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Review

Early life influences on emotional reactivity: Evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels

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ABSTRACT

During the early post-natal phases the brain is experience-seeking and provided by a considerable plasticity which allows a fine tuning between the external environment and the developing organism. Since the early work of Seymour Levine, an impressive amount of research has clearly shown that stressful experiences exert powerful effects on the brain and body development. These effects can last throughout the entire life span influencing brain function and increasing the risk for depression and anxiety disorders. The mechanisms underlying the effects of early stress on the developing organism have been widely studied in rodents through experimental manipulations of the post-natal environment, such as handling, which have been shown to exert important effects on the emotional phenotype and the response to stress. In the present paper we review the relevant literature and present some original data indicating that, compared to handling, which imposes an external manipulation on the mother–infant relationship, social enrichment, in the form of communal rearing, in mice has very profound effects on animal's emotionality and the response to stress. These effects are also accompanied by important changes in central levels of brain-derived neurotrophic factor. The present data indicate that communal rearing has more pervasive effects than handling, strengthening previous data suggesting that it is a good animal model of reduced susceptibility to depression-like behavior. Overall, the availability of ever more sophisticated animal models represents a fundamental tool to translate basic research data into appropriate interventions for humans raised under traumatic or impoverished situations.

Contents

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1. Introduction

In humans, quality of family life influences the development of individual differences in vulnerability to psychopathology throughout life (Heim and Nemeroff, 2001). Not only severe conditions such as physical or sexual abuse, but also persistent emotional neglect or family conflict, can compromise growth, intellectual development and lead to increased risk for adult obesity, depression and anxiety disorders (Cicchetti and Toth, 1995; Heim and Nemeroff, 2001; Rutter et al., 2001).

Animal and human studies have provided a wealth of data showing the negative effects of chronic exposure to stress and/or adversity on the developing brain (Plotsky and Meaney, 1993; Liu et al., 1997; Heim and Nemeroff, 2001; Pryce et al., 2001; Roceri et al., 2004; Levine, 2005; Cirulli et al., 2009a,b). However, while there is no doubt that the developing brain is "experience-sensitive" and "experience-dependent", the mechanisms that render a particular experience "good" or "bad" are still in the process of being defined. We are still away from fully understanding why the same experience can be detrimental for some individuals, while having no effect in others. A stressful experience cannot be considered as being necessarily negative, as it represents an adaptive physiological response to the threats faced by each individual (Lupien et al., 2009). Understanding these mechanisms, and how they can be affected by the family environment is extremely important if one wants to provide interventions early enough to individuals who are the most likely to respond to them.

Early affective and social interactions are extremely important in psychological development, especially when we consider 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). Research performed in humans and primates has clearly shown that social deprivation, be it institutionalization in children or social isolation in primates, has long-term consequences on emotional functioning and social behavior (Champoux et al., 1989; Suomi, 1997; Chugani et al., 2001; Gunnar et al., 2001; Cirulli et al., 2009a).

This paper will present some background literature and describe some original data to address the issue of the potential positive impact of early interventions on brain development. These results could help devise new strategies to buffer the problem of early-life stress using the potentiality present in the family and social environment of each individual.

2. Manipulating the mother–infant relationship

Animal models have indicated that plasticity is a fundamental characteristic that can allow brain development to be led through different trajectories that ultimately result, at adulthood, in different adaptations, depending upon the ecological niche of the subject.

Studies performed in altricial rodents (e.g. mice and rats) have clearly shown that maternal care is crucial for an adequate development of the pups, representing the most relevant source of early stimulation. Thus modifications of the maternal environment may result in long-term changes in the pattern of neuroendocrine and emotional/behavioral responses later in life (Cirulli et al., 2009a).

Wild rodents having pups are often forced to leave the nest for variable periods (hours) of time to provide their themselves food. This pattern of maternal attendance to the nest has been modeled in the laboratory settings by early handling (H), which consists of removing the pups from the mother and their cage and placing them in individual compartments for up to 15 min until weaning (Levine, 1957). Animals handled during infancy show important changes in the functionality of the hypothalamic–pituitary–adrenal (HPA) axis (Levine, 1957) in a way such that the ability of the adult organism to respond, cope, and adapt to novel and/or stressful stimuli is increased (Meaney et al., 1991). For example, immediately after the exposure to an electric shock, H rats (tested at adulthood) show a faster peak in the release of the stress hormones glucocorticoids (GC), and a rapid return to basal levels when compared to non-handled (NH) controls. The speed and short duration of the neuroendocrine responses characterizing the H subjects appear to be extremely adaptive preventing the organism to be exposed to high circulating GC levels that can result, especially under chronic stressful conditions, in neurotoxicity through different mechanisms (Lupien et al., 1998; McEwen and Seeman, 1999). These changes in neuroendocrine responses to stress are also accompanied by important changes in emotionality and in those neurotransmitter systems which regulate it such as the GABAergic one (Giachino et al., 2007).

These long-term effects of the H procedure appear to depend upon changes in the phenotype of those neurons involved in the stress response (Meaney et al., 1996). As an example H subjects show an increased number of glucocorticoid receptors (GR) expression in the hippocampus, a brain region strongly implicated in glucocorticoid feedback regulation (Meaney et al., 1989). In addition, hypothalamic corticotropin-releasing hormone (CRH) mRNA and protein levels are higher in NH compared with H animals already under basal conditions (about 2.5-fold) as well as in response to stress (Plotsky and Meaney, 1993).

The HPA axis is not the only system affected by early experiences. Rats exposed to an early H regime show, as adults, greater amplitude of hippocampal long-term potentiation (LTP; a long-lasting form of synaptic plasticity) following brief, tetanic stimulation (Wilson et al., 1986). In addition, the function of the immune system also appears to be affected by handling since H animals show higher survival rate than NH following tumor implantation and are differentially affected by experimental allergic encephalomyelitis, an animal model of multiple sclerosis

(Solomon et al., 1968; Columba-Cabezas et al., 2009). Likewise, the reward value of amphetamine in a conditioned place preference paradigm has been shown to be decreased in adult subjects which were subjected to early H, supporting a role for the early manipulation of the mother–infant relationship also in shaping central neurotransmitter activity (Owens and Nemeroff, 1991; Campbell and Spear, 1999).

It is important to point out that, depending on the duration of the dam–offspring separation, the effects on the HPA axis responses to stress are qualitatively different. Animals that have been exposed to repeated maternal separation (MS) of 180–360 min per day, for the first 2 weeks of life show, at adulthood, increased plasma ACTH and corticosterone (CORT) levels in response to a stressful challenge (when compared to NH controls) an effect opposite to that of the H procedure. This longer period of separation also results in decreased GR binding in both the hippocampus and the hypothalamus (Plotsky and Meaney, 1993).

The effects of prolonged MS are not always consistent and a number of papers indicate that even 3–4 h daily separations performed from birth until weaning result in behavioral and endocrine changes in the same, rather than in the opposite, direction as handling (Lehmann and Feldon, 2000; Pryce et al., 2001; Lehmann et al., 2002; Giachino et al., 2007). In addition, in a recent experiment we have shown the same magnitude of CORT secretion in response to a novelty stress in mice that had undergone 3 h daily MS from post-natal days 2–14, compared to unhandled controls (Venerosi et al., 2003). Subjects that had undergone maternal separation also showed reduced neophobia, suggesting a generalized reduction of behavioral arousal. The apparent discrepancies present in the literature might be due to differences in the experimental procedures used in different laboratories. Sometimes, even subtle differences in weaning procedures can produce different long-term effects. In fact, it has been shown that, following exposure to post-weaning environmental enrichment, the effects of maternal separation on both HPA and behavioral responses to stress can be completely reversed (Francis et al., 2002). This evidence suggests an important role of the environment in compensating, at least functionally, the influence of adversity in earlier stages of development (Francis et al., 2002).

The effects so far summarized can be ascribed to changes in maternal care resulting from the manipulation procedures (both in the case of the H and MS). Indeed, both human data and animal studies suggest that the relation between the quality of the early environment and emotional responses at adulthood appears to be mediated by parental/maternal influences on brain development (Francis and Meaney, 1999; Caldji et al., 2000b; Champagne et al., 2003). The so called "maternal mediation" hypothesis first proposed in 1970s (Smotherman et al., 1974) has been later supported and a direct relationship between variations in the levels of maternal care and the development of individual differences in the behavioral and neuroendocrine responses to stress of the offspring has been described (Caldji et al., 2000b; Pryce et al., 2001; Cirulli et al., 2003; Sale et al., 2004). In particular, high levels of maternal care appear to be directly related to reduced behavioral and neuroendocrine responses to novelty in the offspring (Champagne et al., 2003).

This relationship has been recently questioned (Pryce et al., 2001; Macri and Wurbel, 2006). Indeed, both early handling and longer maternal separations (3–6 h) which have been shown to have opposite effects on the offspring's behavioral and physiological reactivity to stress, result in increased maternal behavior (Pryce et al., 2001; Macri et al., 2004; Cirulli et al., 2007). Thus, the equation linking early environmental variables and offspring behavior at adulthood through changes in maternal care presents some missing variable, which might be revealed using more ethologically relevant paradigms.

3. Communal nesting as a form of early social enrichment

An experimental manipulation that has been exploited to investigate the role of the early social environment on adult behavior is the communal nest (CN) (Branchi, 2009). CN consists of females that combine their pups in a single nest, sharing care-giving behavior. Rearing pups in a CN occurs very frequently in feral mouse populations: semi-naturalistic and field studies demonstrated that up to 80% of females may rear their offspring in communal nests (Crowcroft and Rowe, 1963; Manning et al., 1995). Relatedness and familiarity among females are key factors in determining the creation and success of the CN (König, 1994). Sharing of parental responsibilities by multiple individuals occurs in a number of social species (Gittleman, 1985; Konig, 1997). Indeed, communal nesting and nursing leads to a number of benefits, including allogrooming, cooperative foraging and feeding, improved defense and enhanced thermoregulation. However, it also involves a number of costs, such as higher risk for infanticide, increased competition for food, risk to be spotted by predators and parasite transmission (Hayes, 2000; Ebensperger, 2001).

The social stimulation provided by the CN has a major impact on development of a number of adult behaviors, ranging from social behavior to the emotional response. Adult CN mice, compared to those reared in the standard laboratory condition, display a higher propensity to interact socially and more promptly achieve a well-defined social role when tested in a social interaction test. In this test, which exploits the need of an adult male mouse to defend its own territory and establish a hierarchy, mice lacking communal nesting experience need several encounters to fully display a behavioral profile typical of a dominant or subordinate male, while mice reared in a communal nest display a well-defined social role already on the first encounter (Branchi et al., 2006a). Furthermore, home-cage observations have indicated that CN mice display social responses more appropriate in an eco-ethological perspective: CN mice are more aggressive than standard reared non-handled controls (NH) mice, but only when social hierarchy needs to be established (D'Andrea et al., 2007). The social competencies of CN mice, and in particular the relevance of social environment, have also been investigated in association with the emotional response (Branchi et al., 2006b). In one paradigm, experimental subjects are exposed to an anxiogenic environment (i.e. an elevated plus-maze), in two different social conditions: alone or paired with a familiar conspecific. While NH mice do not behave differently when exposed to the apparatus in the two social conditions, CN mice show significantly less anxiety-like behavior in the paired than in the alone condition. Thus, the social context appears to influence more markedly the emotional response of CN than NH mice (Branchi et al., 2006b).

Being reared in a CN also affects the emotional response at adulthood. However, such modifications appear to be dependent on the procedure used, in particular on the timing of placing the mouse mothers together in a cage. So far, two different procedures have been used: the mothers are either placed together before parturition, or, as in the present study, they are put together immediately after delivery. In particular, at adulthood the CN offspring shows greater emotionality in an open-field and in an elevated plus-maze (but not in a social interaction test) when the mothers are put together 5 days before parturition (Branchi et al., 2006a,b; Branchi, 2009), while opposite effects are observed when the mothers are put together on the day of parturition in a condition referred to as "communal rearing" (CR) (Sayler and Salmon, 1971; Curley et al., 2009). It is possible to hypothesize that these differential effects arise from the stress levels imposed by the two procedures to the mothers occurring during two different time windows (Andersen, 2003). Finally, CN mice show a reduced depression-like behavior (Branchi et al., 2009b).

4. Neurotrophins as transducers of early experiences

Early-life stressful events, such as childhood trauma and neglect, are associated with depression and anxiety disorders and sustained changes in the HPA axis (Heim and Nemeroff, 1999, 2001). Furthermore, depression is accompanied by a dysfunctional HPA system (Holsboer, 2000). These associations demonstrate that developmental environmental factors can produce enduring changes in HPA system physiology and emotional behavior, although the molecular mechanisms underlying such effects have only just started to be elucidated (Szyf et al., 2005). Neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are among the most prominent factors involved in brain development and plasticity and are good candidates for mediating some of the effects of early experiences on brain function since they are involved in synaptic and morphological plasticity with maximal levels at times of neuronal growth, differentiation and synaptogenesis (Thoenen, 1995). Several lines of evidence suggest that changes in BDNF signaling in different areas of the adult brain may be implicated in the pathophysiology of psychiatric disorders, such as depression (Altar, 1999; Castren, 2005; Berton et al., 2006; Duman and Monteggia, 2006; Castren et al., 2007; Sen et al., 2008). BDNF itself does not control mood, but plays an important functional role in the modulation of networks which ultimately determine how a plastic change influences mood (Castren et al., 2007). It has been, in fact, hypothesized that successful antidepressant treatments promote activity-dependent neuronal plasticity by activating BDNF systems, possibly inducing proliferative or survival effects on neural stem cells (Castren, 2005).

In the brain, the expression of NGF and BDNF has been localized in different areas, including the hippocampus and neocortex (Large et al., 1986; Yan et al., 1997). The hippocampus, in particular, is one of several limbic brain structures implicated in the pathophysiology of mood disorders. It expresses high levels of receptors for GCs and plays a significant role in negative feedback regulation of the HPA axis, which is often disinhibited in depressed subjects (Sapolsky, 2001). Because of the fundamental role played by these neurotrophic factors in shaping brain function, a pathological alteration in their activation early during post-natal life could exert long-term effects on synaptic plasticity, impairing the ability of the organism to cope with novel/stressful situation, leading to psychopathology (Zubin and Spring, 1977).

Previous work has shown that manipulations of the early environment can affect the expression of these neurotrophins both during development and at adulthood (Liu et al., 1997; Cirulli et al., 1998, 2000, 2003; Roceri et al., 2004; Sale et al., 2004; Alleva and Branchi, 2006). In particular, daily maternal separations of 3 h performed over the first two post-natal weeks affect BDNF mRNA levels in limbic regions of rats (Roceri et al., 2004). BDNF gene expression is increased shortly (on post-natal day 17) after maternal deprivation stress in the prefrontal cortex and hippocampus, while at adulthood a long-term depression in the expression of this neurotrophin in the same brain area can be found (Roceri et al., 2004). These changes in BDNF expression are accompanied by reduced HPA axis responses to repeated swimming stress (Roceri et al., 2004). Altered BDNF expression may contribute to the generation of individual differences in stress neurocircuitry, providing a substrate for altered vulnerability to depressive disorders at adulthood (Castren et al., 2007; Nair et al., 2007; Cirulli and Alleva, 2009; Cirulli et al., 2009a).

While some effect on NGF levels has been reported, so far no data are available on potential changes in BDNF levels in the central nervous system as a result of handling procedures (Cirulli et al., 2007). By contrast, there is evidence to show that the early social stimulation provided by the CN condition does affect the levels of neurotrophins (Branchi et al., 2006a,b). NGF and BDNF levels have been found to be markedly increased in different brain areas, including the hippocampus and hypothalamus in CN mice compared to NH mice. According to the literature (Castren, 2005), an increase in neurotrophin levels in the hippocampus is suggestive of changes in the neurogenesis rate in the dentate gyrus. Indeed, CN mice show a marked increase in the number of newly generated brain cells (Alleva and Branchi, 2006). In particular, when compared to NH mice, subjects raised in the CN condition show no difference in brain cell proliferation but an increase in survival, in line with the hypothesis that BDNF signaling is required mainly for the long-term survival and less for proliferation of newborn brain cells (Sairanen et al., 2005).

5. Comparing handling vs communal rearing paradigms: evidence that social enrichment has more profound and differential effects than handling

While there is no doubt that experiences early during development have important effects on adult emotionality, the quality and quantity of stimulation resulting in a certain degree of emotional responding, and the underlying mechanisms, still needs to be defined. The purpose of the present study was to compare the effectiveness of a naturalistic type of enrichment, in which mice are reared "communally" by more than one mother (Branchi et al., 2009a; Curley et al., 2009), with the most commonly used early handling paradigm, which involves only physical manipulation of the nest environment (Levine, 1957). Compared to handling, which involves selected changes in maternal behavior (Pryce et al., 2001; Cirulli et al., 2007), CR and CN are characterized by high levels of both maternal behavior and social interactions offered by the nest peers. In particular we assessed the short- and long-term effects of handling vs CR on emotionality, response to a physical stress and levels of BDNF in selected brain areas. The choice of the specific paradigm of CR offered us the opportunity to exclude potential prenatal influences on brain development afforded by the presence of multiple females in the nest area before birth.

Long-term effects of early manipulations, such as neonatal handling, mainly involve changes in behavioral responding to fear-provoking situations (Caldji et al., 2000a; Macri et al., 2004). However, few attempts have been made to assess modifications in the emotional response early during the post-natal period. In mammals, brief separations between the mother and the infant result in "isolation" or "distress" calls. During the first two post-natal weeks mouse pups socially isolated from the mother and siblings emit vocalizations in the ultrasonic range (ultrasonic vocalizations, UVZ) that have been extensively characterized (Elwood and Keeling, 1982; Cirulli et al., 1994; Branchi et al., 1998, 2001). These calls have been effectively used as markers of neurodevelopment and it has been suggested that they might represent an early indicator of adult emotionality (Hofer, 1996; Brunelli, 2005). Indeed, a number of drugs affecting central neurotransmitter systems can modify UVZ rate in infant rodents, including benzodiazepines (Gardner, 1985; Insel, 1989; Insel et al., 1989). It has been previously shown that early-life events influence the development of the GABAA receptor system, thus altering the expression of fearfulness at adulthood (Caldji et al., 2000b; Giachino et al., 2007). So far, only a few studies have tested the functionality of the GABAergic system in the mouse in relation to early manipulations of the mother–infant relationship (Cirulli et al., 1994, 2007). Compared to previous investigations, here we compared the response to a selective GABAergic agonist (chlordiazepoxide – CDP) in both H and CR mice early during development. In addition, we assessed whether possible short-term modifications in emotionality would be maintained up to adulthood and would be correlated with more adaptive coping strategies in response to stressful challenges. We expected manipu-

lated subjects to show reduced emotionality in response to diverse challenges and that these might also be reflected in BDNF levels as measured in selected brain regions (hippocampus, hypothalamus, striatum). In particular, we were interested in assessing whether there might be a relationship between hypothalamic BDNF levels and the neuroendocrine response to stress, since a previous work has indicated an important role of BDNF in the hypothalamus and anterior pituitary, besides the more known effects on the hippocampus (Tapia-Arancibia et al., 2004).

5.1. Materials and methods

5.1.1. Animals and breeding procedures

Seventy-four adult nulliparous females and 30 males of the outbred CD-1 mouse strain without prior breeding experiences were purchased from a commercial breeder (Harlan Italia, San Pietro al Natisone, Udine, Italy). Upon arrival, the animals were housed in an air conditioned room (temperature $21 \pm 1\,^{\circ}C$ and relative humidity: $60 \pm 10\%$) with a reversed 12 h light–dark cycle (lights on at 7 p.m. until 7 a.m.). Males and females were housed in groups of six in 42 cm × 27 cm × 14 cm Plexiglas cages with metal tops and sawdust as bedding. Pellet food (enriched standard diet purchased from RIPIER, Molino, Italy) and water were available *ad libitum*. After 1 week of acclimatization, mice were assigned to one of the three experimental groups: NH, H or CR. For the NH and H groups, 32 females and 16 males and were used to form breeding pairs housed in 33 cm × 13 cm × 14 cm Plexiglas boxes. For the CR group, 42 females and 14 males were used to form breeding groups, made up by 1 male and 3 females, housed in 42 cm × 17 cm × 14 cm Plexiglas boxes. Females were inspected daily at 9 a.m. for the presence of a vaginal plug. The male was removed 10 days following the formation of the breeding pairs and NH, H and CR females were housed individually in a new cage. Females were checked for delivery twice daily at 9 a.m. and 5 p.m. Day of birth was designated as post-natal day 0 (PND 0). On PND 1 pups of the NH and H groups were culled to four males and four females. After the culling procedure, pups and mothers were placed in a clean cage. When needed, pups from other litters were fostered to reach the appropriate gender ratio. In the CR group, when the female trio was formed, there was a discrepancy of up to 6 days in the age of the pups and the trios were rearranged on the day of birth so as to accommodate the correct birth spacing. Litters that did not meet this requirement were discarded. In the CR group, each litter of the trio was culled to four males and four females (for a total number of 24 pups). In the CR litters only pups that differed ±3 days from their littermates were used as focal animals in order to have a homogeneous group of subjects. These were marked on the day following birth by means of injecting a minimal amount of ink in the thigh. Weaning occurred on PND 21. All male mice were group housed with their siblings in 42 cm × 17 cm × 14 cm Plexiglas boxes. Females were used only to assess ultrasonic vocalizations on the first post-natal week but were not used in all subsequent experimental phases.

All experimental procedures have been carried out in accordance with the EC guidelines (EC Council Directive 86/609 1987) and with the Italian legislation on animal experimentation (Decreto L.vo 116/92).

5.1.2. Experimental groups and manipulation procedures following birth

1. Standard reared non-handled group (NH): animals belonging to this group were sexed and weighed on PND 1 and then left undisturbed in their home cages until weaning.
2. Handled group (H): animals belonging to this group were sexed and weighed on PND 1. From PND 2–14 all pups were removed from the home cage and moved into a clean cage with clean sawdust for 15 min; during this time water and food were not available. During the handling procedure pups were placed always in the same holding cages. Dams remained in the home cages during the handling procedure. After 15 min, the entire litter was put back in the home cage. Handling occurred always at the same time of the day (between 1 and 3 p.m.).
3. Communal rearing group (CR): animals belonging to this group were sexed and weighed on PND 1 and then left undisturbed in their home cages until weaning.

5.1.3. Ultrasonic vocalizations and drug treatments

Pups were tested on PND 8, an age when UVZ show a peak in emission (Branchi et al., 2001). On the day of testing four pups in each litter were assigned to the following treatments, according to a split-litter design (see also Cirulli et al., 1994):

1. Vehicle (VEH): Pups were weighted and injected i.p. with vehicle (0.9% NaCl containing two drops of Tween 80 per 10 ml of solution in a volume of 5 μl/g body weight).
2. Chlordiazepoxide: Pups were weighted and injected i.p. with a 2 mg/kg of CDP (Sigma–Aldrich, Italy) or vehicle. Drug dosages have been selected based upon previous studies (Cirulli et al., 1994). After the i.p. injection VEH and CDP pups were kept on clean sawdust together on a heating pad for 30 min. Thus pups were exposed to novelty (clean cage) before UVZ recording. At the end of this period the animal's UVZ were recorded for 5 min in a soundproof chamber (Amplisilence, I-10070 Robassomero, Italy). Pups were removed from the litter and placed individually on a glass dish (20 cm × 20 cm) for 5 min. Room temperature was about $28 \pm 2\,^{\circ}C$. Ultrasonic calls were recorded using a S-25 Bat Detector (Ultrasound advice, London, UK) tuned at 70 kHz as previously described (see also Cirulli et al., 1994; Branchi et al., 1998). Litters used to assess UVZ were not used later on.

5.1.4. Plus-maze test

5.1.4.1. Drug. CDP was dissolved in a physiological saline (0.9% NaCl containing two drops of Tween 80 per 10 ml of solution in a volume of 10 ml/kg) and administered i.p. 30 min prior to testing. Drug dosage (7.5 mg/kg) was selected on the basis of previous research (Cole and Rodgers, 1993; Holmes and Rodgers, 1999; Rodgers et al., 2002).

5.1.4.2. Plus-maze apparatus. The day before the plus-maze test, when animals were 3 months of age, two mice of each litter were placed in a plastic restrainer fitted closely to body size (a 50 ml Falcon tube with holes drilled to allow ventilation and an opening for the tail). After a 30-min period, animals were removed from restrainers and placed alone in a novel cage for 24 h (Martijena et al., 1997).

The elevated plus-maze comprised two open arms (30 cm × 5 cm × 0.25 cm) and two closed arms (30 cm × 5 cm × 15 cm) extended from a common central platform (5 × 5). The apparatus was constructed from Plexiglas (black floor, transparent walls) and elevated to a height of 60 cm above the floor level on a central pedestal. Experimental groups: No Stress-Vehicle; Stress-Vehicle; No Stress-CDP; Stress-CDP.

5.1.4.3. Procedure. Animals were transported to a dimly illuminated room and left undisturbed for at least 1 h prior to testing. Thirty minutes following injection, mice were individually placed on the central platform of the maze facing an open arm. Animals were allowed to freely explore the maze for 5 min. Test order was fully counterbalanced for post-natal condition, Stress and Drug. Immediately after each session, the maze was thoroughly cleaned with cotton pads wetted with 40% ethanol and all sessions were video-recorded for subsequent analysis. The test was conducted

under dim red light during the early dark phase, that is at a time when the animals show high levels of activity.

5.1.4.4. Behavioral analysis. Test videotapes were scored blind to treatment conditions by a trained observer using a commercial software ("The Observer 3.0"; Noldus, 1991). *Percent time* spent and *Percent entries* in the open vs the closed arms were used as a measure of anxiety levels. In addition, frequency and duration of *head dipping* (exploratory movement of head/shoulders over the side of the maze) and *stretched-attend posture* (SAP: exploratory posture in which the body is stretched forward, then retracted to the original position without any forward locomotion) were assessed (Fernandes and File, 1996; Rodgers et al., 1999). Furthermore, the number of fecal boli was scored at the end of the test for each subject.

5.1.5. Exposure to repeated swimming stress

A repeated swimming stress paradigm devised for rats was adapted to adult mice to investigate whether different early experiences could affect the neuroendocrine response to a repeated stress procedure as well as affecting the levels of neurotrophins (Roceri et al., 2004). Stress was administered to adult mice (5 months of age) twice daily at variable times, with a random time interval between treatments. To induce stress, animals were individually placed for 5 min in a bucket filled with cold water (17 °C) that was deep enough so that the animals' paws and tail did not touch the bottom. After forced swimming, they were dried with a towel and returned to their home cage. To ensure the manifestation of repeated stress, animals were stressed over a 2-week period. Mice were sampled twice to assess blood levels of CORT, on day 1 and on day 13, and in both cases blood was collected from the tail before (basal CORT levels) and 30 min after the cold swim procedure. For the bleeding procedure, a small cut in the tail was made to collect a blood sample. Blood was placed in centrifuge vials filled with ethylenediamine tetra-acetate (EDTA; 0.16 mol/l), centrifuged at 2000 rpm for 20 min to obtain cell-free plasma, frozen at −20 °C, and later assayed for CORT using a commercial radioimmunoassay kit (ICN Biomedicals, Costa Mesa, California). On sampling days, mice underwent a single swimming test. In order to assay CORT levels during the trough, and since animals were kept on a reversed light/dark cycle, they were sampled in the late afternoon (around 6 p.m.). Body weight data were collected on experimental days 1 and 13. On the same days behavioral data (latency, frequency and duration) concerning *Swimming*, *Floating* (the animal floats passively in the water making only those movements necessary to keep its head above water) and *Struggling* (active forepaws movements directed against the walls or vigorous movements of all four limbs with the forelimbs breaking the surface of the water) were video-recorded and later analyzed using a commercial software ("The Observer 3.0"; Noldus, 1991). Upon sacrifice, which occurred 30 min following the onset of the last swimming stress session, brain regions (hypothalamus, hippocampus and striatum) were rapidly dissected out, frozen on dry ice and stored at −80 °C for the analysis of BDNF levels. A separate group of untested subjects was used as control for the BDNF data.

5.1.6. BDNF determination

BDNF protein levels were measured in different brain areas such as the hippocampus, striatum and hypothalamus following the procedure suggested by the manufacturer (Emaxtm ImmunoAssay System number G6891 by Promega, Madison, WI, USA). A monoclonal anti-mouse-BDNF antibody was used. BDNF concentration was determined from the regression line for the BDNF standard curve (ranging from 7.8 to 500 pg/ml-purified mouse BDNF) incubated under similar conditions in each assay. The sensitivity of the assay is about 15 pg/ml of BDNF, and the cross-reactivity with other related neurotrophic factors (NGF, NT-3, and NT-4) is considered nil (Aloe et al., 1999).

5.1.7. Statistical analysis

Data were analyzed using parametric analysis of variance (ANOVA) (with repeated measures, when appropriate). Pup's weight were analyzed taking into account litter effects: males and females in each litter were averaged and analyzed considering the average "male" and "female" value for each litter as repeated measure. For ultrasonic vocalizations, Drug and Sex were added to Condition as between-subjects variables.

As for CORT levels, since data did not follow a normal distribution, they were normalized by transforming them into the square root of the raw data. ANOVA analysis was performed on the difference (delta) obtained between CORT values at time 30 and at time 0 both for day 1 and for day 13 while to assess basal values it was performed separately on CORT values at time 0. Condition was always a between-subjects factor while Stress was considered as within-subjects variable only when the comparison between day 1 time 30-0 vs day 13 time 30-0 was taken into account (repeated measures).

In the case of BDNF determination, post-natal Condition and stress (no swimming stress vs swimming stress) were between-subjects variables. Post hoc comparisons were performed using the Tukey's test.

5.2. Results

5.2.1. Weight gain

On PND 8 no significant effect of Post-natal Condition ($F(2, 20) = 0.431$; $p = 0.6557$), Sex ($F(1, 20) = 3.886$; $p = 0.0627$) or interaction between Post-natal Condition and Sex ($F(1, 20) = 0.721$; $p = 0.4985$) was found on the offspring's weight gain.

5.2.2. Ultrasounds

Once it was determined that Sex was not a significant factor (main effect of Sex ($F(1, 78) = 0.319$; $p = 0.5740$), data were collapsed across this variable. For the number of ultrasounds emitted by pups at PND 8, ANOVA analysis failed to show an effect of Post-natal Condition ($F(2, 84) = 2.930$; $p = 0.0589$), while a main effect of Drug ($F(1, 84) = 5.280$; $p = 0.0241$) and a significant interaction between Condition and Drug ($F(2, 84) = 6.015$; $p = 0.0036$) were found. In particular, post hoc analysis revealed that CDP reduced significantly the number of ultrasounds in the NH ($p < 0.01$) but not in the H and CR groups, and that saline injected CR pups were characterized by reduced basal levels of ultrasounds in comparison with similarly-treated NH pups ($p < 0.05$) (Fig. 1).

5.2.3. Plus-maze test

When percent entries in the Open arms were considered, we found a main effect of Post-natal Condition (frequency: $F(2, 84) = 4.294$; $p = 0.0168$), with the CR group showing a higher frequency of entries when compared to NH subjects ($p < 0.05$), but no effect of Stress or Drug or any interaction between the two variables. No significant effect was found as for the percent time spent in the same part of the maze ($F(2, 84) = 2.596$; $p = 0.0805$) (Fig. 2 and Table 1). General activity was found to be affected by Post-natal Condition ($F(2, 84) = 15.634$; 119; $p < 0.0001$; $p < 0.0001$ for frequency and duration, respectively), CR subjects showing a higher frequency and duration of this behavior when compared both to H and NH mice ($p < 0.01$ for both frequency and duration). In the case of activity duration, a main effect of Stress was also found ($F(1, 84) = 7.102$; $p = 0.009$) (Table 1), stressed subjects spending overall more time exploring the maze. Analysis of head dipping revealed a main effect of Post-natal Condition and Drug for frequency only (main effect of Post-natal Condition:

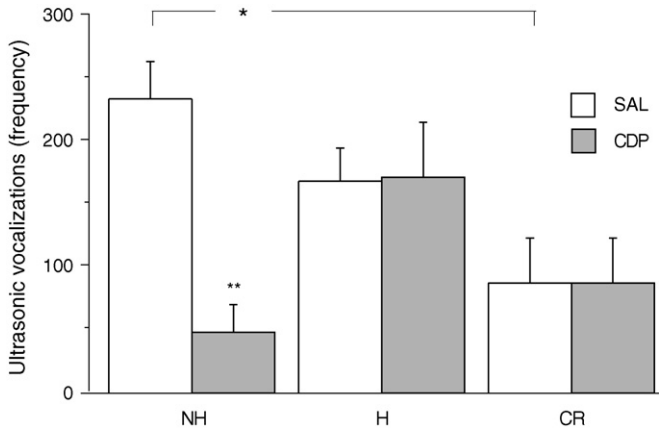

Fig. 1. Ultrasounds vocalization (UVZ) emitted by PND 8 pups in the three post-natal conditions. Mice were i.p. injected either with vehicle (saline solution) or CDP (2 mg/kg) 30 min before testing and UVZ registered during a 5 min period. CDP reduced significantly the number of ultrasounds emitted only in the NH group (**$p < 0.01$). Under basal conditions (saline injection) the CR group was characterized by a lower amount of UVZ when compared to the NH pups (*$p < 0.01$). Data represent means ± S.E.M. $N = 18$ NH, 15 H and 12 CR pups in each final group.

$F(2, 84) = 5.018$; 2.831; $p = 0.0087$; $p = 0.0646$, respectively for frequency and duration; main effect of Drug: $F(1, 84) = 4.981$; 2.216; $p = 0.0283$; $p = 0.1404$, respectively for frequency and duration). In particular, the CR group performed less head dipping than the NH group ($p < 0.01$) but did not differ from the H, while CDP treated subjects performed this behavior more often than the control group. In particular, CDP increased the amount of head dipping in all groups ($p < 0.05$) (Table 1). As for SAP, a measure of risk assessment, a main effect of Post-natal Condition was found both for frequency and duration ($F(2, 84) = 53.356$; 80.655; $p < 0.001$; $p < 0.001$, respectively for frequency and duration), CR subjects performing this behavior significantly less ($p < 0.01$) (Fig. 2 and Table 1). A main effect of Drug on SAP duration was also found, CDP reducing the duration of this behavior in all groups ($F(1, 84) = 16.232$; $p < 0.001$). The CR group was also characterized by reduced levels of fecal boli (main effect of Post-natal Condition ($F(2, 84) = 3.156$; $p = 0.0477$), which, together with reduced SAP is a good index of reduced emotionality (Fig. 2).

5.2.4. Chronic swimming stress

Concerning *Swimming*, when placed in the bucket, all subjects swam more (both frequency and duration) on the first, compared to the last day of testing but no difference was found between the different Post-natal Conditions (main effect of Day, $F(1, 21) = 11.320$; 37.619; 11.328; $p < 0.01$; $p < 0.01$, respectively for frequency and duration; see Table 2). Latency to swim did not differ among Post-natal Conditions or Days. Frequency of *Struggling* was higher on the first, compared to the last day ($F(1, 21) = 7.720$; $p = 0.0113$), the opposite being true for latency and duration of this behavior, which were longer on the last day ($F(1, 21) = 6.591$; 4.461; $p = 0.0189$; 0.0468, respectively for latency and duration) (Wilcoxon Signed rank test for *Struggling* latency confirmed analysis with parametric statistics: $p = 0.0520$). As for *Floating*, latency to exhibit this behavior, as expected, was lower on the last day, compared to the first ($F(1, 21) = 6.291$; $p = 0.0204$) (Wilcoxon Signed rank test for *Floating* confirmed analysis with parametric statistics: $p = 0.0017$). No significant interaction between Post-natal Condition and Day was found ($F(2, 21) = 2.813$; $p = 0.0827$) (Kruskal–Wallis test for independent groups performed on the difference between the first and the last day of testing confirmed the analysis performed with parametric statistics: $p = 0.0767$). Nonetheless, we observed that the CR group already showed low *Floating* latencies on the first day and did not modify it significantly, while both H and NH subjects showed

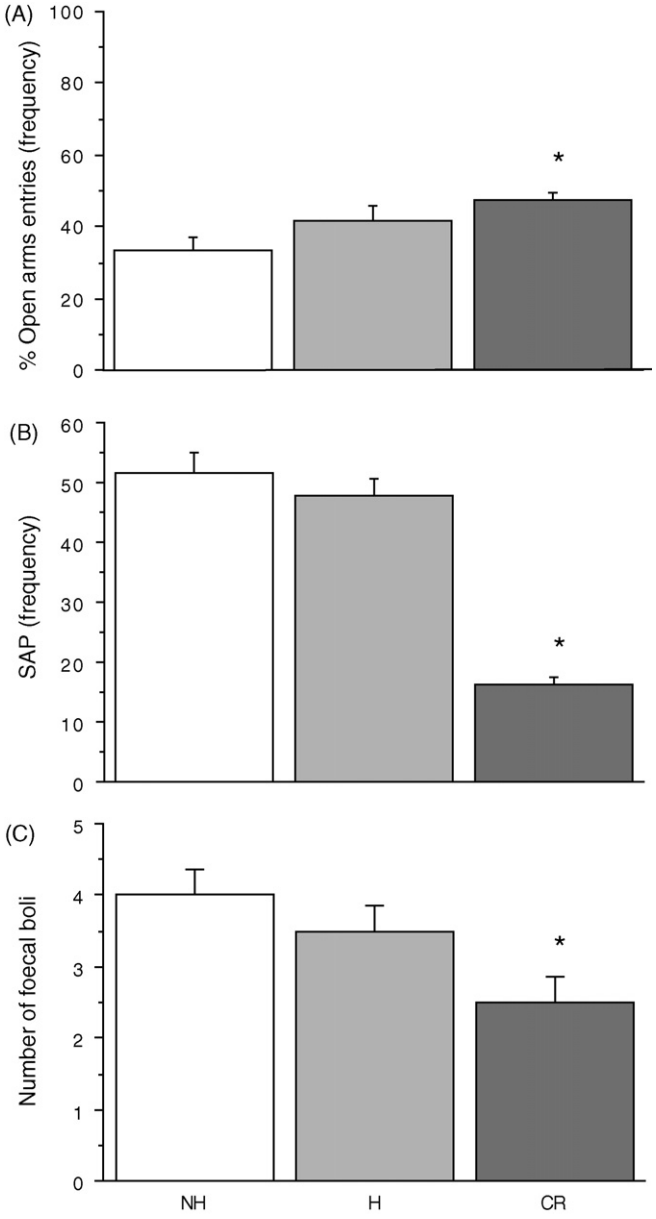

Fig. 2. Emotional reactivity in the elevated plus-maze test at adulthood. Compared to the NH control group, CR mice were characterized by a lower emotional profile as shown by the higher frequency of visits to the open arms (panel A, *$p < 0.05$, CR vs NH). In addition, CR mice performed less stretched-attend (risk assessment) postures (B, *$p < 0.05$, CR vs H and NH) and produced a lower amount of fecal boli during the test (C, *$p < 0.05$, CR vs NH). Data represent means ± S.E.M. $N = 32$ subjects in each final group.

high latencies on the first day and reduced them when tested on day 13. While no significant difference was found for the frequency of *Floating* ($F(2, 21) = 0.008$; $p = 0.9918$), duration of this behavior was significantly higher in the NH group, compared to both the CR and H groups, only on the last day of testing (Day × Post-natal Condition $F(2, 21) = 3.275$; $p = 0.0578$) (post hoc, Tukey $p < 0.01$) (see Table 2 and Fig. 3).

As for CORT levels, the three Post-natal Conditions differed in their basal values (day 1), i.e. before the administration of the stress procedure ($F(2, 15) = 8.308$; $p = 0.0037$) (Fig. 4A and Table 3). In particular, post hoc comparisons showed that the NH group was characterized by higher CORT levels than both the H and the CR groups ($p < 0.01$). When considering the differential increase in CORT (delta: t30 − t0), while no main effect of Pos-natal Condition

Table 1
Behaviors shown in the elevated plus-maze test by experimental subjects in the different post-natal conditions.

Behavior	Cond	No stress		Stress	
		Ctrl	CDP	Ctrl	CDP
% Open (frequency)	NH	25.4 ± 7.2	31.13 ± 8.24	39.27 ± 5.45	38.18 ± 7.69
	H	42.72 ± 8.63	37.61 ± 11.42	41.46 ± 6.05	45.45 ± 5.35
	CR*	46.82 ± 5.99	49.13 ± 5.88	45.29 ± 2.32	48.94 ± 2.08
% Open (duration)	NH	23.38 ± 8.41	31.21 ± 9.33	39.40 ± 8.09	36.88 ± 10.25
	H	42.74 ± 10.40	41.09 ± 14.54	47.96 ± 7.77	41.85 ± 7.15
	CR	46.32 ± 5.91	47.49 ± 9.01	41.25 ± 4.25	48.42 ± 3.29
Activity (frequency)	NH	55.88 ± 4.69	54.75 ± 3.92	56.13 ± 6.63	58.25 ± 6.05
	H	52.50 ± 8.39	42.50 ± 7.99	54.50 ± 6.81	57.38 ± 3.48
	CR#	33.88 ± 2.32	35.75 ± 1.53	37.63 ± 2.01	38.75 ± 2.46
Activity (duration)	NH	107.00 ± 4.26	119 ± 8.80	118.88 ± 7.60	130.00 ± 10.70
	H	107.38 ± 14.60	84.50 ± 16.85	117.50 ± 15.82	136.63 ± 12.26
	CR§	214.25 ± 10.51	214.25 ± 8.33	220.13 ± 5.14	224.13 ± 7.83
Head dipping (frequency)	NH	11.25 ± 3.30	15.25 ± 2.79	11.50 ± 2.09	16.63 ± 3.59
	H	11.00 ± 2.49	11.50 ± 3.16	8.38 ± 1.50	14.50 ± 2.10
	CR**	8.38 ± 1.16	8.00 ± 1.96	6.88 ± 1.64	10.00 ± 1.41
Head dipping (duration)	NH	16.00 ± 5.37	24.63 ± 6.69	19.63 ± 5.12	23.00 ± 5.10
	H	13.50 ± 3.38	15.63 ± 5.04	10.63 ± 2.43	19.13 ± 3.13
	CR	15.88 ± 4.14	14.50 ± 4.49	12.63 ± 3.12	13.88 ± 1.78
SAP (frequency)	NH	52.75 ± 7.92	48.13 ± 6.78	53.25 ± 6.88	52.38 ± 5.62
	H	51.38 ± 7.12	39.88 ± 6.01	51.88 ± 5.60	47.75 ± 3.26
	CR	16.25 ± 2.80	16.25 ± 2.01	20.63 ± 1.92	11.75 ± 2.68
SAP (duration)	NH	138.38 ± 8.11	111.75 ± 16.07	126.13 ± 11.39	107.75 ± 13.70
	H	139.13 ± 16.58	96.63 ± 12.08	141.25 ± 16.97	96.25 ± 8.78
	CR§	33.63 ± 8.02	28.50 ± 4.32	41.25 ± 6.68	19.00 ± 4.48

Data are mean values ± S.E.; $N = 8$ in each final group.
* $p < 0.05$, main effect of post-natal condition (CR vs NH).
** $p < 0.01$, main effect of post-natal condition (CR vs NH).
# $p < 0.05$, main effect of post-natal condition (CR vs NH and H).
§ $p < 0.01$, main effect of post-natal condition (CR vs NH and H).

Table 2
Behaviors shown during the first and the last swimming stress sessions.

Behavior	Cond	Day 1	Day 13
Swimming (latency)	NH	0 ± 0	0 ± 0
	H	0 ± 0	0 ± 0
	CR	0 ± 0	37.5 ± 37.5
Swimming (frequency)	NH	18.0 ± 3.7	7.3 ± 1.4
	H	10.4 ± 1.8	8.0 ± 2.7
	CR	12.5 ± 1.7	7.3 ± 2.2
Swimming (duration)	NH	166.1 ± 20.9	73.9 ± 27.0
	H	101.4 ± 26.7	69.5 ± 21.8
	CR	133.0 ± 27.5	58.6 ± 17.4
Struggling (latency)	NH	10.8 ± 4.1	96.3 ± 45.1
	H	10.3 ± 3.1	53.0 ± 25.0
	CR	13.0 ± 2.9	23.1 ± 8.1
Struggling (frequency)	NH	16.1 ± 3.5	6.0 ± 2.2
	H	9.0 ± 1.6	8.1 ± 1.8
	CR	10.3 ± 1.3	7.3 ± 1.5
Struggling (duration)	NH	99.4 ± 16.1	91.5 ± 35.4
	H	139.1 ± 28.0	174.8 ± 31.0
	CR	100.4 ± 17.0	186.9 ± 32.6
Floating (latency)	NH	168.3 ± 43.2	105.0 ± 45.5
	H	190.4 ± 32.5	40.4 ± 18.8
	CR	117.3 ± 15.0	125.4 ± 48.0
Floating (frequency)	NH	2.8 ± 1.1	5.6 ± 2.8
	H	3.8 ± 1.1	4.5 ± 1.5
	CR	4.0 ± 1.0	4.6 ± 1.6
Floating (duration)	NH	35.0 ± 15.1	135.0 ± 46.9
	H	60.0 ± 19.6	56.3 ± 23.3
	CR	67.4 ± 15.5	54.9 ± 19.2

Data are mean values ± S.E.; $N = 8$ in each final group.

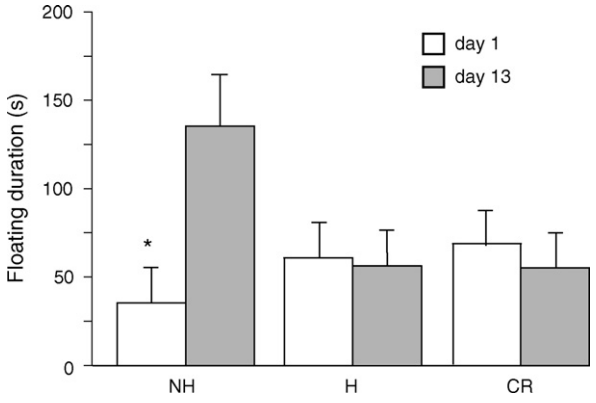

Fig. 3. Susceptibility to depression-like behaviour in the forced swimming stress test. No change in the frequency of immobility characterized the H and the CR groups following 13 days of chronic swimming stress, suggesting that they might be characterized by a better coping strategy compared to the NH control group (*$p < 0.05$). Data represent means ± S.E.M. $N = 8$ subjects in each final group.

was found ($F(2, 15) = 0.849$; $p = 0.4474$), a significant interaction between the Post-natal Condition and Days was revealed by ANOVA ($F(2, 15) = 3.900$; $p = 0.0433$). In particular, post hoc comparisons showed that on day 1 subjects from the CR group were characterized by a robust increase in CORT levels ($p < 0.05$: day 1, CR vs NH and H), while they showed a more efficient habituation to the repeated stress procedure on day 13 ($p < 0.05$: CR day 1 vs CR day 13) (Fig. 4B).

5.2.5. BDNF levels

The CR group was overall characterized by higher levels of BDNF in the hypothalamus (main effect of Post-natal Condition:

Table 3
Corticosterone levels in subjects undergoing repeated swimming stress.

	Day 1		Day 13	
	t0	t30	t0	t30
NH	6.5 ± 0.3	13.1 ± 0.9	4.4 ± 0.8	11.4 ± 0.8
H	4.7 ± 0.5	11.3 ± 0.8	2.9 ± 0.4	9.6 ± 0.2
CR	4.4 ± 0.3	14.0 ± 0.4	6.1 ± 0.7	11.9 ± 1.2

Data are means of the square root of raw data ± S.E.; $N = 6$ in each final group.

($F(2, 34) = 9.227$; $p < 0.01$). Swimming stress increased hypothalamic BDNF levels (main effect of Stress: $F(1, 34) = 5.954$; $p = 0.0201$) in all Post-natal Conditions (interaction between treatment and condition: $F(2, 34) = 0.759$; $p = 0.4760$) (Fig. 5A). In addition a significant correlation was found between the hypothalamic BDNF content and CORT output at 30 min post stress, suggesting that BDNF in this brain region might be involved, as previously indicated, in pituitary activation following stress (Fig. 6).

As for the hippocampus, no difference was found as a result of Stress and Post-natal Condition (main effect of Stress: $F(1, 34) = 1.763$), main effect of Post-natal Condition ($F(2, 34) = 0.253$; $p < 0.1931$), interaction between stress and post-natal Condition ($F(2, 34) = 1.773$; $p = 0.1852$) (Fig. 5B).

When BDNF levels were measured in the striatum, a significant effect of Post-natal Condition was found ($F(2, 34) = 5.736$, $p < 0.01$).

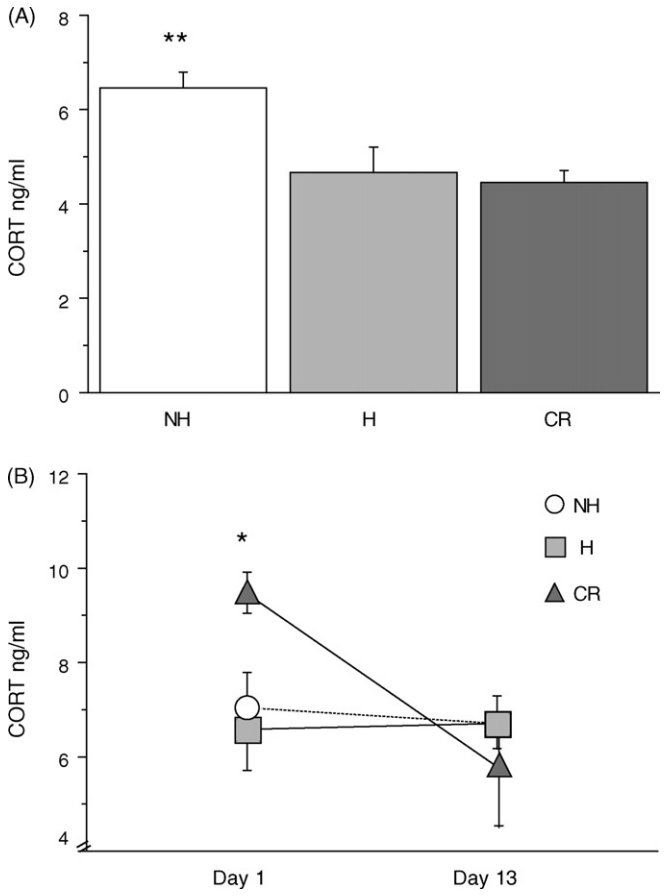

Fig. 4. Neuroendocrine responses in the forced swimming test. Both CR and H mice showed lower basal levels of CORT compared to non-manipulated subjects (panel A, $^{**}p < 0.01$). On panel B are represented the overall cortical output (delta: t30 – t0) for the first and the last day of testing. On day 1 the CR group was characterized by a peculiar, though adaptive, adrenal response showing an initial high surge of CORT (day 1, panel B) followed by a sharp decrease as a result of the chronic exposure (day 13, panel B, $^{*}p < 0.05$ CR day 1 vs CR day 13). Data represent means obtained on the square root of raw values ± S.E.M. $N = 6$ subjects in each final group.

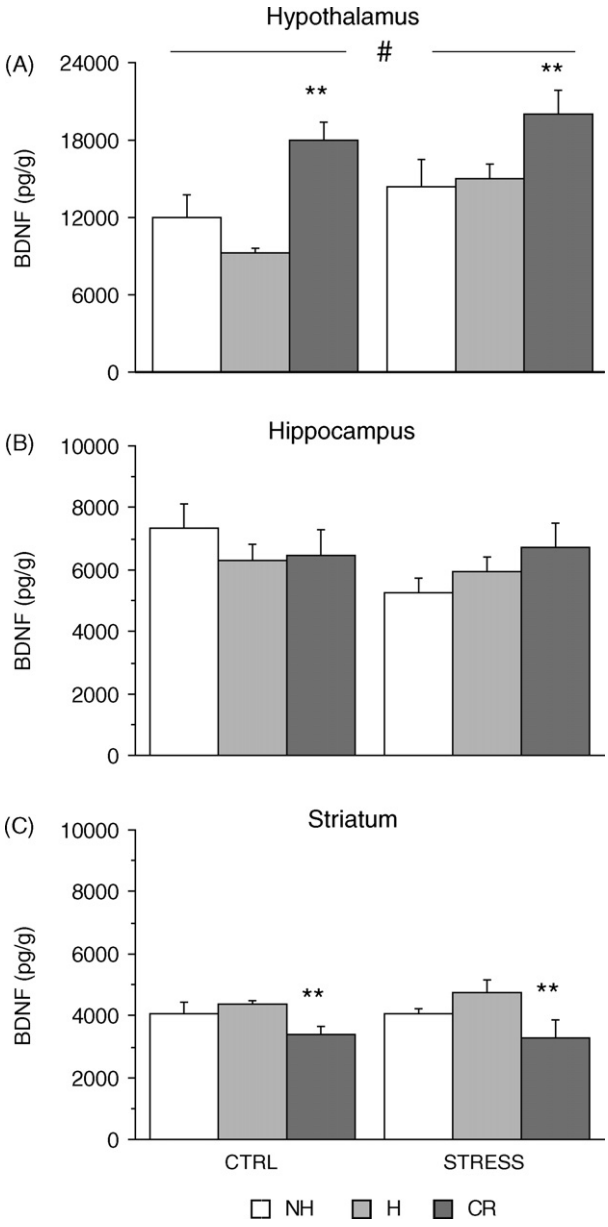

Fig. 5. Long-term effects of early manipulations on BDNF protein levels in different brain regions. Subjects were sacrificed following the forced swimming stress procedure. The effects of stress on BDNF levels were region-dependent and were modulated by early manipulations. In particular, the CR group showed the highest hypothalamic levels of BDNF ($^{**}p < 0.01$). In addition, regardless of post-natal condition, swimming stress overall increased BDNF levels in this region ($^{\#}p < 0.05$, see panel A). Post-natal condition or stress did not affect BDNF levels in the hippocampus (panel B), while the CR condition resulted in overall decreased BDNF levels in the striatum ($^{**}p < 0.01$). Data represent means ± S.E.M. $N = 8$ NH, 6 H and 6 CR subjects in each final group.

In particular, the CR group was characterized by lower BDNF levels compared both to the H and NH subjects (post hoc comparisons: CR vs H, $p < 0.01$ and CR vs NH $p < 0.05$) (Fig. 5C). The stress procedure did not affect levels of this protein in the three Post-natal Conditions (main effect of Stress: $F(1, 34) = 0.132$; $p = 0.7184$; interaction between stress and Post-natal Condition: $F(2, 34) = 0.248$; $p = 0.7821$).

5.3. Discussion

Overall these data indicate that early manipulations, such as handling and communal rearing, have both short- and long-term

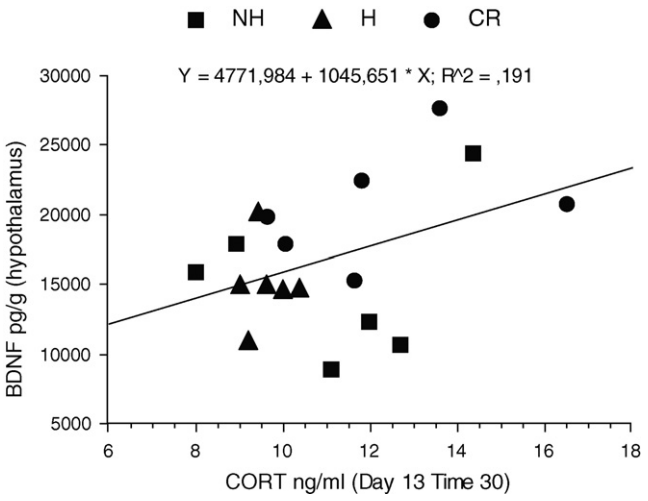

Fig. 6. Correlation between hypothalamic BDNF levels and the HPA axis response to stress. A direct relationship was found between BDNF hypothalamic levels and CORT assessed at the end of the chronic stress procedure suggesting that this neurotrophin might participate in the HPA axis response to stress. Data represent the correlation between BDNF values of all post-natal conditions and CORT levels measured on day 13 at time 30. $N = 18$ subjects.

effects on emotionality, the neuroendocrine response to stress and BDNF levels. Although the effects of these two manipulations are in the same direction, leading to reduced emotionality, social enrichment in the form of communal rearing appears to have more pervasive effects than a physical manipulation of the nest area, such as handling.

5.3.1. Short-term effects of early manipulations on mouse emotionality

In this study we tested pup's emotionality by measuring UVZ emitted as a result of a brief isolation from the mother and siblings. UVZ are an ethologically–ecologically relevant behavior, playing an important role in mother–offspring dyadic communication in rodents, strongly contributing to survival of the pup and to its physiological and behavioral development. These vocalizations are one of the few response patterns emitted by young rodents which can be quantitatively analyzed (Elwood and Keeling, 1982; Branchi et al., 1998, 2001). Results of this study are quite striking since they indicate that CR pups showed reduced basal UVZ production. In addition, and most importantly, together with the H group, CR pups did not reduce their calling rate following administration of CDP, an anxiolytic compound that has been previously shown to reduce UVZ in CD-1 mice of this age (Cirulli et al., 1994). This is one of the few reports indicating important changes in mouse emotionality during the first post-natal week as a result of early manipulations (D'Amato et al., 2005; Cirulli et al., 2007).

As for the mechanisms underlying the lack of an effect of CDP in the CR and H groups, it has been previously shown that the offspring of mothers expressing high levels of maternal care show increased GABAA/benzodiazepine receptor levels in the basolateral and central nucleus of the amygdala as well as in the locus coeruleus (Caldji et al., 2000b, 2003). In addition, we have previously shown that handling in rats increases the density of GABAergic interneurons in selected brain regions involved in the regulation of the stress response and emotional behavior, including the hypothalamus and the amygdala (Giachino et al., 2007). Although it is also possible to hypothesize that changes in GABAA receptor density/neuronal number might affect the threshold for responsiveness to agonists acting at the benzodiazepine site, a floor effect might be at work here. Thus overall these data indicate that important changes in emotional behavior can already be detected early during development. In addition, they indicate a much stronger effect of CR than H.

5.3.2. Long-term effects on emotionality and the neuroendocrine responses to stress

Long-term effects of handling and social enrichment on emotional behavior were assessed using the elevated plus-maze test which indicated a decreased level of anxiety, with greater effects in CR subjects compared to the H group. In particular, CR mice entered more frequently in the open "anxiogenic" arms of the maze, performed less SAP (risk assessment) postures and were characterized by reduced levels of fecal boli during the test. Taken together, and with the exception of head dipping, these behavioral responses suggest reduced emotionality in the CR condition.

Previous studies have already shown that the social stimulation provided by being reared communally has a major impact on emotional reactivity. However, the direction of the effects appears to depend upon the timing of placing the mothers together. According to the protocol used in the present study (i.e. CR), that is when mothers are put together on the day of parturition, an overall reduction in emotionality is found in the plus-maze (Sayler and Salmon, 1971; Curley et al., 2009). By contrast, CN mice are more emotional in an open-field or an elevated plus-maze (but not in a social interaction test) when the mothers are put together 5 days before parturition (Branchi et al., 2006a,b; Branchi, 2009).

In this study we found that the H group was characterized by an emotional response in the same direction, but of much smaller intensity, than the CR group. It is also worth noticing that no main interaction between drug administration (CDP, the same anxiolytic used in the UVZ test) and post-natal manipulations was found, suggesting that the unresponsiveness to anxiolytic drugs, shown by the CR and H subjects early during development, is a transient effect, which is no longer evident at adulthood.

The response to a repeated physical stressor was assessed in all experimental groups at adulthood. From a behavioral point of view, while the H group, and to a lesser extent the NH, reduced significantly their latency to show *Floating*, the CR group showed lower *Floating* latencies already on the first day and did not modify this behavior significantly (Branchi et al., 2009b). In addition, a reduced immobility characterized both H and CR after 13 days of stress, suggesting that they might be characterized by an active coping (escape-driven) response, compared to the NH control group.

From a neuroendocrine point of view, both CR and H mice showed lower basal levels of CORT compared to non-manipulated subjects. This is an interesting piece of data since previous work assessing neuroendocrine activity following early manipulations, such as handling, only evidenced changes in the stress-induced neuroendocrine activity, but not on basal CORT secretion, in rats (Meaney et al., 1985). When challenged by swimming stress, H mice, as expected, showed reduced adrenocortical output, while CR mice showed a somewhat different, although still adaptive response, being characterized by a very high surge of CORT following the first stress episode, then reducing this response following repeated exposure to stress. This pattern of responding appears highly adaptive since an initial very high level of CORT is needed to mobilize body resources or fight infections following a threat. However, this response needs to be terminated to prevent the deleterious effects of chronic neuroendocrine activation (Sapolsky et al., 2000). Previous studies employing a social stressor had shown an overall lower adrenocortical output in CN mice (Branchi et al., 2009b). This can be well explained taking into account that CN are characterized by greater social competences than NH mice, thus a social/aggressive encounter may be experienced as less stressful than an inescapable swimming stress by this group. Overall, these data indicate that early manipulations can modulate neuroendocrine responses to stress at adulthood, an effect that appears

to be dependent upon the type of challenge the animal is facing (i.e. social vs physical).

5.3.3. Long-term effects of early manipulations on BDNF levels

Neurotrophins, such as BDNF, are involved in adaptive brain plasticity and their expression is modified as a result of stressful challenges (Cirulli and Alleva, 2009). Recent data suggest that, in addition to acting as possible repair mechanism, they might directly participate in the physiological response to a stressor. In particular, it has been hypothesized that BDNF might participate in the hippocampus-hypothalamus-pituitary-adrenal axis response to stress since this neurotrophin increases rapidly following stressful challenges in all these regions (Smith et al., 1995; Tapia-Arancibia et al., 2004; Cirulli and Alleva, 2009). Together with the hippocampus, the hypothalamus is also the brain structure that contains the highest BDNF mRNA and protein levels (for a review see Tapia-Arancibia et al., 2004). BDNF mRNA is expressed in the paraventricular nucleus of the hypothalamus (PVN) and anterior and neurointermediate lobes of the pituitary gland, areas important in mediating the endocrine response to stress (Smith et al., 1995; Tapia-Arancibia et al., 2004).

In this study we found that the effects of a repeated swimming stress procedure on BDNF protein levels were region-dependent and were modulated by early manipulations, such as CR. The most striking effect was the high levels of this neurotrophin found in the hypothalamus, a brain region which represents the final common pathway integrating a number of stress inputs. It has been suggested that BDNF could participate in the stress response. Indeed, there are data to show that in the parvocellular portion of the PVN, neurons coexpress BDNF with CRH while in the magnocellular portion BDNF is coexpressed with vasopressin (Tapia-Arancibia et al., 2004). BDNF is a potent stimulator of neuropeptide synthesis in hypothalamic neurons in vitro. In particular, BDNF might play an important role as co-secretagogue of CRH and vasopressin, the main regulators of ACTH secretion (Tapia-Arancibia et al., 2004). This might explain the meaningful correlation we found between overall CORT output on the last day of the stressing procedure and BDNF protein levels measured in the whole hypothalamic region.

It has also been suggested that, in the hypothalamus, BDNF could help to re-establish the hormonal pool (Givalois et al., 2004; Tapia-Arancibia et al., 2004). Indeed, increased levels of BDNF were found in the NH and H groups following the repeated stress procedure. Thus, this neurotrophin could contribute – likely through increased hypothalamic neurohormones synthesis – to replenish the cellular compartments, once they have been exhausted by a strong demand. This process might help neurons to sustain the demand underlying the stress response. Thus, compared to NH and H groups, CR subjects, which show increased basal BDNF levels, might be endowed by a greater capacity to react to stress and maintain neuroendocrine activity in the face of repeated stimulation.

Apart from very traumatic situations, the brain shows some resilience to stress due to its ability to adapt through plastic changes. BDNF might act as a plasticity mediator enhancing this trait which seems to be crucial in adaptive processes. Changes in hypothalamic BDNF levels might thus represent another mechanisms through which early experiences could exert long-term effects on the stress axis, in addition to well described changes in GR (Meaney et al., 1989; Plotsky and Meaney, 1993). A greater reactivity of the HPA axis under conditions of stress might indeed be adaptive for individuals living in crowded conditions, as it would be expected in the case of subjects raised in a communal nest due to increased competition for space and resources.

Compared to previous studies here we found no change in hippocampal BDNF levels, which might depend upon the peculiar CR protocol used. By contrast, lower BDNF levels characterized the striatum of the CR group. It has been previously shown that selective, viral knockdown of BDNF in the mesolimbic dopamine pathway can obliterate most of the effects of repeated stress on gene expression within this circuit, with similar effects being produced by chronic treatment with antidepressants. These results have established an essential role for mesolimbic BDNF in the complex pathophysiology of depression, in addition to the hippocampus and hypothalamus (Berton et al., 2006). Thus, the lower BDNF levels characterizing the CR group could be related to reduced susceptibility to develop a depressed state.

6. Conclusions

The effects of early environmental stimulation on brain structure and function have long being exploited. In particular, early experiences, including handling, have been shown, since the very first investigations, to accelerate the development of the central nervous system and to affect behavior at adulthood. According to the "maternal mediation" hypothesis, changes in maternal behavior are the key factor underlying the long-term effects of early manipulations on the offspring's behavior. Compared to the H paradigm, which results in extra bouts of maternal behavior (Cirulli et al., 2007), being reared in a communal nest is a much more complex situation, in which subjects experience both increased levels of maternal care and increased peer interactions. The discrepancies on the maternal mediation hypothesis present in the literature are further underlined by the findings that a minor change in the communal nesting paradigm (thus producing the "communal rearing" condition), which has the same effects of increasing maternal care and social interactions, results in different effects on the emotional phenotype (Branchi et al., 2006a; Curley et al., 2009). In the case of the CR paradigm used in this study, it can be hypothesized that being put together at the time of parturition might modify levels of stress hormones in the mothers, which could be transferred, through the milk, to the offspring, influencing the emotional phenotype (Macri et al., 2007).

The studies discussed and the original findings presented in this paper highlight the potential of social enrichment as a promising strategy to promote central nervous system maturation, leading to more adaptive coping styles and, possibly reduced vulnerability to psychopathology. Previous studies using a different form of communal rearing have indicated important effects of such manipulation on brain plasticity, showing increased BDNF levels and accelerated development of the visual system (Sale et al., 2004). Although animal data cannot translate directly to the human situation, they may help devise analytical strategies and interventions to be tailored on the specific needs of children raised under traumatic or impoverished situations.

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References

Alleva, E., Branchi, I., 2006. NGF: a social molecule. Psychoneuroendocrinology 31, 295–296, author reply 297–298.

Aloe, L., Properzi, F., Probert, L., Akassoglou, K., Kassiotis, G., Micera, A., Fiore, M., 1999. Learning abilities, NGF and BDNF brain levels in two lines of TNF-alpha transgenic mice, one characterized by neurological disorders, the other phenotypically normal. Brain Res. 840, 125–137.

Altar, C.A., 1999. Neurotrophins and depression. Trends Pharmacol. Sci. 20, 59–61.

Andersen, S.L., 2003. Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci. Biobehav. Rev. 27, 3–18.

Berton, O., McClung, C.A., Dileone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W., Nestler,

E.J., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864–868.
Bowlby, J., 1982. Attachment and loss: retrospect and prospect. Am. J. Orthopsychiatry 52, 664–678.
Branchi, I., 2009. The mouse communal nest: investigating the epigenetic influences of the early social environment on brain and behavior development. Neurosci. Biobehav. Rev. 33, 551–559.
Branchi, I., Santucci, D., Alleva, E., 2001. Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. Behav. Brain Res. 125, 49–56.
Branchi, I., Santucci, D., Vitale, A., Alleva, E., 1998. Ultrasonic vocalizations by infant laboratory mice: a preliminary spectrographic characterization under different conditions. Dev. Psychobiol. 33, 249–256.
Branchi, I., D'Andrea, I., Gracci, F., Santucci, D., Alleva, E., 2009a. Birth spacing in the mouse communal nest shapes adult emotional and social behavior. Physiol. Behav. 96, 532–539.
Branchi, I., D'Andrea, I., Cirulli, F., Lipp, H.P., Alleva, E.,;1; 2009b. Shaping brain development: mouse communal nesting blunts adult neuroendocrine and behavioral response to social stress and modifies chronic antidepressant treatment outcome. Psychoneuroendocrinology, in press [Epub ahead of print].
Branchi, I., D'Andrea, I., Fiore, M., Di Fausto, V., Aloe, L., Alleva, E., 2006a. Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. Biol. Psychiatry 60, 690–696.
Branchi, I., D'Andrea, I., Sietzema, J., Fiore, M., Di Fausto, V., Aloe, L., Alleva, E., 2006b. Early social enrichment augments adult hippocampal BDNF levels and survival of BrdU-positive cells while increasing anxiety- and "depression"-like behavior. J. Neurosci. Res. 83, 965–973.
Brunelli, S.A., 2005. Development and evolution of hidden regulators: selective breeding for an infantile phenotype. Dev. Psychobiol. 47, 243–252.
Caldji, C., Diorio, J., Meaney, M.J., 2000a. Variations in maternal care in infancy regulate the development of stress reactivity. Biol. Psychiatry 48, 1164–1174.
Caldji, C., Diorio, J., Meaney, M.J., 2003. Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. Neuropsychopharmacology 28, 1950–1959.
Caldji, C., Francis, D., Sharma, S., Plotsky, P.M., Meaney, M.J., 2000b. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 22, 219–229.
Campbell, J., Spear, L.P., 1999. Effects of early handling on amphetamine-induced locomotor activation and conditioned place preference in the adult rat. Psychopharmacology (Berl.) 143, 183–189.
Castren, E., 2005. Is mood chemistry? Nat. Rev. Neurosci. 6, 241–246.
Castren, E., Voikar, V., Rantamaki, T., 2007. Role of neurotrophic factors in depression. Curr. Opin. Pharmacol. 7, 18–21.
Champagne, F.A., Francis, D.D., Mar, A., Meaney, M.J., 2003. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol. Behav. 79, 359–371.
Champoux, M., Coe, C., Schanberg, S., Kuhn, C.M., Suomi, S.J., 1989. Hormonal effects of early rearing conditions in the infant rhesus monkey. Am. J. Primatol. 19, 111–117.
Chugani, H.T., Behen, M.E., Muzik, O., Juhasz, C., Nagy, F., Chugani, D.C., 2001. Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans. Neuroimage 14, 1290–1301.
Cicchetti, D., Toth, S.L., 1995. A developmental psychopathology perspective on child abuse and neglect. J. Am. Acad. Child Adolesc. Psychiatry 34, 541–565.
Cirulli, F., Alleva, E., 2009. The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front. Neuroendocrinol. 30, 379–395.
Cirulli, F., Micera, A., Alleva, E., Aloe, L., 1998. Early maternal separation increases NGF expression in the developing rat hippocampus. Pharmacol. Biochem. Behav. 59, 853–858.
Cirulli, F., Alleva, E., Antonelli, A., Aloe, L., 2000. NGF expression in the developing rat brain: effects of maternal separation. Brain Res. Dev. Brain Res. 123, 129–134.
Cirulli, F., Santucci, D., Laviola, G., Alleva, E., Levine, S., 1994. Behavioral and hormonal responses to stress in the newborn mouse: effects of maternal deprivation and chlordiazepoxide. Dev. Psychobiol. 27, 301–316.
Cirulli, F., Capone, F., Bonsignore, L.T., Aloe, L., Alleva, E., 2007. Early behavioural enrichment in the form of handling renders mouse pups unresponsive to anxiolytic drugs and increases NGF levels in the hippocampus. Behav. Brain Res. 178, 208–215.
Cirulli, F., Francia, N., Berry, A., Aloe, L., Alleva, E., Suomi, S.J., 2009a. Early life stress as a risk factor for mental health: role of neurotrophins from rodents to non-human primates. Neurosci. Biobehav. Rev. 33, 573–585.
Cirulli, F., Francia, N., Branchi, I., Antonucci, M.T., Aloe, L., Suomi, S.J., Alleva, E., 2009b. Changes in plasma levels of BDNF and NGF reveal a gender-selective vulnerability to early adversity in rhesus macaques. Psychoneuroendocrinology 34, 172–180.
Cirulli, F., Berry, A., Alleva, E., 2003. Early disruption of the mother–infant relationship: effects on brain plasticity and implications for psychopathology. Neurosci. Biobehav. Rev. 27, 73–82.
Cole, J.C., Rodgers, R.J., 1993. An ethological analysis of the effects of chlordiazepoxide and bretazenil (Ro 16-6028) in the murine elevated plus-maze. Behav. Pharmacol. 4, 573–580.
Columba-Cabezas, S., Iaffaldano, G., Chiarotti, F., Alleva, E., Cirulli, F., 2009. Early handling increases susceptibility to experimental autoimmune encephalomyelitis (EAE) in C57BL/6 male mice. J. Neuroimmunol. 212, 10–16.
Crowcroft, P., Rowe, F.P., 1963. Social organization and. territorial behavior in the wild house mice. Proc. Zool. Soc. Lond. 140, 517–531.
Curley, J.P., Davidson, S., Bateson, P., Champagne, F.A., 2009. Social enrichment during postnatal development induces transgenerational effects on emotional and reproductive behavior in mice. Front. Behav. Neurosci. 3, 25.
D'Amato, F.R., Scalera, E., Sarli, C., Moles, A., 2005. Pups call, mothers rush: does maternal responsiveness affect the amount of ultrasonic vocalizations in mouse pups? Behav. Genet. 35, 103–112.
D'Andrea, I., Alleva, E., Branchi, I., 2007. Communal nesting, an early social enrichment, affects social competences but not learning and memory abilities at adulthood. Behav. Brain Res. 183, 60–66.
Duman, R.S., Monteggia, L.M., 2006. A neurotrophic model for stress-related mood disorders. Biol. Psychiatry 59, 1116–1127.
Ebensperger, L.A., 2001. A review of the evolutionary causes of rodent group-living. Acta Theriologica 46, 115–144.
Elwood, R.W., Keeling, F., 1982. Temporal organization of ultrasonic vocalizations in infant mice. Dev. Psychobiol. 15, 221–227.
Fernandes, C., File, S.E., 1996. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol. Biochem. Behav. 54, 31–40.
Francis, D.D., Meaney, M.J., 1999. Maternal care and the development of stress responses. Curr. Opin. Neurobiol. 9, 128–134.
Francis, D.D., Diorio, J., Plotsky, P.M., Meaney, M.J., 2002. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J. Neurosci. 22, 7840–7843.
Gardner, C.R., 1985. Distress vocalization in rat pups. A simple screening method for anxiolytic drugs. J. Pharmacol. Methods 14, 181–187.
Giachino, C., Canalia, N., Capone, F., Fasolo, A., Alleva, E., Riva, M.A., Cirulli, F., Peretto, P., 2007. Maternal deprivation and early handling affect density of calcium binding protein-containing neurons in selected brain regions and emotional behavior in periadolescent rats. Neuroscience 145, 568–578.
Gittleman, J.L., 1985. Functions of communal care in mammals. In: Greenwood, P.J., Harvey, P.H., Slatkin, M. (Eds.), Evolution: Essays in Honour of John Maynard Smith. Cambridge University Press, Cambridge, pp. 187–205.
Givalois, L., Naert, G., Rage, F., Ixart, G., Arancibia, S., Tapia-Arancibia, L., 2004. A single brain-derived neurotrophic factor injection modifies hypothalamo-pituitary-adrenocortical axis activity in adult male rats. Mol. Cell Neurosci. 27, 280–295.
Gunnar, M.R., Morison, S.J., Chisholm, K., Schuder, M., 2001. Salivary cortisol levels in children adopted from romanian orphanages. Dev. Psychopathol. 13, 611–628.
Hayes, L.D., 2000. To nest communally or not to nest communally: a review of rodent communal nesting and nursing. Anim. Behav. 59, 677–688.
Heim, C., Nemeroff, C.B., 1999. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. Biol. Psychiatry 46, 1509–1522.
Heim, C., Nemeroff, C.B., 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol. Psychiatry 49, 1023–1039.
Hofer, M.A., 1996. Multiple regulators of ultrasonic vocalization in the infant rat. Psychoneuroendocrinology 21, 203–217.
Holmes, A., Rodgers, R.J., 1999. Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. Neurosci. Biobehav. Rev. 23, 971–980.
Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477–501.
Insel, T.R., 1989. Decreased in vivo binding to brain benzodiazepine receptors during social isolation. Psychopharmacology 97, 142–144.
Insel, T.R., Gelhard, R.E., Miller, L.P., 1989. Rat pup isolation distress and the brain benzodiazepine receptor. Dev. Psychobiol. 22, 509–525.
Konig, B., 1997. Cooperative care of young in mammals. Naturwissenschaften 84, 95–104.
König, B., 1994. Fitness effects of communal rearing in house. Mice: the role of relatedness versus familiarity. Anim. Behav. 48, 1449–1457.
Large, T.H., Bodary, S.C., Clegg, D.O., Weskamp, G., Otten, U., Reichardt, L.F., 1986. Nerve growth factor gene expression in the developing rat brain. Science 234, 352–355.
Lehmann, J., Feldon, J., 2000. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Rev. Neurosci. 11, 383–408.
Lehmann, J., Pryce, C.R., Jongen-Relo, A.L., Stohr, T., Pothuizen, H.H., Feldon, J., 2002. Comparison of maternal separation and early handling in terms of their neurobehavioral effects in aged rats. Neurobiol. Aging 23, 457–466.
Levine, S., 1957. Infantile experience and resistance to physiological stress. Science 126, 405.
Levine, S., 2005. Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology 30, 939–946.
Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., Meaney, M.J., 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. Science 277, 1659–1662.
Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445.
Lupien, S.J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N.P., Thakur, M., McEwen, B.S., Hauger, R.L., Meaney, M.J., 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat. Neurosci. 1, 69–73.

Macri, S., Wurbel, H., 2006. Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. Horm. Behav. 50, 667–680.
Macri, S., Mason, G.J., Wurbel, H., 2004. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. Eur. J. Neurosci. 20, 1017–1024.
Macri, S., Pasquali, P., Bonsignore, L.T., Pieretti, S., Cirulli, F., Chiarotti, F., Laviola, G., 2007. Moderate neonatal stress decreases within-group variation in behavioral, immune and HPA responses in adult mice. PLoS One 2, e1015.
Manning, C.J., Dewsbury, D.A., Wakeland, E.K., Potts, W.K., 1995. Communal nesting and communal nursing in-house mice, mus-musculus-domesticus. Anim. Behav. 50, 741–751.
Martijena, I.D., Calvo, N., Volosin, M., Molina, V.A., 1997. Prior exposure to a brief restraint session facilitates the occurrence of fear in response to a conflict situation: behavioral and neurochemical correlates. Brain Res. 752, 136–142.
McEwen, B.S., Seeman, T., 1999. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Ann. N. Y. Acad. Sci. 896, 30–47.
Meaney, M.J., Aitken, D.H., Bhatnagar, S., Sapolsky, R.M., 1991. Postnatal handling attenuates certain neuroendocrine, anatomical, and cognitive dysfunctions associated with aging in female rats. Neurobiol. Aging 12, 31–38.
Meaney, M.J., Aitken, D.H., Bodnoff, S.R., Iny, L.J., Sapolsky, R.M., 1985. The effects of postnatal handling on the development of the glucocorticoid receptor systems and stress recovery in the rat. Prog. Neuropsychopharmacol. Biol. Psychiatry 9, 731–734.
Meaney, M.J., Aitken, D.H., Viau, V., Sharma, S., Sarrieau, A., 1989. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. Neuroendocrinology 50, 597–604.
Meaney, M.J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seckl, J.R., Plotsky, P.M., 1996. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. Dev. Neurosci. 18, 49–72.
Nair, A., Vadodaria, K.C., Banerjee, S.B., Benekareddy, M., Dias, B.G., Duman, R.S., Vaidya, V.A., 2007. Stressor-specific regulation of distinct brain-derived neurotrophic factor transcripts and cyclic AMP response element-binding protein expression in the postnatal and adult rat hippocampus. Neuropsychopharmacology 32, 1504–1519.
Owens, M.J., Nemeroff, C.B., 1991. Physiology and pharmacology of corticotropin-releasing factor. Pharmacol. Rev. 43, 425–473.
Plotsky, P.M., Meaney, M.J., 1993. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res. Mol. Brain Res. 18, 195–200.
Pryce, C.R., Bettschen, D., Feldon, J., 2001. Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev. Psychobiol. 38, 239–251.
Roceri, M., Cirulli, F., Pessina, C., Peretto, P., Racagni, G., Riva, M.A., 2004. Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol. Psychiatry 55, 708–714.
Rodgers, R.J., Davies, B., Shore, R., 2002. Absence of anxiolytic response to chlordiazepoxide in two common background strains exposed to the elevated plus-maze: importance and implications of behavioural baseline. Genes Brain Behav. 1, 242–251.
Rodgers, R.J., Haller, J., Holmes, A., Halasz, J., Walton, T.J., Brain, P.F., 1999. Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. Physiol. Behav. 68, 47–53.
Rutter, M.L., Kreppner, J.M., O'Connor, T.G., 2001. Specificity and heterogeneity in children's responses to profound institutional privation. Br. J. Psychiatry 179, 97–103.
Sairanen, M., Lucas, G., Ernfors, P., Castren, M., Castren, E., 2005. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. J. Neurosci. 25, 1089–1094.
Sale, A., Putignano, E., Cancedda, L., Landi, S., Cirulli, F., Berardi, N., Maffei, L., 2004. Enriched environment and acceleration of visual system development. Neuropharmacology 47, 649–660.
Sapolsky, R.M., 2001. Depression, antidepressants, and the shrinking hippocampus. Proc. Natl. Acad. Sci. U.S.A. 98, 12320–12322.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.
Sayler, A., Salmon, M., 1971. An ethological analysis of communal nursing by the house mouse (*Mus musculus*). Behavior 40, 60–85.
Sen, S., Duman, R., Sanacora, G., 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol. Psychiatry 64, 527–532.
Smith, M.A., Makino, S., Kim, S.Y., Kvetnansky, R., 1995. Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 136, 3743–3750.
Smotherman, W.P., Bell, R.W., Starzec, J., Elias, J., Zachman, T.A., 1974. Maternal responses to infant vocalizations and olfactory cues in rats and mice. Behav. Biol. 12, 55–66.
Solomon, G.F., Levine, S., Kraft, J.K., 1968. Early experience and immunity. Nature 220, 821–822.
Suomi, S.J., 1997. Early determinants of behaviour: evidence from primate studies. Br. Med. Bull. 53, 170–184.
Szyf, M., Weaver, I.C., Champagne, F.A., Diorio, J., Meaney, M.J., 2005. Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. Front. Neuroendocrinol. 26, 139–162.
Tapia-Arancibia, L., Rage, F., Givalois, L., Arancibia, S., 2004. Physiology of BDNF: focus on hypothalamic function. Front. Neuroendocrinol. 25, 77–107.
Thoenen, H., 1995. Neurotrophins and neuronal plasticity. Science 270, 593–598.
Venerosi, A., Cirulli, F., Capone, F., Alleva, E., 2003. Prolonged perinatal AZT administration and early maternal separation: effects on social and emotional behaviour of periadolescent mice. Pharmacol. Biochem. Behav. 74, 671–681.
Wilson, D.A., Willner, J., Kurz, E.M., Nadel, L., 1986. Early handling increases hippocampal long-term potentiation in young rats. Behav. Brain Res. 21, 223–227.
Yan, Q., Rosenfeld, R.D., Matheson, C.R., Hawkins, N., Lopez, O.T., Bennett, L., Welcher, A.A., 1997. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience 78, 431–448.
Zubin, J., Spring, B., 1977. Vulnerability—a new view of schizophrenia. J. Abnorm. Psychol. 86, 103–126.