

Influence of prenatal stress and postnatal maternal behaviour on child temperament and coping with stress

Eugenia Baibazarova

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Thesis Summary

There is some evidence from both animal literature and human studies to suggest that maternal emotional well-being during pregnancy, as well as maternal behaviour during early childhood, can have important implications for the development of child temperament and children's distress reactivity and recovery (Albers, Riksen-Walraven, Sweep & de Weerth, 2008; Huizink, Mulder & Buitelaar, 2004; Leerkes, Blakson, and O'Brien, 2009). This thesis consists of two parts. The first part explored what measures of prenatal stress and anxiety are related to infant birth outcomes, temperament and cognition. The second part explored the role of maternal sensitivity and intrusiveness in child behavioural and endocrinological coping with stress across the first three years of life, concurrently and longitudinally.

It was found that even relatively small fluctuations in prenatal stress and pregnancy-related anxiety in a normal sample of healthy women can still have an impact on infant birth outcomes and temperament in the first six months postnatally. In addition, maternal and amniotic fluid cortisol levels during early pregnancy were positively associated, suggesting that increases in maternal cortisol can influence cortisol concentrations in the amniotic fluid and the foetus.

In the studies on early postnatal maternal behaviour and child coping with stress, maternal sensitivity had different effects on child behavioural and cortisol reactivity in response to stress across early childhood. For example, maternal behaviour had a direct influence on child behavioural and cortisol reactivity to separation and novelty as well as recovery from distress; however these effects were only observed when children were two years old, and not when they were aged one or three years. Nevertheless, the results of the longitudinal study revealed that *early* maternal sensitivity can influence child behavioural

and cortisol distress reactivity when children are three years old. Children of more sensitive mothers were more reactive to separation and novelty. In addition, the longitudinal results revealed that maternal sensitivity and intrusiveness are not fixed and stable traits, but are behaviours that change over time and across different emotional states of the child during the course of early childhood.

Collectively, the findings of this thesis demonstrate that small variations in prenatal stress and postnatal maternal behaviour in a normal low-risk sample can influence child temperament and coping with separation and novelty in early childhood. These findings indicate that a multi-method approach to studying maternal prenatal stress is necessary in order to obtain a better understanding of what aspects of prenatal stress are more important for child outcomes. In addition, the findings on the instability of maternal behaviour during early childhood highlight the importance of multiple assessments of sensitivity and intrusiveness in order to better capture maternal caregiving behaviour across time and its influence on child coping with stress.

Chapter 1. Introduction

Child temperament

Child temperament is one of the most widely researched and important aspects of child development (e.g. Rothbart, 2007). Difficult temperaments, characterized by high negative reactivity and difficulty to sooth, have been linked to various emotional problems later in childhood, adolescence and even adulthood (Pauli-Pott, Mertesacker & Beckmann, 2004). Child reactivity to novelty and the ability to recover from emotionally arousing events are important aspects of child temperament. Opposite and extremely intense reactions to novel events have been linked to different types of emotional problems. Intense negative reactivity to novelty has been associated with the development of excessive behavioural inhibition, which is characterized by restraint and high fearfulness when faced with unfamiliar situations, events and people (Harshfeld-Becker et al., 2003). Extreme behavioural inhibition in toddlerhood has been linked to the development of internalizing problems in childhood and adolescence (Kagan, Snidman, Zentner & Peterson, 1999; Biederman et al., 1993). On the other hand, extreme low reactivity to novelty, fast approach and a lack of restraint have been linked with behavioural disinhibition (Harshfeld-Becker et al., 2003). Various studies have linked low behavioural and physiological reactivity to novelty to development of externalizing problems later in childhood (Colder, Mott & Bergman, 2002; Van Goozen, Fairchild, Snoek & Harold, 2007).

There is accumulating evidence, both in animal research and human studies, that the emotional state of the mother during pregnancy can have important and long-lasting effects on child temperament, with some studies providing evidence for foetal programming effects (e.g. O'Connor et al., 2005). 'Programming' or 'imprinting' refers to the associations between events taking place prenatally and altered foetal growth and

development, and later psychopathology (Seckl, 2004). It reflects an action of a factor in the prenatal environment during a sensitive developmental window to affect the development of specific tissues, producing effects that last through lifetime (Seckl, 2004). However, animal studies as well as some human research suggest that the negative effects of prenatal experiences can be overridden by a positive early postnatal environment (Wakshlak & Weinstock, 1990).

There is some literature demonstrating the importance of the early postnatal environment for the development of child emotional reactivity and regulation (e.g. Albers, Riksen-Walraven, Sweep & de Weerth, 2008; Hane & Fox, 2006). It has been suggested that the early postnatal environment, and the early mother-child relationship in particular, have important effects on child reactivity and recovery, making it an important aspect to study in addition to the influence of prenatal environments.

The structure of this thesis consists of two parts. The first part includes Chapters 2 and 3, and focuses on the prenatal influence of the mother on subsequent birth outcomes, and infant temperament and cognition in the first year of life. The second part, which consists of Chapters 6 and 7, investigates the postnatal influence of the mother on child emotional reactivity and regulation.

Prenatal stress and child development

Studies in rodents and non-human primates demonstrate the clear and long-lasting influence of stress during pregnancy on offspring emotional, social and cognitive development (Estanislau & Morato, 2006; Seckl, 2004; Ward, 1984; Weinstock, 2005). This research has shown that the prenatal maternal environment can influence the development of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous

systems of the foetus, which can lead to altered postnatal stress reactivity and recovery in the offspring (Weistock, 2005).

Similar patterns of results emerge from human studies. There is accumulating evidence to suggest that the emotional state of the mother-to-be during pregnancy can influence the child's emotional and cognitive development (Van den Bergh, 1990; Huizink, Mulder & Buitelaar, 2004; Austin, Hadzi-Pavlovic, Leader, Saint & Parker, 2005; O'Connor, Bel-Shlomo, Heron, Golding, Adams & Glover, 2005). For example, Gutteling et al. (2003) found that pregnancy anxiety was associated with more attention problems in children at 27 months. O'Connor et al. (2003) found that children of more anxious mothers had more emotional and behavioural problems in the 6th year of life and Gutteling et al. (2005) found that perceived stress during pregnancy was related to problem behaviour in toddlerhood. Maternal prenatal depression has also been identified as a risk factor for subsequent child development. Hay, Pawlby, Waters, Perra and Sharp (2010) found prenatal depression to predict violence and antisocial behaviour in adolescence. Thus, an increasing body of research shows that the emotional state of the mother-to-be can influence subsequent child development, providing evidence for foetal programming.

Although the HPA system is proposed to be an important mediator of the influence of prenatal stress on foetal development, the mechanisms through which prenatal maternal stress influences child development are not fully understood (Talge, Neeal, Glover et al., 2007). It has been proposed that heightened levels of cortisol, the end product of the HPA system activity, in maternal plasma, may affect cortisol levels in the amniotic fluid, which is indeed what was found in a small number of studies (Gitau, Cameron, Fisk & Glover, 1998; Glover, Bergman, Sarkar & O'Connor, 2009). However, these studies are few in numbers, and more research is needed to gain a better understanding of the mechanisms underlying the effects of maternal HPA-axis activity on the foetal cortisol concentrations.

The study in Chapter 3 was set up with two important goals. The first goal was to assess the extent to which maternal and foetal cortisol concentrations are associated. Secondly, we were interested to investigate whether and how maternal self-reports of stress and anxiety, maternal cortisol levels and cortisol levels in the amniotic fluid were related to birth outcomes, infant temperament and cognitive development. Chapter 2 consists of a review of the past findings on the role of prenatal stress on child emotional and cognitive development, while Chapter 3 presents the findings of a study investigating the influence of maternal self-reports of stress and anxiety as well as more objective assessments of stress on birth outcomes, temperament and cognition.

Maternal behaviour and child development

A more extensive literature has examined the effects of the early postnatal environment on child temperament and the ability to cope with stress. Research has focused particularly on early maternal interactive behaviour, the formation of the mother-child relationship and the role of attachment in child distress reactivity and recovery. Early maternal interactive behaviours, such as her sensitivity and intrusiveness, have been found to be associated with child negative emotionality and stress reactivity (Pauli-Pott et al., 2004; Hane & Fox, 2006), including infant HPA-axis functioning (Blair, Granger, Willoughby, Kivlighan et al., 2006; Albers et al., 2008; Blair, Granger, Kivlighan, Mills-Koonce, Willoughby, Greenberg et al., 2008). Taken together, these findings demonstrate the importance of the early postnatal environment, specifically the role of the mother, for the child's emotional reactivity and regulation.

Maternal interactive behaviour, however, is complex, and there is existing debate on when and how it should be best assessed. A relatively large amount of research has focused on maternal sensitivity and intrusiveness, concepts derived from the original scales

developed by Mary Ainsworth (1969; 1971). However, currently there are suggestions that these maternal behaviours may not be stable across different situations and emotional states of the child (McElwain & Booth-LaForce, 2006). For example, Leerkes, Blankson and O'Brien (2009) proposed that maternal sensitivity in response to infant distress has particular importance for child emotional reactivity and regulation. In addition, it is not yet clear whether maternal behaviour is stable across time as well as across different emotional states of the child. Although the majority of research assumes relative stability of maternal behaviour there is some evidence to suggest that maternal sensitivity is neither stable across child emotional states nor across time (Kempimenn et al., 2004). The issue of maternal stability was examined in Chapter 6 and Chapter 7. In addition, we were interested in investigating the influence of maternal behaviour on child emotional reactivity and recovery concurrently as well as across time in order to gain better understanding of the influence of maternal interactive styles on child outcomes.

The overarching aim of the present thesis is to separately investigate the prenatal and postnatal influence of the mother on child temperament and coping with stress. The study in Chapter 3 was set up to improve our understanding of the influence of prenatal stress on child outcomes. The studies on postnatal influence of maternal sensitivity and intrusiveness (Chapters 6 and 7) aimed to not only explore the role of the mother in child coping with stress, but also to investigate the stability of maternal behaviour across different emotional states of the child as well as across time.

Thesis outline

As mentioned earlier, the thesis consists of two parts. The first part consists of Chapters 2 and 3, and focuses on the role of prenatal maternal experiences, prenatal stress in particular, on infant birth outcomes, temperament and cognition. Chapter 2 reviews the

literature on prenatal stress and child outcomes. Chapter 3 reports on a study investigating the role of maternal self-reported stress, maternal plasma and foetal amniotic cortisol, and their joint effects on child temperament. One specific goal of this study was to test whether foetal cortisol concentration mediated the link between prenatal maternal stress, birth weight, and infant temperament and cognition in the first year of life.

The second part of the thesis covers Chapters 4 to 7, and focuses on the influence of the postnatal environment, specifically maternal sensitive and intrusive behaviour, on child emotional reactivity and recovery across the first three years of life. Chapter 4 consists of a literature review of the influence of maternal behaviour on child coping with stress. Chapter 5 reviews the methodology of investigating maternal behaviour and child temperament and stress reactivity. The influence of the mother at child age one, age two and age three years was examined cross-sectionally in Chapter 6, whereas the longitudinal development of both maternal and child characteristics across the first three years of life was studied in Chapter 7.

Chapter 2. Effects of prenatal stress and anxiety on child development:

A literature review

An increasing amount of research suggests that the prenatal environment can have profound effects on child outcomes. The importance of physical well-being of the pregnant woman (e.g., infectious illness, smoking, alcohol intake) has been relatively well-established to affect the developing foetus with implications for development after birth; however more recently researchers have reported that prenatal emotional well-being of the mother also plays an important role in the development of the child (Huizink, Mulder & Butelaar, 2004; Talge et al., 2007). Specifically, stress and anxiety during pregnancy became a main focus of research investigating the effects of emotional state of a pregnant woman on birth outcomes and subsequent child emotional and cognitive development.

It has been suggested that a foetus can be 'programmed' in prenatal life for different postnatal characteristics. The principle of foetal programming is that prenatal endocrine and metabolic environment provided by the mother has lasting or lifelong significance for the child (Huizink et al., 2004) . This chapter will examine the evidence for the effects of prenatal stress on the foetus and the persistence of these effects.

Animal research has clearly demonstrated foetal programming effects (Huizink et al., 2004). Studies with rats, rodents and non-human primates have demonstrated links between natural exposure to stress and subsequent offspring outcomes, such as reduced attention spans and neuromotor capabilities (Schneider, Moore, Kraemer, Roberts, & DeJesus, 2002), taking longer to initiate social play than control animals (Takahashi, Haglin, & Kalin, 1992), displaying higher fear and anxiety in novel situations (Weinstock, Matlina, Maor, Rosen, & McEwen, 1992). These findings provide initial evidence that prenatal stress can affect future child characteristics. It has been suggested that animal studies provide direct support for harmful effects of prenatal stress on offspring

development because it is easier to control the timing and the magnitude of the stressors as well as controlling for genetic factors and maternal pre- and postnatal environments (Weinstock, 2005).

In humans, many studies also claim to have found a link between prenatal stress and child outcomes, where higher levels of stress lead to more adverse temperament, cognitive and motor development of the child (Austin, Hanzi-Pavlovic, Leader, Saint & Parker, 2005; Huizink, de Medina, Mulder, Visser, & Buitelaar, 2003; O'Connor et al., 2003; Gutteling et al, 2005; Wadhwa, Sandman, Porto, Dunkelschetter, & Garite, 1993).

However, in contrast to animals, maternal stress in humans is a complex construct that cannot be measured or controlled easily (Huizink et al., 2000). The studies in this area vary greatly in their choice of different measures to assess prenatal stress. There is one line of research that focuses on the assessment of stress by using self-report questionnaires.

Another category of studies however makes use of more objective measures by looking at the levels of maternal stress hormones in the blood. This review will consider and evaluate both types of studies with suggestions of possible missing mechanisms in both types of research with implications for future studies.

Self-Reports of stress and anxiety

A number of studies has focused on investigating prenatal stress through maternal self-reports of experienced stress during pregnancy. Only the studies that have assessed prospective measurements of stress can be considered worthy of mentioning, since retrospective recall of stress over a period of time is prone to many biases and therefore its validity is under question (Wadhwa, 2005).

There are several studies that assessed self-reports of prenatal stress during pregnancy and have demonstrated a link between prenatal stress and child outcomes

(O'Connor et al., 2003; Austin et al., 2005; Sjostrom, Valentin, Thelin, & Marsal, 2002; Hedegaard, Henriksen, Sabroe, & Secher, 1993). The studies however, have used different measures of stress and anxiety, varying from assessing occurrence of stressful events to feelings of general anxiety. At present, there seems to be no consensus on what types of self-report measurements are associated with child outcomes. For example, Austin et al. (2005) conducted a study with 970 subjects, where women in the 3rd trimester reported their trait anxiety, perceived stress relating to life events, and antenatal depression. Child temperament was assessed by maternal and paternal reports between 4 and 6 months of age. It was found that trait anxiety and not perceived life events predicted maternal ratings of difficult child temperament. In a large Danish study, where 8,719 women participated, Hedegaard et al. (1993) found significant associations between self-reported general distress at 30 weeks gestation and increased risk for premature delivery (considered to be less than 37 weeks gestation), which then puts the child at numerous health risks. O'Connor et al. (2003) conducted a longitudinal study where they found that self-reports of general anxiety in late pregnancy (around 32 weeks gestation) affected behavioural and emotional problems of children in middle childhood (up to 81 months age). The fact that the effects have been found both at 47 and 81 months have been interpreted by the authors as support for a persistent effect of prenatal stress found in the animal literature. These studies suggest that both general feelings of stress and anxiety during pregnancy may be associated with child outcomes and development.

On the other hand, Huizink (2000) argued that it is more important to consider specific pregnancy-related anxiety rather than general anxiety, as only looking only at general anxiety during pregnancy may actually be an underestimation of anxiety that is specifically related to pregnancy, which in turn may be more closely related to child outcomes. Huizink et al. (2003) found that pregnancy anxiety, in particular the aspect of

fear of giving birth in mid-pregnancy emerged as the strongest predictor of child motor and mental outcomes at 3 and 8 months. They contrasted their findings with those of Van den Bergh (1990) who found measures of general anxiety to be unrelated to infant mental and motor development. Furthermore, Huizink, Mulder, Robles de Medina, Visser and Buitelaar (2004) developed a 3-factor model of pregnancy anxiety through confirmatory factor analysis, reflecting 'fear of giving birth', 'fear of bearing a handicapped child' and 'concern about own appearance' as the 3 factors. They found that general anxiety and depression explain only a small part of variance of these factors, providing support for the view that pregnancy anxiety is a distinctive syndrome (Huizink et al., 2004). The researchers therefore strongly suggest that pregnancy anxiety reflects a unique dimension of human pregnancy and should be considered in future studies investigating the effects of prenatal stress on child development.

Even though self-reports of prenatal stress have been widely used, it is clear that prenatal stress is a subjective concept, and there is still no consensus about what aspects of prenatal stress or anxiety should be measured. Furthermore, it can be suggested that psychological stress influences physiological functioning of the mother, which can then cause physiological alterations in the prenatal environment that might have an effect on the foetus and child. Simply focusing on questionnaire measures alone would miss this important link in investigating the effects of prenatal stress on child outcome.

Measuring Maternal HPA axis activity

As it has been mentioned earlier, some studies have focused on more objective measures of stress, specifically investigating the activity of maternal hypothalamic-pituitary-adrenal (HPA) axis as a primary mechanism that underlies the effects of prenatal stress on the foetus. The HPA axis is one of the body's major stress systems, and it has

been hypothesized that high levels of maternal stress are associated with alterations of normal activity of maternal HPA axis (Davis & Sandman, 2006). Its activity is regulated by the release of corticotropin-releasing hormone (CRH) from the hypothalamus. CRH then in turn stimulates the anterior pituitary to release adrenocorticotropin hormone (ACTH). The ACTH then stimulates glucocorticoid (GCs) production and release from the adrenal cortex. In humans, one of the released glucocorticoids is cortisol, which is considered to be an end-product of the HPA axis.

Therefore, increased activity of maternal HPA axis with experienced stress is associated with an increase in cortisol levels, which is suggested to put the foetus at a greater risk for a variety of developmental problems, starting with premature delivery (Moawad et al., 2002; Wadhwa et al., 2004), which is in turn associated with long-term developmental and motor impairments (Knoches & Doyle, 1993). Therefore, the activity of maternal HPA axis has been put forward as a possible mediator of the effects of maternal stress on foetus development, birth outcome and subsequent child development (Wadhwa, 2005). It is important to remember however, that even though this review will focus mainly on HPA axis activity, there are other possible mechanisms, such as activity of sympathetic-adrenal system (Tagle et al., 2007) and as it has been pointed out by Rice, Jones and Thapar (2006) it is important to assess the genetic effects of a mother on the foetus in order to understand to what extent the influence of prenatal stress is due to genetic inheritance as opposed to the provided prenatal environment. However, Rice, Harold, Boivin, van den Bree, Hay and Thapar (2010) attempted to disentangle the hereditary influence of prenatal anxiety from the environmental influence. They looked at prenatal stress levels in women pregnant with own child and those pregnant with a genetically unrelated child (either through egg or embryo donation through *in vitro* fertilization). If stress during pregnancy in women who were genetically unrelated to their offspring was found to influence infant

outcomes, this would provide support for the environmental influence of prenatal stress on child development. This is what was found: the association between prenatal stress and child outcomes such as gestation age, anxiety and antisocial behaviour was established in both genetically related and unrelated mother-child pairs. This study provides evidence for the idea that maternal stress does not influence the child only through genetic factors, but also through other factors, one of which could be the HPA axis activity.

It has been possible to observe how pure prenatal exposure to GCs would affect child outcome to investigate whether exposure to these elements can have adverse effects on future child development. This was carried out by looking at children whose mothers have been prescribed with prenatal course of GCs. A course of antenatal GCs is at times proscribed to stimulate foetus lung maturation (Crowley, 2000). Unlike maternal natural cortisol, synthetic GC's are not oxidized by placental 11 *B*-hydroxysteroid dehydrogenase (11 *B*-HSD) into inactive cortisone and can cross the placenta easily (King, Smith, & Nicholson, 2001).

A study by French, Hagan, Evans, Godfrey, and Newnham (1999) looked at a total sample of 477 pregnancies where women were administered a number of courses of GCs because they were at risk for a pre-term delivery. It was found that a greater number of GC's courses in pregnancy (greater or equal to 3) were associated with significant reductions in birth weight and head circumference, irrespective of any pregnancy complications. The study followed up 327 children out of initial sample when they were 3 years old, but found no differences in growth and development in the children who were exposed to different numbers of GCs courses, suggesting the influence of GCs is short-lived and could diminish over time.

However, more long-lasting influence of prenatal GCs was found in a pilot study by Trautman, Meyerbahlburg, Postelnek and New (1995). They looked at a sample of children

who were exposed to prenatal GCs treatment (DEX-treatment) during early stages of pregnancy because they were at risk for adrenal hyperplasia, but none went on to develop the disorder. They compared this sample of 26 treated pregnancies with 14 pregnancies where the risk was untreated. The children were followed up from six months postnatally up to the age of five years and six months. It was found that even though the groups did not differ in their cognitive abilities, differences in children's temperaments were found. The GCs-treated group was found to display more shyness, less sociability, greater emotionality and greater avoidance than the non-treated group.

However, it would be mistaken to make concrete conclusions based on these studies. First of all, there is contrasting evidence about the effects of GCs exposure on cognition. Some studies suggest that multiple courses in GCs produced an elevated risk for neurodevelopmental delays in childhood (Spinillo et al., 2004) while other studies have found no such effects (Dessens, Smolders-de Haas & Koppe, 2000). Similarly, Trautman et al. (1995) failed to find any differences in cognitive problems between the treated sample and non-treated one. Second, most of these studies are based on some form of clinical population, i.e. children already at risk for some developmental or genetic problem, and that is why they have to be administered the GCs treatment prenatally. Therefore, this may produce problems generalizing to non-clinical population. Nevertheless, these studies are important in attempting to demonstrate a direct effect of prenatal stress hormones on subsequent child development.

It is now possible to look at studies that apart from or instead of including self-reports of prenatal stress have considered investigating the activity of maternal HPA axis by measuring levels of stress hormones in maternal blood plasma. It has been hypothesized that such objective measures of stress should be closely linked to self-reports of stress. For example, Wadhwa, Dunkel-Schetter, ChiczDeMet, Porto and Sandman (1996) looked at a

sample of 54 women and measured their experienced stress in pregnancy before 28 weeks of gestation. They included psychosocial measures (life events stress, perceived stress and pregnancy-related anxiety) and physiological measures (cortisol and ACTH) to investigate how these are related. Overall the study found that psychosocial measures were significantly related to the physiological ones, and that ACTH turned out to be the most sensitive indicator of maternal psychological stress.

Similarly, Sarkar, Bergman, Fisk and Glover (2006) investigated a sample of 254 women awaiting amniosentesis during their pregnancy between 15 to 37 weeks gestation. The women completed questionnaires about their state and trait anxiety and provided blood samples immediately before this stressful procedure. It was found that state (and not trait) anxiety was positively correlated with plasma cortisol independent of gestation and the time of collection. These findings further suggest that it would be important to include these more objective measures of prenatal stress in the investigations of its effects on birth outcomes and infant development.

Indeed, it is an approach that a number of studies have adopted. For example, a study by Diego et al. (2006) has looked at the effects of maternal stress hormones on the developing foetus in 98 women in mid-gestation (16-29 weeks). They found that maternal cortisol and norepinephrine levels were negatively related to foetal biometry measures and estimated foetal weight. Furthermore, after controlling for different psychological distress measures (such as daily hassles, state and trait anxiety) and biochemistry measures they found that levels of prenatal cortisol were the only significant predictors of foetal weight. A study by De Weerth, Van Hees and Buitelaar (2003) examined the effects of late pregnancy cortisol level (weeks 37-38 of gestation) on child behaviour and temperament in the first 5 months of life. It was found that women with high cortisol levels were at higher risk for earlier delivery than women in low cortisol group. Infants of mothers in the high-cortisol

group were also found to display more crying and fussing in observations when the mother gave them a bath. The mother reports of their infant's temperament have also shown that these children had higher emotionality and activity scores. Similarly, Huizink et al. (2000) found that higher levels of ACTH in a sample of 170 women were related to temperamental problems at 8 months.

Despite some evidence supporting the relation between maternal stress hormones and infant outcomes, studies often failed to find such a relationship. For example, Gutteling et al. (2005) found no relationship between prenatal cortisol levels and infant temperament or problem behaviour. Their sample included 103 women, whose cortisol samples were collected at early (15-17 weeks), mid- (27-28 weeks) and late (37-38 weeks) pregnancy where the samples were collected throughout the day, and the mean values of the morning and day cortisol values were used. They explain the non-significant findings by suggesting that it could either be the case that cortisol levels does not reliably reflect perceived stress experienced by the mother, or that there are more sensitive measures of physiological experience of stress than cortisol (e.g. ACTH). Alternatively, maternal cortisol might only affect child's HPA activity rather than affecting temperament and behaviour. The explanations seem plausible, but not always applicable considering the studies that did find an association between maternal cortisol levels and child outcomes. Overall, however, it is possible to suggest that more evidence points to the existence of at least a weak to moderate link between maternal HPA axis activity and child outcomes.

Combining psychosocial and physiological measures

Given the findings discussed do we still consider it necessary to use questionnaire measures to assess stress if a more objective predictor is also available? The problem with using only endocrinological measures of stress is that it would fail to provide insight into

what kinds of stress are experienced by the mother, and which are most harmful for infant outcome. This information is clearly important for assessment of stress risk factors and future interventions in pregnant women who experience elevated stress. In fact, several researchers have become increasingly interested in what kinds of stress are most harmful in pregnancy for infant outcomes. For example, as was mentioned above, Huizink et al. (2000) pointed out the importance of pregnancy-related anxiety for child outcome. They showed that this anxiety is unique to human pregnancy and could therefore be an important risk factor for infant outcome. Similarly, others suggested that problems in partner relationship during pregnancy can also hold particular importance for infant outcomes. For example, Bergman, Sarkar, O'Connor, Modi and Glover (unpublished) found that on a questionnaire that assesses stressful life events, the items that measured partner relationship strain were the strongest predictors of mental development and fearfulness in a sample of 103 19-months old infants. It is unlikely that these items reflected the most stressful events since the other items included similarly stressful events such as serious illness, trouble with law, accidents and death in family.

One of the few studies that combined objective and subjective measures of stress during pregnancy was conducted by Huizink et al (2003). In their study of 170 women they took measures of pregnancy-specific anxiety, occurrence of daily hassles and cortisol levels at early (15-17 weeks), mid- (27-28 weeks) and late (37-38 weeks) pregnancy. They found that pregnancy-specific anxiety at mid-pregnancy predicted lower mental and motor development of the infant at 8 months. Higher amounts of daily hassles in early pregnancy were associated with decreased mental development at 8 months. In turn only late pregnancy cortisol measures were associated with mental and motor development at 3 and 8 months. In addition, it was found that cortisol measures in this study correlated with psychosocial measures of stress only in late pregnancy, when cortisol levels seem to rise.

This study provides insight into the type of stress and anxiety related to infant development, as well as into the influence of maternal HPA axis on infant outcomes. Future investigation of both physiological and psychosocial measures would also allow researchers to gain better understanding about the types of stress associated with elevations in cortisol levels and subsequent infant outcomes and development.

Moving to assessing the effects of prenatal stress on foetal HPA axis

From the discussion above it is possible to conclude that there appears to be some evidence to suggest that stress experienced by the mother during pregnancy can have adverse effects on the child development posnatally, supporting the programming hypothesis. However, it still remains relatively unclear as to *how* such effects can take place. Most studies seem to focus on demonstrating the link between prenatal stress and child outcomes but relatively few attempts to investigate the possible mediating factors that might be important in this link.

One of such factors has been suggested to be the influence of maternal HPA axis activity on foetal cortisol levels. It has been argued that in a stressed mother, elevated HPA axis activity will set higher foetal axis activity which then may lead to possible adverse developmental outcomes. For example, a study by O'Connor et al. (2005) found that maternal reports of general anxiety in mid- and late pregnancy were predictive of awakening cortisol concentrations of the child 10 years later, with increased mother's prenatal anxiety leading to higher child cortisol concentrations. Therefore, it would be possible to hypothesize that the effects of elevated levels of stress hormones in the mother could affect the stress hormones levels of the foetus, which in turn would affect its brain development and maturation.

However, some debate exists as to whether maternal stress hormones levels can have influence foetal stress hormones levels. Placental enzyme 11 β -HSD is considered to protect the foetus from maternal cortisol levels by converting it into inactive cortisone (Benediktsson & Seckl, 1998). On the other hand, the amount of such conversion is disputed, with Shwartz (1997) suggesting it is highly possible for maternal cortisol to contribute to the foetal cortisol concentrations. Gitau, Cameron, Fisk and Glover (1998) conducted a study to investigate whether maternal cortisol levels are associated with cortisol levels of the foetus. They measured plasma cortisol concentrations in maternal and foetal venous samples of 43 women between 13 to 35 weeks of gestation and found a linear relation between maternal and foetal cortisol concentrations ($r=0.62, p<0.001$). It was found that the major part (80-90 %) of cortisol concentrations in maternal plasma does seem to be metabolized by the placental enzymes, with the other 10-20% being transferred to the foetus. But since foetal concentrations are generally very low, even this small contribution by the mother can greatly affect foetal cortisol levels.

More recently, Sarkar, Bergman, Fisk and Glover (2007) looked at a larger sample of women who were undergoing amniocentesis (data on both maternal and foetal cortisol concentrations was available for 267 participants). In this study, gestational age ranged from 15 to 37 weeks, with median age of 17 weeks. A positive relationship was found between amniotic fluid cortisol and maternal plasma cortisol ($r=0.32, p<0.001$), which persisted after controlling for gestation, time of collection and maternal age. The researchers also found that the correlation between maternal and foetal cortisol levels was moderated by gestation. They found that at 15-16 weeks it was $r(107)=0.14$ (ns), then at 17 weeks it increased to $r(71)=0.28$ ($p<0.05$), at 18 weeks it was $r(31)=0.46$ ($p<0.001$) and at 19-20 weeks gestation $r(21)=0.56$ ($p<0.001$). These results therefore provide

support that the effects of maternal stress stronger influence the foetal cortisol concentration in the second half of second trimester.

A question therefore arises as to if cortisol concentrations in the amniotic fluid are associated with those of the mother, then perhaps amniotic fluid cortisol concentrations can be considered to be better predictors of future child outcomes when considering the effects of maternal stress. It can be hypothesized that stress experienced by the mother activates maternal HPA axis which in turn affects the concentrations of stress hormones in the amniotic fluid which could in turn influence foetal HPA axis development. It could therefore be hypothesized that prenatal stress affects child outcome via affecting foetal cortisol concentrations. Therefore considering this possible mediator could be important in predicting child outcomes and might as well be a more accurate predictor than both objective and subjective measures of stress of the mother. Indeed, a study by Glover et al. (2010) is among the only studies to investigate the influence of cortisol concentration in the amniotic fluid on child outcomes. It was found that higher levels of amniotic cortisol were associated with lower cognitive scores at 17 months. These findings call for more research to be carried out to investigate the influence of amniotic fluid cortisol on a wider range of outcomes such as birth outcomes and temperament development.

Conclusions and future study objectives

Overall it is possible to conclude that future studies should make use of both subjective and objective measures of maternal stress, and they should ideally include some measures of foetal cortisol levels as a possible mediator of the effects of maternal stress on a variety of developmental outcomes. So far this link has been relatively unexplored in this area of research and establishing a mediating model should provide more insight into the concept of prenatal programming and allow better understanding of the effects of prenatal

stress on child development and therefore allow construction of more effective interventions. This is addressed in the study on the influence of prenatal stress and anxiety on child temperament and cognition in Chapter 3.

Chapter 3 Effects of prenatal stress, and cortisol concentration in maternal plasma and amniotic fluid on infant birth outcome, temperament and cognitive development. ¹

Introduction

From ancient times, the emotional state of the woman during pregnancy has been believed to affect the child she is carrying (Ferreira, 1965). Contemporary empirical evidence supports this claim. For example, prenatal maternal stress can negatively influence future child development (Buitelaar, Huizink, Mulder, Robles de Medina, & Visser, 2003; Austin, Hadzi-Pavlovic, Leader, Saint & Parker, 2005; Huizink, Mulder & Buitelaar, 2004, O'Connor, Ben-Shlomo, Heron, Golding, Adams & Glover, 2005). The study described in this chapter examines links between indices of prenatal stress (maternal self-reports and physiological assessment of stress in the mother and the foetus) and infant birth outcomes and emotional and cognitive development.

Assessment of prenatal stress

Various non-human animal studies show clear adverse effects of stress in pregnancy on offspring behaviour, and mental and motor development (Weinstock, 1997). Studies with animals generally enable control of timing and severity of stress, as well as genetic and postnatal environments (Weinstock, 2005). Clearly, maternal stress in humans is a complex construct, which cannot be as easily measured or controlled (Huizink et al., 2000). Empirical studies investigating the effect of emotional well-being during pregnancy often use psychosocial assessments of feelings of stress and anxiety and/or physiological assessments of stress.

¹ As part of the thesis, the role of the candidate was to analyze and report on the glucocorticoid data in relation to already collected perinatal data

Psychosocial (or questionnaire) assessments of stress include measures that investigate different aspects of stress and anxiety. For example, some measures look at the prevalence of stressful life events or daily hassles during pregnancy (e.g. Buitelaar et al., 1999). However, it has been hypothesized that maternal perception of stress, rather than the occurrence of stressors, better represents stress experience and could therefore have a greater influence on infant outcomes. Cohen (1986) suggested that measures of perceived stress better represent a person's perception that demands exceed the ability to cope.

Some of the research in this area has also looked at feelings of anxiety experienced in pregnancy. Various studies focused on the potentially harmful effects of general anxiety on infant outcomes (O'Connor et al., 2005; Austin et al., 2005). The most widely used questionnaire to assess anxiety is the State-Trait Anxiety Inventory (STAI, Spielberger, Gorsuch & Lushene, 1970). However, it has been suggested that the use of general anxiety measures in pregnancy might fail to capture some worries and anxieties that are specifically associated with pregnancy and child birth, and these may have a unique influence on infant development (Huizink et al., 2004). In order to address this, Huizink et al. (2004) revised the Pregnancy Related Anxiety Questionnaire by Van den Berg (1990) into the Pregnancy Related Anxiety Questionnaire-revised. It consists of three scales: fear of giving birth, fear of bearing a handicapped child, and concerns about own appearance. Huizink et al. (2004) found that general anxiety and depression explained only a small portion of the variance in these fears and concluded that pregnancy anxiety is a distinctive symptom, which may have independent effect on child outcomes. Thus, there are different ways of assessing stress and anxiety during pregnancy with no consensus on a gold standard approach.

Various researchers have expressed concern about the problem of the lack of concrete definition what aspects of stress during pregnancy may be harmful future child

development, and proposed to incorporate more objective assessments of stress and anxiety, such as the physiological assessment of prenatal stress.

Maternal HPA axis activity during pregnancy

The hypothalamic-pituitary-adrenal (HPA) axis is proposed to be one of the major systems involved in stress response and its regulation. The HPA system is activated during stress and threat (Weinstock, 2005), and studies have looked at the concentrations of its end product, the hormone cortisol, as an endocrinological marker of stress and anxiety (Weinstock, 2008). It has been suggested that the activation of the HPA axis is one of the main biological mechanisms underlying the effects of prenatal stress (Talge et al., 2007). Various studies have included measures of maternal cortisol concentrations during pregnancy in addition to, or instead of, subjective assessments of stress and anxiety (e.g. Buitelaar et al., 2003).

However, the relationship between maternal reports of stress and anxiety and her cortisol concentrations is not straightforward. Generally, it is considered that elevations in maternal feelings of stress and anxiety should influence HPA axis activity and hence be positively related to elevated levels of cortisol. This is indeed what some studies have found. Wadhwa, Dunkel Schetter, Chicx De Met, Porto and Sandman (1996) found perceived stress during pregnancy to be marginally associated with cortisol concentrations in maternal blood plasma at 28 weeks gestation. However, Harville, Savitz, Dole, Herring and Thorp (2009) found no association between perceived stress and cortisol, nor between pregnancy-related anxiety and cortisol at 14-19 weeks and 24-29 weeks of gestation. Sarkar, Bergman, O'Connor and Glover (2008) found a modest association between feelings of state anxiety in a group of women undergoing amniocentesis cortisol levels ($r = .19$). They suggested that the association between state anxiety and cortisol level could be

influenced by gestational time. After having divided their participants into those undergoing amniocentesis before and after week 17 of gestation they found that the association between anxiety and cortisol was only significant in early pregnancy. It is possible that the maternal HPA axis becomes more attenuated as pregnancy progresses (Sarkar et al., 2008) and that psychosocial measures of prenatal stress are only related to cortisol in early pregnancy. Collectively, these studies seem to suggest that there is a need to combine both psychosocial and physiological measures of stress to obtain a more complete picture of the experience of stress in pregnancy.

However, it has been proposed that the influence of maternal prenatal anxiety and other prenatal factors that impact infant development are influenced by heritable maternal characteristics, this questioning the role of the HPA axis as a mechanism of effect (Rutter, Pickles, Murray & Eaves, 2001). In other words, the associations between prenatal stress and infant development may be explained by heritable factors that influence both maternal anxiety and offspring outcomes (Rice, Harold, Boivin, van den Bree, Hay & Thapar, 2010). The study by Rice et al. (2010) was among the first to attempt to disentangle the environmental and hereditary influence of prenatal anxiety in influencing infant outcomes. They looked at prenatal stress levels in women pregnant with own child and those pregnant with a genetically unrelated child (either through egg or embryo donation through *in vitro* fertilization). It was proposed that if stress during pregnancy in women who were genetically unrelated to their offspring was found to influence infant outcomes, this would provide support for the environmental influence of prenatal stress on child development. Indeed this link was found: the association between prenatal stress and child outcomes such as gestation age, anxiety and antisocial behaviour was established in both genetically related and unrelated mother-child pairs. These findings provide evidence for the idea that

maternal stress does not influence the child only through genetic factors, but through other factors, one of which could be the HPA axis activity.

Prenatal stress and foetal HPA axis

It has been proposed that with elevated stress, maternal cortisol levels increase and that maternal cortisol is able to pass through the placenta and can have an effect on the foetal cortisol concentration and HPA axis activity. These mechanisms are not yet fully understood in humans (Talge et al., 2007). It has also been suggested that activity of placental enzyme 11 β -hydroxysteroid-dehydrogenase protects the foetus from maternal cortisol (e.g. Benediktsson & Seckl, 1998) by converting it into inactive cortisone. In opposition to this claim, Gitau, Cameron, Fisk and Glover (1998) compared maternal and foetal cortisol concentrations in women undergoing clinically-indicated foetal testing, and found foetal concentrations to be linearly related to maternal cortisol concentrations ($r = .62$). They found that maternal cortisol accounts for about 40% of the variance of foetal concentrations in high stress conditions. Furthermore, Glover, Bergman, Sarkar and O'Connor (2009) looked at maternal and foetal cortisol in a sample of women undergoing amniocentesis. It was again found that maternal and foetal cortisol concentrations were significantly correlated ($r = .32$), and that in the sub-sample of more anxious women the correlation was even higher ($r = .59$). These studies support the claim that elevations in maternal cortisol can have an impact on concentrations of cortisol in the amniotic fluid. Therefore, it can be proposed that the effect of maternal stress and anxiety is mediated through elevations in maternal and foetal cortisol concentrations. This study therefore aimed to combine both psychosocial and cortisol assessment of maternal prenatal stress as well as foetal cortisol levels in order to better capture prenatal stress experience.

Prenatal stress and perinatal outcomes

Prenatal stress and anxiety were found to be associated with infant birth outcomes. For example, Lobel, Dunkel-Schetter and Scrimshaw (1992) found that higher anxiety and perceived stress scores in pregnancy predicted lower infant birth weight. Similarly, Wahdwa et al. (1993) found that stress caused by life events was related to lower birth weight. Lower birth weight is associated with developmental delays in infancy and childhood (e.g. Als et al., 2009), making it important to examine whether prenatal stress affects later development via lower birth weight.

Maternal pregnancy cortisol concentrations have been found to be related to infant birth weight. Valladares et al. (2009) found that stress-related increases in cortisol were associated with decreased birth weight. Similarly, Diego et al. (2006) found maternal cortisol in mid-gestation to be significantly related to lower birth weight. Findings from studies like these indicate that prenatal stress can adversely affect perinatal outcome.

Child Temperament

Before discussing current findings on the influence of prenatal stress on child temperament, a brief review of temperament dimensions and research will be included. An in-depth review of temperament measures can be found in Chapter 5 (page 77).

Child temperament is generally defined as constitutionally based individual differences in reactivity and self-regulation (Rothbart & Derryberry, 1981). According to this theory, *constitutional* refers to individual's enduring biological make-up which is influenced by heredity, maturation and experience. *Reactivity* refers to physiological and behavioural arousability of the individual, while *self-regulation* refers to the neural and behavioural processes working towards modulating reactivity (Rothbart, 1981). Various temperament dimensions can be systematically studied through observational and caregiver

report measures. Thomas and Chess (1977) made one of the first large-scale attempts to develop assess child temperament. They looked at 9 temperament dimensions of activity, rhythmicity, approach, adaptability, threshold, intensity, attention span, distractibility and persistence. One of the further temperaments assessments that followed was the Infant Behavioural Questionnaire (IBQ; Rothbart, 1981). It was developed on the basis of Rothbart and Derryberry's (1981) temperament theory and aimed to investigate differences in infant reactivity and regulation. The scale focused on 6 dimensions of temperament: activity level, soothability, fear, distress to limitations, smiling and laughter, and duration of orientation. However, the IBQ has been further revised to create IBQ-Revised (Garstein & Rothbart, 2003) in order to capture more specific rather than global temperament dimensions. The final version of the questionnaire included 14 subscales: activity level, distress to limitations, approach, fear, duration of orienting, smiling, vocal reactivity, sadness, perceptual sensitivity, high intensity pleasure, low intensity pleasure, cuddliness, soothability, and rate of recovery.

Individual differences in temperament are evident from very early infancy, and even in newborns. Temperament can be studied in terms of activity level, anger-frustration, distress and smiling and laughter (Rothbart, Deryberry & Hershey, 2000). Studying early development of temperament has been a focus of recent research, as it has been proposed that early focus could contribute to identification of individuals at risk for later emotional and behavioural problems (Crockenberg & Leerkes, 2006).

Prenatal stress and child temperament

Some prospective studies have investigated the effects of prenatal stress and anxiety on infant temperament and emotional development in infancy and childhood. There is some

evidence to suggest that stress during pregnancy may have long-lasting negative effects on child temperament.

Van den Bergh (1990) found that feelings of general anxiety in the third trimester of pregnancy were positively correlated with difficult infant temperament at 10 weeks and 7 months postnatally. O'Connor et al. (2003) looked at maternal self-reported general anxiety at 32 weeks gestation and child difficult temperament and behavioural problems at 81 months in a sample of 6, 493 mothers and infants and found that children of mothers who experienced higher levels of anxiety were reported to have more emotional and behavioural problems, after controlling for maternal postnatal anxiety and depression. Austin et al. (2005) looked at the effects of maternal trait anxiety and perceived stress at 32 weeks gestation on maternal reports of infant temperament at 4 and 6 months in 970 subjects. It was found that antenatal trait anxiety was associated with difficult infant temperament at 4 and 6 months, but contrary to researchers' expectation, perceived stress was not significantly correlated with infant temperament. Buitelaar et al. (2003) found that maternal pregnancy anxieties (fear of giving birth and fear of having a handicapped child) in mid-pregnancy were related to infant exploration, affectivity and goal-directedness at 3 and 8 months in a sample of 170 participants. Gutteling et al. (2003) also looked at pregnancy anxiety in the form of fear of bearing a handicapped child in a sample of 103 women. It was found that higher levels of pregnancy anxiety led to a greater chance of children being more restless and having more attention problems at 27 months. The evidence from these different studies suggests that pregnancy anxiety could be an important component of emotional state during pregnancy with a lasting influence on child temperament.

Perceived stress during pregnancy has also been found to be related to child temperament. For example, Gutteling et al. (2005) found that perceived stress during pregnancy was related to more mother-reported problem behaviour in 27-month old

children. Buitelaar et al. (2003) also found perceived stress in early pregnancy to predict mother-reported infant difficulty and inadaptability at 3 and 8 months. These studies highlight the significance of perceived stress during pregnancy for child temperament.

Maternal cortisol level, as a more objective measure of prenatal stress, has been included in some studies. De Weerth et al. (2003) looked at the influence of maternal cortisol levels on infant temperament in the first year of life. Mothers with higher awakening salivary cortisol levels at 37-38 weeks gestation had babies who showed more crying, fussing, and negative facial expressions in the neonatal period. The authors reported that although most of these effects had disappeared by 4-5 months, infants born to mothers with relatively higher cortisol levels still displayed more fussing during interaction.

Other studies, however, have not found a relationship between maternal cortisol levels and child development. For example, Gutteling et al. (2005) did not find an association between maternal cortisol in early, mid- and late pregnancy and infant temperament at 27 months. The influence of maternal prenatal cortisol levels on infant temperament is therefore not yet clear. No study, to this author's knowledge, has investigated the influence of foetal cortisol concentration on infant temperament. It could well be that foetal cortisol levels have a more direct relation to later temperament than maternal prenatal cortisol levels. One of the aims of this study was to address this issue specifically.

Prenatal stress and child cognitive development

Although non-human animal studies show an effect of prenatal maternal stress on infant cognitive outcome, there has been very little research with human infants. The few studies conducted in the area seem to provide some support for the hypothesis that higher prenatal stress and anxiety may have an adverse effect on infant cognition. Huizink et al.

(2003) investigated this with a sample of 170 pregnant women by collecting their self-reports of experienced daily hassles and pregnancy-related anxiety, as well as taking salivary cortisol measures in early, mid- and late pregnancy. Subsequent infant cognitive development was measured by the Bayley Scales of Infant Development (BSID; Bayley, 1969). It was found that maternal daily hassles in early pregnancy and fear of giving birth in mid- pregnancy were associated with lower mental and motor development at 8 months post-natally. Furthermore, higher cortisol levels in late pregnancy were related to infant motor development at 3 and 8 months, and mental development at 3 months.

There was one study that looked at the association between amniotic fluid cortisol levels as a marker of prenatal stress and subsequent cognitive development of infants at 17 months (Bergman et al., 2010). It was found that higher amniotic fluid cortisol concentration was associated with lower cognitive scores of children. This is the first study to investigate the association between foetal cortisol concentration and child outcomes, suggesting it to be an important mediator of prenatal stress experienced by the mother. The results suggest that maternal stress and anxiety in pregnancy can influence infant cognitive development in the first year of life. The results of this study seem to provide evidence for the importance of amniotic fluid cortisol levels for subsequent cognitive development of children. In this study we want to explore this further by investigating whether cortisol levels in the amniotic fluid may also be associated with child temperament development in addition to cognition.

Collectively, previous research suggests that prenatal stress and anxiety can have a negative influence on infant neonatal outcomes, temperament and cognition. However, as explained above, prenatal stress and anxiety are complex constructs which can be operationalised and measured in different ways. It seems that more research is needed to clarify the relationships between maternal psychosocial measures of stress and anxiety and

the physiological indicators of stress, as well as the relationship between maternal HPA axis activity and its influence on foetal HPA axis. Then it is necessary to investigate whether each one of these measures has an influence on child birth outcomes, temperament and cognition. This study aimed to address these issues by looking at experienced stress and prenatal anxiety in a sample of women undergoing amniocentesis early in pregnancy, and collecting maternal and foetal cortisol concentrations during this potentially stressful procedure.

It was hypothesized that:

- a) Subjective reports of prenatal stress and pregnancy-related anxiety would be positively associated with maternal and foetal cortisol;
- b) Maternal plasma cortisol and amniotic fluid (i.e., foetal) cortisol would be positively associated;
- c) Psychosocial and hormonal measures of prenatal stress would be related to infant birth outcome (i.e., birth weight)
- d) Psychosocial and hormonal measures of prenatal stress would be related to infant temperament and cognitive outcomes in the first year of life.

Method

Subjects and sampling

All participants in the study took part in a prospective longitudinal project into the effects of prenatal hormones on development in children. Participants were recruited from a consecutive series of referrals, between January 1999 and August 2000, to the Department of Obstetrics at the University Medical Centre in Utrecht (UMCU), the Netherlands, to undergo an amniocentesis because of prenatal diagnostic screening. All possible candidates were approached by written information, and in the end 185 women declared a willingness

to participate. Only data from continued healthy singleton pregnancies were used. Abnormalities of the thyroid gland, if well treated, were allowed (n = 3). Cases of twin pregnancies (n = 4), diabetes (n = 2), chronic use of steroid ointment (n = 3), and the intake of hormonal medication because of asthma (n = 6) were excluded. Two women who turned out to carry a female foetus with a sex chromosome deviation (triple-x) were excluded, as well as one woman who had general anaesthesia during her pregnancy. Four pregnancies were terminated because of an induced abortion and one because of a miscarriage. The final sample comprised 158 pregnant women (consisting of 74 male, and 74 female foetuses and 9 cases where gender information was missing). Each patient gave informed consent to the procedure and the UMCU Medical Ethical Committee approved of the study. The majority (96%) was referred because of their age (36 or older), others had an amniocentesis because of a deviate serum screening (0.7 %) or their medical history (3.3%). Age ranged from 28 to 45 years (with a mean age of 37.6 years). Amniotic fluid samples were provided by 153 participants and collected between weeks 15.3 and 18.2 of pregnancy. The length of gestation was determined by the last menstrual period or ultrasonic measurement of crown-rump length (CRL) (Daya, 1993). Maternal serum was collected immediately following the amniocentesis. Serum and amniotic fluids were stored at -30°C until assayed.

Predictors

Maternal Questionnaires

Maternal Perceived stress was assessed with a Dutch translation of the 14-item Perceived Stress Scale (Cohen & Williamson, 1987). The scale measures perceived stress over a 4 week period, covering the 2 weeks before the puncture until the day of the result on a 4-point scale, ranging from 'never' to 'always'. Cronbach's alpha was .92.

Pregnancy anxiety was assessed by means of the Pregnancy Related Anxieties Questionnaire-Revised (PRAQ-R; Huizink, 2000). This questionnaire was developed by confirmatory factor analysis from the PRAQ of Van den Bergh (Van den Bergh, 1990) and consisted of 10 items that fitted to a three-factor model: fear of giving birth (3 items; scores ranging from 3 to 15), fear of bearing a physically or mentally handicapped child (4 items; scores ranging from 4 to 20), and concern about one's own appearance (3 items; scores ranging from 3 to 15). Examples of items are: 'I am worried about the pain of contractions and the pain during delivery' (fear of giving birth) and 'I am afraid the baby will be mentally handicapped or will suffer from brain damage' (fear of bearing a physically or mentally handicapped child). The items were answered on a 5-point scale, ranging from 'never' to 'very often'. Cronbach's alpha's of the subscales were all >.76 throughout pregnancy. It has been shown that pregnancy anxiety rather than general anxiety was related to birth outcome and activation of the neuroendocrine axis in pregnancy (Killingsworth Rini, Dunkel-Schetter, Sandman & Wadhwa, 1999; Wadhwa et al., 1993). For the purposes of present study, only the 'fear of giving birth' scale was used due to past findings of its particular relevance to infant outcomes (Huizink et al., 2003).

Hormone assays

Cortisol in amniotic fluid was determined in a radioimmunoassay after heat denaturing of the bindingprotein CBG. A polyclonal cortisolantibody was used and [1,2-³H(N)]-Hydrocortisone (NEN - DUPONT, Dreiech, Germany) as a tracer. The lower limit of detection was 0.5 nmol/l and interassay variation was on average 6%. Maternal cortisol samples were collected immediately following the amniocentesis. Cortisol in serum was measured using a competitive technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection

was 0.01 $\mu\text{mol/L}$ and within run precision was 4% at 0.55 $\mu\text{mol/L}$. All samples were analyzed in one batch.

Cortisol Binding Globulin was measured using the CBG RIA kit (BioSource Europe S.A., Nivelles, Belgium). The lower limit of detection was 10 mg/L for serum and 1,6 mg/L for amnionfluid. Interassay variation was on average 4%.

Outcome variable

Perinatal outcome. Infant birth weight (grams) was used as a perinatal outcome.

Infant temperament. Temperament was assessed at 3, 6 and 9 months when mothers filled in the Dutch translation of the short version of Infant Behaviour Questionnaire-Revised (IBQ-R, Rothbart & Garstein, 1998). Three scales were used in present study: activity level, distress to limitations and fear. Infant activity level refers to infant's movement of arms and legs, squirming and locomotor activity. Distress to limitations refers to baby's fussing and crying in confining positions, during caretaking activities and when unable to perform a desired action. Fear looks at baby's startle or distress to changes in stimulation, novel physical objects and social stimuli, and inhibited approach to novelty. Mothers reported on infants' behaviour in the two week period prior to assessment.

Bayley Scales of Infant Development. Children were seen at the age of 13 months. A home visit took place and the Bayley Scales of Infant Development II (BSID II) (Bayley, 1969) were administered to assess motor and mental development.

The examinations were performed by a psychologist who was blind to the data on stress during pregnancy. The BSID has been translated and validated in a Dutch population of infants (Van der Meulen & Smrkovsky, 1983) and results in a Mental Developmental Index (MDI) and a Psychomotor Developmental Index (PDI).

Missing Data

Before starting data analysis, variables were examined for missing values. The following variables had missing values: maternal cortisol levels (14%), amnion cortisol levels (6.35%), infant birth weight (11%), Maternal concerns about giving birth (7%), maternal perceived stress (12.7%), infant activity level at 3 months (11.4%), at 6 months (10.1%), and at 9 months (9.5%); infant distress to limitation at 3 months (11.4%) at 6 months (10.1%) and 9 months (9.5%); infant fear at 3 months (11.4%), at 6 months (10.1%) and at 9 months (9.5%). The pattern of missingness of the data was tested and found to be Missing Completely at Random (MCAR; Acock, 2005) with Little's MCAR test, which was not significant $\chi^2(887) = 910.67, p = .28$, indicating that the data were MCAR. This shows that there was no systematic missing pattern in the dataset and that missing values were randomly distributed across all observations. Missing data were imputed using the SPSS software built-in procedure Replace Missing Value (PMV) with linear trend at point estimation method. This imputation method uses the regression slope between variables to replace the missing values with the linear trend for that point. The missing values were replaced with their predicted values.

Statistical Analysis

Normality of distribution of the variables was checked using a histogram with a normal curve. The moderately positively skewed variable of maternal cortisol underwent natural log (ln) transformation, which is recommended for this type of skewness (Tabachnick & Fidel, 2007), after which normal distribution was attained for this variable. The non-transformed variable was used in the descriptive statistics table and the frequency distribution, but the transformed variable was used in all further analyses.

In order to investigate individual contribution of each prenatal factor in predicting infant birth weight, temperament and cognitive and motor scores, linear multiple regressions were carried out for each outcome measure. When predicting birth weight, gestational age of the infant was controlled for. When predicting infant temperament at 3 months, we controlled for birth weight, as it can have an impact on early infant development. When predicting temperament at 6 months, the 3 months scores on the specific temperament parameter were controlled for, and when predicting temperament at 9 months, the 6 months scores were controlled for in the regression analysis.

Results

The characteristics of the sample are presented in Table 1. Correlations between the variables in the study are shown in Table 2.

Relationship between maternal plasma and amniotic fluid cortisol and psychosocial assessment of stress

There was a positive correlation between maternal plasma and amniotic fluid cortisol concentrations ($r = .18, p < .05$). The two stress questionnaire measures, concerns about giving birth and perceived stress in pregnancy, were also positively correlated ($r = .27, p < .01$). As can be seen in Table 2, both questionnaire measures were not correlated with either maternal or foetal cortisol levels.

Table 1 Descriptive statistics for the variables during prenatal and postnatal assessment of mothers and infants (N=158).

Variables	Mean	SD	Range
<i>Prenatal predictors</i>			
Maternal cortisol (microMol/l)	.46	.13	.25–1.06
Amniotic cortisol (nmol/l)	42.11	6.62	44–167
Gestation (weeks)	39.84	1.63	30.71–42.73
Time of puncture (weeks)	16.41	.46	15.30–18.20
Concerns birth	5.52	2.32	3.0–13.0
Perceived stress	6.04	4.93	15.0–40.0
<i>Birth Outcomes</i>			
Birth Weight	3484.36	519.17	1435.0– 4772.0
<i>IBQ 3 months</i>			
Activity	2.49	.61	1.13–5.00
Distress to limitation	3.11	.96	1.36–6.33
Fear	1.97	.80	1.00– 5.10
<i>IBQ 6 months</i>			
Activity	3.22	.82	1.29–5.65
Distress to limitation	2.71	.77	1.15–5.15
Fear	2.41	.77	1.00–5.08
<i>IBQ 9 months</i>			
Activity	3.52	.88	1.31–5.94
Distress to limitation	3.07	.81	1.50–5.40
Fear	2.66	.78	1.0–4.50

Table 2: Correlation matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>Prenatal Measures</i>																	
1 Maternal cortisol	--																
2 Amniotic fluid cortisol	.18*	--															
3 Mother concerns birth	.13	-.04	--														
4 Total Stress	.01	.08	.27**	--													
<i>Birth Outcomes</i>																	
5 Gestation weeks	-.07	-.17*	.07	.07	--												
6 Birth Weight	-.11	-.21**	-.10	.03	.41**	--											
<i>IBQ measures 3 months</i>																	
7 Activity	-.02	.19*	.16*	.33**	-.01	-.09	--										
8 Distress to limitation	.01	.06	.09	.18*	-.06	-.18*	.37**	--									
9 Distress to novelty	.02	-.01	-.03	.09	-.13	-.16*	.33**	.33**	--								
<i>IBQ measures 6 months</i>																	
10 Activity	-.11	-.01	.05	.10	.03	-.02	.43**	.23**	.24**	--							
11 Distress to limitation	-.15	-.01	.14	.22**	-.08	-.07	.31**	.55**	.35**	.37**	--						
12 Distress to novelty	-.17*	-.09	.15	-.02	-.06	-.08	.19*	.12	.34**	.22**	.33**	--					
<i>IBQ measures 9 month</i>																	
13 Activity	-.02	.06	.07	.07	-.01	-.04	.44**	.26**	.19*	.65**	.30**	.15	--				
14 Distress to limitation	-.10	-.05	.09	.06	.03	-.05	.29**	.37**	.23**	.36**	.60**	.28**	.43**	--			
15 Distress to novelty	.02	-.05	.05	-.01	-.05	-.03	.13	.17*	.31**	.31*	.36**	.46**	.31**	.42**	--		
<i>Bayley scores 13 months</i>																	
16 Mental development	-.12	-.00	-.06	.08	.05	-.01	-.04	.10	.06	-.00	.21**	-.01	.10	.13	.02	--	
17 Motor development	-.12	-.07	.05	-.03	.06	-.04	.10	.03	-.05	.23**	.15	-.07	.21**	.22**	.08	.28**	--

Predictors of infant birth weight

Infant birth weight was negatively correlated with amniotic cortisol levels ($r = -.21, p < .01$). To examine the relative role of the prenatal variables in the prediction of birth weight, all four predictors (concerns about giving birth, perceived stress, maternal cortisol and amniotic cortisol) were entered into a linear multiple regression, whilst controlling for gestational age. The results are presented in Table 3. Both amniotic cortisol and concerns about giving birth approached significance as predictors of birth weight after controlling for gestational age, with higher concentrations of amniotic cortisol and more concerns about birth leading to lower birth weight.

Table 3: Summary of a multiple regression analysis for variables predicting infant birth weight (N=158)

Variable	B	SE B	β
Gestation time	125.05	23.39	.39**
Concerns birth	-32.23	16.94	-.14^
Total Stress	5.90	7.94	.06
Maternal cortisol	-217.22	377.14	-.04
Amnion cortisol	-11.04	5.86	-.14^

Note. $R^2 = .04$ * $p < .05$, ** $p < .01$, ^ $p = .06$

Stability of postnatal temperament in the first year

Before examining which prenatal factors affect infant temperament, continuity and change of each temperament characteristic were assessed. A repeated measures one-way ANOVA revealed that there was a significant main effect of time on infants' activity level ($F(2,314) = 145.71, p < .001$), and this effect was moderate ($\eta^2 = .48$). Post-hoc Bonferroni comparisons showed that all three means were significantly different from each other, with a significant increase in mean activity level from 3 months (Mean=2.49) to 6 months (Mean=3.22) to 9 months (Mean=3.52) (Figure 1).

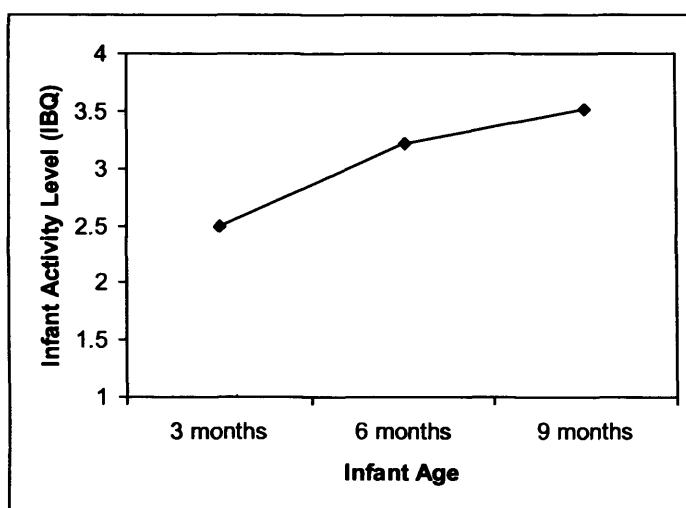


Figure 1: Infants' activity levels at 3, 6 and 9 months.

There was also a significant effect of time on distress to limitation, $F(1,157) = 21.19, p < .001$, ($\eta^2 = .12$). Post-hoc Bonferroni test comparisons showed that distress to limitation at 3 months was significantly higher than at 6 months, but not from distress at 9 months, and that distress to limitation at 6 months was significantly lower than that at 9 months. There was a U-shaped trajectory in the development of distress to limitation from 3 months (Mean=3.11) to 6 months (Mean=2.71) to 9 months (Mean=3.10).

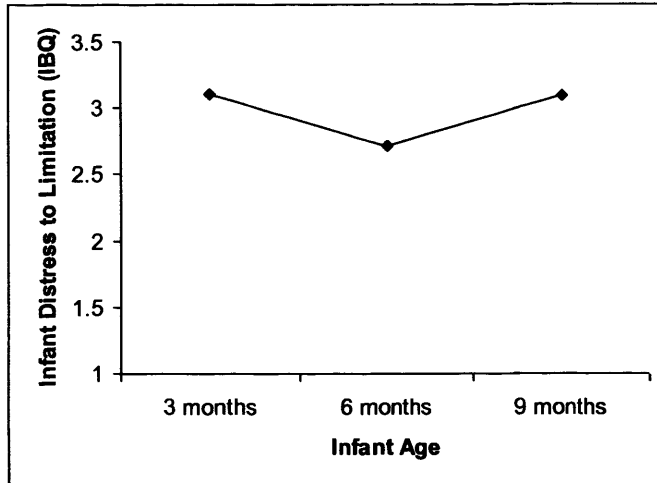


Figure 2: Infants' distress to limitation levels at 3, 6 and 9 months.

Finally there was a significant effect of time on fear, $F(2, 314) = 50.39, p < .001$, ($\eta^2 = .24$). Bonferroni test comparisons showed that fear at 3 months was significantly lower than at 6 months, and 9 months, and that fear at 6 months was significantly lower than fear at 9 months. There was an overall increase in the development of fear from 3 months (Mean=1.97) to 6 months (Mean=2.41) to 9 months (Mean=2.66).

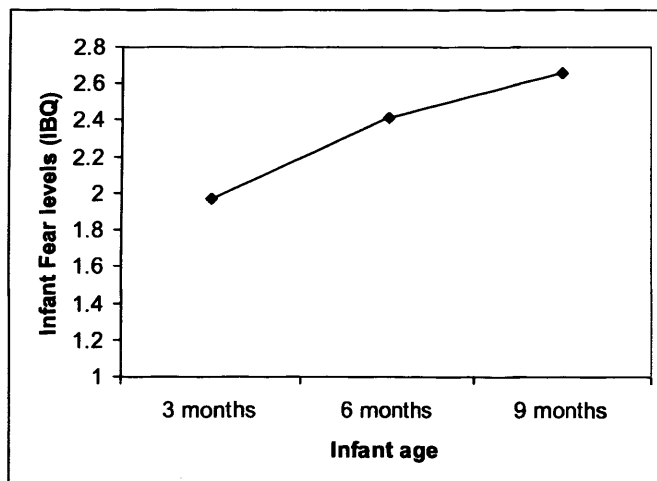


Figure 3: Infants' fear levels at 3, 6 and 9 months.

Which prenatal factors predict infant temperament?

Predicting infant activity levels at 3, 6 and 9 months

The results of a multiple linear regression with four predictors (concerns about birth, total perceived stress, maternal cortisol and amniotic cortisol) of IBQ activity score at 3 months, while controlling for birth weight, are shown in Table 4. Perceived stress by the mother and amniotic cortisol emerged as significant predictors of early activity level, with higher perceived stress and amniotic cortisol levels leading to more active infants at 3 months.

Table 4: Summary of a multiple regression analysis for variables predicting infant activity as scored on the IBQ at 3 months (N=158)

Predictor	B	SE B	β
Birth weight	-7.03E-05	.00	-.06
Concerns about birth	.02	.02	.09
Total stress	.04	.01	.29**
Maternal cortisol	-.41	.46	-.07
Amniotic cortisol	.02	.01	.17*

Note. $R^2 = .12$, $*p < .05$, $**p < .01$,

The results of a multiple regression with the same 4 prenatal predictors of infant activity levels at 6 months, while controlling for infant activity level at 3 months, are presented in Table 5. None of the prenatal variables predicted activity

level and it was concluded that the effect of perceived stress and amniotic cortisol at 3 months had ceased to exist by 6 months.

Table 5: Summary of a multiple regression analysis for variables predicting infant activity as scored on the IBQ at 6 months (N=158)

Predictor	B	SE B	β
Activity 3 months	.62	.11	.46**
Concerns about birth	.00	.03	-.01
Total stress	-.01	.01	-.04
Maternal cortisol	-.67	.61	-.08
Amniotic cortisol	-.01	.01	-.08

Note. $R^2 = .12$, $*p < .05$, $**p < .01$,

From the correlation matrix (Table 2) it can be seen that none of the prenatal predictors was significantly related to activity levels at 9 months, and this was confirmed by the multiple linear regression (with only activity level at 6 months being a significant predictor of infant activity at 9 months ($\beta = .65$, $p < .001$)).

Predicting infant distress to limitation at 3, 6 and 9 months

The results of a multiple regression with the four prenatal predictors of infant distress to limitation at 3 months, while controlling for infant birth weight, are presented in Table 6. Apart from birth weight, maternal perceived stress significantly contributed in predicting infant distress to limitation at 3 months ($\beta = .19$, $p < .01$), with higher stress leading to more infant distress at 3 months.

Table 6: Summary of multiple regression analysis for variables predicting infant distress to limitation as scored on the IBQ at 3 months (N=158)

Predictor	B	SE B	β
Birth Weight	.00	.00	-.18*
Concerns birth	.01	.03	.03
Total Stress	.04	.02	.19**
Maternal cortisol	-.18	.76	-.02
Amnion cortisol	.00	.01	.01

Note. $R^2 = .04$, * $p < .05$, ** $p < .01$

Infant distress to limitation at 6 months, while controlling for distress at 3 months, was predicted by maternal cortisol ($\beta = -.17$, $p < .05$) (Table 7). However, there was an inverse relationship between maternal cortisol and infant distress to limitation with higher cortisol leading to less infant distress.

Table 7: Summary of a multiple regression analysis for variables predicting infant distress to limitation as scored on the IBQ at 6 months (N=158)

Predictor	B	SE B	β
Distress to limit 3 m	.42	.05	.52**
Concerns birth	.03	.02	.09
Total Stress	.02	.01	.10
Maternal cortisol	-1.25	.51	-.17*
Amnion cortisol	-.00	.01	-.02

Note. $R^2 = .33$, * $p < .05$, ** $p < .01$,

Finally, as shown in the correlation matrix, none of the prenatal variables was associated with distress to limitation at 9 months. A multiple regression analysis showed that the only significant predictor of distress at 9 months was distress at 6 months ($\beta=.61, p<.001$).

Predicting fear at 3, 6 and 9 months

As shown in Table 8, birth weight was the only significant predictor of infant fear at 3 months ($\beta=-.18, p<.05$), with lower birth weight associated with higher fearfulness. Thus none of the prenatal variables was related to infant fearfulness at 3 months of age.

Table 8: Summary of regression analysis for the variable predicting infant fear as scored on the IBQ at 3 months (N=158)

Predictor	B	SE B	β
Birth Weight	.00	.00	-.18*
Concerns about birth	-.03	.03	-.09
Total stress	.02	.01	.13
Maternal cortisol	.10	.64	.03
Amniotic cortisol	-.01	.01	-.07

Note: $R^2=.01, *p<.05$

After controlling for fear at 3 months, maternal concerns about giving birth ($\beta=.21, p<.01$) and maternal cortisol ($\beta= -.20, p<.01$) emerged as significant predictors of infant fear at 6 months. Greater concern about giving birth led to more fearful children at 6 months, but there was an inverse relationship between maternal cortisol and fear with higher levels of maternal cortisol in pregnancy being related to less fear at 6 months.

Table 9: Summary of multiple regression analysis for variables predicting infant fear as scored on the IBQ at 6 months (N=158)

Predictor	B	SE B	β
Fear at 3 months	.35	.07	.36**
Concerns about birth	.07	.03	.21**
Total stress	-.02	.01	-.10
Maternal cortisol	-.15	.57	-.20**
Amniotic cortisol	-.00	.01	-.03

Note: $R^2=.17$, ** $p<.01$, * $p<.05$

As can be seen from the correlation matrix, none of the prenatal variables was associated with fear at 9 months. The only significant predictor of fear at 9 months was infant fear at 6 months ($\beta=.48, p<.001$).

What predicts infant motor and mental development?

Table 2 shows that there were no associations between the prenatal predictors and/or birth weight on the one hand and the child's mental or motor development scores at 13 months, on the other (see Table 2). Multiple regression revealed that none

of the perinatal variables predicted child mental or motor development at 13 months as measured by Bayley scale.

Discussion

The aim of this study was to examine relationships between psychosocial and physiological measures of stress and to assess their joint effects on infant birth weight, temperament, and cognitive and motor development. The study looked at different assessments of prenatal stress (perceived stress and pregnancy-related anxiety) around amniocentesis in early pregnancy and maternal cortisol levels during the procedure. Cortisol concentrations in the amniotic fluid were used as an indicator of foetal cortisol concentration. To this author's knowledge, this is the first study to investigate the influence of amniotic cortisol on infant birth weight, temperament and cognition.

Relationship between measures of prenatal stress

Contrary to prediction, maternal self-reports of perceived stress and pregnancy-related anxiety were not associated with either maternal or foetal cortisol levels, suggesting a discrepancy between self reports and more objective assessment of stress. Even though an association was predicted, various previous studies failed to establish a relation between self-reports of stress and anxiety, on the one hand, and cortisol levels, on the other (e.g. Harville et al., 2009). Perhaps one of the reasons for the current finding is that cortisol concentrations collected in this study reflect a specific stress response to the amniocentesis procedure, whereas reports of perceived stress and anxiety about giving birth assess more dynamic feelings of stress and anxiety. It might have been useful to obtain other cortisol samples during pregnancy that reflect more trait-like experience of stress, in addition to the state response to the stressful procedure. This would help clarify the association between psychosocial reports of stress and anxiety and their physiological indices. However, the discrepancy between self-reports of stress and anxiety and physiological assessment of stress is not

uncommon, highlights that self-reports of stress and anxiety should be combined with more objective assessments of stress in order to capture a more complete picture of stress experience.

As predicted, maternal cortisol was significantly correlated with amniotic fluid cortisol. This finding, in addition to work of Gitau et al. (1998), Sarkar, Bergman, Fisk and Glover (2006) and Glover et al. (2009) provides further evidence that maternal cortisol may be related to foetal cortisol concentrations, and hence the effects of prenatal stress could have an impact on foetal cortisol and HPA-axis activity. The correlation in present study was considerably lower ($r = .18$) than those found in previous studies. For example, Glover et al. (2009) found the correlation to be .32 in a sample of women of similar background undergoing amniocentesis. Gitau et al. (1998) found their correlation to be even higher at .62. One possible explanation for the lower association could be the fact that the samples were collected in early pregnancy in present study (weeks 15 to 18), while the age of collection in studies by Gitau et al. (1998) and Glover et al. (2009) ranges from 13 to 35 weeks gestation and 15 to 37 weeks respectively. Diaz, Brown and Seckl (1998) report that in the rat, the foetal brain is protected from glucocorticoids by 11 β -HSD, which is highly expressed in the brain around mid-pregnancy. This expression is dramatically reduced in the late period of pregnancy, which allows glucocorticoids to interact with their receptors and influence foetal brain development (Diaz et al., 1998). Furthermore, Huizink et al. (2003) collected maternal salivary cortisol in early, mid and late pregnancy, and found only late pregnancy cortisol to be related to infant mental and motor development. This suggests that in normal healthy samples the association between maternal and foetal cortisol may be stronger in late pregnancy, and that late pregnancy cortisol in particular might explain infant outcome. However, the finding that a positive

association has been established even in early pregnancy provides further support that foetal cortisol concentrations are influenced by maternal HPA-axis functioning and is not fully protected by 11 β -HSD as has been assumed by some past studies (e.g. Benediktsson & Seckl, 1998).

Prenatal stress and anxiety and infant birth weight, temperament and cognition

The second aim of this study was to investigate the influence of prenatal factors on child birth outcomes and subsequent cognition and temperament. Overall, the influence of maternal stress and anxiety was relatively small and were not found to exist by 9 months of age.

Before discussing these findings, it is worth noting that there were developmental changes in temperament of children between 3 and 9 months. The three infant temperament dimensions (i.e., activity, distress to limitation and fear) were not static in the first year of life, and similar patterns of development have been observed in previous studies (Rothbart, 1981; 1986). This underlines the importance of assessing infant temperament at multiple time points, because some prenatal predictors may influence temperament neonatally, while the influence of others might emerge later in infancy (see also, Buitelaar et al., 2003, for similar patterns of findings).

In terms of psychosocial predictors of infant outcomes, it was found that concerns about giving birth were marginally associated with infant birth weight, where higher worries about giving birth were associated with lower birth weight. In addition, higher concerns about giving birth were also associated with higher fearfulness of infants at 6 months only. It was anticipated that pregnancy-related anxiety will be associated with more aspects of child temperament and cognition than

presently found, as Huizink et al. (2004) identified it as a important unique contributor to child development, and research by for example Buitelaar et al. (2003) have found pregnancy-related anxiety to be associated with child mental and motor development in the first year of life.

Perceived stress during pregnancy was found to be associated with some early aspects of child temperament. Higher perceived stress was associated with higher activity levels of the child and higher distress to limitation at 3 months. These effects were not found at 6 months.

The third aim of this study was to consider the unique contribution of physiological assessment of stress through maternal plasma cortisol and the novel measure of cortisol concentration in the amniotic fluid for postnatal development of the child. In terms of maternal plasma cortisol, there were two significant associations with child temperament outcomes, both of which, however, were in an unexpected direction. It was found that higher maternal cortisol levels were associated with lower infant distress to limitation at 6 months as well as lower infant fearfulness at 6 months. These results do not correspond with the existing literature (De Weerth et al. 2003; Huizink et al., 2003) and are not easy to explain. There were no associations between maternal cortisol and child temperament at 3 or at 9 months, suggesting that the association emerged at about 6 months in the present sample.

We were further interested in the influence of amniotic cortisol concentration, as it was hypothesized that it could be an important unique predictor and mediator of maternal stress on child outcomes. It was found that higher amniotic fluid cortisol concentration was marginally associated with lower birth weight over and above gestation age. It was further found that it was associated with higher activity levels at 3 months. We expected amniotic cortisol concentrations to be one of the best

predictors of infant outcomes, as it is considered to reflect the foetal HPA-axis functioning. A possible reason for the lower influence could be the fact that the single measurement of foetal cortisol during amniocentesis does not adequately reflect the influence of ongoing stress of the mother on foetal HPA-axis. This problem can be applied also the single measure of maternal plasma cortisol taken during amniocentesis, which is a state measure of stress, rather than a more trait-like assessment of stress and anxiety, which is assessed in the questionnaires. It is advised that multiple assessments of maternal plasma or salivary cortisol at different time points in pregnancy might create a better picture of hormonal stress experience. Unfortunately, this is not easily applied to amniotic fluid cortisol collection, as it is carried out only during amniocentesis procedures.

Another possible reason for the lack of association between cortisol in the amniotic fluid and temperament in present study is the fact that temperament was assessed by maternal self-report. It would further be interesting to investigate whether amniotic cortisol levels are positively associated with child *cortisol* reactivity in infancy as a physiological assessment of temperament in addition to maternal reports.

Overall, it seems that in the present sample of women, the effects of prenatal stress are subtle, and even though some influence of perceived stress, pregnancy-related anxiety and maternal plasma cortisol levels is present in early infancy, all of these effects cease to exist by 9 months postnatally. In past studies, where associations have been found between prenatal stress and infant outcomes, these effects are also subtle and majority of the effects cease to exist by 12 months. These findings could be explained by the possible effects of postnatal environment, and the possibility that optimal postnatal environment (e.g. harmonious mother-infant relationship, high levels of social support of the mother) may overrule negative prenatal experience. In

the animal literature, it has been found that postnatal maternal care is an important predictor of infant outcomes, overriding effects of prenatal stress (Liu, Tannenbaum, Francis, Freedman et al., 1997; Caldji et al., 1998). With human infants, a study by Kaplan, Evans and Monk (2008) investigated the joint influence of maternal depression and anxiety in pregnancy and postnatal mother-infant relationship on infant outcomes. It was found that it was only maternal sensitivity postnatally that was predictive of child outcomes such as heart rate variability and infant responsivity to the mother. It was also found that there was an interaction between prenatal anxiety/depression and maternal sensitivity in predicting infant baseline cortisol levels, where infants of women without prenatal diagnosis had relatively low cortisol levels regardless of maternal sensitivity, whereas infants of mothers with a prenatal diagnosis had higher cortisol levels only if mothers were insensitive. In addition to this, Bergman et al. (2010) looked at the influence of amniotic fluid cortisol levels on child cognitive development at 17 months. Initially a negative association between two factors was established. However, it was found that this relationship was moderated by attachment style. In a group of non-securely attached children the association between foetal cortisol levels and child cognition was negative, whereas in securely attached children the association was nonexistent.

These results demonstrate the importance of early postnatal caregiving environment of infant development, and the interaction between prenatal anxiety and postnatal maternal behaviour and mother-child relationship. From these findings, it can be concluded that maternal behaviour can modulate the effects of prenatal factors on infant outcomes, suggesting that foetal programming can be modified by early postnatal environments. Overall, participants of present study are of a low-risk, middle class background sample, and the majority of them are likely to develop

optimal relationships with their infants. Therefore it could be that the possible influence of prenatal stress and anxiety were overridden by postnatal environments.

This brings the discussion to some of the limitations of present study and suggestions for future studies. It would be useful to obtain postnatal measures of maternal well-being and maternal caregiving behaviours to better understand the joint effects of prenatal and postnatal maternal factors on infant outcomes and development. The remaining chapters of this thesis describe investigations of the unique influence of postnatal maternal care on child emotional development. More generally however, future research should combine prenatal and postnatal factors that together can have an impact on child outcomes.

This study is among the first to combine measures of questionnaire assessment of stress and anxiety, maternal plasma cortisol levels and amniotic fluid cortisol levels to have a more complete measure of stress experience and to disentangle what measures are better linked to child outcomes. As discussed earlier, it would have been useful to include multiple assessments of maternal cortisol (in early, mid and late pregnancy), either in blood plasma or through saliva samples in order to assess more trait-like physiological experience of prenatal anxiety.

Overall, the study makes an important contribution by including an additional measure of amniotic fluid cortisol levels, which reflects cortisol levels of the foetus. This study further supports the findings by Gitau et al. (1998) and Glover et al. (2009) that maternal HPA-axis functioning influences cortisol concentrations in the amniotic fluid. Overall, the findings of this study indicate a relatively small variation in prenatal stress in a normal, low-risk sample of mothers is associated infant birth weight and temperament, but these effects cease to exist beyond 6 months postnatally. To conclude, it would be beneficial to combine both psychosocial and physiological

assessment of prenatal stress as each index of stress may have a unique contribution to child outcomes. Furthermore, it would be important to investigate the joint effects of prenatal and postnatal environments to better understand the process of foetal programming and whether and how it can be modified by postnatal experience.

Chapter 4. The role of the mother in child emotional development.

A literature review.

It is generally considered that children differ greatly in their moods and behaviour from early on in life. It has been of specific interest in developmental research to investigate these early differences in infants' underlying traits. Over the last few decades, child temperament has been one of the most researched aspects of individual differences in childhood, because of its relationship to children's emotionality, stress reactivity and regulation (Rothbart & Dewberry, 1981). It is generally agreed that variability in temperament can be observed in children's latency, intensity and frequency of emotional reactions as well as the strategies children use to deal with and manage those reactions (Rothbart & Dewberry, 1981).

Research into temperamental differences between children is important because more difficult temperaments are considered to be a risk factor for child behavioural problems later in life (Pauli-Pott, Mertesacker & Beckmann, 2004). A child is considered to have a 'difficult' temperament if he or she displays high negative emotionality and has persistent difficulties in adapting to new situations (Pauli-Pott et al., 2004). Various studies have reported that different aspects of difficult temperament, and negative emotionality and fearfulness in particular are related to the development of both internalizing and externalizing behaviour disorders.

Emotional reactivity to novelty is a particular temperament dimension, usually characterized by child's response to unfamiliar or potentially threatening situations. High or low levels of reactivity to novelty have been linked to various behavioural and emotional problems (Hirshfeld-Becker et al., 2003). This chapter will review existing literature on young children's emotional reactivity and recovery and will focus on the role of the mother in the development child coping with distress.

Fear reactivity and regulation in early childhood

As described above, child distress reactivity is an important aspect of child temperament. Extreme levels of distress reactivity and difficulty in recovery from distress can be related to different psychopathologies in children. High levels of distress to novel events can lead to behavioural inhibition (BI). Behaviourally inhibited children show restraint and fearfulness with unfamiliar people, situations and events (Harshfeld-Becker et al., 2003). This construct has different manifestations during different times in development. For example, BI manifests itself through displays of fear, distress and avoidance in toddlerhood, quiet restraint and decreased affect with unfamiliar figures in pre-school children, and reserved behaviour and reticence with unfamiliar peers in school children (Hirshfeld-Becker et al., 2003). Behavioural inhibition has been linked to various anxiety problems. Kagan, Snidman, Zentner and Peterson (1999) found that infants who were highly reactive to novel stimuli at 4 months were significantly more likely to be inhibited at 4 years and displayed more anxious symptoms at 7 years. In a study by Biederman et al. (1993), inhibited children showed higher rates of various anxiety disorders than non-inhibited children three years later. Moreover, Moehler et al. (2008) proposed that emotional reactivity and fearfulness in infancy may be a predictor of behavioural inhibition in the second year of life. They found that infants who were more reactive to novelty (i.e. cried more), were more likely to develop inhibited temperament later on in childhood. The implications of these findings are demonstrated by Biederman et al. (1993), who found that higher rates of anxiety disorders were seen in inhibited children rather than uninhibited children.

On the other hand, very low distress reactivity to novelty may also be a risk factor for future psychopathology. Children with behavioural disinhibition (BD) show

high approach, lack of restraint to novel and unfamiliar people and situations (Hirshfeld-Becker et al., 2003). Colder, Mott and Berman (2002) found that low levels of reactivity to novel stimuli along with high activity levels were related to increases in externalizing symptoms among boys.

Child reactivity and recovery from distress are proposed to be two distinct, biologically based aspects of temperament (Rothbart, 1989; Ramsey & Lewis, 2003). However, since reactivity affects the need for regulation and recovery, and regulation influences reactivity levels, it is proposed that the two parameters are intertwined, making it important to study both aspects of temperament (Gloggler & Pauli-Pott, 2008; Calkins & Johnson, 1998). Highly reactive children were reported to have a lower threshold of initial response to distress, a slower recovery and a higher level of arousal with repeated exposure to stress (Compas et al., 2001).

Behavioural expression of distress and recovery, however, are only one aspect of emotional reactivity. A growing number of researchers use more than one parameter in the study of child emotionality, for example, a combination of behavioural and physiological measures (Buss, Davidson, Kalin, & Goldsmith, 2004). Most of the studies in the area have focused on associations between behavioural expression of fear and responses of two physiological systems: cardiovascular (including measures of heart rate, heart rate variability, and respiratory sinus arrhythmia) and the neuroendocrine hypothalamic-pituitary-adrenocortical (HPA) system, which usually includes the measure of cortisol. For the purposes of this review, findings relating to the HPA system will be discussed.

Neuroendocrine and behavioural responses to fear-eliciting situations

It has been found that emotional stressors such as novelty and uncertainty that evoke behavioural distress also result in cortisol increases (Gunnar, 1989). Cortisol is the main glucocorticoid and the end product of the HPA axis, and is an important element in human survival. Gunnar (1992) reports that organisms deprived of cortisol can survive only in protected environments. Cortisol levels and general HPA-axis activity play an important role in distress regulation and resistance by increasing energy available for action in stressful conditions, modulating activity of other stress-sensitive systems and affecting emotions and learning (Gunnar, 1992).

Nachmias et al. (1996) suggest that in order for a potentially threatening event to activate the HPA axis, it must not only be perceived as threatening by the individual, but it must also be perceived that it can be potentially realized, which would then trigger the HPA axis. The activity of the HPA axis then starts with the release of corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) by the paraventricular nuclei of the hypothalamus (reviewed by Gunnar & Quevedo, 2007). The released CRH and AVP travel to the anterior pituitary via small blood vessels, and stimulate release of adrenocorticotrophic hormone (ACTH). The release of ACTH stimulates the release of glucocorticoids (GCs) (cortisol in humans) to general circulation in the cortex of the adrenal gland. Cortisol production and feedback to the hypothalamus and other brain structures inhibits further release of CRH and therefore acts as a negative feedback mechanism.

In human adults, cortisol is produced in frequent pulses under basal conditions just prior to awakening in the early morning hours (Gunnar, 1992). These pulses then become more infrequent throughout the day and decrease greatly by midnight. Newborns do not show the adult rhythm in cortisol production over the day, but

exhibit two peaks 12 hours apart, not correlated with the time of day (Sippell et al., 1978). At two or three months, however, infants start demonstrating the decrease from morning cortisol levels to the night ones (Gunnar & Donzella, 2002).

Cortisol reactivity is considered to be an important factor in child emotional development. Overly heightened reactivity of the HPA axis is considered to be damaging for physiological homeostasis. Hyperactivity of the HPA axis has been linked with psychopathology in adulthood, hyperactivity of the HPA axis in adulthood has been linked with depression (Pariante, 2003; Pariante, Thomas, Lovestone, Makoff & Kerwin, 2003). On the other hand, extremely low responsivity of the HPA axis to stress has been proposed to be one of the factors that may lead to future aggressive and antisocial behaviour in children (VanGoozen et al., 2007).

Gunnar, Larson, Hertsgaard, Harris, and Broderson (1992) found that elevations in cortisol correlated with distress levels in 9-months-olds during an episode of maternal separation. Furthermore, Nachmias et al. (1996) found cortisol levels to be positively correlated with withdrawal and amount of crying in novel situations. However, some studies failed to find this association between behavioural and cortisol reactivity (Buss et al., 2003; Kagan, Reznick, Snidman, et al, 1988). Ramsay and Lewis (2003) found no association between cortisol and behavioural reactivity to inoculation in 6-months-old infants.

Studies differ in their findings on the relationship between behavioural and cortisol reactivity to stressful events. The studies that found no association between child behavioural reactivity and cortisol levels suggest that low emotional expression of distress does not necessarily indicate an absence of physiological stress response. Because of this, research into distress reactivity should combine both behavioural and

physiological measures of distress in order to gain a more complete understanding of distress reactivity in early childhood.

Post-natal Experience and distress reactivity

Some research findings suggest that elevated levels of emotional reactivity and the ability to recover from stress are stable in childhood and lead to behavioural and anxiety problems later in life. However, there are various factors in children's postnatal environment that can influence and/or moderate initial reactivity levels. Kagan (1997) reports that only a very small percentage of children classified as highly reactive to novelty at 4 months displayed high reactivity to novelty at 4.5 years. Kagan (1997) therefore suggested that intervening family experiences may partly account for these observations. Colder et al. (2002) report that various studies of temperamental risk for future development find that parenting is an important moderator of initial temperamental characteristics in infants. In addition, Kaplan et al. (2008) have shown that early postnatal maternal sensitivity can override influence of prenatal stress and anxiety for future child emotionality and cortisol levels. Studies have shown that difficult child temperament in combination with low levels of parental control can predict high levels of problem behaviour (Bates, Pettit, Dodge, & Ridge, 1998).

Therefore, early relationship with the primary caregiver may be a factor that can buffer or exacerbate the effects of early temperament of the child. The infant's relationship with the mother or primary caregiver has been considered an important influence on infants' physical and emotional well-being for a long period of time (Bowlby, 1988).

Animal models show that individual differences in maternal caregiving behaviour have an impact on offspring stress reactivity through programming and epigenetic processes. It has been demonstrated that the amount of licking and grooming and arched-back nursing provided by the mother are associated with offspring behavioural and HPA-axis responses to stress (Weaver, Cervoni, Champagne et al., 2004). Initially, Levine (1994) found that early handling (separation from the mother for brief periods during the first three weeks of life) was associated with decreased adrenal gland weights after glucose injection. Some authors proposed that the effects of early handling are mediated by changes in mother-offspring interactions (Levine, 1995). Early handling has been found to stimulate maternal care behaviour and spontaneously increase maternal behaviour that is associated with decreased behavioural and endocrine responses to stress (Liu, Diorio, Tannenbaum, Caldju, Francis, Freedman et al., 1997). It has been proposed that handling alters maternal behaviour and these changes in mother-pup interaction mediate the effect of handling on endocrine and behavioural stress response (Levine, 1995). These effects show how early maternal behaviour can permanently alter development of HPA axis responses to stress through tissue-specific effects on gene expression in the offspring (Weaver et al., 2004). *In vitro* and *in vivo* studies have found that maternal caregiving behaviour increased the glucocorticoid receptor (GR) gene expression in the offspring through increasing serotonin (5-HT) activity and the subsequent expression of cAMP and cAMP-dependent protein kinase activity (Weaver et al., 2004; Weaver et al., 2001).

The mother-infant relationship in humans, however, is a complex concept, and there are various approaches to its investigation. The next section of the review will

discuss various ways of conceptualizing the quality of the mother-child relationship and its importance for child development.

Early mother-child relationship

There is general agreement in the developmental literature that the early mother-child relationship is an important aspect in children's development. Drake, Humenick, Amankwaa, Younger and Roux (2007) summarize that sensitive and responsive caregiving in the first year of the child's life is important for the neurophysiological, physical and psychological development of the child. Attachment has been put forward by Bowlby (1982) as one of the most important aspects of the mother-child relationship in early childhood. An individual's attachment style can be best observed when a person is frightened, ill or fatigued. The theory has developed from the objects relations theory developed by Melanie Klein in psychoanalysis, but has also utilized concepts from evolution theory, ethology, control theory and cognitive psychology (Bowlby, 1988). Attachment in childhood is described as an emotional long-lasting bond that the child forms with the attachment figure (Ainsworth, 1989). Securely attached children use their caregivers as a base from which to explore and a haven of safety when needed (Brumariu & Kerns, 2010).

The work of Mary Ainsworth has made it possible to empirically measure attachment quality using the well-known Strange Situation Task. During this procedure, mother and child go through a series of separation/reunion episodes and the child's encounter with a stranger. The technique allows categorization of child attachment style as secure, insecure-avoidant, insecure-ambivalent or disorganized. Securely attached children use the caregiver as an exploratory base when the caregiver is in their proximity, but become distressed upon separation from the

caregiver and try to search for them. When they are reunited, however, the child is happy to see their caregiver and they establish emotional stability by engaging in bodily contact with them (Spangler & Schieche, 1998). Children classified as insecure-avoidant show little distress during separation. Upon reunion, they do not seek contact with the caregiver, and generally ignore their presence and even avoid physical contact with them. Insecure-ambivalent children demonstrate high distress when the caregiver leaves, but display both seeking physical contact upon return and angry resistance at the same time. This group of children is not able to establish emotional stability for a long time (Spangler & Schieche, 1998). Finally, children with a disorganized pattern of attachment are characterized by a lack of a coherent pattern of behaviour towards their mother; their behaviour is often confused and contradictory (Spangler & Schieche, 1998).

It is suggested that secure attachment is a core milestone in providing a foundation for subsequent development (McElwain, & Booth-LaForce, 2006). The interplay of various factors may be important in the formation of attachment, including both maternal and child factors. However, the literature tends to point to the importance of maternal behaviour in the mother-child interactions for the development of a secure attachment (Ainsworth, Blehar, Waters, & Wall, 1978; Fish & Stifter, 1995). Ainsworth et al. (1978) proposed that maternal sensitivity and intrusiveness during child during interactions are important prerequisites of attachment security. Ainsworth et al. (1978) described a sensitive mother as being responsive to her child's needs and able to read her child's signals promptly and adequately, while also respecting the child's separateness, and not considering them to be a projection of herself and her own moods/desires. On the other hand, an insensitive mother is not able to correctly recognize the child's signals, and can either

be non-engaging or intrusive during interaction. An intrusive mother is said to act with little respect for the baby as a separate and autonomous individual, whose wishes and activities do not simply have to follow those of the mother.

Mother-child play interactions are suggested to be a good setting of observation of maternal behaviour. Fish and Stifter (1995) report that there is considerable evidence linking the quality of mother-child interaction in infancy to future attachment styles as measured in the Strange Situation. Ainsworth et al. (1978) found more sensitive, responsive and synchronomous interactions to be related to subsequent secure attachment style. Bakermans-Kranenburg, van IJzendoorn and Juffer (2003) found that interventions most effective at enhancing maternal sensitivity were also most effective in encouraging attachment security, suggesting that maternal sensitivity is an important determinant of attachment.

Some studies, however, failed to find a link between early maternal sensitivity and attachment security (Seifer, Schiller, Sameroff, Resnick & Riordan, 1996; Ward & Carlson, 1995), and have put forward suggestions that there are other important determinants of attachment security than maternal sensitivity, such as infant temperament (Kagan, 1992). Others have suggested that maternal sensitivity is a concept that is too general and that there is a need to have more specific measurements of maternal behaviour (e.g. Meins, Fernyhough, Fradley and Tuckey, 2001; Page, Wilhelm, Gamble & Card, 2010). Maternal mind-mindedness was proposed to be a more specific assessment of the mothers' understanding of infant's mental states. The measure included scales such as *maternal responsiveness to change in infant's direction of gaze* and *appropriate mind-mindedness comments*. However, when investigating the relationship between maternal sensitivity, mind-mindedness and attachment, it was found that only the *appropriate mind-mindedness comments*

scale was associated with attachment, while maternal sensitivity remained associated with attachment security.

It is suggested that the reason why some studies found maternal sensitivity to be associated with attachment security while others have not established this link is because this relationship is not a simple one and it is moderated by various factors. Vendelman, Bakermans-Kranenburg, Juffer and van IJzendoorn (2006) evaluated the effects of interventions with respect to maternal sensitivity on child attachment and examined whether child temperament moderated this relationship. It was found that when infants were classified as high or low irritable, increases in maternal sensitivity led to attachment security in highly irritable infants, but not in low irritable ones.

Furthermore, Fish and Stifter (1995) suggested that infant behaviour during mother-child interaction is an important additional determinant of attachment. The authors suggest that attachment is based on the relationship between mother *and* child, rather than maternal behaviour alone. In their study they split mothers and their 5- and then 10- month-old infants into three clusters based on maternal behaviour (sensitivity and intrusiveness) and infant behaviour (responsiveness to the mother). Cluster one consisted of sensitive and non-intrusive mothers with highly responsive infants. Cluster two included less sensitive non-intrusive mothers and less responsive infants. Finally, cluster three included dyads of insensitive and intrusive mothers and non-responsive infants. The authors rated cluster one as most optimal for child development. Cluster three, where the mothers were not only insensitive but also intrusive in the infants' activities, was rated as the least optimal for child development. These findings suggest that it would be important to consider both maternal sensitivity and intrusiveness in the study of attachment and subsequent child emotional development.

Finally, McElwain and Booth-LaForce (2006) claim that some specific aspects of maternal sensitivity are more important for attachment security and subsequent development and they suggested that sensitivity to distress rather than general sensitivity is one such aspect. They explained that sensitivity to distress is better related to attachment as Bowlby (1982) proposed attachment to be a biobehavioural safety-regulating system. Instances of infant distress activate and reflect attachment patterns more than non-distress episodes. In support of this, maternal sensitivity to distress but not non-distress during an interaction at 6 months emerged as a significant predictor of attachment security at 15 months. Further support for this hypothesis comes from a study by Gunnar, Brodersen, Nachmias, Buss and Rigatuso (1996) who found that maternal responsiveness to infant distress during baby check-ups and inoculations at 2 and 6 months led to attachment security at 18 months.

To summarize, maternal sensitivity is an important prerequisite in the development of attachment security, and is an important feature of a harmonious mother-child relationship. Many studies have utilized measures of attachment security or maternal sensitivity as predictors of different child developmental outcomes. This thesis investigates the role of maternal sensitivity and intrusiveness in child emotional development. However, for the purposes of this chapter, some attachment literature will also be reviewed as there are relatively few studies that specifically focused on maternal sensitivity and/or intrusiveness. The next section will review a range of literature looking at the influence of mother-infant relationship and maternal behaviour on child coping with stress.

The role of the mother in child emotional reactivity and regulation

Harmonious mother-child relationships have a positive effect on emotional development in children. The literature supports the idea that maternal sensitivity and having a secure attachment may act as buffers against negative emotional development. Pauli-Pott et al. (2004) report that the caregiver's role in modulating infant's levels of arousal is important in the development of negative emotionality and fear.

These researchers conducted a study to investigate the role of the mother in the development of emotional reactivity in children. In this study 101 mother-child dyads were tested at infants' 4, 8 and 12 months. On each testing occasion mother-infant interaction during bathing (at 4 months) and dressing (8 and 12 months) was observed and scored for maternal sensitivity. Child emotional reactivity was assessed at 8 and 12 months in the laboratory during a stranger approach. It was found that maternal sensitivity at 4 months was a significant predictor of withdrawal/fear at 12 months, after controlling for negative emotionality at 4-months. Higher levels of maternal sensitivity at 4 months were related to lower levels of fear at 12 months during stranger approach. In relation to general negative emotionality it was found that higher sensitivity with 8-months old infant was related to lower negative emotionality at 12 months, controlling for previous infant negative emotionality. This study supports the hypothesis that early temperament is moderated by sensitive parenting in infancy.

Hane and Fox (2006) also investigated the effect of maternal behaviour on infant fearfulness. Mothers were assessed for maternal sensitivity and intrusiveness during interaction at home when infants were 9 months old. Infant distress reactivity was assessed during a laboratory visit, where infants underwent the masks paradigm

of the Lab-TAB (Goldsmith & Rothbart, 1999). It was found that infants of mothers with low sensitivity expressed significantly more distress than those with high sensitivity. These findings suggest that maternal care during the first year of life is an important factor in infant distress reactivity. This study, however, did not control for previous child distress reactivity, and is therefore unable to infer the causality of the directions of effect between maternal care and child distress.

This problem was overcome in a longitudinal study by Kochanska (2001) on the influence of attachment style on the development of negative emotionality in the first three years of children's lives. The children were assessed for distress reactivity and other emotionality concepts (anger and joy) at 9, 14, 22 and 33 months by using the Lab-TAB and mother-child attachment security was assessed at 14 months. Attachment security was a significant predictor over and above existing distress reactivity and fearfulness at 33 months. There were also interesting developmental patterns of fear reactivity across the attachment groups. For example, avoidant children displayed the lowest distress at 14 months, but they had much higher distress at 33 months, significantly higher than the more secure children. Because of its longitudinal nature, Kochanska's (2001) study is an important contribution to the study of the effects of mother-child relationship on emotional reactivity in early childhood, as it was possible to control for past child emotional reactivity.

Some authors, however, do not believe that maternal sensitivity or attachment security can buffer the child from behavioural distress, but suggest that it may be protective of physiological distress reactivity. It has been proposed that a good mother-child relationship may buffer children from elevations in cortisol levels, despite the child showing high levels of behavioural distress (Gunnar & Donzella, 2007). Sensitive, responsive and attentive caregiving allows the child to express their

feelings and communicate these emotions to the caregiver so that they can provide help, without the child having an increase in glucocorticoids. Spangler and Schieche (1998) suggest that both securely and insecurely attached children would express behavioural distress in a stressful or a potentially threatening situation, but only secure attachment buffers children from elevated cortisol reactivity. They tested this hypothesis by investigating behavioural and cortisol reactivity during the Strange Situation in 12 month-old infants and found that securely attached children displayed high levels of behavioural distress upon separation with their mother, but not elevations in cortisol level. Insecure-ambivalent children, in contrast, displayed both high levels of behavioural distress and elevations in cortisol. Insecure-avoidant children showed neither behavioural distress nor heightened adrenocortical reactivity.

These findings are complicated by the fact that some studies did not find an effect of maternal care on child cortisol reactivity. Blair, Granger, Willoughby, Kivlighan and colleagues (2006) looked at the effect of maternal sensitivity on infant glucocorticoid reactivity in a series of tasks that were aimed to elicit negative affect (i.e. mask presentation, barrier, toy reach and arm restraint). The sample included 1,292 mothers and infants assessed at the age of 6 months. They found that infants of sensitive mothers had lower baseline cortisol levels than those of less sensitive mothers, but there were no differences between these groups in their cortisol response to stress. However, the groups did differ in cortisol regulation with children of sensitive mothers showing lower cortisol levels 40 minutes after the tasks than did infants of less sensitive mothers. These findings suggest that maternal sensitivity influences baseline cortisol concentrations and cortisol recovery from stress, but not necessarily cortisol response to stress.

Similarly, Albers et al. (2008) were interested in the effects of maternal sensitivity on 3-month-old infants' cortisol reactivity and recovery from a bathing session. They found that infants responded with a significant increase in cortisol to bathing, and that there were no differences between infants of low and high sensitive mothers. It was found, however, that infants of more sensitive mothers had better cortisol recovery from the stressor than did infants of less sensitive mothers, similar to Blair et al. (2006).

A further study by Blair, Granger, Kivlighan, Mills-Koonce, Willoughby, Greenberg et al. (2008) examined maternal and child contributors to cortisol response to emotional challenge and found different effects of maternal sensitivity/responsivity on cortisol reactivity in infancy (7-15 months) and toddlerhood during a 10-minute free play and emotional challenge. Maternal engagement during free play had an effect on child cortisol reactivity *and* regulation in infancy. Specifically, it was found that infants of high engaging mothers had lower baseline cortisol levels, higher cortisol reactivity, and faster regulation (faster return to baseline) than infants of low engagement mothers. However, maternal engagement at 15 months did not predict child reactivity. Interestingly it was found that toddlers of mothers who had been more engaged during play in infancy generally had lower cortisol levels (baseline, reactivity and recovery) than children of less engaged mothers. The results of this study suggest that maternal engagement may be especially important during infancy and has lasting effects on children's HPA axis functioning in toddlerhood. More research on the long-term effects of maternal sensitivity in infancy should be conducted in order to better elucidate this issue.

It can be concluded that mother-infant relationship and maternal behaviour could have a buffering effect on infant behavioural and physiological emotional

reactivity and recovery. However, the studies discussed are greatly varied in their methodology, for example in how maternal behaviour is measured and what emotion-eliciting paradigms are used, which can result in mixed findings.

It has been proposed that maternal behaviour is measured in a relatively global way in the majority of studies, i.e. behaviour such as sensitivity and intrusiveness are observed in lengthy interactions that incorporate different emotional states of the child. McElwin and Booth-LaForce (2006) proposed that maternal sensitivity to infant distress is a separate aspect of maternal interactive behaviour, one that could potentially be more important for attachment security and other aspects of infant emotional development than general sensitivity (McElwin & Booth-LaForce, 2006; Goldberg, Grusec & Jenkins, 1999). In support of this, Leerkes et al. (2009) found that maternal sensitivity to infant distress at 6 months predicted fewer behavioural problems and greater social competence at 24 and 36 months, while sensitivity to non-distress was not predictive. These findings provide some evidence that maternal sensitivity is not a global and stable concept, as it is generally assumed to be, but may vary with different emotional states of the child.

This brings us to the issue of timing of measurement of maternal behaviour. Not only has it generally been assumed that the behaviour of the mother is stable across different emotional states of the child, but also that it is a stable characteristic over time. The majority of studies discussed above include multiple assessments of child emotionality but, with very few exceptions, have only one measure of maternal behaviour. It is possible that maternal behaviour changes throughout the child's first years of life. The few studies that looked at stability of maternal interactive behaviour have found low to moderate stability at best (Kemppinen, Kumpulainen, Raita-Hasu, Moilanen & Ebeling, 2006). Maternal behaviour can be related to different factors

such as infant development and family situations. For example Feldman, Greenbaum, Mayes and Erlich (1997) found an overall decrease in maternal sensitivity and intrusiveness from 3 to 9 months, and found the changes in maternal behaviour to be linked with changes in infant difficulty and father involvement. These findings suggest that it is necessary to include multiple measures of maternal behaviour in order to understand its developmental patterns and how it is related to infant development.

Future research should therefore attempt to eliminate various shortcomings from previous research. Ideally, the studies should use a prospective, longitudinal design, because maternal engagement during infancy may be of special importance in later emotional reactivity (see Blair et al., 2008) and to enable a control for existing child emotionality. Furthermore, in order to better understand which aspects of emotional coping with stress are influenced by maternal sensitivity, measures of both behavioural and cortisol reactivity and recovery should be included. It should also be investigated which specific parameters of mother-child relationship are important, for example is it general sensitivity to the child or sensitivity to child distress which is a more crucial factor for distress reactivity. Studies in Chapters 6 and 7 aimed to address these shortcomings to gain better understanding of the role of the mother in child ability to cope with distress in early childhood.

Chapter 5: Methods of studying child temperament and mother-child relationship

Part I: Child Temperament and Fear

Temperament has been studied using three main methods: questionnaires, observation, and physiology assessment. This section will review the methods for the measurement of young children's temperament, and fear reactivity and recovery in particular, together with their advantages and disadvantages.

Questionnaires

Questionnaire assessments of children's temperament have been widely used in developmental research (Rothbart & Mauro, 1990). The main benefits of using questionnaires to assess child temperament are their ease of use and the relatively low cost of implementation. Parents, who are usually asked to fill in the questionnaires, rely on a broad and deep information base about their child's temperament in their reports (Rothbart & Mauro, 1990). Parent reports therefore provide information of child typical temperament characteristics at home (Rothbart, 1981).

One of the first large-scale attempts in assessing temperament via questionnaires was conducted by Thomas and Chess (1977) as part of the New York Longitudinal Study (NYLS) sample. It was a detailed investigation of 9 temperament dimensions (activity, rhythmicity, approach, adaptability, threshold, intensity, attention span, distractability, and persistence) in which parents were interviewed during the first two years of their children's lives.

Even though highly influential, the Thomas and Chess (1977) scales have been criticized for some shortcomings, such as the high variation in age during first

assessment (3 to 6 months), difficulty to determine homogeneity in the behavioural scales (Rothbart, 1981), and the lack of independence between scales (Rothbart & Mauro, 1990).

The problem of scale interdependence exists in various questionnaires developed on the basis of NYLS dimensions, such as the widely used Infant Temperament Questionnaire (ITQ; Carey, 1970) and the Revised Infant Temperament Questionnaire (RITQ; Carey & McDevit, 1978). Both suffer from overlap of conceptual definitions for the temperament scales (Rothbart & Mauro, 1990). Another possible problem with the scales derived from NYLS is their internal consistency and whether it is possible to generalize them across different response classes and sensory modalities (Rothbart & Mauro, 1990).

Another widely used questionnaire measure is the Infant Characteristic Questionnaire (ICQ; Bates, Freeland & Lounsbury, 1979) that attempted to combine findings from NYLS and items from questionnaires by Robson and Moss (1970) and Prechtl (1963). The authors used a principal-axis factor analysis with varimax rotation which yielded 4 factors of child temperament: *Fussy-Difficult*, *Unadaptable*, *Dull*, and *Unpredictable*. Their work produced an important distinction between two distress items: the *Unadaptable* factor related to distress to novel events, things and people, and the *Fussy-Difficult* factor related to all other distress (Rothbart & Mauro, 1990).

Further questionnaire development aimed to go beyond the scales produced by the NYLS study, and among these was the Infant Behaviour Questionnaire (IBQ; Rothbart, 1981). This questionnaire was partly developed on the basis of Rothbart and Derryberry's (1981) temperament theory, and aimed to look at individual differences in reactivity and regulation in infancy. The measure examines 6 domains of infant

temperament: activity level, soothability, fear, distress to limitations, smiling and laughter, and duration of orientation. Since its development, the IBQ has been one of the most widely used temperament measures (Garstein & Rothbart, 2003).

In the years following the introduction of the IBQ, there has been considerable development in temperament research, and Garstein and Rothbart (2003) felt this should be taken into account in parent-report measures of temperament. Therefore a new, revised version of the IBQ was developed, the IBQ-Revised (IBQ-R; Garstein & Rothbart, 2003). Of particular relevance to its development was the introduction of Child Behaviour Questionnaire (CBQ; Rothbart, Ahadi, Hershley, & Fisher, 2001), a highly differentiated assessment of temperament for children aged 3 to 7 years, containing 15 scales. Similarly, the IBQ-R was developed to assess more specific dimensions of temperament, rather than broad traits such as difficulty (Parade & Leerkes, 2008). After administration of the IBQ-R to 360 families, a final version of the questionnaire included 14 subscales: activity level, distress to limitations, approach, fear, duration of orienting, smiling and laughter, vocal reactivity, sadness, perceptual sensitivity, high intensity pleasure, low intensity pleasure, cuddliness, soothability, and rate of recovery. There was moderate agreement between primary and secondary caregivers on the IBQ-R scales. Even though the majority of inter-item correlations were low to moderate, some coefficients were moderately high, thus implying non-independence between some of the scales (e.g., between smile/laughter and vocal reactivity, and between approach and vocal reactivity). This was in line with the expectation of the authors (Garstein & Rothbart, 2003). Overall, it can be concluded that IBQ-R is a valid and reliable development of the widely-used IBQ measure (Garstein & Rothbart, 2003; Parade & Leerkes, 2008).

However, despite the described advantages of using mother-reports of infant temperaments, some have expressed doubts whether mothers are able to objectively report on their children's characteristics (Kagan, 1994). It has been suggested that parental reports of child temperament can be influenced by the subjective perceptions of the child, the parent's own personality, their educational background and the parent's sensitivity to their child's expressions (Kagan, 1994).

Rothbart, Evans and Ahadi (2000) defended the format of the IBQ by proposing that it asks very specific questions about concrete behaviours that happened in the past 2 weeks, and not general perceptions of the child. However, it does not completely shield the report from parental subjectivity and the effects of caregiver's sensitivity and maternal emotional well-being (Leerkes & Crockenberg, 2003).

Associations between maternal reports and actual observations of infant temperament have been found to be low (Seifer, Sameroff, Barrett, & Krafchuk, 1994).

It seems that although parent reports may provide some useful information about infants' characteristics, it is less clear how well they reflect the infants' actual temperament (Seifer et al., 1994). It therefore seems useful to combine parental reports with more objective measures in order to obtain a more valid impression of infant temperament.

Observation of child temperament and fearfulness

Home observations

Observations of infants in their home settings have been widely used in temperament research (Bates, Freeland, & Lounsbury, 1979; Rothbart, 1986; Seifer et al., 1994). These observations assume that the home environment is the most natural and influential environment for young children to express their temperament

characteristics (Strelau, 1998). Strelau (1998) reports that home observations usually take no longer than 3 hours, and should ideally be conducted on two or more visits. During these visits trained observers carry out moment-to-moment coding of infant behaviour and the context in which it occurs (Rothbart, 1986). The most common situations used to observe infants' behaviours in terms of their temperament are bathing, feeding, dressing and play interactions (Strelau, 1998). For example, Rothbart (1986) carried out three 40-45 minute long home observations of infants' behaviours during bathing, dressing and play. Trained coders observed the behaviours and coded them according to both frequency and intensity during each episode.

Generally, moderate to good inter-rater reliability can be established during home observations, with reliabilities ranging from .56 to .90 (Rothbart, 1986). However, Strelau (1998) reports that intersession reliabilities are generally much lower, not exceeding .30.

Because of the uncontrolled nature of the setting, home observations have some limitations. It is difficult to control the situation in which infants' behaviour is recorded (Strelau, 1998), as well as the physical intensity of the various stimuli during observations (Rothbart et al., 2000). Partly because of these shortcomings, home observations are rarely used in isolation (Strelau, 1998). It has been proposed that observations of infants under controlled laboratory environment might solve some of these problems.

Laboratory observations

Laboratory observations of infant temperament are used in order to overcome some of the limitations of home observations as they allow researchers to use controlled stimuli and situations that are expected to provoke manifestations of

various temperament characteristics (Strelau, 1998). Laboratory studies also allow easier video-recording of the sessions, and more standardized scoring of behaviours. Carranza, Perez-Lopez, Honzalez and Martinez-Fuentes (2000) proposed that laboratory measures of temperament can help avoid the confounding effects of home settings and characteristics.

One of the first major contributions to the laboratory study of temperament was the Louisville Longitudinal Twin Project (Matheny & Wilson, 1981), which utilized different vignettes to measure specific aspects of temperament. The vignettes were a standardized battery of tasks (mother-infant interactions and separations, age-related challenges, and play) that were used to elicit and record infants' behaviour in response to these situations (Strelau, 1998). The video recordings of the session were coded by trained observers to yield composite measures of infant temperament profile (Matheny et al., 1985). This method allowed assessment of dimensions like emotional tone, activity, attentiveness, social orientation to staff, and resistance to restraint (Strelau, 1998). It was reported that the assessment yielded satisfactory inter-rater reliability in infants (.65-.92) and toddlers (.72-.94) (Strelau, 1998).

The Laboratory Temperament Assessment Battery (Lab-TAB; Rothbart & Goldsmith, 1992) was developed more recently and is one of the most widely used instruments for the study of child temperament. The Lab-TAB enables the assessment of five temperament dimensions: activity level, fearfulness, anger proneness, interest/persistence, and joy/pleasure. There are 15 episodes in Lab-TAB for 6-months-olds, and 20 for 12-to-18-months olds, and each characteristic is measured by a number of episodes. Lab-TAB allows for standardized measure of temperament, with detailed descriptions of procedures and apparatus in the manual and is therefore

considered to be the most standardized procedure for measuring temperament (Strelau, 1998).

With respect to the measurement of fear reactivity, the Lab-TAB includes a number of procedures, such as Parasol Opening, Masks, and the Unpredictable Mechanical Toy. Each episode is scored on emotional parameters such as the latency to act, behavioural acts, and peak and average intensity of emotion.

Although generally accepted as an important and valid assessment of temperament, laboratory observation measures still suffer from some shortcomings. Rothbart, Chew and Gartstein (2001) report that carryover effects can be a problem, when the child's reactions to one of the tasks affects their mood and behaviour for the rest of the assessment. They also suggest that children are often unable to use their usual coping strategies, such as crawling away, in the relatively restrained conditions of a laboratory. The novelty of the laboratory can also make fear responses especially potent (Rothbart et al., 2001). Finally, there is a scoring problem in relation to detecting and interpreting ambiguous reactions of children from video recordings. To address the latter point, a growing number of studies now also look at children's physiological reactions to various situations, instead of, or in addition to, behavioural measures in order to obtain more objective measure of early temperament.

Physiological measures

Since biological predispositions are part of the definition of temperament (Rothbart & Derryberry, 1981; Gunnar, 1990), various researchers have proposed to investigate children's physiological reactions to the stimuli that tap into various temperamental dimensions. These measures include brain activity (Hane & Fox, 2006), skin conductance, vagal tone (Stifter & Fox, 1990), and heart rate variability

(Gilissen, Koostra, van IJzendoorn, Bakermans-Kranenburg & van der Veer, 2007).

Another widely used physiological measure is to examine the activity of the body's main stress response system, the Hypothalamus-Pituitary-Adrenal (HPA)-axis system. The end product of the activation of this system, secretion of the stress hormone cortisol, is assessed in response to various challenges, particularly distress and fear (Gunnar & Donzella, 2002).

Cortisol assessment in adult and animal studies began with the development of radioimmunoassay (RAI) techniques, which allowed assessment of cortisol concentrations in blood plasma and urine samples (Gunnar & White, 2001). Further refinement of RAI in the mid 1980's allowed assessment of cortisol in saliva which led to a large increase in the investigation of cortisol in developmental research (Gunnar & White, 2001).

Saliva sampling is a relatively easy and non-invasive procedure in studies with young children. There are various methods of obtaining saliva samples, the majority of which involve placing a cotton dental roll attached to a plastic end into a child's mouth, and then placing it into a needleless plastic syringe where it can be stored (Gunnar & White, 2001). The samples are usually stored at -20°C until they are assayed.

There is some debate in the literature as to when cortisol collection should be carried out during testing. For example, it was believed that cortisol release in saliva in response to an emotional challenge can be detected about 25 minutes after the onset of the event and that levels start to decrease again about 40 minutes after the onset of the emotional challenge (i.e., recovery). In accordance with this, Ramsey and Lewis (2003) found that most 6-months-olds had peak cortisol concentrations at 20-25 minutes following challenge, while some had their peak at 30 minutes. However,

Goldberg, Levitan, Leung et al. (2003) found that about 50% of the infants aged 12 to 18 months had their peak response to fear challenge at 40 minutes.

Usually, infant cortisol reactivity is assessed by calculating the difference between cortisol baseline and cortisol post-stimulation values (Alberts et al., 2008; Blair et al., 2006). However, these calculations should be interpreted with caution. Baseline cortisol levels should be evaluated separately, as higher baseline levels may lead to lower cortisol increase in some infants (Gunnar, 1990). It has been proposed that infants with higher baseline cortisol concentrations in the normal range may be better prepared to cope with novel stimulation; however, the findings are inconclusive (Gunnar, 1990).

Caution should also be taken to obtain valid and representative findings. For example, because of the circadian rhythm in cortisol production, cortisol collection should take place at the same time for all participants (Gunnar & White, 2001). Finally, infant sleeping and feeding patterns on the day of testing should also be taken into account, since they are reported to affect cortisol concentrations (Gunnar & White, 2001).

Convergence between methods

Low associations have been reported between questionnaire measures of temperament and direct observation of children's behaviour (Lancaster, Prior, & Adler, 1989). Carranza et al. (2000) suggested that observation and questionnaires are non-equivalent measures of temperament, and that each has its advantages and disadvantages. The same can be said about physiological measures. It can be suggested that physiological measures only tap into state reactions, not trait predispositions. For these reasons, it seems that using a combination of different

methods is the most optimal way of gaining a better understanding of temperament and fear development in childhood.

Part II: Measuring mother-infant relationship

Attachment

Quality of mother-child relationship is usually assessed by examining attachment security in early childhood as proposed by Bowlby (1969). Since Mary Ainsworth developed the well-known Strange Situation, it became possible to empirically measure the security of a child's attachment to the mother. Behaviour of children during a series of separations and reunions with the mother and stranger encounter allowed researchers to group children into four attachment categories: Secure, Insecure-Ambivalent, Insecure-Avoidant and Disorganized.

Even though attachment security has been established as an adequate measure of mother-child relationship, it will not be used in present study for the following reasons. Firstly, present research is more interested in maternal behaviour towards her child rather than in the general relationship between them. Attachment research does not take the behaviour of the mother into account, as the focus lies on the child's reactions. Secondly, measuring attachment security is done in a categorical way, and the majority of normal healthy children coming from a middle-class sample are likely to be securely attached. One needs a large sample of children coming from very different family backgrounds to obtain enough children in each of the different attachment categories. On the other hand, within the securely attached group of children there is likely to be variation in the quality of care and emotional support the mother provides for her child. It is this variation in maternal behaviour and the effects it has on child temperament that we want to study in the current research. However,

there are different ways of measuring maternal behaviour during interaction and the next section will provide a brief discussion of some of these measures.

Maternal behaviour during mother-child interaction

Mother-child interaction across various situations has been used as a setting for measuring maternal and/or child behaviours. Mother-infant interactions are important in different areas of infant development since they serve cognitive, linguistic, social and emotional functions for the infant (Bornstein & Tamis-LeMonda, 2004).

There is a range of interaction contexts that can be used depending on the interests of specific studies, such as free or structured play interaction, interaction during bathing or feeding, and during inoculation. Studies also vary in the length of time they use for assessing these interactions, ranging from a few minutes to about an hour.

There are many aspects of maternal behaviour towards the child that are important not only for establishing a harmonious relationship between mother and child, but also for future child development. 'Maternal sensitivity' is probably the most widely used aspect of quality of maternal care. It was developed by Ainsworth, Bell and Stayton (1971) and relates to the ability of the mother to perceive signals of the infant promptly and correctly, while respecting the infant's individuality. Their measure consisted of a 9-point scale where a highly sensitive mother is able to perceive things from her baby's point of view, to tune with her baby's signals, and to respond to them correctly and on time. Sensitive mothers almost always provide the baby with what he or she wants, and if she does not, she is tactful and offers them an acceptable alternative (Ainsworth et al, 1971).

In addition to maternal sensitivity, Ainsworth et al. (1971) also developed a measure of interference-cooperation by the mother, assessing the interference/intrusiveness of the mother during interaction with the child. The authors portray an interfering mother as someone with little respect for the baby as a separate and autonomous being, whose wishes and activities have little validity of their own, and who have to simply follow the orders of the mother. On the other hand, a cooperative mother would guide rather than control the infant's activities, and would integrate her own moods and activities with those of the infant.

In addition, Ainsworth's original scales also included constructs of availability/ignoring and acceptance/rejection. Availability/ignoring scale included parameters such as awareness of the baby and the ability to divide her attention between the baby and other persons and activities without losing the awareness of the child. The acceptance/rejection scale looks at the balance between the positive and negative feelings about the baby, with positive feelings such as love and acceptance at one end of the scale and negative feelings such as anger and irritation toward the baby on the opposite end. Even though important constructs, it seems that these should be measured in naturalistic environments over relatively lengthy periods of time.

Fish and Stifter (1995) simplified the 9-point sensitivity and intrusiveness scales of Ainsworth et al. (1971) by shortening it to a 4-point scale for ease of scoring. The ratings were made for maternal sensitivity and maternal intrusiveness separately, with the 4-point scale scores reflecting none, low level, moderate level and high level of each behaviour during 30-second periods over 5 minutes of free play, with a composite score for each behaviour. The authors found that children of highly sensitive and low intrusive mothers were most likely to be securely attached at 18

months, children of low sensitive/low intrusive mothers were more likely to become insecurely-avoidant attached, and children of low sensitive/highly intrusive mothers were more likely to be insecurely-ambivalent attached. This study demonstrates that both maternal sensitivity *and* intrusiveness are important parameters of maternal behaviour, and that both are important in the development of attachment. It is worth mentioning that the scales developed by Fish and Stifter (1995) also included a variable of infant responsiveness to the mother, which looked at infant engagement and involvement with toys and the mother. The authors included this measure because of the rationale that infant behaviour can be an important contributor to the development of attachment security in addition to maternal behaviour.

Overall, maternal sensitivity and intrusiveness scales can be used to measure maternal behaviour during free play in a time-constrained setting, and the shorter scales allow for easier scoring and better inter-rater agreement. Finally, they are suitable for children across early childhood and not restricted to infancy, making it possible to use them in longitudinal research.

There are other measures of maternal behaviour during interaction apart from maternal sensitivity. For example, Donovan, Leavitt, Taylor and Broder (2007) proposed the concept of 'maternal sensory sensitivity', which looks at how well mothers can recognize various positive and negative emotions in photographs of infants. Meins, Fernyhough, Fradley and Tuckey (2001) considered maternal sensitivity as a measure is too general, and proposed a measure of maternal mind-mindedness, which involves the mother's ability to treat her infant as an individual with a mind, rather than simply a young creature with needs that must be satisfied. Their measure distinguishes between general maternal sensitivity to infant's physical

and emotional needs (as measured by Ainsworth's construct) and more specific sensitivity to child mental states. However, all of the proposed mind-mindedness categories were positively correlated with maternal sensitivity as measured by Ainsworth et al. (1971) scale.

McElwan and Booth-LaForce (2006) proposed a measure of maternal sensitivity to distress. They suggested that maternal attention to infant's episodes of distress might be especially important for attachment security and general infant development. They examined maternal sensitivity during an interaction when the infant was and was not distressed on a 4-point scale. *Sensitivity to non-distress* captured the extent to which the mother interacted and responded to her infant's gestures and signals in an appropriately-paced manner, whereas *sensitivity to distress* captured the extent to which the mother responded to the infant's cries, frets and general distress in a consistent, timely and appropriate manner (McElwan & Booth-LaForce, 2006). Sensitivity to distress, not to non-distress, at 6 months was predictive of attachment security. These results suggest that maternal sensitivity to the infant when he or she is emotionally upset or in physical danger are important contributors to attachment security, and possibly general child development, and therefore should be considered in future research.

Because of the findings discussed above, the studies in this thesis will use the 4-point scale developed by Fish and Stifter (1995) to score maternal sensitivity and intrusiveness during two mother-child interactions. We scored both maternal sensitivity and intrusion, to have a positive and a negative aspect of maternal behaviour, because both have been shown to differently affect attachment security (Fish & Stifter, 1995). These maternal behaviours were scored during a 3-minute free

play session at the beginning of the testing, and during a 3-minute interaction following a distressing episode. To the best of our knowledge, an examination of maternal behaviour following reunion after an episode of infant distress has not been studied before. We aimed to further develop the findings by McElwan and Booth-LaForce (2006) in investigating the importance of maternal sensitivity during emotional distress for child emotional development.

Chapter 6. The role of the mother in children's behavioural and hormonal coping with stress: Stability and change across the first 3 years of life

Introduction

A good mother-child relationship has been reported to be associated with positive social and emotional development of children (Ainsworth, Blehar Waters & Wall, 1978; Kochanska, 2001; McElwain & Booth-LaForce, 2006). Sensitive and responsive caregiving during early childhood is important for the neurophysiological, physical and psychological development of the child (Amankwaa, Younger & Roux, 2007). There is a considerable amount of research that investigates the role of maternal characteristics in child emotional development. Positive maternal characteristics have been linked with the development of a child who is better able to cope with stress and to recover from it more quickly. However, it is not yet clear which maternal characteristics (e.g. her general sensitivity, her intrusiveness, or her specific sensitivity to the child when in distress) are most important for child emotional reactivity and regulation. A series of three studies are presented in this chapter that collectively serve to examine the relationship between maternal sensitivity and intrusiveness with child emotional reactivity and recovery when children are one, two and three years old. The aim of these studies is to investigate the relationship between maternal behaviours during different emotional states of the child and their influence distress on child emotional reactivity and recovery.

Child reactivity and regulation

Rothbart (1989) identified reactivity and regulation as two biologically based components of temperament. Distress reactivity encompasses individual differences in

physiological and emotional response to stress, while recovery is the regulation of a response to a stressor (Barr & Gunnar, 2000; Compas, Connor-Smith, Salzman, Thomsen & Wadsworth, 2001) in order to keep the arousal within an individually optimal range (Gloggler & Pauli-Pott, 2008). Though proposed to be distinct systems (Ramsey & Lewis, 2003), reactivity and regulation are intertwined, since reactivity affects the need for regulation and regulation then influences reactivity levels (Gloggler & Pauli-Pott, 2008; Calkins & Johnson, 1998), making it important to study both aspects when investigating child emotionality. Highly reactive children were reported to have a lower threshold of initial response to distress, a slower recovery and a higher level of arousal with repeated exposure to stress (Compas et al., 2001).

Stress response and recovery involve multiple complex behavioural and physiological systems which help an organism restore and maintain homeostasis (Haley & Stansbury, 2003). It has been shown that emotional stressors such as novelty and uncertainty evoke behavioural distress and also result in the activation of the hypothalamic-pituitary-adrenocortical (HPA) system, with the secretion of cortisol (among others) into circulation (Gunnar, 1989). It is believed by various researchers that both behavioural and physiological measures of stress are needed in order to provide a reliable picture of individual differences in stress response (Lewis & Ramsey, 1999; Gunnar, 1986). The association between behavioural distress and cortisol increase are not yet clearly understood, with the majority of studies finding at best a moderate relation between cortisol and behavioural distress (Lewis & Ramsey, 1995; Ramsey & Lewis, 2003). This indicates that an absence of behavioural distress does not necessarily mean that children are not experiencing any distress (Lewis & Ramsey, 1999). Theoretically, this may be truer for older children, for example in the

second and third year of life, as children learn to control their behavioural expression of emotion. Overall, current evidence seems to suggest that it is important to combine both behavioural and hormonal responses to stress in order to obtain a more complete understanding of the child's distress experience.

Maternal characteristics and child reactivity and regulation

It is considered that development of stress-response and regulation systems of the child may in part depend on the caregiver's interactive behaviour (Haley & Stansbury, 2003; Gloggler & Pauli-Pott, 2008). Higher levels of sensitive, reliable and appropriate maternal behaviour are theorized to lead to more secure and self-efficient children who explore novel environments more freely (Pauli-Pott et al., 2004).

It is worth noting that studies interested in the influence of the mother on child emotionality investigate this by using different measures of maternal behaviour. Some studies look at mother-child *relationship*, the majority focusing on attachment styles, which reflects both child and parent contributors. The concept of a 'secure base' is one of the most important aspects of attachment theory, where to 'be attached' means to use someone, in this case the mother, preferentially as a secure base from which to explore the world (Waters & Cummings, 2000). It is said that a secure attachment refers to the use of the secure base in different contexts and over time, and the feeling of confidence in the availability and responsiveness of the caregiver (Waters & Cummings, 2000).

Other studies, on the other hand, focus more on *maternal* interactive behaviour, by examining characteristics such as sensitivity, responsivity and intrusiveness during mother-child interactions. Maternal sensitivity and responsivity involves how well maternal behaviour is tuned to her child's signals, whereas

intrusiveness reflects the degree of maternal interference with her child's activities during interactions (Ainsworth et al., 1978). Even though different concepts, maternal characteristics such as sensitivity and intrusiveness have been shown to be related to future attachment style development in various studies (Ainsworth et al., 1978; Fish & Stifter, 1995; Bakermans-Kranenburg, van IJzendoorn and Juffer, 2003). The present study investigates the role of both maternal sensitivity and intrusiveness in child emotional development. However, for the purposes of this chapter, some attachment literature will also be reviewed as there are relatively few studies that specifically focused on maternal sensitivity and/or intrusiveness.

As mentioned above, some studies found maternal characteristics and child attachment style to be related to future child distress reactivity and recovery. Pauli-Pott et al. (2004) observed that higher maternal sensitivity was related to lower child negative emotionality, frustration and fear reactivity at 12 months. Hane and Fox (2006) found that less maternal caregiving behaviour at 6 and 9 months was related to more child fearfulness during novelty as well as less positive joint attention with the mother. Egeland, Pianta and O'Brien (1993) also observed long-term effects of intrusiveness on child development, with children of intrusive mothers at 6 months doing emotionally, socially and behaviourally poorly in the first and second grade. In terms of mother-child relationship, Kochanska (2001) found that attachment security at 14 months was associated with child fear and anger reactivity at 33 months, with securely attached children being less fearful and angry. Collectively, these studies indicate the importance of maternal characteristics and mother-child relationship for the development of adaptive emotional reactivity in early childhood. These findings support the idea that sensitive parenting and attachment security enable the child to

explore the world more freely and to learn to regulate their arousal in a more adaptive way.

There are fewer studies explicitly looked at the influence of maternal care on child speed of recovery from distress, but some studies did investigate its influence on stress regulation. For example, Gloggler and Pauli-Pott (2008) found that maternal sensitivity at 8 and 12 months was related to active distress regulation behaviours such as self-soothing and active avoidance at 30 months. However, the findings in recovery are not clear-cut, where Lewis and Ramsay (1999) did not find maternal soothing to influence infant recovery after inoculation and an every-day stressor. It is therefore important to further investigate whether maternal sensitivity influences speed of recovery from distress as it is an important aspect of the stress experience. Whereas the experience of distress is a normal aspect of development, lingering distress after termination of the stressful episode is not desirable from a homeostatic perspective. Thus the ability to recover behaviourally and physiologically quickly is important for the child's emotional equilibrium, and sensitive parenting could influence the child's ability to recover from stress more quickly and efficiently.

As mentioned earlier, *behavioural* distress and recovery are only one aspect of the distress experience, physiology being the other. It has been suggested that the quality of the mother-child relationship may be more important for the child's physiological reactivity and recovery than for the intensity of their behavioural distress. Gunnar and Donzella (2007) suggested that sensitive caregiving allows children to express their feelings and communicate emotions (that is, show distress) without necessarily having an increased glucocorticoid reaction. Consistent with this view, Spangler and Schieche (1998) found that securely attached children displayed appropriate levels of behavioural distress upon maternal separation, but no rise in



cortisol level. Insecure-ambivalent children showed behavioural distress and elevations in cortisol, while insecure-avoidant children showed neither behavioural distress nor heightened adrenocortical activity.

However, some studies found no influence of maternal sensitivity on child physiological reactivity during a negative affect challenge (Blair et al., 2006), but more sensitive mothers did have children who recovered faster than children of less sensitive mothers. Albers et al. (2008) also found that maternal sensitivity did not influence 3-month-old infants' cortisol response to stress, but did influence stress recovery. It was found that infants of more sensitive mothers had better recovery from distress. These results suggest that maternal sensitivity is more important in regulating cortisol recovery from stress than in influencing cortisol stress response. On the other hand, a study by Blair et al. (2008) found maternal sensitivity to influence both cortisol reactivity *and* recovery at 15 months, with greater sensitivity being associated with lower cortisol reactivity and faster recovery. One of the possible reasons for the discrepancy in results could be the fact that these studies use different methods of stress assessment at different ages. The Albers et al. (2008) study looked at 3-month-old infants, whereas Blair et al. (2008) focused on outcomes in toddler years. Second, Albers et al. (2008) looked at the influence of maternal sensitivity on an everyday mild stressor (i.e., being taken out of the bath), whereas the Blair et al. (2008) study focused on structured stress-eliciting episodes, where maternal sensitivity may perhaps play a more important role for distress reactivity.

Overall, the findings discussed above seem to be relatively inconclusive with respect to the role of maternal characteristics and mother-child relationship in child emotionality. Some studies found an influence on child behavioural reactivity, some found an effect on children's physiological reactivity during stress exposure, and

others found an effect on children's physiological recovery only, while some did not find any influence of the mother (Lewis & Ramsay, 1999).

Part of the reason for these different outcomes may be related to differences in the assessment of mother-child relationship. Some studies focused on attachment security while others assessed maternal characteristics such as maternal sensitivity, responsiveness, intrusiveness or maternal soothing. The current thesis will focus on maternal sensitivity and intrusiveness in particular, as one of its main aims is to examine the relationships between maternal characteristics and child behavioural and cortisol reactivity and recovery.

McElwain and Booth-LaForce (2006) pointed out that the majority of studies to date investigate maternal caregiving behaviour in a global way, without differentiating between maternal sensitivity to different emotional states of the child. They and others have proposed that maternal sensitivity to distress is a separate aspect of maternal interactive behaviour, one that could potentially be more important for attachment security and other aspects of infant emotional development than general sensitivity (McElwain & Booth-LaForce, 2006; Goldberg, Grusec & Jenkins, 1999). In support of this, Leerkes et al. (2009) found that maternal sensitivity to infant distress at 6 months predicted fewer behavioural problems and greater social competence at 24 and 36 months, while sensitivity to non-distress was not predictive. However, in this study, maternal sensitivity to distress and non-distress were measured during play, when children are not likely to get intensely distressed. Mothers and infants were observed during a 15-minute home interaction, and maternal sensitivity to distress was coded at moments when and if the child became distressed during the interaction, which may undermine the validity of this measure, as not all children experienced distress during the interaction.

This calls for a specific measure of maternal sensitivity after an episode which is likely to cause some distress to the child (Leerkes et al., 2009). The present study introduces measures of both positive and negative aspects of maternal caregiving behaviours (sensitivity and intrusiveness) after a separation and fear-eliciting episode that is designed to cause some distress and discomfort for the child and their mother. This would enable us to compare and contrast maternal characteristics after these qualitatively different emotional events and to examine the associations between these maternal behaviours and child distress reactivity and recovery. Using this design, we can assess whether it is true that maternal characteristics are stable across different emotional states of the child, which is the view that has been generally accepted in past research. It can be theorized that maternal interaction is ‘easier’ when the child is calm and playful than when the child is distressed and more demanding.

We were further interested in the child’s ability to cope with novelty in the absence of the mothers. As the review of literature indicates, there is currently no consensus on the role of the mother in child cortisol reactivity to stress and its recovery. We therefore used a combination of two well-known stressors (i.e., exposure to a novel toy and maternal separation) to investigate a range of individual differences in cortisol and behavioural reactivity. As such, this is one of the first studies to investigate children’s reactivity to novelty in the absence of their mother. Ahnert et al. (2004) looked at the association between attachment style and child stress reactivity to separation and novelty of childcare. They found that securely attached children displayed higher behavioural distress and cortisol reactivity in response to separation and attending childcare. Children’s ability to cope with distress in the absence of their caregivers seems to be an important area of research as many

children face novelty stress in the absence of their parents. A clearer understanding of children's reactivity to novelty and separation is necessary.

The present chapter consists of three repeated studies investigating maternal characteristics (sensitivity and intrusiveness) at three major time points in early childhood during the first, second and third years of life. Repeating the same design with the same sample of participants enables us to investigate the influence of maternal characteristics on child emotionality separately at each year of early childhood. This is a unique design, as past studies either focused on the influence of maternal characteristics on child development at one assessment, or looked at the influence of *early* maternal characteristics on later child development. The design of the studies of this chapter allows us to assess the unique contribution of maternal sensitivity and intrusiveness on child emotionality at each year of assessment. This further sets the sample for the longitudinal study in Chapter 7 that looks at the stability and change of maternal characteristics and their continuous effects on child distress reactivity and recovery throughout early childhood.

The aims and hypotheses of the studies in the present chapter are as follows:

- a) To investigate the association between maternal sensitivity and intrusiveness.

It is hypothesized that sensitivity and intrusiveness will be negatively associated.

- b) To investigate whether maternal sensitivity and intrusiveness are affected by child's emotional state. It is hypothesized that levels of maternal sensitivity and intrusiveness are different when comparing normal, playful interaction and interaction after distress.

- c) To investigate the influence of maternal sensitivity and intrusiveness on child behavioural and endocrinological stress response and recovery. It is

hypothesized that greater maternal sensitivity predicts less behavioural distress, a smaller cortisol stress response and a faster recovery. Greater maternal intrusiveness, on the other hand, is hypothesized to predict more behavioural distress, a larger cortisol stress level and a slower recovery from stress.

Study 1: Maternal sensitivity and intrusiveness and their influence on child behavioural and physiological distress reactivity and recovery in one-year-olds

Method

Participants

Participants were 72 infants (34 male, 38 female) and their mothers. The infants' ages ranged from 6 to 14 months ($M = 9.9$ months, $SD = 1.9$). All participants were born in Cardiff, South Wales and were recruited from local nurseries and leisure centres.

The mothers' ages ranged from 22 to 41 years ($M = 32.6$ years, $SD = 4.26$). About 78% of them were married, 10% were living with their partner and 4% were single. Families were predominantly of white British and middle-class origin (90%) with some participants of Asian-Indian origin (7%), White-Black origin (1.4 %) and White-Asian origin (1.4%). The majority of children who took part of the study were first-born (60%).

Full ethical approval for the current and subsequent studies was granted by Cardiff University's School Research Ethics Committee (Ethics Codes EC.05.04.05.434, EC.05.04.434 & EC.07.01.09.1181G)

Measures

Maternal Behaviour

Maternal behaviour towards her infant was assessed during two free-play interactions. Mother and baby were left alone in the room and asked to play together with a standard selection of toys. The first interaction took place upon arrival after the procedures of the study had been explained, and the second was immediately after the distress task; each interaction lasted 3 minutes.

Maternal behaviour was assessed on parameters of maternal sensitivity (MS) and intrusiveness (MI) using the scoring system of Fish and Stifter (1995) (Appendix 2). Both behaviours were rated at 30 sec intervals on 4-point (0-3) scales designed to reflect none, a low level, a moderate level, or a high level during each 30 sec period (i.e. 6 scores for each interaction). The summed scores for maternal sensitivity or intrusiveness during one interaction episode could range from 0 to 18. We did not use the scale of infant responsiveness also included by Fish and Stifter (1995) because our study focuses on the role of the mother and her behaviour.

Fish and Stifter (1995) defined highly sensitive maternal behaviour as well-timed and providing an appropriate level of response/stimulation, following the infant's lead and signals, and facilitating the infant's self-regulation. Behavioural examples are contingent responses to the infant's signals and actions, positive verbal encouragement, and providing assistance for the child to reach his/her goals.

Maternal behaviour rated as intrusive is overly stimulating, poorly timed, not responsive to the child's pace and signals, and appears to address the mother's agenda rather than the infant's (Fish & Stifter, 1995). Examples of such behaviour are removing a toy that the infant is interested in, persisting in play when the infant shows protest or an averted gaze, overloading the child with toys, making developmentally inappropriate demands, and continuing to manipulate the toy the child is interested in handling himself/herself.

The inter-rater reliability (Cohen's kappa) between two trained coders on 11% of the sample was .71 for maternal sensitivity, and .73 for maternal intrusiveness.

Assessment of infant distress

Behavioural distress

Infant distress was assessed using the unpredictable mechanical toy episode of the Lab-TAB (Rothbart & Goldsmith, 1992). It closely followed the protocol of the Lab-TAB, however, for purposes of present study the mechanical toy dog used in Lab-TAB was replaced with a mechanical remote-controlled robot. Additionally, the mother was asked to leave the room.

After the mother had left the room, an unfamiliar experimenter dressed in a white lab coat and goggles entered the room and placed the robot approximately 1.5 meters away from the child, who was strapped in a car-seat. The experimenter made the robot walk towards the child, stopping approximately 15 cm away from the child, while making movements with its arms and producing some noise. The robot would then walk backwards and stop at the back for about 10 seconds before moving

forward again. This trial was repeated for the total of three times in line with the Lab-TAB protocol.

The Lab-TAB's guidelines were followed for the behavioural coding of the episode, which was carried out using video recording of the session. It was important to gain clear and full frontal shots of the infant's face. Each of the three trials of robot approach and movement in front of the child was separated into three epochs (robot walk towards child, robot moving in front of child part 1, robot moving in front of child part 2) (Appendix 3). This created a total of 9 epochs that were scored separately. Each epoch was scored on the following dimensions and scales: intensity of facial fear (0-3), intensity of facial sadness (0-3), intensity of distress vocalisation (0-5), intensity of bodily fear (0-3), intensity of escape (0-3), and presence/absence of startle response (0-1). The high reliability between these variables (Cronbach's $\alpha = .84$) enabled us to create a composite score by adding the individual ratings for these behavioural variables across the distress episode, to indicate an overall level of distress. The possible range for the composite score was 0 to 162.

Four coders scored the episodes independently. Intra-correlation coefficients between coders ranged between .70 and .99 across the behavioural variables for 11% of the sample.

Cortisol reactivity

In order to measure the stress hormonal response to the distressing event four salivary samples for the assessment of cortisol were collected from each child: two baseline and two stress reactivity samples. The first saliva sample was taken shortly after mother and infant's arrival at the laboratory; the second sample was collected 15 minutes after the first. The third sample was taken 5 minutes after the termination of the distress challenge, and the fourth sample was taken 25 minutes after the third.

The samples were collected between 9 and 11 am, and each sample collection took approximately 1 minute. Sorbettes and cryovials (Salimetrics, State College, USA) were used for collecting saliva from the infant's mouth. Because of the evidence that milk can interfere with the cortisol assay (Maganon, Diamond & Gardner, 1989) mothers were asked not to feed their children during the procedure.

After collection, samples were frozen at -20°C and stored until they were shipped in dry ice for analysis. All samples were analysed with Elisa cortisol assays. The samples were spun at 15000 rpm for 15 minutes at 4°C and assayed in duplicate. The data were transferred to a computer using the assay software KC4, creating a standard curve. The concentration of cortisol present in each sample was then calculated from the standard curve. A standard curve was generated for every plate of samples assayed. The average intra- and interassay coefficients of variation were 4.33 % and 9.25 %, respectively.

We used a mean of the two pre-stress cortisol values as the index of baseline cortisol levels. For cortisol stress index we used the peak post-stress cortisol sample. For each individual cortisol reactivity was calculated in two ways: Cortisol Δ (i.e., the cortisol peak stress value minus the mean baseline value), and the percentage increase (%increase) between the peak cortisol value and mean baseline level, with higher Cortisol Δ and %increase values indicating greater cortisol reactivity (Ramsay & Lewis, 2003)

Infant Recovery

In order to investigate infant recovery from stress, the time it took the child to recover from distress was measured. The coder observed the infant's behaviour from the video-recording and measured the time (in seconds) it took the child to return to a normal emotional state, defined as the child stopping vocal distress and/or crying, an

absence of facial sadness and being able to engage in activity with the mother and/or any toys.

Procedure

Participants were recruited by distributing leaflets (Appendix 1) in nurseries and leisure centres containing information about the procedure and purposes of present study. The mothers received a £10 gift voucher in return for their participation and their travel costs were reimbursed. The infants were presented with an age-appropriate book as a gift. The testing took place in a child laboratory in the School of Psychology at Cardiff University.

Upon arrival, the experimenter explained the procedure of the study while mother and child got familiarized with the environment, and the infant was free to play with a range of toys. Full informed consent was obtained from the mothers before the study began. Because it was anticipated that infants would become distressed by the presentation of the unpredictable toy, the mother was informed about this issue before agreeing for her child to take part in the study. The mother was assured throughout the study that she was free to withdraw her child from the study at any time without the loss of payment or gift. Her permission to start video recording was also obtained (information about video recording the session was also included in the consent form).

The first cortisol sample was collected shortly thereafter by swiping the child's mouth with the sorbet. Once the infant appeared to have settled, the dyad was left alone to interact in the room for three minutes. They were left with a selection of toys. After this interaction, the second cortisol sample was taken.

The child was then carefully placed in a car seat and strapped in safely by the mother. The mother was asked to leave and go to an adjacent room from which she could observe her child through a one-way mirror. She was reassured that she could terminate the episode at any point. An unfamiliar experimenter, dressed in a white lab coat and goggles, entered with the robot, placing it at the back of the room. The experimenter operated the robot by remote control and remained unresponsive to the child. The whole episode lasted four minutes.

The mother returned to the room and was told that she could take the child out of the chair. They were left to interact for three minutes. At the end of the three minutes the 3rd cortisol sample was taken. The main experimenter offered the robot to the child to investigate and play with. The 4th and final cortisol sample was taken approximately 20 minutes after the 3rd sample. Mother and child were given their presents/vouchers, and left the laboratory soon after.

Missing Data

Before starting data analysis, variables were examined for missing values. Overall missingness was relatively small at 4.69%. The following variables had missing values: the individual 30 sec scores for maternal sensitivity at baseline, maternal sensitivity after distress, maternal intrusiveness at baseline, maternal intrusiveness after distress, cortisol baseline concentration and cortisol peak stress response concentration. The pattern of missingness of the data was tested and found to be Missing Completely at Random (MCAR; Acock, 2005) with Little's MCAR test, which was not significant $\chi^2(97) = 86.20, p = .78$, indicating that the data were MCAR. This shows that there was no systematic missing pattern in the dataset and that missing values were randomly distributed across all observations.

Data imputation was used because of the small proportion of the missing values and in order to retain the sample size of $N=72$, because listwise deletion would decrease the sample to $N=63$. Because of the small percentage of missing data ($< 5\%$) single data imputation was reasonable (Schafer, 1999). Missing data were imputed using the SPSS software built-in procedure Replace Missing Value (PMV) with linear trend at point estimation method for the missing 30 sec interval scores for maternal sensitivity at baseline, maternal intrusiveness at baseline, maternal sensitivity after distress, maternal intrusiveness after distress, infant baseline cortisol concentration and infant cortisol peak response. This imputation method uses the regression slope between variables to replace the missing values with the linear trend for that point. The missing values are replaced with their predicted values. The imputed values were then used to calculate composite scores for each of the maternal behaviours at baseline and after distress for participants with missing values.

Statistical Analysis

Normality of distribution of the variables was checked in a histogram with a normal curve and skewness assessment. The moderately positively skewed variables of infant recovery time, infant cortisol baseline level, Cortisol Δ and %increase all underwent natural log (ln) transformation, which is recommended for this type of skewness (Tabachnick & Fidel, 2007), after which normal distribution was attained for this variable. The non-transformed variable was used in the descriptive statistics table and the frequency distribution, but the transformed variable was used in all further analyses.

We first investigated the associations between variables by looking at the inter-correlations between maternal and child variables. Then, in order to test the

unique contribution of infant distress in addition to maternal baseline behaviour on maternal behaviour after distress, a step-wise multiple regression was used. Furthermore, in order to test the possible moderating effects of infant distress on maternal baseline behaviour in influencing her behaviour after distress, an interaction effect of the two variables was tested in a multiple regression. In order to test interaction effects, the two independent variables were centered in order to reduce multicollinearity (Aiken & West, 1996). Centering a variable involves subtracting the mean for that variable for each participant, creating a mean of zero for each variable. Centering variables reduces multicollinearity but does not affect the variables' simple correlation with other variables (Tabachnick & Fidell, 2007). The interaction term was created as the product of the centered predictor variables. The centered variables and their interaction term were then entered in the regression analysis.

Preliminary Analysis

Since the ages of infants in present sample were quite varied (range between 6 and 14 months), we checked whether the age of the child played a role in child emotional reactivity and recovery. Children were separated into groups of younger (6-9 months) and older (10-14 months) infants. For behavioural stress reactivity, and independent samples t-test revealed that there was a difference between two groups ($t(2, 69) = -3.18, p < .01$) where younger infants ($M = 47.55, SD = 34.12$) were significantly less distressed than older infants ($M = 73.08, SD = 33.56$). Infant age is therefore included in further analyses of infant behavioural reactivity. In terms of behavioural recovery, the difference between the two groups was marginally significant ($t(2, 69) = -1.88, p = .06$) with younger infants ($M = 42.64, SD = 69.97$) recovering marginally faster than older infants ($M = 71.35, SD = 58.31$). In terms of

cortisol reactivity (delta) however, there was no significant difference between younger and older infants ($t(2, 69) = .05, p = .96$). Furthermore, there were no difference between boys and girls on any of the maternal characteristics or behavioural/cortisol reactivity and behavioural recovery.

Results

The descriptive statistics of the sample in Table 1 and the correlations in Table

2. The frequency distributions of the variables in the study can be seen in the Appendix section (Appendices 4- 7).

Table 1: Descriptive statistics for the infant and maternal variables (N=72)

Variables	Mean	SD	Range
<i>Maternal Variables</i>			
MS normal	10.44	2.38	6.00 – 17.00
MS after distress	10.63	2.51	5.00 – 17.00
MI baseline	3.85	3.21	0.00 – 13.00
MI after distress	2.69	2.41	0.00 – 11.00
<i>Infant variables</i>			
Infant behavioural distress	60.12	36.25	6.00 – 134.00
Infant recovery time (sec)	56.81	65.19	0.00 – 295.00
Infant cortisol baseline	2.11	2.65	.08 – 19.81
Infant cortisol peak	3.66	2.94	.70 – 19.01
Infant cortisol increase (delta)	1.54	2.53	-8.44 – 12.99
Infant cortisol %increase	190.49	531.10	- 61.37 – 4360.00

Table 2 Correlation matrix for the maternal and infant variables (N=72)

	1	2	3	4	5	6	7	8	9	10
<i>Maternal Variables</i>										
1. MS normal	-									
2. MS after distress	.51**	-								
3. MI normal	-.39**	-.21^	-							
4. MI after distress	-.15	-.32**	.54**	-						
<i>Infant variables</i>										
5. Behavioural distress	-.17	-.31**	.15	.23^	-					
6. Recovery time (sec)	-.15	-.19	.06	.17	.65**	-				
7. Cortisol baseline	.12	.08	-.19	-.25*	.02	-.18	-			
8. Cortisol peak	.14	-.07	-.03	-.01	.30**	.25*	.55**	-		
9. CortΔ	.14	-.18	.15	.12	.24*	.31**	-.23	.55**	-	
10. Cortisol %increase	.00	-.11	.17	.22^	.27*	.45**	-.51**	.37**	.69**	-

Note: ** $p < .01$, * $p < .05$, ^ $p = .05-.06$

Relationship between maternal sensitivity and intrusiveness before and after infant distress

There were significant positive correlations between maternal sensitivity at normal interaction (MS_{normal}) and after distress (MS_{distress}) ($r = .51, p < .01$), and between maternal intrusiveness at normal interaction (MI_{normal}) and after distress (MI_{distress}) ($r = .54, p < .01$). Maternal sensitivity during normal interaction was negatively associated with maternal intrusiveness ($r = -.39, p < .01$), and maternal sensitivity after infant distress was negatively correlated with maternal intrusiveness after distress ($r = -.32, p < .01$).

Influence of infant distress on maternal sensitivity and intrusiveness

In order to investigate whether maternal sensitivity and intrusiveness changed as a result of their child's distress, the normal and post-stress scores were compared. A paired-samples t-test revealed that there was no significant difference between maternal sensitivity during normal interaction (Mean=10.44, $SD=2.38$) and after distress (Mean=10.63, $SD=2.51$), $t(71) = -.69, p=.49$. However, it was thought that there may be a different pattern of scores of MS_{normal} and $MS_{distress}$. More specifically, we expected maternal sensitivity to be relatively stable when the child is calm, but less so when the child is distressed. This was examined by investigating the effect of time on maternal sensitivity during normal interaction and after infant distress. A one-way repeated measures ANOVA with six MS_{normal} scores revealed no significant effect of time ($F(5,355) = .76, p=.58$). However, for $MS_{distress}$, a significant effect of time was revealed ($F(5,355) = 4.98, p<.01$). Pairwise mean comparisons between the scores at each time point were conducted with the Sidak adjustment for multiple comparisons. It was found that there was a significant difference between mean MS scores at 30 sec (Mean=1.87) and at 180 sec (Mean=1.57), $SE_{diff} = .09, p<.05$, and between mean scores at 120 sec (Mean=1.90) and 180 sec (Mean=1.57), $SE_{diff} = .07, p<.01$. These results suggest that there was a significant drop in sensitivity from the first 30 seconds to the last 30 seconds of interaction, as well as from the scores at 120 seconds to the last 30 seconds.

The pattern of maternal sensitivity scores across six 30-second intervals during normal interaction and after infant distress can be observed in Figure 1. The results suggest that general sensitivity may be relatively stable across time, whereas sensitivity after distress begins at a high level but drops significantly towards the end of the interaction.

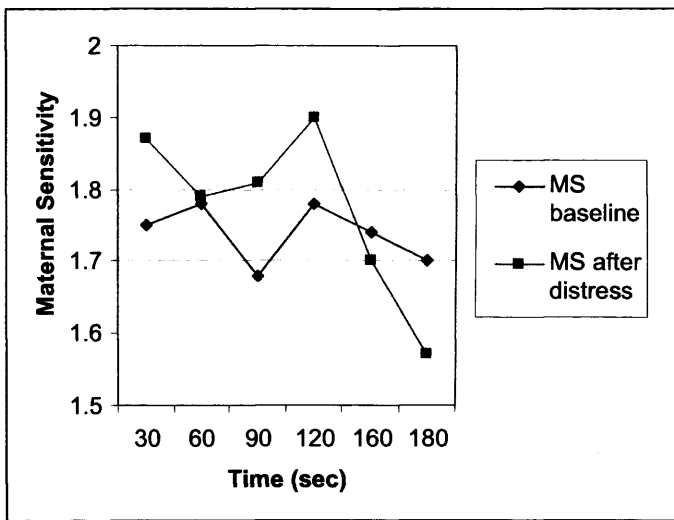


Figure 1. Maternal sensitivity at six 30-seconds intervals during normal interaction and after infant distress.

Mothers seem to be more sensitive in the first two minutes of interaction when the child is distressed compared to when the child is calm, but become less sensitive in the 3rd minute of interaction. A paired-samples t-test with Bonferroni correction comparing the first 120 seconds of baseline and post-stress interaction revealed no significant difference between MS scores in both conditions ($MS_{\text{normal}} = 1.77$, $SD = .41$, $MS_{\text{distress}} = 1.84$, $SD = .42$, $t = -1.28$, $p = .21$). A paired-samples t-test with Bonferroni correction comparing MS in the final 3rd minute of interaction also revealed no significant difference ($MS_{\text{normal}} = 1.72$, $SD = .46$, $MS_{\text{distress}} = 1.63$, $SD = .58$, $t = -1.30$, $p = .20$).

A possible reason for the drop in MS in the last minute of post-stress interaction could be because some infants needed a longer recovery time than others. This was tested by creating a low MS (0-1) and high MS (>1) group based on the mothers' post-distress MS scores in the last 30 seconds of interaction. An independent samples t-test revealed a significant difference in infant recovery between these groups ($MS_{\text{high}} = 1.59$, $SD = .74$, $MS_{\text{low}} = 1.12$, $SD = .85$, $t(2, 79) = 2.37$, $p < .05$). This

suggests that mothers who were less sensitive in the last minute of interaction had children who took longer to recover. This could be an explanation of the drop in sensitivity, as the last 30 seconds of interaction might have become overly demanding for the mothers whose infants were slow to recover.

In terms of maternal intrusiveness, a paired samples t-test revealed that there was a significant difference between intrusiveness during normal interaction (Mean=3.80, SD=3.34) and after infant distress (Mean=2.73, SD=2.39), $t(71) = 3.23$, $p < .01$, indicating that maternal intrusiveness decreased after the distress episode.

Predictors of maternal sensitivity and intrusiveness after distress

It can be seen in Table 2 that infant distress and maternal sensitivity after distress are negatively associated ($r = -.31$, $p < .01$), suggesting that greater distress is associated with lower sensitivity levels. A step-wise regression was conducted in order to investigate whether maternal and/or infant variables predicted maternal sensitivity after distress. The results are presented in Table 4. It can be seen that both maternal sensitivity during normal interaction and infant distress predicted maternal sensitivity after infant distress. Infant distress explained an additional 5% of variance in maternal sensitivity after distress after controlling for normal sensitivity. Higher levels of MS_{normal} were associated with higher levels of MS_{distress} , whereas higher levels of infant distress were associated with less maternal sensitivity after distress.

We next investigated whether infant distress moderated the influence of maternal sensitivity at baseline in predicting her sensitivity after infant distress. The effect of the interaction between MS_{normal} and infant distress was added as a predictor of maternal sensitivity after infant distress in step 3 of the multiple regression (Table 4). The interaction was not significant.

A step-wise regression was carried out to investigate whether MI_{normal} and infant distress predicted maternal intrusiveness after infant distress. The results are presented in Table 5. Only maternal intrusiveness during normal interaction emerged as a significant predictor of intrusiveness after distress.

Table 4: Summary of a step-wise regression analysis for variables predicting maternal sensitivity after infant distress (N=72)

Variable	B	SE	B	B
<i>Step 1:</i>				
MS normal	.54	.11		.51**
<i>Step 2:</i>				
MS normal	.49	.11		.47**
Infant behavioural distress	-.02	.01		-.23*
<i>Step 3:</i>				
MS normal	.48	.11		.45**
Infant behavioural distress	-.02	.01		-.22*
MS normal x Infant behavioural distress	.00	.00		.08

Note: for step 2 R^2 change = .05, F change (1, 69) = 5.02, $p < .05$; for step 3 R^2 change = .01, F change (1, 68) = .65, $p = .42$

** $p < .01$, * $p < .05$

Table 5: Summary of a step-wise regression analysis for variables predicting maternal intrusiveness after infant distress (N=72)

Variable	B	SE B	β
<i>Step 1:</i>			
MI normal	.41	.08	.54**
<i>Step 2:</i>			
MI normal	.39	.08	.52**
Infant behavioural distress	.01	.01	.15

Note: R^2 change = .02, F change (1, 69) = 2.23, p = .14

** p < .01, * p < .05

Infant Distress

A multiple regression was carried out to investigate maternal sensitivity and intrusiveness during normal interaction as predictors of infant distress. The results of the regression are presented in Table 6. Contrary to our hypothesis, neither maternal sensitivity nor intrusiveness emerged as significant predictors of infant distress.

Table 6: Summary of a multiple regression analysis for variables predicting infant distress (N=72)

Variable	B	SE B	β
MS normal	-2.04	1.96	-.13
MI normal	1.09	1.45	.10

Note: R^2 = .01, F (2, 71) = 1.33, p = .27

Infant Recovery

A multiple regression examined the predictive value of maternal characteristics (sensitivity and intrusiveness after distress) and infant distress on infant

recovery time. The results are presented in Table 7. Infant distress but not maternal behaviour was a significant predictor of recovery, with higher infant distress leading to longer recovery time.

Table 7: Multiple regression analysis for variables predicting infant recovery time (N=72)

Variable	B	SE B	β
MS after distress	.00	.03	.02
MI after distress	.01	.03	.03
Infant behavioural distress	.02	.00	.65**

Note: $R^2=.40$, $F(3, 71) = 16.47$, $p<.01$

** $p<.01$

Infant Cortisol Reactivity

After comparing infant baseline and peak post-stress cortisol concentrations, a Wilcoxon Signed Rank Test revealed that the stress episode resulted in an overall significant increase in cortisol in infants ($z = -5.85$, $p<.001$). The median score of cortisol concentration increased from 1.40 at baseline to 2.96 after distress. There were no differences between younger and older infants in cortisol reactivity ($t(2, 70) = .05$, $p=.96$).

None of the maternal characteristics were significantly associated with infant baseline cortisol levels (see Table 2). None of maternal characteristics were associated with Cortisol Δ . However, the association between maternal intrusiveness and child cortisol %increase approached significance ($r=.22$, $p=.06$). Therefore we entered it as a predictor of %increase while controlling for child behavioural distress (Table 8). It was found that only behavioural distress was a significant predictor of cortisol %increase.

Furthermore, contrary to previous research, it was found that infant behavioural distress and cortisol reactivity were significantly positively associated ($r=.24, p<.05$). Similarly, cortisol stress increase and behavioural recovery time were positively related ($r=.32, p<.05$ between recovery and Cortisol Δ and $r = .45, p<.01$ between recovery and cortisol %increase), with stronger cortisol stress response being associated with a longer recovery time.

Table 7: Multiple regression analysis for variables predicting infant cortisol %increase (N=72)

Variable	B	SE B	β
Child Behavioural distress	.00	.00	.24*
MI after distress	.03	.02	.17

Note: $R^2=.08$, $F(2, 71) = 3.99$, $p<.05$

* $p<.05$

Discussion

The present study had two main aims. First, to examine the association between maternal sensitivity and intrusiveness and their stability across qualitatively different emotional states of the infant. Second, to study the relationship between these maternal characteristics and infant distress reactivity and recovery in the first year of life.

As hypothesized, maternal sensitivity and intrusiveness were negatively associated, both during normal interaction and after infant distress, supporting the theory that greater sensitivity is associated with less intrusiveness across different emotional states of the infant (Fish & Stifter, 1995). We were also interested in the stability of these maternal characteristics across different emotional states of the child. In terms of maternal sensitivity, it was found that in the first year of life, sensitivity was stable between normal and distress conditions. However, maternal intrusiveness was found to be reduced after infant distress. This suggests that this characteristic is not stable across different emotional states of the infant and that in the present sample mothers became less intrusive following a distressing episode. These findings support the claim of McElwain and Booth-LaForce (2006) that some maternal characteristics are not stable across different emotional states of her child.

We were further interested in the stability of maternal sensitivity within each condition (i.e., normal play, recovery from distress). It was hypothesized that sensitivity would be relatively stable during normal interaction, since there are no emotional demands on the mother. However, we also expected that sensitivity would be less stable when the child was distressed, especially if the child fails to recover quickly. In support of this, we found that sensitivity during a 3 minute normal interaction was relatively stable, but that it dropped after distress from the first to the last 30 seconds of interaction. Specifically, mothers became less sensitive when they

had children who needed more time to recover. These findings suggest that maternal sensitivity may not be stable but could decrease in interactions with a distressed infant, especially when the child takes longer to recover. To our knowledge, this issue has not been investigated in previous research.

Overall, the findings of our first study suggest that maternal sensitivity and intrusiveness are not stable characteristics, but change as a result of different emotional states of the child. In the majority of previous studies maternal interactive behaviours were measured as global characteristics during lengthy interactions which would have included different emotional states of the child. Present findings and research by McElwain and Booth-LaForce (2006) and Leerkes et al. (2009) seem to indicate that a more detailed investigation of maternal characteristics is necessary, specifically one that differentiates between maternal sensitivity and intrusiveness during interaction with a normal and happy vs. a distressed child.

The second aim of present research was to investigate the influence of maternal sensitivity and intrusiveness on child emotional reactivity and recovery. It was hypothesized that more maternal sensitivity and less intrusiveness would be associated with less behavioural and cortisol distress reactivity of the child and a faster recovery. Unfortunately, these effects could not be confirmed.

In terms of behavioural reactivity, maternal characteristics during the normal interaction were not associated with child distress. The absence of an influence of maternal characteristics on child emotionality contradicts some findings of past research, where maternal interactive behaviour was found to influence child emotional reactivity (Pauli-Pott et al., 2004, Hane & Fox, 2004), however there were other studies who also did not find maternal care or attachment security to be associated with behavioural distress (Spangler & Schieche, 1998).

There was, however, an association between maternal sensitivity during the interaction after child distress and child emotional reactivity ($r = -.31$). Because of the design of present study it was not possible to make inferences about this relationship, as mother-child interaction took place after the child was distressed. However, the association between maternal sensitivity after child distress and child emotional reactivity can be investigated further in our longitudinal study (Chapter 7) where it will be possible to investigate whether maternal sensitivity after distress in the first year of life is associated with child distress and recovery across time (i.e., at two or three years of age).

We also investigated whether maternal sensitivity and intrusiveness during normal interaction and after distress were related to increase in cortisol. Contrary to predictions, a clear association was not found. We did find that maternal intrusiveness after distress was positively associated with percentage increase in cortisol, but this association was not significant after controlling of the behavioural distress. It should be noted that some past studies have also failed to find an association between maternal characteristics and infant cortisol reactivity. For example, Blair et al. (2006) and Albers et al. (2009) did not find maternal sensitivity to be predictive of infant cortisol reactivity in response to distress. Both studies, however, did find maternal sensitivity to have an impact on child cortisol recovery of distress, with infants of more sensitive mothers having faster cortisol recovery. This was not assessed in the present study, but it would be useful to investigate this aspect further in the study with children at 2 or 3 years, as recovery is an important part of stress experience, and as some studies indicate maternal characteristics may be more important for cortisol recovery from stress than cortisol reactivity (Blair et al., 2006; Albers et al., 2008).

However, in terms of *behavioural* recovery, we also failed to find an influence of maternal sensitivity and intrusiveness after distress on how quickly children recover from stress. This finding contradicts our hypothesis, and some previous findings, for example those by Haley and Stansbury (2003), who found maternal responsiveness during interaction to be associated with infant recovery from stress. However, relatively few studies looked at the influence of maternal characteristics on behavioural recovery from stress. Another study by Lewis and Ramsey (1999) also did not find maternal soothing behaviour to help child recovery from different stressors in the first year of life. Therefore future investigation of possible relationship between maternal characteristics and child behavioural recovery from stress is needed. For example these effects may arise later in childhood, which is addressed in Studies 2 and 3.

One possible reason for the lack of association between maternal characteristics during normal interaction and child reactivity and regulation in the present study may be the difference in the design of present distress episode with previous studies. In the present design children were faced with a distressing event in the absence of their mothers, while in the majority of past studies the mothers were present during similar fear provocation episodes, therefore making it a separation as well as novelty paradigm. Therefore, the mother was not present to support the child during stress. Another possible reason for the absence of a relationship between maternal characteristics and child distress is that maternal characteristics might become more important over time, as children get older.

Aside from the main hypotheses of this study, the present research also contributes to the study of the association between child emotional behaviour and cortisol reactivity. Contrary to findings of Ramsey and Lewis (2003), behavioural

reactivity and recovery were positively associated, and infant behavioural distress was moderately positively associated with cortisol stress response. A possible reason for the discrepancies with previous research could be the different nature of the stressors used. For example, whereas Ramsey and Lewis (2003) focused on inoculation stress, the present study looked at novelty stress and fear challenge.

The results of the present study do not show a clear role for maternal sensitivity in predicting child distress and recovery, even though there was a significant negative association between infant distress and maternal sensitivity after distress. It will be interesting to find out how stable these maternal and infant characteristics are across time, and whether their associations become stronger as the child gets older. We will examine these issues in our next studies.

In conclusion, this study investigated the relationships between maternal intrusiveness and sensitivity, and their stability and change within and across different emotional states of the infants. The absence of a clear influence of maternal characteristics on child emotional reactivity and recovery may be due to the design of the present study, or may reflect the fact that maternal characteristics become more important for the development of children's emotion reactivity and regulation over time. In the next study, we will investigate whether these maternal characteristics have become more important in how children cope with stress when they are in their second year of life.

**Study 2: Maternal sensitivity and intrusiveness and their influence on
child behavioural and physiological distress reactivity and recovery
in two-year-olds**

Method

Participants

Participants were 48 children (23 male, 25 female) and their mothers who also took part in Study A. Child age ranged from 16 to 30 months (Mean age= 23.1 months, $SD = 2.8$).

Of the initial sample of 72 children, 12 declined participation for a number of reasons (for example, because they had moved away, mother going back to work, mother having another child) while twelve other children were too young to be tested. We checked whether the sample that dropped out was in any way different from the current sample. No differences were found between participating and non-participating mothers with respect to ethnicity ($\chi^2 (1) = .11, p = .74$), marital status ($\chi^2 (1) = .07, p = .78$) and age ($t (2, 64) = 1.02, p = .31$). We also found that there was no difference in emotional reactivity in Year 1 between those children who returned to the study in Year 2 and those that did not ($t (2, 70) = -1.14, p = .25$).

Measures

The procedure and measures used in the present study were identical to the ones used in the first study, with the exception of a small change in the collection of cortisol. The inter-rater reliability (Cohen's kappa) between two trained coders on 12% of the sample was .73 for maternal sensitivity, and .78 for maternal intrusiveness. In terms of child behavioural distress, intra-correlation coefficients between 4

independent coders ranged between .70 and .99 across the behavioural variables for 11% of the sample.

Additional cortisol measures

Some studies have shown that there is great variation in the timing of child cortisol response to stress (Ramsey & Lewis, 2003; Alberts et al., 2008). It is generally considered that the peak cortisol response takes place approximately 20 minutes following exposure to a stressor. However, studies by Ramsey and Lewis (2003) and Albers et al. (2008) found that there is great variability in cortisol response to stress, with some children having a peak response earlier than 20 minutes, while others may have it as late as 30-35 minutes after the distress episode. We therefore included an extra measure of cortisol taken 35-40 minutes after termination of the stressor in addition to the timing of the four samples described in Study 1 (with one at 5 and 20 minutes after distress) in order to capture the peak stress response for those children who may have a later response.

Missing Data

Before starting data analysis, variables were examined for missing values. Missingness was small for some variables (2.1% for maternal and intrusiveness after distress) and moderate for others (10.5% for maternal sensitivity and intrusiveness during normal interaction) and higher for others (18.8% for cortisol baseline concentrations and 22.9% for cortisol peak). The missingness of data was tested to be Missing Completely at Random (MCAR; Acock, 2005) with Little's MCAR test, which was found to be significant $\chi^2(22) = 75.55, p < .01$, indicating that the data is *not* MCAR. However, because of the high percentage of missing values for cortisol

concentrations, we tested the data to be MCAR without the cortisol missing values, which was not significant $\chi^2(41) = 41.12, p = .47$, indicating that the missing data for maternal variables is MCAR. This shows that there was no systematic missing pattern in the dataset and missing values were randomly distributed across observations.

For missing cortisol data, it could be hypothesized that distress levels of the child during the procedure could have an impact of child willingness to engage in cortisol collection. This was tested by comparing children who did have cortisol samples to those who did not on dimensions of behavioural distress and recovery. It was found that there were no difference between children from whom it was and it was not possible to collect cortisol on dimensions of behavioural distress ($t(2, 46) = -.23, p = .82$) and behavioural recovery ($t(2, 46) = .58, p = .57$).

Data imputation was used in order to retain the sample size of $N=48$, because listwise deletion would decrease the sample to $N=38$. Missing data imputation was identical to the one used in Study 1.

There were two children with cortisol outliers, with values more than three standard deviations away from the mean for both baseline and peak cortisol. Therefore, these values were replaced by values of three standard deviations above the mean to preserve the high-end of the original outlier value.

Statistical Analysis

Normality of distribution of the variables was checked in a histogram with a normal curve. The moderately positively skewed variables of maternal intrusiveness during normal interaction and after distress, cortisol increase (Cortisol Δ) and cortisol %increase underwent natural log (ln) transformation, which is recommended for this type of skewness (Tabachnick & Fidel, 2007), after which normal distribution was

attained for these variables. The non-transformed variables were used in the descriptive statistics table and the frequency distributions, but the transformed variable was used in all analysis of the variable. In order to test the unique contribution of infant distress levels in addition to maternal behaviour at baseline on maternal behaviour after distress, step-wise multiple regression was used.

Since some of the children at the age of 24 months attend day care, we wanted to check that children do not differ in their emotional reactivity/regulation based on the time they spend in day care, since differences in cortisol concentration have been found in previous studies (Ouellet-Morin et al., 2010). Children were separated into three groups based on how many hours of day care they attend: low (under 10 hours), medium (between 11 and 20 hours) and high (over 21 hours). Information on day care attendance was available for 41 children. A single measures ANOVA revealed no significant difference between the three groups in distress reactivity ($F(2, 38) = .80$, $p = .46$), recovery time ($F(2, 38) = .51$, $p = .60$) or cortisol reactivity ($F(2, 38) = 1.22$, $p = .31$). These results suggest that children do not differ in how they coped with stress as a function of how many hours of day care they attend. Furthermore, there were no differences between boys and girls on any of the maternal, behavioural or cortisol measures.

In order to calculate cortisol increase (Cortisol Δ), the mean of the two baseline values was subtracted from the peak cortisol value. As anticipated, there was considerable variation in the timing of cortisol peak response, where 2 children had no increase in cortisol, 7 children showed their peak response at 5 minutes after distress, 8 children had their peak at 20 minutes after distress and 8 children had their peak at 35 minutes after distress. For remaining 12 children the last sample of cortisol (35 minutes after distress) was not available for reasons of refusal of the child, or the

study running too long for the mother, and the dyad having to leave. These 12 children had their peak at 20-minutes post-distress; however it is possible that they would have had it at 35-minute sample. For the purposes of analysis the 20-minute peak was used to calculate cortisol increase for this sub-sample. Furthermore, similarly to Study 1, we used these measures to calculate cortisol %increase in addition to Δ Cortisol.

Results

The descriptive statistics of the sample in Table 1 and the correlations in Table

2. The frequency distributions of the variables in the study can be seen in Appendices 8- 11.

Table 1 Descriptive statistics for the child and maternal variables (N=48)

Variables	Mean	SD	Range
<i>Maternal Variables</i>			
MS normal	11.50	1.91	5.00 - 15.00
MS after distress	12.07	2.17	5.00 – 17.00
MI normal	1.77	2.88	0.00 – 17.00
MI after distress	.79	1.56	0.00 – 7.00
<i>Child variables</i>			
Child behavioural distress	78.21	42.76	3.00 – 153.00
Child recovery time (sec)	107.83	99.60	0.00 – 320.00
Child cortisol baseline	1.45	.80	.29 – 3.71
Child cortisol stress peak	4.32	3.94	.61 – 19.01
Child Cortisol Δ	3.15	4.77	-.55 – 15.86
Child cortisol %Increase	234.08	376.58	-18.26 – 2373.29

Table 2 Correlation matrix for the maternal and child variables (N=48)

	1	2	3	4	5	6	7	8	9
<i>Maternal Variables</i>									
1. Maternal sensitivity normal	-								
2. Maternal sensitivity after distress	.50**	-							
3. Maternal intrusiveness normal	-	-.31*	-						
4. Maternal intrusiveness after distress	.49**	-	.58**	-					
	.51**	.40**							
<i>Child variables</i>									
5. Child behavioural distress	-	-	.02	.03	-				
6. Child recovery time (sec)	.38**	.39**							
	-.14	-.31*	-.13	-.16	.33*	-			
7. Child cortisol baseline	-.25	-	.13	.17	.09	.00			
8. Child CortisolΔ	-.33*	-.26^	.13	.07	.31*	.23	-		
9. Child cortisol %increase	-.26	-.22	.12	.09	.23	.21	-.11	.83**	-

Note: ** $p < .01$, * $p < .05$, ^ $p = .08$

Relationship between maternal sensitivity and intrusiveness before and after infant distress

There were significant positive correlations between maternal sensitivity during normal interaction (MS_{normal}) and after distress ($MS_{distress}$) ($r = .53$, $p < .01$), and between maternal intrusiveness during normal interaction (MI_{normal}) and after distress ($MI_{distress}$) ($r = .64$, $p < .01$). As predicted, MS_{normal} was significantly negatively correlated with MI_{normal} ($r = -.52$, $p < .01$), and $MS_{distress}$ was negatively correlated with $MI_{distress}$ ($r = -.45$, $p < .01$).

Influence of infant distress on maternal sensitivity

A paired-samples t-test revealed that maternal sensitivity during normal interaction (Mean = 11.51, $SD = 1.92$) was marginally lower than sensitivity after distress (Mean = 12.07, $SD = 2.17$), $t(47) = -1.89$, $p = .07$, suggesting that mothers

became more sensitive after the distress episode. We were also interested in whether there were differences in maternal sensitivity between the normal and distress conditions in the first and second halves of interaction. It was hypothesized that maternal sensitivity after distress would be greater immediately after distress, because the mothers would feel the need to comfort their child. A paired samples t-test revealed no difference in maternal sensitivity scores in the first 90 seconds of interaction between the normal (Mean= 5.89, $SD=.95$) and distress conditions (Mean= 6.10, $SD= 1.17$, $t(47) = -1.20$, $p=.24$). However, a paired-samples t-test revealed that there was a marginally significant difference in maternal sensitivity between these conditions in the final 120 sec ($MS_{\text{normal}} = 5.63$, $SD= 1.30$, $MS_{\text{distress}} = 5.99$, $SD=1.21$, $t(47) = -1.79$, $p=.08$) with more maternal sensitivity after distress in the last 120 seconds of the interaction.

Furthermore, we were interested in patterns of change in maternal sensitivity and in order to investigate this, the sensitivity scores at normal interaction and after distress were separated into high (score > 11) or low (score ≤ 11) based on the frequency distribution (see Appendix 4). Cross-tabulation analysis revealed that 58% of mothers in the low sensitivity group during normal interaction (40%) remained low, while 42% increased in their sensitivity. Out of the mothers who were in the high sensitivity group during normal interaction (60%), 76% remained high, while 24% decreased in sensitivity after distress.

We were interested in the patterns of maternal sensitivity within the normal and post-distress interactions. As in the 1st study, we expected that MS_{normal} would be relatively stable, but MS_{distress} would be less stable and could drop with time.

A repeated measures ANOVA revealed that there was no significant effect of time on MS_{normal} , $F(5,325) = 1.17$, $p=.32$ or MS_{distress} , $F(5,235) = .58$, $p=.71$. These

results suggest that when the child is about 2 years old, maternal sensitivity is relatively stable across different emotional states of the child. The patterns of maternal sensitivity across each interaction episode can be observed in Figure 1.

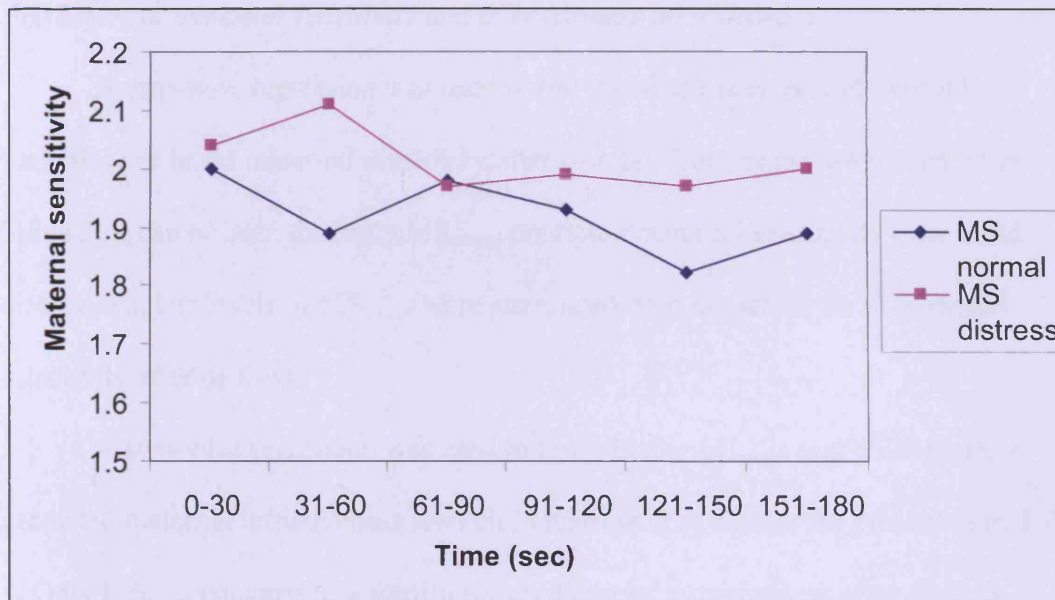


Figure 1: Pattern of maternal sensitivity across the 3-minute interaction at baseline and after child distress.

Influence of infant distress on maternal intrusiveness

A paired-samples t-test revealed that MI_{normal} (Mean= .31, $SD=.31$) was significantly higher than MI_{distress} (Mean= .16, $SD= .25$), $t(47) = 3.91, p < .001$, suggesting that mothers became less intrusive after a distress episode. We were further interested in the percentages of mothers who changed in their intrusiveness levels. Based on the frequency distributions (see Appendix 5), maternal intrusiveness during normal interaction and after distress were separated into high (score > 2) or low (scores ≤ 2). Cross-tabulation analysis revealed that that 90% or mothers who were low in intrusiveness during normal interaction (63%) remained low, while 10%

increased in intrusiveness. Of the mothers high in intrusiveness at baseline (37%), 39% remained high, whereas 61% decreased in intrusiveness.

Predictors of maternal sensitivity and intrusiveness after distress

A step-wise regression was used to test whether maternal and/or child variables predicted maternal sensitivity after distress. The results are presented in Table 3. It can be seen that only MS_{normal} predicted maternal sensitivity after child distress. Higher levels of MS_{normal} were associated with higher levels of maternal sensitivity after distress.

A step-wise regression was used to test whether MI_{normal} and child distress predicted maternal intrusiveness after child distress. The results are presented in Table 4. Only MI_{normal} emerged as a significant predictor of intrusiveness after distress. These results suggest that at 24 months, the child's emotional state does not seem to have a significant impact on maternal behaviour.

Table 3: Summary of a step-wise regression analysis for variables predicting maternal sensitivity after infant distress (N=48)

Variable	B	SE	B	β
<i>Step 1:</i>				
MS normal	.56	.14		.50**
<i>Step 2:</i>				
MS normal	.46	.15		.41**
Child distress	-.01	-.01		-.23

Note: R^2 change = .05, F change (1, 45) = 3.00, p = .09

** p < .01

Table 4: Summary of a step-wise regression analysis for variables predicting maternal intrusiveness after child distress (N=48)

Variable	B	SE B	B
<i>Step 1:</i>			
MI normal	.47	.10	.58**
<i>Step 2:</i>			
MI normal	.47	.10	.58**
Child distress	.00	.00	.02

Note: R^2 change = .00, F change (1, 45) = .03, p = .87

** p < .01

Child Distress

A multiple regression was used to assess the influence of maternal sensitivity and intrusiveness during normal interaction on child distress. The results of the regression are presented in Table 5. Maternal sensitivity emerged as a significant predictor of child distress. It was found that lower levels of sensitivity were associated with higher distress of the child.

Table 5: Summary of a multiple regression analysis for variables predicting child distress (N=48)

Variable	B	SE B	β
MS normal	-10.99	3.43	-.49**
MI normal	-31.04	21.25	-.23

Note: R^2 = .15, F (2, 45) = 5.11, p < .05

Child Recovery

A multiple regression was used to investigate the influence of maternal characteristics (sensitivity and intrusiveness after distress) and child distress on child

recovery. Child behavioural distress was entered as a predictor in step 1 of the regression analysis in order to investigate its unique contribution to child recovery and to investigate whether its influence would remain after maternal characteristics were entered in step 2 of the analysis. The results are presented in Table 6. In step 1, child distress was a significant predictor of recovery. However, when maternal sensitivity and intrusiveness of the post-stress interaction were entered as predictors, the influence of child distress was reduced to not significant. Maternal sensitivity and maternal intrusiveness after distress emerged as significant predictors of infant recovery. As hypothesized, it was found that higher levels of maternal sensitivity led to faster recovery. Perhaps surprisingly, it was also found that higher levels of maternal intrusiveness led to faster recovery.

Table 6: Summary of a multiple regression analysis for variables predicting child recovery time (N=48)

Variable	B	SE B	β
<i>Step 1:</i>			
Child behavioural distress	.78	.32	.33*
<i>Step 2:</i>			
Child behavioural distress	.46	.34	.20
MS after distress	-15.78	7.25	-.34*
MI after distress	-20.49	9.09	-.30*

Note: R^2 change = .11, F change (2, 44) = 3.22, p = .05

* p < .05

Child Cortisol Reactivity

After comparing child baseline and peak post-stress cortisol concentrations, a Wilcoxon Signed Rank Test revealed that the stress episode resulted in an overall significant increase in cortisol in children ($z = -5.67$, $p < .001$). The median score of

cortisol concentration increased from 1.24 ng/ml at baseline to 2.86 ng/ml after distress.

We did not find an association between maternal sensitivity during normal interaction and baseline cortisol levels. There was a significant negative correlation between maternal sensitivity after child distress and baseline cortisol ($r = -.34, p < .05$) however, because of the study design we could not imply causality of this association.

Maternal sensitivity during normal interaction was positively associated with child Cortisol Δ ($r = -.33, p < .05$), indicating that higher levels of sensitivity during normal interaction were associated with lower cortisol reactivity. The correlation between sensitivity after distress and child cortisol reactivity approached significance ($r = -.26, p = .08$), suggesting that higher sensitivity after distress was associated with a lower cortisol stress response. Intrusiveness before or after distress was not associated with cortisol reactivity. We therefore entered maternal sensitivity before and after distress as predictors of Cortisol Δ , while controlling for child behavioural distress. The results are presented in Table 7. It was found that maternal characteristics were no longer associated with child Cortisol Δ . Similarly, none of the maternal characteristics were found to be associated with child cortisol %increase.

Table 7: Summary of a multiple regression analysis for variables predicting child recovery time (N=48)

Variable	B	SE B	β
<i>Step 1:</i>			
Child behavioural distress	.00	.00	.31*
<i>Step 2:</i>			
Child behavioural distress	.00	.00	.19
MS normal	-.04	.03	-.21
MS after distress	-.01	.02	-.07

Note: R^2 change = .06, F change (2, 44) = 1.48, p = .24

* p < .05

Discussion

Consistent with Study 1, this study had two main aims: first, to investigate the association between maternal sensitivity and intrusiveness and their stability across different emotional states of 2-year old children. Second, we wanted to investigate the influence of maternal sensitivity and intrusiveness on child coping with stress.

In terms of the first aim, as hypothesized, maternal sensitivity and intrusiveness were negatively associated during a normal, playful interaction and after distress, suggesting that higher sensitivity is associated with lower intrusiveness across different emotional states of the child in the 2nd year of life. Further, we were interested to find out whether these maternal characteristics were stable across different emotional states of the child. With respect to maternal sensitivity, it was found that, on average, mothers became more sensitive in response to a distressed state of the child than during a normal interaction. One possible reason for this finding is that mothers become more attentive and sensitive to their children when they are distressed. Research by McElwain and Boothe-LaForce (2006) and Leerkes et al. (2009) has suggested the necessity for a clarification of the relationship between maternal sensitivity during child distress and non-distress, and the present study adds to past research in finding that sensitivity is not stable but differs depending on the emotional state of the child. This could have important theoretical and methodological implications for the study of maternal sensitivity. It has been suggested that sensitivity to distress may be a more important contributor to the development of attachment security (McElwain & Booth-LaForce, 2006) and child emotional development (Leerkes et al., 2009) than sensitivity during normal, non-emotional interactions, making this an important target for future studies.

Differences were also found between maternal intrusiveness during normal interaction and after distress, with mothers being more intrusive during normal

interaction than when their child was recovering from distress. The results indicate that in the present sample, on average, mothers responded with more sensitive and less intrusive behaviour towards their children after their distress. These results support the proposal that maternal behaviour towards the child is not fixed but modulated by the emotional state of her child.

Maternal sensitivity during normal interaction and after distress was found to be relatively stable within these periods of interaction. It was hypothesized that sensitivity during normal interaction would be stable, whereas it could be less stable and go down when the child continued to remain emotionally distressed and the interaction was more challenging to the mother. However, this effect was not found; sensitivity after distress was found to remain stable during their interaction. These results are in contrast with the findings of Study 1, in which we found that maternal sensitivity decreased during the post-distress interaction. A possible reason for this finding could be that as children get older mothers encounter different emotional states in their child, to which they adapt, and their behaviour ultimately attains stability across different states.

The second aim of present research was to investigate the influence of maternal sensitivity and intrusiveness on child emotional reactivity and recovery. It was hypothesized that greater sensitivity and less intrusiveness would be predictive of less distress of the child and a faster recovery. As hypothesized, it was found that maternal sensitivity at baseline was predictive of child distress, with more sensitive mothers having less distressed children. However, contrary to prediction, maternal intrusiveness was not found to influence child distress. It was also found that maternal sensitivity during normal interaction was negatively associated with child's cortisol stress response, with more sensitive mothers having children whose cortisol levels

increased less during stress exposure. However, this association was not confirmed in a regression analysis.

These associations between maternal sensitivity and reactivity found in the present study support findings from some previous studies (Hane & Fox, 2004; Pauli-Pott et al., 2004; Blair et al., 2008) in which maternal sensitivity was found to be associated with less emotional reactivity in the child. Importantly, the present study combined observed and physiological distress measures of the child, and found that maternal sensitivity during normal interaction was related to both aspects of stress experience. Past studies have been inconsistent in their findings of the influence of maternal sensitivity on child behavioural and physiological distress, with some studies finding only an association for child behavioural distress but not for cortisol reactivity, or only for cortisol recovery but not for behavioural reactivity (Albers et al., 2008). Therefore our findings contribute to previous research in providing evidence that during the second year of life more sensitive mothers tend to have less distressed children.

The present findings support the hypothesis that sensitive parenting buffers the child from strong reactions to novel and potentially distressing situations (Pauli-Pott et al., 2004). With high maternal sensitivity, children experience reliable and appropriate maternal behaviour, which leads to feelings of security and self-efficacy for the child, allowing the child to explore novel environments without being restrained by too much anxiety. Furthermore, it has been suggested that when the child is comforted by sensitive parents when distressed, s/he would be expected to be less frightened or distressed during new distressing situations, because the child has learned to feel safe, secure and comforted in previous interactions with their caregiver (Gilissen et al., 2007).

We were also interested in the influence of maternal sensitivity and intrusiveness after distress on the child's rate of recovery from distress. It was hypothesized that greater maternal sensitivity after the distress episode would lead to faster recovery, whereas greater intrusiveness would be associated with slower recovery. We were able to confirm that greater maternal sensitivity was associated with faster child recovery. This finding is in agreement with findings by Hayley and Stansbury (2003) who found that 6-months-old infants of more responsive mothers had faster behavioural recovery from distress. However, contrary to our expectation, we also found that greater maternal intrusiveness was associated with faster recovery after distress. One possible reason could be that even though there was an unexpected negative association between intrusiveness and regulation, intrusiveness scores were on average very low (with an average of about 65% of mothers having a score of 0 for intrusiveness). It is suggested that low levels of intrusiveness are acceptable and could be beneficial for child recovery from distress. This can especially be applied to times after an episode of distress, where low to moderate levels of intrusiveness could reflect the mother taking initiative and actively engaging in attempting to calm her child down or distract them from distress. Overall, the findings that more sensitive mothers have children who recover faster adds further to the hypothesis that sensitive parenting is associated with less distress reactivity in children and a better ability to cope with distress.

Overall, maternal behaviour seemed to have a clearer effect on child emotional behaviour at 2 than at 1 year of age. Because the design of the two studies was identical, it can be argued that the influence of the mother in year 2 seems more apparent than in year 1. However, this does not imply that early maternal characteristics are not important for the child's emotional development, as maternal

sensitivity and intrusiveness in the 1st year of life may still have an effect on emotional development later in childhood, for example when the children are 2 or 3 years old. This question will be addressed in our longitudinal study presented in Chapter 7.

Aside from the main hypothesis of this study, the present research also adds to the study of the relation between child behavioural and physiological coping with stress. In terms of behavioural reactivity and recovery, it was found that the two constructs were moderately positively associated, which is in contrast to findings by Ramsey and Lewis (2003). Our findings are also in accordance with those of Study 1 when children were one year old, even though the associations at age two were slightly weaker. Some researchers have proposed that even though stress reactivity and recovery are separate constructs, they are inevitable intertwined, since reactivity affects the need for regulation, and regulation influences reactivity levels (Glogglar & Pauli-Pott, 2008; Calkins & Johnson, 1998). The current results support this claim.

Furthermore, again unlike the findings of Ramsey and Lewis (2003), we found that child behavioural and cortisol distress reactivity were positively associated. These findings are in agreement with those in Study 1, where similar associations were found. The possible reason for discrepancies with some previous research could be the different nature of stressors used, since research by Ramsey and Lewis (2003) focused on response to inoculation, whereas present study looked at the response to novelty and emotional challenge.

To summarise, the study indicates that maternal interactive behaviours in the child's second year of life vary as a result of the emotional state of the child. Specifically, maternal sensitivity increases and maternal intrusiveness decreases when the child is distressed. These findings suggest that sensitive maternal care giving

behaviour is not stable across different emotional states of the child. Secondly, the present findings also support the hypothesis that sensitive maternal care is associated with less distress (both behavioural and hormonal) and a faster recovery in children. These findings could have important implications because in this sample of relatively stable, middle-class families with moderately highly sensitive mothers, minor fluctuations in maternal sensitivity were found to have a significant impact on their children's emotionality and stress response. The next step in this chapter is to investigate whether the associations found at two years of age continue to be present when children are three years old. Study 3 further investigates the relationships between maternal characteristics and child reactivity and regulation at our final time point in early childhood.

Study 3: Maternal sensitivity and intrusiveness and their influence on child behavioural and physiological distress reactivity and recovery in three-year-olds

Participants

Participants were 38 children (20 male, 18 female) and their mothers who also took part in Studies 1 and 2. Child age ranged from 33 to 42 months (Mean age= 37.1 months, $SD = 2.4$).

Of the sample in Study 2 of 48 children, 5 mothers declined participation for a number of reasons (e.g., moved away, going back to work, having another child), while 5 other children were too young to be tested. We checked whether the sample that dropped out was in any way different from the current sample. No differences were found between participating and non-participating mothers with respect to ethnicity, marital status and age. We also found that there was no difference in emotional reactivity in Year 2 between those children who returned to the study in Year 3 and those who did not ($t(2, 46) = .25, p = .80$)

Measures

The procedure and measures used in the present study were identical to the ones used in Study 2. However, the last cortisol sample collected in Study 2 (35-40 min. after distress) was used in the present study as a measure of cortisol recovery, since the majority of children showed dampening of cortisol response (see below). The inter-rater reliability (Cohen's kappa) between two trained coders on 12% of the sample was .72 for maternal sensitivity, and .74 for maternal intrusiveness. In terms of child behavioural distress, intra-correlation coefficients between 4 independent coders ranged between .70 and .99 across the behavioural variables for 11% of the sample.

Missing Data

Before starting data analysis, variables were examined for missing values. There were missing values for both maternal and child variables: 15.8% for maternal sensitivity and intrusiveness during normal interaction, 7.9% for sensitivity and intrusiveness after distress, 7.9% for cortisol baseline, 10.5% for first post-stress cortisol sample (5 min. after distress), 7.9% for second post-stress cortisol sample (20 min. after distress) and 13.2% for cortisol recovery sample (40 min. after distress). The missingness of data was tested to be Missing Completely at Random (MCAR; Acock, 2005) with Little's MCAR test, which was found to be non-significant $\chi^2(63) = 71.05, p=.23$, indicating that the data is MCAR, therefore there is no systematic missingness pattern.

Data imputation was used in order to retain the sample size of $N=38$, because listwise deletion would decrease the sample to $N=23$. Missing data imputation was identical to the one used in Studies 1 and 2.

Statistical Analysis

Normality of distribution of the variables was checked in a histogram with a normal curve and skewness assessment. The moderately positively skewed variables of maternal intrusiveness during normal interaction and after distress, baseline cortisol levels, cortisol peak response, cortisol %increase, cortisol decrease and cortisol %decrease underwent natural log (ln) transformation, which is recommended for this type of skewness (Tabachnick & Fidel, 2007), after which normal distribution was attained for these variables. The non-transformed variables were used in the descriptive statistics table and the frequency distributions, but the transformed variable was used in all further analyses. In order to test the unique contribution of

infant distress levels in addition to maternal behaviour at baseline on maternal behaviour after distress, step-wise multiple regression was used.

Since some of the children at the age of 3 attend day care, we wanted to check that children do not differ in their emotional reactivity/regulation based on the time they spend in day care, since differences in cortisol concentration as a result of day care attendance have been found (Ouellet-Morin et al., 2010). Children were separated into three groups based on how many hours of day care they attend: low (less than 10 hours), medium (between 11 and 20 hours) and high (more than 21 hours). Information on day care attendance was available for 35 children. A single measures ANOVA revealed no significant difference between the three groups in distress reactivity ($F(2, 34) = .36, p = .70$), recovery time ($F(2, 34) = .09, p = .92$) or cortisol reactivity ($F(2, 34) = 2.9, p = .08$). These results suggest that children do not differ in how they coped with stress as a function of how many hours of day care they attend. Furthermore, there were no differences between boys and girls on any of the maternal, behavioural or cortisol measures.

In order to calculate cortisol increase (Cortisol Δ), the mean of the two baseline values was subtracted from the peak cortisol value. We also looked at the %increase in cortisol levels from baseline to the peak post distress value, which is calculated by subtracting cortisol baseline value from the peak response, dividing the outcome by the original baseline value and multiplying that outcome by 100. These two measures allow us to investigate cortisol increase while taking the baseline levels of cortisol into account. Higher cortisol delta and %increase indicate greater cortisol reactivity (Ramsay & Lewis, 2003).

Novel measure of cortisol recovery

In Year 3 we were able to obtain a sample of cortisol about 40 minutes after distress. Ramsay and Lewis (2003) proposed that cortisol response begins to dampen between 30 to 40 minutes after challenge. Therefore we refer to this sample as 'recovery'. Cortisol recovery from distress was calculated by subtracting cortisol recovery values from the peak values, with higher difference scores indicating better recovery. We also calculated %decrease in cortisol from peak to recovery, where higher percentage indicates better recovery.

Results

The descriptive statistics of the sample in Table 1 and the correlations in Table

2. The frequency distributions of the variables in the study can be seen in Appendices 12- 15.

Table 1 Descriptive statistics for the child and maternal variables (N=38)

Variables	Mean	SD	Range
<i>Maternal Variables</i>			
MS normal	10.86	2.34	6.00 - 17.00
MS after distress	11.71	2.00	7.00 – 17.00
MI normal	1.56	2.04	0.00 – 8.00
MI after distress	.67	1.67	0.00 –10.00
<i>Child variables</i>			
Child behavioural distress	79.96	41.72	4.00 – 162.00
Child recovery time (sec)	58.65	71.40	0.00 – 300.00
Child cortisol baseline	1.29	1.26	.10 – 7.72
Child cortisol stress peak	2.80	1.80	.48 – 7.78
CortisolΔ	1.49	1.94	-4.05 – 7.17
Child cortisol %increase	254.01	428.32	-60.00 – 2270.00
Child cortisol decrease from peak	.98	1.11	0.00 – 5.27
Child cortisol %decrease from peak	161.55	366.68	0.00 – 1800.00

Table 2 Correlation matrix for the maternal and child variables (N=38)

	1	2	3	4	5	6	7	8	9	10	11
<i>Maternal Variables</i>											
1. Maternal sensitivity normal	-										
2. Maternal sensitivity after distress	.47**	-									
3. Maternal intrusiveness normal	-.53**	-.20	-								
4. Maternal intrusiveness after distress	-.10	-.27	.39*	-							
<i>Child variables</i>											
5. Child behavioural distress	-.05	-.06	.11	.25	-						
6. Child recovery time (sec)	.03	.13	.15	.18	.79**	-					
7. Child cortisol baseline	-.12	-.27	-.17	-.17	.18	.05	-				
8. Child cortisol delta	-.02	.10	-.15	-.03	.11	-.03	-.35*	-			
9. Child cortisol %increase	.10	.16	.04	-.02	.16	.24	-.52**	.76**	-		
10. Child cortisol decrease from peak	-.10	-.09	.02	-.06	.27	.21	.25	.25	.14	-	
11. Child cortisol %decrease from peak	-.14	.03	.12	-.14	.00	-.01	.13	-.18	-.26	.63**	-

Note: ** $p < .01$, * $p < .05$

Relationship between maternal sensitivity and intrusiveness before and after infant distress

As in year 1 and year 2, there were significant positive correlations between maternal sensitivity at normal interaction (MS_{normal}) and after distress (MS_{distress}) ($r = .47, p < .01$), and between maternal intrusiveness at normal interaction (MI_{normal}) and after distress (MI_{distress}) ($r = .39, p < .05$). Maternal sensitivity during normal interaction was negatively associated with maternal intrusiveness ($r = -.53, p < .01$). However, maternal sensitivity after infant distress was not significantly associated with maternal intrusiveness after distress ($r = -.27, p = .10$).

Influence of infant distress on maternal sensitivity and intrusiveness

In order to investigate whether maternal sensitivity and intrusiveness changed as a result of their child's distress, the normal and post-stress scores were compared. A paired-samples t-test revealed that maternal sensitivity increased significantly from normal interaction (Mean=10.87, $SD=2.34$) to distress (Mean=11.71, $SD=2.00$), $t(38) = -2.32, p < .05$. In addition, we wanted to investigate whether the patterns of maternal sensitivity during normal interaction and after child distress were different. We expected maternal sensitivity to be relatively stable when the child was calm, but less so when the child was distressed. This was examined by investigating the effect of time on maternal sensitivity during normal interaction and after distress. Contrary to prediction, a one-way repeated measures ANOVA with six MS_{normal} scores revealed a significant effect of time ($F(5,185) = 5.38, p < .001$) during normal interaction. Pairwise mean comparisons between the scores at each time point were conducted with the Sidak adjustment for multiple comparisons. It was found that there was a

significant difference between mean MS scores at first 30 sec (Mean=2.00) and at 150 sec (Mean=1.54), $SE_{diff} = .08$, $p < .01$, and between mean scores at 150 sec (Mean=1.54) and 180 sec (Mean=1.93), $SE_{diff} = .09$, $p < .01$. These results suggest that there was a drop in maternal sensitivity from the first 30 seconds to the 150 seconds of interaction, with a final increase in sensitivity from the 150 seconds to the last 180 seconds time point.

Similarly, for $MS_{distress}$, a significant effect of time was revealed ($F(5,185) = 4.94$, $p < .001$). Pairwise mean comparisons between the scores at each time point were conducted with the Sidak adjustment for multiple comparisons. It was found that there was a significant difference between mean MS scores at 30 sec (Mean= 2.20) and at 60 sec (Mean= 2.05), $SE_{diff} = .11$, $p < .05$, and between mean scores at 30 sec (Mean=2.20) and 150 sec (Mean=1.88), $SE_{diff} = .10$, $p < .05$, as well as between the mean scores at 30 seconds and 180 seconds (Mean= 1.77) $SE_{diff} = .11$, $p < .01$. These results suggest that there was a significant continuous drop in sensitivity from the first 30 seconds through to the end of the interaction following distress. The pattern of maternal sensitivity scores across six 30-second intervals during normal interaction and after infant distress can be observed in Figure 1.

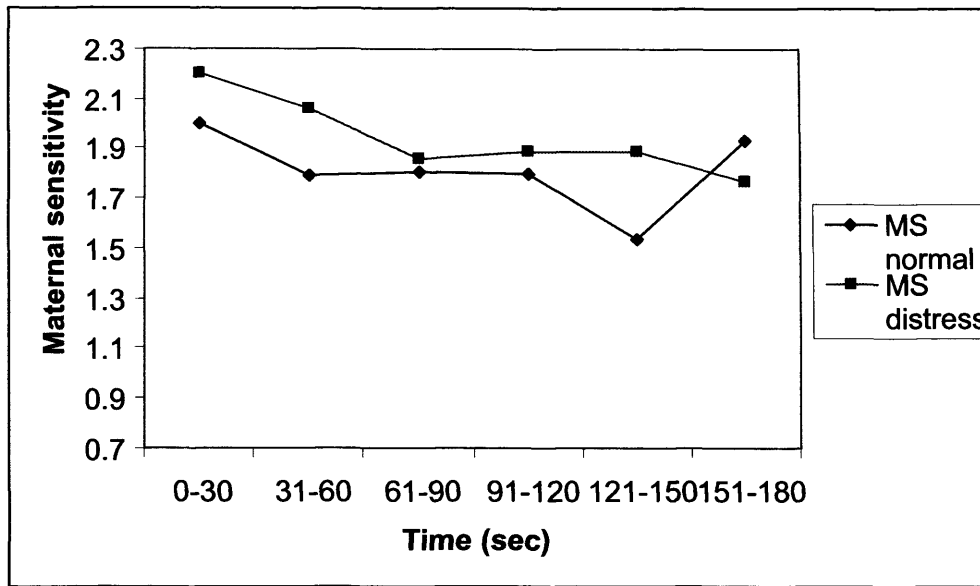


Figure 1: Pattern of maternal sensitivity across the 3-minute interaction during normal interaction and after child distress

A paired-samples t-test revealed that MI_{normal} (Mean= 1.57, $SD=2.03$) was significantly higher than $MI_{distress}$ (Mean= .67, $SD= 1.68$), $t(47) = 3.39, p<.001$, suggesting that mothers became less intrusive following a distress episode.

A significant effect of time was revealed for MI_{normal} ($F(5,185) = 4.59, p<.001$). Pairwise mean comparisons between the scores at each time point were conducted with the Sidak adjustment for multiple comparisons. There was a significant difference between mean MI scores at 90 sec (Mean= .16) and at 150 sec (Mean= .45), $SE_{diff} = -.29, p<.05$, between mean scores at 120 sec (Mean=.18) and 150 sec (Mean=.45), $SE_{diff} = -.26, p<.05$, and between the mean scores at 150 seconds and 180 seconds (Mean= .17) $SE_{diff} = -.27, p<.01$. These results suggest that MI around 150 seconds of interaction was higher than at other points of the interaction. In terms of $MI_{distress}$, no significant effect of time was revealed ($F(5, 185) = .79, p=.56$), suggesting that maternal intrusiveness after an episode of child distress is relatively stable. The pattern of maternal sensitivity scores across six 30-second

intervals during normal interaction and after infant distress can be observed in Figure

2.

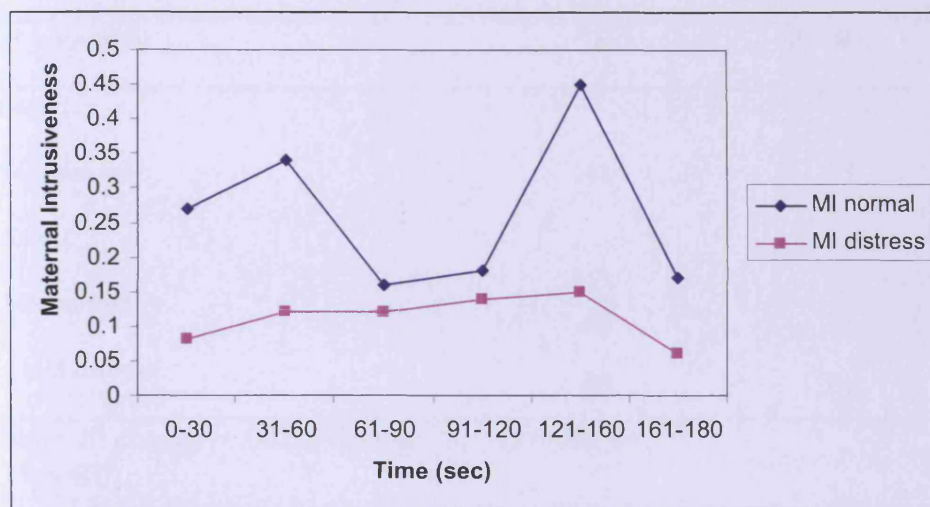


Figure 2: Pattern of maternal intrusiveness across the 3-minute interaction during normal interaction and after child distress

Predictors of maternal sensitivity and intrusiveness after distress

A step-wise regression was used to test whether maternal and/or child variables predicted maternal sensitivity after distress. The results are presented in Table 3. It can be seen that only MS_{normal} predicted maternal sensitivity after child distress. Higher levels of MS_{normal} were associated with higher levels of maternal sensitivity after distress.

A step-wise regression was used to test whether MI_{normal} and child distress predicted maternal intrusiveness after child distress. The results are presented in Table 4. Only MI_{normal} emerged as a significant predictor of intrusiveness after distress. These results suggest that in year 3 the level of child's emotional distress levels do not seem to have a significant impact on maternal behaviour.

Table 3: Summary of a step-wise regression analysis for variables predicting maternal sensitivity after infant distress (N=38)

Variable	B	SE B	β
<i>Step 1:</i>			
MS normal	.41	.13	.47**
<i>Step 2:</i>			
MS normal	.40	.13	.47**
Child distress	-.00	.01	-.04

Note: R^2 change = .002, F change (1, 35) = .08, $p=.77$

** $p<.01$

Table 4: Summary of a step-wise regression analysis for variables predicting maternal intrusiveness after child distress (N=38)

Variable	B	SE B	β
<i>Step 1:</i>			
MI normal	.29	.12	.39*
<i>Step 2:</i>			
MI normal	.27	.12	.36*
Child distress	.00	.00	.21

Note: R^2 change = .04, F change (1, 35) = 1.83, $p=.19$

* $p<.05$

Child Distress

A multiple regression was used to assess the influence of maternal sensitivity and intrusiveness during normal interaction on child distress. The results of the regression are presented in Table 5. None of the maternal characteristics in Year 3 were found to be associated with child behavioural distress.

Table 5: Summary of a multiple regression analysis for variables predicting child distress (N=38)

Variable	B	SE B	β
MS normal	.28	3.53	.02
MI normal	16.11	27.76	.12

Note: $R^2 = -.05$, $F(2, 35) = .21$, $p = .82$

Child Recovery

A multiple regression was used to investigate the influence of maternal characteristics (sensitivity and intrusiveness after distress) and child distress on child recovery. Child behavioural distress was entered as a predictor in step 1 of the regression analysis in order to investigate its unique contribution to child recovery and to investigate whether its influence would remain after maternal characteristics were entered in step 2 of the analysis. The results are presented in Table 6. It was found that higher levels of distress were associated with longer recovery and that maternal sensitivity after distress approached significance in predicting child recovery rate. However, surprisingly, higher levels of maternal sensitivity after distress were also associated with longer recovery of the child.

Table 6: Summary of a multiple regression analysis for variables predicting child recovery time (N=38)

Variable	B	SE B	β
<i>Step 1:</i>			
Child behavioural distress	.02	.00	.79**
<i>Step 2:</i>			
Child behavioural distress	.02	.00	.79**
MS after distress	.08	.04	.19^
MI after distress	.15	.40	-.01

Note: R^2 change = .03, F change (2, 26) = 2.46, p = .10

** p < .01, ^ p = .07

Child cortisol reactivity

After comparing child baseline and peak post-stress cortisol concentrations, paired samples t-test revealed a significant increase from baseline cortisol (Mean = 1.29, SD = 1.25) to the peak response after child distress (Mean = 2.80, SD = 1.80) suggesting a significant effect of manipulation on child cortisol reactivity.

We did not find an association between maternal sensitivity during normal interaction and baseline cortisol levels.

We were interested in whether any of the maternal behaviours before or after distress were associated with child cortisol reactivity (Cortisol Δ and %increase) over and above child distress levels. The predictors were entered into a multiple regression (Table 7). In terms of Cortisol Δ , neither child distress levels nor any of the maternal variables were associated with Cortisol Δ .

We were further interested in the influence of maternal behaviours on child cortisol %increase. Because of the low association between child distress and

Cortisol Δ , this variable was not included in the analysis (Table 8). None of maternal predictors were found to be associated with cortisol %increase.

Table 7 Summary of a step-wise regression analysis for variables predicting child Cortisol Δ (N=38)

Variable	B	SE B	β
<i>Step 1:</i>			
Child distress	.01	.01	.11
<i>Step 2:</i>			
Child distress	.01	.01	.12
MS normal	-.21	.19	-.24
MI normal	-1.85	.1.45	-.29
MS after distress	.18	.20	.19
MI after distress	.69	1.74	.08

Note: R^2 change = .002, F change (1, 32) = .56, p =.69

Table 8 Summary of a step-wise regression analysis for variables predicting child cortisol %increase (N=38)

Variable	B	SE B	β
MS normal	.03	.06	.11
MI normal	.25	.45	.12
MS after distress	.04	.06	.13
MI after distress	-.05	.53	-.02

Note: R^2 = -.09, F (1, 33) = .32, p =.86

Cortisol Recovery

A paired samples t-test revealed that on average, there was a significant decrease in cortisol between the peak sample (Mean= 2.80, *SD*= 1.80) and the recovery sample (Mean= 1.89, *SD*= 1.53) ($t(38) = 8.89, p < .01$). There was an overall decrease in cortisol levels between the peak cortisol and the recovery sample of 166%. The figure demonstrating the pattern of cortisol response and recovery is shown in Figure 3.

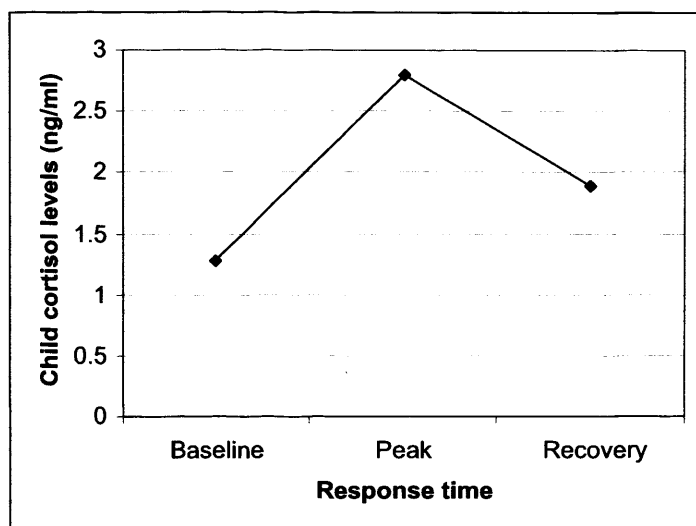


Figure 3: Pattern of cortisol stress response and recovery.

We were interested in whether any of the maternal variables had an influence on child cortisol recovery. First, we looked at the influence of maternal variables on child cortisol decrease from peak response to the recovery sample, with higher cortisol decrease values representing better cortisol recovery. All maternal predictors were entered in a multiple regression analysis, controlling for child cortisol reactivity (Table 9). None of maternal predictors were associated with cortisol decrease following distress. Similarly, none of the maternal predictors were found to be associated with child cortisol %decrease.

In addition, we did not find an association between any of the child reactivity and recovery measures, suggesting that higher reactivity does not lead to longer cortisol recovery. These findings suggest that at the age of three physiological reactivity and recovery are separate aspects of coping with stress.

Table 9 Summary of a step-wise regression analysis for variables predicting child cortisol decrease (N=38)

Variable	B	SE B	β
<i>Step 1:</i>			
Child cortisol delta	.03	.02	.25
<i>Step 2:</i>			
Child cortisol delta	.03	.02	.25
MS normal	-.00	.02	-.08
MI normal	.04	.39	.05
MS after distress	-.00	.06	-.01
MI after distress	-.07	.17	-.07

Note: R^2 change = .01, F change (1, 32) = .11, p = .98

Discussion

Consistent with Studies 1 and 2, this study had two main aims: first, to investigate the association between maternal sensitivity and intrusiveness and their stability across different emotional states of 3-year old children. Second, we wanted to investigate the influence of maternal sensitivity and intrusiveness on child coping with stress.

In terms of the first aim, as hypothesized, maternal sensitivity and intrusiveness were significantly negatively associated during a normal, playful interaction, suggesting that greater sensitivity is associated with less intrusiveness during normal interactions in the third year of life. This association became non-significant during interaction after child distress. This finding suggests that greater maternal sensitivity is not necessarily associated with less intrusiveness during an emotionally challenging interaction. It is possible to have mothers who are highly sensitive and show some intrusiveness, and mothers who are low in sensitivity are not necessarily high in intrusiveness. This finding is in agreement with Fish and Stifter (1995), who found separate caregiving groups of mothers low in sensitivity and intrusiveness, and those low in sensitivity and high in intrusiveness. These findings combined suggest that the two concepts are not orthogonal.

We were interested to find out whether these maternal characteristics were stable across different emotional states of the child. With respect to maternal sensitivity, it was found that, on average, mothers became more sensitive in response to a distressed state of the child than during a normal interaction. These findings support the results of Study 2, where a similar effect was found, suggesting that mothers in present sample become more sensitive and attentive to children after an emotional challenge. These findings support claims made by McElwain and Boothe-

LaForce (2006) and Leerkes et al. (2009) that sensitivity is not a stable characteristic but differs depending on the emotional state of the child. This could have important theoretical and methodological implications for the study of maternal sensitivity.

We also found differences in maternal intrusiveness during normal interaction and after distress, with mothers becoming less intrusive after the child had been in distress. These findings are in line with the results of Studies 1 and 2 that both maternal sensitivity and intrusiveness change as a function of the child's emotional state. It seems that on average, across the three years, the mothers in the present sample responded with more sensitive and less intrusive behaviour towards their children after an episode of separation and distress.

In year 3, we found some instability of maternal sensitivity, not only between interactions, but also within both normal and after-distress interactions. It was found that maternal sensitivity during normal interaction decreased significantly from first 30 seconds of interaction towards the end of the interaction, and increased again during the last 30 seconds. We hypothesized that normal interaction would be relatively stable, as it was found to be in 2-year old children. These findings therefore suggest that there may be some fluctuations in maternal sensitivity levels in year 3 even when children are calm and playful. Perhaps a possible reason could be that as children get older, they may lead the interaction more and the mother has to adapt to the demands of the child more so than when the child was younger.

It was hypothesized that sensitivity would be less stable and go down when the child continued to remain emotionally distressed and the interaction was more challenging to the mother. We indeed found maternal sensitivity after child distress to be unstable and to steadily decrease from the first 30 seconds of interaction to the end of the play episode. These results are in line with the findings in year 1, when

sensitivity also decreased during the post-distress interaction, but in contrast with year 2, where both sensitivity before and after distress were found to be stable.

On the other hand, in terms of maternal intrusiveness, we found mothers to show some instability in their intrusive behaviour during normal interaction, with an overall increase in intrusiveness around the 150 seconds of interaction. Maternal intrusiveness was found to be relatively stable following an episode of child distress. This could be explained by the fact that majority of mothers decreased in their intrusiveness levels after the episode of separation and child distress, and differences in intrusiveness patterns during this episode were negligible.

The second aim of present research was to investigate the influence of maternal sensitivity and intrusiveness on child emotional reactivity and recovery. It was hypothesized that greater sensitivity and less intrusiveness would predict less distress in the child and a faster recovery. However, we found no influence of both maternal behaviours on child emotional distress. Similarly, we did not find an association between maternal behaviours and child cortisol reactivity in response to distress. These findings are in contrast to those of Study 2, where maternal factors were related to both emotional and cortisol response to stress, but mirror those of Study 1, where mothers of 1-year olds did not seem to play a role in child distress experience. Even though the literature points to the importance of the mother in child behavioural and physiological reactivity to distress, various studies also failed to establish a link with child distress reactivity (e.g. Albers et al., 2008). The results of the three present studies combined suggest that the timing of maternal influence may be important. We found the mother to play an important role in child reactivity at age 2, while previous studies found that sensitivity in early infancy may be of particular

importance (e.g. Pauli-Pott et al. 2004). None of the studies known to us systematically assessed maternal behaviour across different ages of the child, assuming that these are stable characteristics. However, the findings that maternal sensitivity and intrusiveness are neither stable across time, nor between different emotional states of the child suggests that these are not stable traits, and future studies should therefore aim to obtain multiple measures of maternal behaviour to better understand its influence on child emotionality.

We were also interested in the influence of maternal sensitivity and intrusiveness after distress on the child's recovery from distress. It was hypothesized that greater maternal sensitivity after the distress episode would lead to faster recovery, whereas greater intrusiveness would be associated with slower recovery. Although we found maternal sensitivity after distress to be marginally associated with child recovery, the association was in the unexpected direction; more sensitive mothers had children who took more time to recover from distress. The reason for this finding is unclear. One possible explanation could be that with more sensitive mothers, children express their behavioural distress for longer as they rely more on the comforting of the mother than children of less sensitive mothers. We also examined whether children of more sensitive mothers had a better cortisol recovery, but found this not to be the case in the present sample. Other studies also failed to find an influence of maternal behaviour on child recovery from distress. For example, Hubbard and van IJzendoorn (1991) did not find that greater maternal responsiveness to the child led to less infant crying. They actually found that a more frequent *delay* in responsiveness was associated with reduced frequency of infant cry bouts. Similarly, Lewis and Ramsay (1999) failed to find maternal soothing to be effective in reducing

infants' behavioural or cortisol reactivity in response to inoculation and an every-day stressor.

Overall, maternal behaviour seemed to have a clearer effect on child emotional behaviour at age 2 than at age 1 and 3. Because the designs of the three studies were identical, it can be argued that in the present sample the influence of the mother is more apparent during the second year of life. This is known to be a challenging year for children, because they have to adapt to more frequent separations from their mother and are also able to understand better what is going on in their environment (e.g. novelty, separation) without yet having developed the necessary skills to deal with this effectively. It could therefore be hypothesized that the role of the mother is most apart direct during this year. However, it should be remembered that these results are cross-sectional and that a study with a longitudinal design is required to properly assess the role of the mother on child development over time. Such a study is described in Chapter 7. However, the non-significant results in Study 3 should be interpreted with some caution because of the small sample size in the study, which could result in a low power to detect possible effects.

Aside from the main hypothesis of this study, the present research also adds to the literature on the relation between child behavioural and physiological coping with stress. In terms of behavioural reactivity and recovery, it was found that these two constructs were highly positively associated, which is in contrast to findings by Ramsey and Lewis (2003). Our findings in Year 3 are in accordance with results of Study 1 and 2 which also found behavioural reactivity and recovery to be positively associated. These findings demonstrate that with the present emotional challenge, on

average it takes children longer to recovery if they have been more distressed, suggesting that distress and recovery are interlinked (Gloggler & Pauli-Pott, 2008; Calkins & Johnson, 1998).

We were further interested in the association between behavioural and cortisol reactivity and recovery. Unlike Studies 1 and 2, we found no association between behavioural distress and cortisol reactivity. These findings suggest that by the age of 3 there is a discrepancy in the extent of behaviourally and hormonally expressed distress. This is a common finding in the literature on older children and adults (Van Goozen et al., 2000), and could be explained by the more advanced cognitive development of children and their ability to better control their emotional expressions as they get older.

In addition, in the present study we were able to investigate the association between cortisol reactivity and recovery. We found a clear dampening of cortisol response about 40 minutes after distress, with the large majority of children showing a reduction in cortisol levels. We found no association between cortisol reactivity and recovery, suggesting that the level of cortisol response does not influence the recovery rate. This finding is in agreement with Ramsey and Lewis (2003), who also found cortisol reactivity and recovery to be unrelated. These findings demonstrate that it is important to include both behavioural and physiological assessments of stress reactivity and recovery in early childhood, because they are not strongly associated and therefore could tap into different processes that are relevant to obtain a proper understanding of the development of stress and coping in young children.

In summary, the study found that maternal interactive behaviours at age 3 differed across different emotional states of the child. Specifically, maternal

sensitivity increased when the child was distressed, while maternal intrusiveness decreased. These findings suggest that sensitive maternal care giving behaviour is not stable across different emotional states of the child. We also found no effect of maternal characteristics on child behavioural and hormonal distress reactivity and regulation at this age. It is possible that maternal behaviour – given that we found that it changes over time - only exerts these effects when children are younger. It is also possible that it is specifically *early* maternal behaviour that has a lasting effect on the development of stress regulation in the child. In Chapter 7 we will, among others, investigate whether early maternal behaviours are associated with later child emotionality.

Chapter 7. Developmental patterns of maternal characteristics and child emotional reactivity and recovery across the first three years of life:
The role of the mother as a moderator of child coping with stress.

Introduction

Although there is some stability in child temperamental characteristics such as emotional reactivity and regulation, various studies report developmental change in these characteristics over childhood (Lewis & Ramsey, 1995). This highlights the importance of assessing children's emotional reactivity and regulation across time in order to gain a better understanding of their development. Maternal behaviour has been implicated in these aspects of children's emotional development (e.g. Pauli-Pott et al., 2004; Lewis & Ramsey, 1999). The majority of research generally assumes stability and consistency of maternal care giving behaviours across childhood. However, some literature points to the limited amount of evidence for stability of maternal characteristics such as sensitivity and intrusiveness across time (Kempainen, Kumpulainen, Raita-Hasu, Moilanen & Ebeling, 2006) and across different emotional states of the child (Leerkes et al., 2009). The present longitudinal study was designed to investigate developmental change of maternal sensitivity and intrusiveness across the first three years of children's lives. We were also interested in assessing developmental change in child behavioural and cortisol coping with stress and to investigate whether early maternal behaviour may moderate the developmental patterns of these characteristics.

Stability of maternal behaviour

As mentioned earlier, maternal behaviour during mother-child interactions has generally been assumed to remain stable across different emotional states of the child and across time, i.e. a sensitive mother is expected to be sensitive across different situations and throughout childhood. However, relatively few studies have assessed the stability of maternal behaviours and the few studies that did look at maternal stability across time found low to moderate stability at best (Kemppinen et al., 2006), with the majority only focusing on stability across the first year of life. Bohlin and Hagekull (2000) were among the few to assess stability of maternal sensitivity from infancy to 4 years and they only found modest. Landry, Smith, Swank and Miller-Locar (2000) also found moderate stability of maternal behaviour across the same period. Kemppinen et al. (2006) assessed stability of maternal sensitivity between 4 and 24 months, finding it to be moderately stable ($r = .57$). Besides the moderate stability, maternal sensitivity was found to increase slightly from infancy to toddlerhood. They also found slight stability of maternal control during interactions and that this decreased from infancy to two years. These findings suggest that even though some stability is found, maternal behaviour also changes over time, with parenting maturing as children develop (i.e., becoming more sensitive and less controlling).

Feldman, Greenbaum, Mayes and Erlich (1997) also acknowledged the possibility of change of maternal behaviours, and were interested to examine whether factors such as maternal trait anxiety, child difficulty and father involvement would account for changes in maternal interactive behaviour. To that end, they assessed maternal sensitivity and intrusiveness during mother-child interactions at three and nine months and found that there was moderate stability in maternal behaviour, with

both maternal sensitivity and intrusiveness decreasing from 3 to 9 months. They also found that a reduction in maternal trait anxiety, a decrease in infant difficult temperament and greater father involvement were associated with positive changes in maternal behaviour from 3 to 9 months (Feldman et al., 1997). These findings suggest that changes in maternal behaviour are associated with changes in both parental and infant traits, i.e., that there are environmental influences on maternal behaviour across time.

Furthermore, other studies (Leerkes et al., 2009; McElwain & Booth-LaForce, 2006) suggest that maternal behaviour may differ depending on the emotional state of the child (e.g. the child being playful and calm vs. distressed). McElwain and Booth-LaForce (2006) found that maternal sensitivity to child distress but not to non-distress was associated with subsequent attachment security. Similarly, Leerkes et al. (2009) found that sensitivity to distress and not non-distress at 6 months was associated with child temperament. Only sensitivity to infant distress was associated with fewer behaviour problems at 24 and 36 months as well as better social competence at 24 months. Sensitivity to non-distress was associated with child affect dysregulation at 36 months, but only among the children of mothers who were sensitive to children's distress. In addition, the findings of Studies 1, 2 and 3 in Chapter 6 of this thesis also demonstrated that maternal sensitivity and intrusiveness are unstable between a normal interaction and following child distress. These results suggest that maternal interactive behaviour is complex, that it can vary across different emotional states of the child, and that sensitivity to distress and non-distress can be differentially related to child development. This calls for a more in-depth assessment of the development and stability of maternal sensitivity, which this study aimed to address.

Child Reactivity and Recovery

In previous chapters it was explained that the distress experience involves both behavioural and physiological reactivity, and a recovery from distress, making it a relatively complex concept to study. It has been suggested that child reactivity and recovery are unrelated temperamental characteristics or traits, with greater reactivity not necessarily being associated with longer recovery (Rothbart, 1989; Ramsey & Lewis, 2003). Others have reasoned that the individual's reactivity affects his/her need for regulation and recovery, and that the ability to regulate and recover affects the reactivity levels, making these parameters intertwined (Glogglar & Pauli-Pott, 2008; Calkins & Johnson, 1998). Compas et al. (2001) report that generally highly reactive children have a lower threshold for initial stress response, a slower recovery and a higher level of arousal with repeated exposure to stress. On the other hand, Ramsey and Lewis (2003) found no association between cortisol reactivity and recovery in response to inoculation in the first year of life, nor between behavioural reactivity and recovery. Research is required to investigate the association between reactivity to stress and recovery from stress across early childhood in order to gain a better understanding of the association between these different parameters of temperament and to obtain a more complete picture of stress experience.

Furthermore, at present there is no clarity on the association between child behavioural and endocrinological stress experience (both reactivity and recovery). Ramsey and Lewis (2003) found no association between behavioural distress reactivity and recovery, on the one hand, and cortisol reactivity and recovery in response to inoculation stress, on the other. The findings of Chapter 6, however, seem to indicate that behavioural and cortisol response to distress are associated when children were one and two-years old, but not by the age of three. These findings could

indicate that behavioural and cortisol response are not associated by the time the child develops more advanced cognitive abilities to suppress and control their expression of emotion.

In terms of stability of stress response, Lewis and Thomas (1994) and Ramsey and Lewis (1994) found little stability in cortisol stress response before 6 months of age. It is suggested that there is a developmental decrease in adrenocortical response to distress from early infancy to 6 months, with some stability established after that age. In support of this, Lewis and Ramsay (1995) found cortisol stress (i.e., inoculation) responses to decrease from 2-4 months to 6 months of age, and to remain relatively stable between 6 and 18 months. However, no stability in behavioural distress response was observed between 2, 4, 6 and 18 months. Furthermore, they found little individual stability in behavioural and cortisol response across time. These findings suggest that there is low stability in behavioural stress response, but that there might be some stability of the cortisol response after 6 months. The present study aimed to investigate this by assessing the developmental pattern of physiological and behavioural coping with stress across the first three years of life.

Given the low to moderate stability in child reactivity and recovery found in previous studies, it seems relevant to examine the role of possible moderators of developmental patterns of emotionality. Kagan (1997) reported that only a very small percentage of children classified as highly reactive to novelty at 4 months also displayed high reactivity to novelty at 4.5 years, and suggested that intervening family experiences, such as the quality of maternal care, may partly be the reason for these observations. Colder et al. (2002) reports that various studies of temperamental risk for future development find that parenting is an important moderator of early temperament in infants. In addition, Kaplan, Evans and Monk (2008) showed that

postnatal maternal sensitivity can override the effect of prenatal stress and anxiety for future child emotionality and cortisol levels. These findings suggest that the quality of postnatal environment may be an important moderator of initial temperamental predispositions of the child.

The literature on the role of the mother on child coping with stress was discussed in detail in Chapters 4 and 6. The design of this longitudinal study allowed us to investigate whether earlier maternal behaviours may have an impact on the child's development of the ability to cope with stress. Some literature suggests that it is particularly the early characteristics of the mother (i.e. during infancy) that may be important for child development. For example, Kochanska (2001) found that it was attachment security in the first year of life that was associated with child fear reactivity at 33 months. This suggests that maternal behaviour during the first year of life may be of particular importance for the development of emotional reactivity and the ability to cope with distress. However, comparatively few studies assessed maternal behaviour across different ages to investigate whether it is maternal behaviour during infancy rather than during toddlerhood that is important for child development. In addition, the majority of previous studies interested in the role of the mother on child emotional development focused on the predictive value of maternal behaviour during a specific point of assessment on child outcomes later in life. However, there is evidence for change in child characteristics in the early years, as was reviewed in Chapter 4, indicating that snapshots of associations between maternal behaviour and child outcomes could be misleading. It is important therefore to investigate the role of the mother as a moderating factor in the developmental patterns of child emotional reactivity and regulation as these characteristics unfold across time.

Even though no gender difference were observed in emotional coping with stress and the ability to recover across the first three years of life in the studies in Chapter 6, it could nevertheless be possible that gender differences may be apparent in the developmental patterns of these variables. This is an interesting aspect to investigate. However, because Ktistaki (2009) also found no differences in behavioural fear reactivity between boys and girls between the ages of 1 and 2, and because of the small sample size in present study, we did not look at gender differences in patterns of coping with stress at this stage.

The present study was designed to address shortcomings of past research by investigating the developmental patterns of both maternal and child behaviours and by examining the role of early maternal behaviour as a potential moderator of child emotional development.

Aims and hypotheses of present chapter:

- a) To investigate stability and change of maternal sensitivity and intrusiveness across early childhood. It was hypothesized that maternal sensitivity and intrusiveness would not be stable across early childhood.
- b) To investigate stability and change of child emotional reactivity and recovery across early childhood. It was hypothesized that distress reactivity and recovery would not be stable and increase from infancy to toddlerhood.
- c) To investigate associations between child behavioural reactivity and recovery across the first three years of life. We expected reactivity and recovery to be associated.
- d) To investigate associations between child behavioural and cortisol reactivity and recovery in early childhood. We expected cortisol and

behaviour to be associated, with the association reducing in magnitude over time.

- e) To investigate whether maternal behaviours moderate the developmental trajectories of child reactivity and recovery across the first three years of life. We hypothesized that more sensitive and less intrusive maternal behaviours would be associated with better coping with stress later in childhood. We were further interested whether maternal behaviour during normal interactions and following episodes of child distress may be differentially related to child emotional development.

Method

Sample

The sample and procedure are the same as that described in Study 3 of Chapter 6. Participants were 38 children (20 male, 18 female) and their mothers. Participant ages in Year 1 ranged from 6 to 14 months (Mean age = 9.95 months, $SD = 2.42$), while in Year 2 the range was 16 to 30 months (Mean age = 23.1 months, $SD = 2.8$) and in Year 3 the age ranged from 33 to 42 months (Mean age = 37.09 months, $SD = 2.17$). For additional details, please refer to the Methods section in Chapter 6 (see p. 97).

Measures

All measures used in this study are identical to those of Study 3 in Chapter 6. Some variables were found to be non-normally distributed: infant cortisol baseline levels Year 1 ($skewness = 3.91$, $kurtosis = 18.05$), $Cort\Delta$ Year 1 ($skewness = 2.41$, $kurtosis = 7.70$), recovery time Year 1 ($skewness = 1.91$, $kurtosis = 4.32$), $Cortisol\Delta$ Year 2 ($skewness = 2.92$, $kurtosis = 10.50$), cortisol baseline levels year 3 ($skewness =$

3.89, *kurtosis*= 18.78), cortisol recovery time Year 3 (*skewness* = 2.30, *kurtosis* = 5.77). Therefore, some non-parametric tests were used to investigate comparisons between some of these variables.

Results

The descriptive statistics of the sample across the first three years are presented in Table 1.

Table 1: Descriptive statistics for the infant and maternal variables (N=38)

Variables	Mean	SD	Range
<i>Maternal Variables</i>			
MS _{normal} Y1	9.82	2.29	6.00 – 15.00
MS _{normal} Y2	11.37	2.08	5.00 – 15.00
MS _{normal} Y3	10.87	2.34	6.00 – 17.00
MS _{distress} Y1	10.12	2.31	5.00 – 16.00
MS _{distress} Y2	11.92	2.32	4.00 – 17.00
MS _{distress} Y3	11.71	2.00	7.00 – 17.00
MI _{normal} Y1	4.17	3.38	0.00 – 13.00
MI _{normal} Y2	2.06	3.26	0.00 – 15.00
MI _{normal} Y3	1.57	2.04	0.00 – 8.00
MI _{distress} Y1	2.58	2.69	0.00 – 11.00
MI _{distress} Y2	0.95	1.56	0.00 – 17.00
MI _{distress} Y3	0.67	1.68	0.00 – 10.00
<i>Child variables</i>			
Behavioural distress Y1	58.37	33.84	7.00 – 130.00
Behavioural distress Y2	79.03	44.48	3.00 – 153.00
Behavioural distress Y3	79.86	41.88	4.00 – 162.00
Recovery time Y1	58.95	63.84	0.00 – 270.00
Recovery time Y2	110.50	102.89	0.00 – 320.00
Recovery time Y3	58.64	71.40	0.00 – 300.00
Cortisol baseline Y1	2.61	3.40	.08 – 19.81
Cortisol baseline Y2	1.53	.84	.29 – 3.71
Cortisol baseline Y3	1.28	1.31	.10 – 7.72
CortisolΔ Year 1	1.74	3.09	-8.44 – 12.99
CortisolΔ Year 2	3.53	5.28	-.55 – 27.07
CortisolΔ Year 3	1.49	1.94	-4.05 – 7.17
Cortisol %increase Y1	249.24	710.82	-61.73 – 4360.00
Cortisol %increase Y2	312.32	722.46	-18.35 – 4437.70
Cortisol %increase Y3	254.01	438.33	-60.00 – 2270.70
Cortisol decrease Y3	0.98	1.11	0.00 – 5.27
Cortisol %decrease Y3	151.55	366.68	0.00 – 1800.00

Stability of maternal sensitivity and intrusiveness

Maternal Sensitivity

One of the first aims of present chapter was to investigate the stability and change of maternal behaviours across the first three years of children's lives. A 2x3 Repeated Measures ANOVAs (2 conditions: normal interaction and after distress, and 3 time assessments: Year 1, Year 2 and Year 3) were used to investigate the effect of time on maternal behaviour during normal and post-distress interactions. There was a significant main effect of condition on MS ($F(1, 37) = 5.97, p < .05, power = .82$). Pairwise mean comparisons between the two conditions were conducted with the Sidak adjustment for multiple comparisons. It was found that MS was significantly higher after distress (Mean = 11.25) than during normal interaction (Mean = 10.69; $SE_{diff} = .23, p < .05$) across the three years. Furthermore, there was a significant main effect of time ($F(2, 74) = 10.48, p < .001$). Pairwise mean comparisons between the two conditions with the Sidak adjustment for multiple comparisons revealed an overall increase in MS from Year 1 (Mean = 9.97) to Year 2 (Mean = 11.64), $SE_{diff} = .40, p < .01$ and between Year 1 and Year 3 (Mean = 11.29), $SE_{diff} = .42, p < .01$. There was no significant difference in MS between Year 2 and Year 3. There was no interaction between time and condition.

The results suggest that there is little stability in MS between different emotional states of the child, and also from infancy to toddlerhood (Figure 1). Overall, for MS_{baseline} it was found that 9 mothers (23.7%) decreased in sensitivity from Year 1 to Year 3, while the remaining 29 mothers (76.3%) increased in sensitivity. In terms of MS_{distress}, 7 mothers (18.4%) decreased in sensitivity from Year 1 to Year 3, while the remaining 31 mothers (81.6%) increased in sensitivity.

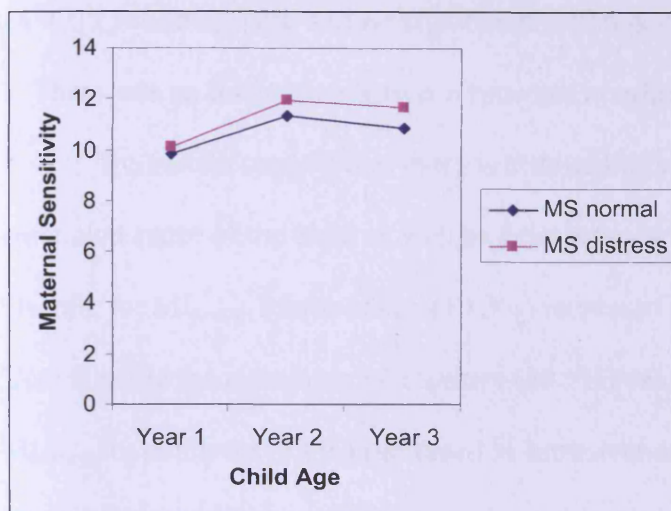


Figure 1: Maternal sensitivity during normal and post-distress interaction across the first three years of life.

Maternal Intrusiveness

A 2x3 design Repeated Measures analysis of variance (2 conditions: normal interaction and after distress and 3 time assessments: Year 1, Year 2 and Year 3) were used to investigate the effect of time on maternal intrusiveness during normal and post-distress interactions. There was a significant main effect of condition ($F(1, 37) = 38.06, p < .001, power = 1.00$). Pairwise mean comparisons with the Sidak adjustment for multiple comparisons revealed a significant increase in MI from normal interaction (Mean = 2.60) to after child distress (Mean = 1.40), $SE_{diff} = .03, p < .001$ across the three years. Furthermore, there was a significant main effect of time ($F(2, 74) = 24.41, p < .001, power = 1.00$). Pairwise mean comparisons between the two conditions were conducted with the Sidak adjustment for multiple comparisons. It was found that there was an overall increase in MI from Year 1 (Mean = 3.37) to Year 2 (Mean = 1.50), $SE_{diff} = .05, p < .001$ and between Year 1 and Year 3 (Mean = 1.12), SE

diff = .05, $p < .001$. There was no significant difference in MI between Year 2 and Year

3. There was no interaction between time and condition.

The results suggest that there is little stability in MI between different emotional states of the child as well as from infancy to toddlerhood (Figure 2).

Overall, for $MI_{baseline}$ four mothers (10.5%) increased in intrusiveness from Year 1 to Year 3, while the remaining 34 mothers (89.5%) decreased in intrusiveness. For $MI_{distress}$ six mothers (15.8%) increased in intrusiveness, while 32 mothers (84.2%) decreased in intrusiveness.

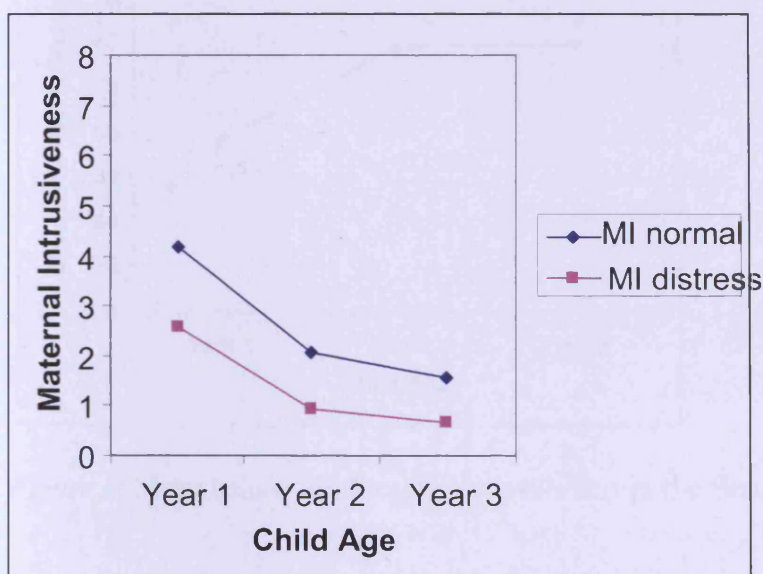


Figure 2: Maternal intrusiveness during normal and post-distress interaction across the first three years of life.

Stability of child emotional reactivity and recovery

Behavioural Reactivity

A mixed between-within analysis of variance was conducted to assess the influence of time (Year 1, Year 2 and Year 3) and gender on child behavioural stress reactivity. A significant effect of time was revealed ($F(2, 74) = 4.51, p < .05$)

indicating that child distress reactivity was not stable over time. Pairwise mean comparisons with Sidak adjustment for multiple comparisons revealed that the difference in reactivity levels between Year 1 (Mean = 58.74) and Year 2 (79.07) approached significance, $SE_{diff} = 8.68$, $p = .06$ and that there was a significant increase between Year 1 and Year 3 (Mean = 80.44) reactivity, $SE_{diff} = 8.14$, $p < .05$. The results suggest that there was an overall increase in reactivity from infancy, with some stability established between Year 2 and 3 (Figure 3).

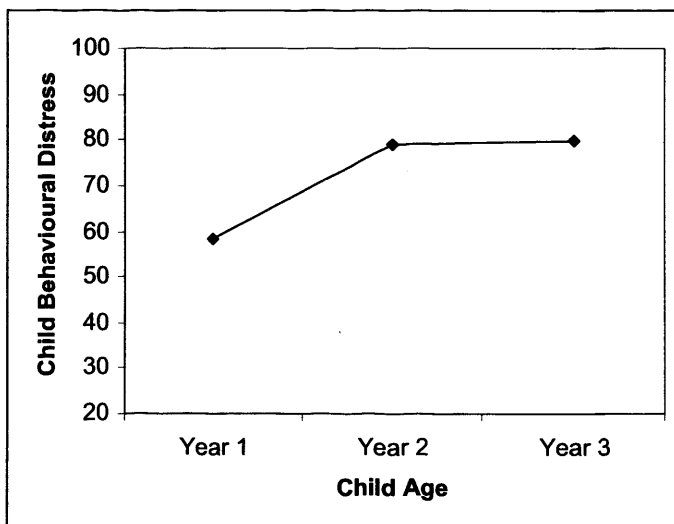


Figure 3: Mean behavioural reactivity levels across the first three years of life.

Behavioural Recovery

Because behavioural recovery was not normally distributed in Year 1 and Year 3, but was in Year 2, a non-parametric Friedman for repeated measures was used to compare the scores across the three years. It was revealed that there was an overall significant change in child recovery rate ($\chi^2(2) = 9.17$, $p < .05$). Inspection of the median values revealed an increase in recovery time from Year 1 ($Md = 44.50$) to Year 2 ($Md = 72.50$) but a decrease from Year 2 to Year 3 ($Md = 46.50$). The results suggest that length to recovery peaks in Year 2 (see Figure 4 for mean increases).

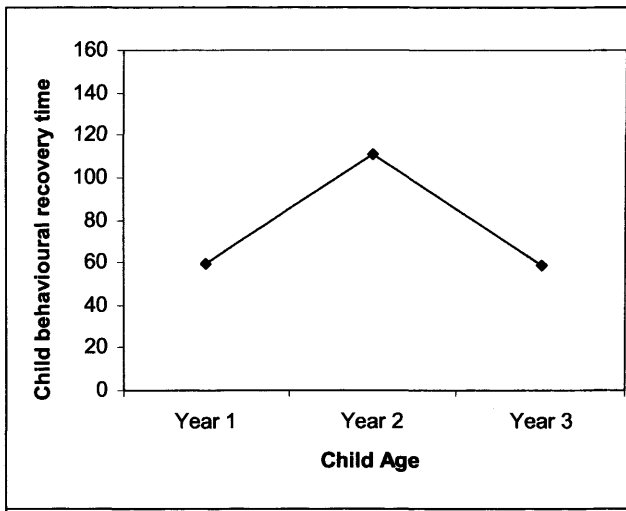


Figure 4: Patterns of mean time of child behavioural recovery across the first three years of life.

Cortisol baseline levels

Because cortisol baseline concentration was only normally distributed in Year 2, and not in Years 1 and 3, a non-parametric Friedman test for repeated measures was used to compare the scores across the three years. It was revealed that there was an overall significant change in baseline cortisol levels ($\chi^2 (2) = 8.26, p < .05$). Inspection of the median values revealed a gradual decrease in cortisol baseline levels from Year 1 ($Md = 1.78$) to Year 2 ($Md = 1.33$) to Year 3 ($Md = 1.06$). Post-hoc comparisons using the Wilcoxon Signed Rank tests (using Bonferroni adjusted alpha values) revealed that there was no significant difference between baseline cortisol levels in Year 1 and Year 2 ($z = -1.72, p = .09$), but that there was a significant difference between baseline levels at Year 1 ($Md = 1.78$) and Year 3 ($Md = 1.06$), $z = -3.11, p < .002$. The difference in baseline levels between Year 2 ($Md = 1.32$) and Year 3 ($Md = 1.06$) was no longer significant after Bonferroni correction. The results demonstrate that there is a significant drop in baseline cortisol levels between the first and 3rd year of life.

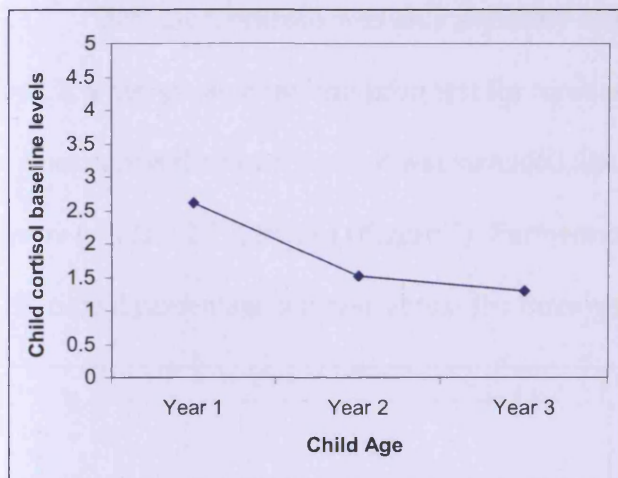


Figure 5: Mean cortisol baseline levels across the first three years of life.

Cortisol Reactivity

Across the three years there was an overall effect of stress exposure on cortisol levels. Wilcoxon Signed Ranks test revealed that there was a significant increase from baseline levels to peak cortisol response after distress in Year 1 ($z = -4.92, p < .001$), Year 2 ($z = -4.21, p < .001$), and Year 3 ($z = -4.40, p < .001$) (Figure 6).

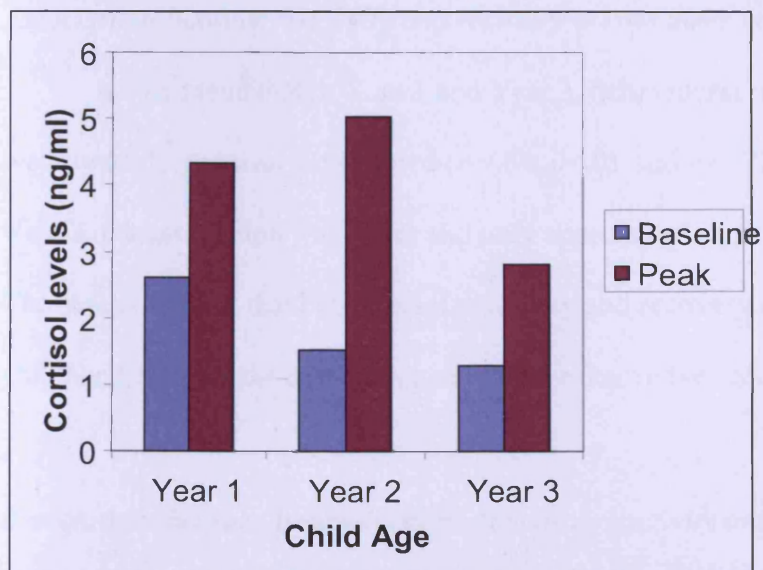


Figure 6: Mean cortisol baseline and peak stress response levels across the first three years of life.

Because Cortisol Δ was only normally distributed in Year 3, and not in Year 1 and 2, a non-parametric Friedman test for repeated measures was used to compare the values across the three years. It was revealed that Cortisol Δ the same across the three years ($\chi^2 (2) = 2.74, p=.26$) (Figure 7). Furthermore, we found no significant change in cortisol percentage increase across the three years ($\chi^2 (2) = 2.11, p=.90$).

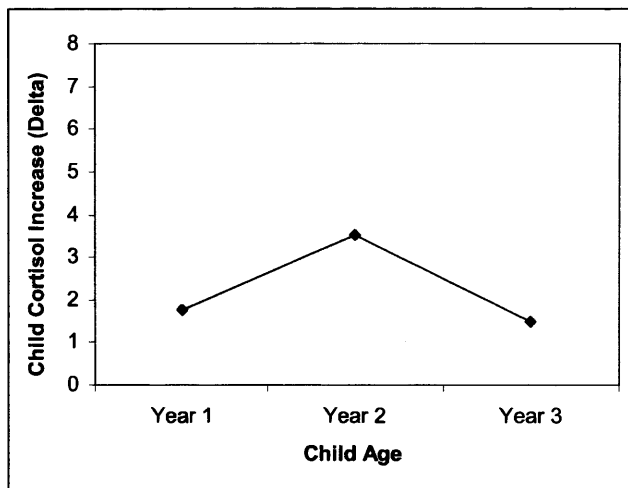


Figure 7: Mean cortisol Δ reactivity levels across the first three years of life.

Associations between reactivity and recovery across three years

It was found that in Year 1 and Year 3, behavioural reactivity and recovery were strongly positively associated ($r = .64, p < .01$ and $r = .72, p < .01$ respectively). In Year 2, the association was lower and only approached significance ($r = .30, p = .06$). The results suggest that behavioural reactivity and recovery are associated in early childhood, but that the association may be weaker in the second year of life.

Associations between behavioural and cortisol reactivity and recovery

In Year 1 behavioural reactivity was not associated with Cortisol Δ ($r = .05, p = .78$). This association was significant in Year 2 ($r = .33, p < .05$) but dropped again in Year 3 ($r = .11, p = .11$). These results indicate that behavioural and cortisol reactivity

seem to be unrelated aspects of distress experience in early infancy and later toddlerhood.

Because a recovery measure of cortisol in Year 3 but not in Years 1 and 2, it was only possible to investigate the association between behavioural and cortisol recovery at this time point. The association between cortisol and behavioural recovery was not found to be significant ($r=.21$, $p=.21$), indicating a discrepancy in physiological and behavioural recovery in addition to reactivity, for example children experiencing endocrinological arousal do not necessarily display high behavioural distress.

The role of the mother in the changes of child emotional reactivity and recovery

Child Reactivity

Since the findings of this study show change from infancy to toddlerhood in children's behavioural reactivity to distress, we were interested in establishing whether early maternal behaviour might moderate the development of child reactivity. Mothers were separated into high and low sensitive groups. The groups were based on the principle of mothers receiving two or more 'low' scores (score 1 or 0) per interaction (low sensitivity group) and if they had one or no 'low' scores they were grouped into the 'high' group. These groups were also reflected in the frequency distribution for the variables. This principle was applied to both normal and post-distress MS scores. A mixed between-within subjects analysis of variance revealed a significant interaction between time and maternal sensitivity group (*Wilks Lambda* = .70, $F(2, 72) = 7.46$, $p < .01$, $power = .87$). Children of high MS mothers showed less reactivity in infancy (Mean = 42.53, $SD = 22.92$) than children of low MS mothers (Mean = 68.50, $SD = 31.18$), independent samples $t(2, 36) = 2.48$, $p < .05$). However,

children of high MS mothers showed more reactivity in Year 3 (Mean= 96.43, $SD=40.28$) compared to children of less sensitive mothers (Mean=69.04, $SD= 40.10$), independent samples $t(2, 36) = -2.06, p < .05$ (Figure 8). There were no differences in reactivity between children of more and less sensitive mothers in Year 2.

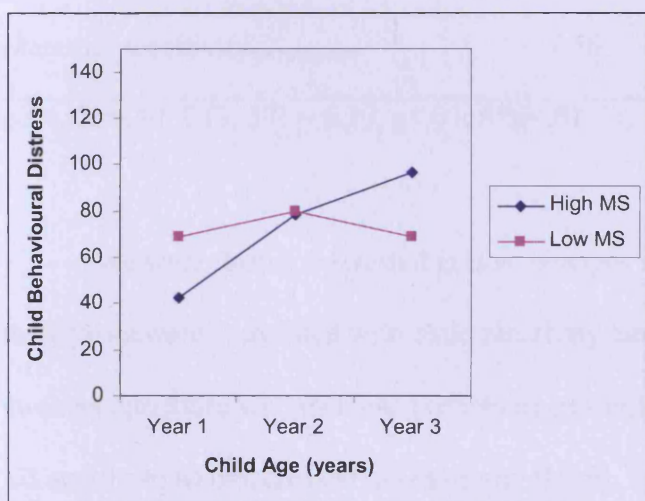


Figure 8: Development of behavioural reactivity in children of high and low $MS_{normal-year1}$ mothers.

Furthermore, we were interested in whether MS_{normal} in Year 1 influenced child behavioural reactivity in Year 3. A multiple linear regression was carried out in order to assess this influence, while controlling for previous child reactivity (Table 2). It can be seen that both child distress levels aged 2 and maternal sensitivity at baseline in the first year of life have an influence on child behavioural reactivity aged 3. Higher behavioural distress in Year 2 and higher maternal sensitivity in Year 1 are associated with higher behavioural distress in Year 3.

Table 2: Multiple regression analysis for variables predicting child behavioural reactivity aged 3 (N=38)

Variable	B	SE B	β
Behavioural distress _{year1}	.25	.18	.20
Behavioural distress _{year2}	.44	.13	.47**
Maternal sensitivity _{normal-year1}	7.56	2.61	.41**

Note: $R^2=.30$, $F(3, 37) = 6.29$, $p<.01$, ** $p<.01$

We were further interested in how changes in maternal sensitivity across the three years were associated with child reactivity later in childhood. We separated mothers into those who increased or remained stable in sensitivity (N=29) from Y1 to Y3 and those who decreased in sensitivity (N=9). Overall, mothers who decreased in sensitivity had children who had higher behavioural reactivity in Y3 (Mean= 92.28, $SD= 48.35$) than those who increased or remained stable in sensitivity (Mean =76.00, $SD=39.81$). However, an independent samples t -test revealed the difference to be non-significant ($t(2, 36) = 1.02$, $p=.31$).

Next, the question of whether maternal sensitivity after distress in Year 1 moderated the development of distress reactivity was investigated. A mixed between-within subjects analysis of variance revealed no significant interaction between time and MS_{distress} group ($Wilks\ Lambda = .99$, $F(2, 72) = .26$, $p=.77$). Because of the small sample size the power to detect an effect was low (.10) and the null result should therefore be interpreted with considerable caution. The pattern of reactivity across the first three years for children of high and low MS_{distress} mothers can be observed in Figure 9.

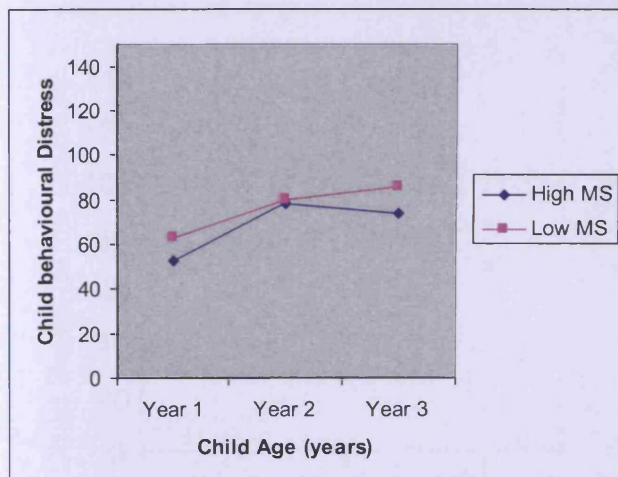


Figure 9: Development of behavioural reactivity in children of high and low MS_{distress-}

year1 mothers

We were further interested in the influence of early maternal intrusiveness on development of child reactivity. Two groups of high and low intrusiveness mothers were created for both interactions. Mothers in the 'high' intrusiveness group received more than two 'high' scores for the interaction, whereas mothers in 'low' group received two or less 'high' scores, which also corresponded to the frequency distribution groups for the variables. For intrusiveness at baseline, a mixed between-within subjects analysis of variance revealed no significant interaction between $MI_{baseline}$ and time, suggesting that the level of maternal intrusiveness did not impact the development of behavioural reactivity ($Wilks\ Lambda = .90$, $F(2, 72) = 1.98$, $p = .15$). The power of detecting an effect was low (.38), suggesting that the null result should be interpreted with caution. The pattern of reactivity across the first three years for children of high and low intrusive mothers can be observed in Figure 10.

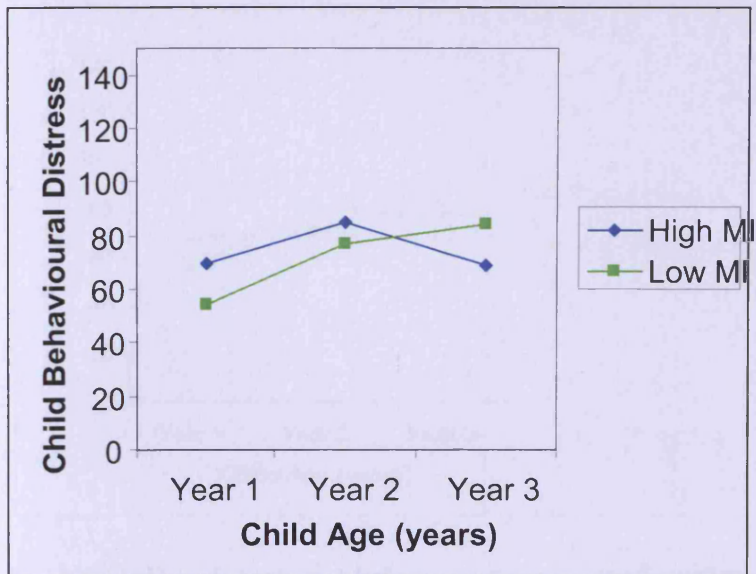


Figure 10: Development of behavioural reactivity in children of high and low

MI_{normal-year1} mothers

Further we investigated whether MI_{distress} moderated the effect of time on child behavioural reactivity. A mixed between-within subjects analysis of variance revealed no significant interaction between MI_{distress} and time, suggesting that the level of maternal intrusiveness did not impact the development of reactivity (*Wilks Lambda* = .96, $F(2, 72) = .30, p = .64$). Because of the small sample size the power to detect an effect was low (.09) the null results should be interpreted with caution. The pattern of reactivity across the first three years for children of high and low intrusive mothers can be observed in Figure 11.

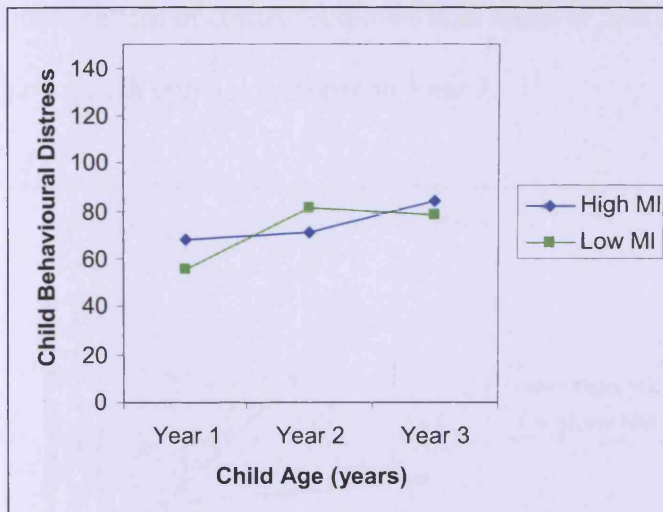


Figure 11: Development of behavioural reactivity in children of high and low

MI_{distress-year1} mothers

Cortisol Reactivity

We wanted to investigate whether there was an interaction between maternal factors and time on cortisol reactivity. Because the indicators of cortisol reactivity (Cortisol Δ and %increase) were highly correlated (Year 1 Spearman $\rho = .89$, $p < .001$, Year 2 $\rho = .92$, $p < .001$, Year 3 $\rho = .90$, $p < .001$), it was decided to use the Cortisol Δ as an indicator of cortisol reactivity since it reflects the increase in cortisol levels from baseline to peak sample after distress.

In terms of MS_{baseline} and time, mixed between-within subjects ANOVA revealed no significant interaction ($Wilks\ Lambda = .98$, $F(2, 72) = .45$, $p = .64$). The power for detecting an effect was small (.12), therefore making it difficult to make inferences. The patterns of cortisol reactivity across the 3 years for children of low and high sensitive mothers are shown in Figure 12. It can be seen that even though not statistically significant, children of more sensitive mothers seemed to have more

stable pattern of cortisol response than those of low sensitive mothers, who seem to have a peak cortisol response in Year 2.

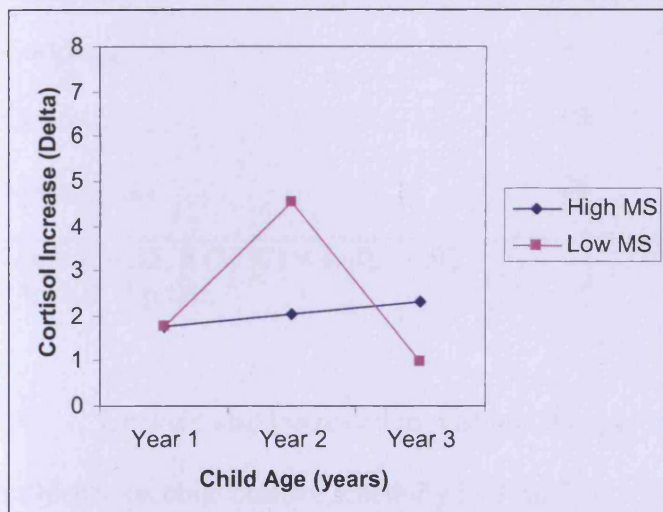


Figure 12: Development of cortisol reactivity in children of high and low MS_{normal} -year1 mothers

Next, we were interested in whether MS_{normal} in Year 1 influenced child cortisol reactivity in Year 3. A multiple linear regression was used to test this effect, while controlling for previous child cortisol reactivity (Table 3). It can be seen that both child Cortisol Δ at Year 1 and maternal sensitivity at baseline in the first year of life have an influence on child Cortisol Δ in Year 3. Higher Cortisol Δ in Year 1 and higher maternal sensitivity were associated with greater Cortisol Δ in Year 3.

Table 3: Multiple regression analysis for variables predicting child Cortisol Δ aged 3 (N=38)

Variable	B	SE B	β
Cort Δ year1	.31	.09	.49**
Cort Δ year2	.02	.05	.06
MS _{normal-year1}	.25	.12	.30*

Note: $R^2=.31$, $F(3, 37) = 6.40$, $p<.01$

** $p<.01$, * $p<.05$

We were also interested in whether changes in maternal sensitivity had an influence on child cortisol reactivity in Year 3. It was found that even though children of mothers who decreased in sensitivity had higher Cortisol Δ (Mean= 2.34, $SD=2.18$) than those of the mothers who increased/remained stable (Mean=1.22, $SD=1.82$), the difference was not statistically significant ($t(2, 36) = 1.54$, $p=.13$).

In terms of the influence of MS_{distress} in Year 1, a mixed between-within subjects ANOVA revealed no significant interaction between MS_{distress} and time, suggesting that the level of maternal sensitivity did not have an impact on child cortisol reactivity ($Wilks\ Lambda=.93$, $F(2, 72) = 1.42$, $p=.26$). Again, the power was small (.28) calling for caution of interpretation of effects. There was no difference in the pattern of Cortisol Δ of children of high and low sensitive mothers.

In terms of the influence of MI_{baseline} on patterns of Cortisol Δ , a mixed between-within subjects ANOVA revealed no significant interaction between MI_{baseline} and time, suggesting that the level of maternal intrusiveness did not affect child cortisol reactivity development ($Wilks\ Lambda=.98$, $F(2, 72) = .46$, $p=.63$). Again, the power was small (.12). The pattern of Cortisol Δ for the two groups was not different.

Similarly, there were no differences in $\Delta\text{Cortisol}$ between mothers high and low in $\text{MS}_{\text{distress}}$ in Year 1.

Child Behavioural Recovery

We were further interested in whether early maternal sensitivity in response to child distress ($\text{MS}_{\text{distress}}$ in Y1) was important for the development of the ability to recover from stress. A mixed between-within subjects ANOVA revealed no significant interaction between $\text{MS}_{\text{distress}}$ and time, suggesting that the level of maternal sensitivity did not have an impact on the development of the ability to recover ($\text{Wilks Lambda} = .98, F(2, 72) = .30, p = .75$). Because of the small sample size the power to detect an effect was low (.09) and the null result should be interpreted with caution. The pattern of recovery speed across the first three years for children of high and low sensitivity mothers can be inspected in Figure 13. Similarly, there was no effect of maternal intrusiveness after distress on the patterns of child recovery.

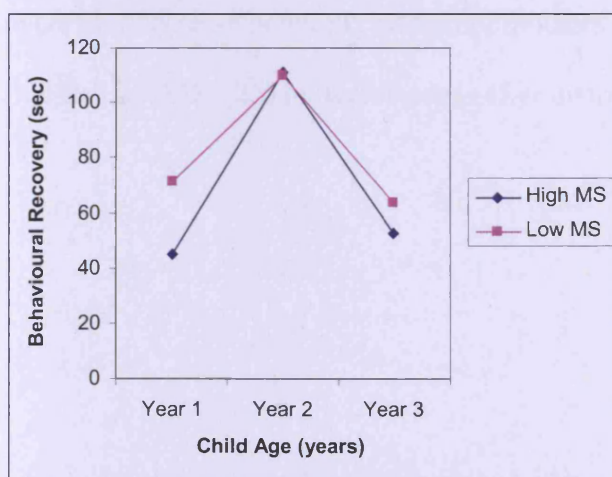


Figure 13: Development of behavioural reactivity in children of high and low $\text{MS}_{\text{distress-year1}}$ mothers

Child cortisol recovery in Year 3

Since a cortisol recovery sample was collected only in Year 3, we were not able to investigate the developmental pattern of endocrinological recovery. In Year 3 we found an overall decrease in cortisol level from peak value to recovery sample (Paired-samples $t(37) = 9.13, p < .01$). We were interested in whether maternal behaviour in infancy was associated with child cortisol recovery. In terms of MS_{baseline} , an independent-samples t -test revealed no difference in cortisol decrease following stress between children of mothers high (Mean = .26, $SD = .23$) and low (Mean = .22, $SD = .20$) in baseline sensitivity ($t(2, 36) = -.52, p = .61$). In terms of MS_{distress} , there was also no difference in cortisol decrease between children of mothers high (Mean = .28, $SD = .24$) and low (Mean = .20, $SD = .18$) in sensitivity after distress ($t(2, 36) = -1.18, p = .25$).

For MI_{baseline} , there was no difference in cortisol decrease between children of mothers low (Mean = .24, $SD = .20$) and high (Mean = .24, $SD = .25$) in baseline intrusiveness ($t(2, 36) = -.11, p = .91$). Similarly, for MS_{distress} , there was no difference in cortisol decrease between children of mothers high (Mean = .24, $SD = .20$) and low (Mean = .25, $SD = .25$) in intrusiveness after distress ($t(2, 36) = -.18, p = .86$).

Discussion

The aims of the present study were to investigate the developmental trends of both maternal behaviours and child behavioural and physiological reactivity and recovery across early childhood and to assess whether early maternal behaviour moderated the patterns of child emotionality.

As hypothesized, we found relatively low stability of maternal sensitivity and intrusiveness across time and different emotional states of the child. In terms of development over time, we found that maternal sensitivity tended to increase from infancy to toddlerhood and intrusiveness tended to decrease. These effects were found for normal interactions and interactions after child distress. These findings are in accord with Kemppinen et al. (2006), who also found mothers to become more sensitive and less controlling from infancy to 24 months. The finding of this study concurs with the idea that parenting behaviour is not stable but develops over time (Feldman et al., 1997). Little stability was found between maternal sensitivity in infancy and years 2 and 3; however, some stability was observed between year 2 and year 3. A similar pattern of results was established for maternal intrusiveness.

In support of the suggestions by Leerkes et al. (2009), it was also found that maternal sensitivity in early childhood was not stable across different emotional states of the child. In the present sample, mothers generally became more sensitive and less intrusive in response to an episode of separation and child distress. These findings are strengthened by the fact that maternal behaviours were studied in identical environments before and after distress, therefore the changes in maternal behaviour cannot be attributed to variation in the testing environment. This supports the proposal by Leerkes et al. (2009) that maternal behaviour is not only subject to change across time but also across different emotional states of the child. This calls for more

specific and separate assessments of maternal behaviour in response to a calm and playful child as opposed to when a child is distressed. These observations of mothers becoming more sensitive and less intrusive following child distress were done in a normal sample. It would be interesting to conduct a similar study in a more at-risk sample of mothers, for example of lower SES or those experiencing marital problems or instability. It can be suggested that some mothers can become less sensitive and more intrusive with children during episodes of distress if there are more emotional demands on the mother in her life. Overall, our findings provide evidence that maternal behaviours are not stable across early childhood and across different emotional states of the children, as it is often assumed in research on maternal behaviour (Kempinnen et al., 2006). Our findings highlight the importance of conducting longitudinal assessments of maternal behaviour across childhood and different situations. Collectively, these findings indicate that maternal behaviour towards her child is not static but subject to change and development during the first years of life. It could be hypothesized that changes in family life, maternal employment, maternal mental health, and attendance to childcare may influence maternal behaviour. Indeed, Feldman et al. (1997) found that factors such as maternal trait anxiety, child difficulty and paternal involvement to all be important moderating factors of maternal behaviour. Ahnert et al. (2004) also found attachment security to be unstable and to be subject to change following child attendance to childcare. Taken together, these results suggest that both mother and child change and adjust their behaviour styles in response to changes in their environment.

Secondly, we were interested in the developmental patterns of children's stress reactivity and regulation across time. In terms of behavioural reactivity, little stability was found in children's reactions to distress from infancy to toddlerhood. We found

that child distress in response to separation and novelty generally increased from first year to the second and third years of life, but remained relatively stable between year two and three. This developmental pattern was anticipated because children tend to become more fearful from infancy to toddlerhood (Gunnar et al., 1996; Ollendick et al., 2001). Although the underlying reasons for these increases are not fully understood and identified, it has been suggested that the developing cognitive abilities of older children, particularly the ability to identify and appraise unfamiliar events as potentially dangerous, can explain the increased fearfulness (Ollendick et al., 2001).

In terms of cortisol, even though child cortisol reactivity in response to distress tended to peak in year 2, this difference was not statistically significant, suggesting that cortisol reactivity to separation and novelty across early childhood was relatively stable. This provides evidence for the stability of child HPA axis activity across early childhood. Our findings extend the work of Lewis and Ramsey (1995) who found emerging stability in child cortisol reactivity between 6 and 18 months. In addition to cortisol reactivity, we were also interested in the development of cortisol baseline levels across early childhood. It was found that cortisol baseline levels changed over time, with generally decreasing levels from infancy to childhood. Although this is an unexplored issue, the current finding is in accord with at least one previous study (Watamura, Donzella, Kertes & Gunnar, 2004).

We were also interested in the development of children's ability to recover from stress. It was found that children's time to recover from distress peaked in Year 2 and dropped when they were 3 years old, despite the levels of distress being similar at age 2 and 3 years. This suggests that with age, children are better able to recover from stress, which may partly be due to their developing cognitive abilities and coping strategies.

We also investigated the associations between child physiological and behavioural indices of stress across early childhood. Overall, we found few associations between cortisol and behavioural reactivity in early childhood, supporting results of Lewis and Ramsey (2003). Interestingly, however, at age two behavioural distress and cortisol reactivity were positively related. Children in the present sample had their highest cortisol values when they were two years old, and therefore it is perhaps at this age of heightened sensitivity to distress that behavioural distress and cortisol reactivity are associated.

We were also interested in the relationship between child reactivity to and recovery from stress, since some research suggests that these are different aspects of temperament (Rothbart, 1989; Ramsey & Lewis, 2003), while others suggest that the two concepts are intertwined (Calkins & Johnson, 1998). We found that child reactivity to and speed of recovery from separation and novelty stress were significantly associated across the three years, with higher distress being associated with a longer recovery time. These findings suggest that reactivity and recovery are not independent structures in early childhood and that higher distress levels are linked with longer recovery time. It is worth noting that the different stressors used across studies, ranging from inoculation to separation, may to some extent be responsible for the discrepancy in results (Albers et al., 2008; Ramsey & Lewis, 2003).

Overall, we found little to moderate stability in child emotionality across the first three years of life. Behavioural reactivity and recovery, as well as baseline cortisol levels, showed low stability, whereas cortisol reactivity had relatively moderate stability. Collectively, the results indicate that emotional development in early childhood is subject to change, and we were therefore interested in whether the

mother played a moderating role in this process of emotional development in the early years of life.

Maternal sensitivity during normal interaction in year 1 was a moderator of child behavioural reactivity across time. Specifically, children of highly sensitive mothers were less distressed to begin with (at one year old), but became increasingly distressed over time. The distress pattern of children of low sensitive mothers was different: it was higher in Year 2 and decreased in Year 3 (see Figure 8, p. 181). In addition, higher early maternal sensitivity (when children were one year old) was found to be related to higher behavioural distress and greater cortisol reactivity in children when they were 3 years old. It can be suggested that children whose mothers were highly sensitive in infancy may still rely on their mothers' presence and comfort in distressing situations when they are three years old.

There are relatively few studies that have assessed the influence of maternal sensitivity or intrusiveness on child reactivity to separation and novelty across early childhood. One study, however, looked at the relationship between attachment security and child emotional and cortisol reactivity in response to maternal separation and attendance at nursery (Ahnert et al., 2004). Even though sensitivity and attachment are different structures, higher sensitivity has often been linked with development of secure attachments (Bakermans-Kranenburg, van IJzendoorn and Juffer, 2003; Fish & Stifter, 1995), and is an indicator of positive mother-child relationship; therefore some parallels with this study were made. Ahnert et al. (2004) looked at the influence of the mother in child adjustment to novelty of childcare and separation from the mother. They examined two stages in their study: first a transition phase where mothers accompanied children to the nursery to help them adjust to the novelty of childcare and second a separation phase, where children were left in

childcare without their mother. It was found that when the mother was present, securely attached children did not display increases in behavioural or cortisol distress in response to being in an unfamiliar environment. However, during the separation phase, it was the securely attached children who displayed higher distress levels and greater cortisol increase. It has been suggested that securely attached children, who receive sensitive parenting, show more distress in situations of separation and novelty because the child is left to cope without their mother; this event 'violates their expectations' that their mother will respond and their signals of distress are meant to bring about the return of the mother (Hennessy & Levine, 1979; Ahnert et al., 2004).

The findings of this study and of the study by Ahnert et al. (2004) suggest that children of more sensitive mothers, who may be or become securely attached, use their mother as a reference base when faced with novelty, which may prevent children from getting distressed when their mother is in proximity, but may lead to higher levels of behavioural distress during an episode of separation. Overall, these findings suggest that general maternal sensitivity to the child in the first year of life may be an important moderator and predictor of the developmental patterns of child emotional reactivity in the first three years of life.

Apart from this moderating effect and the influence of maternal sensitivity on child behavioural and cortisol reactivity we did not find maternal characteristics to moderate the patterns of child cortisol reactivity or behavioural recovery. There were some interesting patterns of results, where, for example, children of more sensitive mothers in Year 1 were found to have more stable cortisol reactivity levels than those of less sensitive mothers. However, this and other effects were not statistically significant. There appeared to be no differences in the patterns of behavioural recovery between children of high and low sensitive/intrusive mothers. There is some

evidence that children of more sensitive mothers may have a better cortisol recovery following stress (Albers et al., 2008), but we found no differences in cortisol recovery between high and low sensitive mothers. The stress manipulation used in present study was different from the one used by Albers and colleagues, who used an everyday bathing situation as a stress stimulus. Therefore our findings appear more consistent with the results from the Ahnert et al. (2004) study, which found no evidence that securely attached children regulate stress better than insecurely attached children in the absence of their mothers. The results of present study suggest that early levels of maternal sensitivity and/or intrusiveness are not associated with three-year-olds' ability to recover from stress in the absence of their mothers.

However, the non-significant results of present study should be interpreted with caution. Because of the small sample size the power to detect effects was very small, making it difficult to interpret the non-significant findings. It therefore remains unclear whether maternal behaviours do not moderate developmental trajectories of child physiological reactivity and regulation or whether it was not possible to detect these effects because of the small sample size.

The small sample size is a clear limitation of the present study and is a consequence of the timing of the ongoing data collection for the larger study from which these data derive. An important goal of future research should be to analyze the developmental patterns of both maternal and child behaviours, and the moderating role of mother behaviour, in the larger sample of approximately 70 children.

Support was not found for the hypothesis that maternal behaviour after child distress would be related to the child's coping with stress. As mentioned before, the failure to find support for our hypotheses could be attributable to the low power to detect effects. In addition, in the present sample, the large majority of mothers

increased in sensitivity and decreased in intrusiveness following child distress, and it would be interesting to compare these patterns to the ones observed in a more at-risk sample of mothers. Therefore, even though no influence of maternal behaviour after distress was found in our children, it may be an important factor to consider in at-risk samples.

Overall, the results of present study highlight the importance of considering the *development* of maternal characteristics in addition to, and in parallel with, the development of the child's coping with stress. It seems that the majority of current studies assume that such a development of child factors occurs even though actual data are scarce. Even less attention has been devoted to study of the developmental of maternal characteristics. The present study shows that maternal behaviour is dynamic. The development of maternal sensitivity may be influenced by many different kinds of factors in her environment (Feldman et al., 1997). One of the main strengths of the present study is that it was possible to assess maternal behaviours as a possible moderator of the developmental trends of child coping with stress, as well as a predictor of future distress reactivity. A possible next step in our current research is to examine those factors that impact on maternal behaviour in more detail (e.g., father involvement, child day care attendance, birth of second child, work involvement). In addition, the findings of the present study highlight the role of maternal sensitivity as an important moderator of child reactivity to stress.

Chapter 8. General Discussion

The main aims of the thesis were to assess the influence of prenatal maternal stress and postnatal maternal behaviour on child temperament and the development of emotional reactivity and recovery in early childhood. The thesis consists of two parts: the first part examined the prenatal influence of the mother on birth outcomes and infant temperament and cognition; the second part investigated the role of the mother in the development of child emotional reactivity and recovery in the first three years of life. This chapter describes and discusses the main findings of this research and puts forward suggestions for future research.

Influence of prenatal stress on child development

The study described in Chapter 2 aimed to gain a better understanding of the influence of prenatal stress on infant birth outcomes, temperament and cognition in the first year of life. More specifically, we were interested in how prenatal stress might influence these outcomes. The activity of maternal HPA axis as reflected by the concentration of its end product, cortisol, has been proposed to influence the development of the foetal HPA axis (Talge et al., 2007). One of the strengths of the current study was the multi-method approach used to investigate the influence of prenatal stress and anxiety on child outcomes by including a combination of maternal self-reports of perceived stress and pregnancy-related anxiety together with the more objective indices of prenatal stress, such as maternal and amniotic fluid cortisol levels collected during amniocentesis in early pregnancy. We were particularly interested in the association between maternal and foetal cortisol levels. There has been some debate as to how strongly maternal cortisol can influence foetal cortisol concentrations (Benediktsson & Seckl, 1998; Gitau et al. 1998; Glover et al., 2009). It

has been suggested that the foetus is protected from maternal cortisol elevations by 11 beta-hydroxysteroid-dehydrogenase enzyme (11 β -HSD), which converts cortisol into inactive cortisone (Benediktsson & Seckl, 1998). However, the activity of this enzyme may be downregulated during stress (Glover et al. 2009), making it easier for cortisol to pass from mother to foetus. Gitau et al. (1998) found maternal and foetal cortisol concentrations to be highly correlated ($r = .62$) during the process of amniocentesis, a stressful procedure. In a later study Glover et al. (2009) found this association be much lower at .32. In our study the association was also low ($r = .18$), but significant nevertheless.

A possible reason for the lower association in our study could be the fact that cortisol concentrations were collected in early pregnancy, while collection ranged from early to later pregnancy in studies by Glover et al. (2009) and Gitau et al. (1998). Even though the collection of cortisol in these studies was also carried out during amniocentesis, the time period for collection was considerably larger (13-35 weeks) as opposed to the range in our study (15-18 weeks). In the rat, 11 β -HSD is highly expressed around mid-pregnancy, protecting the foetus from elevations in maternal glucocorticoids (Diaz, Brown & Seckl, 1998). This enzyme expression is then greatly reduced in late pregnancy, which would then allow the glucocorticoids to interact with receptors and influence the foetus (Diaz et al., 2003). Huizink et al. (2003) found that only late pregnancy cortisol levels were related to infant outcomes. This may explain the lower association found in the current study. In addition, various moderating factors could influence the association between plasma and amniotic fluid concentrations. For example, Glover et al. (2009) found that the association between plasma and amniotic fluid cortisol was only significant in a sub-sample of highly anxious women, suggesting that the levels of anxiety can moderate this relationship. It

is possible that overall anxiety levels of women taking part in our study were relatively low, resulting in a lower association between plasma and amniotic fluid cortisol levels.

We were interested in the influence of subjectively experienced stress and anxiety, as well as maternal and amniotic fluid cortisol levels on child outcomes. This multi-method approach aimed to provide a better insight into the pathways through which prenatal stress can have an impact on child outcomes. We hypothesized that amniotic fluid cortisol levels would have particular importance because of its direct association with foetal development. It was found that higher amniotic fluid cortisol levels were marginally significantly related to lower birth weight and positively related to child activity levels at three months. However, apart from these effects, no other effect of amniotic fluid cortisol was found. Similarly, maternal plasma cortisol levels significantly predicted infant 'distress to limitation' and 'fear' at six months. Maternal self-reported stress and pregnancy-related anxiety also predicted different parameters of infant temperament at three and six months. Overall, it can be concluded that self-reported stress was a better predictor of child outcome than biologically based predictors. This could be due to the fact that maternal self-reports reflect enduring feelings of stress and anxiety (our questionnaires assessed feelings of stress and anxiety over a 4 week period) instead of a single time-point assessment, as is the case with the biological sampling. Therefore, maternal reports may be better indicators of maternal well-being through that period of her pregnancy.

However, an alternative reason for the fact that maternal self-reports were related to more child temperament dimensions could be the fact that temperament was also mother-reported. More associations between maternal reports and infant temperament could therefore be due to mother-report effect. Although maternal

reports can give valuable insight into child temperament, they are also subjective because they can be influenced by maternal sensitivities and her emotional well-being (Leerkes & Crockenberg, 2003). For example, maternal reports of child temperament are weakly associated with independent observations of temperament (Seifer, Sameroff, Barrett, & Krafchuk, 1994). In order to clarify this confound, future studies should aim to combine maternal reports of infant temperament with observations of temperament as well as physiological assessments of dimensions such as fearfulness and distress to limitation.

Another possible reason for fewer associations between cortisol and child outcomes is that some past research has shown that maternal cortisol in late pregnancy is more strongly associated with child outcomes than cortisol collected in early pregnancy (Huizink, De Medina, Mulder, Visser, Buitelaar, 2002). The amniocentesis procedure is usually carried out in the 2nd trimester of pregnancy, and later amniocentesis assessments are usually associated with higher risk for poor obstetric outcome. Thus we might have carried out our procedure at a time when maternal cortisol levels do not yet have the most profound influence on foetal development and subsequent child outcomes.

Overall, we found that some prenatal indices of stress and anxiety influenced infant birth outcome and temperament between 3 and 6 months. It is worth noting that no influence of prenatal stress on child temperament was detected at 9 months of age. This suggests that whatever effect there was, it was short-lived and limited to the early postnatal period. It is worth keeping in mind that our sample was a normal healthy one and that good quality maternal care (e.g., a positive mother-child relationship) may attenuate any negative influence of prenatal stress (Kaplan, Evans & Monk, 2008). Indeed, Kaplan et al. (2008) found that early maternal sensitivity attenuated the

negative influence of prenatal stress on child temperament and baseline cortisol levels. These results suggest that early maternal behaviour may moderate the influence of prenatal stress and foetal programming on child temperament, which may explain why the influence of prenatal stress was not found after six months of age.

Limitations of prenatal study

One of the limitations in assessing the influence of maternal HPA axis activity on foetal and child development was the fact that only a single assessment of maternal cortisol level was obtained. Therefore, in order to gain a better understanding of the influence of andenocortical markers of prenatal stress on child development, future research should include multiple assessments of maternal cortisol levels throughout the pregnancy, as well as including an assessment of amniotic fluid cortisol levels. Therefore, future studies should aim to obtain cortisol samples throughout pregnancy at least capture cortisol levels during early, mid- and late pregnancy in addition to the assessment during amniocentesis in order to gain a better understanding of maternal HPA axis activity in relation to self-reported stress and anxiety. However, multiple assessments of amniotic fluid cortisol concentrations are not possible, as multiple amniocentesis samplings are associated with increased risk for poor obstetric outcomes. However, there could be other indirect assessments of foetal weight and development by looking at foetal heart rate and motor development through ultrasound scans for example (DiPietro, Ghera & Costigan, 2008).

There is evidence to suggest that postnatal maternal well-being as well as the developing mother-child relationship may moderate the influence of prenatal maternal well-being on child outcomes (Bergman, Sarkar, Glover & O'Connor, 2008; Hay, Pawlby, Waters & Sharp, 2008). For example, it has been shown that attachment

security in the first year of can moderate the influence of prenatal stress on child outcomes (Bergman et al., 2008). The present study did not include assessments of postnatal maternal well being or mother-child relationship as moderators of the influence of prenatal stress on child development.

To summarize, our study adds to the scarce literature on the role of prenatal maternal stress on foetal and infant outcome. It was also found that both maternal self-reported stress and anxiety, and her cortisol stress response had an effect on birth outcome and early temperament. This highlights the importance of combining both subjective and objective measures of stress and anxiety in order to derive a more complete picture of prenatal stress experience. In addition, it was found that relatively small variations in prenatal stress in a sample of normal healthy women affected infant birth outcomes and early temperament.

The role of the mother in child emotional reactivity and recovery in early childhood

The second main aim of the present thesis was to assess the influence of postnatal maternal sensitivity and intrusiveness on child behavioural and physiological coping with stress in the absence of their mother. In Chapter 6 we reported on three cross-sectional studies that assessed the influence of maternal behaviour when children were one, two, and three years old. These studies used Fish and Stifter's (1995) measure of maternal sensitivity and intrusiveness in different situations, first during normal, playful interaction and again after an episode of child distress. This design allowed us to assess maternal behaviours across different emotional states of the child, since there is some evidence to suggest that maternal behaviour may not be stable across different situations (McElwin & Booth-LaForce, 2006).

We were interested in the influence of two maternal behaviours: sensitivity and intrusiveness. Consistent with our hypotheses, sensitivity and intrusiveness were found to be negatively associated across the first three years of life, demonstrating that greater sensitivity was associated with less intrusiveness. However, when children were three years old, the negative association between maternal sensitivity and intrusiveness during interaction after distress was found to be non-significant. This finding suggests that around the age of three, greater sensitivity is not necessarily associated with less intrusiveness in an emotionally challenging situation and that mothers can be highly sensitive and intrusive at the same time. It also means that mothers can be low in sensitivity but not necessarily high in intrusiveness. Fish and Stifter (1995) indeed found separate caregiving groups of mothers low in sensitivity and intrusiveness and low in sensitivity and high in intrusiveness.

In addition and as hypothesized, sensitivity and intrusiveness were not stable characteristics but changed depending on child's emotional state. Mothers became more sensitive and less intrusive when the child was in distress. This finding suggests that maternal behaviour is not stable across different emotional states of the child across early childhood, calling for more in-depth assessment of maternal behaviour that takes different emotional states of the child into account.

Overall, we found the influence of maternal behaviour on child stress reactivity and recovery to be different at one, two and three years. In one-year-olds, maternal sensitivity or intrusiveness had no effect on the child's behavioural distress, the child's cortisol stress response or his/her recovery from distress. However, when the children were two years old, maternal sensitivity (during normal interaction) was associated with less behavioural distress, a weaker cortisol response, and a faster recovery from stress. These results suggest that sensitive mothers help two-year-olds

to cope more effectively with stress. However, these effects subsided by the age of three; maternal behaviour was not found to be associated with child reactivity or recovery at this age.

The results of these cross-sectional studies suggest that maternal sensitivity and intrusiveness only have a direct influence on child coping with stress around the age of two. It is still possible that early maternal behaviour is important for the development of child coping with stress over time (e.g. Pauli-Pott et al., 2004), and we addressed this issue in our longitudinal study. In Chapter 7 we examined the influence of early maternal behaviour on later child stress reactivity and recovery, as well as on the longitudinal patterns of their development over the first three years of life. Additionally, we investigated the stability and change of maternal behaviour over time.

In terms of stability of maternal behaviour, little stability was found across the first three years of life, with only some stability between the ages of two and three. These findings make an important contribution to the study of maternal interactive behaviour, since most studies assume that maternal sensitivity and intrusiveness are relatively stable. The current results suggest that maternal behaviour is complex and subject to change, because of ongoing development in her child or because of changes in the mother's environment. For example, events like the mother returning back to work or the child starting childcare could cause changes in maternal behaviour (Brooks-Gunn, Han & Waldfogel, 2010). Our results suggest that various factors outside the mother-infant dyad may have an important influence on the behaviour of the mother towards her child.

Our results suggest that maternal behaviour should ideally be studied across different emotional states of the child and across time in order to gain a better

understanding of maternal behaviour and its potential influence on child development. Because of its apparent instability, it is possible that maternal behaviour in response to varying emotional states of the child and at different times during childhood may exert different influences on subsequent child coping with stress, as has been found in our cross-sectional studies. Acknowledging that mothers change in their behaviour across childhood is important for those interventions that focus on maternal behaviour and mother-child relationship.

In addition to investigating the development of maternal behaviour longitudinally (Chapter 7) we were also interested in the development of child's emotional reactivity and recovery in response to separation and novelty in early childhood. Low stability in child reactivity to distress and recovery from distress were found; however, cortisol reactivity was relatively stable across time. We found that behavioural distress reactivity increased from infancy to toddlerhood, and remained relatively stable between the ages of two and three. Interestingly, however, the development of children's ability to recover followed a different pattern. Children were relatively fast in recovery during infancy, while taking increasingly longer to recover by the age of two. At the age of three however, even though distress levels between two and three-year olds were the same, three-year olds were considerably faster in their ability to recover from distress. This suggests that with age, children develop more effective coping strategies, allowing them to recover from distress faster. These results, like those observed in the mother, suggest that the development of children's coping with stress is a process that evolves in a dynamic and complex way.

As mentioned earlier, one of the main aims of the present thesis was to look at the role of the mother in child behavioural and adrenocortical coping with separation

and distress. More specifically, we were interested in finding out whether early maternal sensitivity and/or intrusiveness were associated with later child emotional reactivity and recovery, and whether maternal behaviour moderated the developmental patterns of the infant characteristics. Early maternal sensitivity was indeed found to be associated with later child behavioural and cortisol reactivity. It was found that maternal sensitivity at age one predicted more behavioural distress in children aged three. In addition, infants of sensitive mothers were less distressed when they were one year old, but they were more distressed when they were three years old. In addition, greater maternal sensitivity early in life predicted stronger cortisol reactivity to distress at the age of three. Taken together, these findings indicate that maternal sensitivity early in life has important implications for the child's coping with stress later in life; specifically, maternal sensitivity in the first year seems to have a long-lasting influence on the child's behavioural and cortisol reaction to separation and novelty two years later.

The finding that early sensitivity was associated with more behavioural and physiological distress was somewhat unexpected. It seems to indicate that children of mothers who were more sensitive during their first year of life are more reactive to novelty and still need their mothers' presence when faced with a challenge when they are older. The combination of maternal separation and exposure to novelty in her absence may cause more distress in children of more sensitive mothers because they feel more out of control without their mother and their distress is a protest against her absence (Field, 1985). Both animal and human studies show that the presence of an attachment figure or a sensitive caregiver prevents or buffers elevations of cortisol in response to stress (Levine & Wiener, 1988; Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996; Spangler & Grossmann, 1993). The findings of this study are also

consistent with those of Ahnert et al. (2004) who found that securely attached children showed more behavioural and physiological distress in response to separation from the mother in the context of the novelty of childcare. These results suggest that perhaps children of sensitive mothers who are, or become, securely attached experience a 'violation of expectation' when faced with distressing situations in their mother's absence. We know that in our sample by the age of three, the majority of children attend day care. It is possible that these children are used to separations from their mothers and encountering novel environments. However, the situation in our study is nevertheless a novel and different situation for children, as we observe child coping with distress not only in the absence of their mother, but also in the absence of another adult that the child may look to for protection. For example, even though facing novelty in the absence of their mother in day care, children are rarely unattended. In addition, we found no difference in distress reactivity between children who do or do not attend day care across the years, suggesting that our paradigm of separation and distress still poses novelty for children.

More research on the role and the presence of the mother in child coping with stress across different ages is needed. We propose that the reason why children of more sensitive mothers are more reactive to separation and novelty is because they seek proximity and protection from their mothers. However, alternative explanations are plausible. First of all, it can be proposed that the reason why some children of more sensitive mothers get more distressed is because heightened sensitivity may be associated with 'over-protectiveness', i.e. the children of more sensitive mothers are not exposed to many stressors as mothers usually protect the children from such situations. However, this explanation cannot account for our findings because in the first year of children's lives the average maternal sensitivity scores were only

moderately high (i.e. just above the middle of the scale). As mentioned earlier, little research has been carried out in the area of children's ability to cope with distress in their mothers' absence. Therefore, in order to better understand the role of the mother in preparing her child to cope with separation and distress, further research is necessary. It would be interesting to investigate whether through experiencing more similar situations, children of more sensitive mothers become better able to cope with distress when they are slightly older, for example when they are 4 years old, or whether they may still rely on their mothers' presence.

Even though maternal sensitivity was found to influence behavioural and physiological reactions to stress, it did not seem to affect behavioural or cortisol recovery from stress. Very few studies have looked at the influence of the mother on child speed of recovery from distress. Most of this literature has focused on the effectiveness of maternal soothing during inoculations on the child's ability to recover from distress. For example, Lewis and Ramsey (1999) found no influence of maternal soothing on child behavioural or cortisol recovery from stress. Other studies, however, did find maternal sensitivity to influence child recovery. For example, Albers et al. (2008) and Blair et al. (2008) found that maternal sensitivity was positively associated with a faster cortisol recovery in infants. A possible reason for the lack of an effect is that we used a different stressor than Albers et al. (2008) and Blair et al. (2008), who used milder, more every-day stressors. Our findings suggest that in our sample and using a moderately strong stressor maternal behaviour did not affect child stress recovery.

Maternal intrusiveness did not have any influence on her child's coping with stress, neither when assessed concurrently nor longitudinally. This could be due to the

fact that the majority of mothers in our study displayed low levels of intrusiveness and all scores were in the very low range across the three years of assessments.

In addition, we expected maternal sensitivity in response to child distress to have an important influence on the child's emotional reactivity and recovery, over and above of the influence of her general sensitivity (Leerkes et al., 2009). However, this effect was not found and it is not clear what the reason for the lack of association was. To our knowledge, only the study by Leerkes et al. (2009) addressed this issue directly, and found maternal sensitivity to child distress to be more important than general sensitivity in predicting child outcomes. Our results seem to demonstrate that it is the sensitivity of the mother during normal playful interactions that holds particular importance for child distress reactivity.

The studies in this thesis were carried out in a low-risk, community sample. The rationale for using this sample was to gain better understanding of the effects of everyday variations in prenatal stress and early maternal behaviour on normal development of child reactivity and regulation. It is important to gain a better understanding of the development of behavioural and cortisol reactivity in a normal, healthy and low-risk sample because we first need to comprehend the development of and association between behavioural and endocrinological reactivity to stress in early childhood before we can make use of this knowledge when we start investigating high-risk children and abnormal development. In addition, current studies add to the limited research on the development of maternal sensitivity and intrusiveness across early childhood. Findings from a low-risk sample could serve as a control sample for future work on prenatal stress, child stress reactivity and maternal behaviour in high-risk samples.

Limitations of the postnatal chapters

The research as reported in Chapter 6 and 7 had some limitations. A limitation that is applicable to most of the studies is the small sample size. The problem is most potent in the longitudinal study as reported in Chapter 7, where the sample consisted of 38 children. The small sample size resulted in a low power to detect effects. Because of this, the non-significant results of the longitudinal study should be interpreted with caution, since it is not clear whether there is indeed no influence of maternal behaviours on child recovery or the lack of significant findings is due to the small sample size.

In addition, it was possible to collect cortisol recovery sample only when children were three years old. We were therefore unable to investigate the developmental patterns of children's endocrinological ability to recover from stress across early childhood as well as to investigate what role the mother might play in helping child cortisol recovery when they are one and two years old.

Implications and future directions

The results of this thesis highlight the importance of maternal prenatal and postnatal influence on child temperament and coping with stress. In a series of studies involving normal samples relatively small variations in maternal prenatal stress and postnatal sensitivity were found to be associated with variations in child temperament and emotional reactivity. In future it would be interesting to examine the influence of more adverse experiences of prenatal stress and/or more profound problems in mother-child interaction on child temperament and coping with stress. However, our findings on the effects of normal variations in prenatal stress and postnatal maternal behaviour on child temperament provide insight for the development of interventions

that minimize the effects of prenatal stress and promote positive maternal behaviour in early childhood.

It has been well documented that interventions aimed at improving child emotional development achieve best results when starting early. For example, the Early Head Start intervention program, developed by the Administration on Children, Youth and Families in the United States, was aimed at improving child cognitive, emotional and language development as well as parenting skills and maternal behaviour in low-income families and it started working with families during pregnancy, through to the age of three. The report on this program states that at risk, low-income families should enrol at the program as early as possible, preferably during pregnancy (Love et al., 2009). This report showed that although the intervention improved outcomes among the children whose parents enrolled after birth, the strongest pattern of impacts was observed if parents joined the program during pregnancy (Love et al., 2002).

The studies in this thesis aimed at taking new steps in understanding how and why this early role of the mother prenatally and postnatally is important for child emotional development in early childhood. More specifically, we attempted to disentangle what aspects of prenatal stress are best predictors of child birth weight and early temperament. The findings that the activity of maternal HPA axis is associated with the cortisol levels of the foetus add to the very small number of studies that investigated this relationship (e.g. Bergman et al., 2010). Furthermore, the findings of our study seem to indicate that different assessments of stress and anxiety (self-reports of stress, pregnancy-related anxiety, plasma and amniotic fluid cortisol levels) all carry important information about prenatal stress experience and can be associated

with child outcomes. We found further confirmation that prenatal stress and anxiety can have an influence on infant birth outcomes and early temperament.

Our postnatal studies aimed at gaining better understanding of how and why early maternal behaviour influences child ability to cope with stress across early childhood. We were interested in investigating the stability of maternal behaviours during normal playful interactions and following an episode of child distress as well as across time in order to gain a better understanding of maternal behaviour and its relationship to child coping with stress. The findings of low stability of maternal interactive behaviours across different emotional states as well as across time are important for possible interventions that focus on maternal interactive behaviour. Early interventions concerned with maternal sensitivity should acknowledge this instability and incorporate information on changes in maternal behaviour in order to provide the best support for mothers. This applies to when mothers have changes in their immediate environments, such as returning back to work, experiencing changes in family circumstances and child attendance to day care, all of which could have an impact on maternal interactive behaviour.

Our studies show that child temperament dimensions as well as emotional reactivity and regulation change over time. Therefore, future research should focus on this development as well as educating families about instability of child characteristics and developmental stages. For example, the fact that the children in our study became significantly more distressed over time came as a surprise to the majority of mothers, who expected them to be less upset as they grew older. It seems important to inform parents about the heightened reactivity to separation and novelty when aged two and three years in order to facilitate more adaptive separations between mother and child, for example when the child enters childcare.

For obvious reasons, there is very little research on the development of children's ability to cope with distressing situations in the absence of their mother. Although informative, there are many occasions, especially in the life of two and three year olds, which they will have to face novel and potentially stressful situations while their mother is not around. Our results show that three year olds still get quite distressed while being separated from their mothers, but that potentially important individual differences become apparent in how quickly they recover from these events. It seems that more research should be conducted in order to understand how and at what age a child learns to cope with stress in the absence of their mothers. This can have important implications for informing policies of better transition into day care and other scenarios involving mother-child separation.

The findings of the thesis provide important insights into prenatal stress and postnatal maternal behaviour in a normal, low-risk sample. We saw that small variation in prenatal stress and maternal sensitivity can influence child temperaments and emotional reactivity. It can be hypothesized that greater variation in both prenatal stress and maternal behaviour in high-risk samples will result in more profound and possibly long-lasting influences of the mother on child temperament. Our findings provide an important foundation for understanding the principles of how and why the role of the mother is important in child temperament and ability to cope with stress.

To conclude, the studies in this thesis provide significant insights into the mechanisms underlying the influence of prenatal stress and postnatal maternal behaviour on child emotional development. It was found that the prenatal and postnatal role of the mother in child emotional development is complex. In terms of prenatal stress, it has been demonstrated that different assessments of stress and anxiety (i.e. maternal self-reports and cortisol assessments) have different effects on

child outcomes, and that both objective and subjective reports capture important information about the prenatal stress experience and should be included as part of future research. In addition, the postnatal studies in this thesis demonstrate the complexity and instability of maternal behaviour, which is often overlooked in the literature. Our postnatal chapters inform developmental research as well as child health-care practitioners that maternal separation and novelty still pose a stressful challenge for toddlers, and that children still need their mother as a reference base when facing novelty at the age of three.

References

- Acock, A.C. (2005). Working with missing values. *Journal of Marriage and Family*, 67, 1012-1028
- Ainsworth, M.D.S. (1989). Attachments beyond infancy. *American Psychologist*, 44, 709-716
- Ainsworth, M.D.S., Blehar, M.C., Waters, E., & Wall, S. (1978). *Patterns of attachment: A psychological study of the strange situation*. Hillsdale, NJ: Erlbaum
- Ahnert, L., Gunnar, M.R., Lamb, M.E., Barthel, M. (2004). Transition to child care: Associations with infant-mother attachment, infant negative emotion, and cortisol elevations. *Child Development*, 75, 639-650
- Albers, E.M., Riksen-Walraven, J.M., Sweep, F.C.G., de Weerth, C. (2008). Maternal behavior predicts infant cortisol recovery from a mild everyday stressor. *Journal of Child Psychology and Psychiatry*, 49, 97-103
- Austin, M. P., Hadzi-Pavlovic, D., Leader, L., Saint, K., & Parker, G. (2005). Maternal trait anxiety, depression and life event stress in pregnancy: relationships with infant temperament. *Early Human Development*, 81(2), 183-190.
- Bakermans-Kranenburg, M.J., Van IJZendoorn, M.H., & Juffer, F. (2003). Less is more: Meta-Analyses of sensitivity and attachment interventions in early childhood. *Psychological Bulletin*, 129, 195-215
- Bates, J.E., Freeland, C.A.B., & Lounsbury, M.L. (1979). Measurement of infant difficulty. *Child Development*, 50, 794-803
- Bates, J.E., Pettit, G.S., Dpdge, K.A., & Ridge, B. (1998). Interaction of temperamental resistance to control and restrictive parenting in the

development of externalizing behaviour. *Developmental Psychology*, 34, 982-995

Barr, R.G., & Gunnar, M. (2000). Colic: The 'transient responsivity' hypothesis. In R.G. Barr & B. Hopkins (Eds), *Crying as a sign, a symptom & a signal: Clinical emotional and developmental aspects of infant and toddler crying* (pp.41-66). New York: Cambridge University

Bayley, N. (1969). *Bayley scales of infant development: Birth to two years*. New York: Psychological Corp

Belsky, J. (1997). Theory testing, effect size evaluation, and differential susceptibility to rearing influence: The case of mothering and attachment. *Child Development*, 68, 598-600

Belsky, J. (2005). Differential susceptibility to rearing influence: An evolutionary hypothesis and some evidence. In B. Ellis & D. Bjorklund (Eds.), *Origins of the social mind: Evolutionary psychology and child development* (pp. 139-164). New York: Guilford

Benediktsson R., & Seckl, J. R. (1998). Understanding human parturition. *Lancet*, 344, 77-81

Bergman, K., Sarkar, P., O'Connor, T. G., Modi, N., & Glover, V. (unpublished). Maternal stress during pregnancy predicts cognitive ability and fearfulness in infancy

Biederman, J., Rosenbaum, J.F., Bolduc-Murphy, E.A., Faraone, S.V., Chaloff, J., Hirshfield, M.A., Kagan, J. (1993). A 3-year follow-up of children with and without behavioral inhibition. *Journal of American Academy of Child and Adolescent Psychiatry*, 32, 814-821

Blair, C., Granger, D.A., Wiloughby, M., Kivlighan, K., & Family Life Project

investigators (2006). Maternal sensitivity is related to Hypothalamic-Pituitary-Adrenal axis stress reactivity and regulation in response to emotion challenge in 6-month-old infants. *Annals of New York Academy of Sciences*, 1094, 263-267

Blair, C., Granger, D.A., Kilvighan, K.T., Mills-Koonce, R., Wiloughby, M., Greenberg, M.T., Hibel, L., Furtano, C.K., & Family Life Project investigators (2008). Maternal and child contributors to cortisol response to emotional arousal in young children from low-income, rural communities. *Developmental Psychology*, 44, 1095-1109

Bohlin, G., & Hagekull, B. (2000). Behavior problems in Swedish four-year-olds. The importance of maternal sensitivity and social context. P.M. Crittenden & A.H. Claussen (Eds), *The organization of attachment relationships. Maturation, culture, and context*. Cambridge: Cambridge University Press.

Bowlby, J. (1969). *Attachment and loss, Vol. 1: Attachment*. New York: Basic Books

Bowlby, J. (1982). *Attachment*, 2nd edition of vol.1 of *Attachment and Loss*. London: Hogarth Press

Bowlby, J. (1988). *A Secure Base: Clinical Applications of Attachment Theory*. London: Routledge

Bornstein, M.H., & Tamis-LeMonda, C.S. (2004). Mother- Infant interactions. In Bremer, G., & Fogel, A. (Eds.) *Blackwell handbook of infant development*. Wiley-Blackwell

Buitelaar, J.K., Huizink, A.C., Mulder, E.J., Robles de Medina, P.G., Visser, G.H.A. (2003). Prenatal stress and cognitive development and temperament in infants. *Neurobiology of Aging*, 23, 53-60

Buss, K.A., Davidson, R.J., Kalin, N.H., & Goldsmith, H.H. (2004). Context-specific

freezing and physiological reactivity as a dysregulated fear response.

Developmental Psychology, 40, 583-584

Buss, K.A., Malmstadt Schumacher, J., Dolsky, I., Kalin, N.H., Goldsmith, H.H., & Davidson, R.J. (2003). Right frontal brain activity, cortisol and withdrawal behavior in 6-months old infants. *Behavioral Neuroscience*, 117, 11-20

Brumariu, L.E., & Kerns, K.A. (2010). Parent-child attachment and internalizing symptoms in childhood and adolescence: A review of empirical findings and future directions. *Development and Psychopathology*, 22, 177-203

Carey, W.B. (1970). A simplified method of measuring infant temperament. *Journal of Pediatrics*, 77, 735-739

Carey, W.B., & McDevitt, S.C. (1978). Revision of the infant temperament questionnaire. *Pediatrics*, 61, 735-739

Carranza, J.A., Perez-Lopez, J., Gonzalez, C., & Martizez-Fuentes, M.T. (2000). A longitudinal study of temperament in infancy: Stability and convergence of measures. *European Journal of Personality*, 14, 21-37

Cohen, S. (1986). Contrasting the hassle scale and the perceived stress scale. *American Psychologist*, 41, 159-163

Cohen, S., & Williamson, G.M. (1987). Perceived stress in a probability sample of the United States. In S. Spacapan, S. Oskamp (Eds.), *The social Psychology of Health* (31-47). Newsbury Park, CA: SAGE publications

Colder, C.R., Mott, J.A., & Berman, A.S. (2002). The interactive effects of infant activity level and fear on growth trajectories of early childhood behavior problems. *Development and Psychopathology*, 14, 1-23

Compas, B.E., Connor-Smith, J.K., Salzman, H., Thomsen, A.H., Wadsworth, M.E. (2001). Coping with stress during childhood and adolescence: Problems, progress, and potential in theory and research. *Psychological Bulletin*, 127, 87-127

- Craig, R., Mott, J.A., & Berman, A.S. (2002). The interactive effects of infant activity level and fear on growth trajectories of early childhood behaviour problems. *Development and Psychopathology*, 14, 1-23
- Davis, E. P., & Sandman, C. A. (2006). Prenatal exposure to stress and stress hormones influences child development. *Infants and Young Children*, 19(3), 246-259.
- Daya, S. (1993). Accuracy of gestational age estimation by means of fetal crown-rump length measurement. *American Journal of Obstetrics & Gynecology*, 168, 903-908
- De Kloet, E.R. (1991). Brain corticosteroid receptor balance and homeostatic control. *Frontiers in Neuroendocrinology*, 12, 95-164
- Dessens, A. B., Smolders-de Haas, H., & Koppe, J.G. (2000). Twenty-year follow-up of antenatal corticosteroid treatment. *Pediatrics*, 105, 77-84
- De Wolff, M.S., & Van IJzendoorn, M.H. (1997). Sensitivity and attachment: A meta-analysis of parental antecedents of infant attachment. *Child Development*, 68, 571-591
- De Wreeth, C., Van Hees, Y., Buitelaar, J.K. (2003). Prenatal maternal cortisol levels and infant behavior during the first 5 months. *Early Human Development*, 74, 149-151
- Diaz, R., Brown, R.W., Seckl, J.R. (1998). Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11beta-hydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. *Journal of Neuroscience*, 18, 2570-2580
- Diego, M.A., Jones, N.A., Field, T., Hernandez-Reif, M., Schanberg, S., Kuhn, C., & Gonzalez-Garcia, A. (2006). Maternal psychological distress, prenatal cortisol, and fetal weight. *Psychosomatic Medicine*, 68, 747-753

- Drake, E.E., Humernick, S.S., Amankwaa, L., Younger, J., & Roux, G. (2007). Predictors of maternal responsiveness. *Journal of Nursing Scholarship*, 39, 119-125
- Egeland, B., Pianta, R., & O'Brien, M. (1993). Maternal intrusiveness in infancy and child maladaptation in early school years. *Development and Psychopathology*, 5, 359-370
- Fish, M., & Stifter, C.A. (1995). Patterns of mother-infant interaction and attachment: A cluster-analytic approach. *Infant Behavior and Development*, 4, 435-446
- French, N. P., Hagan, R., Evans, S. F., Godfrey, M., & Newman, J. P. (1999). Repeated antenatal corticosteroids: Size at birth and subsequent development. *American Journal of Obstetrics and Gynecology*, 180, 114-121
- Garstein, M.A., & Rothbart, M.K. (2003). Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behavior and Development*, 26, 64-86
- Gilissen, R., Bakermans-Kranenburg, Van IJzendoorn, van der Veer (2008). Parent-child relationship, temperament, and physiological reactions to fear-inducing film clips: Further evidence for differential susceptibility. *Journal of Experimental Child Psychology*, 99, 182-195
- Gilissen, R., Koolstra, C.M., Van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., & Van der Veer, R. (2007). Physiological reactions of preschoolers to fear-inducing film clips: Effects of temperamental fearfulness and quality of the parent-child relationship. *Developmental Psychobiology*, 49, 187-195
- Gitau, R., Cameron, A., Fisk, N. M., Glover, V. (1998). Fetal exposure to maternal cortisol. *Lancet*, 352, 707-708

- Glover, V., Bergman, K., Sarkar, P., & O'Connor (2009). Association between maternal and amniotic fluid cortisol is moderated by maternal anxiety. *Psychoneuroendocrinology*, 34, 430-435
- Gluckman, P., & Hanson, M. (2005). *The fetal matrix: Evolution, Development and Disease*. Cambridge, Cambridge university press
- Goldberg, S., Levitan, R., Leung, E., Masellis, M., Basile, V.S., Nemeroff, C.B., & Atkinson, L. (2003). Cortisol concentrations in 12- to 18-months-old infants: Stability over time, location, and stressor. *Biological Psychiatry*, 54, 719-726
- Goldsmith, H.H., & Lemery, K.A. (2000). Linking temperamental fearfulness and anxiety symptoms: A behavioural-genetic perspective. *Biological Psychiatry*, 48, 1199-1209
- Goldsmith, H.H., & Rothbart, M.K. (1999). *Laboratory Temperament Assessment Battery, Prelocomotor Version 3.1*. University of Wisconsin, Madison
- Gunnar, M.R. (1989). Studies of the human infant's adrenocortical response to potentially stressful events. In M. Lewis & J. Woroby (Eds.), *Infant stress and coping* (Vol. 45, pp 3-18). San Francisco: Jossey-Bass
- Gunnar, M.R. (1990). The psychobiology of infant temperament. In Colombo, J., & Fagen, J. (Eds.), *Individual differences in infancy: Reliability, stability, prediction*. New Jersey: Lawrence Erlbaum Associates Inc.
- Gunnar, M. R. (1992). Reactivity of the Hypothalamic-Pituitary-Adrenocortical system to stressors in normal infants and children. *Pediatrics*, 90, 491- 597
- Gunnar, M.R., Brodersen, L., Nachmias, M., Buss, K., & Rigatuso, J. (1996). Stress reactivity and attachment security. *Developmental Psychology*, 29, 191-204
- Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, 27, 199-220

- Gunnar, M.R., Larson, M., Hertsgaard, L., Harris, M., & Broderson, L. (1992). The stressfulness of separation among 9-month-old infants: Effects of social context variables and infant temperament. *Child Development, 63*, 847-857
- Gunnar, M. R., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Reviews in Psychology, 58*, 145-173
- Gunnar, M.R., & White, B.P. (2001). Salivary cortisol measures in infant and child assessment. In Singer, L.R., & Zeskind, P.S (Eds.), *Biobehavioral assessment of the infant*. New York: The Guilford Press
- Gutteling, B. M., de Weerth, C., Willemsen-Sinkels, S. H. N., Huizink, A. C., Mulder, E. J. H., Visser, G. H. A., et al. (2005). The effects of prenatal stress on temperament and problem behavior of 27-months-old toddlers. *European Child & Adolescent Psychiatry, 14*, 41-51
- Hane, A.A., & Fox, N.F. (2006). Ordinary variations in maternal caregiving influence human infants' stress reactivity. *Psychological Science, 17*, 550-556
- Hay, D.F. (2007). The gradual emergence of sex differences in aggression: alternative hypotheses. *Psychological Medicine, 37*, 1527-1537
- Hay, D.F., Pawlby, S., Waters, C.S., Perra, O., Sharp, D. (2010). Mothers' antenatal depression and their children's antisocial outcomes. *Child Development, 81*, 149-165
- Hayley, D.W., & Stansbury, K. (2003). Infant stress and parent responsiveness: Regulation of physiology and behaviour during still-face and reunion. *Child Development, 74*, 1534-1546
- Hedegaard, M., Henriksen, T. B., Sabroe, S., & Secher, N. J. (1993). Psychological distress in pregnancy and preterm delivery. *British Medical Journal, 307*, 234-239.

- Hirshfield-Becker, D.R., Biederman, J., Calltharp, S., Rosenbaum, E.D., Faraone, S.V., & Rosenbaum, J. F. (2003). Behavioral inhibition and disinhibition as hypothesized precursors to psychopathology: Implications for paediatric bipolar disorder. *Biological Psychiatry*, 53, 985-999
- Hubbard, F.O.A., & van IJendoorn, M.H. (1991). Maternal unresponsiveness and infant crying across the first 9 months: A naturalistic longitudinal study. *Infant Behavior and Development*, 14, 299-312
- Huizink, A. C. (2000). Prenatal stress and its effect on infant development. Unpublished PhD Thesis, Utrecht University, The Netherlands
- Huizink, A. C., de Medina, P. G. R., Mulder, E. J. H., Visser, G. H. A., & Buitelaar, J. K. (2003). Stress during pregnancy is associated with developmental outcome in infancy. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 44(6), 810-818.
- Huizink, A. C., Mulder, E. J. H., & Buitelaar, J. K. (2004). Prenatal stress and risk for psychopathology: Specific effects or induction of general susceptibility? *Psychological Bulletin*, 1, 115-142
- Huizink, A. C., Mulder, E. J. H., de Medina, P. G. R., Visser, G. H. A., & Buitelaar, J. K. (2004). Is pregnancy anxiety a distinctive syndrome? *Early Human Development*, 79(2), 81-91.
- Kagan, J. (1994). *Galen's prophecy: temperament in human nature*. New York: BasicBooks
- Kagan, J. (1997). Temperament and the reactions to unfamiliarity. *Child Development*, 68, 139-143
- Kagan, J., Reznick, J.S., Snidman, N., Gibbons, J. (1989). Inhibited and uninhibited types of children. *Child Development*, 60, 838-845

- Kagan, J., Reznick, J.S., Snidman, N., Gibbons, J., Johnson, M.O. (1988). Childhood derivatives of inhibition and lack of inhibition to the unfamiliar. *Child Development*, 59, 1580-1589
- Kagan, J., Snidman, N., Zentner, M., & Peterson, E. (1999). Infant temperament and anxious symptoms in school age children. *Developmental Psychopathology*, 11, 209-224
- Killingsworth Rini C., Dunkel-Schetter, C., Wadhwa, P.D., & Sandman, C.A. (1999). Psychological adaptation and birth outcomes: The role of personal resources, stress, and sociocultural context in pregnancy. *Health Psychology*, 18, 333–345
- King, B. R., Smith, R., & Nicholson, R. C. (2001). The regulation of human corticotrophin-releasing hormone gene expression in the placenta. *Peptides*, 22(5), 795-801.
- Klein Velderman, M., Bakermans-Kranenburg, M.J., Juffer, F., & van IJzendoorn, M.H. (2006). Effects of attachment-based interventions on maternal sensitivity and infant attachment: Different susceptibility of highly reactive infants. *Journal of Family Psychology*, 20, 266-274
- Knoches, A. M. L., & Doyle, L. W. (1993). Long-Term Outcome of Infants Born Preterm. *Baillieres Clinical Obstetrics and Gynaecology*, 7(3), 633-651.
- Kochanska, G. (2001). Emotional development in children with different attachment histories: The first three years. *Child Development*, 72, 474-490
- Kochanska, G., Aksan, N., & Joy, M.E. (2007). Children's fearfulness as a moderator of parenting in early socialization: Two longitudinal studies. *Developmental Psychology*, 43, 222-237
- Ktistaki, G. (2009). The psychophysiology of fearful temperament: A follow-up

- study. Unpublished PhD thesis, Cardiff University, United Kingdom
- Lancaster, S., Prior, M., & Adler, R. (1989). Child behavior ratings: The influence of maternal characteristics on child temperament. *Journal of Child Psychology and Psychiatry*, 30, 137-149
- Leerkes, E.M., Blankson, A.N., O'Brien, M. (2009). Differential effects of maternal sensitivity to infant distress and non-distress on social emotional functioning. *Child Development*, 80, 762-775
- Levine, S. (1994). The ontogeny of the hypothalamic-pituitary-adrenal axis. The influence of maternal factors. *Annual New York Academy of Science*, 746, 275- 293
- Lewis, M., & Ramsey, D.S. (1995). Developmental change in infants' responses to stress. *Child Development*, 66, 657-670
- Lewis, M., & Ramsey, D.S. (1999). Effect of maternal soothing on infant stress response. *Child development*, 70, 11-20
- 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
- Matheny, A.P., Riese, M. L, & Wilson, R.S. (1985). Rudiments of infant temperament: Newborn to 9 months. *Developmental Psychology*, 21, 486-494
- Matheny, A.P., & Wilson, R.S. (1981). Developmental tasks and rating scales for the laboratory assessment of infant temperament. *JSAS Catalog of Selected Documents in Psychology*, 11, 81-82
- McElwain, N.L., Booth-LaForce, C. (2006). Maternal sensitivity to distress and

- nondistress as predictors of infant-mother attachment security. *Journal of Family Psychology*, 20, 247-255
- Meins, E., Fernyhough, C., Fradley, E., & Tuckey, M. (2001). Rethinking maternal sensitivity: Mothers' comments on infants' mental processes predict security of attachment at 12 months. *Journal of Child Psychology and Psychiatry*, 42, 637-648
- Moawad, A. H., Goldenberg, R. L., Mercer, B., Meis, P. J., Iams, J. D., Das, A., Caritis, S. N., Miodovnik, M., Menard, M. K., Thurnau, G. R., Dombrowski, M., Roberts, J. M. (2002). The preterm prediction study: The value of serum alkaline phosphatase, alpha-fetoprotein, plasma corticotropin-releasing hormone, and other serum markers for the prediction of spontaneous preterm birth. *American Journal of obstetrics and gynecology*, 186, 990-996
- Moeler, E., Kagan, J., Oelkers-Ax, R., Brunner, R., Poutska, L., Haffner, J., & Resch, F. (2008). Infant predictors of behavioural inhibition. *British Journal of Developmental Psychology*, 26, 145-150
- Nachmais, M., Gunnar, M., Mangelsdorf, S., Parritz, R., Buss, K. (1996). Behavioral inhibition and stress reactivity: The moderating role of attachment security. *Child Development*, 67, 508-522
- O'Connor, T. G., Heron, J., Golding, J., & Glover, V. (2003). Maternal antenatal anxiety and behavioural/emotional problems in children: a test for a programming hypothesis. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 44, 1025-1036
- O'Connor, T. G., Ben-Shlomo, Y., Heron, J., Holding, J., Adams, D., & Glover, V. (2005). Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biological Psychiatry*, 58, 211-217

- Oullet-Morin, I., Tremblay, R.E., Boivin, M., Meaney, M., Kramer, M., & Cote, S.M. (2010). Diurnal cortisol secretion at home and in child care: a prospective study of 2-year old toddlers. *Journal of child psychology and psychiatry*, 51, 295-303
- Parade, S.H., & Leerkes, E.M. (2008). The reliability and validity of the Infant Behavior Questionnaire-Revised. *Infant Behavior and Development*, 31, 637-646
- Pariante, C.M. (2003). Depression, stress and the adrenal axis. *Journal of neuroendocrinology*, 15, 811-812
- Pariante, C.M., Thomas, S.A., Lovestone, S., Makoff, A., Kerwin, R.W. (2003). Do antidepressants regulate how cortisol affects the brain? *Psychoneuroendocrinology*, 29, 423-447
- Pauli-Pott, U., Mertesacker, B., & Beckmann, D. (2004). Predicting the development of infant emotionality from maternal characteristics. *Development and Psychopathology*, 16, 19-42
- Prechtl, H.F.R. (1963). The mother-child interaction in babies with minimal brain damage (a follow-up study). In B.M. Foss (Ed.), *Determinants of infant behavior* (Vol.2). New York:Wiley
- Ramsay, D., & Lewis, M. (2003). Reactivity and regulation in cortisol and behavioural responses to stress. *Child Development*, 74, 456-464
- Rice, F, Jones, I., Thapar, A. (2006). The impact of gestational stress and prenatal growth as measures of the prenatal environment on emotional problems in offspring: a review. *Acta Psychiatrica Scandinavica*, 1-13
- Rice, F., Harold, G.T., Boivin, J., van den Bree, M., Hay, D., Thapar, A. (2010). The links between prenatal stress and offspring development and psychopathology:

- disentangling environmental and inherited influences. *Psychological Medicine*, 40, 335-345
- Robson, K.S., & Moss, H.A. (1970). Patterns and determinants of maternal attachment. *Journal of Pediatrics*, 77, 976-985
- Rothbart, M.K. (1981). Measurement of temperament in infancy. *Child Development*, 52, 569-578
- Rothbart, M.K. (1986) Longitudinal observation of infant temperament. *Developmental Psychology*, 22, 356-365
- Rothbart, M.K., Ahadi, S.A, Hershey, K.L., & Fisher, P. (2001). Investigations of temperament at three to seven years: The children's Behaviour Questionnaire. *Child Development*, 72, 1394-1408
- Rothbart, M.K., Chew, K.H., & Garstein, M.A. (2001). Assessment of temperament in early development. In L. Singer & P.S. Zeskind (Eds.), *Biobehavioral assessment of the infant*. New York: Guilford
- Rothbart, M.K., & Derryberry, D. (1981). Temperament and the development of self-regulation. In L.C Hartlage & Telzrow (Eds.), *The neuropsychology of individual differences: A developmental perspective*. New York: Wiley
- Rothbart, M.K., & Mauro, J.A. (1990). Questionnaire approach to the study of infant temperament. In J. Colombo, & J. Fagen (Eds.), *Individual differences in infancy: Reliability, stability, prediction*. New Jersey: Lawrence Erlbaum Associates Inc.
- Sapolsky, R.M., Romero, L.M., Munch, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrinology review*, 21, 55-89

- Sarkar, P., Bergman, K., Fisk, N. M., & Glover, V. (2006). Maternal anxiety at amniocentesis and plasma cortisol, *Prenatal diagnosis*, 505-509
- Schneider, M. L., Moore, C. F., Kraemer, G. W., Roberts, A. D., & DeJesus, O. T. (2002). The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model. *Psychoneuroendocrinology*, 27(1-2), 285-298
- Schwartz, L. B. (1997). Understanding human parturition. *Lancet*, 350, 1792-93
- Seckl, J. R., & Meane, M. J. (2006). Glucocorticoid 'programming' and PTSD risk. *Annals of New York Academy of Sciences*, 1071, 351-378
- Seifer, R., Sameroff, A.J., Barrett, L., & Krafchuk, E. (1994). Infant temperament measured by multiple observations and mother report. *Child Development*, 65, 1478-1490
- Seifer, R., Schiller, M., Sameroff, A.J., Resnick, S., & Riordan, K. (1996). Attachment, maternal sensitivity, and infant temperament during the first year of life. *Developmental Psychology*, 32, 12-25
- Sippell, W.G., Becker, H., Versmold, H.T., Bidlingmaier, F., Knorr, D. (1978). Longitudinal studies of plasma aldosterone, cortisone, deoxycosterone, progesterone 17-hydroxyprogesterone, cortisol and cortisone determined simultaneously in mother and child at birth and during the early neonatal period. I. Spontaneous delivery. *Journal of Clinical Endocrinology and Metabolism*, 46, 971-985
- Sjostrom, K., Valentin, L., Thelin, T., & Marsal, K. (2002). Maternal anxiety in late pregnancy: effect on fetal movements and fetal heart rate. *Early human development*, 67, 87-100
- Spangler, G., & Schieche, M. (1998). Emotional and adrenocortical responses of

- infants to the strange situation: The differential function of emotional expression. *International journal of behavioural development*, 22, 681-706
- Spielberger, C. D., Gorsuch, R.L., and Lushene, R.E. (1970). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press
- Spinillo, A., Viazzo, F., Colleoni, R., Chiara, A., Cerbo, R. M., & Fazzi, H. (2004). Two-year infant neurodevelopmental outcome after single or multiple antenatal courses of corticosteroids to prevent complications of prematurity. *American Journal of Obstetrics and Gynecology*, 191(1), 217-224.
- Stifter, C.A., & Fox, N.A. (1990). Infant reactivity: Physiological correlates of newborn and 5-months old temperament. *Developmental Psychology*, 26, 582-588
- Strelau, J. (1998). *Temperament: a psychological perspective*. New York: Plenum Press
- Tabachnik, B.G., & Fidel, L.S. (2001). *Using multivariate statistics (4th Ed)*. Boston: Allyn & Bacon
- Takahashi, L. K., Haglin, C., & Kalin, N. H. (1992). Prenatal Stress Potentiates Stress-Induced Behavior and Reduces the Propensity to Play in Juvenile Rats.
- Talge, N. M., Neal, C., & Glover, V. (2007). Antenatal maternal stress and long-term effects on child neurodevelopment: How and why? *Journal of child psychology and psychiatry*, 48, 245-261
- Thomas, A., & Chess, S. (1977). *Temperament and development*. New York: Brunner/Mazel
- Trautman, P. D., Meyerbahlburg, H. F. L., Postelnek, J., & New, M. I. (1995). Effects of Early Prenatal Dexamethasone on the Cognitive and Behavioral-

Development of Young-Children - Results of a Pilot-Study.

Psychoneuroendocrinology, 20(4), 439-449.

Physiology & Behavior, 51(2), 319-323.

Van den Bergh, B. (1990). The influence of maternal emotions during pregnancy on fetal and neonatal behavior. *Pre- and Peri-Natal psychology Journal*, 5, 119-130

Van der Meulen, B.F., & Smrkovsky, M. (1983). BOS 2-30. *Bayley ontwikkelingsschalen: Handleiding* [Bayley Developmental series in Dutch: A manual]. Lisse, The Netherlands: Swets and Zeitlinger B.V.

VanGoozen, S.H.M., Fairchild, G., Snoek, H., & Harold, G.T. (2007). The evidence of a neurobiological model of childhood antisocial behavior. *Psychological bulletin*, 133, 149-182

Velderman, M.K, Bakermans-Kranenburg, M.J., Juffer, F., Van IJzendoorn, M.H. (2006). Effects of attachment-based interventions on maternal sensitivity and infant attachment: differential susceptibility of highly reactive infants. *Journal of Family Psychology*, 20, 266-274

Wadhwa, P. D. (2005). Psychoneuroendocrine processes in human pregnancy influence fetal development and health. *Psychoneuroendocrinology*, 30(8), 724-743.

Wadhwa, P. D., DunkelSchetter, C., ChiczDeMet, A., Porto, M., & Sandman, C. A. (1996). Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. *Psychosomatic Medicine*, 58(5), 432-446.

Wadhwa, P.D., Garite, T. J., Porto, M., Glynn, L., Chicz-DeMet, A., Dunkel-Schetter, C., & Sandman, C. A. (2004). Placental corticotropin-releasing hormone

- (CRH), spontaneous preterm birth, and fetal growth restriction: A prospective investigation. *American journal of obstetrics and gynecology*, 191, 1063-1069
- Wadhwa, P D., Sandman, C. A., Porto, M., Dunkelschetter, C., & Garite, T. J. (1993). The association between prenatal stress and infant birth-weight and gestational age at birth- a prospective investigation. *American Journal of Obstetrics and Gynecology*, 169, 858-865
- Ward, M.J., & Carson, E.A. (1995). Associations among adult attachment representations, maternal sensitivity, and infant-mother attachment in a sample of adolescent mothers. *Child Development*, 66, 69-79
- Weaver, I.C., et al. (2001). Early environmental regulation of hippocampal glucocorticoid receptor gene expression: characterization of intracellular mediators and potential genomic target sites. *Molecular cellular endocrinology*, 185, 205-218
- Weaver, I.C.G., Cervoni, N., Champagne, F.A., Alesion, A.C.D., Sharma, S., Seckl, J.R., Symov, S., Szyf, M., Meaney, M. (2004). Epigenetic programming by maternal behaviour. *Nature*, 847-854
- Weinstock, M. (2005). The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behavior and Immunity*, 19(4), 296-308.
- Weinstock, M., Matlina, E., Maor, G. I., Rosen, H., & McEwen, B. S. (1992). Prenatal stress selectively alters the reactivity of the hypothalamic-pituitary adrenal system in the female rat. *Brain Research*, 595, 195-200

Appendix 1



Emotions and Coping in Children

We would like to invite you and your child to take part in a study of children's emotions and early coping behaviour. This would take up about 1 hour of your time, and would involve a visit to a specially designed playroom in the School of Psychology on Park Place, in central Cardiff. We can arrange parking or pay your bus or taxi fare.



We would like to find out why some children are born with a more easy going temperament than others. Temperamental differences in children are generally seen from birth onwards but especially also when children start to crawl and explore their environments. You will probably know that some children approach new people or new toys quite readily, whereas others prefer to stay closely to their parent and take time before cautiously approaching or responding. The reason is that children differ in fearlessness. Children who are less fearful react less strongly to novel events, and are more prepared to approach new objects and take risks.



We are particularly interested in the development of fear and risk taking in 6-12 months old children. If you decide that you want to participate, we will video your child's behaviour and record his/her bodily responses while we present him/her with different objects. We measure sweat reaction by attaching two metal stickers to the sole of your child's foot.

We also want to collect some saliva from your child. Some children are less likely than others to produce certain hormones that help them deal with new situations. We can measure that from their saliva.

In the session we will record your child's reaction to calming music, to novel sounds and to a robot toy, that slowly approaches your child.

To show our appreciation for your participation, we will give your child a present and give you a £10 gift voucher. In addition, we will pay for your travel to and from the School of Psychology.

For further details, you may ring the project coordinator at Cardiff University on 029 20 876191. Thank you for taking the time to read this information sheet. We hope that you will be able to help us.



Appendix 2 Coding sheet for maternal sensitivity and intrusiveness

Participant Number:

Date:

Gender:

Name of coder:

Free Play before Stress Eliciting Event (Normal Interaction) (3 min)

	Maternal Sensitivity	Maternal Intrusiveness
0-30 sec		
30- 60 sec		
60- 90 sec		
90-120 sec		
120-150 sec		
150-180 sec		

Free Play after Stress Eliciting Event (3 min)

	Maternal Sensitivity	Maternal Intrusiveness
0-30 sec		
30- 60 sec		
60- 90 sec		
90-120 sec		
120-150 sec		
150-180 sec		

Appendix 3 Coding sheet for child behavioural distress (Lab-TAB)

Unpredictable Toy Scoring EC: 1, 2

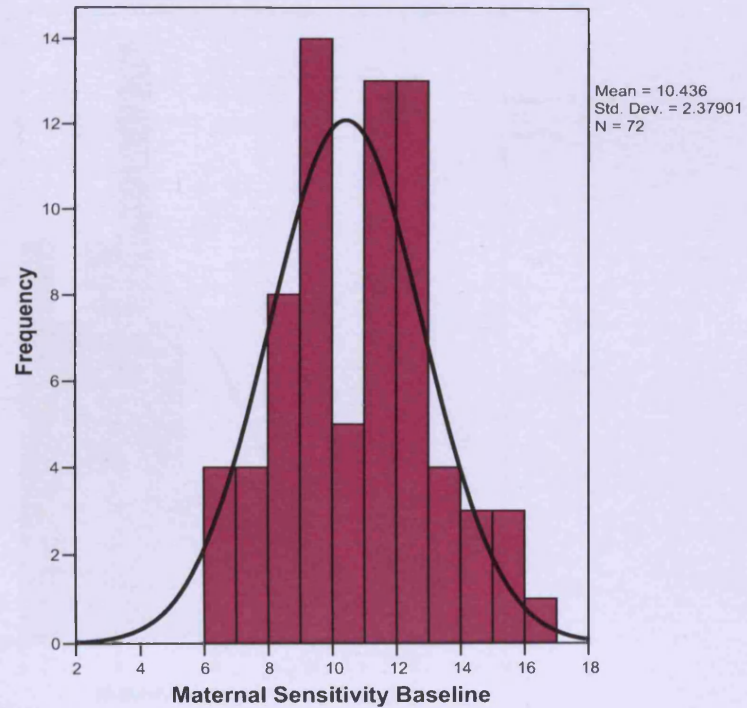
Subject# _____
 Subject Name _____
 Visit 1 or 2 Tape# _____
 Counter# _____
 DOB _____

Scorer _____
 Date Scored _____
 Episode Order _____
 Experimenter Code _____
 DOV _____

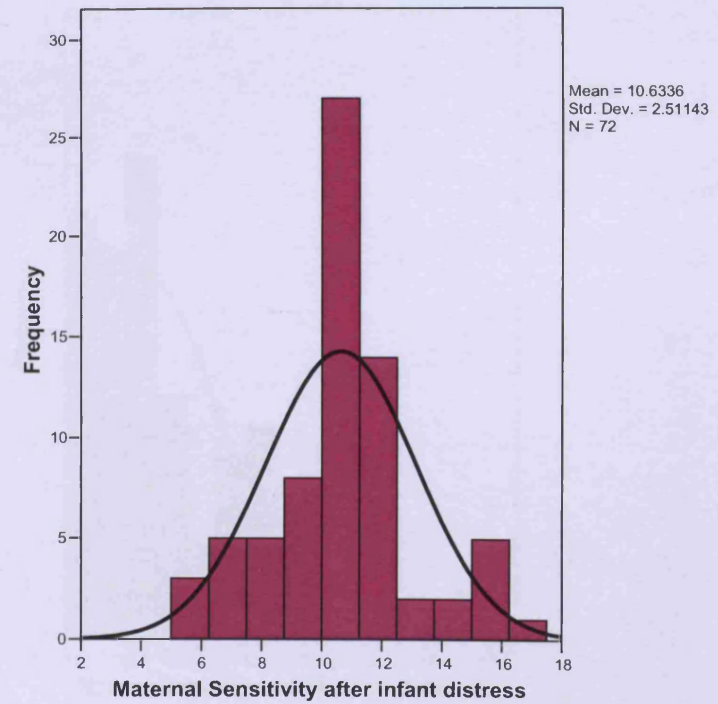
Latency to fear Response					Latency to sadness response			
T1 _____ s					T1 _____ s			
T2 _____ s					T2 _____ s			
T3 _____ s					T3 _____ s			
	Trial 1				Trial 2			
Time Begin/End				Avg.				Avg.
Intensity of facial fear (0-3)								
Intensity of facial sadness (0-3)								
Intensity of vocal distress (0-5)								
Intensity of bodily fear (0-3)								
Presence of startle response 0=yes 1=0								
Intensity of escape behavior (0-3)								

	Trial 3			
Time Begin/End				Avg.
Intensity of facial fear (0-3)				
Intensity of facial sadness (0-3)				
Intensity of vocal distress (0-5)				
Presence of startle response 0=yes 1=0				
Intensity of escape behaviour (0-3)				

Appendix 4 Frequency distributions of maternal sensitivity in Year 1

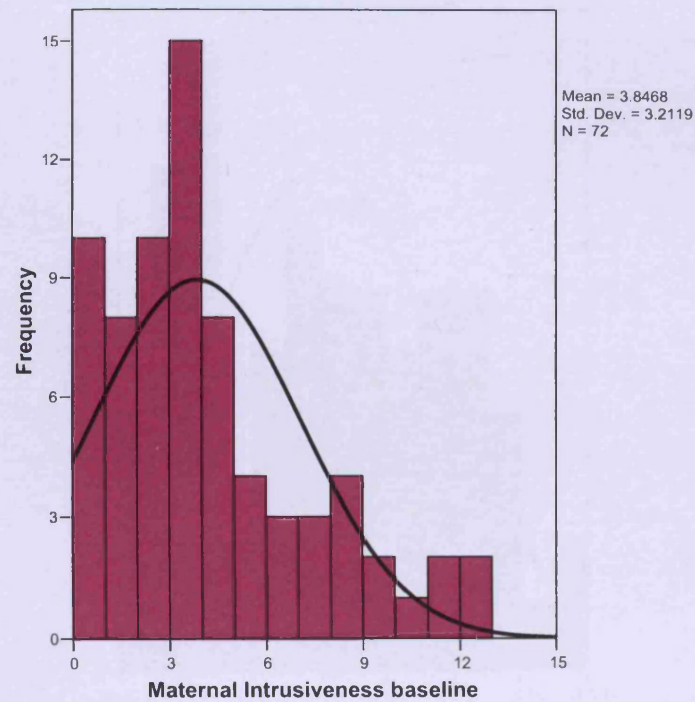


Frequency distribution of maternal sensitivity during normal interaction in Year 1.

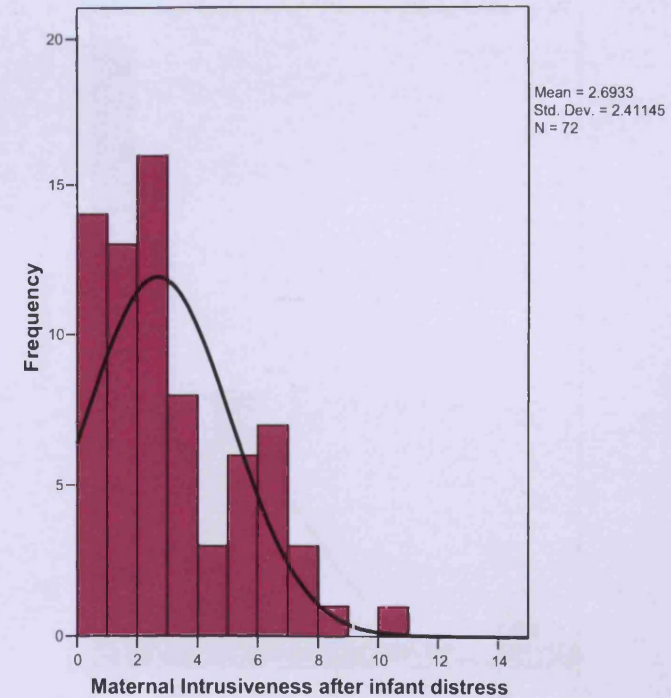


Frequency distribution of maternal sensitivity after child distress in Year 1.

Appendix 5 Frequency distributions of maternal intrusiveness in Year 1

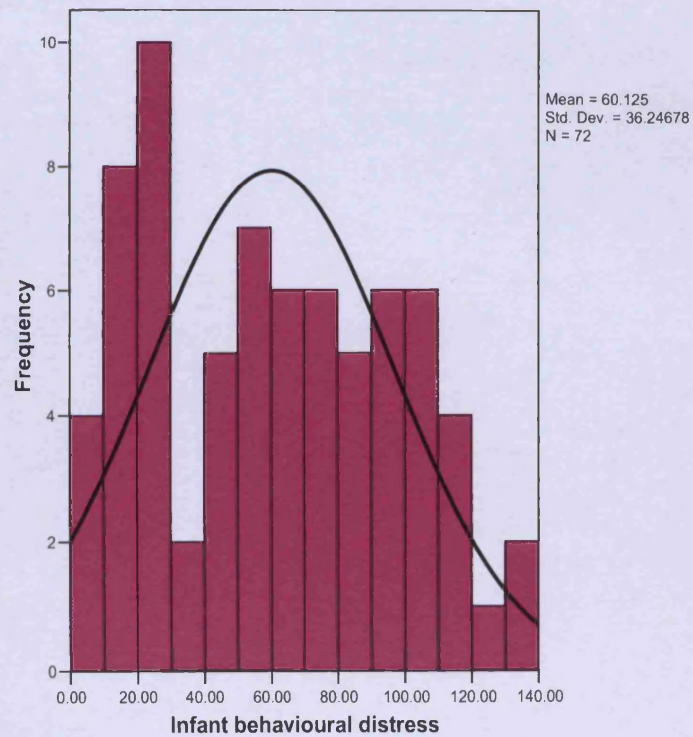


Frequency distribution of maternal intrusiveness during normal interaction in Year 1.

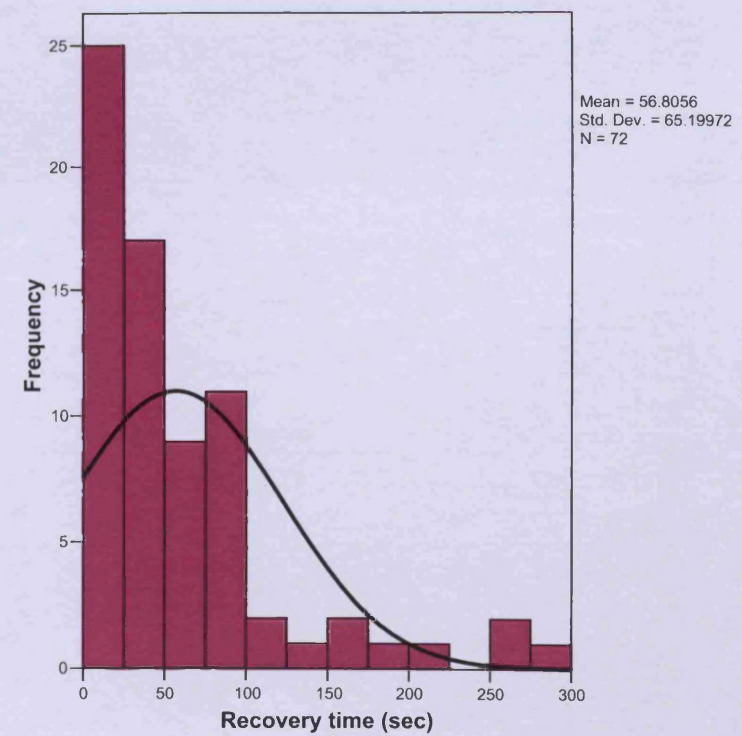


Frequency distribution of maternal intrusiveness after infant distress in Year 1.

Appendix 6 Frequency distributions of child behavioural distress and recovery in Year 1

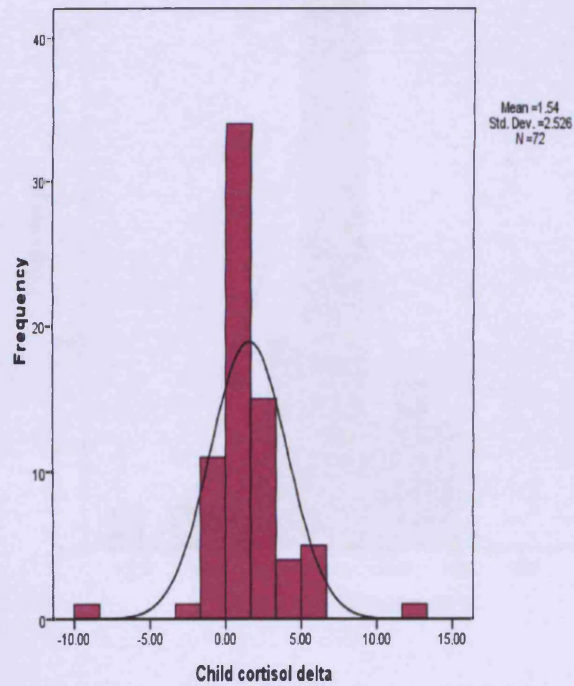


Frequency distribution of infant Lab-TAB composite distress scores in Year 1.

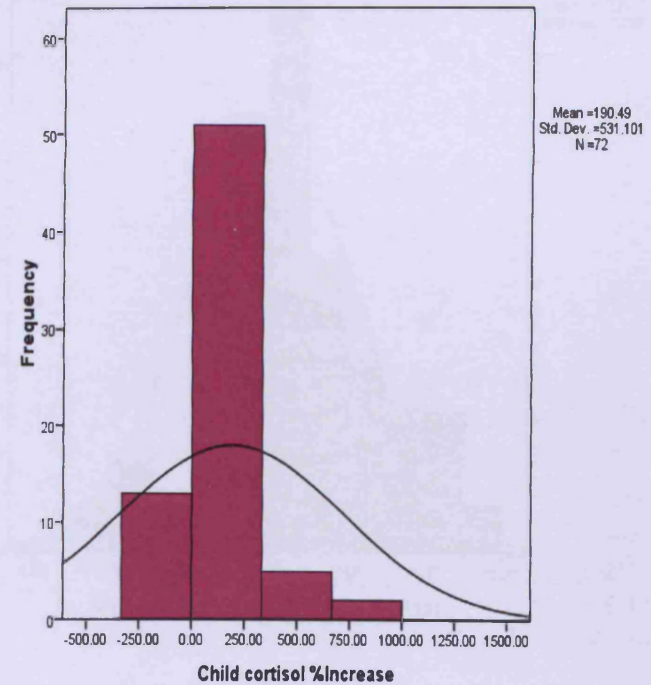


Frequency distribution of infant recovery time from distress in Year 1.

Appendix 7 Frequency distributions of child cortisol reactivity (delta) and cortisol percentage increase in Year 1

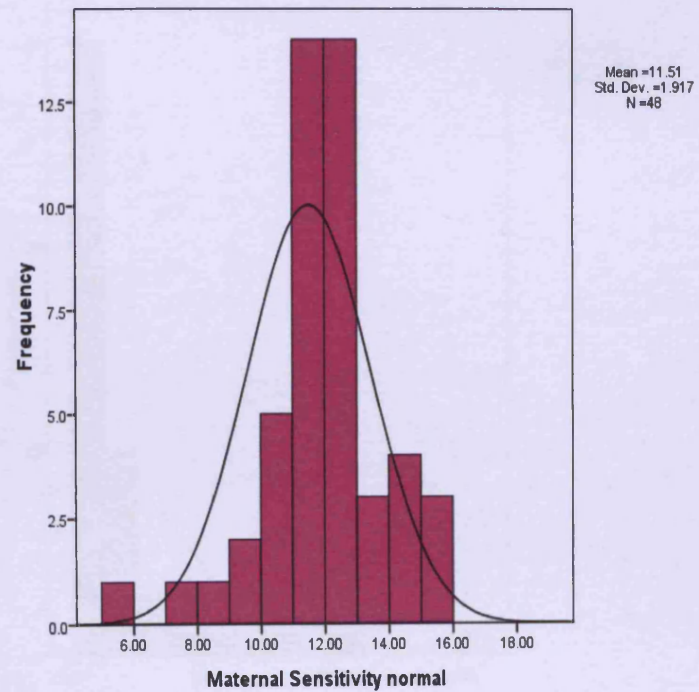


Frequency distribution of infant cortisol increase (delta) in response to distress episode.

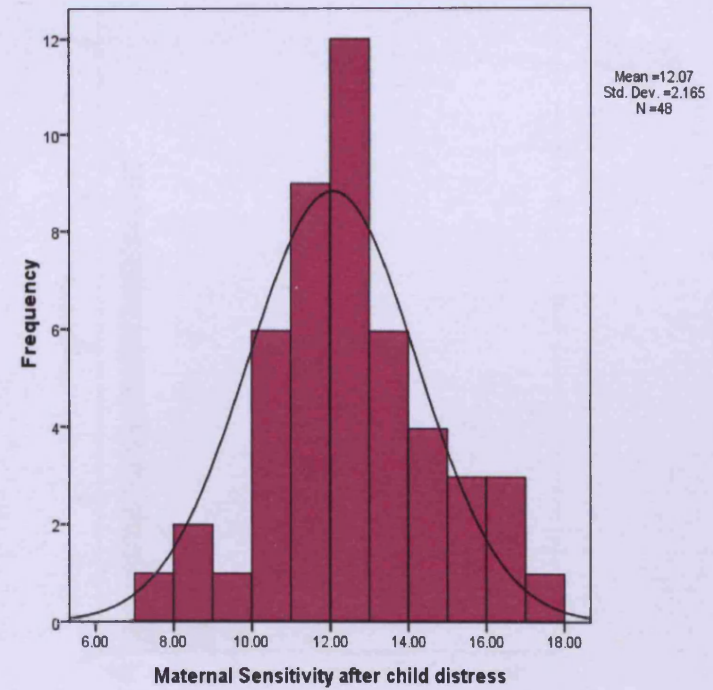


Frequency distribution of infant cortisol %increase in response to distress episode.

Appendix 8 Frequency distributions of maternal sensitivity in Year 2

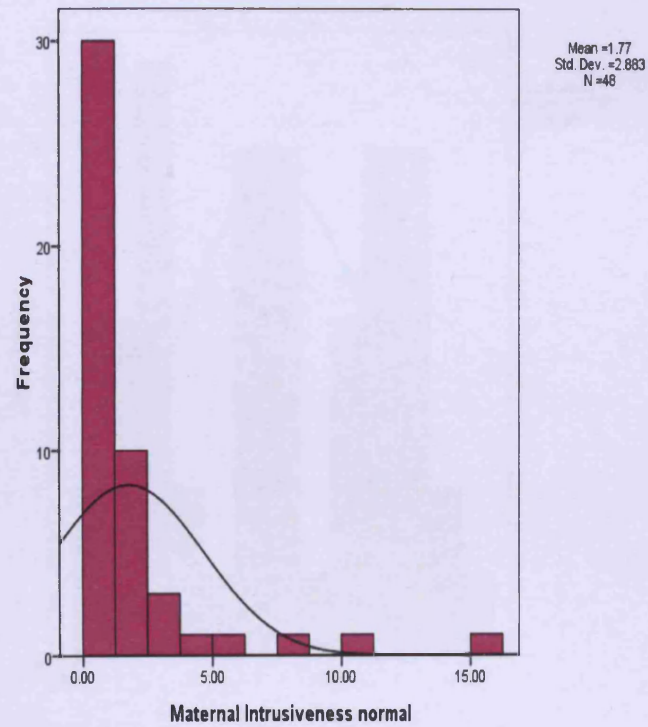


Frequency distribution of maternal sensitivity during normal interaction in Year 2.

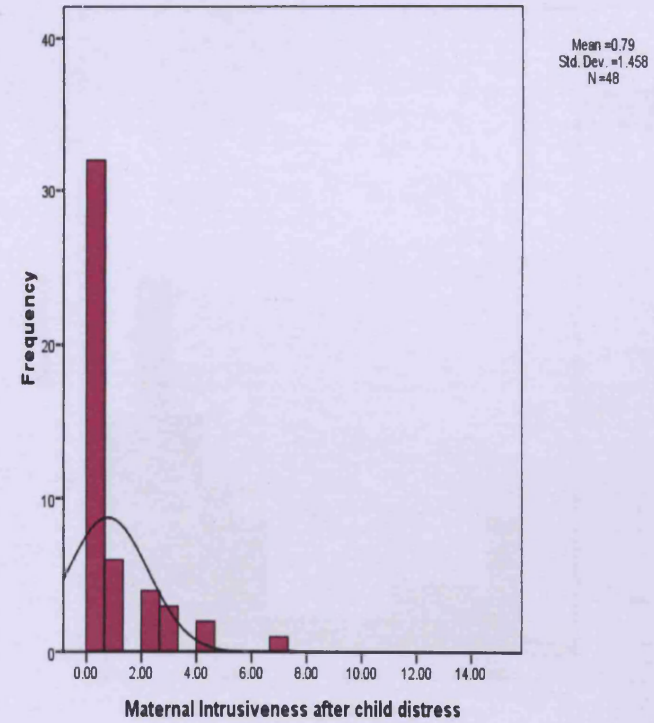


Frequency distribution of maternal sensitivity after child distress in Year 2.

Appendix 9 Frequency distributions of maternal intrusiveness in Year 2

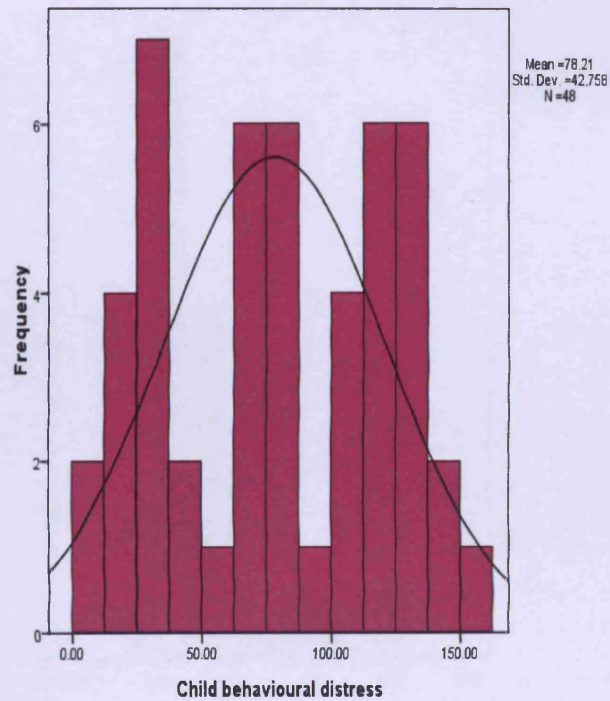


Frequency distribution of maternal intrusiveness during normal interaction in Year 2.

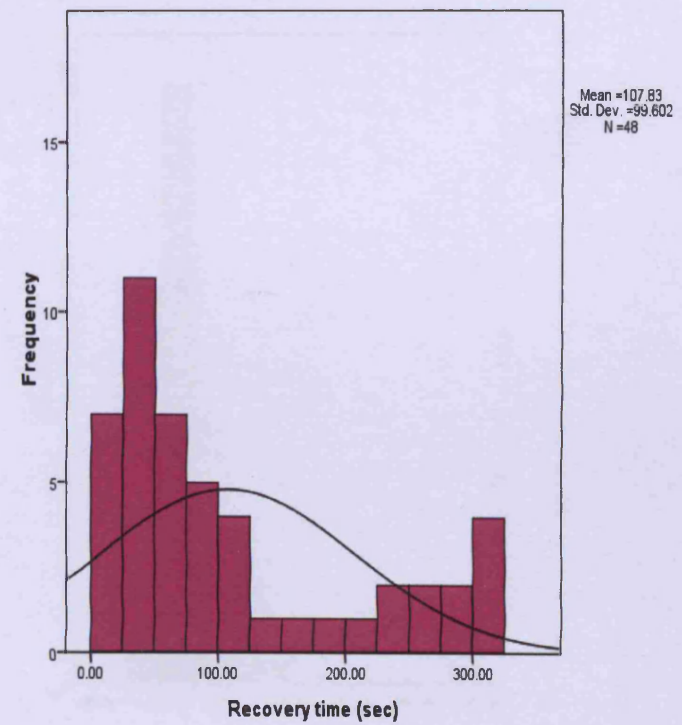


Frequency distribution of maternal intrusiveness after child distress in Year 2.

Appendix 10 Frequency distributions of child behavioural distress and recovery in Year 2

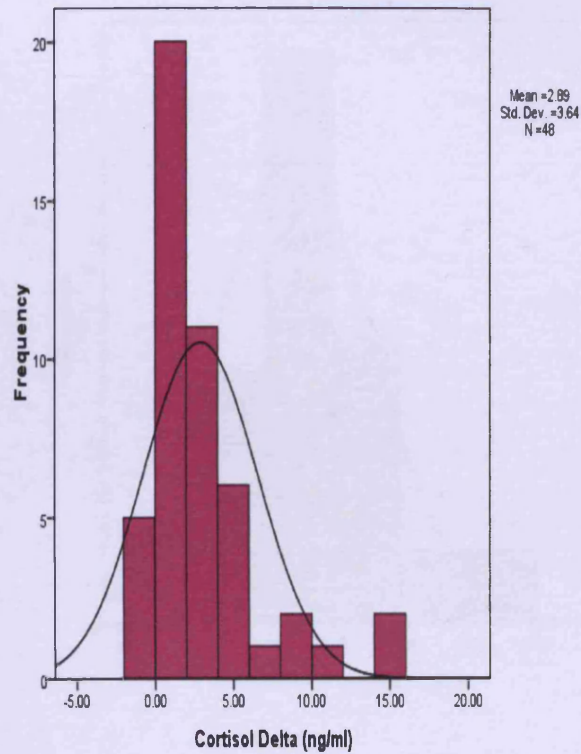


Frequency distribution of child Lab-TAB composite distress scores in Year 2.

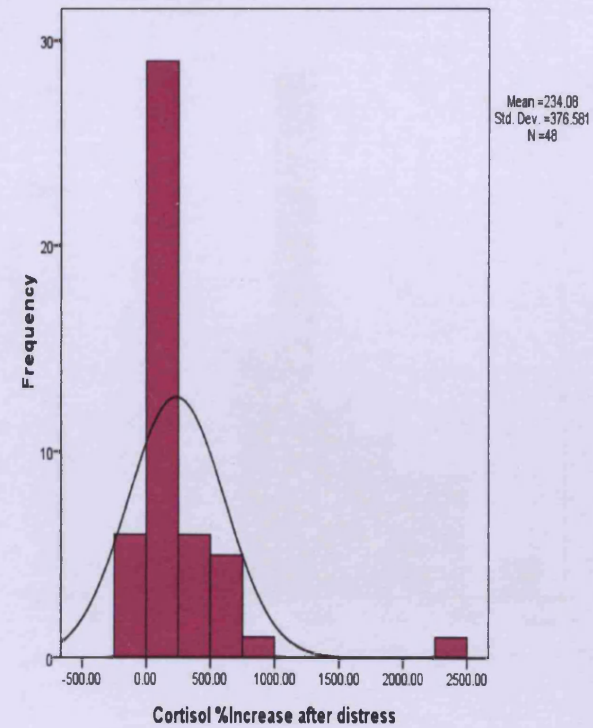


Frequency distribution of child recovery time from distress in Year 2.

Appendix 11 Frequency distributions of child cortisol reactivity (delta) and cortisol percentage increase in Year 2

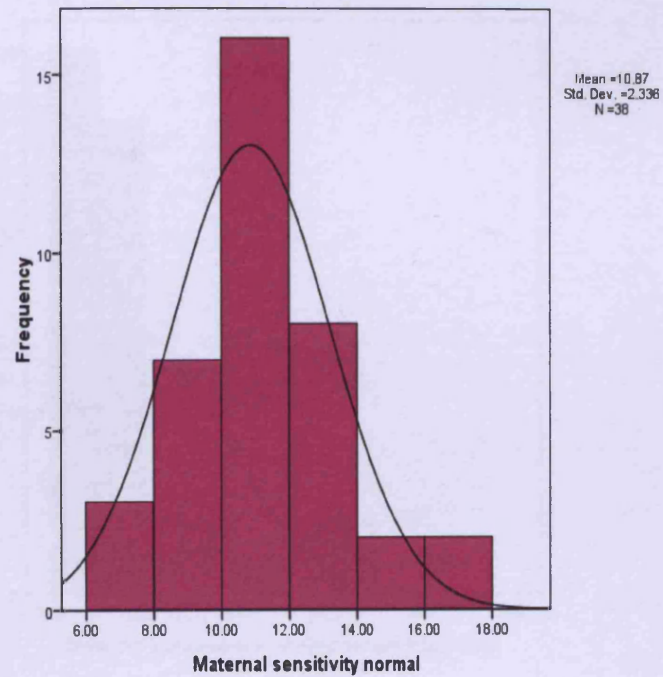


Frequency distribution of infant cortisol increase (delta) in response to distress episode in Year 2.

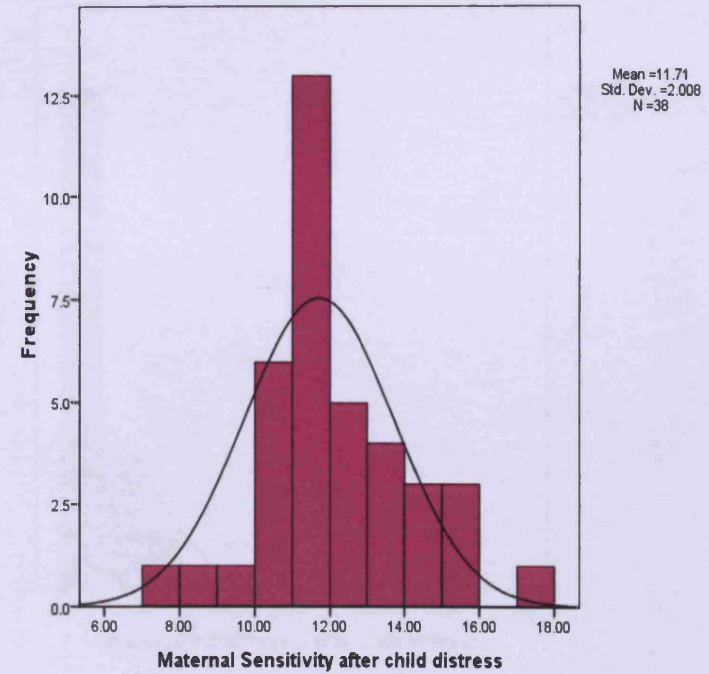


Frequency distribution of infant cortisol %increase in response to distress episode in Year 2.

Appendix 12 Frequency distributions of maternal sensitivity in Year 3

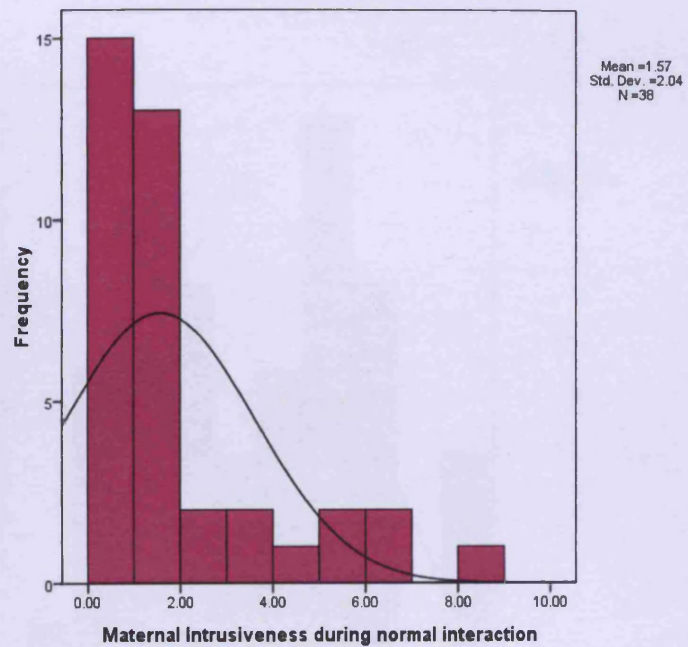


Frequency distribution of maternal sensitivity during normal interaction in Year 3.

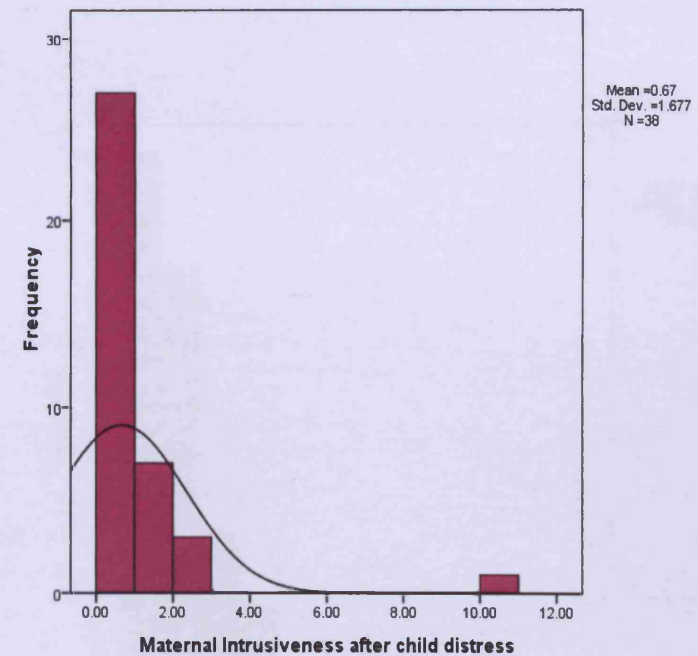


Frequency distribution of maternal sensitivity after child distress in Year 3.

Appendix 13 Frequency distributions of maternal intrusiveness in Year 3

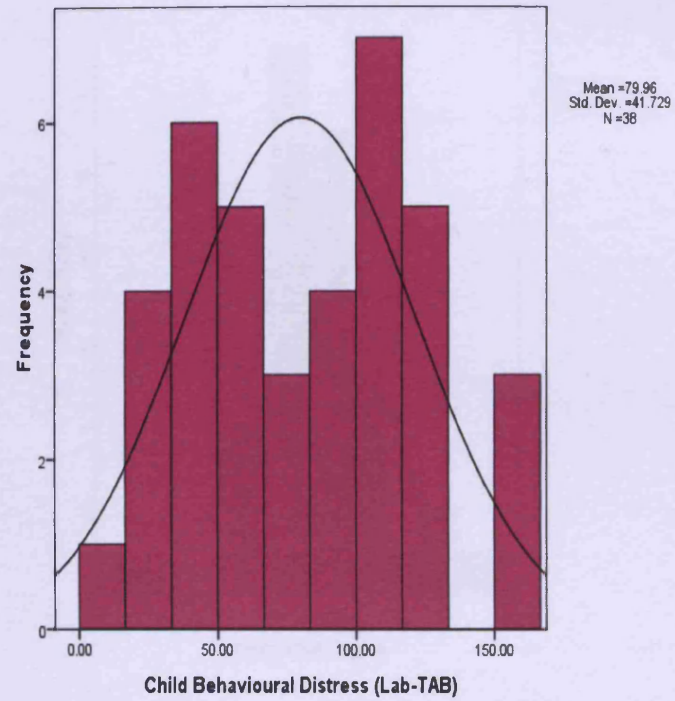


Frequency distribution of maternal intrusiveness during normal interaction in Year 3.

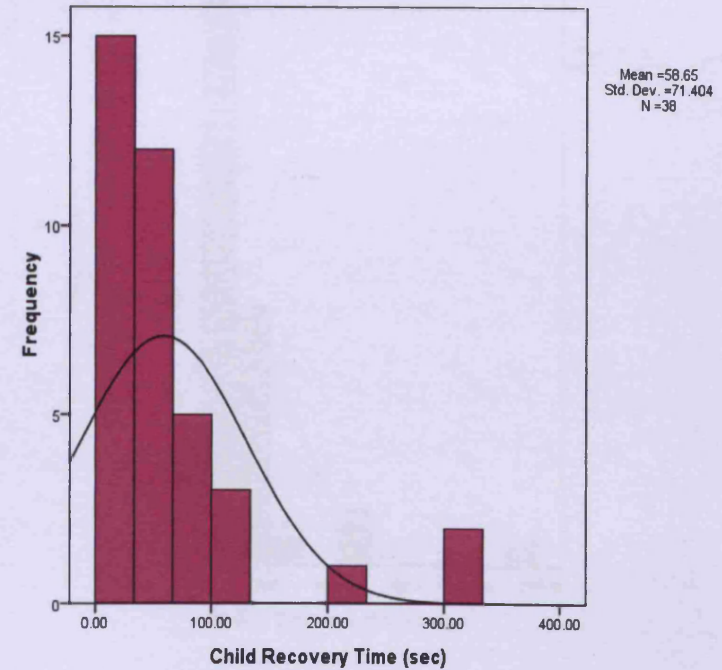


Frequency distribution of maternal intrusiveness after child distress in Year 3.

Appendix 14 Frequency distributions of child behavioural distress and recovery in Year 3

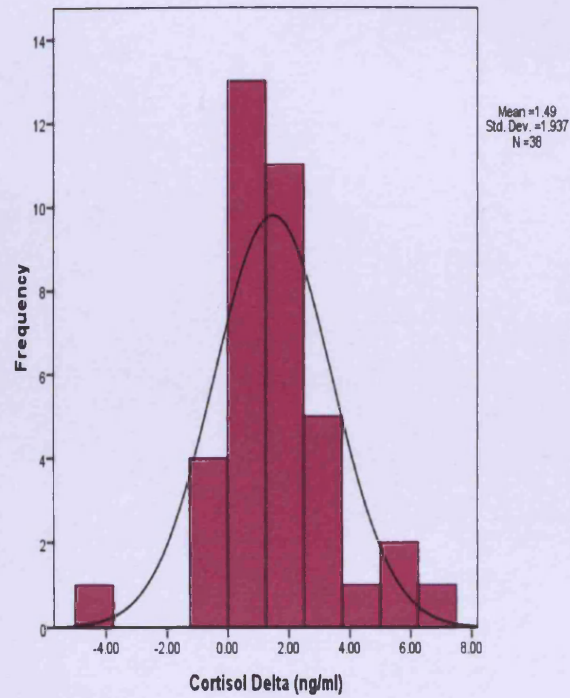


Frequency distribution of child Lab-TAB composite distress scores in Year 3.

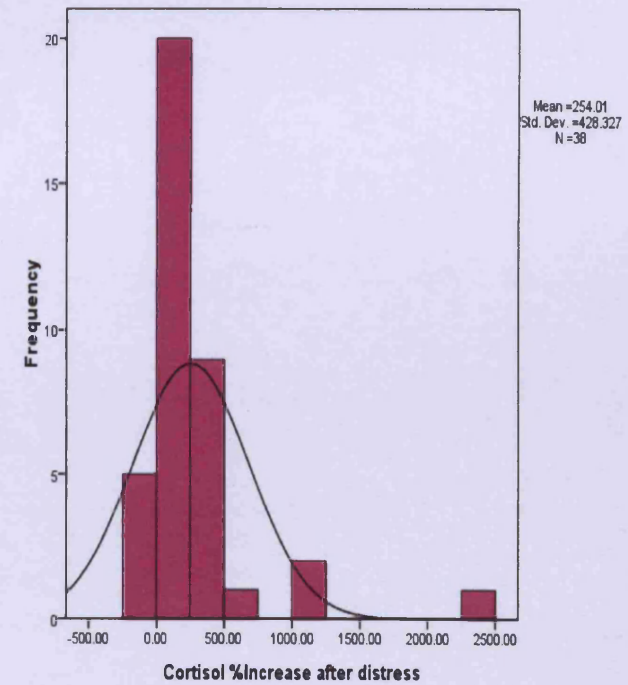


Frequency distribution of child recovery time from distress in Year 3.

Appendix 15 Frequency distributions of child cortisol reactivity (delta) and cortisol percentage increase in Year 3



Frequency distribution of infant cortisol increase (delta) in response to distress episode in Year 3.



Frequency distribution of infant cortisol %increase in response to distress episode in Year 3.

