NEUROTROPHINS AND ATTACHMENT

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Abstract

Object: Over the past decade, studies in different animals, including humans, have addressed the neural, molecular and cellular basis of social attachment, focusing on the role of the central neuropeptides (especially oxytocin and vasopressin) and their interactions with the hypothalamic-pituitary adrenal axis. Recent investigations have suggested neurotrophins as important modulators of social bonding mechanisms.

Method: MEDLINE and Pub-Med (1970-2007) databases were searched for English language articles using the keywords *attachment, pair bonding, social behavior, and neurobiology, neuropeptides, neurotrophins*. We reviewed papers that addressed the relationship between neurotrophins and attachment both in animal and human studies.

Results: Several neural and endocrine factors, most of which are still largely unknown, may modulate reproductive behaviors, mother-infant attachment and adult-adult bonding. In particular, oxytocin and vasopressin are supposed to be involved in attachment process, influencing the onset of maternal and paternal behavior, respectively; moreover, both neuropeptides may contribute to pair-bonding process by stimulating limbic areas known to integrate social informations with reward pathways. Recently, evidence for a role of neutrophins in social bonding has been arising from animal models of maternal deprivation: in these studies, abnormal levels of the Brain-Derived Neurotrophic Factor (BDNF) were found in different brain areas, as well as significant deficits in hippocampal circuits involved in memory and learning. In addition, Nerve Growth Factor (NGF) has been implicated in molecular mechanisms of romantic love, while acting as a modulator of different endocrine functions.

Conclusions: Preclinical studies suggest the involvement of HPA axis in regulating social behaviour and attachment. Recent observations coming also from humans suggest an important role for neurotrophins, that, while acting on HPA axis activations, may regulate social bonding.

Key Words: Attachment – Social Bonding – Maternal Deprivation – Neurotrophins – Nerve Growth Factor – Brain-Derived Neurotrophic Factor

Declaration of interest: None

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Introduction

Attachment is a dimension of the human mind developing from the first relationships between the infant and the caregivers, which includes emotional, cognitive and behavioral processes. It remains almost unchanged in the lifetime, while influencing all social relationships. Attachment is the most important interpersonal process for humans as, at birth, we are very immature and could not survive if we were not "social".

The attachment theory, originally proposed by Bowlby (Bowlby 1969, 1973, 1980), integrated the psychoanalitic model with behavioral observations of animals, while focusing particularly on the mother-infant relation. Bowlby considered attachment as the main motivational system: mother-child links would already begin during pregnancy and shape the system which regulates interactions in the adulthood.

The attachment system can be defined from the presence of three characteristics: first, the *search of proximity* to one preferred person, the *secure base effect*, and, finally, the *protest for the separation.* The *secure base* represents for the child a springboard in order to develop curiousity and exploration. The function of *secure base,* that in the first years of life is carried out from the mother, becomes then, through the internalization of the behaviors and the emotions, an

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inner structure which is a source of self-comfort and self-protection. In this way, the child and then the adult can feel free to go away and differentiate him-or-herself gradually from the caregiver and begin to explore the external world.

The *protest for separation* represents the primary answer to the separation from the caregiver. The protest includes two aims: to repair the tie of attachment and to punish the caregiver in order to avoid further separations.

The ontogenesis of attachment consists of three phases:

– the first phase (guideline and pattern of acknowledgment), corresponds to the period that goes from birth to the first six months of life. At birth, children are not able to distinguish the others from themselves, but they react intensily to the human contact; at the forth week, they smile in front of a human face, while evoking the smile in the others; at the third month, the attachment relation starts to be set up;
– the second phase ("set-goal" attachment), spans from six month to three years, period in which "the anxiety for stranger" appears (Spitz 1965) and in which the *set-goal* of the child is to mantain him or herself sufficiently close to the mother who becomes the secure bases for explorations, and to protest for the separation, or in case of danger;
– the third phase (formation of a mutual relation) begins with the third year of life and it is characterized by more complex patterns because of the development and consolidation of the language: the child begins to consider the parents like separated individuals, with personal aims and plans in order to influence them.

Bowlby hypothesized that the attachment has solid genetic and biological bases, but only in the last 30 years, several studies in animals and also in humans have begun to investigate its anatomical and neuro-biological bases.

Social attachment is fundamental for life of mammals and, in particular, of the human species; however, its exploration in higher animals turns out particularly complex. The attachment system implies multisensory perceptions, mostly olfactive in rodents and visual in primates, and complex motor answers (vicinity search, defence and handling behaviors). Attachment moreover, underlies many cognitive processes, such as attention, memory, social acknowledgment and, particularly, the motivational system, while linking the sensory to the motor outputs. With the exception of pharmacological studies in maternal monkeys (Martel et al. 1993) and recent human imaging studies (Bartels and Zeki 2000), investigations of neural systems involved in attachment have so far used non primate mammals. Recent evidences show that two neurohypophyseal peptides, oxytocin and vasopressin, are relevant in the formation of social relationships. Moreover the role of these hormones seems to be gender-specific: oxytocin mediates behavioral aspects in females, while vasopressin in males. Several other neurotransmitters are, however, certainly implicated in the regulation of attachment mechanisms: prolactin, opioids, dopamine and GABA play a role in maternal behaviour (Numan 1994), GABA, opioids and serotonin modulate infant attachment (Winslow and Insel 1990), but no one functions in independent way. Oxytocin receptors are localized in the lactotroph cells of hypophysis (Breton at al. 1995); opioids regulate oxytocin release (Bicknell and Leng 1982); oxytocin and vasopressin present targets of actions in many autonomic nuclei of the brain stem. These interactions are region specific, depending on the presence of receptors and are influenced by sexual steroids. The implication of oxytocin and vasopressin in the types of attachment (infant and maternal) raised the hypothesis of the existence of a single circuit, already present at birth, involved in the attachment. Recent data show that regions of lateral amigdala and lateral septum and their projection to rostral hypothalamus (medial preoptic area), through the bed nucleus of stria terminalis, play an important role in parental behaviour and in pair bonding formation. These circuits probably integrate social informations with reward pathways; interestingly, many of these regions are rich in oxytocin and vasopressin receptors. Limbic areas implicated in attachment behaviours process olfactory stimuli in rodents and visual stimuli in primates.

Neurotrophins and attachment

In the last years a growing body of research has been investigating the role of neurotrophins (NTs) in the neurobiology of attachment.

The neurotrophin family comprises some structurally similar proteins that play an important role in the regulation of survival, differentiation and functioning of neuronal populations both of the central and the peripheral nervous system. The Nerve Growth Factor (NGF), the Brain-Derived Neurotrophic Factor (BDNF), the neurotrophin-3 (NT3), the neurotrophin-4 (NT4), the neurotrophin-5 (NT5) and the neurotrophin-6 (NT6) currently belong to the neurotrophin family (Patapoutian and Reichardt 2001). These different proteins are closely related in terms of sequence homology and receptor specificity binding; a low-affinity trasmembrane receptor $p75^{NTR}$ binds all neurotrophins, and in addition, each neurotrophin binds with high affinity to one of the tirosin-kinasi (trk) family of transmembrane receptors: NGF to trkA, BDNF and NT4/5 to trkB, NT3 to trkC (and to trkA with a lower affinity) (Patapoutian and Reichardt 2001, Poo 2001). BDNF is the most widespread neurotrophin in the brain of mammals, both during development and in adult life. It is a polypeptide consisting of two chains of 119 amino acids, with total molecular mass of 29684 Da, which is encoded by a gene located on the short arm of chromosome 13 whose expression is modulated by the transcription factor CREB.

The expression of BDNF in the CNS is positively regulated by neuronal activity (Lindholm et al. 1994): evidence derives from studies investigating hippocampus functioning that is strongly influenced by the physiological and pathophysiological neuronal activity (Isackson et al. 1991). *In vitro* and *in vivo* analyses mainly focused on rat hippocampus, cerebral cortex and hypothalamus showed that the activity-dependent regulation of BDNF is mediated by the

classical neurotransmitters: in general, BDNF mRNA is up-regulated by glutamate, acetylcholine and serotonin while it is down-regulated by the GABA. This regulation is functional to provide a positive feedback on synaptic function through a localized increase of BDNF and to protect neurons from excito-toxicity following a neuronal damage (Lindholm et al. 1994, Lindvall et al. 1994).

Besides BDNF expression in neurons, BDNF mRNA is also synthesized in several tissues and peripheral organs. In animal studies BDNF mRNA has been found in the aorta wall and generally in endothelial cells, heart, kidneys, sub-mandibulary glands, ovaries, dorsal root ganglia, retina, muscles and lungs. In these tissues, BDNF, released by cells following neuronal stimulation, has the role of promoter of the neuronal survival. BDNF is also present in the bloodstream as shown for the first time about ten years ago (Rosenfeld et al. 1995). Serum levels of BDNF are about ten times higher than plasma concentrations and this difference is mainly due to platelet release of large amounts of BDNF during activation. Platelets do not synthesize BDNF, but take it from plasma and internalize it through a still unknown mechanism (Fujimura et al. 2002).

Effects of maternal behaviour on neurotrophin expression

Early life experiences, such as handling, can influence neural development of rodents, while modulating physiological and behavioral reactivity to stress. A growing body of data from animals and humans suggests that the relation between early experiences and emotional response in adults may be mediated by maternal behaviour (Francis and Meaney 1999, Caldji et al. 2000, Champagne et al. 2003). Experimental models of early manipulation, such as neonatal handling and male intruder test, allow to assess variations in parental behaviour (e.g., nest building, licking and nursing) and pups' emotional responses (Cirulli et al. 2007). Offspring of mothers subjected to stressful challenges, e.g., by the exposure to the odour of a potentially infanticidial male, with increased arousal during lactation, showed an enhanced emotionality in adulthood (Moles et al. 2004). Handling manipulation has been demonstrated to progressively increase licking behaviours in postnatal days: this pattern differs from the natural tendency of maternal behaviours to decrease in non-handled litters. Furthermore, handled pups showed significantly lower weight than non-handled subjects. When pup's reactivity to novelty was tested by measuring ultrasonic vocalization (UVZ) emitted as a result of a brief isolation from the mother, in handled litters the number of UVZ was not decreased after chlordiazepoxide administration, an anxiolytic compound that reduced UVZ in non-handled pups.

However, from a neurobiological point of view the molecular mechanism underlying the effects of maternal care on the behaviour of the offspring in adulthood are still to be clarified. Previous studies found that the levels of maternal care were negatively correlated with the behavioral and hormonal responses to stress in the offspring (Champagne et al. 2003). Additionally, handling-evoked augmentation of maternal care of pups reduced hypothalamic corticotrophin releasing hormone (CRH) expression and upregulated hippocampal glucocorticoid receptor levels, thus promoting a lifelong attenuation of hormonal stress responses (Plotzky and Meaney 1993, Fenoglio et al. 2004). It has been observed that the peripheral thyroid hormone release and the activation of ascending serotoninergic pathways are required for the handling effect on hippocampal glucocorticoid receptor expression (for review, see Meaney et al. 1996).

The study of these long-lasting, handling-evoked changes has more recently involved neurotrophins, such as NGF, which are involved in promoting neuronal survival, brain differentiation and function. In rodents, an hypothalamic and cortical increased expression of the immediate early genes *c*-fos and the NGF-inducible gene NGFI-B was found after maternal deprivation (Smith et al. 1997). Further studies showed that the effects of maternal separation on the increased NGF expression are stronger with longer separation (Cirulli et al. 1998, 2000). The handling procedure was shown to increase *c*-fos mRNA levels in amygdala and thalamus (Fenoglio et al. 2006) and hippocampal NGF protein levels in response to an acute stress (Cirulli et al. 2008). These data suggest an important role for NGF and, perhaps, other neurotrophins in acting as transducers of early environmental conditions in permanent changes in brain structure and function.

Neurotrophins and attachment in animal studies

A growing body of data suggests BDNF involvement in stress-related hippocampal degeneration (Duman et al. 1997, Duman and Monteggia 2006). Mother deprivation is an animal model of early stress, which is used to study the development of depression-like behaviours in adults (Hall 1998, Ladd et al. 2000, Arborelius et al. 2004). Rats separated from their mother for long periods show when adults anxious-like abnormal behaviours and an exaggerated endocrine response to acute stress (Plotsky and Meaney 1993, Ladd et al. 1996, Biagini et al. 1998, McIntosh et al. 1999, Wigger and Neumann 1999, Ladd et al. 2000, Huot et al. 2001, Kalinichev et al. 2002, Ladd et al. 2004). Recent studies showed that stress can decrease BDNF mRNA expression in hippocampus, neocortex and amygdala, while increasing its synthesis in other brain areas such as the hypothalamus (Smith et al. 1995a, Smith et al. 1995b, Russo-Neustadt et al. 2001). Analysis of BDNF mRNA levels in the brain of rats separated from their mother at birth showed different results in short-and long-term, respectively. A short-term increase in gene expression of BDNF was observed in prefrontal cortex and hyppocampus (Cirulli et al. 2003, Roceri et al. 2004), while a reduced gene BDNF expression was detected in long-term observations, specifically in prefrontal cortex (Roceri et al. 2004). On the contrary, others (Greisen et al. 2005) found a highly significant increase in hippocampal BDNF concentrations in adult rats separated from the mother for a time of 180 minutes per day, compared to rats kept separate from the mother for only 15 minutes a

day. BDNF levels in frontal cortex and hypothalamus/paraventricular nucleus did not differ between the two groups. BDNF mRNA expression in CA1, CA3 areas, dentate gyrus of hippocampus and paraventricular nucleus of hypothalamus was not influenced by maternal separation. Bromo-deossiuridine repeated administrations in dentate gyrus did not result in any significant effect in neurogenesis and cell survival of adult rats, both when they had been separated from his mother for 180 minutes and for 15 minutes. Similarly, both groups did not differ, with regard to the age-dependent decline in the neurogenesis process between the third and seventh months of life. According to the BDNF up-regulation of neurogenesis and cellular repair, the high hippocampal concentration of BDNF found in rats longer separated from mother may represent a compensatory response to the separation during the neonatal period, thus maintaining the neurogenesis in adults at same levels as those of rats breaded by their mother.

Chronic separation from the mother may lead to the production of abnormal levels of BDNF both in the mature and immature form (pro-BDNF) in different brain areas, as well as significant deficits in hippocampal circuits involved in memory and learning (Smith et al. 1995b, Huot et al. 2002). Chronic reduction of BDNF levels in hippocampus could result in changes of neuronal plasticity leading, clinically, to depressive-like behaviors such as changes in locomotor activity and behavioural stereotypes observed in maternal separation rats.

A recent study showed that rats, subjected to daily maternal separation, developed behavioral deficits, impaired functioning of the HPA axis in response to acute stress and long-term changes in BDNF levels (Lippman et al. 2007). It has been suggested that the pro-BDNF has different and partly opposed effects than those of the mature form of BDNF on cell survival and synaptic plasticity. It seems that the increased levels of pro-BDNF induces apoptosis and a long-term down-regulation of hippocampal functioning, while the increased mature BDNF expression leads to longer cell survival and long-term potentiation (Ghosh et al. 1994, Korte et al. 1998, Teng et al. 2005, Woo et al. 2005).

Animals following chronic maternal separation showed a decreased mature BDNF expression which may lead to a reduced cell survival and deficiency in hippocampal functioning: these changes would sustain the development of depressive-like behaviours (Duman and Monteggia 2006). An increase in the levels of mature and pro-BDNF has been observed in ventral tegmental area (VTA) (Lippman et al. 2007) whose dopaminergic neurons are involved in reward circuits. Stressful events activate dopaminergic neurons of VTA and upregulate dopaminergic transmission in rats (Horger and Roth 1996, Di Chiara et al. 1999, Yadid et al. 2001, Nieoullon and Coquerel 2003), while the injection of BDNF in LTV is followed by an increase of depressive-like behaviors (Eisch et al. 2003). Significant decreased mature BDNF levels have been recently found in the striatum of adult rats following repeated postnatal maternal separation (Lippman et al. 2007).

A recent study examined the effects of early isolation rearing on cell proliferation, survival and differentiation in the dentate gyrus of the guinea pig, while showing that it decreases hippocampal cell proliferation, probably by reducing BDNF expression, and hampers migration of the new neurons to the granule cell layer, likely by altering density and morphology of radial glia cells (Rizzi et al. 2007). The wide reduction of the number of granular cells following isolation rearing emphasizes the role of environmental stimuli as key modulators in neurogenesis.

Being reared in a communal nest (CN), consisting of a single nest where three mothers keep their pups together and share care-giving behavior from birth to weaning, provides an highly socially stimulating environment to the developing pup (Branchi et al. 2006). At adulthood, CN reared mice show increased BDNF protein levels and longer survival of BrdU-positive cells in the hippocampus, compared to mice reared in standard nesting laboratory condition (SN). CN mice, although showing levels of exploratory and locomotor activity similar to those of SN mice, display increased anxiety-like behavior. These results suggest that an highly-stimulating early social environment leads to an increase of adult neuronal plasticity, which seems to be associated with an increased anxiety and depression-like behaviors at adulthood.

Early social manipulations may then affect the development of SNC, and lead to individual differences in the physiological and behavioral responses to the environment. Specifically, maternal deprivation, even for a short period, results in neuroendocrine, neurochemical and behavioral changes, but the underlying mechanisms are still to be clarified (Kuma et al. 2004). Recent data suggest that neurotrophins could act as mediators to translate the effects of external manipulation on brain development. NGF and BDNF play an important role in brain development, while in adult animals they are mainly involved in maintaining structural and functional integrity of neurons. Changes in the neurotrophins level during the critical stages of development may result in long-term changes of neuronal plasticity and lead to greater vulnerability to aging and psicopathology (Cirulli et al. 2003).

The ontogenetic pattern of BDNF gene expression has been studied throughout the course of development in prairie and montane voles which show an early BDNF mRNA appearance and a transient expression in a regionally specific manner (Liu et al. 2001). BDNF mRNA was found neonatally in the dentate gyrus and CA3 region of the hippocampus, increased gradually during development, and reached a peak at weaning, then showed a subsequent decline to the adult level. In the paraventricular nucleus of the hypothalamus, levels of BDNF mRNA persisted until weaning, then increased significantly to the adult levels at 3 months of age. BDNF mRNA also demonstrated a species-specific developmental pattern: in the cingulate cortex, BDNF mRNA labeling showed a transient increase in the second and third postnatal weeks and a subsequent decrease to the adult level in prairie voles, but persisted throughout the course of development in montane voles. In general, in montane voles an adult pattern of BDNF mRNA expression was achieved earlier than in prairie voles. These data suggest that the function of BDNF is probably different in infant and adult brains, and that the ontogenetic pattern of BDNF mRNA expression of

the two species of voles differs in a regional-specific manner, which may be associated with their different life strategy and brain and behavioral development.

In the mammalian brain, adult neurogenesis seems to occur primarily in the sub-ventricular zone (SVZ) and dentate gyrus of the hippocampus (DG) and to be influenced by both exogenous and endogenous factors. A study examined the effects of male exposure or social isolation on neurogenesis in adult female prairie voles (Fowler et al. 2002). By means of a cell proliferation marker, 5-bromo-2'-deoxyuridine (BrdU), neurogenesis was detected in various brain areas including the SVZ, DG, amygdala, hypothalamus, neocortex and caudate/putamen. Two days of male exposure significantly increase the number of BrdU-labeled cells in the amygdala and hypothalamus of female prairie voles in comparison to social isolation. This difference remains constant in the amygdala, while in the hypothalamus, male exposure determined a higher increase in neurogenesis compared to female exposure. In SVZ, two days of social isolation stimulated cell proliferation compared to female exposure, but this difference vanished over time. Group differences in the number of cells undergoing apoptosis seemed irrelevant to account for the observed differences in neurogenesis. Overall, these data indicate that the effects of social environment on neuron proliferation in adult female prairie voles are stimulus- and site-specific.

Neurotrophins and romantic attachment: human studies

A recent study investigated for the first time the peripheral levels of neurotrophins in subjects in love (Emanuele et al. 2006), looking at the hypothesis that the early romantic phase of a loving relationship could be associated with alterations in circulating levels of neurotrophins (NTs). Subjects who had recently fallen in love and two control groups, consisting of subjects who were either single or were already engaged in a long-lasting relationship, were compared for plasma NGF, BDNF, NT-3 and NT-4 levels. NGF levels were significantly higher in the subjects in love than in the control subjects. A significant positive correlation was found between NGF levels and the intensity of romantic love as assessed with the passionate love scale. No differences in the concentrations of other NTs were detected. In 39 subjects in love who maintained the same relationship after 12-24 months but were no longer in the same mental state as during the initial evaluation, plasma NGF levels decreased and became indistinguishable from those of the control groups. Overall, these results suggest that some behavioral and/or psychological features associated with falling in love could be related to increased NGF levels in the bloodstream. This neurotrophin might play a role in molecular mechanism of romantic love, and act as a modulator of different endocrine functions. From a larger prospective, neurotrophins may be involved in social relationships, directly or modulating stress-related symptoms.

Conclusions

The neurobiological approach to human attachment is just at its dawn, as most of the available data have been collected in animal models. Preclinical studies suggests the involvement of the HPA axis in regulating social behaviour and attachments, and a growing body of evidence supports the role of central neuropeptides, such as oxytocin and vasopressin, in increasing positive social behaviour in animals: the hypothesized mechanism links oxytocin and vasopressin, stimulated by social interactions, to dopamine pathways associated with reward. Latest investigations are focusing on neurotrophins, while suggesting their putative role as modulators of HPA axis activation. As discussed by Alleva and Branchi (2006), neurotrophins may play a crucial role in many human behaviours dealing with emotions and passions, such as social competition, proudness and just love.

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