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Early life stress as a risk factor for mental health: Role of neurotrophins from rodents to non-human primates

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Abstract

Early adverse events can enhance stress responsiveness and lead to greater susceptibility for psychopathology at adulthood. The epigenetic factors involved in transducing specific features of the rearing environment into stable changes in brain and behavioral plasticity have only begun to be elucidated. Neurotrophic factors, such as Nerve Growth Factor (NGF) and Brain-derived neurotrophic factor (BDNF), are affected by stress and play a major role in brain development and in the trophism of specific neuronal networks involved in cognitive function and in mood disorders. In addition to the central nervous system, these effectors are produced by peripheral tissues, thus being in a position to integrate the response to external challenges. In this paper we will review data, obtained from animal models, indicating that early maternal deprivation stress can affect neurotrophin levels, suggesting that they might be involved in the mechanisms underlying the mother-infant relationship. Maladaptive or repeated activation of NGF and BDNF, early during postnatal life, may influence stress sensitivity at adulthood and increase vulnerability for stress-related psychopathology.

Keywords

Maternal deprivation; Stress; Brain development; Nerve growth factor; Brain-derived neurotrophic factor; Vulnerability; Depression; Anxiety

1. Introduction

Stressful events experienced early during postnatal life can influence the development of individual differences in vulnerability to psychopathology throughout life (Heim and Nemeroff, 2001). Severe conditions such as physical or sexual abuse, in addition to persistent emotional neglect or family conflict, can compromise growth, intellectual development and

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lead to increased risk for adult obesity, depression and anxiety disorders (Cicchetti and Toth, 1995; Heim and Nemeroff, 2001).

The relationship between quality of early life and health in adulthood is still an open question. During the early postnatal phases the brain is experience-seeking and provided with a considerable plasticity which allows a fine tuning between the external environment and the developing organism (Greenough, 1987). One possible hypothesis posed is that adversity early in life is able to enhance or inhibit the experience-dependent maturation of structures underlying emotional functioning and endocrine responses to stress, such as the cortico-limbic system, leading to increased stress responding at adulthood (Tronick and Weinberg, 1997; Heim et al., 2000; Schore, 2000; Heim and Nemeroff, 2001; Meaney, 2001; Seckl and Meaney, 2004). Depressed patients with a history of childhood abuse are often characterized by a hyperactive hypothalamic-pituitary-adrenal (HPA) axis, a major component of the stress response (Heim and Nemeroff, 2001). In addition, childhood abuse or neglect has been associated with abnormalities in brain regions involved in emotional disorders including an overall volume loss in hippocampus, corpus callosum and prefrontal cortex, altered cortical symmetry in cortical regions and reduced neuronal density and integrity in the anterior cingulate (Bremner et al., 1997; Stein et al., 1997; Driessen et al., 2000; Carrion et al., 2001; De Bellis et al., 2002; Teicher et al., 2004).

In order to explain how psychopathology comes about at adulthood, a diathesis-stress model has been proposed. According to this model, a genetic vulnerability or predisposition (diathesis) interacts with the environment and life events (stressors) to trigger behaviors or psychological disorders (Zubin and Spring, 1977). Many psychiatric disorders can be accounted for by this ‘two hit model’ in which genetic or environmental factors disrupt early central nervous system (CNS) development leading to a long-term vulnerability to a “second hit” that then leads to the onset of psychiatric symptoms (Maynard et al., 2001). The signaling pathways involved in cellular differentiation, could be targets for a “first hit” during early development. These same pathways, redeployed for neuronal maintenance and plasticity, may be targets for a “second hit” in the adolescent or adult brain. Thus, if the same pathways in both the developing and the mature organism appear as targets of stress we have a way of integrating genetic, developmental, and environmental factors that contribute to vulnerability and pathogenesis of psychopathology (Norman and Malla, 1993; Pani et al., 2000; Maynard et al., 2001). In this context, the cascade of events initiated by stressful experiences at adulthood represents a major vulnerability factor and its deleterious effects can be aggravated in individuals who have experienced adversities early in life.

The importance of the interaction between genetic and experiential factors in the development of psychopathology has become recognized by preclinical and clinical researchers over the last ten years (Yehuda et al., 1997; Heim and Nemeroff, 1999). The latest theoretical approaches are based on the notion that genes influence the susceptibility to environmental “pathogens” (Caspi and Moffitt, 2006). In one of the most influential studies involving gene-environment interactions, Caspi and coworkers (Caspi et al., 2003) have shown that a functional polymorphism in the promoter region of the serotonin transporter (*5-HTT*) gene would moderate the influence of stressful life events on vulnerability to depression. Individuals with one or two copies of the *5-HTT* ‘short’ allele exhibited more depressive symptoms, diagnosable depression, and suicidality following stressful life events than individuals with two copies of the ‘long’ allele (Caspi et al., 2003). Additional support for the importance of gene-environment interactions in susceptibility to psychopathology is emerging. In two studies of attention-deficit hyperactivity disorder, polymorphisms in the dopamine system interacted with antenatal risk factors (such as low birth weight and maternal use of alcohol) to predict key symptoms associated with the disorder (for a review see (Caspi and Moffitt, 2006).

The study of gene-environment interactions has been the province of epidemiology, in which genotypes, environmental pathogens exposures and disorder outcomes are studied as they naturally occur in the human population (Caspi and Moffitt, 2006). However, research in the neuroscience field has now a major goal to achieve, that is to complement psychiatric genetic epidemiology by specifying the more proximal role of nervous system reactivity in the gene-environment interactions. Indeed, there are a number of still open questions that need to be addressed, including the quality and quantity of experience that can predispose an individual towards psychopathology and the specific neural substrates affected. Appropriate animal models, in which early environmental and experiential factors can be manipulated under controlled conditions are needed to answer these questions (Suomi, 1991; Cirulli, 2003a). In recent years there has been a growing emphasis on developing complex models that incorporate a number of variables which can be manipulated by the experimenter providing new opportunities for translation from basic to clinical research. In this review we will provide examples of studies performed with the common aim of understanding the effects of the early rearing environment in shaping brain development and emotional functioning. While rodents offer a great opportunity to ask questions that can be answered in a short-time scale and allow for the analysis of neurobiological substrates, non-human primates, although offering a number of challenges in terms of understanding brain function, provide the closest match to humans in terms of genetic, behavioral, biological and social similarity. In addition, non-human primates' relatively long lifespan, extended infancy, and socio-affective behavior parallel many aspects of human development (Suomi, 1997). The challenge that basic science needs to meet is to make use of a comparative approach to benefit the most from what each model, notwithstanding its constraints, can tell us about the mechanisms that lead from environmental adversity to increased risk for mental health.

2. Development and vulnerability for anxiety and mood disorders: role of early social relationships

While there is now clear evidence documenting the relationship between childhood abuse and neglect (or other early adverse events) with individual vulnerability to psychiatric diseases, such as anxiety and depression, clinical studies cannot produce sufficient evidence on cause-effect relationships. (Cirulli, 2003a #49). A major goal of these studies is to define times in development and strategies for intervening to prevent or reverse the effects of adverse early life experiences.

The majority of the models that have been developed so far have relied on mimicking the effects of early trauma by means of disrupting the mother-infant bond. Indeed, human data and animal studies have suggested that the relationship between the quality of the early environment and emotional responding at adulthood appears to be mediated by parental/maternal influences on brain development (Tronick and Weinberg, 1997; Schore, 2000; Trevarthen and Aitken, 2001).

The importance of early affective and social interactions in psychological development, although already present in the work of Freud and other pioneers of the study of development, has been given specific attention only in recent years (Rutter, 1993). For a long time developmental theories have not taken into account children's individuality and their social life, such as friendship and play with other children, relationships with siblings as well as love relationships (Rutter and Rutter, 1993). We now know that the early environment is fundamentally a social environment and that the primary social object mediating infant's approach with the external environment is the mother (Bowlby, 1982). The mother's modulatory function upon environmental input is essential for the facilitation (and inhibition) of the experience-dependent maturation of the child's developing biological (particularly neurobiological) structures. Such a concept of "regulation" is particularly important since it is

one of the few theoretical constructs that is now being used by most developmental disciplines and is a central linking concept that could potentially elucidate the “hidden” processes in development (Hofer, 1970; Schore, 2000).

Research conducted in humans has indicated the precocious emergence of an active ‘self-and other’ awareness which plays an important role for infant communication and cognition. (Trevarthen and Aitken, 2001). These interactions make up the process of mutual regulation in a reciprocal feedback system (Tronick and Weinberg, 1997). It has been suggested that the mother’s external regulation of the infant’s developing immature emotional systems during selected critical periods may represent the essential factor that influences the experience-dependent growth of brain areas, particularly cortico-limbic and subcortico-limbic structures that can self-regulate emotional states (Schore, 2000).

Studies of institutionally-reared children have been highly instrumental in understanding the long-term consequences of childhood social deprivation on cognitive and behavioural functioning (Gunnar et al., 2001). In particular, studies of children following removal from the orphanages and adoption from families in the United Kingdom and North America have revealed the presence of cognitive, social and physical deficits (Rutter, 1998). Longitudinal studies have demonstrated that, although these children show some degree of recovery, the behavioural abnormalities are qualitatively similar to those seen in socially deprived non-human primates (Champoux et al., 1997).

Although post institutionalized children have experienced a variety of extreme early adversities, including poor nutrition and poor prenatal care, there is good reason to consider emotional neglect as playing a major role in the ontogenesis of the social difficulties apparent in these children. A prominent lack of emotional and physical contact from caregivers is consistently found throughout institutional settings in Eastern Europe (Human Rights Watch, 1998); although any individual child’s experience may be different, the probability of a child receiving warm, consistent care giving in these settings is quite low (Gunnar et al., 2001). This is because in orphanage settings children receive minimal communication or attention from caregivers, and experience little responsiveness to their individual needs (Rutter, 1998).

The results of these studies are consistent with the view that early social experience plays a significant role in the development of basic affective processes. Post institutionalized children had significant difficulty matching appropriate facial expressions to happy, sad, and fearful scenarios. Thus, the contingencies that children experience in the course of social interactions appear to support learning through connections between cues, situations, and emotional experiences (Fries and Pollak, 2004). In addition, the group of Chugani has reported that post institutionalized children from Romania show decreased glucose metabolic rates in distributed regions including the orbital frontal gyrus and infralimbic prefrontal cortex, consistent with changes in cognitive and emotional functioning in these children (Chugani et al., 2001).

Together these considerations underscore the need for compelling research addressing the consequences of treatment or intervention strategies in subjects exposed to adverse early experiences.

3. Animal models of early stressful experiences

Animal models are fundamental to gain insights into the behavioural and physiological mechanisms underlying the short- and long-term effects of early experiences on emotional reactivity, the stress response and susceptibility to disease. Successful animal models have been generated targeting experiential factors with robust effects that are relatively consistent across species. Amongst these we can enlist studies involving separating mother and infants in mammals (Table 1). Animal models have been developed both using rodents (Levine,

1957; Denenberg et al., 1967; Hofer, 1970) and non-human primates (Harlow and Zimmermann, 1959; Harlow et al., 1965; Rosenblum and Kaufman, 1968; Suomi, 1997). Early experiences, involving some manipulation that results in disruption of the mother-infant relationship, have been shown to have long-term influences on the behavioral and endocrine responses to stress. In the rodent, brief periods of separation result in an attenuated adrenal response to stress (reduced secretion of corticosterone). In contrast, longer periods of separation result in an exaggerated response and several behavioral anomalies i.e. increased alcohol consumption, increased startle response etc (Levine, 2005).

3.1. Maternal influences on infant's neurobehavioral development

The pattern of changes in the infant following maternal separation is not a unitary syndrome (Hofer, 1994b). The complex behavioural and physiological response that occurs reveals the existence of several discrete regulators, which operate on different physiological and behavioural systems of the infant. For example, separated infant rats show lower cardiac rate than normally mothered controls. Normal heart rate can be maintained by feeding the rat pup, a mechanism dependent upon milk interaction with gastrointestinal receptors (Hofer, 1970, 1994a). On the other hand, non-nutritive sucking, in particular, the rhythmicity of milk delivery, influences the infant's sleep pattern. Furthermore, the continuous tactile stimulation provided by the mother to the pup through licking, retrieving, and nursing keeps the activity levels of the pups to a certain degree, which is otherwise increased in her absence. Tactile stimulation by the mother is also responsible for maintaining basal levels of activity of enzymes necessary for normal growth, such as ornithine decarboxylase (ODC) as well as growth hormone levels (Kuhn et al., 1978).

The mother plays also an important role in regulating stress responsiveness of the offspring (Walker et al., 1986; Rosenfeld et al., 1992). Although most research on HPA system regulation during ontogeny has focused on intrinsic regulatory factors, it appears as though extrinsic factors also play an important role. In particular, maternal factors appear to exert a strong inhibitory effect on the infant's HPA system. A number of studies have clearly shown that the infant rat is characterized by a markedly reduced adrenocortical response to stimuli which are able to elicit a strong response in the adult. This time period has been termed stress-hyporesponsive period (SHRP) (Sapolsky and Meaney, 1986; Rosenfeld et al., 1992). It must be emphasized that neonatal rats can secrete adrenocorticotropin hormone (ACTH) in response to certain types of stressors, although this response appears to be stimulus-specific and developmentally regulated. The adrenal gland, on the other hand, shows minimal corticosterone (CORT) output even when stimulated by high levels of ACTH (Rosenfeld et al., 1992; Okimoto et al., 2002). In the rat, the SHRP ensues around postnatal day (PND) 4 and lasts until about PND 14, although the mechanisms underlying it have only been partially elucidated. What is clear, so far, is that a partial immaturity of the system, combined with active inhibitory processes, results in a period during which circulating CORT remains at low, relatively imperturbable levels (Rosenfeld et al., 1992). This can be demonstrated by removing the source of the regulation. Following prolonged (24 h) maternal deprivation, the infant rodent shows a marked increase in adrenal responsiveness to ACTH and, at certain ages, in basal- and stress-induced CORT and ACTH secretion. The endocrine responses are dependent upon the age of the pup and the duration of maternal separation.

Data from other experiments clearly indicate that maternal contact in the absence of suckling and/or feeding is not able to down regulate the HPA system as measured through CORT secretion (Cirulli et al., 1992). In addition, these results suggest that the processes responsible for maintaining the SHRP differ from those that modulate the stress response in an infant that has been rendered responsive by 24 h of maternal deprivation. Thus, the mother appears to regulate HPA responsiveness in the infant in at least two different ways: (1) maintaining the

HPA axis relatively ‘unresponsive’ to external stimulation and (2) suppressing HPA axis activity when this has been activated. It has been later shown that the disinhibition of the HPA axis is a process characterized by a slow onset and occurs reliably after prolonged separation periods (about 8–24 h; (Levine et al., 1991)), resembling the time course of other processes under maternal regulation (Hofer, 1994a). The effects of maternal separation on CORT secretion can be generalized also to other rodent species, such as the mouse (Cirulli et al., 1994; Schmidt et al., 2002). Metabolic signals, particularly reduction in glucose levels play an important role in triggering the HPA response of the neonate rodent to maternal separation (van Oers et al., 1998b; Schmidt et al., 2006).

3.2. Rodent models of early life stress

The seminal work of Levine (Levine, 1957) and Denenberg (Denenberg et al., 1967) has clearly demonstrated that manipulations of the mother-infant relationship have long-term consequences on neuroendocrine and behavioural responses later in life. While attempting to model the effects of early “trauma” it was found that lack of any stimulation resulted in subjects that, as adults, were much more fearful and inhibited compared to neonatal rats exposed to a mild electric shock (Levine, 1957). Thus stimulation during infancy is an important mechanism by which the response to the external environment can be adapted to the ecological niche characterizing a specific individual. These effects were counterintuitive since it was at that time believed that young mammals, including children, were rather unresponsive to the external environment.

Since then, handling has been the most common manipulation used, consisting of removing the animals from the mother and their cage and placing them in individual compartments for up to 15 minutes until weaning. Animals handled during infancy (H) show increased exploration, less defecation and urination in an open field (Levine, 1957), a high degree of exploration in the hole board test, and a reduced taste neophobia and conditioned taste aversion (Weinberg et al., 1978).

Stimulation during infancy markedly affects the activity of the endocrine system (Levine, 1957). Handled subjects show higher levels of glucocorticoids (GC) immediately after shock exposure, and a more rapid return to basal levels. In contrast, non handled (NH) subjects show a much slower rise and a higher peak in the post-shock secretion of adrenal hormones. Basal levels of GC do not differ between neonatally manipulated and non-manipulated animals. When H subjects are tested in an open field, they show significantly lower increases in GC, compared to controls (Levine, 1957; Meaney, 2001). The differences previously described are long-lasting and can persist for the entire life of the animal. It has been so far hypothesized that the modified endocrine response of H animals would be extremely adaptive for the body: the speed and short duration of response would enable the organism to respond to a challenging situation rapidly, while avoiding the effects of prolonged exposure to adrenal steroids, which have been shown to exert deleterious effects on the nervous system (McEwen, 2007). It must be emphasized, however, that, given the role played by GC e.g. as immunosuppressors, a blunted HPA axis activity would unleash the immune system making the organism more vulnerable to autoimmune diseases (Cirulli et al. manuscript in preparation).

It has been reported that the long-term effects of handling on HPA responses to stress are qualitatively different, depending upon the duration of the maternal separation (DEP), very short separation intervals resulting in a sort of “emotional immunization”, longer separations being detrimental for the developing animal, leading to increased responses to stress (Plotsky and Meaney, 1993; Wigger and Neumann, 1999; Ladd et al., 2000; Huot et al., 2001; Pryce, 2001). It has been reported that, as adults, animals exposed to maternal separations of 180–360 min per day for the first 2 weeks of life show significantly increased plasma ACTH and CORT response to stressful stimuli, compared to unseparated controls, an effect that has been claimed

to be opposite to handling (Plotsky and Meaney, 1993). The longer period of separation also results in decreased glucocorticoid receptor binding in both the hippocampus and the hypothalamus (Plotsky and Meaney, 1993). However, the effects of these prolonged, but still relatively brief, maternal separations are not always consistent. A few papers are currently available indicating that even 3-4 hr daily separations performed from birth until weaning result in behavioural and endocrine changes in the same, rather than in the opposite, direction as handling (Pryce, 2001; Lehmann et al., 2002; Giachino et al., 2007). In particular, we have shown that early environmental manipulations of the mother-infant relationship induce lasting changes in the density of gabaergic interneurons in selected brain regions involved in the regulation of the stress response and emotional behavior (Giachino et al., 2007). Rats exposed to handling (H, 15 min daily) and maternal separation (DEP, 3 hr daily) from postnatal day 2 to 14 were compared to non manipulated (NH) controls during periadolescence (ranging from postnatal day 35-45), a peculiar developmental period of anatomical and neurochemical remodeling of different stressor-sensitive brain areas (Spear, 2000). Taking advantage of the high degree of affiliative and playful social interactions displayed in adolescence (Cirulli et al., 1996; Spear, 2000), this study assessed whether two different manipulations would differentially affect emotionality in the social interaction test, a behavioral test which is sensitive to modifications in the stress-related brain regions studied, especially the lateral amygdala (File and Seth, 2003). We found decreased emotionality in both H and DEP periadolescent rats. Indeed, when exposed to a social challenge, they readily engaged in social interactions with an unfamiliar conspecific. By contrast, mice never manipulated as infants were less prone to explore the unfamiliar partner directing their attention to the cage environment. Higher frequency of play soliciting behaviors, such as mutual circle and push under, characterized both H and DEP subjects, indicating reduced neophobia in a social situation (Giachino et al., 2007). These data are consistent with other findings in periadolescent mice undergoing the same separation protocol (3 hr from PND 2-14) (Venerosi et al., 2003) which showed higher levels of aggressive social interactions, compared to unmanipulated controls, an effect that could depend upon reduced social phobia (Venerosi et al., 2003). These data overall suggest that maternal separations of a length of up to 3 hrs reduce emotionality in both rats and mice during the critical period of periadolescence. Discrepancies present in the literature might be related to differences in the experimental procedures used in the different laboratories, particularly in reference to the sex of the experimental subject since it has been recently shown that maternal separation results in greater stress responsiveness only in females (Desbonnet et al., 2008).

Differently from brief separations, maternal separations lasting 6-24 hrs lead to a number of long-term detrimental changes in the HPA axis activity and behaviour (van Oers et al., 1998a; Pryce, 2001). Data on longer separations appear more consistent and possibly rely on the fact that following 6-8 hrs of maternal separation an increase in ACTH and CORT occurs in both rats and mice, even during the during the SHRP (see below) (Levine et al., 1991; Cirulli et al., 1994; Schmidt et al., 2004).

It appears as though it is not always easy to discriminate what a truly 'adverse' experience is or to predict its behavioural and/or physiological consequences. Indeed, although stress has always been associated with an increased activity of the HPA axis, a number of recent studies performed in humans suggest that the neuroendocrine axis can be hyporesponsive in a number of stress-related states. Such a state, named hypocortisolism, is characterized by a paradoxical suppression of the HPA axis under conditions of trauma or prolonged stress. As an example, children exposed to adverse rearing conditions are characterized by an overall dampening of the HPA axis response (Gunnar and Vazquez, 2001). Thus, a reduced neuroendocrine activity needs not always to reflect a better-adapted organism but could be the long-term consequence of early adverse events, implying that behavioural variables should always accompany neuroendocrine data when assessing the long-term effects of early experiences.

These data overall indicate that the differentiation of neural structures is sensitive to a variety of environmental signals during the postnatal period suggesting a high degree of plasticity through which neuroendocrine and behavioural responses to stress can be finely adjusted in response to external events.

3.3. The “Maternal mediation” hypothesis

In the original experiments by Denenberg and others, it was clearly found that the mother-infant relationship has decisive effects on the later emotional development of the infant (Denenberg et al., 1967). In particular, it was found that the “emotional state” of the mother affected the emotional state of the offspring. (Denenberg et al., 1967).

The so called “maternal mediation” hypothesis first proposed in the ‘70s (Smotherman, 1974) states that changes in maternal behaviour underly the effects of early manipulations on the offspring. This hypothesis has been later confirmed and a direct relationship between variations in the levels of maternal care and the development of individual differences in the behavioural and neuroendocrine responses to stress of the offspring has been described (Meaney, 2001; Pryce, 2001). In particular, high levels of maternal care appear to be directly related to reduced behavioural and neuroendocrine responses to novelty in the offspring (Liu et al., 1997).

Over development, a steady decline occurs in the proportion of daily time spent in maternal care (Grotz, 1969). Mothers of H litters have shorter and more frequent nesting bouts and spend significantly more time licking the offspring and performing ‘arched-back’ nursing compared to NH (Meaney, 2001; Pryce, 2001). Handling ultimately results in a persistent alteration of maternal care, while longer separations (4 hr) are characterized by an intense phase of maternal care when pups are returned to the mother, with increased licking and arched-back nursing and low levels of dam off pups, relative to controls. Thus it appears as though, differently from H, longer separations induce an acute, rather than a chronic alteration of pup elicitation of maternal care (Pryce, 2001). However, other researchers have shown that 3 hrs of maternal separation induce an increase of activities similar to handling (Macri et al., 2004).

But are these changes in maternal behavior playing a role in the effects of early manipulations on the development of endocrine and behavioral responses to stress? A causal relationship between maternal behavior and reaction to stress in the offspring as well as the transmission of such individual differences in maternal behavior from one generation of females to the next has been shown (Francis et al., 1999; Francis and Meaney, 1999). Other literature data clearly indicate which specific aspects of maternal behavior can affect the development of individual differences in HPA responses to stress in rats (Liu et al., 1997). This study was based on the notion that there are substantial, naturally occurring variations in maternal licking/grooming in rat dams. As adults, the offspring of mothers that exhibit more licking and grooming of pups during the first 10 days of life show reduced plasma ACTH and CORT responses to restraint stress, increased hippocampal GR mRNA expression, enhanced glucocorticoid negative feedback sensitivity, and decreased hypothalamic levels of corticotrophin-releasing factor (CRF) mRNA, thus resembling handled animals. These rats also show increased CRF receptor levels in the locus coeruleus and decreased central GABA/benzodiazepine receptor (Liu et al., 1997). Results from these studies thus suggest that, at least in the rat, changes in maternal behavior are one of the critical factors mediating the effects of handling on HPA development. However, this one to one relationship between maternal behaviour and offspring's emotionality has been recently questioned. Indeed, both early handling and longer maternal separations (3-6 h) which have been shown to have different effects on the offspring's behavioural and physiological reactivity to stress, result in increased maternal behaviour (Pryce, 2001; Macri et al., 2004). In addition, some reports have described that both handling and maternal deprivation have the same effects on the formation of neural circuits providing limbic and

cortical control over autonomic emotional motor output (Card et al., 2005). Thus, the equation linking early environmental variables and offspring behaviour at adulthood through changes in maternal care still presents some unknown intervening variable (Macri and Wurbel, 2006).

Of course, we cannot disregard the fact that maternal factors are likely to interact with genetic influences in determining the long-term effects on stress reactivity of the offspring at adulthood. Indeed, while comparisons between different inbred strains of mice expose remarkable differences in measures of anxiety-related behaviour, such as performance in the open field or elevated plus maze paradigm, differences within strains can be attributed to environmental influences. Inbred and recombinant inbred strain studies are highly efficient in dissecting genetic influences, for investigating interactions between genotype and environment, and for testing the disposition-stress model (Eley and Plomin, 1997). In addition, embryo transfer and cross fostering have been successfully employed to identify epigenetic mechanisms at work during pre- and postnatal life (Francis et al., 1999; Francis et al., 2003).

3.4. Neural mechanisms underlying the long term effects of early experiences on emotionality

The molecular mechanisms by which early environmental influences alter circuits that may mediate vulnerability to mood disorders and emotional regulation still await to be investigated properly. Structural changes, including dendritic debranching and hypertrophy, cell proliferation, and synaptic remodeling could be the result of the combined hyper- activity of stress hormones and endogenous neurotransmitters (Heim and Nemeroff, 1999; McEwen, 2007).

These effects appear to be mediated primarily by changes occurring in brain areas such as the amygdala and the temporal and prefrontal cortices. As for the neurochemical systems, the monoaminergic one plays a significant role because drugs that affect monoamines, such as serotonin, are effective in treating anxiety and depression. The current challenge in the field is to move from a general understanding that these systems are important to a specific understanding of the mechanisms by which alterations in these systems result in pathology in some individuals more than in others. The 5-HT1A receptor has been implicated in mediating the effects of serotonergic agents in anxiety and depression. Mice that have been genetically engineered to be lacking the 5HT1A receptor show increased anxiety in a number of tests. Repression of the receptor expression until four weeks of age is sufficient to produce adult mice with increased anxiety-related behaviour, thus the critical period for establishment of the knockout phenotype is probably in the third and fourth postnatal weeks, a period of dramatic synaptogenesis and dendritic growth in the forebrain (Gross and Hen, 2004). This period might also be important for the adjustment of anxiety circuits in response to experience-dependent signals.

Like the serotonin system, dysfunction in the HPA axis has been implicated in the pathogenesis of mood disorders. Reduced hippocampal volume in adult subjects that have experienced abuse as children is an important piece of evidence calling for a role of stress hormones in setting the stage for psychopathology (Bremner et al., 1997). Significant evidence suggests that early environmental factors establish an HPA reactivity that can be set for life and be transmitted epigenetically to subsequent generations. Some of this evidence comes from studies in which non human primates are raised under conditions that alter maternal stress levels. The effects of early life stress are manifested at both behavioural and biochemical levels, including changes in HPA axis, which are also correlated with functional modification of the serotonergic system.

Human data have also established a genetic contribution of the HPA axis in anxiety and depression (Leonardo and Hen, 2006). CRF neurons in limbic and brain stem regions appear to mediate, at least in part, anxiety. There is evidence for increased CRF activity in both patients

with depression and patients with anxiety disorders. Patients with panic disorder have been shown to demonstrate a blunted ACTH response in a standard CRF stimulation test. Because blunted ACTH responses were also observed in the presence of basal eucortisolemia, these findings support pituitary CRF-receptor down-regulation secondary to hypothalamic CRF hypersecretion as underlying this endocrine abnormality in panic disorder. Patients with obsessive-compulsive disorder, in contrast, have been reported to exhibit increased CSF CRF concentrations which normalize upon clinical recovery (Kluge et al., 2007).

Data from rodent models indicate that the long-term effects of handling appear to depend upon changes in the differentiation of those neurons known to be involved in the stress response (Meaney et al., 1996). Handled subjects show an increased number of glucocorticoid receptors (GR) expression in the hippocampus, a region strongly implicated in glucocorticoid feedback regulation (Meaney et al., 1989). The differences in negative-feedback are also reflected in differences in hypothalamic synthesis of various ACTH secretagogues. For example, hypothalamic corticotropin-releasing hormone (CRH) mRNA and protein levels, under basal conditions, are about 2.5-fold higher in non-handled compared with handled animals (Plotsky and Meaney, 1993). In addition, preclinical studies have suggested that stress during development may result in persistently increased activity of one or more CNS CRF systems and a sensitization of the HPA axis to stress.

Results from our own work and other studies have pointed out that manipulations of the mother-infant interaction can induce in the offspring neurochemical changes in various brain regions that might result in long term effects on behavior (Cameron et al., 2005). As an example, both DEP and H lead to changes of subpopulations of GABAergic neurons in brain areas involved in the regulation of the HPA axis and stress response, thus confirming and extending the role played by early experiences in shaping the development of neuronal circuits involved in the emotional response (Giachino et al., 2007).

3.5. Epigenetic factors transducing the effects of early experiences and shaping adult social behaviour: role of neurotrophins

Early adverse experiences in humans are associated with an increased risk for developing psychiatric disorders such as anxiety and major depression, although little is known of the neurobiological mediators (Kaufman et al., 2000; McEwen, 2000; Heim and Nemeroff, 2001). Neurotrophins, such as Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF), which play a fundamental role in brain function and neuroprotection and are affected by stress, are good candidates for transducing the effects of adverse events in changes in brain function (Smith et al., 1995a; Thoenen, 1995; Duman et al., 1997).

The neurotrophin family comprises several polypeptides including, in addition to NGF and BDNF, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) (Reichardt, 2006). NGF was the first trophic factor to be discovered more than 50 years ago as a target derived trophic molecule that regulates the survival and maturation of sympathetic neurons in the peripheral nervous system (Levi-Montalcini, 1987). However it is now evident that these neurotrophins not only support the survival of postmitotic neurons (Lewin and Barde, 1996), but are important mediators of synaptic and morphological plasticity (Thoenen, 1995). The expression of these neurotrophins and their receptors is developmentally regulated with significant increases in their expression at times of maximal neuronal growth, differentiation and synaptogenesis. Physiological stimuli, such as visual input, or stressful events can affect the expression of neurotrophins possibly through activity-dependent mechanisms (Castren et al., 1992; Smith et al., 1995b; Smith et al., 1995a; Thoenen, 1995; Russo-Neustadt et al., 2001).

Neurotrophins are also produced by cells outside the nervous system, thus being in a position to integrate neural, immune and endocrine responses to stress (Aloe et al., 1986; Nisoli et al.,

1996; Nockher and Renz, 2005). NGF is increased in anxiety-loaded situations, such as in male soldiers experiencing their first parachute jump (Aloe et al., 1994), in spouses caring for Alzheimer's patients (Hadjiconstantinou et al., 2001) or following smoking cessation (Lang et al., 2002). By contrast, decreased blood levels of BDNF characterize subjects diagnosed as major depressives, antidepressants reverting this change (Shimizu et al., 2003; Karege et al., 2005). BDNF, in particular, is a neurotrophin central to the neurotrophic theory of depression (Duman et al., 1997). The gene for this neurotrophin is a risk locus for depression (Neves-Pereira et al., 2002; Sklar et al., 2002). In humans, changes in peripheral levels of BDNF, as well as the presence in some individuals of selected gene variants, have been associated with mood disorders, also in interaction with early trauma (Karege et al., 2002; Karege et al., 2005; Kaufman et al., 2006; Castren et al., 2007; Kauer-Sant'Anna et al., 2007). Recent studies have also suggested a direct link between the efficacy of antidepressants and increased levels of this neurotrophin (Vaidya and Duman, 2001; Shimizu et al., 2003; Karege et al., 2005). A specific dimorphism in peripheral BDNF levels has been reported in humans, women showing higher levels of this neurotrophin than men (Lommatzsch et al., 2005). This piece of data is especially interesting since depression is reportedly more prevalent in women than men, although the neurobiological bases for this difference have not been exhaustively explored (Grigoriadis and Robinson, 2007).

Studies performed in rodents have shown that during development, the expression of NGF and BDNF is localised to the hippocampus and prefrontal cortex, two regions playing a key role in psychiatric disorders and which are well-studied sites of both developmental and adult synaptic plasticity (Large et al., 1986; Yan et al., 1997).

Changes in the expression of these neurotrophins at critical times during development could promote a cascade of events interfering with maturations of these regions, setting the stage for altered responsiveness to stress at adulthood (Cirulli, 2003a). In preclinical studies modelling early adversity and adult vulnerability to psychopathology, maternal separation has been used as a potent stressor able to produce long-lasting changes on emotional and depression-like behavior (Plotsky and Meaney, 1993; Ladd et al., 2000; Meaney, 2001; Cirulli, 2003a). Studies performed in rodents have shown that neurotrophins are sensitive to manipulations of the mother-infant relationship and, more in general, of the rearing environment (Cirulli et al., 1998; Cirulli et al., 2000; Cirulli, 2001; Roceri et al., 2004; Sale et al., 2004; Branchi et al., 2006b). Changes in neurotrophin levels as a result of early manipulations might be functional to counteracting the negative impact of stress on selected brain regions, such as the hippocampus, as well as on other body organs (Thoenen, 1995).

In a pioneering attempt to assess experience-dependent changes in brain function, it was shown that early environmental stimulation results in an increased number of dendritic spines on rat cortical pyramidal cells, thus suggesting that structural changes in axons and dendrites might underlie plastic rearrangements of brain circuits during development (Schapiro and Vukovich, 1970). Since NGF has been involved in the control of dendritic growth in a highly specific fashion, it might be a good candidate for mediating the effects of early manipulations on brain development (McAllister et al., 1999). Indeed, previous work had shown that environmental stimulation has long-term effects on hippocampal NGF levels (Pham et al., 1997). In a series of experiments we have addressed the question as to whether disruption of the mother-infant relationship might affect the expression of neurotrophins, such as NGF, in the CNS of neonatal rats. In a first study, it was found that following a brief (1 h) maternal separation NGF expression was increased in the hippocampus of 3-day-old rats (Cirulli et al., 1998). This region was chosen because NGF and its receptors are reliably expressed by hippocampal neurons already early on during development (Cirulli et al., 1997). In a subsequent study, a more extended separation time was used (up to 3 h) and changes in NGF expression assessed in a number of CNS regions, including the hypothalamus, in 9- and 16-day-old rats (Cirulli et al.,

2000). Results indicate that, during development, NGF expression can be increased in the hippocampus, cerebral cortex and hypothalamus in a time-dependent manner, by means of a simple manipulation, i.e. following a brief maternal separation of rat pups (Cirulli et al., 1998; Cirulli et al., 2000). It has been recently shown that an increase in NGF gene expression in hippocampus and cerebral cortex also characterizes 24 h maternally separated 12-day-old rats (Zhang et al., 2002). Concomitantly, there was a noticeable increase in the rate of cell death in the neocortex, white matter, and granule cells of the dentate gyrus. Whether the increase in cell death results in a permanent reduction in cell number is not known (Zhang et al., 2002).

The increase in NGF expression represents yet another physiological response to maternal separation. The specific aspects of maternal behaviour involved in this induction need to be investigated, although stroking alone did not prevent the effects of a 24 h separation on cell death (Zhang et al., 2002). A number of studies suggest that, in contrast to the periphery, neurotrophins are synthesized in the central nervous system in an activity-dependent manner and that they are released upon depolarization of CNS neurons (Zafra et al., 1990). Although the direct mechanism responsible for the increase in NGF expression observed during the maternal separation procedure is still unclear, it is unlikely that GCs may play a role since significant elevations in CORT can be seen only after longer separation periods (Levine et al., 1991).

In a further study we have focused on the short-term effects (first two postnatal weeks) of repeated manipulations of the rearing environment in CD-1 mouse pups (Cirulli et al., 2007). Litters were removed from the animal facility and either underwent 15 min of neonatal handling or were taken to another room and exposed to an unfamiliar male intruder for a total manipulation time of 15 min (MI) from PND 2-14. Compared to the H group, MI pups were not removed from the cage while the dam was exposed to an unfamiliar, potentially infanticidal male. We expected that repeated exposure to a male intruder would disrupt the mother-infant relationship, resulting in greater responding to arousing stimuli and increased levels of NGF, compared to the H group. Results of this study contradicted our original hypothesis: compared to NH and MI, only H subjects showed a significant increase in NGF protein levels in response to an acute novelty stress in the hippocampus (Cirulli et al., 2007). Thus, active pup manipulations in the form of handling are able to increase the expression on NGF. Given the effects of handling on behavioural and neural plasticity at adulthood, increased NGF levels in response to an acute manipulation could favour neuronal plasticity early on, adjusting the phenotype of each individual to its environment. Whether changes in NGF expression are long-lasting or limited to the acute maternal separation event is currently under investigation.

Maternal separation has also been shown to affect BDNF mRNA levels in limbic regions in rats subjected to repeated 3hr maternal separations during the first two weeks of postnatal life (Roceri et al., 2004). Interestingly, different short- and long-term effects were found: a short-term increase in BDNF gene expression was found in prefrontal cortex and hippocampus, while a long-term depression in this neurotrophin characterized the prefrontal cortex (Roceri et al., 2004). At adulthood, maternally-deprived subjects also showed impaired HPA axis responses to chronic swimming stress (Roceri et al., 2004). BDNF expression was found to be increased on PND 17, approximately 3 days after the last maternal deprivation, a time point that was chosen to distinguish the cumulative effect of repeated separations from the effect of an acute separation. The effect observed in hippocampus and frontal cortex at this early age represents an adaptive consequence of the maternal deprivation previously occurred. In fact, a single 3h deprivation on PND 17 did not alter the expression of the neurotrophin in control as well as in deprived rats, in agreement with previous work showing a lack of change in BDNF expression after 2, 6 or 24 hours of maternal deprivation on PND 9 (Roceri et al., 2002). The basal changes in BDNF expression observed on PND 17 are transient and normalize within the end of puberty. Interestingly, a significant reduction of BDNF mRNA and protein has been recently observed

in the dorsolateral prefrontal cortex of schizophrenic subjects (Weickert et al., 2003). These data, together with previous results (Molteni et al., 2001; Roceri et al., 2002), suggest that the timing and duration of the postnatal manipulation are important for the anatomical specificity of the long-lasting changes in brain function.

Other data indicate an anatomical specificity in the effects of maternal separation and suggest differential modulation of the mature and immature forms of BDNF (pro-BDNF) (Lippmann et al., 2007). A recent study has shown that chronic maternal separation leads to a decrease in the levels of mature BDNF in hippocampus and striatum, and an increase in the ventral tegmental area in rats, while levels of pro-BDNF were significantly increased in the ventral tegmental area (Lippmann et al., 2007). From a behavioural point of view, these subjects were characterized by behavioural deficits and impaired functioning of the HPA axis in response to acute stress. These data show that maternal separation induces long-term changes in BDNF expression, and more specifically the processing of BDNF, in the hippocampus, striatum and ventral tegmental area. A reduction in the expression of the mature form of BDNF has been suggested to induce reduced cell survival (Lippmann et al., 2007).

Reduced levels of BDNF could lead to important effects on cell proliferation, survival and differentiation. In a recent study early isolation rearing was found to be associated with reduced levels of BDNF cell proliferation, survival and differentiation in the dentate gyrus of the guinea pig (Rizzi et al., 2007). The wide reduction of the number of granular cells following isolation rearing emphasizes the role of environmental stimuli as key modulators in neurogenesis. Further data in support to this hypothesis come from studies on communally raised mice (Branchi et al., 2006a; Branchi et al., 2006b). Indeed, changes in BDNF levels in association with reduced neurogenesis and increased depression-like behaviour have been found following early social enrichment in CD-1 mice (Maynard et al., 2001; Branchi et al., 2006b).

Overall, changes in the expression of neurotrophins as a result of early adverse experiences, such as maternal separation, could exert both short- and long-term effects on neurobehavioral plasticity, resulting in important changes in social competences or response to stressful stimulations at adulthood (Venerosi et al., 2003; Cirulli, 2003a; Branchi et al., 2004; Roceri et al., 2004; Branchi et al., 2006b; Giachino et al., 2007).

3.6. Non-human primate models of early stress

Many years of work in non-human primates have shown that various forms of social impoverishment have important long-lasting consequences in non-human primates. The mother-infant bond is the most fundamental early relationship in primates and is critical to developing the social skills necessary to succeed in finding mates, resources and to create social bonds and alliances (Suomi, 1997). Primate infants spend their first weeks of life either in constant physical contact or very close to the mother who provides a secure base in order to develop emotional stability. Work performed in the laboratory of Harry Harlow with rhesus macaques has shown that isolation rearing from birth leads to alterations in an array of physiological and behavioral processes (Harlow, 1965; Harlow et al., 1965). Monkeys reared without conspecifics for the first few months of life show inadequate development of aggressive, affiliative, play, and sexual behavior (Harlow et al., 1965; Capitanio et al., 1986). Infant monkeys removed from their mothers shortly after birth will establish attachment bonds with age-mate peers or even artificial surrogates or other inanimate objects (Suomi and Harlow, 1972). Peer-rearing compensates for many of the deficits seen in infants raised without conspecifics (Harlow and Harlow, 1965). As infants, however, these subjects show important emotional and social disturbances and behavioral abnormalities, such as motor stereotypies (Suomi, 1991; Champoux et al., 2002; Barr et al., 2003). Peer-reared macaques are highly reactive and aggressive and, as adults, rank at the bottom of the dominance hierarchy and show increased voluntary alcohol consumption (Suomi, 1991; Barr et al., 2003).

The non-human primate model is particularly useful for studying the role of environmental influences in the development of psychopathology (Barr et al., 2003). Indeed, most psychopathology revolves around social functioning and, compared to rodents, non-human primates are endowed by complex social behaviors and social structures that more closely approximate those characterizing humans. Moreover, the rearing environment of non-human primates can be tightly controlled (Suomi, 1991).

Over the past 15 years, the Laboratory of Comparative Ethology (LCE) in Poolsville (MD, USA) has developed and refined a compelling rhesus monkey model for studying the origins, developmental course, and long-term consequences of individual differences in physiological reactivity. Dramatic individual differences have been described in rhesus monkey infants exposed to environmental novelty and social challenge (Higley and Suomi, 1996). The physiological patterns that characterize high reactivity (such as increased adrenocortical output, increased monoamine turnover) and impulsive aggressiveness (impaired serotonergic function) in rhesus monkeys mirror those seen in highly reactive and aggressive children, respectively (Higley et al., 1996; Higley and Suomi, 1996). Such differences among rhesus monkeys seem to be: i) highly heritable, appear early in life and remain relatively stable throughout development when environments do not change dramatically; ii) affected by early rearing experiences; and iii) associated with differential risk for developing anxiety- and depressive- like disorders (in the case of high reactive monkey infants) and extreme aggressiveness (for unusually impulsive young monkeys) later in life.

Recent studies have revealed a significant interaction between histories of early-life stress and the genetic background in this primate model (Barr et al., 2003). More in detail, variations in the serotonin transporter gene-linked polymorphic region have been shown to increase the likelihood of developing a vulnerable phenotype (Bennett et al., 2002; Champoux et al., 2002). These effects are highly dependent upon the early life history of the monkeys: infant rhesus macaques exposed to early stress (reared in the presence of peers, rather than by the mother) show exaggerated adrenocortical responses to stress at adulthood if they carry a copy of the short allele of the serotonin transporter gene (*l/s rh5-HTTLPR*), compared to subjects carrying two long alleles (*l/l rh5-HTTLPR*; (Barr et al., 2003).

3.7. Changes in peripheral levels of neurotrophins in a non-human primate model

The serotonin transporter is a protein critical to regulating serotonin function in the brain. Serotonin plays a pivotal role in many forms of psychopathology, with the specific serotonin reuptake inhibitors and other serotonin agents being some of the most widely prescribed psychotropic medications. Although much research up to date has been concentrating on this neurotransmitter, new approaches and new molecular targets are needed to develop effective therapeutic drugs for the treatment of depression and mood disorders. The main hypothesis underlying these studies is that, because of their involvement in brain development and function, neurotrophins might be important factors involved in mediating the effects of early stressful or traumatic experiences on behavioural dysfunctions and psychopathology. Indeed, in addition to neurons, neurotrophins are produced by a variety of cell types including immune cells, adipocytes, endocrine and endothelial cells, thus being in a position to affect and integrate neural, immune, endocrine and metabolic responses to stressful challenges (Aloe et al., 1986; Bonini et al., 1996; Nisoli et al., 1996; Nockher and Renz, 2005). In addition, changes in peripheral levels of neurotrophins, as well as the presence in some individuals of selected gene variants, have been associated with mood disorders, also in interaction with early trauma (Aloe et al., 1994; Hadjiconstantinou et al., 2001; Karege et al., 2002; Karege et al., 2005; Kaufman et al., 2006; Castren et al., 2007; Kauer-Sant'Anna et al., 2007).

Preliminary results obtained assessing peripheral and cerebrospinal fluid (CSF) levels of NGF and BDNF in normally-reared rhesus macaques indicate that, independently from rearing

condition, plasma concentrations of NGF increase significantly with age in rhesus macaques, while an opposite trend has been observed for plasmatic levels of BDNF, 1 month-old monkeys showing higher plasma BDNF concentrations than 1-year or 7-years-old subjects (Cirulli et al., manuscript in preparation). While to date, no clear findings have been reported on changes in NGF levels in human serum as a function of age, levels of BDNF are in line with findings in humans showing reduced concentrations in older individuals (Lommatzsch et al., 2005).

When NGF and BDNF levels were measured in the CSF, no age difference emerged, nor a correlation between CSF and plasma levels for these neurotrophins. However, CSF BDNF levels were found to decrease with age similarly to plasma concentrations (Cirulli et al., manuscript in preparation). There is evidence that BDNF serum levels are closely related to BDNF concentrations in the central nervous system (Karege et al., 2005). Large amounts of BDNF are stored in human platelets, as reflected by high serum levels of BDNF (Lommatzsch et al., 2005). Notably, BDNF is not produced by platelets but it is acquired from external sources. Platelet BDNF could originate from the central nervous system, since this neurotrophin readily crosses the blood–brain barrier (Pan et al., 1998). Thus, serum BDNF might reflect the amount of BDNF taken up by circulating platelets, which might represent a unique BDNF transportation system in the human body (Fujimura et al., 2002). Immune cells, particularly lymphocytes, could represent another important source of blood neurotrophins (Torcia et al., 1996).

In a further analysis, NGF and BDNF plasma levels of 5-months-old subjects reared in the absence of the mother and with the continuous (Peer-reared, PR) or intermittent (Surrogate-peer-reared, SPR) presence of social companions were compared with normally reared infants (Mother-reared, MR). CSF samples from these subjects were also assayed for concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), the noradrenalin metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), and the dopamine metabolite, homovanillic acid (HVA). Results indicate that early stress affected plasma levels of neurotrophins in 5-month-old infant rhesus monkeys. As concerns NGF, no significant difference was found at this age as a function of rearing condition, except for a tendency in the SPR group to show higher NGF levels (Anova: $F_{(2, 12)} = 2.694$, $P = 0.1080$) (SPR>PR>MR). By contrast, MR subjects showed higher levels of plasmatic BDNF ($F_{(2, 16)} = 4.42$; $p = 0.0296$), especially when compared with SPR monkeys (Table 2). The MR group showed also higher CSF levels of MHPG than both the PR and SPR groups ($F_{(2, 22)} = 13.37$; $p = 0.0002$; Table 3). Moreover, CSF levels of 5-HIAA measured in MR monkeys were higher than PR ones ($F_{(2, 22)} = 3.26$; $p = 0.0576$; Table 3). Finally, CSF concentrations of HVA were higher in SPR monkeys when compared to PR ones ($F_{(2, 229)} = 5.68$; $p = 0.0102$; see Table 3), while no differences were found between SPR and MR groups or between PR and MR groups.

Infants separated from their mothers at birth and reared with a peer group show a delay in the development of appropriate social behavior, compared to normally reared monkeys (Novak and Suomi, in press). A fine grain behavioral analysis indicated that, as previously detailed, peer-reared subjects in this study were characterized by high levels of social contact but minimal amounts of play, in addition to showing high levels of passivity and self directed and stereotypic behaviors (Fig. 1). By contrast high levels of play were measured in SPR infants. The tendency to increased levels of NGF shown by the SPR group could be due to more complex social interactions with peers resulting from the lack of contact-comfort by the mother (Fig. 1).

Playful interactions could result in higher NGF plasma concentrations, in line with findings in the literature indicating an involvement of this neurotrophin both in social interactions and in emotional/stressful situations (Aloe et al., 1994; Hadjiconstantinou et al., 2001). Thus, higher levels of NGF might reflect greater social arousal in monkeys reared in the absence of the

mother. We cannot exclude the interesting possibility that increased NGF levels could indicate the stress of establishing a strong social bond, as has been shown in humans (Alleva and Branchi, 2006; Emanuele et al., 2006). More in detail, a positive correlation between peripheral NGF levels and the intensity of the bond established with a partner has been previously shown (Emanuele et al., 2006). Although nursery rearing results in the inability of young subjects to establish an attachment relationship with the mother, subjects interact and do attempt to establish primary attachment relationships with peers. It is possible to hypothesize that the tendency of PR and SPR subjects to show high levels of NGF could reflect these efforts, suggesting that NGF could be involved in the establishment of early social relationships (Alleva and Branchi, 2006).

These same groups also showed reduced BDNF plasma levels, especially the SPR. This finding, as previously suggested in the rodent literature, could indicate reduced neuroplasticity as a result of exposure to early chronic stress (Cirulli, 2003a; Roceri et al., 2004). Data on CSF 5-HIAA concentrations suggesting reduced serotonin function in monkeys reared in the absence of the mother confirm previous results obtained in this primate model and are in line with the BDNF data (Higley et al., 1996; Shannon et al., 2005). The reduction in peripheral BDNF levels induced by early nursery rearing suggests that low levels of this neurotrophin could be an enduring, stable biological marker that may have roots in early infancy, as previously demonstrated for serotonin (Shannon et al., 2005).

Thus, this preliminary study indicates that a reduction in both BDNF and serotonin metabolites characterizes overall subjects exposed to early stressful conditions, indicating BDNF as a novel neuroendocrine factor involved in the response to early stress also in human primates and confirm the important relationship between serotonin function and BDNF (Mattson et al., 2004).

4. General conclusions

The comparative approach used in these studies has revealed important changes in the levels of NGF and BDNF both as a function of age and as a consequence of the quality of the rearing environment.

The preliminary findings obtained in rhesus macaques suggest that changes in plasma levels of neurotrophins might function as peripheral markers of early adversity, being differentially affected by changes in the rearing environment. Peripheral levels of NGF in monkeys reared without adults might reflect stressful social interactions with their peers, in the absence of the mother. As for BDNF, reduced peripheral levels characterize subjects exposed to early stress. BDNF levels in human serum have been shown to correlate with the severity of depression and low serum BDNF concentrations are currently discussed as a risk factor for the development of mood disorders and as a potential biological marker for depression (Karege et al., 2002; Shimizu et al., 2003). The finding that monkeys experiencing maternal separation show overall reduced BDNF levels suggest a major impact of the disruption of the mother-infant relationship on this marker of brain plasticity and suggest that this procedure might result in long-term effects on behavior.

Further studies are currently in progress to validate the use of NGF and BDNF as peripheral markers of brain plasticity in this primate model. The availability of easy-to-collect and easy-to-screen neurobiological indices could be important tools to predict meaningful gene x environment interactions underlying increased susceptibility to psychopathology. Indeed, BDNF polymorphisms impact on the incidence of anxiety-related personality traits and mood disorders in humans (Sen et al., 2003; Schumacher et al., 2005) and a possible role in this model still needs to be investigated.

Overall, these studies could be relevant to identify effective behavioral strategies as well as suggesting possible pharmacological targets for the prevention and cure of mood disorders, also according to the individual life histories and individual differences in the genotype. Animal models of early stress provide an important avenue for translational research aimed at developing prevention, intervention, and treatment strategies for humans affected by early childhood adversity.

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Fig. 1.

Social and non social behavior assessed in 150-days-old rhesus macaques. All data expressed as means + SEM. Tukey HSD test, $P < 0.05$; MR vs †PR, or *SPR; ‡PR vs SPR. MR = Mother reared; PR = Peer-only reared; SPR = Surrogate-peer reared.

Table 1
Most commonly used manipulations of the mother-infant relationship in rodents and non-human primates

Manipulation	Methodology	Animal model
Handling (H)	3-15 min. removal of pups from the home cage (variables: temperature, mother stays in the cage). Most commonly performed daily from postnatal day 2-14	Rat, mouse
Maternal separation (DEP)	3-24 hr. Relatively short (3-6 hrs) separations can be performed daily. Long (24 h) separations are usually performed only once.	Rat, mouse, non-human primate
Isolation rearing	Rearing from birth without mother or siblings	Non-human primate
Peer-rearing (PR)	Removal from mother at birth and raised with siblings	Non-human primate
Surrogate-peer-rearing (SPR)	Removal from mother at birth and contact with siblings only for limited time over the day	Non-human primate
Control conditions		
Non-handling (NH)	Litters not cleaned, not handled until	Rat, mouse
Animal facility rearing (AFR)	Normal colony room handling	Rat, mouse
Mother rearing (MR)	Normal colony rearing	Non-human primate

Table 2

Plasma levels (pg/ml) of NGF and BDNF in 5 months-old Mother- (MR), Peer-only- (PR) and Surrogate-peer-reared (SPR) monkeys. Data are mean values $\pm$ S.E.

	NGF	BDNF
		*
MR	11.7 $\pm$ 3.0	230.1 $\pm$ 85.0
PR	38.5 $\pm$ 18.0	173.1 $\pm$ 54.0
SPR	55.0 $\pm$ 42.0	38.1 $\pm$ 9.0

Post-hoc: *MR vs SPR; $p < 0.05$

Table 3

CSF levels (pmol/ml) of 5-HIAA, HVA and MHPG in 5 months-old Mother- (MR), Peer-only- (PR) and Surrogate-peer-reared (SPR) monkeys. Data are mean values $\pm$ S.E.

	5-HIAA	HVA	MHPG
	#	§	# *
MR	412.9 $\pm$ 26.0	1568.3 $\pm$ 72.0	159.9 $\pm$ 5.0
PR	327.3 $\pm$ 21.0	1241.3 $\pm$ 122.0	112.5 $\pm$ 7.0
SPR	381.2 $\pm$ 12.0	1722.7 $\pm$ 77.0	120.7 $\pm$ 6.0

Post-hoc: #MR vs PR; *MR vs SPR; §PR vs SPR; $p < 0.05$