Early disruption of the mother–infant relationship: effects on brain plasticity and implications for psychopathology

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Abstract

Early environmental manipulations can impact on the developing nervous system, contributing to shape individual differences in physiological and behavioral responses to environmental challenges. In particular, it has been shown that disruptions in the mother–infant relationship result in neuroendocrine, neurochemical and behavioral changes in the adult organism, although the basic mechanisms underlying such changes have not been completely elucidated. Recent data suggest that neurotrophins might be among the mediators capable of transducing the effects of external manipulations on brain development. Nerve growth factor and brain-derived neurotrophic factor are known to play a major role during brain development, while in the adult animal they are mainly responsible for the maintenance of neuronal function and structural integrity. Changes in the levels of neurotrophic factors during critical developmental stages might result in long-term changes in neuronal plasticity and lead to increased vulnerability to aging and to psychopathology.

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1. Introduction

In most mammalian species development is a gradual process that can be envisioned as the continuous accumulation of small changes or events leading to long term modifications in the adult animal. In some cases experiences occurring early during postnatal life can affect not only the existence of the single individual, but also population biology, breaking the behavioural reproductive barriers between species. For example, it has been shown that early social preferences and mate choice in female rodents are highly dependent upon early environmental exposure to selected olfactory cues linked to the presence of the father [1].

One of the most important characteristics of mammalian brains is their plasticity, that is the ability to undergo functional adjustments to changes in the internal or external milieu. This is especially true for the developing nervous system, characterized by a series of important growth and differentiation events that offer the possibility for experience-dependent modifications of brain function. While there is no doubt that the postnatally developing brain is ‘experience-sensitive’ and ‘experience-dependent’ the characteristics of this experience are still in the process of being defined [2]. This appears especially relevant since adverse events can hinder brain maturation, leading to permanent brain damage and psychiatric illness. This paper attempts to review some work performed both in our and in other laboratories dealing with this question.

2. Mother–infant interactions and brain development in mammals

It is important to emphasize that, for the mammalian infant, the mother (or the primary caregiver) is the most important source and modulator of stimulation [3]. Attachment in humans has been defined as the interactive regulation of biological synchronicity between organisms [4]. For such a ‘synchrony’ to occur we have to hypothesize that the infant, rather than being a passive recipient of
maternal stimulation, is an active component of the mother–infant dyad. Infants appear highly motivated to communicate with human beings and to establish intersubjective states. 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: the brain of the newborn infant is capable of producing an intermodal sensory-motor coordination allowing him to orient to preferred stimuli and to learn from them [5]. Successful engagement with the world of people and things depends on the status and effectiveness of the child–caregiver communicative system in facilitating child’s motivated intentions. These interactions make up the process of mutual regulation: the capacity of the two interactants to express their intentions, appreciate those of their partner, so that both can achieve their goals in a reciprocal feedback system [6]. As an example, the mother participates in ‘interactive repair’, a regulatory process utilized after her own induction of stress in her infant [7]. These dyadic transactions serve to regulate the infant’s affect state in the short term and lead to structural changes in the long-term. It has been suggested that the mother’s external regulation of the infant’s developing immature emotional systems during particular 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 [8].

But how do we go about pinning the basic neurobiological mechanisms underlying such fine maternal regulation of infant brain development? There is much controversy concerning whether non-human primates and rodents actually express attachment the way this concept applies to humans. In particular, while some rodent species, such as the guinea pig, do show some form of attachment, as indicated by their hormonal responses to separation, attachment theory is not easily applied to rodents such as rats and mice. By separating mother and infant, Hofer demonstrated that specific maternal behaviors can affect specific physiological systems in the immature rodent [3,9]. In these studies Hofer has stressed the notion that studying the sensory and nutritional aspects of the mother–infant relationship (which are likely to be involved in bond formation in early life in some species) is fundamental as a mean to understand the role played by the mother for a developing organism, independently from any role these aspects may play in attachment. Indeed, basic neurobiological research has firmly established that the mother acts as an external regulator of the infant’s states. The pattern of changes in the infant following maternal separation is not a unitary syndrome: the complex behavioral and physiological response that occurs reveals the existence of several discrete regulators which operate on different physiological and behavioral 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 [3]. 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 [3]. 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 [10]. More importantly, some indications exist that changes in the mother infant relationship can affect the function, structure and neurochemical architecture of the brain [11]. For example, an increase in the proliferation of hippocampal cells has been associated with maternal touch and this has enduring effects on memory during senescence [8]. Since the mutual regulation occurring between mother and infant can shape the organization of the infant’s brain, disruptions in the mother infant relationship could affect brain plasticity. We could hypothesize that, if a certain amount of stimulation was required for correct brain development, prolonged interruption in mother–infant contact might have negative effects, leading to an increased vulnerability to psychopathology [12].

3. Long term effects of early manipulations

Numerous studies conducted in rodents have clearly indicated that manipulations of the mother–infant relationship have long-term consequences on neuroendocrine and behavioral responses later in life. The most common manipulation, and the first to be used, consisted of removing the animals from the mother and their cage and placing them in individual compartments for up to 15 min until weaning. Animals handled during infancy (H) show increased exploration, less defecation and urination in an open field [13], a high degree of exploration in the hole board test, and a reduced taste neophobia and conditioned taste aversion [14]. Thus, early manipulations result in less emotional and better adapted animals, at least as tested in the laboratory.

In addition, stimulation during infancy markedly affects the activity of the endocrine system. 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 and when H subjects are tested in a milder situation, such as in an open field, they show significantly lower increases in GC, compared to controls [13,15]. The differences previously described are long-lasting and can persist for the entire life
of the animal. The modified endocrine response of H animals appears to 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 [16,17].

These long-term effects of handling appear to depend upon changes in the differentiation of those neurons known to be involved in the stress response [15]. Handled subjects show an increased number of glucocorticoid receptors (GR) expression in the hippocampus, a region strongly implicated in glucocorticoid feedback regulation [18]. Indeed, in the absence of a glucocorticoid negative-feedback signal, such as in adrenalectomized animals, adrenocorticotropic hormone (ACTH) secretion in response to stress is comparable in H and NH rats [19]. 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 [20]. Furthermore, CRH release from the median eminence in response to stress is significantly greater in NH compared with H rats [20].

The HPA axis is not the only system affected by early experiences. Early handling has been shown to affect long-term potentiation (LTP), a long-lasting increase in synaptic efficacy following brief, tetanic stimulation. Rats exposed to an early handling regime show, as adults, a greater amplitude of LTP in the hippocampus [21]. Immune system function also appears to be affected by early handling, H animals showing higher survival rate, following tumor implantation, than NH [22]. In addition, stimulation early in life appears to have a long-term impact on central neurotransmitter activity, for example decreasing the reward value of amphetamine in a conditioned place preference paradigm [23,24].

It has been reported that the long-term effects of handling on the hypothalamic-pituitary–adrenal (HPA) responses to stress are qualitatively different, depending upon the duration of the maternal separation, longer separations being detrimental for the developing animal. As adults, animals exposed to repeated maternal separation of 180–360 min/day for the first 2 weeks of life show significantly increased plasma ACTH and corticosterone (CORT) response to stressful stimuli, compared to unseparated controls, an effect opposite to handling. The longer period of separation also results in decreased glucocorticoid receptor binding in both the hippocampus and the hypothalamus [20]. These same results can be obtained exposing rat pups to a small dose of endotoxin (Salmonella enteridis), which produces a not life-threatening illness [15,25]. However, the effects of prolonged maternal separations are not always consistent. A number of papers are currently available indicating 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 [26–28]. We have also generalized this finding to another rodent species, the mouse. In a recent experiment we found the same magnitude of CORT secretion in response to a novelty stress in mice that had undergone 3 h daily maternal separations from postnatal day 2–14, compared to unseparated controls [29]. These apparent discrepancies might be due to differences in the experimental procedures used in the different laboratories, as suggested by a recent report [30]. Results from this study indicate that, following exposure to post-weaning environmental enrichment, maternally separated (3 h from pnd 1–14) subjects do not differ from handled animals in their behavioral and CORT response to restraint stress. Nonetheless, hippocampal glucocorticoid receptor gene expression, which mediates the effects of early experience on CRF expression was unaffected by environmental enrichment. These evidence suggest an important role of the environment in compensating, at least functionally, the influence of adversity in earlier stages of development. They also indicate that compensation at later developmental stages may be obtained through action on later-developing neural systems, or through systems characterized by a more prolonged period of plasticity [30].

It appears as though it is not always easy to discriminate what a truly ‘adverse’ experience is or to predict its behavioral 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 a paradoxical suppression of the HPA axis response [31]. 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 behavioral 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 behavioral responses to stress can be finely adjusted in response to external events.

4. Disruptions in the mother–infant relationship: effects on maternal behavior

It has been suggested that long-term effects of early manipulations indirectly result from changes in maternal
care due to alterations in pup stimulus characteristics following the handling procedure [32]. For example, removal of a rat pup from the nest results in a drop in body temperature and in the emission of ultrasonic vocalizations, in an age-specific fashion, which, in turn, stimulate maternal behavior [33,34]. Rat maternal care occurs in bouts. A nesting bout begins when the mother approaches the litter, gathers the pups under her so that they can attach to the nipples and feed, and ends when the mother licks the pups and leaves the nest [35]. Over development a steady decline occurs in the proportion of daily time spent in maternal care [36]. Mothers of H litters have been reported to present shorter and more frequent nesting bouts and to spend significantly more time licking the offspring and performing ‘arched-back’ nursing compared to NH [15,28]. Handling ultimately results in a persistent alteration of maternal care, while longer separations (4 h) 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 [28].

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? Hennessy and coworkers [37] have first shown that the strain of the mother, and thus the peculiar maternal behavior characterizing it, determines the nature of the handling effect on adrenal glucocorticoid responses to novelty in mice. These data have been recently confirmed in a study showing 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 [38,39]. 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 [40]. 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 CRF mRNA levels, thus resembling handled animals. These rats also show increased CRF receptor levels in the locus coeruleus and decreased central GABA/benzodiazepine receptor levels [40]. 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.

5. Neuroendocrine responses in infant rodents are under maternal regulation

The infant rodent is characterized by a markedly-reduced adrenocortical response to stimuli which are able to elicit a strong response in the adult [41,42]. This time period has been termed stress-hyporesponsive period (SHRP) [41,42]. Although neonatal rats can secrete ACTH in response to certain types of stressors, this response appears to be stimulus-specific as well as age-dependent. The adrenal gland, on the other hand, shows minimal CORT output even when stimulated by high levels of ACTH [42,43]. In the rat, the SHRP lasts from postnatal day (pnd) 4 until about pnd 14. Although the mechanisms underlying it have not been fully elucidated, it is clear that a partial immaturity of the system, together with an active inhibitory process, results in a period during which circulating levels of CORT are relatively low and difficult to elevate [42].

Numerous studies have shown that maternal factors appear to exert a strong inhibitory effect on the infant’ HPA system. This can be demonstrated by removing the source of the regulation: following prolonged 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 [10, 42,44,45]. We investigated the role played by contact with a lactating female, in the absence of nutrition and suckling [46]. Previous data had shown that passive contact with a female was able to prevent further elevations in GC levels once the HPA axis of the pup had been activated by 24 h of maternal deprivation [47]. Thus contact with the mother per se could be the critical variable responsible for keeping CORT secretion of the neonatal rat at relatively low levels. Data from this experiment 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 [46]. 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 activated by 24 h of maternal deprivation. It has been later shown that the disinhibition of the HPA axis is a process characterized by a slow onset occurring after prolonged separation periods (about 8–24 h; [44]), resembling the time-course of other processes under maternal regulation [3]. The effects of maternal separation on CORT secretion can be generalized also to another rodent species, such as the mouse [48,49].

The critical aspect of the mother–infant interaction missing during the deprivation period seems to be related to the feeding process. One of the hypothesis proposed to explain the SHRP is that this hyporesponsiveness results from an increased negative feedback signal of CORT that would lead to a suppression of ACTH release [41]. Small amounts of maternal CORT are transferred to the neonate
through milk. In a subsequent experiment [46] the hypothesis was tested that GC present in the mother’s milk might contribute to the enhanced negative feedback hypothesized to underlay the SHRP. We predicted that pups reared by adrenalectomized (ADX) mothers would be more responsive to exogenous ACTH because of reduced negative feedback signal in the absence of maternal CORT. Data from this experiment do not support the above hypothesis. Nonetheless, they suggest that feeding might be an important variable in the regulation of HPA activity by the mother. Older pups reared by an ADX mother showed a greater response to saline and ACTH injections than normally reared ones. ADX females have been shown to exhibit normal maternal behavior, however, they might produce less milk because of CORT absence [50]. Thus the amount of milk supplied by these dams might have been insufficient to satisfy the nutritional demand of older pups. If milk was a critical factor, then the reduction of milk intake in the 12-day-old animals might be sufficient to explain the partial disinhibition of the HPA system observed at this age. In addition, it has been shown that the effects of maternal deprivation on CORT secretion can be prevented providing the pups with food during the separation period [51]. More interestingly, it has been shown that stroking alone, without feeding, can prevent the stress-induced rise in ACTH in separated subjects, without affecting CORT. In summary, different maternal behaviors appear to regulate different components of the HPA axis: feeding serves to reduce the sensitivity of the adrenal to ACTH, while stimulation of the anogenital region is directly involved in the inhibition of ACTH.

Prolonged maternal separation alters the central components of the stress response system [52,53]. Following a 24 h separation there is an induction of c-fos mRNA in the paraventricular nucleus (PVN) of the hypothalamus. Concomitantly, there is a decrease in basal MR and a GR mRNA level in the CA1 area as well as GR mRNA in the PVN. It appears as though a dichotomy in terms of regulatory processes also occurs in the brain. Specifically, stroking alone has been shown to prevent the reduction in CRH mRNA and the induction of c-fos in the PVN of the hypothalamus, as well as the effects of maternal separation on hippocampal MR mRNA. However, stroking plus feeding can restore GR mRNA hippocampal levels to higher, non-deprived levels [52]. Recent findings also indicate significant alterations in 5HT receptors gene expression following 24 h maternal separation in rats [54]. Thus, in addition to regulating the HPA axis, the presence of the mother also exerts an important regulatory function on CNS neurochemical systems, the cumulative effect of maternal stimulation being that of maintaining behavioral arousal and HPA responses to stressful stimuli at a relatively low level.

6. Role of neurotrophins in brain development and in neuronal plasticity

Neurotrophins, such as nerve growth factor (NGF), play a fundamental role in brain development since they regulate proliferation, survival and neurochemical differentiation of selected neuronal populations both in the peripheral and the CNS (Table 1) [55]. In addition to exerting a long-recognized role on neuronal survival and differentiation, neurotrophins are important mediators of synaptic and morphological plasticity [56,57]. Expression of NGF and of another functionally-related neurotrophin, brain-derived neurotrophic factor (BDNF), for example, has been localised to the hippocampus and neocortex which represent target areas for basal forebrain cholinergic neurons and which are well-studied sites of both developmental and adult synaptic plasticity [58,59]. In addition, 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 [60].

In the CNS neurotrophins are synthesised predominantly by neurons in an activity-dependent manner and are released upon neuronal depolarization. Up-regulation of neurotrophin synthesis is effected by glutamate via N-methyl-D-aspartate (NMDA) and non-NMDA receptors and also by acetylcholine via muscarinic receptors [61,62]. While glutamate receptor stimulation increases NGF mRNA expression, gamma-aminobutyric acid (GABA) down-regulates it. This activity-dependent regulation not only functions under extreme experimental conditions, such a kindling, [62] but is also involved in maintenance of normal physiological levels of NGF and BDNF [63]. In addition, physiological stimuli, such as visual input, have been shown to regulate neurotrophin mRNA levels, since light affects levels of hypothalamic NGF and BDNF [63]. NGF expression can be upregulated by behavioral arousal as reported by Spillantini et al. [65] who have shown increased levels of hypothalamic NGF mRNA following intraspecific aggression in mice [66]. There is evidence for short-term modulation of synaptic transmission by neurotrophins on hippocampal neurons in vitro. For example, NGF potentiates high potassium induced release of acetylcholine and glutamate from hippocampal synaptosomes and increases intracellular calcium levels [67,68]. In addition, both NGF

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**Synaptic plasticity**

**Programmed cell death**

**Axon growth**

**Dendritic arborization**

**Synaptic rearrangement**

**Table 1**

Neuronal properties regulated by neurotrophins

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**Programmed cell death**

**Axon growth**

**Dendritic arborization**

**Synaptic rearrangement**

**Thermogenesis**

**Apoptosis**

**Synaptic plasticity**

**Programmed cell death**

**Axon growth**

**Dendritic arborization**

**Synaptic rearrangement**
and BDNF can cause rapid and lasting changes in stimulus-dependent activity in the adult cortex in vivo [69]. Structural changes in axons and dendrites underly plastic rearrangements in the CNS. For example, the activity-driven rearrangements leading to the formation of ocular dominance columns in visual cortex involve elaboration of synapses in one layer or column and the loss in another layer or column [70]. Because of its action on neurite outgrowth, NGF seems especially well suited to be involved in such structural changes. In addition, neurotrophins control dendritic growth in a highly specific fashion [57]. The developing visual system has been the most widely used model system to test neurotrophin’s function as substrates for activity-dependent synaptic competition [71]. Recently, activity dependent expression of BDNF has been shown to regulate cortical inhibition and duration of the critical period for visual cortical plasticity [72]. Neurotrophins could be involved in the plasticity of postnatal brains which subserve the development of complex behaviors. The mammalian nervous system is still immature at birth. A dramatic increase in the connectivity of the mammalian cerebral cortex occurs during the early postnatal period. In the molecular layer of the hippocampal dentate gyrus of the rat, the number of synapses increases exponentially until reaching adult value on postnatal day 30, while most granule neurons of the dentate gyrus are produced during the first two postnatal weeks of life [73,74]. Thus, the postnatal period of neurogenesis and synaptogenesis offers the possibility for experience-dependent modifications of brain function.

7. Changes in neurotrophin expression following maternal separation

The long-term effects of early manipulations on adrenocortical activity and behavior depend, at least in part, on permanent changes in those brain structures, such as the hippocampus and the hypothalamus, known to be involved in the regulation of the stress response [18,20]. Only a few studies have attempted to study systematically the basic neurochemical and neuronatomical changes resulting from early manipulations of the mother–infant relationship and the mechanisms linking changes in maternal behavior to specific modifications in gene expression. For example, 5-HT has been indicated as a possible mediator of the effects of handling on hippocampal GR binding capacity [75]. However, the mechanisms linking changes in maternal behavior to specific modifications in neurotransmitter levels, such as serotonin, as well as the cascade of events leading from this neurotransmitter to changes in gene expression has not been elucidated. In a pioneering attempt to address this issue 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 [76]. 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 [57]. Indeed, previous work had shown that environmental stimulation has long-term effects on hippocampal NGF levels [77]. 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 [78]. This region was chosen because NGF and its receptors are reliably expressed by hippocampal neurons already early on during development [79]. 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 (Fig. 1) [80]. 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 [78,80]. 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 [81]. Concomitantly, there was a noticeable increase in the rate of cell death in the neocortex, white matter, and granule cells of the dentate gyrus [81]. Whether the increase in cell death results in a permanent reduction in cell number is not known [81].

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 [81]. A number of studies suggest that, in contrast to the periphery, in the central nervous system neurotrophins are synthesised in an activity-dependent manner and that they are released upon depolarization of CNS neurons [62]. 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 [44]. Whether changes in NGF expression are long-lasting or limited to the acute maternal separation event is currently under investigation.

8. Long-term effects of early experiences: implications for neuroplasticity during senescence and Alzheimer’s disease

GCs are fundamental hormones involved in the response to acute physical stressors. However, excessive secretion of
GCs, such as that occurring during chronic stress, can cause a number of deleterious effects, including neurotoxicity, inhibition of neurogenesis, and disruption in neuronal plasticity (reviewed in Ref. [82]). GCs effects on the brain are mainly centered on the hippocampus, a structure which is rich in corticosteroid receptors and thus sensitive to GC action, and which is involved in learning and memory. Because glucocorticoid exposure increases linearly with aging in both rodents and humans, ‘successful brain aging’ might be linked to maintaining low GC levels throughout the individual’s lifespan [82]. Indeed, experimental evidence shows that chronic exposure to high levels of these GCs can result in hippocampal atrophy [83,17]. In particular, aged humans with significantly prolonged cortisol elevations show reduced hippocampal volume and deficits in hippocampus-dependent memory tasks compared to normal-cortisol controls, the degree of hippocampal atrophy being correlated with both the degree of cortisol elevation over time and actual basal cortisol levels [17].

Early, subtle manipulations performed in rodents, such as handling, result in important long-term consequences, such as an attenuation in the neuroendocrine, anatomical and behavioral impairments related to brain dysfunction during aging [41,84]. In comparison to NH animals, H subjects show an increased number of GR receptors throughout their lifespan [82]. Indeed, experimental evidence shows that chronic exposure to high levels of these GCs can result in hippocampal atrophy [83,17]. In particular, aged humans with significantly prolonged cortisol elevations show reduced hippocampal volume and deficits in hippocampus-dependent memory tasks compared to normal-cortisol controls, the degree of hippocampal atrophy being correlated with both the degree of cortisol elevation over time and actual basal cortisol levels [17].

It is possible to hypothesize that changes in neurotrophins resulting from manipulations of the mother-infant relationship might underlie some of the plastic changes characterizing H subjects. In fact, changes in NGF expression resulting from brief maternal separations occur in brain regions involved in the neural control of the stress response, such as the hippocampus and the hypothalamus [52,85]. Changes in NGF levels in the hypothalamus have been reported following highly arousing situations, such as intraspecific fighting in male mice, and have been hypothesized to be involved in neuronal plasticity occurring as a result of stressful events [66]. The hippocampus and the hypothalamus may represent sites of interaction between NGF and the HPA axis in vivo. In the hippocampus NGF and its mRNA have been localized to granule cells of the dentate gyrus and pyramidal neurons, which are also targets for glucocorticoids action [86] while in the hypothalamus, NGF and its mRNA have been detected in the preoptic area and the ventrolateral nucleus, two areas involved in neuroendocrine regulations [65]. Indeed, both NGF and maternal deprivation induce the expression of the NGFI-B gene, which has strong homologies with the glucocorticoid receptor gene family [85,87]. Furthermore, in hippocampal cultures, NGF exerts a dose-dependent negative effect on GR expression, but not MR. Since expression of GRs is decreased in the hippocampus and in the PVN following 24 h maternal separation a causal relationship between increased NGF expression in this region and changes in this GC receptor can be hypothesized [88]. Changes in the levels of neurotrophins occurring early during development might also be reflected in changes in hippocampal plasticity, such as LTP [84,21]. Indeed, BDNF modulates the competence of presynaptic nerve terminals to generate the repetitive exocytotic events needed to modify the responses of postsynaptic neurons (for example promoting docking of

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**Fig. 1. Changes in NGF expression in maternally-separated rat pups.** Results of the data quantitation, each data point represents the mean ± SEM of the percentage of positive cells over total cells counted in each brain area examined in the different experimental groups (n = 3 in each final group). Sections from the separated (1 h vs. 3 h) or non-separated (time 0) subjects were hybridized to an NGF antisense riboprobe. Overall, NGF expression increased with the length of the separation, an effect more pronounced in the hippocampal and hypothalamic areas. Significant intragroup comparisons are marked. *p < 0.05; **p < 0.01. (Reprinted from Developmental Brain Research, Cirulli et al., pp. 129-134 (2000), with permission from Elsevier Science).
synaptic vesicles to the presynaptic membrane) (reviewed in Ref. [57]).

Normal aging and Alzheimer’s disease (AD) have many features in common [89]. Cumulative evidence suggests that the brain, both during the aging process and AD, is actively adapting to a disregulation in energy metabolism to the point that progressive AD has being defined as a metabolic disease [90]. The long-term changes in HPA axis activity resulting from early manipulations have important implications also for this brain pathology. It has been shown that, in addition to lifestyle factors, disregulations in HPA axis function, leading to an increase in circulating GC, can impair energy metabolism in susceptible brain areas resulting in an increased vulnerability for the disease. A defining feature of AD is the presence of neuritic plaques and neurofibrillary tangles both in neocortex and hippocampus. However, a more significant neuropathological finding is perhaps the region-specific degeneration of cholinergic neurons of the septo-hippocampal system [91]. In mammals, these neurons play a functional role in learning and memory [92,93]. On a neurobiochemical level, neurotrophic factors such as NGF and BDNF are likely to be involved in the degeneration of cholinergic neurones since adequate amounts of these neurotrophins are required for maintaining functional and structural integrity of these neuronal populations and experimental evidence indicate that the expression of neurotrophins is modified in both hippocampus and parietal cortex of AD patients [56,94].

Recent evidence suggest that loss of brain plasticity might be a common denominator also for major psychopathologies such as depression and schizophrenia. It is thus possible to hypothesize that early environmental manipulations, leading to adaptive changes in HPA axis activity (and thus to low circulating GCs), and the maintenance of high levels of neuronal plasticity (e.g. through optimal expression of neurotrophins in selected brain regions), might overall ameliorate the aging process as well as reduce the risk to develop mental illnesses.

9. Conclusions

Overall, evolution appears to have set up a series of mechanisms by which the developing nervous system can finely ‘tune’ its activity according to the demands of the environment. This behavioral plasticity is most likely based upon both structural modifications in neural networks as well as long-term changes in the synthesis and release of neuromodulators (including neurotrophins), thus allowing for fast acting biochemical switching in response to appropriate stimuli [95].

While the mother remains the most important source of experience for a mammal we cannot exclude that other stimuli might be important regulators of the developing nervous system. A general notion that emerged from the pioneering studies of Levine [13] is that the critical factor for ‘correct’ development (i.e. in a range of possibilities shaped by species-specific evolutionary processes) is that the animal receives some kind of stimulation. Lack of or minimal stimulation, which is often the rule in the ‘unnaturally impoverished’ laboratory environment, appears to be a truly detrimental condition, leading to a more vulnerable and less adapted individual. Further insights into the role of early experiences in shaping brain function might be relevant for understanding the basis for individual vulnerability to brain aging and psychopathology.

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References


