analysis suggests that this activity measure may reflect several underlying psychological dimensions. Given this multiplicity of interpretation, open field activity may be better viewed as a hallmark measure to

indicate the presence of a neonatal handling effect than as the measure for a single clearly defined psychological construct.

In addition to the open field test, a number of behavioral paradigms have been used to examine a range of psychological functions during the past five decades, with most of the explorations concentrated in the 1950s to 1970s (Daly, 1973). Although these explorations utilized a variety of behavioral protocols designed to measure learning and memory and social functions, the conclusions reached by reviews at the end of that era were not optimistic. The effects of neonatal handling on these learning and memory measures and on social function measures were not replicated, and competing hypotheses existed concerning what was the critical underlying variable that controlled the direction and magnitude of the so-called handling effects. Unfortunately, this criticism was never seriously rebutted and perhaps through both collective forgetting and introduction of new investigative tools, the difficulties in interpretation were put aside.

The introduction of the Morris water task in the early 80s (Morris *et al.*, 1982) added a new behavioral assessment tool for detecting handling-induced changes in learning and memory. Combined with a cross-sectional investigation of aging effects and multileveled analyses across receptors, cell counts, and basal and evoked stress hormone concentrations, Meaney and colleagues rekindled the interest in the neonatal handling paradigm by demonstrating an age-specific handling effect on spatial learning near the end of life (Meaney *et al.*, 1988). Today, at the level of behavior, limitations on the number of replications available, range of functional assessment, and repeated measures across developmental stages within a given individual remain a concern.

These limitations need to be overcome before findings from this type of approach can provide any insights into our understanding of human cognitive and social development. Further, these shortcomings bear critical relevance to our interpretation of brain and neuroendocrine changes associated with measures of behavior.

3.2.2 Neonatal handling and brain

Although the handling procedure involves multiple components, one common feature of these components is that they all increase the novelty or reduce the familiarity of the pups' environment. The physical handling, the time spent in a non-home environment, the absence of the familiar dam and siblings can all contribute to a reduction in environmental familiarity or a relative increase in environmental novelty. As the most robust effects of neonatal handling have been changes in behaviorally measured emotional responses to novelty, we will focus the following review on key brain structures that are known to increase response to novelty.

We first consider the locus coeruleus (LC), the brain structure that contains norepinephrine (NE) neurons and generates the first neuromodulatory responses to novelty in the environment (McEwen and Sapolsky, 1995). The activation of these neurons leads to an increase in NE input to cortical and subcortical structures throughout the brain, which in turn directly affects neuronal excitability as well as synaptic plasticity. GABA_A receptor levels in the LC (Caldji *et al.*, 2000) and NE output from LC to the PVN (Liu *et al.*, 2000) are decreased by the neonatal handling procedure, whereas NE auto-receptors in the LC are increased by handling treatment (Liu *et al.*, 2000). This means that the LC of handled rats may be more capable of

providing local feedback control of its own activity via increased presence of auto-receptors, and possibly a reduced global inhibition via reduction in GABA_A receptors. Although it is difficult to determine the significance of reports of handling-induced reductions in the volume and neuronal number in LC (Lucion *et al.*, 2003), it does confirm that the LC may be an important neural substrate supporting the behavioral expressions of neonatal handling.

Aside from its role in processing spatial and emotional information, the hippocampus is an important structure for detecting novelty, such that novel objects or a novel context are known to increase hippocampal activation (Knight and Nakada, 1998; Kumaran and Maguire, 2007; Nyberg, 2005; Parkin, 1997). Interestingly, much of the investigation on the handling effects on the hippocampus have not dealt with detection or acute responses to novelty. Instead, the most replicated finding across several stages of development is an increase in GR function, long after the neonatal handling has occurred (Meaney and Aitken, 1985; Meaney *et al.*, 1985a; Meaney *et al.*, 1985b; Meaney *et al.*, 1988; Meaney *et al.*, 1989; O'Donnell *et al.*, 1994). This increase in hippocampal GR function is hypothesized to support a greater negative feedback control of the HPA axis via the inhibitory effects of GR binding on hippocampal neuronal excitability (Meaney *et al.*, 1989). While changes in hippocampal cell counts were found between the handled and non-handled rats, this difference was only present later in life (Meaney *et al.*, 1988), and thus, could not explain functional differences between the handled and non-handled rats prior to aging and senescence. An interesting exception to these data is the failure to generalize from the Long Evans hooded rats to the Lewis rats (Durand *et al.*, 1998). The latter is characterized by its high anxiety and hypo-

responsive HPA axis (Durand *et al.*, 1998). For this strain, no increases in hippocampal GR were induced by neonatal handling. Though this strain-specific effect was interpreted as suggesting an unspecified role of genetics (Durand *et al.*, 1998), it is possible that the handling effects on the hippocampus depend on the existing level of circulating CORT, which is different between these two strains.

As regions of the frontal cortex form direct connections with the hippocampus, it is not surprising that the frontal cortex also shows handling induced changes. Earlier studies showed a concurrent observation of increased GR function in the frontal cortex and the hippocampus (Meaney *et al.*, 1985b). This increase may allow the frontal cortex to participate in further feedback control via interaction within the frontal-limbic circuit (Herman *et al.*, 2003). More recently, basal single-unit activity in the mPFC of anesthetized rats was significantly increased by the handling treatment (Stevenson *et al.*, 2008). This change in background activity may support differential patterns of frontal activity in response to novelty. In the dorsal anterior cingulate cortex, a region involved in the perception and regulation of emotions, neonatal handling treatment increased dendritic spine density in layer III cortical neurons, suggesting a change in synaptic function (Helmeke *et al.*, 2001). Although relatively few studies have examined and replicated handling effects on frontal lobe function, these few studies offer converging evidence that neonatal handling-induced changes within the frontal cortex occur at multiple levels, such as receptor and synaptic density and single neuron activity.

Given the role of amygdala in emotional processing, it was somewhat surprising that initial reports regarding handling-induced changes in GR were not observed in the

amygdala of adult rats (Meaney *et al.*, 1985b). Later studies using different techniques revealed that GR expression was reduced in the central nucleus of the amygdala (CeA) in the handled pups as early as PND9, in comparison to the controls (Fenoglio *et al.*, 2004). This change in GR preceded an increase in GR expression in the hippocampus at PND23 (Avishai-Eliner *et al.*, 2001). It is important to note that the direction of change in GR expression is opposite in the amygdala and hippocampus. Therefore, the causes of handling-induced GR up- or down-regulation cannot be explained by changes in the circulating CORT concentration alone.

Furthermore, handling-induced changes in amygdala are not restricted to those that are directly affected by hormones of the endocrine system. Instead, it appears to involve all major neuromodulatory systems. Handled rats showed reduced levels of the mRNA for the gamma 2 subunit of the GABA_A receptor complex in the amygdaloid nuclei (Caldji *et al.*, 2000), and decreased levels of 5-HT, 5-HIAA, dopamine (DA) and NA (Arbroelius and Eklund, 2007). These observations suggest that amygdalar function in the handled rats may be affected via changes in multiple neuromodulatory systems. To account for both up- and down-regulation of GR in the amygdala and the hippocampus, to relate changes across multiple neuromodulatory systems, and more broadly, to explain these handling effects across multiple brain regions, a computational theory that takes into consideration the dynamic interaction between components of the stress circuit is needed.

3.2.3 Neonatal handling and endocrine function

The effects of neonatal handling on endocrine function have received much experimental attention. Our discussion will focus on CORT, while acknowledging that many studies have examined effects on CRH and ACTH secretion and various neuroendocrine manipulations of the HPA axis for the purpose of determining where within the axis handling-induced changes occurred.

Early studies using repeated electric shocks as a stressor showed that rats that experienced neonatal handling showed a faster rise and faster recovery to baseline in the evoked CORT response compared to control rats (Levine, 1960). This careful description of the stress-induced temporal response profile of CORT has been preserved in some, but not all, later studies. This inconsistent practice is reflected in the use of phrases in some studies, such as “handling decreasing corticosterone” or “handling facilitating corticosterone recovery” without referencing when the measure was taken relative to the presence of the stressor. The most robust and perhaps most often replicated finding through the past five decades has been the faster return of CORT to baseline, while the effects of handling on basal levels of CORT, the rate of initial rise and peak CORT levels appeared to be inconsistent, or at least lack consistent replication.

This distinction between CORT measures obtained at different times relative to a stressor is critical, as CORT can play different roles prior to, upon the onset, and during the short or long presence of a stressor. While it may be considered adaptive to mount a fast initial response to a stressor whose level of threat is currently unknown, it would be maladaptive to sustain a high level of CORT output when the stressor is later judged

of little threat (McEwen, 2007). At the level of neuronal activation, the initial fast rise will mainly increase neuronal excitability via the MRs, while a sustained high level of CORT output will reduce neuronal excitability via the GRs (de Kloet *et al.*, 1999; Joels *et al.*, 2006). Hence, the shape of the CORT response profile may determine the balance of excitation and inhibition within neural circuits affecting functions supported by regions of the brain containing MRs and GRs, particularly the hippocampus and frontal cortex (McEwen *et al.*, 1968). To understand how early stimulation induced changes in endocrine function can be linked to functional changes at the level of behavior, it is critical to make necessary methodological improvement such that the effects on basal CORT levels and the initial rise of CORT can be reliably obtained and replicated.

3.3 Neonatal Novelty Exposure

In studies that use the handling procedure, brief maternal separation, maternal stress, experimenter handling, and experience of a relatively novel non-home physical environment may all contribute to the effects of the neonatal handling treatment. It is possible to view all these components as environmental manipulations that increase novelty, surprise, or uncertainty of the pups' environment, or decrease the environmental familiarity in comparison to the background familiar home environment. In this sense, it is possible to view these different components simply as different cases of increased novelty. If we consider a change in environmental novelty or familiarity as the essence of the neonatal handling manipulation, then it might be possible to induce the hallmark changes in emotional reactivity and neuroendocrine function by manipulating environmental novelty via the exposure to a novel physical environment

alone. To do so, the procedure of neonatal novelty exposure was introduced (Tang, 2001).

Neonatal novelty exposure is a procedure performed during the first 3 weeks of life during which half of randomly selected pups from each litter are exposed to a novel cage for 3 min a day (Novel) while the remaining half of the litter stays in the home cage (Home; Figure 4) **<Figure 4 near here>**. During this procedure, the dam is first removed from the home cage and returned only after differential treatment of the Novel and Home pups have been completed. This insures that both the Novel and Home pups are separated from their mother for the same brief duration (< 15 min). The Novel pups are individually transferred from the home cage into their own relatively novel environment consisting of a freshly cleaned small plastic cage lined with bedding similar to the home cage, while Home pups are picked up and put back down into the home cage at approximately the same time. This insures that Novel and Home pups receive the same amount of experimenter handling. As pups within each litter are pseudo-randomly assigned to the Novel and Home treatments, the genetic make up of the Novel and Home pups do not differ systematically. All of these cautions in experimental design are essential for ruling out other factors that might confound the exposure to a novel environment, such as differences in maternal separation, experimenter handling, and individual differences in maternal stress reactivity.

It should be pointed out that different from the majority of the handling studies in which animals are tested only once at one particular age, novelty exposure effects have been demonstrated repeatedly in the same individual at multiple points throughout development. For instance, the earliest novelty effects have been observed at 4 weeks

of age and others as late as 26 months of age. Only with testing of the same individuals at multiple points in development can one give practically meaningful answers to questions concerning the persistence of early life experiences.

3.3.1 Neonatal novelty exposure and behavior

Effects of neonatal novelty exposure on behavior have been investigated across several functionally distinct psychological domains, including emotional response to novelty, learning and memory, and social interaction. Importantly, these behavioral tests involve not only negative reinforcement but positive as well. Different from the handling procedure, findings from neonatal novelty exposure provide unique opportunities for investigating the environmental origin of individual differences within the same family (i.e., between sibling differences), and shed light on why siblings can be so different, even though they appear to be raised in the same family environment.

Rats that experience neonatal novelty exposure differ in several measures of behavior in an open-field compared to rats that stay in their home cages. For instance, Novel rats show a shorter duration of initial freezing upon entering the open field (Tang, 2001), and their activity levels show a greater initial increase across two brief (20 s) trials of exposure (Reeb *et al.*, 2007). Both observations suggest that Novel rats are faster to recover from the initial behavioral inhibition induced by the unfamiliar environment. When exposed to an odor that the rats have never experienced before, Novel rats show shorter approach latencies and higher frequencies of exploration, again suggesting that Novel rats are faster in recovering from behavioral inhibition induced by a novel odor (Yang *et al.*, 2008).

As emotional states are known to influence cognitive processes, one may predict differential performance in learning and memory tasks that inevitably involve varying degrees of novelty. When tested in a working memory version of the Morris water maze task, in which the rats must locate a hidden platform in the water, Novel rats show significantly greater working memory (Reeb *et al.*, 2007; Tang, 2001; Tang *et al.*, 2006). Specifically, this is indexed by a greater reduction in the time to locate the hidden platform after only a single trial exposure to the location of the hidden platform (Reeb *et al.*, 2007; Tang, 2001; Tang *et al.*, 2006). The effects of novelty exposure on working memory can be eliminated by increased familiarity via repeated swim trials involving identical platform location and reinstated by an increase in novelty by testing the rats again after a prolonged period of delay (e.g. several months; (Tang, 2001).

In contrast to learning in the Morris water task, which involves cold water as a negative reinforcer, learning and memory in an odor discrimination learning task that involves sweets as positive reinforcers also differ between the Novel and Home rats (Tang, 2001). Distinct from the findings from negative reinforcement learning, Novel and Home rats show similar performance in their initial learning of the discrimination task in which they learn that one of the two odors is associated with a sweet reward (Tang, 2001). It is only during a retention test conducted six days after the last day of training that Novel rats show complete retention of the task (but the Home rats show significant forgetting). Furthermore, when these rats are tested for reversal learning, which clearly introduces surprise to the testing situation, Novel rats are faster than Home rats at acquiring the new stimulus-reward relationship (i.e., reversing to the previously non-rewarding odor; Tang, *et al.*, unpublished observation).

In the domain of social functions, the effects of neonatal novelty exposure have been found in aggressive behaviors, social competition, social recognition memory and social engagement. Although biting occurred infrequently during free dyadic interactions between a Novel and Home rat, the Home rats bit the Novel rats twice as often as the Novel rats bit the Home rats (Reeb and Tang; unpublished observation). This observation is consistent with the interpretation that Home rats are more likely to perceive threats than Novel rats. When meeting another rat 24h after their initial interaction, Novel rats show a greater habituation than the Home rats in the frequency of social investigation, and this habituation is blocked among the Novel rats only by inserting a meeting with another “stranger” rat between the initial interaction and an interaction taking place 24h later. These findings suggest that Novel rats have better memory of the previously encounter conspecific than the Home rats (Tang *et al.*, 2003a). It is possible that the Home rats are more fearful during their initial social interaction and that this fear contributes to their impaired 24h memory for the previously encountered conspecific.

Similar to the acquisition of the odor-reward association, learning to retrieve a chocolate reward in a testing cage does not differ between the Novel and Home rats. Only when placed in a competitive situation, where only one of the two rats could gain access to a reward (chocolate drops), Novel rats win the competition more frequently than the Home rats (Tang *et al.*, 2006). When the rats are tested in competition without food deprivation, thus with lower levels of stress in general, this greater competitive ability is present only during the initial encounters (first day) and disappears during the later encounters (2nd day). The presence of this novelty effect when the testing situation

is novel may once again reflect a difference in perceived threat between the Novel and Home rats when facing the surprise of seeing another rat.

During free interaction, a greater proportion of Novel rats' social initiations are reciprocated by the Home rats, suggesting that the Novel rats are more able to engage the Home rats. Furthermore, this differential ability in social engagement is found only upon the pair's initial encounter and disappears at the second session of interaction 5 min later, and can be reinstated by priming the rats with a surprise event---spending 2 min in a plastic bottle immediately before social interaction (Tang *et al.*, unpublished observation).

Together these findings demonstrate the impact of early environmental differences in novelty on a wide range of psychological functions and reveal a modified response to novelty and surprise as the key psychological mechanisms mediating the behavioral differences later in life. It is important to note that these different psychological functions are assessed at ages from as early as immediately after weaning (4 weeks of age) to the end of life (26 months of age). Thus, even though the differential treatment during the neonatal novelty exposure only consisted of approximately 1h of total difference, the impact of this early environmental experience appears to be life long.

3.3.2 Neonatal novelty exposure and brain

Effects of neonatal novelty exposure on the brain have been examined at the level of functional brain asymmetry, hippocampal gross anatomy and synaptic plasticity. It has been long known that early stimulation, such as the handling procedure, induces

changes within the brain asymmetrically (Denenberg, 1981). Specifically, in the non-handled rats, left and right cortical damage produce similar effects on open field activity, while handled rats show differential changes in response to left and right cortical damage (Denenberg, 1978). Given this asymmetric effect, the exploration of neonatal novelty effect on brain development has been characterized by separate measures for the left and right brain, whereas the majority of early stimulation studies do not differentiate left and right measures.

Consistent with this finding of an asymmetrical early stimulation effect, Novel and Home rats are found to differ in their “handedness”, with the Novel rats showing a left-shift in their paw preference in a reaching task (Tang and Verstynen, 2002). This left shift is consistently observed across consecutive days and across several months of delay, thus suggesting a persistent increase in right cerebral dominance. Supporting this interpretation is another reliable observation from a second form of functional brain asymmetry measure, the turning asymmetry during spontaneous exploration in a novel environment. Upon initially entering a novel environment, Novel rats have a greater right turn bias than the Home rats (Tang *et al.*, 2003b). As turning towards the right involves stronger pushing by the left front limb, this difference in asymmetry also indicates an increase of right cerebral dominance (Tang and Reeb, 2004). In contrast to the functional asymmetry in “handedness”, this turning asymmetry is transient, appearing to be dependent upon the novelty of the situation. For instance, it shows a clear modulation by time, with the greatest novelty effect found during the first minutes of spontaneous exploration (Tang *et al.*, unpublished observation). Together, these

findings offer clear evidence for a modification of brain asymmetry as a result of neonatal novelty exposure.

As the hippocampus plays a critical role in learning and memory (Eichenbaum, 1997) and in regulating HPA output (Herman *et al.*, 2003; Sapolsky *et al.*, 1984a), effects of neonatal novelty exposure on the anatomy and function of this structure have been investigated. Although there are no overall differences in hippocampal volume, the Novel and Home rats differ in their patterns of volumetric asymmetry with the Novel rats showing a relatively greater right hippocampus than the Home rats by one percent of the total hippocampal volume (Verstynen *et al.*, 2001). Although one percent seems a small effect, computationally it may make a significant difference in dynamics of the neural networks, giving rise to functional asymmetry (Reggia *et al.*, 1998).

Neonatal novelty exposure effects on synaptic plasticity have been examined in the CA1 region of the hippocampus. Long-term potentiation (LTP), the most extensively studied form of synaptic plasticity, differs between Novel and Home rats. Regardless of the side of hippocampus, neonatal novelty exposure leads to enhanced LTP, while synaptic transmission prior to LTP induction shows no significant difference (Tang and Zou, 2002). When the data are further analyzed, taking into consideration the side of the hippocampus, evidence of asymmetric novelty exposure effects are found. This early life stimulation effect is characterized by two forms of selectivity: a selectivity for the right hippocampus and a selectivity for LTP (Tang *et al.*, 2008).

As these asymmetric changes within the brain all involve an increased dominance of right-side function, it begs the question of how these asymmetric effects are achieved and what evolutionary significance this asymmetric environment may

have. As measures of asymmetry have been shown to have predictive power for measures of memory (Tang *et al.*, 2003a; Tang and Reeb, 2004) and the asymmetry in synaptic plasticity is selective for LTP, one may speculate that early experience is critical for the development of normal functional lateralization, or avoidance of abnormal functional lateralization. It should be noted that abnormal functional lateralization has been associated with a range of psychopathologies (Davidson, 2003).

3.3.3 Neonatal novelty exposure and endocrine function

The investigation of neonatal novelty exposure on endocrine function has focused on how circulating CORT, both basal and evoked, relate to other functional measures, and to novelty of the situation in which behavioral measures are obtained. This approach differs from that used in most handling studies where CORT measures are obtained under conditions of shock or restraint that may not allow generalization to learning situations where the maximum level and temporal characteristics of HPA activation are rather different.

The most surprising finding is that no effect of neonatal novelty exposure is found on social interaction evoked CORT responses obtained shortly after social competition, even though the behavioral measures differ between the Novel and Home rats (Akers *et al.*, unpublished observation). This means that if circulating CORT somehow contributes to behavioral differences observed in these tasks, then the behavioral effects cannot be mediated by differential concentrations of CORT alone. Interestingly, Novel and Home rats are found to differ in the plasticity of the evoked CORT response to social competition, with only the Novel rats showing habituation of CORT responses

across two days of repeated social competition against the same competitor. This means Novel and Home rats differ in their ability to down regulate their HPA output according to recent experience.

Similarly, no effect of neonatal novelty exposure is found on evoked CORT after animals have become highly familiarized with the Morris water task. In contrast, seemingly small deviations from the testing routine, such as an unexpected visit to another room where the animal spends a few minutes in an open field, is able to produce differential levels of circulating CORT between the Novel and Home rats. Specifically, we have found that the Novel rats show a greater sensitivity to this surprise manipulation (Tang *et al.*, unpublished observation). This means that the HPA axis of Novel rats is not only better at down regulating its responses when the testing situation has become familiar, but also better at responding to changes in their environment.

The hippocampus provides the major environmentally related driving force for the HPA axis. Thus, clues about how Novel rats might achieve better detection of environmental novelty and down regulation of HPA output as a result of environmental familiarity may be obtained by examining electrophysiological data of population spikes recorded in the CA1 of the hippocampus (Zou *et al.*, 2001; Figure 5) **<Figure 5 near here>**. After the onset of perfusion of the slice by stress levels of CORT, the amplitude of the population spike from hippocampal slices from Novel rats show a small initial, brief increase, followed within a few minutes by a large decrease. Conversely, the slices from Home rats show relatively little change in response to CORT perfusion. This differential effect suggests that Novel and Home may achieve different level of self-regulation via differential levels of functional GRs. Additional pharmacological

experiments further reveal that LTP of the population spikes among the Novel and Home rats is also differentially modulated by stress levels of CORT after the short-term effect on neuronal excitability is washed out. These findings serve to explain how it is possible that the same concentration of circulating CORT could support differential effects on the function of the circuit, hence mediating differential effects on behavioral measures discussed earlier.

One of the possible consequences of this enhanced self-regulation of CORT secretion is that Novel rats might be able to maintain a lower basal level of CORT than Home rats. This was confirmed by the finding that at 16 months of age Novel rats have lower basal CORT than Home rats (Tang *et al.*, 2003a). Most interestingly, even though this measure is temporally remote from behavioral measures of social recognition memory, lower basal CORT retroactively predicted 24h recognition memory 8 months earlier (Tang *et al.*, 2003a).

3.3.4 Neonatal novelty exposure and maternal influence

These neonatal novelty exposure-induced changes in behavioral, neural, and endocrinological functions provide unequivocal evidence that as little as 1h of total difference in early life can induce a wide range of long-lasting effects on development. The transient nature of this environmental manipulation forms a sharp contrast to the omnipresence of the mother to the developing offspring. It begs the question of what role the mother plays in relation to these early stimulation effects. Specifically does the mother discriminate between her Novel and Home pups, and to whom does she show preferential care?

Common wisdom would suggest Novel rats would receive preferential maternal care. However, data from studies of multiple cohorts of rats do not support this idea. The dams either show no preference in her care towards the Novel and Home pups or show preferential care towards the Home pups (Tang *et al.*, 2006). In other words, neonatal novelty exposure treatments lead to enhanced functionality in the Novel rats despite the fact that the mothers show preferential care towards the Home rats. Instead, the state of the dam's stress response system, (e.g., her circulating CORT), can set the stage for differential responses of pups to neonatal novelty exposure. Individual differences in the mother's HPA function can thus lead to differential neonatal novelty exposure effects. This hypothesis has been confirmed by a positive correlation between maternal evoked CORT response to 1 min swim stress and novelty effects on offspring cognitive and emotional measures and a complementary negative correlation between maternal basal CORT and novelty effects (Reeb *et al.*, 2007).

These findings shed light not only on how early stimulation via neonatal novelty exposure interacts with maternal influence, but also suggest alternative interpretations of the handling experiments originally thought to provide clear evidence for supporting the maternal mediation hypothesis (Liu *et al.*, 1997). In response to the stress of handling and separation from her pups, the dams of the handled pups not only increase their care-giving behavior upon reunion, but also more than likely demonstrate elevated circulating CORT levels. A dam's circulating CORT can affect the pups' circulating CORT levels via her milk supply (Catalani *et al.*, 2000; Macri *et al.*, 2007; Meerlo *et al.*, 2001). Thus, it is possible that the increase in maternal care is only an epiphenomena and it is handling-induced maternal stress that provides the pups with low doses of

CORT exposure early in development, which in turn shapes the pups HPA development.

3.4 Maternal Deprivation

In this section, we shift our discussion to the impact of more prolonged phases of separation from the dam and the resulting behavioral, neural and endocrinological changes in the offspring. Specifically, we will discuss maternal deprivation and maternal separation paradigms that remove pups from the nest for relatively extended periods of time (ranging from approximately 3 to 24 hours) either once or multiple times. These paradigms are thought to model infant or childhood neglect. Within a couple of hours of separation, this procedure usually activates the stress axis with increases in CORT and ACTH. Also, robust behavioral responses are evident in the pup. Maternal deprivation has been one of the more prolific procedures in the early life experience literature and the impact of this paradigm on our understanding of early life effects has been critically important. However, due to variability in maternal deprivation procedures between labs, considerable variability in results exist in this literature preventing simplistic statements concerning effects of maternal deprivation. For example, due to pups' reliance on behavioral thermoregulation and insufficient thermogenesis, a minor variation of 1 to 2⁰C in surface or ambient temperature can produce a hyperthermia/hypothermia that can greatly alter organ function, including the infant brain, which is uniquely dependent upon body temperature in pups (Kleitman and Satinoff, 1982; Sullivan and Leon, 1988).

The unique role of sensory stimuli in controlling pup behavior, brain and physiology also contributes to the importance of minor procedural difference in maternal

deprivation. Indeed, sensory stimuli maintain pups' at homeostasis in myriad systems, with different stimuli and its patterning controlling specific systems and referred to as 'hidden regulators' (Hofer, 1995). Thus, the maternal deprivation procedure can be viewed as removal of sensory stimuli, with minor variations between labs removing more or less of these sensory stimuli normally provided by the mother, siblings and the nest. For example, tactile stimulation increases growth hormone, warmth increases NE, maternal odor increases behavioral activity and cold increases CORT (Hofer, 1973; Kuhn and Schanberg, 1998). Together, these data suggest very specific and minor changes in experimental protocols can produce a diversity of behavioral and physiological responses in pups that do not facilitate fine grain interpretations of this literature. However, this literature clearly illustrates that early life separation from the mother produces robust brain changes in specific neural loci and specific modifications in endocrine control that are related to long-term changes in emotion and cognition that are reviewed below.

Recent literature has questioned whether the critical factor in maternal separation experiments is the separation itself or the mother's response to the pups at reunion. The extent of changes in maternal behavior induced by maternal separation is variable across paradigms and laboratories and likely contribute to the varied outcomes reported. For instance, separated, cold pups in the same room as the mother will likely produce different results than a laboratory that keeps separated pups thermoneutral in a room free of maternal odors. Thus, it is likely that both pups' response to separation and the mother's response to reunion contribute to the short-term and long-term effects of maternal deprivation. Regardless of the relative contribution of dam versus pup to the

observed changes, this literature strongly supports the importance of early life experience on later life behavior, brain and endocrine responses.

3.4.1 Maternal deprivation and behavior

The rat pups' immediate behavioral response to maternal separation remains fairly consistent throughout early life and produces increased behavioral activity and vocalizations, including ultrasonic vocalization (Hofer *et al.*, 2001). Interestingly, these immediate responses can be greatly attenuated if pups are provided with adequate warmth and maternal odor (Hofer and Shair, 1978; Sokoloff and Blumberg, 1997). Within approximately an hour, this response changes to hypo-activity, although the immediate response remains more persistent as pups mature (Hofer and Shair, 1991). Separation from the mother also alters the pups' future responses to the mother at reunion (hyper-responsiveness) and subsequent separation (increased ultrasonic vocalization) suggesting maternal separation has profound chronic effects on pups' behavior during the pre-weanling period (Hofer and Shair, 1978).

The long-term effects of maternal separation appear to produce an animal that is more behaviorally responsive to stressful situations (Andersen *et al.*, 1999; Kosten *et al.*, 2005). However, these animals are also thought to exhibit generalized cognitive and contextual fear learning impairments (Bean *et al.*, 2002; Kosten *et al.*, 2006; Kosten *et al.*, 2005), anhedonic (Matthews and Robbins, 2003), decreased or increased food intake based on the context (McIntosh *et al.*, 1999; Penke *et al.*, 2001) and susceptibility to drug and alcohol abuse (Cirulli and Alleva, 2003). The long-term effects of maternal deprivation appear to alter maternal care, which is then transmitted nongenomically

through the next generations (Fleming *et al.*, 2002). In summary, maternal deprivation is considered an early life stressor that produces an animal behaviorally equipped for a stressful adult life and alters specific and global behaviors such as maternal care, food intake and metabolism.

3.4.2 Maternal deprivation and brain

Maternal deprivation produces ubiquitous changes in the brain, although the reciprocally interacting limbic system and stress axis have received particularly intense assessment. A comprehensive review of the maternal deprivation effects on the brain is beyond the scope of this chapter, although we will attempt to highlight the major findings of this area related to stress. Overall, the brain of infant rats with or without maternal deprivation shows a unique response to stress, with the time course and intensity of the neural response diverging from that documented in adults.

Presentation of a stressor to infant rats produce very rapid changes in CRH mRNA in the PVN in non-deprived pups (Dent *et al.*, 2000a). Interestingly, this response in maternally deprived pups is significantly reduced (Dent *et al.*, 2000a). The inability to identify this period of hyper-responsiveness in previous studies appears to be due to a pup's very rapid onset of the stress response compared to adults. Thus, while the adrenal gland shows an SHRP, the central components (i.e., brain) of the HPA axis are responsive, although hypo-responsive with maternal deprivation (Suchecki *et al.*, 1993).

While infant maternal deprivation causes a decrease in PVN CRH in infancy, it shows a marked increase in adulthood (Plotsky and Meaney, 1993). Indeed, neural

changes due to maternal deprivation can be seen throughout the brain in cells containing CRH and GR, especially in areas that integrate the endocrine and behavioral responses to stress. Specifically, compared to non-deprived animals, adult animals that were maternally deprived show a reduction in CRH receptor binding density in the anterior pituitary but increases in CRH receptor binding/ immunoreactivity in the raphe nucleus, parabrachial nucleus, amygdala and bed nucleus of the stria terminalis (BNST). GRs are also modulated following maternal deprivation. Specifically, maternal deprivation is associated with decreased GR in the hippocampus later in life, which suggests maternally deprived pups have impaired HPA negative feedback. Furthermore, the LC, which controls much of the brain's NE and potentiates the HPA axis, is also modified and associated with a down-regulation of NE receptors (Ladd *et al.*, 2000).

The short-term (infant) and long-term (adult) effects of infant maternal deprivation diverge. For example, maternal deprivation has been shown to induce an up-regulation of brain-derived neurotrophic factor (BDNF) expression in hippocampus and prefrontal cortex in pups, although in adulthood there is reduction in prefrontal BDNF expression (Roceri *et al.*, 2004). Finally, connectivity between brain areas appears disrupted by maternal deprivation. For example, compared to non-deprived pups, maternally separated pups show altered responses of the BNST and PVN to amygdala stimulation (Sanchez *et al.*, 1995), though synaptic changes and dendritic branching modifications also contribute to these changes (Braun *et al.*, 2000). Overall, these data suggest that the neural effects of maternal deprivation occur throughout the brain and include anatomical and synaptic changes within and between brain areas. Importantly,

maternal deprivation induces short-term effects in infancy and the long-term effects in adulthood that do not always correspond. It should also be noted that considerable divergence in adult outcome following maternal deprivation appears in the literature suggesting the onset and duration of the maternal deprivation is a critical variable in both the immediate response and the long-term response (Muneoka *et al.*, 1994).

In summary, maternal deprivation alters the HPA axis and brain areas that integrate and control HPA regulation both in infancy and adulthood. Importantly, the infant peripheral hypo-responsiveness is associated with neural hyper-responsiveness. While the causal mechanisms connecting specific stimuli (or the absence thereof) and neural response to maternal deprivation in infant and adult neural consequences still need to be assessed, there is considerable convergence between the maternal deprivation literature and the clinical literature on early life neglect.

3.4.3 Maternal deprivation and endocrine function

Removing pups from the mother for a prolonged period of time has immediate and long-term effects on a pup's endocrine system, although this effect changes depending on the age of manipulation and age of assessment. Overall, the literature indicates prolonged separation from the mother overrides the SHRP and pups show an elevation in baseline CORT, which is potentiated by presentation of a stressor. Thus, while the mechanism is unclear, maternal separation appears to activate the normally quiescent HPA axis to permit a CORT response. In a comprehensive study, pups were separated from the mother for times ranging from 15 min to 24h and the response to stress (CORT, ACTH) assessed at different developmental ages (Dent *et al.*, 2000b).

Overall, pups show a CORT response that became more robust with length of separation from the mother, as well as age. It should also be noted that during early life, normal CORT and ACTH responses can be elicited in neonatal pups injected with an endotoxin, indicating a functioning HPA axis in pups that is stressor-specific (Dallman, 2000; Dent *et al.*, 1999; Stanton *et al.*, 1987; Suchecki *et al.*, 1993; Walker *et al.*, 1991; Witek-Janusek, 1988). Thus, the pituitary-adrenocortical system of the neonatal rat is responsive to stress throughout development in a time-dependent and stressor-specific fashion.

The classic long-term impact of maternal deprivation emerges around weaning and continues into adulthood. For example, weanling aged pups that were separated from their mothers for only one maternal deprivation session at 3 days of age showed heightened ACTH and CORT, although not if the same manipulation was done at 7 days of age. On the other hand, pups maternally deprived at 11 days of age, show an elevated CORT response at weaning but by periadolescence the stress-induced CORT response is attenuated compared to non-deprived controls (Suchecki and Tufik, 1997). Finally, sex differences in response to maternal deprivation occur at both adolescence and adulthood (Rees *et al.*, 2006). These studies suggest the response to early life maternal deprivation is a complex phenomenon, with the causal mechanisms still a mystery.

3.5 Pain, Fear Conditioning and Context of Early Life Adversity

Early life adverse experience alters adult emotionality and cognitive function in clinical populations and has been modeled in infant rodents with moderately painful

electric shock. Early life experience with shock, which does not increase a pup's CORT levels until PND10, appears to activate a pup's nociceptive systems and elicit pain-related behavioral responses (Barr, 1995; Collier and Bolles, 1980; Emerich *et al.*, 1985; Fitzgerald, 2005; Stehouwer and Campbell, 1978). Overall, repeated shock in early life appears to heighten adult emotionality/anxiety and has also been shown to both attenuate and enhance learning, depending on the task. Previous work on infant adverse experiences using shock suggests that unpredictable shock produces greater changes in adult emotionality than predictable shock (Bell and Denenberg, 1962; Denenberg, 1963; Denenberg and Bell, 1960; Henderson, 1965) and varies from the effect typically observed in adults (Shors *et al.*, 1990; Weiss, 1970).

Central processing of pain in infants appears to diverge from adults, as indicated by the ontogeny of fear conditioning using pairings of odors and 0.5mA electric shock to either the tail or hind limb. As a vehicle to understand infant processing of pain, there are several advantages to using the olfactory learning paradigm of fear conditioning. Specifically, the adult fear conditioning circuit is relatively well-defined and permits the direct comparison of the maturing infant brain to the adult circuit (Debiec and LeDoux, 2004; Fanselow and Gale, 2003; Funk and Amir, 2000; Schettino and Otto, 2001; Sevelinges *et al.*, 2007). Additionally, the predictable versus unpredictable nature of stimuli are inherent in the fear conditioning paradigm's use of the experimental (paired odor-shock) and control (unpaired odor and shock) conditioning groups. Finally, pups must learn the odor used for attachment to the mother, with a wide range of stimuli functioning as a reward to support odor preference learning, including painful 0.5mA shock (Camp and Rudy, 1988; Sullivan *et al.*, 2000).

While odor approach/preference learning in early life appears paradoxical, this limitation on aversive learning is seen in species other than rats and is important for attachment. For example, during imprinting in chicks, shock enhances following behavior of the surrogate, although an aversion occurs if presented just hours after the sensitive period closes (Hess, 1962; Salzen, 1970). These learning limitations have also been documented in infant dogs that continue to approach a human attendant that shocks or mishandles the puppies (Rajecki *et al.*, 1978), as well as nonhuman primates that continue to approach a caregiver that handles them roughly (Harlow and Harlow, 1965; Maestriperi *et al.*, 1999; Sanchez *et al.*, 2001). In young rat pups, this limitation on learning also extends to inhibitory conditioning and passive avoidance (Blozovski and Cudennec, 1980; Collier *et al.*, 1979; Myslivecek, 1997; Stehouwer and Campbell, 1978).

We suggest that this model of infant odor-shock conditioning in infancy may be useful as a paradigm to better understand how early life abuse can support and maintain attachment. Furthermore, we suggest that understanding the unique ways in which the infant brain processes painful stimuli, such as shock, we may gain insight into the enduring effects of early life pain.

3.5.1 Odor-shock conditioning and behavior

During a sensitive period in early life (until PND10), an odor paired with a painful stimulus (0.5mA tail or foot shock, tail pinch or mother handling pups roughly) results in pups approaching that odor when it is subsequently encountered (Camp and Rudy, 1988; Haroutunian and Campbell, 1979; Moriceau *et al.*, 2004; Moriceau and Sullivan, 2004; Moriceau *et al.*, 2006; Roth and Sullivan, 2001; Roth and Sullivan, 2005; Spear, 1978;

Sullivan, 2003; Sullivan *et al.*, 2000). This is in sharp contrast to odor-shock conditioning in adults (an auditory stimulus can also be used), which is referred to as fear conditioning, and produces learned freezing responses and odor avoidance in adults with the amygdala being a critical site for plasticity (Davis *et al.*, 2003; Debiec and LeDoux, 2004; Fanselow and Gale, 2003; Fanselow and Poulos, 2005; Hess *et al.*, 1997; LeDoux, 2003; Rosenkranz and Grace, 2002; Sananes and Campbell, 1989; Schettino and Otto, 2001; Sevelinges *et al.*, 2007). The failure of pups to avoid an odor previously paired with pain occurs despite a functional nociceptive system (Barr, 1995; Emerich *et al.*, 1985; Fitzgerald, 2005; Stehouwer and Campbell, 1978).

Considering the necessity of pups learning a preference to their mother's odor for nipple attachment, and other related attachment behaviors, it is certainly beneficial for pups *not* to learn an aversion to their mother's odor or inhibit approach responses to nest odors. Perhaps this attenuated avoidance learning ensures that pups continue to only approach/follow the caregiver (Hofer, 1981; Hofer and Sullivan, 2001). Additionally, this odor-0.5mA shock paradigm supports learning an odor that appears to function similarly to the maternal odor, since it supports nipple attachment (Sullivan *et al.*, unpublished observation).

This early life attachment odor is retained into adulthood where it alters emotion and cognition. Specifically, the odor paired with pain to produce the attachment odor can attenuate adult fear conditioning as well as attenuate amygdala neural activity, as measured by 2-DG autoradiography, that supports this learning (Sevelinges *et al.*, 2007). It should be noted that pups that receive unpaired presentation of the odor-shock do not learn about the odor in infancy, nor does the odor alter adult fear conditioning. However,

these unpaired pups exhibit anxiety-like behaviors in adulthood that are not exhibited by paired odor-shock pups (Tyler *et al.*, 2007).

3.5.2 Odor-shock conditioning and brain

Fear conditioning requires the amygdala, although many additional brain areas are involved in this complex neural circuit (Fanselow and Gale, 2003; Fanselow and LeDoux, 1999; Herzog and Otto, 1997; Maren, 2003; McGaugh *et al.*, 1999; Pape and Stork, 2003; Pare *et al.*, 2004; Rosenkranz and Grace, 2002; Sananes and Campbell, 1989; Schettino and Otto, 2001; Sevelinges *et al.*, 2004; Walker and Davis, 1997). The importance of the amygdala in early life fear conditioning is further suggested by the concurrent emergence of fear conditioning and amygdala plasticity (Sullivan *et al.*, 2000). We have data suggesting the inability of pups to learn this early life version of fear conditioning may be due to the amygdala's failure to participate in early life learning. Specifically, during the sensitive period, when odor-shock produces an odor preference, the olfactory bulb and anterior piriform 'olfactory' cortex show activation during learning, while the amygdala does not appear to participate, as indicated by 2-DG or c-Fos expression (Moriceau and Sullivan, 2006; Moriceau *et al.*, 2006; Roth and Sullivan, 2005). In contrast, similar conditioning in post-sensitive period pups (PND12) that learn odor aversion and freezing, show activation of the posterior piriform cortex, basolateral, lateral and cortical amygdalar nuclei (Moriceau and Sullivan, 2006; Moriceau *et al.*, 2006; Sullivan *et al.*, 2000). Importantly, temporary suppression of the pup's post-sensitive period amygdala during fear conditioning prevents learning (Moriceau and Sullivan, 2006).

3.5.3 Odor-shock conditioning and endocrine function

CORT infused either systemically or directly into the amygdala during odor-0.5mA shock conditioning results in an odor aversion in sensitive period pups, overriding the pups low CORT levels during the SHRP and permitting amygdala plasticity (Moriceau *et al.*, 2004; Moriceau and Sullivan, 2004; Moriceau and Sullivan, 2006; Moriceau *et al.*, 2006). A similar role of CORT has been demonstrated during imprinting in ducklings, with low CORT levels important for following the surrogate .

In pups, CORT levels can be modulated by the environment with early life stress causing a premature increase in CORT levels (Levine, 1962; Levine, 1994), or through CORT received through their mother's milk (Yeh, 1984). Additionally, the mother modulates a pup's CORT concentration based on the level of her maternal care, with decreases in maternal care causing an increase in the pup's CORT levels (Stanton *et al.*, 1987; Suchecki *et al.*, 1993). Indeed, the attenuation of the shock-induced CORT release can be blocked by maternal presence and pup attachment learning to odor-shock conditioning is reinstated (Moriceau *et al.*, 2006). The important role of maternal suppression of shock-induced CORT release in pups odor aversion learning was verified by systemic and intra-amygdala CORT infusions, which permitted pups to learn odor aversions in the presence of the mother. Similar 'social buffering' of CORT release by social cues has been found in other paradigms and species in both infancy and adulthood (Carter and Keverne, 2002; Carter *et al.*, 2003; Dallman, 2000; Deschamps *et al.*, 2003; DeVries, 2002; DeVries *et al.*, 2003; Hennessy, 1984; Hennessy *et al.*, 2006; Wiedenmayer *et al.*, 2003).

3.6 Functional Consequences of Early Life Experiences

Together, there is remarkable convergence across these various early life experience paradigms with long-term effects seen in the HPA axis and brain areas with strong reciprocal interactions over the HPA axis, such as the LC, hippocampus, amygdala and prefrontal cortex. However, it is likely multiple neonatal mechanisms mediating these long-term effects including changes in pups HPA axis and related brain areas in response to early life manipulations, as well as through modifications of maternal care.

4 PUBERTAL EXPERIENCES AND ENDURING BEHAVIORAL AND ENDOCRINE CONSEQUENCES

As reviewed above, there is a vast body of literature that indicates neonatal development is a crucial stage of maturation that, if disturbed, modified or altered, results in changes in the future behavioral, neurological, and endocrinological function of an individual (Gutman and Nemeroff, 2002; Heim and Nemeroff, 2001; Maccari *et al.*, 2003; Zhang *et al.*, 2006). However, the neonatal period is not the only developmental stage when individuals are susceptible to both positive and negative influences. While puberty has long been regarded as a period of rapid physical growth and reproductive maturation, more recently it is also regarded as an additional critical period of neurobehavioral developmental vulnerabilities (Andersen, 2003; Dahl, 2004; Kleinert, 2007; Patton and Viner, 2007; Romeo, 2003; Sisk and Foster, 2004; Spear, 2000). For instance, it is well established that adolescence is marked by increases in the morbidity

of various psychological disorders such as anxiety, depression and schizophrenia (Conger and Petersen, 1984; Costello *et al.*, 2003; Masten, 1987; Patel *et al.*, 2007). The mechanisms mediating the pubertal increase in these disorders are presently unknown.

Recent studies in adolescent boys and girls have indicated that pubertal exposure to stress may be a particularly relevant environmental factor that contributes to an individual's vulnerability to various psychopathologies later in life (Grant *et al.*, 2003; Grant *et al.*, 2004; Turner and Lloyd, 2004). However, it is still unclear how exposure to stress during puberty may lead to various psychological dysfunctions (Romeo and McEwen, 2006). As described above (See Section 2.2.2 *Pubertal development of HPA axis*), in response to acute or repeated stress, pubertal animals demonstrate a more prolonged or higher peak ACTH and CORT response, respectively, compared to adults (Romeo *et al.*, 2006a). Thus, it is possible that this differential responsiveness may contribute to the increase in stress-related psychopathologies during adolescence. In fact, the differential hormonal response in adolescent compared to adult animals may be compounded in the pubertal individual by three additional factors. First, plasma levels of corticosterone-binding globulin (CBG), a protein that binds CORT making it unavailable to target tissues such as the brain (Breuner and Orchinik, 2002), are lower in pubertal than adult animals (Romeo *et al.*, 2006a; Smith and Hammond, 1991). Second, the prepubertal brain may be more sensitive to stress-related hormones, as experiments have shown an equivalent dose of CORT increases hippocampal NMDA receptor subunit expression (e.g., NR2A and NR2B) to a greater degree in prepubertal than adult males (Lee *et al.*, 2003). Finally, brain regions that

continue to mature during adolescence (e.g., hippocampus, amygdala, prefrontal cortex), are also the most sensitive to CORT and are extremely important in modulating emotionality (McEwen, 2005). Thus, upon experiencing a similar stressor, the immature, and possibly more sensitive, pubertal brain is differentially exposed to unbound, bioavailable CORT compared to the adult brain. In the next sections, we briefly review the growing body of literature that supports the assertion that chronic stress during adolescence may be particularly detrimental to both the immediate and future neurobehavioral function of an organism.

4.1 Pubertal Experience and Behavior

Long-term effects of stress on emotionality are noted in rats and mice exposed to a variety of stress paradigms during puberty. Specifically, anxiety- and depressive-like behaviors are greater in adult animals that experience stress during adolescence compared to unstressed controls (Avital *et al.*, 2006; Leussis and Andersen, 2008; Maslova *et al.*, 2002a; Maslova *et al.*, 2002b; Pohl *et al.*, 2007; Schmidt *et al.*, 2007; Stone and Quartermain, 1998; Tsoory *et al.*, 2007; Vidal *et al.*, 2007; Wright *et al.*, 2008). Unfortunately, it remains unclear whether or not changes in adult emotionality after adolescent stress are dependent on the stressors occurring during the adolescent window of development. That is, would similar behavioral changes ensue if exposure to these stressors occurred in adulthood?

Recent data from Richter-Levin and colleagues (Avital and Richter-Levin, 2004) have shown that the timing of the stress exposure is important. In particular, adolescent male rats transiently exposed to elevated platform stress (EPS) exhibit higher levels of

anxiety-like behavior when tested as adults, while adults exposed to same transient EPS treatment do not show any changes in their anxiety levels (Avital and Richter-Levin, 2004). We have also recently shown that exposure to mild physical (1 h or restraint stress every other day) and/or social stress (social isolation) during adolescence leads to increases in depressive-like behaviors and elevated basal levels of plasma CORT in adulthood (Romeo *et al.*, unpublished observation). Moreover, adolescent animals that experience both types of stressors show the greatest increase in depressive-like behaviors, suggesting an additive effect of the stressors on subsequent behavior. It is important to note that when adult animals experience these same mild physical and/or social stressors for the same length of time, no changes in depressive-like behavior or basal HPA function are observed (Romeo *et al.*, unpublished observation). These data strongly argue that, similar to the perinatal period of development, adolescence is a particularly sensitive period to stress, especially in regards to emotional behavior and modulation of the HPA axis.

Though it remains unclear what mechanisms mediate these changes in adult behavior after exposure to stressors during adolescence, it is possible that stress-induced disruptions of developmentally important social interactions occur. For instance, play behavior during adolescent development is integral for the emergence of proper social behaviors in adulthood (Vanderschuren *et al.*, 1997). It has been reported that stressors such as food deprivation (Siviy and Panksepp, 1985), foot shock (Wood *et al.*, 1995), predator odor (Siviy *et al.*, 2006), intense lighting conditions (Vanderschuren *et al.*, 1995), or restraint (Romeo *et al.*, 2006b) inhibit this developmentally important social behavior. Therefore, the stress-induced reduction of

play during the adolescent period may contribute, at least in part, to the behavioral disturbances observed in adult animals after experiencing pubertal stress.

4.2 Pubertal Experience and Brain

Although accumulating data indicate that stress during adolescence may be particularly disruptive in the context of emotional development, the neural substrates that mediate these changes are not well understood. In fact, studies investigating the structural alterations of the brain induced by pubertal stress are sorely lacking. An elegant study by Isgor and colleagues (Isgor *et al.*, 2004) demonstrated that chronic variable stress (CVS) during adolescence leads to long-term changes in hippocampal structure and function. More specifically, they found that compared to non-stressed controls, animals exposed to CVS during adolescence had significantly smaller hippocampal volumes, lower hippocampal GR expression, a hyper-responsive HPA axis to later stressors, and reduced performance on a hippocampal-dependent water maze task (Isgor *et al.*, 2004). Interestingly, these effects of CVS experienced during adolescence were noted 30 days after the stressors had been terminated (Isgor *et al.*, 2004). It is important to note that effects of chronic stress on hippocampal structure in adults return to pre-stress levels within 10-21 days (McEwen and Margarinos, 1997). Thus, the study by Isgor and colleagues (Isgor *et al.*, 2004) suggests stress-induced structural remodeling of the hippocampus during adolescence is longer lasting, and perhaps permanent, compared to stress-induced remodeling in adulthood.

Future studies will need to investigate what ramifications adolescent stress exposure may have on other brain regions that continue to development during

adolescence, that are highly sensitive to glucocorticoids and intimately involved in emotional processing, such as the medial prefrontal cortex and amygdala (McEwen, 2005; Romeo and McEwen, 2006). It should be noted that recent studies have demonstrated stress-induced changes in synaptogenesis and dopamine receptor levels in adolescent prefrontal cortex, which may be associated with the behavioral dysfunction associated with pubertal stress (Leussis and Andersen, 2008; Leussis *et al.*, 2008; Wright *et al.*, 2008).

4.3 Pubertal Experience and Endocrine Function

Relatively few studies have investigated the effects of chronic stress during adolescence on later stress reactivity in adulthood. As mentioned above, we have found that animals exposed to restraint stress and/or social isolation during puberty demonstrate elevated levels of basal CORT in adulthood (Romeo *et al.*, unpublished observation). Furthermore, Isgor and colleagues (Isgor *et al.*, 2004) have found that animals exposed to CVS during puberty demonstrate increased stress-induced HPA responsiveness in adulthood. Specifically, animals stressed during adolescence demonstrate a significantly more prolonged stress-induced CORT response compared to animals not exposed to pubertal stress (Isgor *et al.*, 2004). It was also found that animals exposed to stress during puberty show decreased hippocampal GR expression in adulthood, compared to their unstressed counterparts (Isgor *et al.*, 2004), suggesting decreased hippocampal GR-mediated negative feedback on the HPA axis. Not all experiments have found changes in adult HPA function following adolescent stress. For instance, experiments that employed chronic unpredictable stress, such as restraint

or foot shock, during puberty were unable to find any changes in adult HPA function (Maslova *et al.*, 2002a; McCormick *et al.*, 2005; Overmier and Murison, 1991).

Taken together, it appears that chronic stress during adolescence may lead to subtle changes in HPA function. However, compared to the reliable and long-term effects of neonatal stress on later HPA reactivity, the existing literature does not support such a consistent, robust change in adult HPA function following adolescent stress. Though it is unclear why perturbations of neonatal compared to the pubertal HPA axis may lead to more dramatic changes in later stress responsiveness, it is possible that the constituent neuroendocrine parts of the HPA axis are more malleable during the neonatal period, and thus, more sensitive to perturbations. Future studies will need to compare the relative sensitivities of the developing neonatal and pubertal HPA axes, and also assess possible cumulative effects of both neonatal and pubertal stress exposure on later HPA function in adulthood.

5 ADOLESCENCE AS A PERIOD OF INTERVENTION TO MITIGATE EARLY DEVELOPMENTAL INSULTS

Adolescence is clearly marked by profound changes in an individual's nervous system, physiology and behavior (Andersen, 2003; Dahl, 2004; Romeo, 2003; Romeo, 2005; Romeo *et al.*, 2002; Spear, 2007; Spear, 2000). Although this may render an individual especially vulnerable to harm during this period, it may also allow for interventions to mitigate earlier or concurrent emotional and/or physical trauma (Andersen, 2003; Dahl, 2004). Recent research has explored the ability of environmental enrichment during adolescence to ameliorate the negative influences of

earlier perinatal insults. Environmental interventions may be more attractive than pharmacological interventions to combat adolescent stress-induced neurobehavioral dysfunction, as factors such as cost and unwanted side effects would likely be less with environmental interventions (Raz, 2006; Whittington *et al.*, 2004). Next, we discuss some promising data regarding the role of environmental enrichment in reducing effects of perinatal stress, and even neonatal brain damage.

5.1 Reversals of Perinatal Insults Through Pubertal Environmental Enrichment

Animals derived from stressful pregnancies show increases in anxiety-related behaviors and HPA reactivity and depressed play behavior later in life (Laviola *et al.*, 2004; Morley-Fletcher *et al.*, 2003; Richardson *et al.*, 2006). However, prenatally stressed animals raised in an enriched environment (e.g., larger housing, toys, running wheel) during adolescence do not show these physiological and behavioral changes compared to prenatally stressed offspring raised under standard laboratory conditions (Laviola *et al.*, 2004; Morley-Fletcher *et al.*, 2003). In addition to prenatal stress, postnatal stress in the form of sub-optimal maternal care and maternal separation lead to increased HPA reactivity and emotionality and reduced cognitive function in adulthood (Meaney, 2001; Pryce *et al.*, 2005). Similar to the above mentioned studies, animals exposed to postnatal stress, but raised in enriched environments during puberty, show less HPA reactivity and emotionality and greater cognitive abilities compared to their postnatally stressed counterparts that were raised in normal laboratory environments (Bredy *et al.*, 2003; Bredy *et al.*, 2004; Francis *et al.*, 2002). Taken together, these studies clearly demonstrate that the pubertal period of

development can serve as a time for interventions to reduce or reverse the adverse effects accumulated from earlier developmental insults. The question still remains as to what aspects of the environment during the pubertal enrichment exposure contribute to the changes in later physiology and behavior. Furthermore, it is unclear whether pubertal individuals are equally sensitive to these environmental interventions throughout the entire adolescence period, or whether more circumscribed times during puberty (early-, mid- or late-puberty) would be adequate.

5.2 Mitigation of Perinatal Brain Damage Through Pubertal Environmental Enrichment

A dramatic example of puberty as a window of opportunity to diminish the impact of earlier developmental insults comes from the classic work of Twiggs, Popolow, and Gerall (Twiggs *et al.*, 1978). In this study, prepubertal males were housed in social isolation (solitary) or in groups (social) and then given lesions of the medial preoptic nucleus of the anterior hypothalamus (MPN), an area of the brain critical for the display of male mating behavior (Heimer and Larsson, 1966/1967). Although MPN lesions in adulthood lead to the irreversible elimination of male sexual behavior, males receiving a lesion prior to puberty are able to show copulatory behaviors upon reaching adulthood (Twiggs *et al.*, 1978). Interestingly, however, only the animals raised in the social groups during adolescence demonstrate behavioral reversal of the effects of MPN lesions (Twiggs *et al.*, 1978). These data indicate that pubertal development and social environment can interact to diminish, or even reverse, prior brain damage.

6 CONCLUSIONS

In conclusion, it is clear that early life experiences can have many enduring neurobehavioral consequences. Accumulating evidence highlights the importance of the HPA axis on this often complex interaction of behavior, brain and endocrine function. For instance, the HPA axis is programmable via multiple environmental sources and across multiple developmental stages. Early in development, this modification is jointly programmed by stressors (e.g., handling, novelty, and prolonged lack of maternal care) and stress diminishers (e.g., maternal presence and increases in the stability, quantity and quality of parental care) present simultaneously in the environment. However, it is important to note that in addition to the neonatal period of development, the HPA axis remains plastic and modifiable during later stages of maturation, including adolescence and adulthood. The fact that environmental enrichment during adolescence can offset or mitigate earlier developmental insults, such as neonatal stress, attests to this continued experience-dependent modulation of neurobehavioral and endocrine potentials. These studies indicate that past experiences may not permanently alter the function of an organism, but instead may set the stage for future plastic responses. Though many lines of research have begun to bear fruit regarding how early experiences influence the physiology and behavior of an organism throughout the lifespan, it is important to keep in mind that much work needs to be done. Given the importance of past experiences on the future health and development of an individual, a greater appreciation and understanding of early life experiences on enduring behavioral, neurological and endocrinological consequences remain vital.

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Figure Captions:

Figure 1. A diagram of the HPA axis. Abbreviations, ACTH, adrenocorticotropin hormone; AP, anterior pituitary; AVP, arginine vasopressin; C, cortex; CORT, corticosterone; CRH, corticotropin-releasing hormone; M, medulla; PVN, paraventricular nucleus of the hypothalamus; (+), positive drive; (-), negative feedback.

Figure 2. Mean (\pm SEM) plasma corticosterone (ng/ml) concentrations in prepubertal (28 days of age) and adult (77 days of age) male rats before and after a 30 min session of restraint stress.

Figure 3. Mean (\pm SEM) plasma corticosterone (ng/ml) concentrations in prepubertal (28 days of age) and adult (77 days of age) male rats exposed to a single (acute stress) or a daily 30 min session of restraint stress for 7 days (chronic stress).

Figure 4. Sequential steps in carrying out the within-litter neonatal novelty exposure procedure: (i) dam is removed from the home cage, (ii) Novel pups are transferred to individual non-home cages and yoked Home pups receive a matching amount of experimenter contact, (iii) after 3 min in the non-home cage, Novel pups are returned to the home cage in which the Home pups remain, and (iv) dam is returned to the home cage.

Figure 5. Bath application of 100nM CORT results in greater inhibition of population spikes in slices from Novel than in Home rats. (a) Examples of population spikes

before, during and after 20 min of CORT perfusion in Novel and Home slices. (b) Time course of CORT effect demonstrates the significantly greater reduction of population spike amplitude in Novel slices.

Abbreviations:

ACTH = Adrenocorticotropin Hormone

AVP = Arginine Vasopressin

BDNF = Brain-Derived Neurotrophic Factor

BNST = Bed Nucleus of the Stria Terminalis

CBG = Corticosterone Binding Globulin

CeA = Central Nucleus of the Amygdala

CORT = Corticosterone

CRH = Corticotropin-Releasing Hormone

DA = Dopamine

GR = Glucocorticoid Receptor

HPA axis = Hypothalamic-Pituitary-Adrenal Axis

LC = Locus Coeruleus

LTP = Long-Term Potentiation

MPN = Medial Preoptic Nucleus

MR = Mineralocorticoid Receptor

NE = Norepinephrine

PND = Postnatal Day

PVN = Paraventricular Nucleus of the Hypothalamus

SHRP = Stress Hypo-responsive Period

5-HT = Serotonin