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Infant cortisol and behavioral habituation to weekly maternal separations: Links with maternal prenatal cortisol and psychosocial stress



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Summary

Introduction: Our aim was to examine infants' behavioral and physiological stress responses to three weekly maternal separations, in relation to maternal prenatal psychosocial stress and cortisol. The hypothesis was that more prenatal stress and higher cortisol concentrations would predict smaller decreases in negative behavior and cortisol responses over the separations (i.e. less habituation).

Methods: General and pregnancy-related feelings of stress and anxiety, as well as circadian cortisol levels, were measured in 107 mothers in the third trimester of pregnancy. At 9 months of age, infants were subjected to three weekly 1-h maternal separations in their homes. Salivary cortisol was obtained from the infants prior to the separation and at 35, 75, and 90 min after the mother had left. For each separation, the area under the curve to the ground (AUC_g) was calculated to measure the infants' cortisol response, and the sum of the time spent crying and fussing was calculated to measure the infants' behavioral response.

Results: Maternal pregnancy cortisol awakening response (CAR) significantly predicted infants' cortisol and behavioral responses. A lower CAR was related to a decreasing cortisol response, while a higher CAR was related to a stable cortisol response over all separations, as well as to less crying and fussing over all separations.

Conclusions: Increased maternal prenatal stress, as measured by the CAR, is related to altered behavioral and cortisol responses to a repeated stressor in the 9-month-old infant. These responses might result in prolonged periods with high cortisol levels that may affect the child's development.

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1. Introduction

Research on both animals and humans is providing more and more evidence that maternal prenatal factors can have long-term effects on offspring development. In humans, maternal prenatal stress and cortisol have been related to physical, cognitive, behavioral and temperamental postnatal child outcomes. This process is often referred to as fetal programming (Huizink et al., 2004a; Seckl and Meaney, 2004; Räikkönen et al., 2011), because the effects are often profound, long-term, and also seem to be transmitted from one generation to the next (de Weerth and Buitelaar, 2005b; Matthews and Phillips, 2012).

One mechanism through which maternal prenatal stress and cortisol might be related to later development is by altering the infants' behavioral and physiological stress systems. As a response to a new and stressful situation, infants often react with behavioral signs (e.g. crying and fussing) and physiological signs of distress (e.g. cortisol elevations, Jansen et al., 2010). The hormone cortisol is the end product of the hypothalamic-pituitary-adrenal axis (HPA-axis). Although the HPA-axis is essential for coping with stress, abnormal activity of the HPA-axis is a vulnerability factor for psychopathology later in life (Lupien et al., 2009; Guerry and Hastings, 2011; McCrory et al., 2012). Previous research already showed that prenatal stress and cortisol are related to altered infant cortisol responses upon a stressor (O'Connor et al., 2013; Tollenaar et al., 2011; Velders et al., 2012).

In animal models, exposure to a repeated stressor of the same type (homotypic stressor), such as restraint and maternal separation, is related to decreases in cortisol response (Barnum et al., 2007). This decrement in response that occurs with repeated exposure is often referred to as habituation. By contrast, repeated exposure to more severe homotypic stressors generally does not lead to habituation of the cortisol response, but to an increase, or sensitization, of the cortisol response (Barnum et al., 2007). In accordance with the animal literature, human newborn infants showed habituation of the cortisol response to a repeated discharge exam, while their cortisol response to a repeated heelstick tended to increase (Gunnar et al., 1989, 1991). Moreover, newborns that experienced more obstetric complications failed to show habituation of the cortisol response to the discharge exam, but responded equally to the repeated heelstick, compared to newborns with fewer obstetric complications (Gunnar et al., 1991).

Apparently, environmental conditions early in life can shape infants' cortisol habituation to repeated stress. In this line, environmental conditions during pregnancy, including maternal stress and cortisol concentrations, may also be related to difficulties habituating to stress. Prenatal maternal stress and cortisol may program the offspring's HPA-axis to continue to react to the same type of stressor and/or to habituate less quickly to a stressor, resulting in prolonged and continued physiological stress responses. In turn, these physiological responses may be intricately related to infant behavioral and temperamental difficulties, further shaping behavioral, neuroendocrine and immunological development in the long term (Guerry and Hastings, 2011; McCrory et al., 2012).

There are some indications from the animal literature that maternal prenatal stress is linked to offspring habituation to repeated stressors. In one study in prepubertal and adult rats, corticosterone habituation to a repeated stressor was slower in prenatally stressed rats, as compared to non-stressed controls (Fride et al., 1986). Another study in rats showed that prenatally stressed adult males, in contrast to control males, did not display corticosterone habituation to a repeated physical stressor (Bhatnagar et al., 2005). In female adult rats, the effects were less clear with some habituation in both the prenatally stressed and non-stressed groups. These results suggest that prenatal stress has long-lasting effects on animals' ability to respond to stress in adulthood. In a study of behavioral habituation to repeated tactile stimuli in young adult rhesus monkeys, individuals without prenatal maternal stress habituated across trials with different stimuli, whereas prenatally stressed individuals showed slight behavioral sensitization across trials (i.e. increased withdrawal; Schneider et al., 2008).

To our knowledge, there is only one human study that investigated the effects of maternal prenatal stress and cortisol on offspring cortisol habituation to a repeated stressor. In a small study, our group found that higher maternal pregnancy cortisol and pregnancy-related anxiety predicted higher cortisol on the first day of school after the summer vacation, and on a day a week later in 5-year old children (Gutteling et al., 2005). However, there was no difference between children of higher and lower pregnancy stress with respect to the cortisol habituation to school. This may be explained by the small size of the study ($N = 29$) and by the fact that the group as a whole showed no cortisol habituation over the first week at school.

The aim of the present prospective study was to examine if maternal prenatal stress and cortisol are related to infants' behavioral and cortisol habituation to a repeated stressor in a relatively large, healthy, non-clinical group. We included maternal reports on general stress as well as on specific pregnancy-related anxieties and hassles, as these have been found to be important contributors to infant HPA-axis development in past studies (Gutteling et al., 2005; Tollenaar et al., 2011; Buss et al., 2011). Furthermore, we included maternal circadian cortisol concentrations as a measure of physiological stress levels (Beijers et al., 2010; de Weerth and Buitelaar, 2005a; Pruessner et al., 2003). Behavioral and cortisol stress responses in the infants were measured in reaction to three weekly maternal home separations at 9 months of age. Maternal separations are an ecologically relevant stressor for infants of this age, as many infants are left for shorter or longer periods in the care of babysitters and in center-based childcare. Prolonged maternal separations (i.e. 30 min or longer) produce moderate cortisol elevations in infants at around 9 months of age (reviewed by Jansen et al., 2010). Moreover, the infants were left with an unfamiliar female caregiver, an unobtrusive, unfamiliar assistant that videotaped the session, and unfamiliar toys to play with, to ensure the novelty of the situation and to distinguish these maternal separations from other maternal separations with which the infant might be familiar. In animal models, exposure to mild or moderate stressors, including exposure to a repeated

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