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Prenatal psychosocial and endocrinologic predictors of infant temperament

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9.1 Abstract

Background: Preclinical data in nonhuman primates and rodents indicate that prenatal stress adversely affects the ability of offspring to cope in challenging situations. These effects may be mediated by the hypothalamo-pituitary-adrenal (HPA) axis.

Objective: To examine the effects of psychological stress of pregnant women on temperament of infants in a prospective design.

Methods: Self-report data about various aspects of prenatal stress (pregnancy specific anxiety, daily hassles, perceived stress, and an overall construct of distress) were collected of nulliparous women in early pregnancy. In addition, cortisol saliva and ACTH plasma levels (mid pregnancy) were determined. The temperament of the infant was measured at 3 and 8 months by observation at the administration of the Bayley Scales and by parent reports on the Infant Characteristics Questionnaire (ICQ).

Results: Complete data were available of 170 term-born infants. Pregnancy specific anxieties explained 7% of the variance (p's $< .05$) of test-affectivity and goal-directedness of the infant at the test situation at 8 months. Perceived stress explained 12% of the variance ($p < .01$) of unadaptability of the ICQ at 3 months but not at 8 months. Levels of ACTH explained 29% of the variance ($p < .01$) of unadaptability at 8 months. All results were adjusted for a large number of covariates. Psychological and endocrinologic stress measures were unrelated.

Conclusion: Increased stress in human pregnancy seems to be one of the determinants of temperamental variation of infants at 8 months and may be a risk factor for developing psychopathology later in life. Further systematic follow-up of the present sample is planned.

9.2 Introduction

Harmful events during delivery explain only a small proportion of disorders in child behavior and development (Goodman & Stevenson, 1989; Gillberg et al., 1983; Uljas et al., 99; O'Callaghan et al., 1997; Taylor et al., 2000), and improved obstetric and neonatal care during the last decades has not been able to reduce the rate of these neurodevelopmental disorders (Casaer, 1993; Hjalmorson et al., 1988; Visser & Narayan, 1996).Therefore, prenatal factors and their presumed programming effects on the fetal brain have been incriminated to play a role in developmental psychopathology (Huttunen et al., 1994; van Os & Selten, 1998; Selten et al., 1999). However, prospective studies on the possible involvement of prenatal stress and anxiety are almost lacking in the fields of child psychiatry and developmental psychology.

9.2.1 Long-term effects of prenatal stressors: evidence from animal studies

There is a large body of animal studies to show that prenatal maternal stress may influence offspring behavior. For instance, prenatally stressed rats showed decreased exploration and more defecation (a measure of distress) in an open field test condition (Archer & Blackman, 1971). Long-term effects on behavior have also been demonstrated in prenatally stressed non-human primates. When studied in a novel environment, these infants exhibited more disturbance and fearful behaviors and reduced exploration (Schneider et al., 1992), showed more mutual clinging (Clarke & Schneider, 1993), had less social interactions, and withdrew more often from social interactions (Worlein & Sackett, 1995). Some of the alterations in infant behavior seen after prenatal exposure to noise stress (impairments in attention, and temperament) could also be induced by endocrine activation of the pregnant monkey by means of a 2-week period of adrenocorticotrophic hormone (ACTH) administration (Schneider, 1992a). It has, therefore, been suggested that the maternal hypothalamic-pituitary-adrenal (HPA) axis is one of the mediators of the effects on the developing brain of prenatal stress (Barbazanges et al., 1996).

9.2.2 Long-term effects of prenatal human stressors

Although the belief that the emotional state of the mother may affect the child she is carrying has a very long history (Ferreira, 1965), only recent studies provided some evidence that maternal stress in pregnancy is associated with increased risk for preterm delivery, low birth weight, and a smaller head circumference as a measure of brain development (Dunkel-Schetter, 1998; Wadhwa et al., 1993; Pagel et al., 1990; Hedegaard et al., 1993; Copper et al., 1996; Lou et al., 1994). A few prospective studies have examined the effect of prenatal maternal stress or anxiety on infant behavior. Van den Bergh (1990), in a study on 70 healthy pregnant women, found a significant relationship between high maternal state and trait anxiety scores in late pregnancy and a difficult temperament in 10-week-old and 7-months-old infants. DiPietro et al. (1996) showed that a particular index of fetal maturation predicted

22-60% of the variance in infant temperament scores, such as difficulty, unadaptability, and activity, at the age of 3 and 6 months. Although the latter study did not include maternal stress measures, these findings suggest the existence of some prenatal antecedents of human postnatal behavior.

A difficult aspect of studying stress in human pregnancy is the operationalization of the concept of prenatal stress. Therefore, in the present study we used a multidimensional model of distress in pregnancy. This model includes various stress-provoking factors (life events, secondary appraisal of pregnancy, pregnancy-related anxiety, neuroticism) and a stress-mediating factor (coping) that contribute to the amount of distress a pregnant woman perceives (Huizink et al., 2000b). Neuroticism was found to be the strongest predictor of distress. This model reflects a more detailed picture of prenatal stress than was given in the study of Van den Bergh (1990). Previous studies suggested that distress and daily hassles may be separable concepts, and that pregnancy-related anxieties may be regarded as stress-provoking factors specifically related to pregnancy (Huizink et al., 2000a; Huizink et al., 2000b). Thus, besides a multidimensional concept of distress, daily hassles and pregnancy-related anxieties were regarded as prenatal predictors of infant temperament. In addition, we focused on the effects of perceived stress, which may be regarded as a very subjective measure of prenatal stress, on infant temperament.

9.2.3 The role of possible confounders in the study of prenatal stressors

Various factors, both prenatal and postnatal, complicate the study of possible effects of prenatal stressors on infant behavior. Prenatal confounders include, for instance, smoking and alcohol intake during pregnancy, SES and maternal age (Creasy, 1991; Landry et al., 1997; Pollock, 1996; Hemminki & Gissler, 1996; Fergusson & Lynskey, 1993; Trasti et al., 1999; Makin et al., 1991; Frydman, 1996; Naeye & Peters, 1984; Kaplan-Estrin et al., 1999; Gusella & Fried, 1984; Jacobson et al., 1993). Unlike animal studies, it is impossible to control for the postnatal environment in which the child is raised. Several lifestyles may contribute to the infants' behavior and the mother-infant interaction is likely to influence at least some aspects of the infant's behavior (Cohn & Tronick, 1989; Field, 1992; Lyons-Ruth et al., 1990). If the mother is highly stressed in the postnatal period, her behavior may influence the reactions of her infant and her perceptions of the infant may differ from those of low postnatally stressed mothers. Other environmental factors that may influence infant behavior are family and social risk factors, such as one parent families and low SES.

9.2.4 Infant temperament

Although researchers have focused for many years on environmental variables that are responsible for a child's development and behavior, lately the focus has been shifted to interest in how children's own tendencies affect transactions with caregivers and consequently their own personality development. These behavioral tendencies are often described in terms of temperament (Bates, 1989). The most general definition of temperament is that it consists of biologically rooted individual differences in behavior tendencies that are present in early life and are relatively stable across various kinds of situations and over the course of time (Bates, 1986; Goldsmith et al., 1987). Furthermore, there is general agreement that temperament is manifest largely in the context of social interaction. The concept of temperament is not just a general concept, but also describes specific aspects of individual differences in behavior. Several constructs are frequently mentioned in the literature on temperament, including difficultness, adaptability, and attention regulation. Difficultness refers to a negative mood, withdrawal, high intensity and low regularity of biological rhythms. Adaptability reflects adaptation to novelty, thus individual differences in responding to new people and other new stimuli. Finally, attention regulation reflects the attention span and task persistence (Bates, 1989).

Evidence suggests that temperamentally difficult infants may be at risk for later adjustment problems (Bates et al., 1985; Wolkind & De Salis, 1982; Rutter et al., 1964). In a longitudinal-epidemiological study Caspi et al. (1996) showed that behavioral differences among children in the first 3 years of life are linked to specific adult psychiatric disorders, such as anxiety and mood disorders, antisocial personality disorder, recidivistic and violent crime, alcholism, and suicidal behavior. Thus variations in early temperamental characteristics may be an early sign of or a risk factor for the development of psychopathology in children.

9.2.5 Aims of the study

The present study analyzed the effects of prenatal maternal stress and anxiety on both infant temperament reported by the mother and objectively observed behavior in a standard novel situation. In line with the findings of animal studies, we hypothesize that prenatal stress and anxiety is related to more difficult behavior of the child and to more problematic adaptation to a novel situation. Moreover, we expect less exploratory behavior in prenatally stressed infants in a standard novel test condition, less attention during the test, and more problems in the social interaction with the testleader. Schneider et al. (1999) showed in a study on the ontogenetic vulnerability for prenatal stress in nonhuman primates, that sensitivity to prenatal stress may peak during early gestation and tapers off during mid-late gestation. Therefore, we used planned comparisons for prenatal stress effects on infant temperament derived during early and explored the effect of mid- and late pregnancy stress. The possibly mediating role of the maternal HPA axis was also explored. The following questions were addressed:

1. Does maternal stress or anxiety in early pregnancy have a negative effect on behavioral aspects of the infant at the age of 3 and 8 months?
2. Which aspects of prenatal stress or anxiety have negative effects on behavioral aspects of 3- and 8-months-old infants?
3. Are indices of maternal HPA axis activity (cortisol and ACTH) related to behavioral aspects of 3- and 8-months old infants?

9.3 Methods

9.3.1 Participants

All participants in this study were included in a large prospective longitudinal project which investigated the influence of prenatal psychosocial factors on fetal behavior and the postnatal development of children. Subjects were recruited from a consecutive series of referrals to the Outpatient Clinic of the Department of Obstetrics of the University Medical Center Utrecht (UMCU), which is a first-line referral center for low-risk pregnancies with responsibilities for mid-wives as well, between January 1996 and July 1998. The UMCU is located outside the city of Utrecht and attracts a mixed rural and urban population of patients. From a total of 650 invited women, 230 agreed to participate. The main reason for refusing to participate was the time-consuming aspect of the prenatal part of the study, which included ultrasound recordings of the fetus. The study was approved by the ethical committee of the UMCU; participation was on a voluntary basis but written informed consent was required. Only nulliparous women with a singleton pregnancy were included. Characteristics of participants, such as maternal age, socio-economic status, and biomedical risks did not differ from those of non-participants. However, women with full-time jobs were less likely to participate.

Participants were asked to fill out questionnaires three times during pregnancy; at 15-17 weeks (early pregnancy), 27-28 weeks (mid pregnancy), and 37-38 weeks of gestation (late pregnancy). Of the 230 women who completed the questionnaires on the first occasion, 217 completed the questionnaires on the second occasion and 172 on the third occasion.The main reason for the drop in the number of participants towards late pregnancy was delivery before 37 weeks of gestational age or delivery before the last session of data collection, which was planned near term, had taken place; other reasons were lack of interest, lack of time, stillbirth, pregnancy complications that required intensive follow-up, or relocation to another city.

Only healthy infants born near term (> 37 completed weeks of gestation) were included in the follow-up study after birth to remain free from confounding factors involved with prematurity or health problems of the infant. The total number of participants, both the mothers and their infants, who completed the postnatal part of the study, which included an examination of the infants' development at 3 and 8 months of age, was 170. The sample of participants consisted largely of caucasian middle class women, although both lower and higher social classes were represented (Table 9.3). On average, the women were 31 years old. The majority of women (93.7 %) lived together with their partner, either in wedlock or unmarried. Furthermore, at the time of their inclusion in the study, the majority of women had a paid job (87.4 %), 55.3 % working less than 38 hours a week and 44.7 % working full-time. Of the infants, 84 were boys and 86 were girls.

Postnatally, at the age of 3 months and the age of 8 months, the Bayley Developmental Scales was performed on the infants, while the mother was asked to fill out questionnaires on the infant's behavior and her own stress level.

9.3.2 Measures

9.3.2.1 Psychosocial predictors

To predict infant development we used four aspects of prenatal maternal stress, namely daily hassles, pregnancy anxiety, perceived stress, and distress, which were only moderately intercorrelated (r ranging from -.03 to .26), except for the correlation between perceived stress and distress: r = .41, p <. 005). First, the frequency of daily hassles was assessed with a daily hassles questionnaire, which is a Dutch translation of a selection of items of questionnaires (Alledaagse Problemen Lijst, Vingerhoets et al., 1989), including the Daily Hassles Scale (Kanner et al., 1981), the Everyday Problem Scale (Burks & Martin, 1985) and the Daily Life Experience Questionnaire (Stone & Neale, 1982). It measures the frequency of occurrence of daily hassles in the past month and gives an intensity score which is the subjective experience of the subject of the unpleasantness of the hassles. Examples of items are: 'You could not find important belongings', 'You were trapped in a traffic jam'. In this study, only the frequency score was used in order to stay free from confounding stress provoking and stress resulting factors in the intensity score. In early, mid, and late pregnancy, the frequency of daily hassles was calculated and used as a predictor variable.

Second, pregnancy-related anxiety was shown to reflect another aspect of the emotional status of pregnant women (Huizink et al., 2000a) and was therefore included as predictor of infant development as well. Pregnancy-related anxieties were assessed by means of the Pregnancy Related Anxieties Questionnaire-Revised (PRAQ-R), an abbreviated version of the PRAQ developed by Van den Bergh (1990). We used two subscales in the present study: fear of giving birth (3 items) and fear of bearing a physically or mentally handicapped child (4 items). 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'. This questionnaire was filled out in early, mid, and late pregnancy. The Cronbach's alpha's of the subscales were all > .76 throughout pregnancy.

Third, perceived stress was assessed by means of a Dutch translation of the Perceived Stress Scale of Cohen & Williamson (1987). It contains 14 items on perceived stress of an individual during the last month to be answered on a 4-point scale, ranging from 'never' to 'always'. This scale reflects a very subjective measure of stress and is a part of the higher-order construct of distress. This questionnaire was filled out on each occasion. For the present study, we used only the score derived in early pregnancy.

Fourth, a higher-order distress score was calculated for each period of pregnancy based on multidimensional models of prenatal distress (Huizink et al., 2000b). In short, the distress concept in early pregnancy involved a life event impact score, neuroticism, perceived lack of control over the course of pregnancy, and emotion-focused coping. In mid pregnancy, the life event impact score, neuroticism, daily hassles, and pregnancy-related fears (fear of giving birth and fear of bearing a handicapped child) explained significant parts of the variance in distress. In late pregnancy, the life event impact score, neuroticism, fear of bearing a handicapped child and problem-focused coping were part of the distress concept. Throughout pregnancy, neuroticism was the strongest predictor of distress.The factor score of distress was calculated with LISREL and used as a predictor variable.

9.3.2.2 Endocrinological predictors

In addition to the psychosocial predictors of infant development, we assessed two endocrinologic parameters to determine the relationship between the maternal HPA axis activity during pregnancy and the postnatal development of the infants. Cortisol was measured by determining the concentration of salivary cortisol which has been proven to be a valid and reliable reflection of the unbound hormone in blood (Kirschbaum & Hellhammer, 1989; Meulenberg & Hofman, 1990). Seven saliva samples were collected every two hours between 8:00AM and 8:00 PM, to obtain cortisol day time curves in each of the three periods of pregnancy. All samples were stored at -70^{0}C until assayed. Cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2]-^{3}H(N)-Hydrocortisone (NET 185, NEN-DUPONT, Dreiech, Germany) was used as a tracer following chromatic verification of its purity. The lower limit of detection was 0.5 nmol/L and interassay variation was 11.0%, 8.2%, and 7.6% at 4.7, 9.7 and 14.0 nmol/L, respectively (n = 20). Reference values for adults are 4-28 nmol/L at 8:00 AM. For each cortisol day profile, the mean, and early morning (8 AM) values were chosen to reflect a part of the maternal HPA axis activity.

ACTH. At 24 and 32 weeks of gestation, 30 ml of venous antecubital blood was collected for assessment of ACTH in a subsample of subjects. For the present study, we only used the ACTH collected at 24 weeks of gestational age as predictors of infant temperament (n= 41). ACTH was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 1.0 ng/L and inter-assay variation was 11.4 %, 10.7 % and 6.8% at 11, 68 and 310 ng/L respectively (n = 32).

The correlation coefficients between the psychosocial predictors and cortisol measures assessed in early pregnancy were calculated. No significant associations were found.

9.3.2.3 Infant measures of temperament

The infants were tested in a standard novel situation at 3 and 8 months of age. The Bayley Scales of Infant Development were performed by the first author, or by a trained junior researcher in a standard testing room and provided a mild challenging situation for the infant, since the testleader, the test session and the environment were novel situations to cope with. The testleaders were blinded for the prenatal stress data of the mothers. A temperament questionnaire and two stress questionnaires were filled out by the mother either during the test, or within a week at home. The data gathered during these sessions and analyzed in the present study included.

9.3.2.4 Infant Temperament

Infant temperament at 3 and 8 months was assessed by a Dutch translation of the Infant Characteristics Questionnaire (ICQ; Bates et al., 1979), which was filled out by the mother. The factor structure of the Dutch ICQ has been studied over different ages and reference data have been collected. At the age range 5-6 and 12-14 months, factors were derived that were closely similar to the factors of the original ICQ of Bates (1979). Difficult behavior was assessed by 10 items, unadaptability ws assessed by 5 items. Data was quantified in two factors of Difficult and Unadaptable behavior based on scoring procedures developed through factor analyses by Kohnstamm (personal communication). Cronbach's alpha's of the factors were all > .76 in the present study.

9.3.2.5 Observed behavior of the infant

The behavior of the infants during developmental testing (BSID) was assessed by means of ratings by the testleader on the Infant Behavior Record (IBR), the third component of the Bayley Scales (Bayley, 1969). The ratings were mainly performed by the first author, with contributions from a junior researcher, who was trained by the first author to achieve an interrater reliability of > .85. This test session took place in a standard novel situation. The IBR consists of a number of descriptive rating scales for behavior characteristics of infants up to 30 months of age. The scales included in the present study reflected: exploration, goal-directedness, and test-affectivity. These scales were derived by a factor analytic approach which identifies the IBR scales that cluster together (van der Meulen & Smrkovsky, 1983, 1984).

9.3.2.6 Potential covariates

Data was gathered on various other aspects besides prenatal stress that may influence infant development. Descriptives of these potential covariates are shown in Table 9.1.

Prenatal factors included maternal age (in years), socio-economic status (SES), smoking and alcohol-intake during pregnancy, and biomedical risks. *SES* was defined by educational level and professional level of the pregnant woman and her partner (Westerlaak et al., 1976). *Smoking behavior* was assessed by self-report, expressed as the number of cigarettes per day (cig/day) and categorized in three groups: 1) non-smokers; 2) smoking 1-10 cig/day; 3) smoking > 10 cig/day. The latter group consisted of only 7 subject, and therefore a dichotomous variable was created: 1) non-smokers (n= 141); 2) smokers (n= 29); >= 1 cig/day. *Alcohol-intake* during pregnancy was likewise determined by self-report, and was expressed as the number of alcohol-containing beverages per week. Only 11 subjects consumed more than 2 alcohol-containing beverages per week and therefore a dichotomous variable was created: 1) non-drinkers (n= 144); 2) drinkers (n = 26); > = 1 drink per week. Our sample thus contained very few heavy smokers or drinkers, and only a relatively small number of modest smokers and drinkers. *Biomedical risk factors* included the use of medication during pregnancy, pre-existent health problems, high bloodpressure, fertility problems, gynecological risk factors (DES daughters etc), excessive vomitting and diabetes mellitus caused by pregnancy.The risk factors were added up in a categorical variable; scores ranged from 0 - 5.

Perinatal covariates that may confound the effect of prenatal stress on infant development include birth weight (in grams) and gestational age at birth (in weeks). Also, complications during delivery, the use of medication during delivery, fetal distress, and mode of delivery (elective caesarean section or assisted delivery) were taken into account, by calculating a cumulative score of these perinatal complications (range 0-5).

Potentially postnatal covariates included in the present study are the postnatal stress levels of the mother. *Psychological well-being* was determined by means of the Dutch translation (Koeter & Ormel, 1991) of the General Health Questionnaire (GHQ-30; Goldberg, 1972). This questionnaire contains 30 questions to be answered on a four-point scale and was filled out on both postnatal occasions. *Perceived stress* was assessed with the Perceived Stress Scale of Cohen & Williamson (1988), using a Dutch translation. It contains 14 items on an individual's perceived stress over the last month to be answered on a 4-point scale, ranging from 'never' to 'always'. This questionnaire is the same as used during pregnancy and was filled out on both postnatal occasions.

Table 9.1

Descriptives of potential prenatal, perinatal and postnatal confounders

Confounders	
Prenatal	
Maternal age (years) ± SD	31.3 (4.9)
SES	**Educational level mother *** Low 13.6 % Middle 67.5 % High 18.9 % **Educational level partner** Low 23.4 % Middle 59.8 % High 16.8 % **Professional level mother** Low 8.0 % Middle 54.6 % High 37.4 % **Professional level partner** Low 18.0 % Middle 29.2 % High 52.8 %
Smoking	Smokers: n = 29; > = 1 cigarette per day Non-smokers: n = 141
Alcohol-intake	Drinkers: n = 26; > = 1 drink per week Non-drinkers: n = 144
Biomedical risks	No risk: n = 102 Pregnancy complications: n = 30 Medication during pregnancy: n = 25 Risk for fetus of medication: n = 4 Fertility problems: n = 48 IVF: n = 13 High bloodpressure: n = 15 Diabetus mellitus due to pregnancy: n = 3 Gynecological risk: n = 12 Pre-existent disease: n = 12 Mean score (± SD): 1 (1.2)
Perinatal	
Birth weight (grams) ± SD	3385 (487)
Gestational age at birth (weeks) ± SD	39.6 (1.9)
Perinatal complications	Partus complications: n= 25 Medication during delivery: n=88 Elective caeserean section: n= 24 Artifical delivery due to fetal distress: n=20 Mean score (± SD): 1 (1.3)
Postnatal	
Psychological well-being (GHQ-30) ± SD	3 months postpartum: 4.7 (5.1) 8 months postpartum: 3.6 (5)
Perceived stress ± SD	3 months postpartum: 25.9 (5.8) 8 months postpartum: 25.5 (5.7)

* low level: primary school, high-school education; middle level: secondary school education; high level: college or academic education

9.3.3 Statistical Analysis

First, descriptive analyses were performed on all dependent variables: difficulty, unadaptability, test-affectivity, goal-directedness and exploration scores assessed in 3- and 8-months-old infants. Second, possible categorical or interval scaled covariates (SES, maternal age, gestational age at birth, birth weight, postnatal stress of the mother) were tested for their linear relationship with the dependent variables by means of correlations (Pearson or Spearman when appropriate) and regression analysis. If covariates were significantly related to the dependent variables, they were included in the next steps of analysis. Since we expected to find a small and possibly nonlinear effect of prenatal maternal stress in our sample of healthy fullterm infants, we formed two groups: one group of women who scored in the lower 25 % of a particular questionnaire and one group of women who scored in the upper 25% of that questionnaire. Thereby, high/low (P75/P25; P= percentile) contrasts were set on the predictors and behavioral scores were compared by means of MANCOVA. For ACTH a median split method was used to form two groups, due to the small sample size. Two MANCOVAs were performed for each predictor; one including the observed behaviors at 3 and 8 months (test-affectivity, goal-directedness, exploration) and one including the maternal reports of difficulty and unadaptability at 3 and 8 months. Dichotomous covariates (smoking and alcohol-use during pregnancy, infants' sex) were entered as a factor in the MANCOVAs (see Figure 9.1 step A). Also, perinatal covariates were included in step A. When the MANCOVA showed an overall significant main effect by means of a significant Hotelling's T^2 test for prenatal stress, post-hoc univariate analyses were performed. Next, postnatal stress levels of the mother were added as covariates in subsequently performed ANCOVAs (see Figure 9.1 step B). If the latter were significant, a more detailed analysis of the effect of prenatal stress was performed by means of multiple linear regression analysis to test for a linear association between prenatal stress and temperament of the infant. With all tests, statistical significance was assumed at the level of $p < .05$.

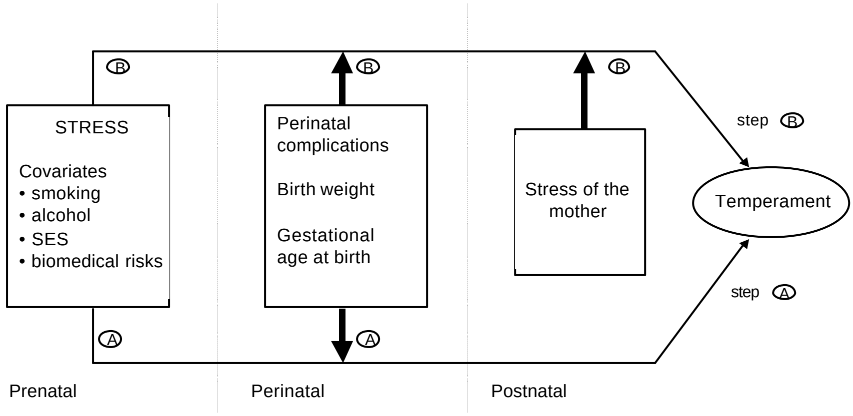

Figure 9.1. Steps in statistical analysis. Step A represents MANCOVA in which prenatal and perinatal confounders are taken into account. Step B is the next step of analysis in which postnatal stress of the mother is added as confounder in the ANCOVA.

9.4 Results

9.4.1 Descriptive analyses

Means, standard deviations and ranges in scores were calculated for all predictors and depending variables (Tables 9.2 & 9.3).

Table 9.2
Means, standard deviations (SD) and ranges in scores of the predictors in early pregnancy

Predictors	*Mean*	*SD*	*Range*	*N*
Psychosocial				
Daily hassles	9.96	6.3	0-45	170
Distress	-.02	1.0	-2.1-2.6	170
Perceived stress	28.07	5.49	18-48	170
Fear of giving birth	6.17	2.9	3-15	170
Fear of handicapped child	9.25	3.5	4-20	170
Endocrinologic				
Mean cortisol	10.57	2.3	5.2-19.8	142
Cortisol 8 AM	19.78	7.4	6-44	142
ACTH 24 weeks	16.95	9.3	5-44	43

Table 9.3

Descriptives of the dependent variables: Difficult behavior, Unadaptability and Reactivity (ICQ) and observed behaviors (IBR) at 3 and 8 months

	Mean (N=170)	*S.D.*	*Range in scores*
ICQ:			
Difficulty 3 months	27.1	6.9	11-46
Difficulty 8 months	29.7	7.1	13-56
Unadaptability 3 months	12.0	4.1	5-26
Unadaptability 8 months	12.3	4.3	5-39
Reactivity 3 months	12.1	2.1	4-18
Reactivity 8 months	12.2	1.9	6-18
Observed behaviors IBR:			
Activity 3 months	16.1	1.9	10-21
Activity 8 months	17.0	2.4	8-23
Exploration 3 months	9.6	3.6	3-18
Exploration 8 months	20.6	1.8	12-24
Test-affectivity 3 months	27.5	4.6	5-38
Test-affectivity 8 months	30.5	4.0	17-37
Goal-directedness 3 months	19.7	3.1	8-27
Goal-directedness 8 months	25.0	2.3	16-31

The two factor scores of infant temperament reported by the mother were significantly correlated in time. That is, difficult behavior at 3 month was positively associated with difficult behavior at 8 months (r = .52, p < .0005), and unadaptability at 3 months was related to unadaptability at 8 months (r = .41, p < .0005). Of the observed behaviors during the BSID, goal-directedness at 3 months was associated with goal-directedness at 8 months (r = .27, p<.001). Likewise, test-affectivity scores determined at 3 months were positively correlated with the test-affectivity scores at 8 months (r = .28, p <. 001).

Temperamental ratings of the infant by the mother were correlated with some observed behaviors. Difficulty of the infant at 3 months of age was negatively correlated with exploration of the infant at 3 months of age (r= -.16, p<.05). Unadaptability of the infant, reported by the mother, was negatively associated with exploration, test-affectivity and goal-directedness at both 3- and 8-months-old infants (r ranging from -.15 to -. 21; p's <.05).

Correlations between prenatal and postnatal stress levels were calculated and ranged from .23 to .61 (p's < .005), with the highest correlation coefficients between late pregnancy stress and stress determined at 3 months postpartum.

9.4.2 Preliminary analyses for potential covariates

Correlation coefficients were calculated between the infant temperament scores and observed behavior at 3 and 8 months and prenatal (SES, maternal age, biomedical risks), perinatal (gestational age at birth, birth weight and perinatal complications), and postnatal (mothers' stress levels) factors. Difficult behavior of the infant at 3 months of age was negatively correlated with birth weight ($r = -.15$, $p < .05$) and the educational level of the mother ($r = -.15$, $p < .05$). Difficult behavior of the infant at 8 months of age was negatively associated with the educational levels of the mother ($r = -20$, $p<.01$). Unadaptability of the infant at 3 and 8 months of age was negatively correlated with the professional level of the mother ($r = -.21$, $p<.01$ and $r = -.39$, $p< .0005$, respectively) and her educational level ($r = -.18$, $p<.05$ and $r = -.28$, $p < .005$, respectively). Unadaptibility of the infant at 8 months of age was furthermore negatively associated with maternal age ($r = -.17$, $p<.05$). Test-affectivity of the infant at 8 months of age was positively associated with the professional and educational levels of the mother ($r = .18$, $p<.05$ and $r = .16$, $p<.05$, respectively). No other linear relationships between potential covariates and the dependent variables were found. Multiple regression analysis showed independent effects on unadaptability at 3 and 8 months of the professional level of the mother ($F= 10.16$, $p < .01$ and $F= 21.64$, $p < .001$, respectively). This variable explained 6.3% and 12.5% of the total variance in the unadaptability scores at 3 and 8 months, respectively. Maternal educational level furthermore contributed to the model of unadaptability at 8 months of age ($F = 13.10$, $p < .005$, $R^2 = 0.02$). Other potential covariates did not significantly contribute to these models, and were excluded from further analysis.

There were no significant main effects or interaction effects with prenatal stress of the infant's sex on the infant's temperamental characteristics. Likewise, no main or interaction effects with prenatal stress and alcohol-intake on infant temperament were found. However, when several high/low contrasts were formed for the psychosocial predictors and the variable 'smoking' was added, a main effect for smoking was found in addition to a main effect of fear of giving birth on test-affectivity of infants at the age of 8 months ($F (1,93) = 4.12$, $p <.05$; non-smoking: 31.40 versus smoking: 28.46).

9.4.3 Infants' observed behavior in relation to measures of prenatal maternal stress

In this section, the test-affectivity, goal-directedness and exploration scores at 3 and 8 months were compared between infants of mothers who had low (<= P25) and high (>= P75) stress levels during pregnancy. This analysis was carried out using MANCOVA (including the observed behavior scores of the infants at 3 and 8 months of age) and univariate analyses for early pregnancy, to test for non-linear effects. For significant univariate tests, the postnatal stress levels of the mother were entered in a subsequently performed ANCOVA (see Figure 9.1 step B), followed by multiple regression analysis, to test for linear effects. The results are summarized in Table 9.4 and Figures 9.2 - 9.4.

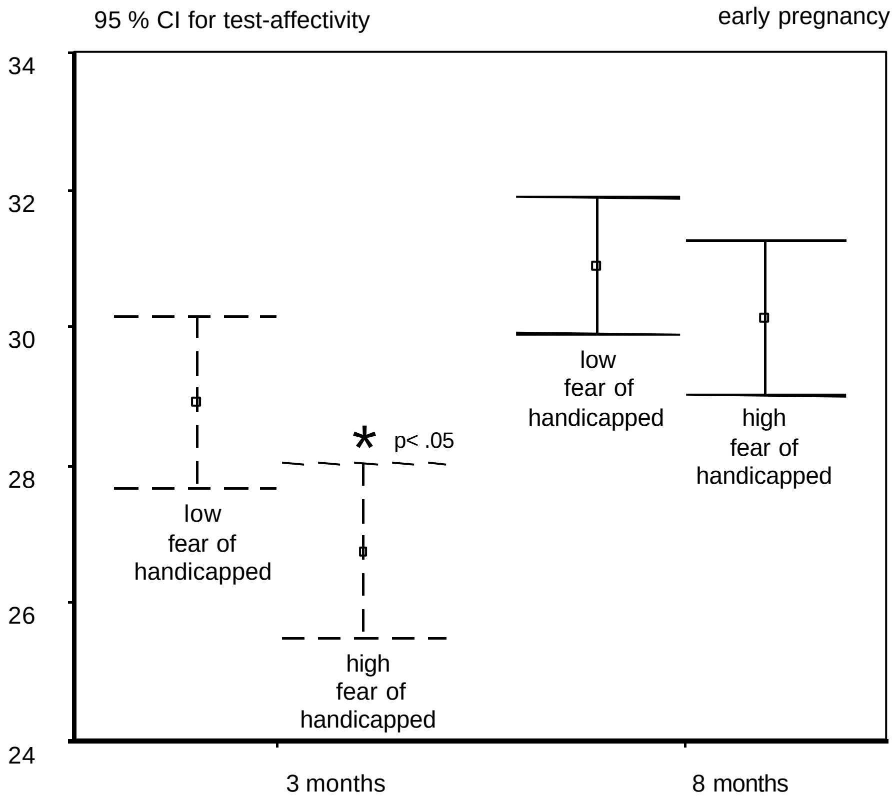

Figure 9.2. The effect of fear of bearing a handicapped child in early pregnancy on test-affectivity of infants at 3 and 8 months of age.

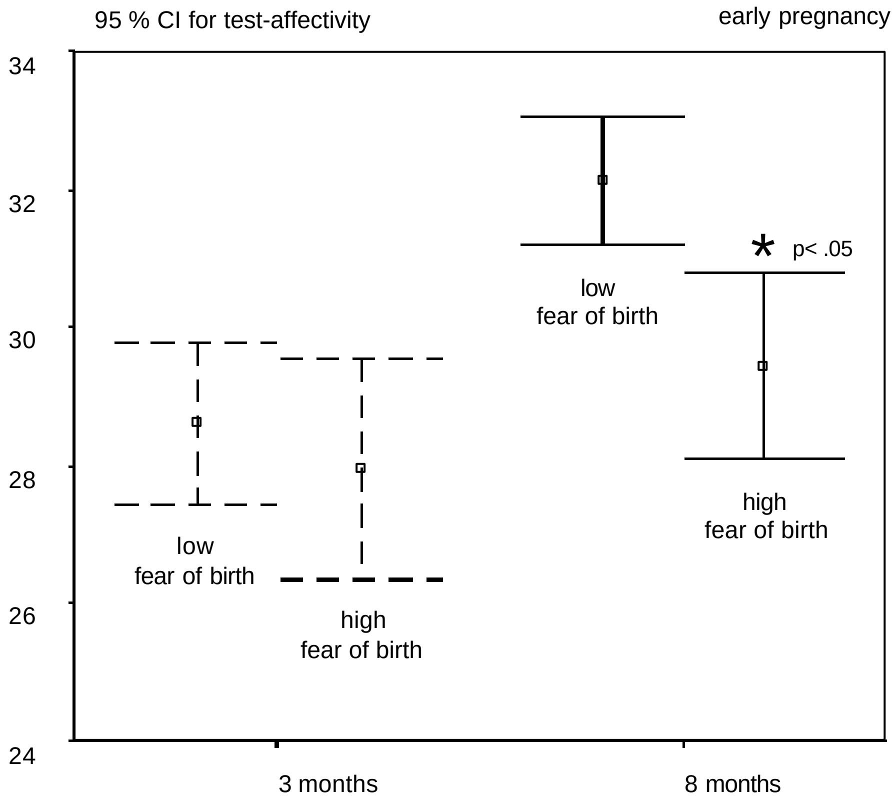

Figure 9.3 The effect of fear of birth in early pregnancy on test-affectivity of infants at 3 and 8 months of age.

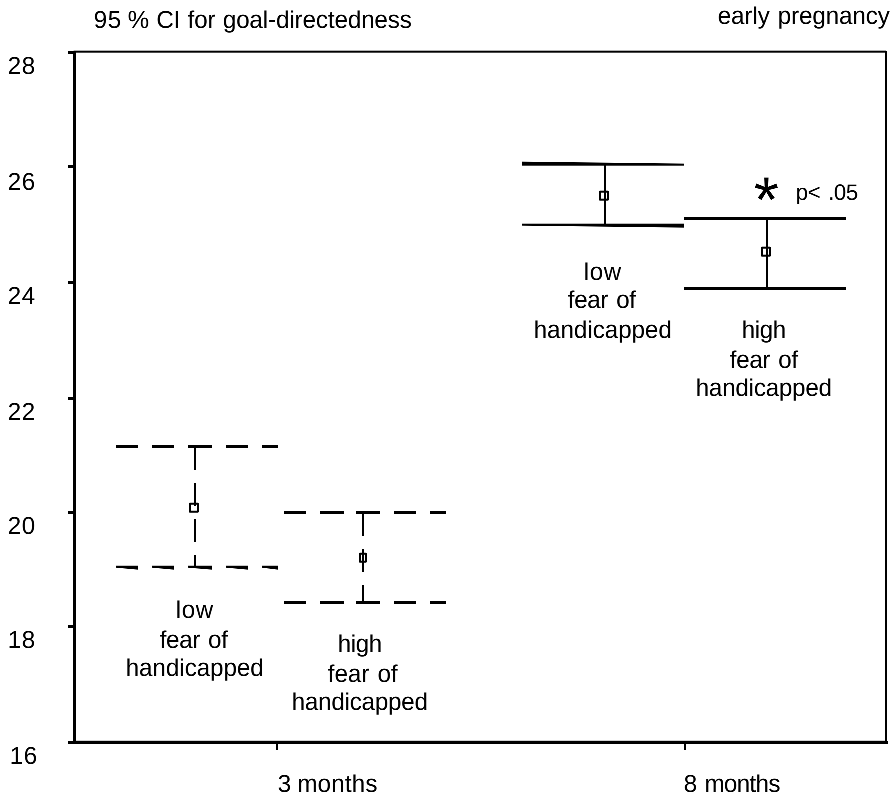

Figure 9.4 The effect of fear of bearing a handicapped child in early pregnancy on goal-directedness of infants at 3 and 8 months of age.

Table 9.4

Results of MANCOVAs, univariate analyses, ANCOVA corrected for postanatal stress and multiple regression analayses

	MANCOVA high/low contrast			*Univariate post-hoc analyses*			*Univariate post-hoc analyses*			*Multiple regression analyses*			
Predictors	*Behavior*	*F*	*P*		*F*	*P*		*F*	*P*	*ß*	*R^2 total*	*F*	*P*
Fear of	Exploration			3 months									
giving	Testaffectivity	2.74	<.05	--	--	n.s.	–	--	--	--	--	--	--
birth	Goal-directedness			8 months			8 months						
				testaffectivity	11.38	<.001	testaffectivity	6.39	<.05	-.21	.07	2.73	<.05
Fear of	Exploration			3 months			3 months						
handicap-	Testaffectivity	2.87	<.05	testaffectivity	5.93	<.05	testaffectivity	5.85	<.05	--	--	--	n.s.
ped child	Goaldirectedness			8 months goal-	6.27	<.05	8 months goal-	5.49	<.05	-.23	.07	2.65	<.05
				directedness			directedness						
Perceived	Exploration			3 months									
stress	Testaffectivity	2.45	<.05	--	--	n.s.	–	--	--	--	--	--	--
	Goaldirectedness			8 months explo-				--	n.s.	--	--	--	--
				ration	7.80	<.01							
Perceived	Difficulty	4.44	<.005	3 months			3 months						
stress	Unadaptability			difficulty	12.32	<.001	difficulty	9.02	<.005	--	--	--	n.s.
				unadaptability	7.80	<.01	unadaptability	7.1	<.05	.28	.12	5.46	<.01
				8 months			8 months						
				difficulty unadapt-	8.11	<.01		--	n.s.	--	--	--	--
				ability	4.55	<.05							
ACTH 24	Difficulty	3.19	<.05	3 months		n.s.	3 months						
week	Unadaptability			difficulty	--	n.s.		--	--	--	--	--	--
				unadaptability			–						
				8 months			8 months						
				difficulty	--	n.s.	–	--	n.s.	--	--	--	--
				unadaptability	5.73	<.05	unadaptability	6.8	<.05	.37	.29	6.12	<.01

MANCOVA showed an overall significant effect of fear of giving birth (F(6,89) = 2.74, p <.05), fear of bearing a handicapped child (F(6,89)= 2.87, p<.05) and perceived stress (F(6,88) = 2.45, p<.05) on the observed behaviors exploration, test-affectivity, and goal-directedness. Subsequently performed univariate analyses showed that test-affectivity was declined in 3-months-old infants after a high level of fear of bearing a handicapped child (F(1,84)= 5.93, p <.05), whereas in infants at 8 months of age, a negative effect on test-affectivity was found for fear of giving birth (F (1,84)=11.38, p <.001). Goal-directedness of 8-months-old infants was reduced in infants of mothers with high levels of fear of bearing a handicapped child (F(1,84)= 6.27, p<.05). Exploration of 8-months-old infants was decreased after exposure to high levels of maternal perceived stress.

After adding postnatal maternal stress levels (see Figure 9.1, step B) only to the significant univariate tests, the negative effects of prenatal stress on test-affectivity and goal-directedness were slightly adjusted and the significant effects of prenatal stress could still be found (see Table 9.4, fourth column), except for the effect of prenatally perceived stress on exploration.

Multiple regression showed a linear negative effect of fear of giving birth on test-affectivity and of fear of bearing a handicapped child on goal-directedness of 8-months-old infants, both explaining 7 % of the total variance (see Table 9.4, fifth column).

No effect on observed behavior was found for daily hassles or distress in early pregnancy. Exploratory analysis with stress measures from mid- and late pregnancy were only carried out for the significant effects found of stress in early pregnancy, after controlling for postnatal stress. Only a trend toward a significant differences in test-affectivity and goal-directedness of infants at 8 months of age was found for fear of giving birth and fear of bearing a handicapped child in mid-pregnancy, respectively. No effects were found for pregnancy related anxiety determined in late pregnancy.

9.4.4 Infants' temperament rated by maternal report in relation to measures of prenatal maternal stress

In this section, analyses are presented analogous to those performed above. The results are presented in Table 9.4 and Figures 9.5 and 9.6.

An overall effect of perceived stress was found with MANCOVA on difficulty and unadaptability at the age of 3 and 8 months (F(4,81)=4.44, p<.005). Univariate analyses showed that more difficult and unadaptable behavior was found in infant at both 3 and 8 months of age (Table 9.4). After correction for postnatal stress levels of the mother, the effects of prenatal stress on difficulty and unadaptability of 8-months-old infants was no longer found, whereas the effect on difficulty and unadaptability of 3-months-old infants remained significant (Table 9.4, fourth column).

Multiple regression showed a linear negative effect of perceived stress on unadaptability at 3-months, explaining 12% of the total variance.

No effect was found for daily hassles, distress, or pregnancy anxiety on temperamental ratings of the infant by the mother. Exploratory analysis carried out with perceived stress in mid- and late pregnancy showed no significant effect on the temperament of the infant.

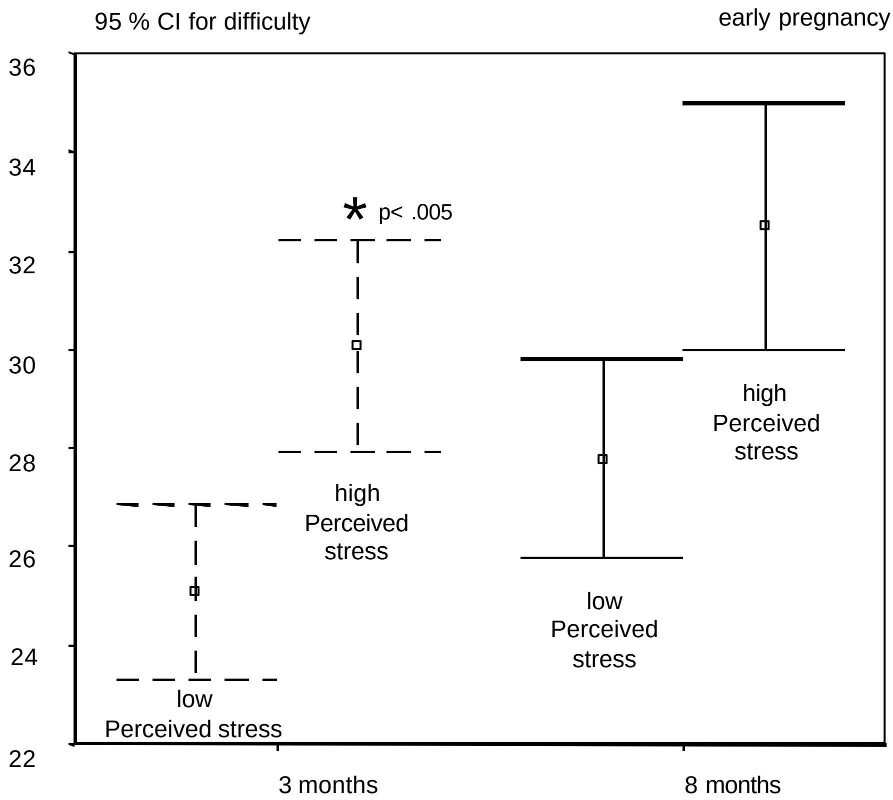

Figure 9.5 The effect of perceived stress in early pregnancy on difficulty based on maternal report of infants at 3 and 8 months of age.

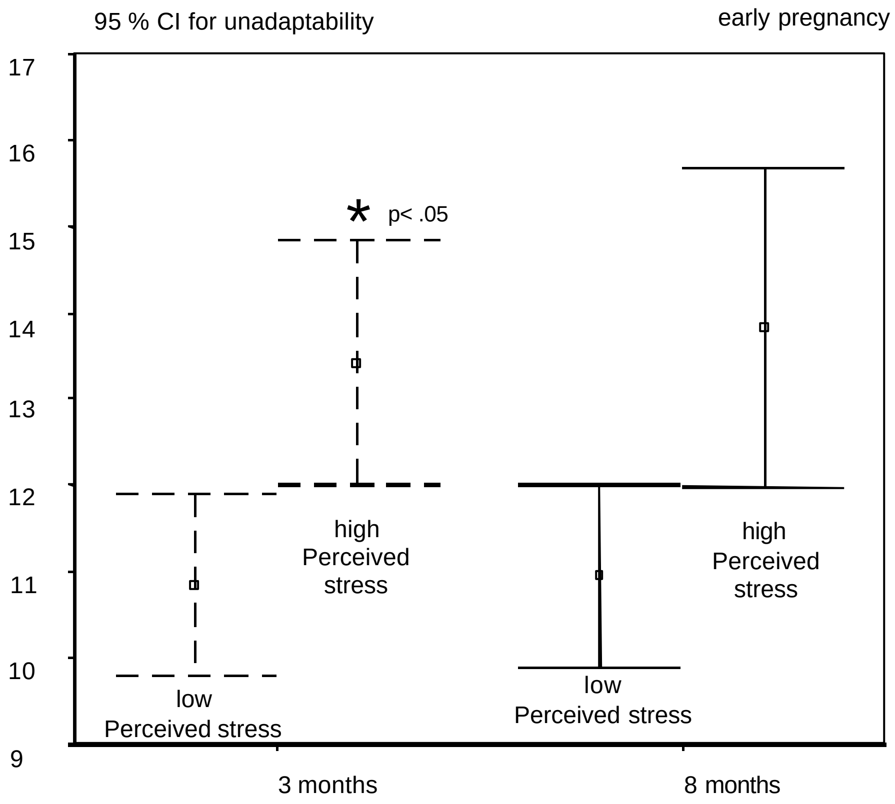

Figure 9.6 The effect of perceived stress in early pregnancy on unadaptability of the infants at the age of 3 and 8 months based on maternal report.

9.4.5 Infants' observed behavior and temperament in relation to measures of maternal HPA axis activity during pregnancy

The results are presented in Table 9.4 and Figure 9.7.

MANCOVA showed an overall effect of maternal ACTH content at 24 weeks of gestation on difficulty and unadaptability of infants of 3 and 8 months of age ($F(1, 41)= 3.19$, $p <.05$). Subsequently performed univariate analyses showed that the effect on unadaptability of 8-months-old infants was significant ($F(1,41)= 5.73$, $p<.05$). After correction for postnatal stress, the effect of ACTH remained significant ($F(1,41)=6.80$, $p<.05$).

It was further found that after correction for the educational level of the mother and the postnatal level of stress, 14.6 % of the total variance in unadaptability at the age of 8 months could be attributed to the level of ACTH at 24 weeks of gestation ($F (1,41)= 6.12$, Beta = .37, p < .01). In total, 29.0 % of the variance was explained by ACTH and the mothers' educational level. Postnatal stress of the mother did not significantly contribute to this model. No other significant contributions of ACTH and no contributions of cortisol to infant temperament or observed behavior were found.

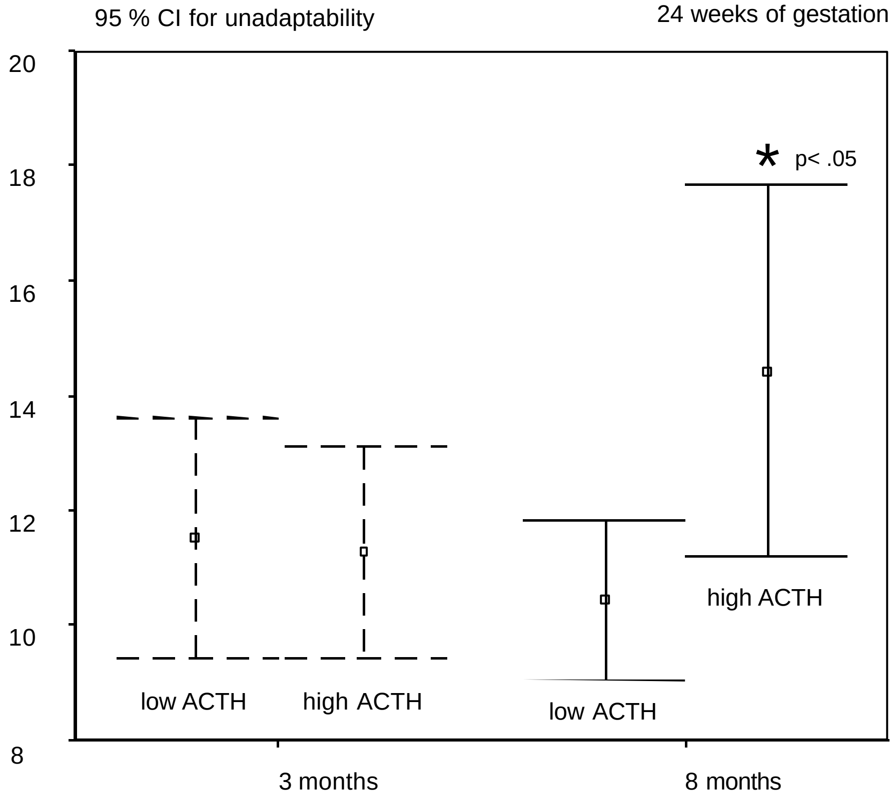

Figure 9.7 The effects of ACTH in plasma at 24 weeks of gestation on unadaptability of the infant at 3 and 8 months of age based on maternal report..

9.5 Discussion

The present study showed that various aspects of prenatal maternal stress or anxiety had a negative effect on the behavior infants at age 3 and 8 months. Pregnancy-related fears were related to a decrease in attention regulation during a standard test situation, as was evident from changes in test-affectivity and goal-directedness. Levels of perceived stress and ACTH were associated with more problems in adaptation to a new situation or to the presence of unfamiliar persons. These findings are in line with animal studies which showed problems in coping with novel situations in offspring of prenatally stressed rats (Archer & Blackman, 1971; Deminière et al., 1992) and nonhuman primates (Schneider, 1992a) and more attentional problems after prenatal stress (Schneider, 1992b). Contrary to our expectations we did not find an effect on observed exploration of the infant during a standard test situation, after we corrected for the postnatal stress levels of the mothers. Although the temperamental characteristic unadaptability was determined by maternal report and thus may be confounded by rater bias, the finding that ACTH was also related to more adaptational problems of the infant suggests that prenatal stress may indeed have an effect on temperament of the infant.

Effects of early pregnancy stress on observed behavior of infants were found at age 8 months rather than at 3 months. This is an interesting finding, when we realize that around the age of 8 months infants start to develop a greated awareness of and interest in the outside world. As a result of this and of concomitant cognitive development as well, infants around 8 months generally are more sensitive to unfamiliarity than at earlier ages. This may be apparent, among others, from increased stranger anxiety. Thus, the test situation at 8 months likely has been much more challenging for the infant than that at 3 months, and therefore may have resulted in a more pronounced effect of prenatal maternal stress on the test-affectivity and goal-directedness of the infant. In contrast, the effects of perceived stress in pregnancy were only significant for 3-months-old infants and were less clear when the infant had reached the age of 8 months. This finding argues against the idea that perceived stress in pregnancy merely reflects a general stress level of women which may influence her perception of the infant. In that case, her infant would also have been significantly more difficult and unadaptable at 8 months.

It is noteworthy to mention that in early pregnancy daily hassles nor the multidimensional construct distress had an effect on infant temperament. Others have suggested that daily hassles could be potentially more harmfull than other stressfactors (DeLongis, 1982; Paarlberg et al., 1999). Our sample consisted of a population of relatively normal risk pregnant women, mostly from a middle class socioeconomic status. Perhaps, the amount of daily hassles in our sample was therefore relatively low. The fact that the prenatal distress level was not associated with temperamental scores of the infant argues against the interpretation that maternal characteristics are mainly responsible for the temperamental scores, since a main predictor of the multidimensional concept of prenatal distress was neuroticism (Huizink et al., 2000b). Pregnancy-specific fears were the most powerful predictors of postnatal behavior of the infant in the present study, and were also found to have an effect on mental and motor development of infants in another study (Huizink et al., 2000a). Moreover, these fears have been found to predict adverse pregnancy outcome (Killingsworth Rini et al., 1999). Clearly, these anxieties of pregnant women deserve more attention in future studies.

It may be that periods of vulnerability exist for behavioral impairments associated with prenatal stress or anxiety. A study of Schneider et al. (1999) showed that in nonhuman primates early gestation stress was associated with more pervasive motor impairments than mid-late gestation. Huttunen (1988) reported a significant relation between maternal stress during the first trimester of pregnancy and temperamental features of the infant (slow adaptability, negative mood and easy distractibility), whereas second and third trimester stress were unrelated to infant temperament. We therefore tested for behavioral problems in infants following early prenatal stress. Our results suggest that for the observed behaviors such as test-affectivitiy, goal-directedness, and the temperament aspects difficulty and unadaptability stress in early pregnancy is harmful, whereas mid- and late prenatal stress did not result in significant effects. ACTH exhibited an effect on postnatal behavior when the concentrations of 24 weeks gestation were analyzed, thus suggesting that mid-pregnancy may be a period with increased vulnerability for increased HPA-axis activity. However, several limitations of the present study warn against strong conclusions. First, we gathered no endocrinologic data on the very early period of pregnancy, which may be especially sensitive to small variations in the fetal environment, since this is the period of neural migration and for instance teratogen exposure during this period can induce gross irreversible malformations. Second, to study the possible vulnerable periods more precisely in pregnancy for prenatal stress effects on the infants, a different design should be used. For instance, exposure to a stressor by nature like the explosion of a fireworks storage located in a residential area in a town of the Netherlands (Enschede) in only early, mid or late pregnancy may result in different outcomes of infant behavior when these three groups divided by the timing of exposure are compared. Third, our stress measures throughout pregnancy were not independent but were correlated over time, which could have biased our results.

With regard to the possibly mediating role of the HPA- axis activity of pregnant women on postnatal behavior it was shown that increased levels of ACTH at 24 weeks of gestation were linearly related to unadaptability at 3 months and explained almost 15 % of this behavior. Thus, the HPA-axis may indeed play a part in explaining the effects of prenatal stress on postnatal behavior in human infants, as has been shown before in animal studies (Weinstock et al., 1997). Evidence is found for an altered HPA axis reactivity in prenatally stressed offspring (Weinstock et al., 1992; McCormick, 1995; Fride et al., 1986), which may result in a general vulnerability for psychopathology later in life (Huizink et al., 2000c). Reactivity of the prenatally stressed infants' HPA axis to novelty or more challenging situations may shed more light on the difficult issue of pathophysiological mechanisms causing the effect of prenatal stress on later altered behavior. However, the exact mechanism by which prenatal distress, prenatal perceived stress or prenatal pregnancy-related anxieties could result in altered postnatal behavior remains rather unclear, since the psychosocial and endocrinologic measures of prenatal stress were unrelated in early pregnancy.

Certain personality factors of the mothers may influence the mother-infant-interaction after birth which could in turn affect the infants' behavioral development. For instance, postnatally depressed women are less responsive and sensitive to their children (Cohn & Tronick, 1989; Field, 1992; Lyons-Ruth et al., 1990) which has an effect on the behavior of the child as well. Moreover, women high on prenatal distress have a tendency to have high overall levels of distress also after birth, which could alter their perception of their infant in a negative way, resulting in higher scores on difficult behavior and unadaptability. Therefore, we performed an analysis in which we controlled for postnatal stress levels and psychological well-being of the mothers. Our results showed that the effect of prenatal stress were declined when postnatal stress was taken into account, but in general the effects of prenatal stress were still noticeable. In human studies, these postnatal influences are hard to control for in an experimental design and it is hardly impossible to conclude from our results that prenatal factors are mainly responsible for a more difficult pattern of behavior in these young infants.

It is important to appreciate that the design of the present study does not allow to examine the relative contribution of genetic versus environmental versus gene by environment interactional influences on infant behavior. Infants could have a high genetic loading on temperamental difficulties, may encounter high environmental demands or may be influenced on a behavioral level by an interaction of these two aspects. Prenatal stress effects may increase the infants' vulnerability for genetic and environmental influences on behavioral problems. This line of research offers a challenge for future research.

The results of the present study show that variation in the exposure to relatively mild prenatal stress is associated with temperamental variation of the infant at 8 months of age. These changes may enhance the risk of developing later behavioral problems, such as anxiety and mood disorders, antisocial personality disorder, recidivistic and violent crime, alcholism, and suicidal behavior (Caspi et al., 1996). Therefore a follow-up study of the infants is warranted. In future studies, we will focus on detailed observation of the mother-infant interaction during a semi-structured play session and the Bayley Scales of Infant Development examination to remain free from the rater bias caused by the mothers'subjective perception of the infant when using parent reports and to perform fine-grained analysis on the (behavioral) mechanisms involved in the effects of prenatal stress on infant temperament.

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