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Changes in plasma levels of BDNF and NGF reveal a gender-selective vulnerability to early adversity in rhesus macaques

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Summary Early stressful events can increase vulnerability for psychopathology, although knowledge on the effectors is still limited. Here we tested the hypothesis that peripheral levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which are involved in the response to stress and in the pathophysiology of anxiety and depression, might be affected in a non-human primate model of adverse rearing. Males and females rhesus macaques reared with their mothers (MR) or in peer-only groups (PR) were used as experimental subjects. BDNF, NGF, adrenocorticotropic hormone (ACTH), cortisol and growth hormone (GH) were determined at baseline on postnatal days (PND) 14, 30 and 60 by means of specific ELISA and RIA procedures. In addition, behavior was assessed on PND 7, 14, 21, 30 (Brazelton test) and 60 (home cage observation). Data indicate gender differences in basal levels of BDNF throughout development. Peer-rearing increased significantly BDNF levels only in females. In addition, while all peer-reared subjects showed high levels of stereotypies and self-directed behaviors, behavioral passivity was selectively increased in females. By contrast, NGF levels were increased in response to peer-rearing only in males, and correlated positively with other "classic" endocrine responses to stress, such as cortisol and GH. Our data identify BDNF and NGF as neuroendocrine markers underlying differential responses to maternal deprivation in males and females rhesus macaques. The selective changes in BDNF levels in females could help explain the greater vulnerability to mood disorders of this gender reported in humans.

1. Introduction

Early adverse experiences in humans are associated with an increased risk for developing psychiatric disorders such as

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anxiety and major depression, although little is known of the neurobiological mediators (Kaufman et al., 2000; McEwen, 2000; Heim and Nemeroff, 2001). Neurotrophins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which play a fundamental role in brain function and neuroprotection, and are affected by stress, are good candidates for transducing the effects of adverse events in changes in brain function (Smith et al., 1995; Thoenen, 1995; Duman et al., 1997). Neurotrophins are also produced by cells outside the nervous system, thus being in a position to integrate neural, immune and endocrine responses to stress (Aloe et al., 1986; Nisoli et al., 1996; Nockher and Renz, 2005). Changes in peripheral levels of neurotrophins, as well as selected gene variants, have been associated with mood disorders, also in interaction with early trauma (Aloe et al., 1994; Hadjiconstantinou et al., 2001; Karege et al., 2002b, 2005; Kaufman et al., 2006; Castren et al., 2007; Kauer-Sant'Anna et al., 2007). NGF is increased in anxiety-loaded situations, such as in male soldiers experiencing their first parachute jump (Aloe et al., 1994), in spouses caring for Alzheimer's patients (Hadjiconstantinou et al., 2001) or following smoking cessation (Lang et al., 2002). By contrast, decreased blood levels of BDNF characterize subjects diagnosed as major depressives, antidepressants reverting this change (Shimizu et al., 2003; Karege et al., 2005). Although a dimorphism in peripheral BDNF levels has been reported in humans, women showing higher levels of this neurotrophin than men (Lommatzsch et al., 2005), information on sex differences related to stress susceptibility are still lacking (Becker et al., 2007).

In preclinical studies modeling early adversity, maternal separation stress has been shown to affect NGF and BDNF levels in limbic areas as well as to produce long-lasting changes on emotional behavior and impaired responses to stress, suggesting that these neurotrophins might modulate mechanisms underlying social bonding (Plotsky and Meaney, 1993; Cirulli et al., 2000, 2003, 2007; Meaney, 2001; Roceri et al., 2004). In non-human primate models, maternal deprivation with some form of social contact, such as access to peers, leads to important emotional and social disturbances and behavioral abnormalities, such as motor stereotypies (Suomi, 1991; Champoux et al., 2002; Barr et al., 2003). Peer-reared macaques develop inadequate social skills, are highly reactive and aggressive and, as adults, show increased voluntary alcohol consumption and rank at the bottom of the dominance hierarchy (Suomi, 1991; Barr et al., 2003).

The hypothesis tested in the present study is that early maternal deprivation would affect peripheral levels of neurobiological mediators of adult affective disorders, which may be related to early life stress. Preclinical studies performed in rodents have shown that neurotrophins are sensitive to manipulations of the mother—infant relationship and, more in general, of the rearing environment (Cirulli et al., 1998, 2000; Roceri et al., 2004; Branchi et al., 2006b). In particular, NGF and BDNF levels are increased in an age- and time-dependent fashion following maternal separation in young rodents (Cirulli et al., 1998, 2000; Roceri et al., 2004; Nair et al., 2007). These changes may program lasting alterations in the circuits regulating the expression of these neurotrophins, leading to the establishment of individual variability in vulnerability to stress-related psychopathology. To this purpose, BDNF and NGF were assessed in the peripheral circulation in rhesus macaques, and related to more common neuroendocrine effectors, such as cortisol and growth hormone (GH). Since there is a strong need for preclinical studies to model sex differences involved in individual susceptibility to psychiatric disorders, aim of this work was to verify differential responses to early adversity in the two genders, particularly in females (Thoenen, 1995; Becker et al., 2007; McEwen, 2007).

2. Methods

2.1. Animals and rearing procedures

Subjects of these studies were 16 males and 17 females rhesus monkey infants (*Macaca mulatta*) born between 2003 and 2004 in the NICHD breeding facility of the Animal Centre in Poolesville (MD, USA). Some subjects were "mother-reared" (MR; 10 males and 9 females), raised in social groups either by their biological mothers or by an unrelated, multiparous foster mother. Some infants were reared without adults, but with constant access to age-mate peers in a "peer-only reared" (PR; 6 males and 8 females) condition. Rearing conditions were randomly assigned at the time of birth balancing the number of males and females in each rearing condition.

An additional group of 12 MR males born in 1999 were included in this study to assess longitudinal changes in basal neurotrophins levels in both plasma and cerebrospinal fluid (1 month, 1 year and 7 years of age, see below).

MR infants remained with their mothers in a stable social group of 8—10 adults and peers. Infants assigned to the PR condition were separated from their mothers following birth, and were subsequently hand-reared in a neonatal nursery. For the first 14 days, PR subjects were kept in an incubator and hand-fed. Each cage contained a blanket and a terry-cloth-covered rocking "surrogate" covered by a heating pad. The infant could see and hear, but did not experience any physical contact with other infants. From day 15 until day 38, infants were moved with their surrogate in individual nursery cages. At 38 days of age all nursery-reared infants were placed into their final condition. PR infants were placed in permanent social groups of 3—4 age-mates, similarly reared peers, with whom they had continuous contact.

Protocols for the use of experimental animals were approved by the Institutional Animal Care and Use Committee of the NICHD. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Behavioral scoring

2.2.1. Neonatal assessment

A 30-min battery was administered on days 7, 14, 21 and 30 of life. This test, derived from the Brazelton Neonatal Assessment scale was administered between 1100 and 1300 h (Champoux et al., 1994). Temperament characteristics were rated after administration of other items, based on the infant's behavior throughout the test period. These measures included the tester's impressions of the animal's overall state