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MATERNAL SEPARATION AS A MODEL OF EARLY STRESS: EFFECTS ON ASPECTS OF EMOTIONAL BEHAVIOR AND NEUROENDOCRINE FUNCTION

Despina A. Tata

Aristotle University of Thessaloniki, Greece

***Abstract*:** A growing body of findings underlines the critical role of the early environment in normal growth and development. Human studies suggest that severe stress during childhood increases vulnerability to development of affective psychopathology in adulthood. Neonatal maternal separation in rodents (rats or mice) is an established model of early stress for studying the neuroendocrine and behavioral effects of early adversity. The article reviews the existing evidence regarding the effects of this postnatal manipulation on the neuroendocrine response to stress and on emotion-relevant behaviors, and attempts to explain the existing inconsistencies seen in the results. Although the prevailing idea is that maternal separation augments emotionality and potentiates the endocrine response to stress, methodological differences among studies prevent us from making definite conclusions. Further studies in which parameters related to neuroendocrine function, behavior, and neuronal plasticity will be concurrently examined are warranted. In addition, the need to investigate whether early stress may increase vulnerability to subsequent challenges during the postnatal period is discussed.

***Key words*:** Early stress, Maternal separation, Neuroendocrine function

Adverse childhood experience is considered to be one of the major risk factors for the development of psychopathology. According to epidemiological studies, children who experienced physical, emotional or sexual abuse are at a higher risk for developing anxiety disorders (e.g., post-traumatic stress disorder) (Feliti, Anda, Nordenbery, Williamson, Spitz, et al., 1998. Gibb, Chelminski, & Zimmerman, 2007) and depression (Springer, Sheridan, Kuo, & Carnes, 2007). Similarly, parental loss due to suicide, accident or sudden death increases the vulnerability of children to depression and substance abuse (Brent, Melhem, Donohoe, & Walker, 2009).

***Address*:** Despina A. Tata, School of Psychology, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece. Tel.: +30 2310 997369, Fax: +30 2310 997384, E-mail: dtata@psy.auth.gr

There is evidence that the effects of early adverse experience on behavior exhibited later in life are mediated by alterations in the neuroendocrine function, specifically dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis (Ehlert, Gaab, & Heinrichs, 2001). The activation of the HPA axis and the resulting release of glucocorticoids constitute the main endocrine reaction to stress. Upon stress, the corticotropin-releasing hormone (CRH) of the hypothalamus stimulates the production and release of the adrenocorticotropin hormone (ACTH) from the anterior pituitary, which in turn enters the blood circulation causing the release of glucocorticoids (cortisol in primates, corticosterone in rodents) from the adrenal cortex (Herman & Cullinan, 1997). Clinical studies of people who experienced early stress showed abnormalities in the function of the HPA axis, such as higher basal cortisol and blunted cortisol responsiveness in the dexamethasone suppression test (Pfeffer, Altemus, Heo, & Jiang, 2007).

The interest for the neurobiological and psychiatric consequences of early adverse experience originated from Seymour Levine's work in the 1950s and 1960s. His pioneering studies demonstrated that separation of neonatal rats from their mother for a brief period of time (15 min), an experimental procedure known as early handling, renders the rodents more resilient to stress: their corticosterone levels in response to stress were lower and returned faster to basal levels compared to the nonhandled animals (Levine, 1967). Interestingly, early handling rendered rats less fearful when exposed to an environmental challenge (Levine, 2005). These findings, although unexpected, demonstrated the importance of early experience on stress reactivity and regulation of the HPA system.

In human studies, early adverse experience can result from various forms of abuse (emotional, sexual, or physical) as well as parental neglect. However, it is often the case that children are exposed to more than a single type of adversity, making difficult to establish cause-effect relationships (Loman & Gunnar, 2010). In addition, the majority of the existing human studies have been conducted on children subjected to sexual abuse in late childhood, rather than on infants or young children that experienced emotional adversity (Pryce, Rüedi-Bettschen, Dettling, Weston, Russig, et al., 2005). As such, it is difficult to apply the findings to cases of early emotional adverse experience which may result from parental neglect.

Over the last three decades scientists developed animal models of early postnatal manipulations in an effort to better understand the neurobiological and behavioral effects of early life adversity and to surpass the limitations mentioned above (Faturi, Tiba, Catallani, Kerstens, & Suchecki, 2010). Although it is extremely difficult, if not unfeasible, to model human life, pre-clinical studies may contribute to a better understanding of the neurobiological underpinnings of the early adversity as well as its consequences on behavior.

Two of the most commonly used paradigms of postnatal manipulations are the maternal separation (MS) and the early deprivation (ED). Both paradigms refer to the separation of pups (usually rodents) from their mothers (dams) for a short period of time. The MS paradigm involves daily separation of the infant rodents from the dam for either short (1hr) (Wilber, Southwood, Sokoloff, Steinmetz, &Wellman, 2007. Wilber & Wellman, 2009) or prolonged periods (3-6 hrs) (Huot, Plotsky, Lenox, & McNamara, 2002. Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007) during the first 1-3 weeks after birth. While in the MS paradigm pups remain together as a litter, in the ED paradigm pups are isolated both from their littermates and their dam, thus being socially isolated (Kosten, Karanian, Yeh, Haile, Kim, et al., 2007. McCormick, Kehoe, & Kovacs, 1998. Zimmerberg, Foote, & Van Kempen, 2009). Typically, both paradigms involve multiple sessions of separations, although there are cases of single 24hr isolation (Rosenfeld, Wetmore, & Levine, 1992. Suchecki, Duarte Palma, & Tufik, 2000).

Given that rodent pups are completely dependent on their mothers during the first three weeks of life, separation from their dams is expected to be particularly stressful. As previously mentioned, experience of stress leads to the activation of the HPA axis and the subsequent release of glucocorticoids. Considering this, we would expect stressful conditions to cause elevations in the basal levels of hormones such as glucocorticoids (e.g., corticosterone)**.** In fact, both MS and ED manipulations increase basal levels of the major stress hormone, corticosterone (Daniels, Fairbairn, van Tilburg, McEvoy, Zigmond,, et al., 2009. McCormick, Kehoe, & Kovacs, 1998. Wilber et al., 2007). These findings support the characterization of these two experimental manipulations as models of early stress.

The aim of the present paper is to provide a review of findings from pre-clinical studies regarding the effects of maternal separation on neuroendocrine function and two aspects of emotional behavior, anxiety and depression. We will explore to which extent early postnatal experience is associated with changes in the activity of the HPA axis as well as alterations on behaviors that may be regulated by this system.

THE EFFECTS OF MATERNAL SEPARATION ON NEUROENDOCRINE FUNCTION

The stress hyporesponsive period

In most studies of postnatal manipulation, maternal separation takes place during the stress hyporesponsive period which extends approximately from postnatal days 4 to 14. During this period the concentrations of basal corticosterone levels, as well

as of hypothalamic CRH and pituitary ACTH are low (Levine, 1967. Walker, Perrin, Vale, & Rivier, 1986). In addition, the responsiveness of the pituitary-adrenal system is attenuated. Indeed, infant rats are unresponsive to mild stressors (e.g., saline injection, ether exposure) which normally induce corticosterone elevations in older rats, and express a reduced adrenal response to ACTH (Rosenfeld et al., 1992. Walker et al., 1986). Although it was suggested that low corticosterone levels found in normally-reared rats during the stress hyporesponsive period may be due to insufficient stimulation of adrenal glands by the low levels of ACTH, recent findings suggest that this might not to be the case (Faturi et al., 2010).

The period of the first two weeks is of critical importance for the developing rat brain. While in humans the brain growth reaches its peaks around the time of birth (9^{th} gestational month), in rats the peak of the brain development occurs during the 2nd week after birth (postnatal days 7-14). Furthermore, the largest portion of myelination as well as neurogenesis of granule cells in specific brain areas (cerebellum, olfactory bulb, dentate gyrus) take place during postnatal days 7-14 (Rodier, 1980).

It has been proposed that the Stress Hyporesponsive Period protects the brain from high levels of glucocorticoids (Sapolsky & Meaney, 1986). Exogenous administration of corticosterone or hydrocortisone to rat pups during the first postnatal week suppresses the proliferation of the granule cells and increases the number of pyknotic cells in the dentate gyrus (Bohn, 1980. Gould, Woolley, & McEwen, 1991). Also, administration of the synthetic glucocorticoid dexamethasone in the early postnatal period suppresses the maturation of astrocytes as well as the expression of genes related to myelination (Tsuneishi, Takada, Motoike, Ohashi, Sano, & Nakamura, 1991). The findings that glucocorticoids administration during this sensitive for the nervous system development period affects negatively neuronal and nonneuronal markers suggest that the stress hyporesponsive period may play a “protective” role.

Pup-dam relationship and hypothalamic-pituitary-adrenal axis

During the Stress Hyporesponsive Period, basal levels of corticosterone are low and stimuli that normally elicit corticosterone elevations in adults are unable to do so in the infant rat (see above). However, early experience related to maternal presence seems to regulate HPA axis function during both the early postnatal period (short term effects) and the adulthood (long term effects).

Early deprivation or maternal separation paradigms potentiates HPA response to stressful stimuli in infant rats. Specifically, repeated pup separations (ED paradigm) from both the dam and the litter for 1 h per day during postnatal days 2-8 leads to significantly higher corticosterone levels compared to controls in response to stress (McCormick et al., 1998). This enhanced stress response is mediated by the

pituitary ACTH since, in addition to corticosterone levels, ACTH levels are also high (Knuth & Etgen, 2005). Significant increases in corticosterone and ACTH levels have been also reported after a *single* 24hr- session of early deprivation (Stanton, Gutierrez, & Levine, 1988).

Postnatal manipulations may also exert long-term effects on the HPA axis response. Early deprivation during the first two postnatal weeks potentiates the corticosterone response to various types of stressors (such as restraint stress, exposure to novelty or food deprivation) in juvenile or young adult rats (Biagini, Pich, Carani, Marrama, & Agnati, 1998. McCormick, Kehoe, Mallinson, Cecchi, & Frye, 2002. Vazquez, Farley, Giros, & Daugé, 2005). In addition, 3hr separation sessions from the dam during the first two postnatal weeks causes a higher increase in the levels of ACTH and/or corticosterone in response to various stressors such as swim stress (Veenema, Blume, Niederle, Buwalda, & Neumann, 2006), restraint stress (Francis, Diorio, Plotsky, & Meaney, 2002), airpuff startle (Huot, Thrivikraman, Meaney, & Plotsky, 2001. Ladd, Huot, Thrivikraman, Nemeroff, & Plotsky, 2004. Lippmann et al., 2007) and mild handling (Kalinichev, Easterling, Plotsky, & Holtzman, 2002). Enhanced corticosterone stress response has been also observed in adult rats that were subjected to only one 24-hr duration session of maternal deprivation (Lehmann, Pryce, Jorgen-Rêlo, Stöhr, Pathuizen, & Feldon, 2002). The above findings indicate that disruption of dam-pup relationship has both immediate as well as long-term effects on responsiveness to stress, thus implying that early life stress alters the HPA system throughout the lifespan.

However, there have been some reports of no significant changes in the HPA axis reactivity of MS rats. Specifically, absence of enhanced corticosterone plasma concentrations has been reported in MS middle-aged female rats after being exposed to airpuff startle (Arborelius & Eklund, 2007) or in young adult rats in response to restraint or novelty stress (Eiland & McEwen, 2010. Hulshof, Novati, Sgoifo, Luiten, den Boer, & Meerlo, 2011). One possible factor contributing to the distinct outcomes regarding the effects of MS manipulation on stress responsiveness may be gender-related since maternal separation tends to affect the HPA-axis response to stress in male rats only (Wigger & Neumann, 1999). In line with this explanation, most of the studies reporting an enhanced neuroendocrine stress response were conducted in male rats (Huot et al., 2001. Ladd et al., 2004. Veenema et al., 2006).

Another potential contributing factor to the discrepant results may be related to the amount of maternal care, specifically maternal licking and/or grooming towards the pup, following separation. Evidence suggests that under normal conditions there is variation in maternal care both among dams as well as within the same lit-

ter, with some pups receiving more care than others (Champagne, Francis, Mar, & Meaney, 2003). Because increased maternal care is associated with attenuation of corticosterone response to stress in adult rats (Liu, Diorio, Tannenbaum, Caldji, Francis, et al., 1997), it has been proposed that it might function as a buffer against the enhanced HPA axis reactivity of MS adult in response to stress (Macrì, Chiarotti, & Würbel, 2008). Based on the above, it is possible to speculate that the corticosterone response of MS rats when exposed to stress as adults may be mediated by the amount of maternal care received during the postnatal period.

THE EFFECTS OF MATERNAL SEPARATION ON ASPECTS OF EMOTIONAL BEHAVIOR

Human data suggest an association between HPA function and emotional behavior related to depression and anxiety during adulthood. Individuals with depression display HPA axis abnormalities, such as a weak glucocorticoids inhibition of the axis as demonstrated by the dexamethasone suppression test (Evans & Nemeroff, 1983). In addition, people with a history of abuse seem to be more vulnerable to develop depression and anxiety disorders (Heim, Shugart, Graighead, & Nemeroff, 2010). Given that maternal separation is also associated with an HPA axis dysregulation in rodents, there has been a strong interest in investigating the effects of early stress on depression and anxiety-like behaviors.

Anxiety-like behavior

A widely used and ethologically relevant test to assess anxiety behavior in rodents is the Elevated Plus Maze (EPM) (Pellow, Chopin, File, & Briley, 1985). The EPM has a "plus" (+) shape, consisting of two open and two closed arms, and is positioned approximately 40-60 cm above the floor. This test relies on the unconditioned fear of rodents towards open spaces and their preference for dark and enclosed areas. Evaluation of anxiety-like behavior is based on estimation of the number of entries in the open/closed arms and the time spent, with more time spent in the closed arms and fewer entries and time spent into the open arms to indicate higher levels of anxiety (Walf & Frye, 2007).

A number of studies indicate that separation of neonatal rats from their mothers increase anxiety-like behavior during adulthood. Adult male rats exposed to a 3-hours daily maternal separation protocol over a 3-week period (postnatal days 2-21) displayed significantly increased anxiety as evidenced by a reduction in the time

spent in the open arms of the EPM compared with control animals (Aisa, Tordera, Lasheras, Del Río, & Ramírez, 2007). Similar anxiety-like behavior was also recorded in adult rats after shorter (2-week) periods of maternal separation (postnatal days 2-14) (Huot et al., 2001. Kalinichev et al., 2002. Lee, Kim, Kim, Ryu, Kim, et al., 2007. Salzberg, Kumar, Supit, Jones, Morris, et al., 2007). Interestingly, it was recently shown that restraint stress during adulthood potentiated anxiety behavior in maternally separated rats when tested 24hrs after the termination of the 3-week restraint stress period (Eiland & McEwen, 2010).

In line with the notion that disruption of mother-infant relationship potentiates anxiety, studies showed that maternal separation affects the behavior of the rats when tested in an Open Field. In this test, originally developed to assess emotional behavior, the rodent is placed into an arena (squared or circular) which is surrounded by a wall to prevent escape, and is allowed to explore it for a period of time. Because rodents don't like large open spaces, exposure to this novel arena triggers an anxiety response which is expressed by fewer entries and less time spent into the central part of the arena. Consequently, time spent in the center of the arena is mostly related to anxiety levels (Lister, 1990; Walsh & Cummins, 1976) and anxiolytic drugs increase the time as well as the entries into the central area (Prut & Belzung, 2003). There is evidence that early adverse experience induces anxiety-like behavior in the Open Field. Adult rats that were exposed to maternal separation or early deprivation paradigms as infants tend to make fewer entries and spend less time in the center area of the maze (Francis et al., 2002. Lambás-Señas et al., 2009. Vazquez et al., 2005).

While the prevailing idea is that experience of early stress caused by disruption of mother-infant relationship enhances anxiety-like behavior, other investigators failed to report a similar effect. Specifically, rats that had been subjected to daily 3-h maternal separation during the first two postnatal weeks did not differ from controls on behavioral aspects of EPM that indicate increased anxiety (Faure, Uys, Marais, Stein, & Daniels, 2007. Grace, Hescham, Kellaway, Bugarith, & Russell, 2009. Hulshof et al., 2011. Slotten, Kalinichev, Hagan, Marsden, & Fone, 2006). In addition, no differences in anxiety-related behaviors as manifested in the Open Field were observed between maternally separated and control rats during adulthood (Eiland & McEwen, 2010. Stevenson, Meredith, Spicer, Mason, & Marsden, 2009) or at an earlier age (Farkas, Reglodi, Gaszner, Szogyi, Horrath, et al., 2009. Zimmerberg & Kajunski, 2004).

This discrepancy regarding the effects of anxiety-like behavior in maternally separated rats may be mediated by the HPA system. In most cases, adult maternally separated rats that spend less time in the open arms or the center of the Open Field

tend also to oversecrete corticosterone when exposed to a stressor as adults (Aisa et al., 2007. Francis et al., 2002. Huot et al., 2001. Kalinichev et al., 2002. Vazquez et al., 2005). On the other hand, rats that did not express enhanced anxiety as adults, failed to exhibit an enhanced neuroendocrine response when exposed to stress (Eiland & McEwen, 2010. Slotten et al., 2006). In support of the contribution of the HPA system, studies have shown that early adverse rearing preferentially potentiates anxiety behavior in male rats, whose neuroendocrine response to stress tend to also be augmented (Aisa et al., 2007. Kalinichev et al., 2002. Wigger & Neumann, 1999). This suggests that gender may also be a contributor to the discrepant results.

Depression-like behavior

A behavioral measure of depression which estimates behavioral despair is the Porsolt Forced Swimming Test (FST), which involves the placement of the rodent in a cylinder filled with water. After trying to escape from it, a normal animal adopts a "despair" behavior which is expressed by an immobile posture, the duration of which is decreased by antidepressants (Porsolt, Le Pinchon, & Jalfre, 1977). Existing evidence supports the idea that an early rearing condition is associated with despair behavior. Specifically, maternal separation for two or three weeks tends to increase immobility and decrease swimming time in adult rats (Lambás-Señas et al., 2009. Lee et al., 2007. MacQueen, Ramakrishnan, Ratnasingan, Chen, & Young, 2003. Veenema et al., 2006).

Early adverse experience affects pleasure-seeking behaviors. Under normal conditions, rats show a preference for sweetened fluids (sucrose or sacharrin) over water. Because anhedonia is a behavior that characterizes depression, reduction in the consumption of sucrose solution intake in rats is considered a sign of depression-like behavior. In fact, administration of antidepressants restores the preference for sucrose solutions as tested by the Sucrose Preference Test (Muscat, Papp, & Willner, 1992). Existing evidence suggests that early adverse experience is associated with gustatory anhedonia. Specifically, findings indicate that the preference for sucrose solution is significantly reduced in adult rats subjected to two or three weeks of maternal separation (Aisa et al., 2007. Huot et al., 2001), although no such anhedonic behavior has been seen at an earlier age (Colorado, Shumake, Conejo, Gonzalez-Pardo, & Gonzale-Lima, 2006).

Existing evidence indicates that, in addition to a dysregulation of the HPA axis (Heim et al., 2000), depression and / or anxiety-like behaviors are associated with alterations in serotonergic neurotransmission. Altered serotonergic function is implicated in the pathophysiology of major depressive episodes (Cannon et al.,

2007) and pharmacological treatments that enhance serotonergic neurotransmission improve mood in both depression and anxiety (Kent, Coplan, & Cornam, 1998. Nutt, 2002). Recently, it has been reported that maternal separation during the neonatal period is associated with reduction in serotonin (5-HT) content in the hippocampus (Arborelius & Eklund, 2007) and decreased serotonergic neurotransmission (Lee et al., 2007).

CONCLUSIONS – IMPLICATIONS FOR FUTURE RESEARCH

Findings from human studies indicate a strong association between adverse experiences during childhood and vulnerability to psychopathology later in life. Seymour Levine's (Levine, 1967) pioneering experiments showed that early handling attenuates stress responsiveness later in life, thus underscoring the importance of early experimental manipulations. His findings created a strong interest in investigating the effects of early adversity in animal models. Since absence of maternal stimulation and care is considered a major stressor early in life (Levine, 2005), researchers used the paradigm of maternal separation in order to investigate effects of early stress on behavior as well as stress-related neuroendocrine systems.

There has been a strong interest in exploring whether early stress combined with subsequent adult chronic stress increased susceptibility to affective disorders. This phenomenon may parallel the increased vulnerability to depression and anxiety disorders observed in individuals with a history of early life adversity (Heim et al., 2008). According to a large number of animal studies, maternal separation may render an organism more vulnerable to future stress. This was supported by findings of an augmented ACTH or corticosterone response as well as enhanced anxiety-like behaviors of maternally separated rats after exposure to adult stress (Aisa et al., 2007. Francis et al., 2002. Huot et al., 2001. Kalinichev et al., 2002. Vazquez et al., 2005). Thus, it would be tempting to conclude that early adverse experience enhances stress reactivity and emotional behavior. However, recent data failed to support such an association. Although maternal separation exaggerates anxiety behavior following 3-weeks of restraint stress as adults, it does not produce an augmented corticosterone response (Eiland & McEwen, 2010). In addition, according to a recent study, maternally separated rats did not exhibit an enhanced stress sensitivity or anxiety in response to various stressors as adults (Hulshof et al., 2011). Unfortunately, methodological differences related to the duration of maternal separation, gender of the animals and type and duration of stress during adulthood prevent us from making direct comparisons and from drawing definite conclusions, thus stressing the need for further investigation.

A finding of great importance is that the effects of early separation on adult emotional behavior and neuroendocrine response to stress later in life may vary as a function of gender. Evidence suggests that exposure to stress caused emotional reactivity and signs of anxiety (open field, elevated plus maze) in male rats that were maternally separated from their mother during the first 1-3 weeks after birth (Renard, Rivarola, & Suárez, 2007) or for a shorter period (24hrs) (Barna, Balint, Baranyi, Bakos, Makara, & Haller, 2003), with the females being unaffected. Even in cases that maternal separation induced a long-term increase in anxiety-related behaviors in both genders, the effect of postnatal stress was more robust in male than in female rats (Wigger & Neumann, 1999). This increase in anxiety-like behaviors was associated with an enhanced HPA axis responsiveness to stress, as indicated by elevations in ACTH and corticosterone in maternally-separated male rats only (Kallinichev et al., 2002. Renard et al., 2007. Wigger & Neumann, 1999). These findings suggest that maternal separation alters not only behavioral, but also endocrine responses to an emotional stressor, thus rendering male rats more vulnerable.

It should be noted, however, that there are some discrepancies among reports regarding interactions between early life experiences, exposure to stressful events in later stages of life, and gender. In fact, there have been reports of either no gender-related differences or higher susceptibility of females regarding the long-term effects of early adverse experience following stress later in life. Specifically, neonatal isolation increases to the same extent anxiety-related behavior in both genders following a stressful challenge (Knuth & Etgen, 2007). As far as the HPA axis function is concerned, experience of stress later in life either causes elevations in plasma corticosterone in maternally-separated female rats only (Desbonnet, Garret, Daly, McDermott, & Dinan, 2008), or has no effect on stress-induced levels of ACTH or corticosterone in maternally-isolated rats of either gender (Knuth & Etgen). These findings are inconsistent with reports of higher vulnerability of maternally-separated males to a stressor later in life (see previous paragraph). Although the exact cause of discrepant results cannot be stated with certainty, inconsistencies may be partly due to differences in the procedures employed across studies (e.g., nature and duration of early adverse experience or stress challenge later in life). For example, studies that reported enhanced emotional behavior and neuroendocrine responsiveness in male rats, have employed the maternal separation paradigm (Kallinichev et al., 2002. Renard et al., 2007. Wigger & Neumann, 1999), while male and female animals that exhibited similar increase in anxiety in response to stress had experienced neonatal isolation which implies separation both from their dam and littermates (Knut & Etgen).

Recent studies have focused on investigating gender differences in the human population regarding HPA axis activation in response to stress challenges. According to research findings, there are links between the HPA axis, depression and psychological stress. Specifically, stressful life events and early adverse experiences have been consistently linked to the onset of depression (Heim & Nemeroff, 1999). In addition, depression has been associated with multiple abnormalities along the HPA axis (e.g., increased basal cortisol levels) (Rubin, Poland, Lesser, Winston, & Blodgett, 1987). Interestingly, women are more likely to have depression and anxiety disorders than men (MacMillan, Fleming, Streiner, Lin, Boyle, et al., 2001), and the impact of stressful life events appears to be greater in women as they appear to be more susceptible to the negative effects of adverse events and trauma during childhood (Breslau & Anthony, 2007). Given the higher rates of depression and anxiety disorders in women, and the links between HPA dysregulation and affective disorders, there has been a strong interest in investigating possible gender differences in HPA responses to stress.

In contrast to the agreement regarding the higher rates of affective disorders in males, findings regarding the HPA axis responses of men and women to stress are not consistent. A determining factor that accounts for the discrepant results appears to be the type of the stress event. In fact, women show greater adrenocortical responses to social rejection stressors (e.g., interpersonal or psychosocial conflict task) compared to males, while exposure to stressors related to achievement (e.g., academic exams) cause higher cortisol levels in men (Kudielka & Kirschbaum, 2005. Stroud, Salovey, & Epel, 2002). One of the questions that these studies raised was whether gender differences in HPA responses to different types of stress mediate the emergence of sex differences in depression. Although future studies are warranted to directly address this question, it appears that gender-related differences regarding HPA responsiveness to stress is only one of the factors that contribute to the higher prevalence of depression in women (Young & Korszun, 2010).

A recent area of interest related to the effects of maternal separation has been the investigation of alterations in markers of synaptic plasticity in the hippocampus along with changes in learning and memory. The hippocampus is a structure with a high concentration of glucocorticoid receptors (Reul & de Kloet, 1985) and one of the main sites that regulate the HPA axis by providing negative feedback (de Kloet, Vreugdenhil, Oitzl, & Joëls, 1998). Importantly, the hippocampus plays a critical role in learning and memory (Poldrack & Packard, 2003) and the negative effects of exposure to high glucocorticoids levels on the memory of adult rats are well established (Conrad, 2008). Recent findings indicate that early stress affects markers of hippocampal synaptic plasticity, such as synaptic density, levels of neurotrophins (e.g., brain derived neurotrophic factor and nerve growth factor) or neurogenesis.

Specifically, there are reports of reduction in synaptophysin levels (Andersen & Teicher, 2004), in the brain derived neurotrophic factor and /or nerve growth factor levels (Aisa, Elizalde, Tordera, Lasheras, Del Río, & Ramírez, 2009. Lippmann et al., 2007. MacQueen et al., 2003. Roceri, Cirulli, Pessina, Peretto, Racagni, & Riva, 2004. Marais, van Rensburg, van Zyl, Stein, & Daniels, 2008) and in neurogenesis (Aisa et al., 2009). At the same time, there is an increasing number of reports regarding impairments in spatial and nonspatial memory (Aisa et al., 2009. Hulshof et al., 2011) and object recognition tasks (Aisa et al., 2007. Marcos, Aisa, & Ramirez, 2008) after maternal separation, although others failed to see any effects on cognitive function in the Morris Water maze (Jones, Kumar, O'Brien, Morris, Rees, & Salzberg, 2009).

Currently, there is a substantial body of work in rodents describing the effects of maternal separation on HPA axis activity, hippocampal plasticity and behavior (cognitive or emotional) mediated by hippocampus or stress-related systems. What remains unclear, however, is to what extent and through what mechanisms they are related to each other. In addition, evidence that maternal separation increased neurotrophin levels seven days after the end of the procedure (Daniels et al., 2009), raises the question of how early stress may interfere with challenges subsequent to maternal separation. Future studies should emphasize the concurrent examination of questions related to HPA activity, performance on behavioral tasks as well as markers of synaptic plasticity in order to contribute to a better understanding of the long-term effects of maternal separation.

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