Nonhuman Primate Models of Depression: Effects of Early Experience and Stress

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Abstract

Depression causes significant morbidity in the human population. The Diathesis-Stress/Two-Hit model of depression hypothesizes that stress interacts with underlying (probably genetic) predispositions to produce a central nervous system that is primed to express psychopathology when confronted with stressful experiences later in life. Nonhuman primate (NHP) studies have been extensively utilized to test this model. NHPs are especially useful for studying effects of early experience, because many aspects of NHP infancy are similar to humans, whereas development occurs at an accelerated rate and therefore allows for more rapid assessment of experimental variables. In addition, the ability to manipulate putative risk factors, including introducing experimental stress during development, allows inference of causality not possible with human studies. This manuscript reviews experimental paradigms that have been utilized to model early adverse experience in NHPs, including peer-rearing, maternal separation, and variable foraging. It also provides examples of how this model has been used to investigate the effects of early experience on later neurobiology, physiology, and behavior associated with depression. We conclude that the NHP offers an excellent model to research mechanisms contributing to the Diathesis-Stress/Two-Hit model of depression.

Key Words: animal models; primate; depression; early experience; Diathesis-Stress; Two-Hit; serotonin; hippocampus

Introduction

Human researchers have long acknowledged the relationship between stress and depression. Stress in the form of early adverse experiences such as abuse or parental loss during childhood is highly predictive of depression both during development and later life. Although underlying genetic, neurophysiologic, and neuroanatomical mechanisms have been hypothesized as contributing to the vulnerability for depression, these factors are not easily investigated in the human population. The nonhuman primate (NHP) model offers the ability to investigate the effects of genetics and early experience in a controlled environment, thus eliminating some of the confounds present in the human population. This article will provide an overview of human depression as well as a developmental model (Diathesis-Stress/Two-Hit) that has been used to explain how underlying vulnerabilities interact with stress to increase the susceptibility to depression. It will also summarize experimental procedures that have been utilized to create early adverse experience in NHPs and provide examples of how they have been used to model genetic vulnerability as well as physiologic, neurobiologic, and behavioral outcomes associated with human depression.

Prevalence and Morbidity of Depression in Human Populations

Depression is an insidious disease that causes significant human suffering. The World Health Organization reports that more than 350 million people worldwide suffer from depression (WHO 2012). Depression has been ranked the fifth leading cause of disability and has been forecasted to be one of the three leading causes of disease burden by the year 2030 (Brundtland 2000; Mathers and Loncar 2006). In the United States, major depressive disorder is one of the most prevalent psychiatric disorders. Although rates of depression can vary by age and sex, an overall estimate of depression indicates that approximately 6.7% of the population is affected within any 12-month period and a lifetime prevalence rate of 16.6% (Kessler et al. 2005a,b). This equates to nearly 2 people in 10 being affected by this debilitating disease one or more times in their lives. Suffering from depression not only severely affects the health and well-being of those afflicted; it also results in an economic burden. Depressed workers have been found to have twice the number of days absent per month compared with their nondepressed counterparts (Kessler et al. 1999; Wang et al. 2003). Depression has also been linked to decrements in work performance (Wang et al. 2004). The total economic burden attributed to depression in the United States was estimated to be 83.1 billion dollars in the year 2000 (Greenberg et al. 2003).
Thus, the economic and human toll exacted by depression is significant. Studying depression in humans is difficult because of many confounding factors, including comorbidities with other psychopathologic disorders and concurrent use of recreational and/or prescription drugs and alcohol as well as economic and nutritional status. Animal models offer a means to eliminate these confounds and experimentally manipulate the variables hypothesized to be associated with depression.

**NHP Models and Translational Research**

NHP biomedical models fall under the general rubric of translational research. The goal of translational research is to relate findings from basic science to practical application for human (or NHP) health and well-being. Although the idea of translational research is certainly not a new concept, it has recently gained renewed interest through its emphasis in the National Institutes of Health Roadmap Initiative (Zerhouni 2003). Translational research was put forth as part of an initiative to “Reengineer the Clinical Research Enterprise” by bridging the knowledge gap between basic and applied science or, as popularly coined, going from “bench to bedside.”

What are the requirements for an animal model of psychopathology to make the leap from bench to bedside? This has been extensively discussed in a number of articles (Belzung and Lemoine 2011; Berton et al. 2012; McKinney and Bunney 1969; Nestler and Hyman 2010; Willner 1984). The general consensus is that for an animal model to be appropriate, it is best if it shares many of the characteristics of the modeled human disease. Ideally, the animal model should arise from the same or similar risk factors or causative agents (providing construct validity) and/or exhibit a substantial degree of neural or behavioral pathology that corresponds to the human disease (providing face validity). In addition, ameliorative therapies should also work similarly (providing predictive validity). However, all of these components are seldom achieved in an animal model, and a strategy often employed by researchers is exploration of the relationship between specific symptoms or symptom clusters and underlying mechanisms hypothesized to contribute to the symptoms. Because, as described below, depression is a multifaceted disease, this strategy provides the opportunity to link specific symptoms to specific neurobiologic or physiologic mechanisms.

One potential drawback of the NHP model is that most symptomology in humans involves mood or thoughts that are not directly measurable in NHPs (see Figure 1). However, some symptoms such as psychomotor slowing and appetitive and sleep disturbances can be directly observed in “depressed” NHPs (Reite and Short 1983). In addition, behaviors such as collapsed posture, social withdrawal, and decreased consumption of a highly preferred reward can strongly imply a state of anhedonia, which is one of the hallmarks of human depression (Felger et al. 2013; Shively et al. 1997, 2005). However, a significant difficulty in modeling depression in any animal model lies not in the model but in the disorder itself. Depression is not a univariate disease but rather a syndrome consisting of a cluster of symptoms reflecting dysregulation in affective, psychomotor, appetitive, and cognitive processes, many of which are not specific to depression. Several authors have noted that depression is extremely heterogeneous and most likely arises from different etiologies and is mediated by different mechanisms (Berton et al. 2012; Chen et al. 2000; Kendler et al. 1996). It should also be noted that depression is frequently comorbid with other neuropsychiatric disorders. For example, depression and anxiety disorders occur together in approximately 50% of depressed patients (Hirschfeld 2001), and depression has a comorbidity rate of 21% with alcohol abuse (Briere et al. 2014).

1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful). (Note: In children and adolescents, can be irritable mood.)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation).
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (Note: In children, consider failure to make expected weight gain.)
4. Insomnia or hypersomnia nearly every day.
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
6. Fatigue or loss of energy nearly every day.
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

**Figure 1** List of symptoms for diagnosis of depression from the DSM IV (American Psychiatric Association 2013). Five (or more) of the above listed symptoms must have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
Despite some drawbacks, NHPs continue to play a major role in examining putative mechanisms and predisposing factors leading to human depression. Ironically, many NHP experimental models begin with a phenomenon observed in humans that is then taken into the laboratory to look for mechanisms, in essence going from bedside to bench. NHPs have proven especially useful in exploring the effects that early experience has on later behavioral and physiologic development in relation to depression. Much of the utility of the model is due to the fact that NHPs are very like humans in that they live in a complex social milieu and are characterized by an extended childhood dependent on parental care long after they are nutritionally self-sufficient. Disruptions in early experience marked by parental absence or insufficiency are similarly salient to both humans and NHPs. In addition, because the genetics of these animals can often be determined and life history experiences can be systematically varied, they are especially useful in studying gene-environment interactions and epigenetic effects. Because of these attributes, NHP models are especially useful in investigating the neurobiology of depression. Although rodents have also been utilized to investigate depressive neurobiology, NHPs appear to be a better model, as their brains more closely resemble humans (Amaral and Lavenex 2007; Preuss 1995; Wise 2008). Although data from human studies may produce compelling correlations, only controlled variation of experimental variables allows inference of causality. Modeling the effect of early experience on subsequent propensity towards depression in humans relies on (often faulty) retrospective recall of distant past events. NHPs, on the other hand, can be assigned to controlled rearing protocols and followed prospectively into adulthood. In addition, NHP infancy is considerably shorter than that of humans, allowing developmental variables to be assessed more rapidly. Studying the relationship between physiologic and/or neurobiologic underpinnings with specific suites of symptoms in a controlled animal model can help in disentangling confounding factors present in humans. A complete review of all studies of NHP models of depression is beyond the scope of this article because of the sheer volume of literature. This review will focus on experimental paradigms that have utilized stressful early experience in NHPs to gauge impact on later development. It will also provide two representative examples of how these paradigms have been used to investigate the behavioral, physiologic, and genetic underpinnings of depression, especially as it relates to the role of early experience.

**Predisposing Factors and the Diathesis-Stress/Two-Hit Model of Depression**

Many theories of the etiology of human psychopathology (including depression) hypothesize a Diathesis-Stress model whereby underlying (probably genetic) vulnerability is acted upon by environmental factors to produce depressive symptoms. Stress in the Diathesis-Stress model can occur at any point in the lifetime. However, the Two-Hit model expands this model to encompass the role of stress in the developing organism by proposing that neuropsychiatric disease, including depression, can be caused by two (or more) adverse events or stressors during the lifetime of an organism. It is hypothesized that events encountered during brain development (the first “hit”) produces a central nervous system (CNS) that is in essence primed to express the psychopathology (Hill et al. 2014; Mc Elroy and Hevey 2014). Later on, the primed CNS encounters a second hit in the form of stress or another triggering event, and the psychopathologic symptoms emerge. This model has been extensively utilized to explain the emergence of later depression in humans who have encountered adverse early experience.

In humans, one of the hallmark predictors for depression is a family history of depression (Chen et al. 2000). Heritability of major depression is estimated to be approximately 31–50% in human populations (Fabbri et al. 2013; Levinson 2006). These data suggest that there is a genetic vulnerability (or diathesis) for depression. In addition, a large number of human studies have found that early-life trauma in the form of neglect, abuse, or parental loss (either through death or divorce) results in an increased propensity for depression as adults (Heim et al. 2004; Heim and Nemeroff 2001; Kivela, et al. 1998; Slavich et al. 2011; Widom et al. 2007). It is also established that stressful events encountered later in life are often associated with the onset of depression (Hammen 2005; Kessler 1997). Therefore, human studies provide correlational support of the Diathesis-Stress/Two-Hit model, indicating that both genetics and early adverse experience (the first hit) are related to later depression that is often associated with a precipitous stressful event (the second hit). The use of NHPs has been of special utility in allowing controlled investigation of the impact of stress on behaviors and physiologic associated with depression, both during development and in adulthood, and has provided further supporting evidence for the Diathesis-Stress/Two-Hit model of depression.

It should be noted that although this model has utility in describing how early experience shapes the predisposition for developing depression, it is only one of a myriad of depression models (for examples of other models, see Holsboer 2000; Raison et al. 2006; Willner 1983; Zunszain et al. 2011). It has also been shown that overwhelming physiological “trigger” events can produce behavioral pathology even in a “non-primed” CNS. For example, physiological states such as Cushing’s syndrome, which results in extremely high levels of circulating cortisol (Tiemensma et al. 2010), or administration of therapeutic treatments such as proinflammatory cytokines (Felger and Miller 2012) have been shown to cause depressive symptoms even in presumably nonvulnerable individuals. Also, following clinical observations in humans, NHP studies have confirmed that administration of certain agents, including corticotrophin releasing factor (CRF), interferon, and reserpine, can, in and of themselves, produce or intensify depressive symptoms (Felger et al. 2007; Kraemer and McKinney 1979; McKinney 1971; Owens and Nemeroff 1988; Strome et al. 2002).
Experimental Models of Early Adversity Utilized in NHP Models of Depression

Several models of early adverse experience and depression have been extensively utilized in NHP models of depression. This section will outline a short history and description of these models, and following sections will describe how these paradigms have been utilized to model mechanisms hypothesized to be associated with depression. These models fall into several categories: (1) rearing without a mother but with contact peers during development (peer-rearing), (2) separating (and reuniting) the infant from the mother at various time points during development (maternal separation), and (3) subjecting the mother to stress-inducing experimental manipulations that affect the mother-infant relationship and alter infant developmental trajectories.

Lack of Maternal Care: Peer-Rearing

Historically, the bulk of studies assessing the effects of rearing only with peers has been conducted in rhesus macaques. Most involve housing animals singly and allowing them to interact for varying periods daily, although some have continuously housed animals together. Peer-rearing produces significant behavioral and physiological changes in NHPs that persist into adulthood when compared with mother-reared controls and are at increased risk for affective responses that are seen as indices of depression-like states (McKinney and Bunney 1969; Mineka and Suomi 1978). Behavioral changes include increases in self-directed, stereotypic, and self-injurious behavior and lower levels of play. Peer-reared animals are also characterized as being more timid, fearful, and anxious compared with mother-reared counterparts (Higley et al. 1996a,b; Kraemer 1997). This is of interest, because, as discussed above, anxiety and depression are often comorbid. Alterations in cerebrospinal fluid (CSF) oxytocin, norepinephrine, serotonin, dopamine, and their metabolites have also been reported (Clarke et al. 1996, 1999; Higley et al. 1992; Kraemer et al. 1983, 1984; Winslow 2005), many of which are consistent with those seen in depressed humans (for review, see Kramer and McKinney 1979). Peer-rearing also produces dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. However, these data are often conflicting, reporting higher, lower, or no significant differences in basal and stress-responsive cortisol and adrenocorticotropic hormone (ACTH) when compared with mother-reared controls (Barr et al. 2004b; Capitanio et al. 2005; Clarke 1993; Fahlke et al. 2000; Higley et al. 1992; Meyer and Bowman 1972; Sackett et al. 1973).

Inconsistency among the effects of early rearing on cortisol could be caused by a host of factors, including sample size, genetics, time of sampling (cortisol has a diurnal rhythmicity), age when the sample was obtained, and differences in rearing and housing, including the amount of time that infants were allowed to interact with peers and, in cases where stress responsivity has been measured, the nature of the stressor (Capitanio et al. 2005; Sanchez 2006). Although the relationship between HPA axis function and peer-rearing is complicated, the relatively consistent finding of all these studies is that this type of early rearing produces HPA axis dysregulation in one form or another. This is important, as it is consistent with human studies of depression that show that HPA axis dysregulation is present in a majority of depressed patients (Aubry et al. 2007). The peer-rearing paradigm has also been extensively utilized to assess the effects of early experience on later anxiety and depressive behaviors exhibited in response to later stress (Barr et al. 2003; Gilmer et al. 2003; Spinelli et al. 2012). It has also been utilized to assess gene-environment interactions relating to excessive alcohol consumption as a NHP model of alcoholism (Fahlke et al. 2000; Schwandt et al. 2010). This is of interest, because, as noted above, alcohol abuse and depression are often comorbid in humans.

At first blush, it might appear that this form of deprivation could never occur in human populations; however, this would be incorrect. Although institutionalization of children, either temporarily or permanently separated from their parents, is standard practice in most developed countries, this is not the case in developing countries. It has been reported that more than 8 million children worldwide currently reside in institutions, mostly orphanages, and therefore in essence are “peer-reared” (Groark and McCall 2011). One of the most publicized examples of human deprivation inadvertently and unfortunately occurred with Romanian orphans who were institutionalized in infancy, and early rearing was in an impoverished environment without a strong caregiver attachment. These infants showed increased depression, attention-deficit/hyperactivity disorder, anxiety, and disruptive behavior compared with controls. Many of these orphans, especially those who remained institutionalized for more than 6 months, showed behavioral and cognitive deficits into adolescence even when adopted into well-functioning British families (Rutter et al. 2010, 2012). Interestingly, the susceptibility to depression and other emotional problems in adolescence in this population has been shown to be moderated by both genetic factors (variability in the serotonin transporter gene discussed later in this manuscript) and later life stresses (Kumsta et al. 2010), thus providing support for the Diathesis-Stress/Two-Hit model of depression. Therefore, studies in both humans and NHPs show that lack of maternal care during development can lead to impaired developmental outcomes, including depression.

Disruption of the Mother-Infant Relationship during Development

Mother-Infant Separations

Studies in NHPs have shown that disruption of early experience in the form of maternal separation causes infant distress and results in both temporary and permanent alterations in infant behavior and physiology, many of them relevant to
depression. These studies have a long rich history and initially were undertaken in macaques beginning in the 1960s (Hinde 1966; Jensen and Tolman 1962; Seay et al. 1962; Seay and Harlow 1965). They have now been replicated in a variety of NHP species (for review, see Gilmer and McKinney 2003; Reite and Short 1983) and represent a major tool in investigating the effects of early experience on infant development. Classically, these studies separate mothers from infants for a period of 10 days to 2 weeks when the infants are about 6 months old (when infants are nutritionally independent), although more recent studies in marmosets (described later in this article) have employed shorter, more frequent separations (Pryce et al. 2011).

The typical macaque response to maternal separation has been described as a two-step process comprised of an initial agitation phase characterized by behaviors indicative of distress, including increased vocalization, vigilance, and locomotor activity accompanied by increased heart rate and body temperature (Reite et al. 1981a). There is also a rise in total and free cortisol, indicating a physiologic stress response (Laudenslager et al. 1995). After 24 to 48 hours, infants typically enter the second step in the process, which has been described as the “despair” or “depression” phase, characterized by disruptions in sleep patterns, circadian rhythms, heart rate, and appetite. Infants also exhibit psychomotor slowing and loss of interest in play and other social interactions and often assume collapsed, depressive-like postures (Kaufman and Rosenblum 1969; Reite and Short 1978; Reite et al. 1981b, 1982; Seiler et al. 1979). Antidepressant pharmacologic interventions have been found to reduce separation responses, thus providing predictive validity for this model (Hrdina et al. 1979). There have been some factors shown to relate to the severity of the separation response; length of separation (the longer the separation, the more severe the response) increased maternal rejection prior to separation (infants who are rejected more often have a more severe response; Harlow and Suomi 1974). Interestingly, differences in age at separation up to 270 days (a point at which young macaques are nutritionally independent) did not produce striking differences in the separation response. This indicates that the mother-infant relationship is similar to humans in its long-lasting importance (Harlow and Suomi 1974). Although the above describes the prototypical response to maternal separation seen in macaques, it should be noted there are significant individual and species differences, including the absence of a “despair” phase in some individuals and some species (Preston et al. 1970; Reite and Short 1983; Rosenblum and Kaufman 1968).

It has been suggested that maternal separation in macaques provides an excellent model of an anakalic depression, a term coined by the psychiatrist Rene Spitz describing the behavioral pathology of children separated from their mothers for an extended period of time (Spitz 1946). The protest/despair responses are strikingly similar to those seen in human infants and young children who are separated from their mothers. The infants exhibit initial agitation followed by inactivity, weepiness, and social withdrawal (Bowlby 1960; Spitz 1946). It is interesting to note that although these responses are commonly viewed as negative, Kaufman and Rosenblum (1967, 1969) hypothesized that, in macaques, they could be adaptive in the proper ecological setting. The protest phase involving agitation and increased vocalization could increase the likelihood of reunion with the mothers. The later despair phase, which is characterized behaviorally by inactivity, could represent the conservation of physiologic resources as well as decrease the likelihood of conspecific aggression and being taken by a predator. Likewise, other researchers have hypothesized that in some cases, depressive symptoms in humans may also be adaptive in that they allow the individual to conserve resources that can be directed toward more productive enterprises when the situation changes (Allen and Badcock 2006).

The maternal separation model received increased interest when it was found that separation from the mother (even for a short period of time) could not only produce transient depressive symptoms in young animals, but could result in sustained alterations in the mother-infant relationship as well as produce behavioral and physiologic changes in the infant that were apparent into adulthood. Spencer-Booth and Hinde (1971a,b) found that infants who had undergone maternal separation spent less time off their mothers as long as 6 months after the separation. In addition, as juveniles, separated macaque infants played less, were less social, were more timid, and had an increased negative response to novelty when compared with nonseparated controls (Caine et al. 1983; Capitanio and Reite 1984; Capitanio et al. 1986). Other studies showed changes in physiological parameters in separated infants into adulthood, including relatively permanent alterations in immune response (Laudenslager et al. 1996; Reite et al. 1981b). The maternal separation model, like the peer-rearing model, has been extensively utilized to examine the effects of early experience on later neurobiology and behavior modeling depression.

Creating Maternal Stress, Thereby Altering the Mother-Infant Relationship

Another set of experimental paradigms has studied the effect of maternal stress on infant development. Rosenblum and Paullly (1984) set up an experimental protocol in which macaque mothers were provided food in one of three conditions: (1) ad libitum (low foraging), (2) adequate amounts of food available, but difficult to obtain (high foraging), or (3) conditions alternated between low and high foraging (variable foraging [VF]). The VF condition was unpredictable, proved to be stressful for the mothers, and negatively altered the mother-infant relationship. Mothers in the VF group made efforts to limit contact with their infants most often. Infants, in turn, made increased efforts to reestablish contact, resulting in a significantly higher “mother leave” and “infant make contact” ratio when compared with the mother-infant dyads in the other conditions. Infants from mothers in the VF condition showed lower levels of play, extremely high levels
of disturbance behaviors, altered autonomic functioning, and depressive episodes (assuming collapsed postures). In adulthood, VF infants showed alterations in behavior as well as alterations in CNS structure and CSF biogenic amines (Coplan et al. 2009; Mathew et al. 2003; Smith et al. 2001). As adults, infants rearved by VF mothers had CRF levels in the CSF that were either chronically elevated levels or lowered, depending on what point in development their mothers had been subjected to VF. VF encountered early in infant development (10–12 weeks) lead to increased levels of CRF, whereas VF later in development (18–20 weeks) lead to lower CRF levels (Coplan et al. 1996, 2001; Mathew et al. 2002).

All of the models described above have utilized experimental interventions to produce stress during development. However, ecological validity has been provided by investigations that show the effects of NHP maternal rejection and abuse on infant development in free ranging, group-living rhesus macaques. NHP mothers vary significantly in the amounts of rejection and abuse that they exhibit towards their infants. These behaviors appear to be trait-like, as rejecting and abusive mothers tend to act consistently towards all of their offspring (Maestripieri et al. 1998a, b; Maestripieri and Carroll 1998a, b). Infants who experienced greater rejection (denial or limitation of suckling, mother breaking or denying infant contact) had lower levels of CSF monoamine metabolites (5-hydroxyindoleacetic acid [5-HIAA], 3-methoxy-4-hydroxyphenylglycol [MHPG], and homovanillic acid [HVA]) and engaged in more solitary play and avoidance of other individuals compared with infants experiencing lower levels of rejection. This was true even when infants from non-related females were cross-fostered to rejecting mothers (Maestripieri et al. 2006a, b). In addition, 5–10% of macaque mothers show patently abusive behavior towards their infants (Maestripieri et al. 1997; Maestripieri and Carroll 1998a, b). These behaviors include dragging the infant by the limb or tail, throwing the infant, or roughly pinning the infant to the ground. Abused infants exhibit a physiological stress response (elevated basal cortisol levels) during their first month of life when abuse is most frequent compared with nonabused infants (Parker and Maestripieri 2011). Data suggest that there are relatively enduring effects on the HPA axis, as abused infants have higher levels of plasma cortisol after exogenous CRF administration up to 36 months of age, suggesting that their HPA axis is hyperresponsive (Sanchez et al. 2010). Behavioral data show that abused infants also take longer to attain independence from their mothers. In addition, there is an intergenerational effect, as females who have been abused also are more likely to abuse their own infants (Maestripieri et al. 1997; Parker and Maestripieri 2011). Given the strong relationship between childhood abuse and later depressive behavior as well as the correlation between HPA axis dysfunction and depression in humans, this model offers a promising, ecologically valid model for future study, especially as it relates to the intergenerational effects of abuse and depression in humans.

Thus, the NHP models of disrupted early experience described here consistently show that stress during development results in deleterious behavioral effects and altered physiology into adulthood that are consistent with those seen in humans. These models have been utilized to identify mechanisms that shed light on how early experience alters the CNS structurally and functionally to produce later pathology in adulthood. Two examples of the utility of these models are described below.

The Hippocampus, the HPA Axis, and Depression: It’s Complicated

NHP studies have elucidated the role that early experience and later stress play in forming the architecture and function of the hippocampus and its relationship to depression. In humans, the hippocampus is associated with regulation of emotional functioning and memory, both of which can be altered during depression (Kaymak et al. 2010). In addition, the hippocampus plays an important role in regulating corticosteroid activity by exerting a general inhibitory influence over the HPA axis and participating in glucocorticoid feedback (reviewed in De Kloet et al. 1998). This is important, as many depressed humans exhibit HPA axis dysregulation, including hypercortisolism and/or impaired negative feedback when challenged with a synthetic glucocorticoid (Akil et al. 1993; Arborelius et al. 1999; Checkley 1996; Halbreich et al. 1985; Heim and Nemeroff 2001; Jareho 2013; Nemeroff 1996; Wong et al. 2000). Human studies on postmortem brains of suicide victims show that they have significantly lower levels of glucocorticoid receptors (GRs) in the hippocampus compared with nondepressed controls, and the authors hypothesize that this contributes to the HPA axis dysregulation seen in major depression (Medina et al. 2013; Webster et al. 2002).

There have been numerous reports of alterations in hippocampal function and structure (including smaller hippocampal volume) in human patients with major depressive disorder, and these changes can be relatively enduring even after depressive symptoms have resolved (Campbell et al. 2004; Frodl et al. 2002a, b; Fuchs and Gould 2000; Kempston et al. 2011; Koolschijn et al. 2009; McKinnon et al. 2009; Sheline et al. 2002). Treatment with antidepressants, which ameliorates behavioral symptoms, also results in hippocampal neurogenesis in both humans and NHPs, implying that hippocampal atrophy is directly related to depressive symptomology (Hanson et al. 2011; Perera et al. 2011). High levels of corticosteroid receptors within the hippocampus make it particularly vulnerable to effects of long-term stress (Joëls 2008). It is known that glucocorticoids are involved in neurodegenerative effects in the hippocampus, which include loss of neurons, dendritic atrophy, and loss of synaptic contacts (Sousa and Almeida 2002). It has been hypothesized that high levels of glucocorticoids caused by stress during development alter the structure and function of the hippocampus, decreasing its regulatory ability on the HPA axis (Heim et al. 2004). Correlational data from human studies support this hypothesis, as hippocampal atrophy has been reported in human adults who have suffered maltreatment and abuse as children...
(Bremner et al. 1997). Studies in NHPs have shown that animals that have experienced prolonged social stress or were experimentally administered high levels of cortisol also develop hippocampal damage (Sapolsky et al. 1990; Uno et al. 1989). Therefore, it is relatively clear that increased corticosteroids result in damage to the hippocampus, and this damage is related to both behavioral and physiological symptoms exhibited during depression. The question, still in debate, is whether hippocampal atrophy is the result of a state (does depression disrupt the HPA feedback loop, increasing corticosteroids, which in turn cause damage to the hippocampus) or a trait (hippocampal atrophy and disruption of the HPA feedback loop are stable characteristics of those individuals who are susceptible to depression and thus precedes the depressive state). The NHP model offers the ability to directly assess the impact of early experience on hippocampal development through experimental manipulation of rearing paradigms and longitudinal assessment.

Effects of Early Experience on the Hippocampus: The NHP Story

Researchers have used the maternal separation model in marmosets, using repeated short-term maternal separations during infancy, to examine the effects of this early life stressor on the hippocampus. As with macaques, marmoset infants separated from their mothers showed typical behavioral and endocrine responses indicative of distress. As juveniles, these animals showed alterations in biochemical, behavioral, and cardiovascular functioning; increased basal cortisol activity; and impaired negative feedback in the HPA axis (Dettling et al. 2007; Pryce et al. 2011). These juveniles also showed behavioral symptoms consistent with depression, including mild anhedonia (indicated by fewer responses for a highly preferred reward on an operant task), impaired behavioral inhibition on an object retrieval task, and deficits in reversal learning, which the authors attributed to deficits caused by an increase in affective responses when the previously rewarded response was no longer rewarded (Dettling et al. 2002a,b, 2007; Pryce et al. 2004a,b).

What were the direct effects of these early stressors on hippocampal volume? Interestingly, Law et al. (2009a) found that marmoset infants exposed to separation stress did not differ from controls in hippocampal volume. What was found, however, was that the neurobiology of the hippocampus was altered by experiencing maternal separation. Pryce et al. (2011) and Arabadzisz (2010) both found that maternal separation during infancy led to reductions in both mineralocorticoid (MR) and glucocorticoid receptor gene expression in the hippocampus when the monkeys were adolescents. Law et al. (2009b) found that maternal separation in infant marmosets also produced long-term effects on other genes expressed in the hippocampus, including decreases in growth-associated protein-43 (GAP-43) mRNA and serotonin 1A receptor (5-HT1a) mRNA and increases in gamma-aminobutyric acid transporter mRNA. The authors concluded that these changes were suggestive of alterations in synaptic plasticity and functioning.

These studies show that the neurobiology of the hippocampus can be altered by early experience, and alterations can include a decrease in corticosteroid receptors. This finding has functional significance, because studies in NHPs have shown that decreased levels of corticosteroid receptors in the hippocampus can lead to impairments in the HPA feedback loop and thereby lead to elevated levels of cortisol (Brooke et al. 1994). However, results differed slightly when modeled in squirrel monkeys. Lyons et al. (2001) found no direct effect on hippocampal volume for adult squirrel monkeys whose mothers were subjected to the VF paradigm while they were infants. These results are consistent with those seen in the marmoset model. However, unlike the marmoset model, they did not find that MR and GR were altered in the hippocampus but instead found alterations in the prefrontal cortex, a brain area that has also been linked to depression (Albert et al. 2014; Lyons et al. 2002). Patel et al. (2008) also reported no difference in MR and GR in the hippocampus in adult squirrel monkeys that, as infants, had undergone maternal separation.

Interestingly, squirrel monkeys exposed to social separation stress as adults show a significant decrease in GRs in the hippocampus compared with nonstressed controls (Lyons et al. 2002; Patel et al. 2008). Although some of the animals in the Lyons study had undergone social separation as infants, the authors did not find any significant interactions by early rearing experience (i.e., there was no greater effect in animals who had undergone separation as infants). However, the number of animals in each group was small (only 2–4) and therefore lacked the statistical power to detect significant interactions.

Therefore, marmosets that experience stress during development appear to exhibit trait-like changes in the neurobiology of the hippocampus, which includes long-lasting downregulation of corticosteroid receptors. Squirrel monkeys do not show these changes in response to developmental stress but do exhibit a state-like downregulation of corticosteroid receptors in response to stress as adults. Although the reason for differences between the marmoset and squirrel monkey models is not known, it is possible that some differences could be attributable to differences in corticosteroid expression during ontogeny (Pryce 2008).

One set of studies has shown that there are frank decreases in hippocampal volume as a result of early adverse experience. Adult bonnet macaques whose mothers had been exposed to VF while they were infants had significantly lower left hippocampal volumes and also evinced indices of decreased hippocampal neuronal viability when compared with controls (Coplan et al. 2010; Jackowski et al. 2011). Differences in hippocampal volume observed in this study could be due to the fact that these animals were measured at an older age than the previous studies. Human studies indicate that hippocampal volumetric differences associated with depression may not be apparent until adulthood (Karl et al. 2006). Differences could also be attributable to the nature of the stressor (intermittent social separation vs. VF) or species.
It is interesting to note that some adult wild-born macaques spontaneously exhibit depressive behaviors even though they have not been subjected to experimentally induced early-life stress (Camus et al. 2014; Shively et al. 1997; Willard and Shively 2012). This model has been most extensively researched in adult cynomologus macaque females who were part of a long-term study on the effects of diet and health outcomes. Depressive behaviors exhibited by some females include a slumped/collapsed body posture with open eyes and a relative lack of responsivity to environmental stimuli (very like the depressive posture assumed by maternally separated infants). They also exhibited higher levels of passive social contact and lower levels of activity. Whereas depressive behavior is seen at a greater rate in socially subordinate (and presumably more stressed) females, subordination and depression were not homologous, because 10% of dominant animals also exhibited depressive behaviors (Shively and Willard 2012). Depressed behavior was also associated with greater mortality and lower body weights compared with non-depressed females. In addition, depressed adult females show alterations in astrocyte and synaptic proteins in the hippocampus indicative of altered synaptic plasticity and postsynaptic integrity (Willard et al. 2014). They also show reductions in hippocampal volume and resistance to dexamethasone suppression that are similar to those seen in depressed humans (Willard et al. 2009, 2013). Thus, the behavioral and physiologic profile of these depressed, adult, NHPs closely resembles that seen in human depression, including significant reductions in hippocampal volume.

Although more research needs to be done, these studies suggest that early stress may not alter hippocampal volume per se, but rather constructs a CNS that is poised to be physiologically hyperreactive to later stressful situations. If the animal does not experience significant stress, the cascade of events leading to hippocampal atrophy and depressive behavior may not occur. However, if significant or prolonged stress occurs later in life (comprising a second hit), it can result in increased corticosteroids and can trigger the cascade effect. Decreased corticosteroid receptors lead to decreased negative feedback and prolonged HPA activation, which in turn lead to hippocampal insult affecting both physiologic and behavioral hippocampal functions.

Serotonin and Depression: Where Genes Meet the Environment

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that is ubiquitous in the brain. The activity of serotonin arises in the brainstem from clusters of neurons known as the raphe nucleus. These neurons extend to virtually all parts of the CNS and are particularly represented in those areas of the brain implicated in emotional regulation (Anbazhagan et al. 2010; Beaudoin-Gobert and Sgambato-Faure 2014; Lowry et al. 2005). Studies have shown that agents that reduce serotonin synthesis can induce depression and that selective serotonin reuptake inhibitors (which make more serotonin available at the synapse) can ameliorate depressive symptoms (reviewed in Beaudoin-Gobert and Sgambato-Faure 2014). Serotonin has enormous influence over many CNS functions, including but not limited to mood, appetite, sleep, memory, learning, anxiety, and aggression (Meneses and Liy-Salmeron 2012). All of these systems can be dysregulated in conjunction with a diagnosis of depression (Lucki 1998). In humans, it has long been established that low levels of central serotonergic activity appear to be trait-like and are associated with a variety of behavioral anomalies, including depression, anxiety, aggression, impulsivity, and suicidal behavior (Kruesil et al. 1990; Malison et al. 1998; Roy et al. 1986). Depressed human patients exhibit decreased levels of CSF 5-Hydroxyindoleacetic acid (5-HIAA, a metabolite of serotonin that is used as a biomarker of serotonergic activity) as well as decreased serotonin receptor binding as measured in postmortem brains when compared with nondepressed controls (Manji et al. 2001). In addition, the serotonergic system (like the hippocampus) also plays an important role in regulation of the HPA axis (Weidenfeld et al. 2002). Therefore, in humans, lower levels of CNS serotonin are implicated in both the behavioral symptomology of depression as well as the dysregulation of the HPA axis and hypercortisolemia seen in depression.

Correlations between behavior and serotonergic profiles are also exhibited by NHPs. Animals with low levels of CSF 5-HIAA have been engaged in more impulsive and aggressive behaviors, engage in lower levels of social interactions, attain lower social rank, experience higher mortality, and consume higher rates of alcohol in an experimental setting (Higley and Linnoila 1997; Higley et al. 1996a,b; Mehlman et al. 1995; Westergard et al. 1999). In addition, experimental administration of agents known to increase central serotonergic levels (fluoxetine, quipazine, tryptophan) results in increased social behaviors and decreased levels of anxiety vigilance behaviors and aggressive behaviors (Higley et al. 1998; Raleigh et al. 1985; Shively et al. 2014).

Effects of Early Experience on CNS Serotonin

NHP models allow prospective study of the effects of early experience on serotonergic profiles. Studies in NHPs have shown that stress during development can lead to alterations in CSF monoamine levels, including serotonin. Peer-rearing paradigms have consistently led to lower levels of CSF 5-HIAA in NHPs as juveniles and adults (Barr et al. 2003, 2004b; Higley et al. 1991, 1996c; Kraemer and Clark 1996; Shannon et al. 2005) as well as decreased serotonin transporter binding in certain areas of the brain (Ichise et al. 2006). In addition, infants from macaque mothers who have been subjected to the VF model had either elevated or lowered CSF 5-HIAA compared with controls, depending on when the mothers were exposed to the stressor (Coplan et al. 1998; Mathew et al. 2002). The relationship of early stress to decreased levels of 5-HIAA does not just occur in response to experimentally induced stressors. Maternal abuse of group-
living infant macaques results in infants who have lower concentrations of CSF 5-HIAA. The lowered concentrations could not be explained by heritability factors, because the effect was present even in nonrelated infants cross-fostered to abusive mothers (Maestripieri et al. 2006b). Therefore, stressful experiences during development can have direct, significant, and long-lasting effects on serotoninergic profiles in NHPs.

Genetic Influences on Serotonin

To further complicate the story, however, there is one widely researched genetic system that is known to be involved in the regulation of central serotonin levels. This is the serotonin transporter gene (5-HTTLPR) that regulates levels of serotonin transporter protein in the CNS. The serotonin transporter protein controls the amount of serotonin that is available in the synaptic space by binding excess serotonin and transporting it back into the cell body for processing. Both humans and rhesus macaques show a repeat-length polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR), yielding a long (l) and a short (s) allele. Like humans, rhesus macaques that possess an s allele show decreased transcriptional efficiency and lower levels of central serotonin and its metabolite 5-HIAA when compared with animals with two long alleles (Bennett et al. 2002; Heils et al. 1996; Hranilovic et al. 2004; Lesch et al. 1997). Furthermore, interindividual differences are stable across time in NHPs, suggesting that the serotoninergic profiles are trait-like (Higley and Linnoila 1997; Kaplan et al. 2000). In humans, the s allele has been associated with an increased incidence of depression and suicide as well as anxiety and impulsivity, especially when individuals have suffered stressful events or maltreatment as children (Caspi et al. 2003; Kaufman et al. 2004; Kendler et al. 2005; Lesch 2011; Wilhelm et al. 2006). Humans who have the s allele also have a greater HPA response to stress (Caspi et al. 2010). In fact, human carriers of the s allele have exhibited a greater propensity for negative physiologic and behavioral responses to a variety of experimentally administered environmental and social challenges (Caspi et al. 2010). It is interesting to note that NHP females with the l/s genotype were significantly more likely to be abusive mothers and evince dysregulation of the HPA axis compared with females with two long alleles (McCormack et al. 2009). In turn, abused infants with the l/s genotype had significantly higher cortisol and more negative behavioral responses to a stressful situation (McCormack et al. 2009). In a rare study that had enough animals to explore the effects of the s/s genotype, Sorenson et al. (2013) found that in response to stress, young rhesus macaques had higher levels of cortisol as well as dexamethasone when compared with l/l or l/s subjects. There has also been interest in this particular polymorphism, as it has been shown to be related to the propensity towards type II alcoholism in humans (Hallikainen et al. 1999; Sander et al. 1998) and has been utilized to explore the role of genetics and early experience on alcohol consumption in NHP models of alcoholism (Higley et al. 1991, 1996a,b). As previously described, depression and alcoholism are often comorbid.

NHPs possessing a short allele also evince behavioral differences when compared with animals possessing two long alleles. These differences emerge very early in life. Champoux et al. (2002) found that nursery-reared infant macaques carrying the s allele exhibited more struggling, were less easy to console, and manifested more severe and frequent signs of emotional distress when tested on a neonatal test battery conducted from 7 to 30 days of age. This is similar to human data that indicate that infants who possess an s allele were rated higher in negative emotionality in a questionnaire filled out by their mothers (Auerbach et al. 1999). Studies of adult NHPs have shown that animals who are carriers of the s allele show reduced gray matter volumes and altered metabolic activity in brain regions related to emotional reactivity (reviewed in Caspi et al. 2010). Therefore, the possession of an s allele appears to be related to behavioral and physiologic indices of greater reactivity and emotionality. It is interesting to note that in the human population, allelic distribution is 32% for the l/l genotype, 49% for the l/s genotype, and 19% for the s/s genotype. Published studies of rhesus macaques and bonnet macaques indicate that the s/s variant is rare, comprising <10% of genotyped animals (Barr et al. 2004a; Champoux et al. 2002; Jackowski et al. 2011; McCormack et al. 2009). This may indicate that having the s/s genotype has negative consequences for survival in NHPs. In the following studies, animals with s/s genotypes are excluded, as they were too rare to include in the analysis.

Gene-Environment Interactions

NHPs have proven to be excellent models for exploring the relationship between the serotonin transporter gene and early adverse experience. The NHP model allows animals of known genetic profiles to be experimentally exposed to controlled environmental stressors and followed longitudinally during development. In experimental studies, there are some indications that gene-environment interactions are apparent early in development. Spinelli et al. (2007) found that during a social separation as infants, peer-reared l/s infants were more likely to exhibit behavioral pathology in the form of stereotypic behaviors and agitation during separation compared with l/l peer-reared or mother-reared infants of either genotype. Therefore, peer-reared l/s infants appear to exhibit a dysfunctional response to stress. Interestingly, peer-reared l/l infants showed increased levels of vocalization, passive behavior, and self-exploration compared with other groups. The authors labeled these as indicative of greater “acute despair,” however, considering the hypothesis of Kaufman and Rosenblum previously discussed, these behaviors should not necessarily be considered maladaptive, as they could increase the probability of reunion and conserve energy until that reunion was possible.
Gene-environment interactions for the l/s genotype are also apparent later in development. Bennett et al. (2002) found that juveniles with the l/s genotype had lower levels of CSF 5-HIAA compared with l/l juveniles, but only if they were peer-reared. Similarly, Barr et al. (2004b) found indications of altered HPA axis function in 6-month-old peer-reared l/s macaques, as indicated by greater increases of ACTH during a social separation. Barr et al. (2003) also found that peer-reared juveniles with the l/s genotype were more likely to engage in aggressive behavior compared with peer-reared l/l juveniles or mother-reared juveniles of either genotype. All these studies indicate that effects associated with the short allele are differentially expressed depending on early experience and are consistent with data from human studies showing a significant relationship between possession of the short alleles, early adverse experience, and depression (Brown et al. 2013). Thus, the serotonin transporter gene appears to be one form of diathesis that interacts with childhood stress to increase vulnerability to depression in adulthood.

In a separate study, however, Barr et al. (2004a) found that juvenile l/s males had higher levels of ACTH in response to social separation regardless of whether they were peer- or mother-reared, indicating a main effect of genotype. Higher levels of ACTH were seen in females only if they were peer-reared. The authors attribute this to females being more affected by early adverse experience. However, an alternate, equally plausible interpretation would be that l/s females were protected from their genetic predisposition by being mother-reared. In essence, competent maternal rearing override their genetic predisposition. This brings up an important issue that has not been considered in any studies that compare mother-reared with peer-reared individuals; that is the effect of the differences in the mother-infant relationship. Differences in maternal treatment of the infant could well introduce variability in outcomes among these studies. This is especially important given that maternal mistreatment has been found to be related to lower levels of 5-HIAA in both human and NHPs (Maestripieri et al. 2006a,b; Roy 2002). Therefore, it would be prudent for future studies to include measures of mother-infant interactions when comparing mother-reared with peer-reared infants.

Conclusions

NHP models of early adverse experience have provided significant support for the Diathesis-Stress/Two-Hit model of depression. Experimental paradigms involving stressors comprising the absence or separation of infants from mothers reliably result in altered behavioral and physiological profiles. These behavioral, neuroendocrine, and neurobiological profiles are consistent with those seen in depressed humans. NHPs provide an excellent model of human depression. They share a close evolutionary history and are similar in both neuroanatomy and neurobiology to humans. They are especially appropriate for studying developmental variables, as many aspects of NHP infancy and social functioning are similar to humans, whereas development occurs at an accelerated rate allowing for more rapid assessment of experimental variables. In addition, the NHP model allows direct manipulation of putative risk factors not ethically possible in human studies. The ability to manipulate variables and longitudinally follow infants has allowed causal attribution of the deleterious effects of stress on behavioral and physiological development.

On a more esoteric note, studies in NHPs have provided evidence that can serve to dispel the stigma that is commonly associated with depression. NHP studies show that experiences during development interact with underlying genetic and/or other physiologic profiles to alter known (and undoubtedly many unknown) aspects of the CNS, thereby predisposing individuals to depression later in life. This takes depression out of the realm of an emotional state and into the realm of organic disease, much like diabetes or cancer, whereby underlying predispositions are acted on by the environment to produce pathology.

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